Use of Exogenous Fibrolytic Enzymes to Improve Feed Utilization by Ruminants[1,2]

K. A. Beauchemin*[3], D. Colombatto*, D. P. Morgavi‡, and W. Z. Yang*

*Agriculture and Agri-Food Canada, Lethbridge, AB, Canada T1J 4B1 and ‡INRA, Centre Clermont-Theix, Unité de Recherche sur les Herbivores 63122 Saint-Genès-Champanelle, France

ABSTRACT: Research has demonstrated that supplementing dairy cow and feedlot cattle diets with fiber-degrading enzymes has significant potential to improve feed utilization and animal performance. Ruminant feed enzyme additives, primarily xylanases and cellulases, are concentrated extracts resulting from bacterial or fungal fermentations that have specific enzymatic activities. Improvements in animal performance due to the use of enzyme additives can be attributed mainly to improvements in ruminal fiber digestion resulting in increased digestible energy intake. Animal responses are greatest when fiber digestion is compromised and when energy is the first-limiting nutrient in the diet. When viewed across a variety of enzyme products and experimental conditions, the response to feed enzymes by ruminants has been variable. This variation can be attributed to experimental conditions in which energy is not the limiting nutrient, as well as to the activities and characteristics of the enzymes supplied, under- or over-supplementation of enzyme activity, and inappropriate method of providing the enzyme product to the animal. A limited number of ruminant enzyme products are now commercially available, and this list of products is expected to grow. However, random addition of enzymes to diets without consideration for specific situations and substrate targets will only discourage or delay on-farm adoption of enzyme technology. Although much progress has been made in advancing enzyme technology for ruminants, considerable research is still required to reduce the variability of response. With increasing consumer concern about the use of growth promoters and antibiotics in livestock production, and the magnitude of increased animal performance obtainable using feed enzymes, there is no doubt that these products will play an increasingly important role in the future. This paper reviews the research on enzyme selection, the animal responses to feed enzymes, and the mechanisms by which these products improve nutrient utilization.

Key Words: Cellulase, Cellulose Digestion, Digestion, Enzymes, Fiber, Ruminants

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Introduction

Over the years, significant improvements in forage cell wall digestibility have been achieved through forage breeding programs and agronomic advances. Despite these improvements, forage digestibility continues to limit the intake of available energy by ruminants, and correspondingly, contributes to excessive nutrient excretion by livestock. The use of exogenous fibrolytic enzymes holds promise as a means of increasing forage utilization and improving the productive efficiency of ruminants.

Recent studies have shown that adding exogenous fibrolytic enzymes to ruminant diets increases milk production (Nussio et al., 1997; Lewis et al., 1999; Rode et al., 1999; Schingoethe et al., 1999; Kung et al., 2000; Yang et al., 2000) and ADG (Beauchemin et al., 1995; 1997; 1999b; Iwaasa et al., 1997; McAllister et al., 1999) in some cases. These increases in animal performance are due to increases in feed digestion. Numerous studies have reported increased digestion of DM and fiber measured in situ, in vitro (Nakashima et al., 1988; Feng et al., 1996; Hristov et al., 1996; Yang et al., 1999; Colombatto, 2000; Colombatto et al., 2002c), or in vivo (Feng et al., 1996; Krause et al., 1998; Rode et al., 1999; Yang et al., 1999; Beauchemin et al., 2000; Kung et al., 2000). However, not all studies report improved animal performance due to the use of exogenous enzymes (Higginbotham et al., 1996; Pritchard et al., 1996; ZoBell et al., 2000), and viewed across a

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[3]Correspondance: Box 3000 (phone: 403-317-2235; fax: 403-317-2182; E-mail: beauchemin@agr.gc.ca).

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variety of enzyme products and experimental conditions the response to feed enzymes by ruminants has been variable. This article reviews the research on the selection of enzymes for use in ruminant diets, the animal responses to feed enzymes and the potential sources that contribute to the variability in response, and the mechanisms by which exogenous fibrolytic enzymes improve nutrient utilization.

Feed Enzymes for Ruminants

Commercial enzymes used in the livestock feed industry are products of microbial fermentation. Feed enzymes are produced by a batch fermentation process, beginning with a seed culture and growth media (Cowan, 1994). Once the fermentation is complete, the enzyme protein is separated from the fermentation residues and source organism. Although the source organisms are, in many cases, similar among enzyme products, the types and activity of enzymes produced can vary widely depending on the strain selected and the growth substrate and culture conditions used (Considine and Coughlan, 1989; Gashe, 1992; Lee et al., 1998).

Compared to the fermentation extract, these enzyme products are relatively concentrated and purified, containing specific, controlled enzyme activities. They usually do not contain live cells. Enzyme products for ruminant diets are of fungal (mostly *Trichoderma longibrachiatum, Aspergillus niger, A. oryzae*) and bacterial (mostly *Bacillus* spp.) origin (Pendleton, 2000). Furthermore, most of the commercially available enzyme products that have been evaluated as ruminant feed additives are produced for nonfeed applications; cellulases and xylanases are used extensively in the food, pulp and paper, textile, fuel, and chemical industries (Bhat and Hazlewood, 2001). Several fibrolytic enzyme products evaluated as feed additives in ruminant diets were originally developed as silage additives (Feng et al., 1996).

In addition to these relatively pure sources of enzymes, crude fermentation products and some nonbacterial direct-fed microbials (**DFM**) are also marketed, at least partly or implicitly, based on their residual enzymic content (Muirhead, 2001). In this case, the enzymes, as well as the entire medium, are recovered complete with metabolites and fermentation substances. Most nonbacterial DFM consist of *A. oryzae* fermentation extract, *Saccharomyces cerevisiae* cultures, or both (Martin, 2000). In comparison to concentrated feed enzyme products, these crude products contain relatively little (<5%) enzyme activity. There is no minimal level of enzyme activity required for products to be registered as feed enzymes, which adds tremendous confusion in the marketplace. Consequently, it can be difficult to distinguish commercially between "true" enzyme products and products with trace levels of activity. The scope of this paper is limited to concentrated fermentation products that have specific, controlled enzyme activities.

Enzyme Activities Involved in Cell Wall Digestion

The focus of most enzyme-related research for ruminants has been on plant cell wall degrading enzymes. Cellulose and hemicellulose, the major structural polysaccharides in plants (Van Soest, 1994), are converted to soluble sugars by enzymes collectively referred to as cellulases and hemicellulases. The types of cellulases and hemicellulases can differ substantially among commercial enzyme products, and differences in the relative proportions and activities of these individual enzymes may have an impact on the efficacy of cell wall degradation by these products. In addition to fiber-degrading enzymes, these products also have secondary enzyme activities, including amylases, proteases, and pectinases.

Cellulose is hydrolyzed through a complex process involving cellulases, and numerous specific enzymes contribute to cellulase activity. The major enzymes involved in cellulose hydrolysis are endocellulase (endoglucanase, endo-β-1,4-glucanase, carboxymethyl cellulase or β-1,4-glucan glucanohydrolase; E.C. 3.2.1.4), exocellulase (exoglucanase, exo-β-1,4-glucanase, cellulose β-1,4-cellobiosidase; E.C. 3.2.1.91), and β-glucosidase (cellobiase or glucohydrolase, E.C. 3.2.1.21). In general, endoglucanases hydrolyze cellulose chains at random to produce cellulose oligomers of varying degrees of polymerization; exoglucanases hydrolyze the cellulose chain from the nonreducing end, producing cellobiose, and β-glucosidases hydrolyze short-chain cellulose oligomers and cellobiose to glucose.

The main enzymes involved in degrading the xylan core polymer to soluble sugars are xylanases (EC 3.2.1.8) and β-1,4 xylosidase (3.2.1.37) (Bhat and Hazlewood, 2001). The xylanases include endoxylanases, which yield xylooligomers and β-1,4-xylosidases, which in turn yield xylose. Other hemicellulase enzymes involved primarily in the digestion of side chains include β-mannosidase (3.2.1.25), α-L-arabinofuranosidase (3.2.1.55), α-D-glucuronidase (3.2.1.139), α-D-galactosidase (3.2.1.22), acetyl xylan esterases (3.1.1.72), and ferulic acid esterase (3.1.1.73) (White et al., 1993; Bhat and Hazlewood, 2001).

Fiber-degrading enzyme activities are generally determined by measuring the rate of release of reducing sugars from pure substrates, with enzyme units expressed as the quantity of reducing sugars released per unit of time per unit of enzyme. Reducing sugars, which include monosaccharides and free sugar ends in oligosaccharides, can be measured colorimetrically using the Nelson/Somogyi copper method (Somogyi, 1952) or the dinitrosalicyclic acid method (Miller, 1959).

The most commonly used substrate for measuring cellulase activity is carboxymethyl cellulose, which

measures endo-β-1,4-glucanase activity (Wood and Bhat, 1988). Exoglucanase activity can be measured using crystalline cellulose preparations, such as Avicel. β-glucosidase activity is determined by measuring the release of glucose from cellobiose, or the release of *p*-nitrophenol from *p*-nitrophenyl-β-D-glucoside (Bhat and Hazlewood, 2001).

Xylanase activity is most commonly measured by determining the release of reducing sugars from prepared xylan, such as oat spelt or birchwood xylan. Xylanases are specific for the internal β-1,4 linkages within the xylan backbone, and are generally considered endoxylanases (Bhat and Hazlewood, 2001). Endoxylanases can be considered to be debranching or nondebranching based on their ability to release arabinose in addition to hydrolyzing the main chain of xylan. β-xylosidase activity can be determined by using *p*-nitrophenyl derivatives.

Enzyme activity measurements must be conducted under conditions closely defined with respect to temperature, pH, ionic strength, substrate concentration, and substrate type, since all of these factors will affect the activity of an enzyme. Enzyme activities of commercial enzyme products are typically measured at the manufacturers' recommended optima. A temperature of approximately 60°C and a pH between 4 and 5 are the optimal conditions for most commercial enzymes (Coughlan, 1985). However, the optimal temperature and pH for assessing enzyme activity are not representative of the conditions in the rumen, which are closer to a pH of 6.0 to 6.7 and 39°C (Van Soest, 1994). Thus, the activities quoted for commercial enzyme products are considerably higher than for those that would be measured at a pH and temperature similar to that of the rumen. Furthermore, because the conditions of the assays and method of expressing enzyme activity vary among manufacturers, it is difficult to compare enzyme products or predict the efficacy of the product in ruminant diets.

Further details of the enzymology of cellulases and xylanases are provided by Ghose (1987), Wood and Bhat (1988), Bhat and Hazlewood (2001), and McCleary (2001).

Animal Responses to Feed Enzymes

The use of exogenous fiber-degrading enzyme additives for ruminants was first examined in the 1960s, as reviewed by Beauchemin and Rode (1996). Many of these early enzyme products were poorly characterized, animal responses were variable, and little to no effort was made to design these products specifically for ruminants. In the last decade, researchers have reexamined the potential use of exogenous enzymes for ruminants due to higher feed costs, lower costs of enzyme production, and the availability of more active and better defined enzyme preparations. However, descriptions of the enzyme products in most research papers are still generic at best. The formulation of enzyme products for ruminants has evolved over time, but it is difficult to follow these changes based on the information provided in the published literature. For example, there is no way of knowing whether an enzyme product from a particular enzyme supplier is the same from study to study. Typically, the enzyme products evaluated for ruminants are blends of various cellulases and xylanases that were originally produced and marketed for other uses. Thus, the components of a ruminant enzyme product can change over time due to improvements in production and strain selections of the enzymes driven by other markets. Also, ruminant enzyme products have been reformulated as new information became available.

Beef Cattle

Several recent studies have examined the use of exogenous enzyme products in high-forage diets fed to growing cattle (Beauchemin et al., 1995; Michal et al., 1996; Pritchard et al., 1996; McAllister et al., 1999; Wang et al., 1999; ZoBell et al., 2000). There is evidence that adding fibrolytic enzymes to forage diets can improve fiber digestibility (Beauchemin et al., 1995; Feng et al., 1996), but whether increased digestibility improves performance of cattle may depend on the physiological status of the cattle and the conditions of the experiment.

The results of adding fibrolytic enzymes to high-grain diets have been surprisingly more consistent than those for high-forage diets. Applying an enzyme product (Xylanase B, Biovance Technologies Inc., Omaha, NE) to a 95% barley-based diet improved feed efficiency by 6 to 12%, depending upon the level of enzyme addition (Beauchemin et al., 1997; 1999b; Iwaasa et al., 1997) (Table 1). Increased feed efficiency was due to an increase in diet digestibility (Iwaasa et al., 1997). Similarly, Krause et al. (1998) reported a 28% increase in ADF digestibility using a similar enzyme product added to a high-concentrate diet. Using another enzyme product (Finnfeeds Int. Ltd., Marlborough, U.K.), McAllister et al. (1999) reported that treating both the forage (ryegrass silage; 30% of the diet) and grain (barley, 70% of the diet) portions of the diet with 3.5 L/t of DM increased ADG by 10% (DM basis). However, ZoBell et al. (2000) reported no effect when what appears to be the same enzyme product was added to a high-grain barley-based feedlot finishing diet containing 17% forage (DM basis).

Despite the potential benefits of using exogenous enzymes, the adoption of enzyme technology by the beef industry has been slow due to the relatively high cost of enzyme products compared with ionophores, antibiotics, and implants. Furthermore, there are few enzyme products commercially available to the beef industry, and most of these have not been widely evaluated under a range of dietary conditions.

Table 1. Effects of adding a commercial feed enzyme product to high-concentrate feedlot finishing diets consisting of barley grain, supplement, and barley silage

Item	Control	Enzyme level 1×	Enzyme level 2×	Change
Beauchemin et al. (1997)[a]				
No. of animals	10	9		
Initial weight, kg	407	414	—	—
DMI, kg/d	9.99	9.53	—	–5%
ADG, kg/d	1.43	1.52	—	+6%
Kilograms of feed DM: Kilograms of gain	7.11[e]	6.33[d]	—	–11%
Iwaasa et al (1997)[b]				
No. of animals	10	10	10	
Initial weight, kg	476	479	481	—
DMI, kg/d	10.6	9.8	9.8	–8%
ADG, kg/d	2.0	2.1	2.2	+1%
Kilograms of feed DM: Kilograms of gain	5.2[g]	4.9[g]	4.6[f]	–6 to 12%
DM digestibility, %	65.7[f]	69.3[g]	68.9[g]	+5%
Beauchemin et al. (1999b)[c]				
No. of animals	86	101		
Initial weight, kg	385[e]	360[d]	—	–6.5%
DMI, kg/d	10.73	10.62	—	–1%
Weight gain, kg	172[e]	188[d]	—	+9%
ADG, kg/d	1.40[e]	1.53[d]	—	+9%
Kilograms of feed DM: Kilograms of gain	7.72	6.95	—	–11%

[a]No ionophore, no implants used.
[b]Ionophores and implants used.
[c]Cattle were vaccinated, implanted, and melangesterol acetate was provided in the supplement.
[d,e]$P < 0.05$.
[f,g]$P < 0.10$.

Dairy Cattle

A number of studies have examined the effects of fibrolytic exogenous enzymes on digestibility and milk production in dairy cows (Stokes and Zheng, 1995; Higginbotham et al., 1996; Nussio et al., 1997; Zheng and Stokes, 1997; Beauchemin et al., 1999a, 2000; Lewis et al., 1999; Nussio et al., 1997; Rode et al., 1999; Schingoethe et al., 1999; Yang et al., 1999, 2000; Kung et al., 2000; Phipps et al., 2000a,b; Bowman, 2001; Sutton et al., 2001; K. A. Beauchemin, L. M. Rode, B. I. Farr, J. A. Shelford, J. Baah, and G. F. Hartnell, unpublished data). Across 20 studies and 41 treatments, the average increase in DMI was 1.0 ± 1.3 kg/d, and the average increase in milk yield was 1.1 ± 1.5 kg/d (3.4% ± 4.7). Thus, when viewed across a variety of enzyme products and experimental conditions, the response is positive, but the variability is also high.

In some studies, the response to enzymes has been substantial. For example, Lewis et al. (1999) treated forage with a cellulase/xylanase mixture (FinnFeeds Int.; supplying 1 mL/kg of total mixed ration [**TMR**], DM basis) and observed that cows in early lactation produced 6.3 kg/d (16%) more milk (Table 2). However, higher and lower levels of the same enzyme product were less effective. Rode et al. (1999) applied an enzyme product (Promote, Biovance Technologies Inc., Omaha, NE) to the concentrate portion of a diet (supplying 1.3 g/kg of TMR on a DM basis) and observed a 3.6 kg/d (10%; $P = 0.11$) increase in milk production for cows in early lactation (Table 2). Yang et al. (2000) added an enzyme mixture (Biovance Technol, Omaha, NE) to the concentrate, and cows in early lactation produced 2 kg/d (5.9%) more milk (Table 2). However, there was no response when the same enzyme was added to the TMR.

It is clear that exogenous enzymes can be effective for ruminants, but it is important to determine the conditions that are most likely to result in positive responses.

Understanding the Variability in Animal Response

In general, results with beef cattle and dairy cows indicate a positive response to enzymes, but the results are variable. Although this variability may be viewed as an indication that feed enzyme additives are not a suitable technology for ruminants, we believe that much of the variability can be attributed to factors such as enzyme type, level of supplementation, method of enzyme application, and the energy balance of the test animals.

Enzyme Activity

There is increasing evidence that plant cell wall-degrading enzymes stimulate fiber digestion in the

Table 2. Effects of supplementing diets fed to cows in early lactation with an enzyme mixture[z]

	Lewis et al., 1999				Rode et al., 1999		Yang et al., 2000		
	Control	Low enzyme	Medium enzyme	High enzyme	Control	Enzyme in conc.	Control	Enzyme in conc.	Enzyme in TMR
DMI, kg/d	24.4[b]	26.2[a]	26.2[a]	26.6[a]	18.7	19.0	19.4	19.8	20.4
Milk production, kg/d	39.6[b]	40.8[b]	45.9[a]	41.2[b]	35.9[f]	39.5[g]	35.3[b]	37.4[a]	35.2[b]
Milk composition, %									
Fat	3.99[a]	3.83[ab]	4.00[a]	3.75[b]	3.87[a]	3.37[b]	3.34	3.19	3.14
Protein	2.95[a]	2.87[b]	2.88[b]	2.85[b]	3.24	3.03	3.18	3.13	3.13
Lactose	4.89[ab]	4.91[ab]	4.92[a]	4.81[b]	4.73[c]	4.62[d]	4.65	4.65	4.56
BW change, kg/d	NA	NA	NA	NA	–0.63	–0.60	0.15	0.04	0.14
DM digestibility, %	NA	NA	NA	NA	61.7[a]	69.1[b]	63.9[a]	66.6[b]	65.7[ab]
NDF digestibility, %	NA	NA	NA	NA	42.5[a]	51.0[b]	42.6	44.3	45.9

[a,b]Means within a study differ ($P < 0.05$).
[c,d]Means within a study differ ($P < 0.10$).
[f,g]Means within a study differ ($P = 0.11$).
[z]Conc = concentrate, TMR = total mixed ration, NA = not available.

rumen (Feng et al., 1996; Yang et al., 1999). The response has been shown to be due to enzymatic activities (Nsereko et al., 1999), but the key enzymes involved have not been identified. Identification of the important enzyme activities would provide a rationale for designing more effective enzyme products for ruminants.

Wallace et al. (2001) used six enzyme products to examine the relationship between enzyme activities and in vitro gas production using grass and corn silage. A significant positive correlation was reported between cellulase activity and gas production from grass silage. In a companion study, it was observed that preparations relatively high in cellulase activity increased the rate of gas production from corn silage compared with the control (no added enzyme). In contrast, products with relatively high xylanase activity did not increase gas production when glucanase activity was low. Based on the results of this study, one may assume that it would be possible to improve the effectiveness of enzyme preparations by increasing cellulase activity. However, it should be noted that the levels of enzymes used in these studies were 20 to 40 times higher than the levels normally (0.5 to 2 mg/g of TMR DM) used as ruminant feed additives. The authors were unable to document any positive effects of exogenous enzymes on rate of gas production when using lower enzyme levels, which may have been a reflection of the techniques used. Caution must be applied when extrapolating from in vitro studies where relatively high levels of enzyme addition are used.

Colombatto et al. (2002a) used 23 commercial enzyme products to determine the relationship between enzyme activity and the in vitro degradation of feeds. The enzyme products were assayed for 16 different activities using pH and temperature conditions similar to those of the rumen (39°C and pH 6.0), and the level of enzyme product added per unit of feed was similar to that used in vivo (1.5 mg/g of DM). For incubations performed in buffer without ruminal fluid, there was a strong relationship between the release of reducing sugars from alfalfa hay or corn silage and the biochemical characteristics of the enzyme products. Protein content of the enzyme product explained about 60% of the variation in reducing sugars released, indicating that concentrated products with higher protein content were most effective. This can be explained by the fact that the same amount of each enzyme product was used even though the enzyme activity varied tremendously among products. β-glucanase activity explained a further 24% of the variation in reducing sugars released from alfalfa hay, whereas endoglucanase, exoglucanase, β-glucosidase, xylanase, and amylase explained a further 37% of the variation for corn silage. Thus, for alfalfa hay, about 84% of the variation, and for corn silage, about 97% of the variation, was explained by these factors.

The same enzyme products were evaluated for their effects on in vitro DM degradation (Colombatto et al., 2002b). Five of the 23 products significantly improved the 18-h degradation of alfalfa hay, and nine of the 23 products improved the degradation of corn silage. There was a significant relationship between xylanase activity and feed digestion. However, the relationship was positive with alfalfa hay, but negative with corn silage. This negative relationship observed for corn silage is perplexing and may not be one of "cause and effect." In addition, it should be noted that whereas the correlations between xylanase activity and digestion were significant, the xylanase activity alone explained less than 30% of the variation in degradation. No other significant relationship was observed between enzyme activities and feed degradation.

Similar observations have been reported in vivo. For example, Kung et al. (2000) compared two different enzyme products with similar cellulase and xylanase activities in the diets of lactating dairy cows. Only one of the two enzyme products resulted in an increase in milk yield.

Although further study is warranted, these results indicate that it may not be possible to predict the potential of increasing cell wall digestion in the rumen

using exogenous feed enzymes based only on their biochemical characterization. This observation is not surprising because enzyme activities are measured on model substrates that do not represent the complexity of plant cell wall material. Enzyme assays are based on the initial rate of reaction with the substrate and do not relate to overall enzyme persistency. Furthermore, the conditions of the assays may not represent those of the rumen. Although enzyme activity units are important for quality control and to ensure that the customer is actually getting the product they expect, it must be recognized that these activity units bear little relationship to the efficacy of the product as a ruminant feed additive.

Enzyme Level

Some of the variability associated with the use of exogenous enzyme products in ruminant diets is due to supplementation with insufficient or excessive enzyme activity. In vivo responses to enzyme addition are typically nonlinear (Beauchemin et al., 1995; Kung et al., 2000), and it is possible to over-supplement. For example, Kung et al. (2000) offered forage (60% corn silage and 40% lucerne hay; DM basis) treated with increasing levels (0, 1, 2.5 mL/kg of TMR) of an enzyme product (FinnFeeds Int.) to cows. Cows fed the low level of enzyme tended ($P < 0.10$) to produce more milk (39.5 kg/d) than those fed the control diet (37.0 kg/d) or those fed the high level of enzyme (36.2 kg/d). Nonlinear responses have also been reported for growing beef cattle (Beauchemin et al., 1995). In that study, ADG of cattle fed alfalfa hay increased by 24 to 30% with lower levels of added enzyme (0.25 to 1 mL/kg of DM) as a result of increased intake of digestible DM, but higher levels of enzyme (2 and 4 mL/kg of DM) were not effective. With timothy hay, a high level (4 mL/kg of DM) of exogenous enzymes increased ADG of cattle by 36% as a result of a 17% increase in ADF digestibility and a 14% increase in digestible DM intake.

These studies demonstrate that high levels of enzyme addition can be less effective than low levels, and the optimal level of enzyme supplementation may depend on the diet. Lack of response to low levels of enzyme addition may indicate an insufficient supply of enzyme activity; however, the rationale for reduced efficacy of added enzymes at high levels of incorporation is not clear. Nsereko et al. (2002) reported a quadratic response in total bacterial numbers in ruminal fluid with increasing levels of an enzyme product from *Trichoderma longibrachiatum* added to a dairy cow diet. The authors speculated that application of a moderate level of enzyme to ruminant feeds caused some beneficial disruption of the surface structure of the feed either before or after ingestion. When excess enzyme was applied, the beneficial disruption of the feed surface structure may have diminished because the excess exogenous enzyme attached to feed may have restricted microbial attachment and limited digestion of feed.

Enzyme-Feed Specificity

The array of enzyme activities required to improve fiber digestion varies according to the composition of the feed. The principle of feed-enzyme specificity is illustrated in a study conducted in our lab (D. Colombatto, K. A. Beauchemin, and D. P. Morgavi; unpublished data) in which 26 enzyme products were used. The products were characterized biochemically, and then assessed in vitro in the presence of rumen fluid to test their ability to influence DM degradation of alfalfa hay or corn silage at 18 h of incubation. The enzyme products that effectively increased degradation were different for both forages (Table 3). Of the candidates that increased degradation of alfalfa, only enzyme E was also effective for corn silage (ranked 10th). These data clearly indicate the importance of matching the enzyme product to the forage.

Enzyme-feed specificity presents a major dilemma for formulating new ruminant feed enzyme products because most commercial ruminant diets contain several types of forages and concentrates. Therefore, to achieve maximal benefit, a number of different enzyme sources would need to be used in a typical diet. A middle approach is to use an enzyme that may not be the best on all forages, but is relatively suitable for most feeds. This generalized approach has been the one taken for the most part in the development of enzyme products for ruminants. To some extent, this approach has limited the rate of progress in terms of bringing effective products to the marketplace. On-farm efficacy of some products may not be high in all situations, contributing to the variability associated with enzyme technology. Because of the relatively high cost of feed enzymes compared to other technologies, livestock producers expect an equally high response in animal productivity. In future, the "one-size-fits-all" approach may be replaced by a more targeted approach in which feed enzyme products are formulated for various types of feeds. Although this "designer enzyme" targeted approach presents an added degree of complexity in the marketplace, it may be the best way to ensure efficacy of feed enzyme technology on the farm.

Method of Providing Enzyme to Animals

Applying fibrolytic exogenous enzymes in a liquid form onto feeds prior to consumption can have a positive effect on animal performance (Rode et al., 1999; Schingoethe et al., 1999; Kung et al., 2000; Yang et al., 2000). In contrast, infusion of enzymes into the rumen has not been effective (Lewis et al., 1996; McAllister et al., 1999; Sutton et al., 2001). The close association of enzymes with feed may enable some form of preingestive attack of the enzymes upon the

Table 3. Top-ranked enzyme products for their ability to increase in vitro dry matter degradation of forages. Letters (A to Z) indicate enzyme products. Xylanase (Xyl) and endoglucanase activity (CMC) of each product is expressed as a percentage of the maximal activity measured across all 26 products

	Alfalfa hay[a]			Corn silage[b]		
Rank	Enzyme	Xyl/CMC	Increase in IVDMD, %	Enzyme	Xyl/CMC	Increase in IVDMD, %
1	A	83.2/2.0	10.3*	Z	88.6/15.4	10.9*
2	B	0.9/0	9.8*	J	15.1/40.2	9.1*
3	C	84.3/21.5	9.5*	N	4.3/40.1	8.7*
4	D	90.7/17.0	9.3*	Y	3.0/12.3	8.3*
5	E	34.6/6.8	8.5*	S	13.3/63.5	7.5*
6	F	62.0/68.0	7.8	P	10.0/57.1	7.3*
7	G	10.5/85.6	7.5	X	2.5/31.7	6.7*
8	H	100/6.9	6.3	Q	2.9/7.1	5.9*
9	I	16.2/100	6.3	I	16.2/100	5.9*
10	J	15.1/40.2	5.6	E	34.6/6.8	5.8*
11	K	4.5/34.0	5.5	G	10.5/85.6	5.6*

[a]In vitro DM digestibility (IVDMD) of alfalfa hay control was 434.9 g/kg.
[b]IVDMD of corn silage control was 424.0 g/kg.
*Increase in IVDMD compared with control silage with no added enzyme ($P < 0.05$).

plant fiber and/or enhance binding of the enzymes to the feed, thereby increasing the resistance of the enzymes to proteolysis in the rumen.

There is apparently little or no requirement for a reaction phase or incubation time between treatment and feeding of forages. Lewis et al. (1996) observed an increase in total-tract NDF digestibility when an enzyme solution was applied to dry hay prior to feeding, but there was no difference between applying the enzyme immediately before feeding and a 24-h incubation period. In vitro studies have reported similar results (Colombatto, 2000).

Enzymes have been applied to TMR (Higginbotham et al., 1996; Beauchemin et al., 1999; Phipps et al., 2000b; Yang et al., 2000), hay (Beauchemin et al., 1995; Lewis et al., 1996; Yang et al., 1999), ensiled forages (Beauchemin et al., 1995; Phipps et al., 2000a), concentrate (Rode et al., 1999; Phipps et al., 2000b; Yang et al., 2000), supplement (Bowman, 2001), or premix (Bowman, 2001). Exogenous enzymes may be expected to be more effective when applied to high-moisture feeds (such as silages) compared to dry feeds because of the higher moisture content. The requirement for water in the hydrolysis of soluble sugars from complex polymers is a fundamental biochemical principle. Furthermore, silage pH values are usually at, or around, the optimal pH for most fungal enzymes. However, in practice, some exogenous enzymes are more effective when applied in a liquid form to dry forage as opposed to wet forage. Feng et al. (1996) applied an enzyme solution directly to grass and observed no effect when added to fresh or wilted forage; however, when it was applied to dried grass, enzymes increased DM and fiber digestibility. Similarly, Yang et al. (2000) reported increased milk production and digestibility of the diet when enzymes were added to the concentrate portion of a dairy cow diet, but not when they were added directly to TMR. In contrast, Phipps et al. (2000b) reported no difference between adding an enzyme product to concentrate or TMR, but the enzyme product used in that study was not effective. The reduced efficacy of exogenous enzymes applied to ensiled feeds may be due to inhibitory compounds in fermented feeds. Nsereko et al. (2000) reported the presence of compounds in whole-crop barley silage that inhibited endo-1,4-β-xylanase activity of an enzyme product from *T. longibrachiatum* by 23 to 50%, although there was no effect on cellulase activity. Also, the application of exogenous enzymes to silages can accelerate their aerobic deterioration. The growth of the ephiphytic microbiota is stimulated by soluble sugars released by enzyme treatment, which could lead to a decrease of the silage feed value if the time elapsed between enzyme application and consumption is sufficiently long (Wang et al., 2002).

Yang et al. (1999) observed no difference between applying an enzyme product to dry forage or to both dry forage and concentrate. Others have also found that adding enzyme to concentrate to be effective (Beauchemin et al., 1997; Iwaasa et al., 1997; Rode et al., 1999; Yang et al., 2000). Bowman (2001) examined the effects of adding an enzyme product (Promote N.E.T., Agribrands International, St. Louis, MO) to various proportions of a TMR fed to dairy cows. The enzyme product was added to the concentrate portion of the TMR (45% of the TMR), to the supplement (4% of the TMR), or to the premix (0.2% of TMR). Diets with enzymes delivered the same quantity of enzyme per cow daily. Total-tract digestibility of NDF was increased from 44.3 to 55.6% (25%) when enzyme was added to concentrate, but the other treatments had no effect. When the same diets were evaluated in vitro, DM degradation of the TMR at 12 h of incubation was increased by 15% when enzymes were added to the

concentrate portion, and by 17% when added to the premix portion, of the diet. At 48 h of incubation, only the treatment with enzyme added to a premix had higher DM digestibility compared to the control. Reasons for reduced response in vivo when enzymes are applied to a smaller portion of the diet are unclear. Beauchemin et al. (1999b) suggested that enzymes should be applied to a large portion of the diet to increase the chances that enzymes endure in the rumen. Adding enzyme to a small portion of the diet may allow rapid passage of enzyme from the rumen, lessening the enzyme effect in the rumen. Rapid passage of the enzyme is not an issue in vitro. Thus, in vitro batch culture assays used as a bioassay to predict the effects of exogenous enzymes on digestibility may not accurately predict variations in the in vivo response caused by method of providing the enzyme to the animal.

Level of Animal Productivity

Animal responses to exogenous enzymes are expected to be greatest in situations in which fiber digestion is compromised and when energy is the first-limiting nutrient in the diet. High-producing dairy cows and growing cattle require high levels of available energy to meet the demands of milk or meat production. It is not uncommon for feed intake of dairy cows to exceed four times the level of intake required for maintenance. In these commercial feeding situations, fiber digestion is often compromised due to low ruminal pH and rapid transit time through the rumen. The NRC (1989) assumes a 4% reduction in digestibility for each multiple increase in feed intake over maintenance. Thus, a dairy cow diet with a potential digestibility of 77% would have an actual digestibility of 68% when fed to high-producing dairy cows. More recently, the NRC (2001) recognized that the decrease in digestibility at high levels of intake is not constant. For high-quality diets, the decrease in digestibility is even greater than previously estimated.

Enzymes help bridge the gap between potential and actual performance of the animal. This concept is illustrated in a study in which dairy cows and sheep were fed a TMR with and without exogenous enzymes (Yang et al., 2000). When measured in dairy cows, the total-tract digestibility of the control diet was 63.9% for DM and 31.8% for ADF. As a result of lower intake as a percentage of BW, digestibility of the control diet was higher in sheep; total-tract digestion was 77.1% for DM and 49.8% for ADF. Use of an enzyme product improved digestibility of the diet when evaluated in dairy cows, but not in sheep. These results indicate that exogenous enzymes improve feed digestion when the potential digestibility of the diet is not attained because digestion is compromised. It is this "loss" in digestible energy that is captured with the use of feed enzymes.

Existing enzyme technology is not likely to benefit ruminants fed at maintenance; rather, the greatest responses will be for ruminants fed for maximal productivity. Similarly, the response to exogenous enzymes is greater for dairy cows in early lactation than for those in later lactation (Nussio et al., 1997; Schingoethe et al., 1999).

Mode of Action: An Integrated Hypothesis

In light of the exceptionally high starch- and fiber-digesting capacity of the rumen, it is difficult to explain why treatment of grain or forage with enzymes prior to consumption further improves feed utilization. It is clear that the mode of action for exogenous enzymes improving digestion of plant cell wall is complex. Based on our experience and the available information, we have developed a putative hypothesis that we feel accounts for the most critical factors that explain the observed increases in fiber and DM digestion.

Exogenous enzymes in the rumen are generally more stable than previously thought (Hristov et al., 1998; Morgavi et al., 2000b, 2001), particularly when applied to feed prior to ingestion. Application of enzymes to feed enhances the binding of the enzyme with the substrate, which increases the resistance of the enzymes to proteolysis and prolongs their residence time within the rumen. In the rumen, the close association between digestive bacteria and feed particles concentrates digestive enzymes close to their specific substrates. However, some ensiled feeds contain compounds that are inhibitory to xylanases (Nsereko et al., 2000); therefore, applying enzymes to dry feeds decreases the variability in response.

Applying enzymes to feed also provides a slow-release mechanism for enzymes in the rumen (Beauchemin et al., 1999a). Thus, the greater the proportion of the diet treated with enzymes, the greater the chances that enzymes endure in the rumen. Without this stable feed-enzyme complex, the enzymes are solubilized in ruminal fluid and flow rapidly from the rumen.

There is evidence for preconsumptive effects of exogenous enzymes causing the release of soluble carbohydrates (Hristov et al., 1996), and in some cases, partial solubilization of NDF and ADF (Gwayumba and Christensen, 1997; Krause et al., 1998). Nsereko et al. (2000) demonstrated compelling evidence that applying enzymes to feed causes structural changes to occur, thereby making feed more amenable to degradation. Cell wall hydrolysis in the rumen proceeds in an erosive manner (White et al., 1993), and it is well recognized that a major constraint to digestion is the limited colonization and penetration of cellulolytic microbes and their hydrolytic enzymes onto the exposed surfaces of feed particles.

It is most likely that the major portion of the positive production responses resulting from the use of enzyme additives is due to ruminal effects. Adding exogenous enzymes to the diet increases the hydrolytic capacity of the rumen mainly due to increased bacterial attachment (Yang et al., 1999; Morgavi et al., 2000c; Wang et

al., 2001), stimulation of rumen microbial populations (Wang et al., 2001; Nsereko et al., 2002), and synergistic effects with hydrolases of ruminal microorganisms (Morgavi et al., 2000a). The net effect is increased enzymatic activity within the rumen, which enhances digestibility of the total diet fed. Thus, improvements in digestibility are not limited to the dietary component to which the enzymes are applied, which explains why fibrolytic enzymes can be effective when added to the concentrate portion of a diet. Increased hydrolytic capacity of the rumen can also lead to an increase in digestibility of the nonfiber carbohydrate fraction, in addition to increasing digestibility of the fiber components of a diet, which explains why fibrolytic enzymes can be effective in high-concentrate diets.

Exogenous enzymes appear to survive in the small intestine for a time sufficient to have an effect on target substrates (Morgavi et al., 2001). However, postruminal effects of exogenous enzymes on digestion are likely only a significant factor when enzymes are infused into the rumen (Hristov et al., 2000) or added to feed in a manner that allows for easy solubilization from feed and rapid passage from the rumen (Beauchemin et al., 1999). Such may be the case when enzymes are applied to wet feeds prior to feeding or when offered as a concentrated premix. Also, the relatively short retention times through the lower gastrointestinal tract means that, postruminally, enzymes are only likely to be effective against the least recalcitrant fraction of dietary fiber. Significant quantities of this easily digestible fiber will only be passing out of the rumen in abnormal circumstances such as extreme ruminal acidosis.

Implications

Adding exogenous fibrolytic enzymes to dairy cow and feedlot cattle diets can potentially improve cell wall digestion and the efficiency of feed utilization by ruminants. Positive responses in milk production and growth rate have been observed for cattle fed some enzyme products, although results have been inconsistent. Some of the variation can be attributed to product formulation, under- or over-supplementation of enzyme activity, inappropriate method of providing the enzyme product to the animal, and the level of productivity of the test animal. Research is needed to understand the mode of action of these products so that on-farm efficacy of ruminant enzyme technology can be assured. With increasing consumer concern about the use of growth promoters and antibiotics in ruminant production, and the magnitude of increased animal performance obtainable using feed enzymes, these products could play an important role in future ruminant production systems.

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Preslaughter intervention strategies to reduce food-borne pathogens in food animals[1,2]

T. R. Callaway[3], R. C. Anderson, T. S. Edrington, R. O. Elder, K. J. Genovese, K. M. Bischoff, T. L Poole, Y. S. Jung, R. B. Harvey, and D. J. Nisbet

Food and Feed Safety Research Unit, Southern Plains Agricultural Research Center, USDA, ARS, College Station, TX 77845

ABSTRACT: Food-borne pathogenic bacteria sicken more than 76 million Americans annually. Many of these illnesses are caused by consumption of foodstuffs produced from animals. Postslaughter intervention strategies effectively reduce bacterial contamination level from the abattoir to the table. However, in spite of these effective strategies, food-borne illnesses and food-related deaths still occur far too frequently. Therefore, strategies that expand the continuum of intervention from the abattoir back to the farm have the greatest potential to reduce pathogenic contamination of meats and resultant human illnesses. A broad range of preslaughter intervention strategies have been contemplated and are currently under investigation. Potential strategies to be discussed include vaccination, competitive exclusion, substrate-adapted competitive exclusion, and the use of pro- and prebiotics (e.g., fructooligosaccharides). Other strategies such as the use of bacteriophage to specifically target certain pathogenic bacteria, and the exploitation of the physiology of specific pathogens will be described. Additionally, the use of antibiotics to specifically reduce pathogens will be examined, as well as the risks incurred by antibiotic usage. The effects of management strategies (e.g., dietary changes), transportation, and stress on food-borne pathogenic bacterial populations of food animals will also be discussed. The parallel application of one or more of these preslaughter strategies has the potential to synergistically reduce the incidence of human food-borne illnesses by erecting multiple hurdles against entry of pathogens into the food chain.

Key Words: Foodborne Diseases, Intervention, Pathogens

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Introduction

Americans expect and demand a safe food supply; yet each year, more than 76 million citizens become ill from consuming foods contaminated with pathogenic bacteria (Mead et al., 1999). Many of these outbreaks have been linked to meat products or to contact with food animals or their waste. Human illnesses caused by the most common food-borne pathogens cost the U.S. economy approximately $7 billion and result in 1,600 deaths each year (ERS/USDA, 2001). The most economically important agents of food-borne illness in the United States are *Campylobacter, Salmonella*, enterohemorrhagic *Escherichia coli* (including O157:H7), and *Listeria* (ERS/USDA, 2001). All of these bacterial species can be found in the gastrointestinal microbial populations of food animals.

Traditionally, much of the research effort aimed at improving the safety of meat products has focused on postslaughter sanitation. Postslaughter antimicrobial treatments in processing plants reduce carcass contamination (Elder et al., 2000), but consumers are still sickened by food-borne pathogenic bacterial outbreaks. Until recently, little emphasis was placed on the development of intervention strategies in the live animal prior to slaughter; however, this has changed, with an increased emphasis on preslaughter intervention strategies.

Human pathogens present in the food animal gastrointestinal tract are often difficult to diagnose on the farm because they often have little or no impact on

[1]Proprietary or brand names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product, or exclusion of others that may be suitable.

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[3]Correspondence: 2881 F & B Rd., College Station, TX 77845. phone: 979-260-9374; fax: 979-260-9332; E-mail: callaway@ffsru.tamu.edu.

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animal health and/or production and are often shed sporadically. Because fecal shedding is correlated with carcass contamination (Elder et al., 2000), the role of the live animal in the production of a safe and wholesome food product is critical. Therefore, strategies that reduce food-borne pathogenic bacterial populations in the animal prior to slaughter could produce "the most significant reduction in human exposures to the organism and therefore reduction in related illnesses and deaths," according to Hynes and Wachsmuth (2000).

Probiotic Methods to Reduce Food-Borne Pathogens

The use of microflora to reduce pathogenic bacteria (including food-borne pathogens) in the gut has been termed a "probiotic" strategy (Fuller, 1989). The overall goal of this strategy is to promote the growth of groups of bacteria that are competitive with, or antagonistic to, pathogenic bacteria. Various probiotic techniques involve introducing a "normal" microbial population to the gastrointestinal tract or providing a limiting nutrient (sometimes termed a "prebiotic") that allows an existing commensal microbial population to expand its role in the gastrointestinal tract. The goal of these methodologies is to fill all microbial ecological niches and thereby prevent the establishment of an opportunistic pathogenic bacterial population. However, probiotics have not always been widely commercially implemented, often due to the concurrent use of noncomplimentary strategies (e.g., antibiotic use can dramatically decrease the effectiveness of competitive exclusion or prebiotics) (Steer et al., 2000). Due to increasing fears over the spread of antimicrobial resistance, it is expected in the future that antibiotics will become more closely regulated and expensive, causing probiotic strategies to become more effective and more widely accepted in the animal industry.

Competitive Exclusion

In neonates, the digestive tract is initially sterile, but it is rapidly colonized by a characteristic gastrointestinal microflora from the environment or the dam (Jayne-Williams and Fuller, 1971; Fuller, 1989). When this population becomes established, the animal is more infection-resistant, especially to bacteria that colonize the gastrointestinal tract (Fuller, 1989). This effect of the natural microbial population has been variously described as "bacterial antagonism" (Freter et al., 1983), "bacterial interference" (Dubos, 1963), the "barrier effect" (Fedorka-Cray et al., 1999), or competitive exclusion (Lloyd et al., 1977).

Competitive exclusion (**CE**), as a technology, involves the addition of nonpathogenic bacterial culture to the intestinal tract of food animals in order to reduce colonization or decrease populations of pathogenic bacteria in the gastrointestinal tract (Fuller, 1989; Nurmi et al., 1992; Steer et al., 2000). The CE culture may be composed of a single specific strain or may be composed of several strains or even several species of bacteria. Depending on the stage of production (maturity of the gut), the goal of CE can be the exclusion of pathogens from the naïve gut of a neonatal animal, or the displacement of an already established pathogenic bacterial population (Nurmi et al., 1992).

Potential Modes of Competitive Exclusion Action. Endogenous gastrointestinal bacteria compete fiercely with one another for available nutrients (Hungate, 1966). The species best adapted to each niche flourishes in the intestinal tract. Introduction of a stable, mixed microbial consortium to the naïve gut can aid in the early establishment of a normal microbial population and can create a highly competitive environment that may prevent the establishment of a pathogenic bacterial population (Nurmi et al., 1992; Crittenden, 1999; Steer et al., 2000).

As the normal (or CE) bacterial population increases throughout the gut, bacteria attach to the surface of the intestinal epithelium (Lloyd et al., 1974). This direct, physical binding can prevent opportunistic pathogens from obtaining a physical attachment site along the intestinal epithelium (Collins and Gibson, 1999). Volatile fatty acids are produced by the gastrointestinal microbial fermentation of carbohydrates or proteins and can be toxic to some species of bacteria, including the pathogenic bacteria *E. coli* O157:H7 and *Salmonella* (Wolin, 1969; Barnes et al., 1979; Prohaszka and Baron, 1983). Some bacteria produce antimicrobial compounds (traditional antibiotics, as well as bacteriocins or colicins) in order to eliminate competitive bacteria (Jack et al., 1995); these antimicrobial-producing species can be used to eliminate food-borne pathogenic bacteria. Intestinal microflora also produce vitamins that aid in the development of a healthy, vascularized intestinal epithelium with increased numbers of microvilli, and therefore, increased nutrient absorptive capacity (Collins and Gibson, 1999), potentially improving animal production efficiency as an added benefit of CE treatment.

Competitive Exclusion Applications. Providing a mixture of bacteria from healthy adult birds to newly hatched chicks (CE) provided an anti-*Salmonella* effect (Nurmi and Rantala, 1973; Nurmi et al., 1992). The beneficial effect in poultry has been widely repeated in many countries, leading to the development of several commercial CE products (Fuller, 1989; Nurmi et al., 1992). Recent studies demonstrating the effectiveness of CE in reducing *Salmonella* colonization of chicks have led to the commercial development in the United States of a mixed-culture CE product, comprised of several defined species of bacteria (Preempt, MS BioScience, Dundee, IL) (Nisbet et al., 1993a,b; 1996).

Treatment of swine with pure cultures of *Streptococcus faecium* reduced enterotoxigenic *E. coli* colonization and diarrhea (Underdahl et al., 1982; Ushe and Nagy, 1985). Other researchers have successfully demonstrated that a mucosal CE culture (mixed-CE culture) could effectively reduce *Salmonella* populations in ex-

perimentally infected piglets (Fedorka-Cray et al., 1999). Recently, a CE treatement for swine has been derived from the colonic contents of healthy pigs that reduces the incidence of *Salmonella cholerasuis* (Anderson et al., 1999) and enterotoxigenic *E. coli* (Genovese et al., 2000).

Competitive exclusion cultures have also been used to reduce *E. coli* O157:H7 in cattle (Zhao et al., 1998). Researchers isolated a defined population of multiple non-O157:H7 *E. coli* strains from naturally *E. coli* O157:H7-free cattle and found that this generic *E. coli* culture could displace established *E. coli* O157:H7 populations from cattle (Zhao et al., 1998). In more recent studies, other CE researchers have found that *Lactobacillus acidophilus* cultures (single strain addition) added to the feed of finishing cattle reduced *E. coli* O157:H7 shedding by more than 50% (Brashears and Galyean, 2002). These results indicate that CE could be a useful compliment to in-plant intervention strategies by reducing the levels of pathogenic bacteria entering the abattoir. In spite of the beneficial results of CE in several species of animals, "real-world" results have often been inconsistent and contradictory, sometimes due to interactions with other incompatible management strategies (e.g., antibiotic treatment) (Steer et al., 2000).

Prebiotics

Sugars or other organic compounds that are not digestible by the host animal, but are digestible by a segment of the microbial population are generally known as prebiotics (Walker and Duffy, 1998; Steer et al., 2000). Prebiotics have been used in humans in an effort to promote intestinal health (Crittenden, 1999). Fructooligosaccharides (**FOS**), for example, are sugars that are not degraded by intestinal enzymes that can pass down to the cecum and colon to become "colonic food" (Willard et al., 2000). Alternatively, other sugars can be used, such as galactooligosaccharides or inulin.

Prebiotics can provide energy and/or other limiting nutrients to the intestinal mucosa and substrates for the colonic/cecal bacterial fermentation to produce vitamins and antioxidants that further benefit the host animal (Collins and Gibson, 1999; Crittenden, 1999). Additionally, some prebiotics can provide specific members of the native microflora (e.g., *Bifidobacteria, Lactobacillus*) a competitive advantage (Willard et al., 2000) that can exclude pathogenic bacteria from the intestine via direct competition for nutrients or for binding sites through the production of "blocking factors" in a fashion similar to CE (Zopf and Roth, 1996). An additional benefit of prebiotic treatment is that some bacterial species that are provided a competitive advantage can produce antimicrobial substances (e.g., bacteriocins, colicins) that can directly inhibit pathogenic bacteria. An additional consideration for ruminants is that prebiotics must be able to bypass ruminal microbial degradation, requiring specific strategies tailored to allow sufficient quantities to reach the ruminant intestine. Coupling the use of CE and prebiotics (known as synbiotics) could yield a synergistic effect in the reduction of food-borne pathogenic bacterial populations in food animals prior to slaughter.

Antimicrobial Strategies to Reduce Food-Borne Pathogens

Antibiotics have often been thought of as a direct method to alter the microbial ecology of the intestinal tract. But the use of medically important antibiotics as growth promoters has become highly controversial in recent years, and is likely to become more so in the near future following recent regulatory action by the European Union. Bacteria have many complex mechanisms to resist antibiotics, and the widespread use of antibiotics in both human medicine and animal agriculture has led to the widespread dissemination of antibiotic resistance genes. Because of concern over the spread of antibiotic resistance, it is likely that the prophylactic use of antibiotics as growth promoters in food animals will become even more highly regulated, or even completely prohibited.

Antibiotics. Antibiotics have been widely used to control disease in both man and animals and to increase animal growth rate and/or efficiency. In spite of the common use of antibiotics in animals, it is sometimes difficult to target bacteria with antibiotics because they fall into diverse groups; therefore, broad-spectrum antibiotics are often included in animal rations. Antibiotic treatment to control gastrointestinal pathogens (including food-borne pathogens) can so disrupt the intestinal microbial ecosystem that opportunistic pathogens can occupy niches from which they would ordinarily be excluded. This can deleteriously impact animal health, performance, and food safety. This consideration, in addition to concerns about the role of subtherapeutic antibiotic treatment in the spread of antibiotic resistance (Witte, 2000), raises further concerns about the use of antibiotics to control food-borne pathogenic bacteria in the animal.

In spite of these potential drawbacks to antibiotic treatment, recent research has found that some antibiotics do have the potential to improve food safety at the live animal level. Neomycin sulfate is an antibiotic approved for use in cattle and has a 24-h withdrawal period. Cattle were fed neomycin for 48 h and went through a 24-h withdrawal period; they shed significantly lower generic *E. coli* and *E. coli* O157:H7 populations in their feces (Elder et al., 2002). After 5 d of neomycin withdrawal, generic *E. coli* populations had returned to near pretreatment levels, but *E. coli* O157:H7 populations remained nearly undetectable (Elder et al., 2002). The use of neomycin sulfate treatment to reduce *E. coli* O157:H7 populations has the benefit of being readily available to the industry at the present time until other strategies become market ready.

Other antimicrobial compounds are routinely incorporated into animal diets to improve animal health and/or growth performance. Ionophores are antimicrobials not related to antibiotics used in human medicine and thus do not appear to lead to an increase in antibiotic resistance. Monensin, the most widely used ionophore, has been used both as a coccidiostat in poultry and as a growth promoter in ruminants (Russell and Strobel, 1989). Because they are potent antimicrobials that are approved for use in food animals, it was hypothesized that ionophores could be used to control food-borne pathogenic bacteria populations. Unfortunately, because of the physiology of some common food-borne pathogens, it does not appear that ionophores reduce food-borne pathogenic bacteria populations (Busz et al., 2002).

Bacteriophages. Bacteria can be infected by bacterial viruses (or bacteriophages) that have very narrow target spectra, and some phages may be active against only a specific strain. This high degree of specificity allows phages to be used against targeted microorganisms in a mixed population without disturbing the microbial ecosystem, and phages have been used instead of antibiotics to treat human diseases in many parts of the world. Bacteriophages are common natural members of the gastrointestinal microbial ecosystem of food animals (Adams et al., 1966; Orpin and Munn, 1973; Klieve and Bauchop, 1988).

Phages recognize specific receptors on the outer surface of bacteria and inject their DNA into the host bacterium, which incorporates phage DNA into its chromosome. Once inserted into the chromosome, the phage "hijacks" the bacterium's biosynthetic machinery to make more phages. When intracellular nutrients are exhausted by phage replication, the host bacterium explodes (lyses), releasing thousands of new phage particles to repeat the process. An exponential increase in the number of phages continues as long as target bacteria are present and allows phages to persist in the gut rather than simply degrade over time as antibiotics do. However, phage populations are limited; if the target bacterium is removed from the environment, then phage populations diminish.

Bacteriophages have been used to control food-borne pathogenic bacteria in several species of food animals, and have been used against specific animal pathogens (Smith and Huggins, 1987; Kudva et al., 1999; Huff et al., 2002). Several research studies have examined the effect of phages on conditions or diseases that impact production efficiency or animal health (Smith and Huggins, 1982; 1983; Huff et al., 2002). The effectiveness of phage treatment in real-world conditions has been variable to date; therefore, more basic work needs to be completed before bacteriophages can be considered a viable method to control populations of food-borne pathogenic bacteria in food animals.

Specific Inhibition of Pathogens via Metabolic Pathways. Salmonella and *E. coli*, among other bacteria, can respire under anaerobic conditions by converting nitrate to nitrite via a dissimilatory nitrate reductase (Stewart, 1988). The intracellular bacterial enzyme nitrate reductase does not differentiate between nitrate and its analog, chlorate, which is reduced to chlorite in the cytoplasm; chlorite accumulation kills bacteria (Stewart, 1988). Chlorate addition to swine diets reduced experimentally inoculated *Salmonella* and *E. coli* O157:H7 fecal and intestinal populations (Anderson et al., 2001a,b). Other studies demonstrated that chlorate administered in drinking water significantly reduced *E. coli* O157:H7 populations in both cattle and sheep in the rumen, intestine, cecum, and feces (Callaway et al., 2002a). Preliminary results examining the use of chlorate in broilers and in turkeys have yielded promising results as well (J. A. Byrd, unpublished data).

Chlorate treatment does not appear to have an impact on the ruminal or the cecal/colonic fermentation in ruminants or monogastrics (Callaway et al., 2002a). It also appears that selection of chlorate-resistant mutants is not likely because chlorate resistant mutants are incapable of competing effectively against the intestinal microbial population (Callaway et al., 2001). Because of the dramatic impact chlorate has on food-borne pathogenic bacterial populations in the gut of food animals, it has been suggested that chlorate could be supplemented in the last meal prior to shipment to the slaughterhouse (Anderson et al., 2000). At the current time, however, the use of chlorate in food animals is under review by the U. S. Food and Drug Administration, but it has not been approved for use in food animals.

Immunization to Prevent Pathogen Colonization. Because food animals can be reservoirs of pathogenic bacteria, methods to exploit the animal's own immune system to reduce pathogen load have been studied. Specific immunization against pathogenic bacteria has shown great promise in reducing the levels of disease-causing pathogens in food animals. Vaccines against *Salmonella* strains responsible for disease have been developed for use in swine and dairy cattle (House et al., 2001). Vaccination has also been successfully used to combat postweaning *E. coli* edema disease in young pigs (Gyles, 1998). The introduction of "edible vaccines" has the potential to make immunization of food animals economically viable for many diseases, including food-borne pathogens.

Recently, a vaccine has been developed for cattle that reduced fecal *E. coli* O157:H7 shedding (B. Finley and A. Potter, personal communication). Preliminary studies have indicated that this vaccine is effective, and large-scale field trials are scheduled to begin in the summer of 2002. However, because *E. coli* O157:H7 and other enterohemorrhagic *E. coli* are shed sporadically by cattle, it appears that natural exposure to *E. coli* O157:H7 does not confer protection to the host (Gyles, 1998). In a similar manner, *Salmonella* can survive in an animal that has developed an antigenic response to *Salmonella* for extended periods of time (Gyles, 1998). Therefore, while some technical issues remain to be

resolved, the use of vaccination to reduce food-borne pathogens appears to hold promise, and has an added benefit in that vaccination could be used synergistically with other pathogen reducing technologies.

Dietary and Management Effects

Good animal management is crucial to the production of healthy, efficient animals. Yet it has not been conclusively demonstrated whether specific management strategies can directly impact shedding or carriage of food-borne pathogens found in animals. However, reducing the multiplication of pathogens in feed and water may reduce exposure and horizontal and vertical transmission of pathogens to and between animals (Hancock et al., 1998).

Dietary Strategies to Reduce E. coli O157:H7 Populations in Cattle. Feeding grain to cattle has a significant effect on the ruminal microbial ecosystem and overall animal health (Russell and Rychlik, 2001). Cattle in the United States are often fed high- grain rations in order to maximize growth efficiency (Huntington, 1997). Some dietary starch bypasses ruminal fermentation and passes through to the cecum and colon where it undergoes microbial fermentation (Huntington, 1997). Studies have indicated that varying the forage to grain ratio in cattle rations can have a marked effect on populations of *E. coli.* Some early studies indicated that reducing hay, over feeding grain, or switching from a better- to poorer-quality forage increased generic *E. coli* and/or O157:H7 populations (Brownlie and Grau, 1967; Allison et al., 1975; Kudva et al., 1995; 1996).

In recent research, cattle fed a feedlot-type ration had generic *E. coli* populations 1,000-fold higher than cattle fed only hay (Diez-Gonzalez et al., 1998). When cattle were abruptly switched from a finishing ration to a 100% hay diet, fecal *E. coli* populations declined 1,000-fold, and the population of *E. coli* resistant to an "extreme" acid shock (similar to that of the human stomach) declined more than 100,000-fold within 5 d (Diez-Gonzalez et al., 1998). Based on these results, the authors suggested that feedlot cattle be switched from high-grain diets to hay prior to slaughter to reduce *E. coli* populations entering the abattoir (Diez-Gonzalez et al., 1998). In a very well-controlled study, Keen et al. (1999) screened cattle on a high-grain diet for natural *E. coli* O157:H7 contamination. These cattle were divided, with one group maintained on a feedlot ration and the other abruptly switched to hay; 52% of the grain-fed cattle were positive for *E. coli* O157:H7 compared with 18% of the hay-fed cattle (Keen et al., 1999). Additional research with experimentally inoculated calves indicated that animals fed a high-concentrate diet consistently shed more *E. coli* O157:H7, and that isolates grown in ruminal fluid from grain-fed animals were more resistant to an acid shock than those grown in hay-fed ruminal fluid (Tkalcic et al., 2000). Gregory et al. (2000) stated that "the most effective way of manipulating gastro-intestinal counts of *E. coli* was to feed hay." However, other research groups have produced contradictory results indicating that forage feeding either had no effect or increased *E. coli* O157:H7 shedding (Hovde et al., 1999; Buchko et al., 2000a,b). Therefore, although it appears from most of the available literature that forage feeding does reduce *E. coli* populations (Callaway et al., 2002b), the debate is by no means complete.

In spite of the benefits potentially offered by feeding forage, the effect of hay feeding on weight gain and carcass characteristics has not been systematically examined. Recent research indicated that a switch to forage did not have a dramatic impact on carcass characteristics or final BW (Stanton and Schutz, 2000). However, other researchers have found that a switch to hay feeding resulted in a lower carcass weight (Keen et al., 1999). Thus, the economic impact of a switch from grain to forage must be carefully considered.

Water Troughs as a Source of Transmission? Cattle, as well as people, can be infected by pathogens via a water-borne route (Jackson et al., 1998; Shere et al., 2002). Researchers have demonstrated that cattle water troughs can be reservoirs of *E. coli* O157:H7 (LeJeune et al., 2001). Although the significance of this route of horizontal transmission has not been conclusively proven, interventions at the pen level offer significant promise to reduce pathogen contamination of animals (LeJeune et al., 2001). Further research into keeping pathogens from surviving in the water supply can potentially increase food safety by reducing the food-borne pathogen horizontal transmission.

Implications

Access to a safe and wholesome food supply is crucial to the American public, and the food supply in the United States is indeed the safest in the history of the world. Yet food-borne illnesses still occur, and these are often associated with products derived from animal agriculture. Although the meat industry has continuously sought improvement in the safety of its products, much of the research has focused on postslaughter strategies. Until recently preslaughter intervention points have not been given full consideration as methods to improve food safety. The use of vaccination, prebiotics, competitive exclusion, antibiotics, antimicrobials, dietary practices, and good animal management can potentially reduce the incidence of food-borne pathogenic bacteria that enter the abattoir. Further research into interventions that take advantage of this preslaughter "critical control point" is crucial to improving overall food safety.

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Genomic and computing strategies in the optimization of the genetic component of specification beef

J. W. Wilton[1]

Centre for Genetic Improvement of Livestock, Department of Animal and Poultry Science, University of Guelph, Ontario, Canada N1G 2W1

ABSTRACT: Genomics and computing are closely interrelated in beef cattle improvement. Both require the prior definition of breeding objectives, both can be used to carry out genetic evaluations of economically important traits, and both can be used in the development of selection tools for sires and dams. Effective use of both requires accurate specification of the desired product, an optimal production program, and crossbreeding structure. Optimizing the genetic component of production requires information on traits of economic importance, identification and relationships of animals, information on candidate and marker genes, and information on economics. Genomic information can be used for strategies involving identified allele deletions, identified allele introgressions, marker-assisted introgression, and marker-assisted selection. Techniques are being developed to combine genotypic data and quantitative data into genetic evaluations, although more developments are needed to optimize the use of these techniques across the range of beef traits varying in economic importance and cost of measurement. The genetic component of economical production of specified products can be optimized with customized selection programs. An example is presented in which performance levels are predicted from genetic evaluations based on quantitative and genomic information. The implications for selection within a seedstock population are also discussed.

Key Words: Beef Production, Genetic Improvement, Genome Analysis

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Introduction

Recent developments in molecular genetics and computing have provided new opportunities for optimizing the genetic component for efficient beef production. At the same time, there is increasing interest in more exactly specified beef products, as indicated for example in the description of certified beef programs by the USDA (2002).

The objectives of this article are to provide a brief review of new developments in molecular genetics and computing, to discuss the interrelationships of these developments, and to discuss strategies for utilizing these new developments in the context of improving the efficiency of producing a clearly specified beef product.

Specification of the Product and Production Program

The importance of specifying the economic value of the carcass in terms of weight and intramuscular fat when selecting sires has been shown by Wade et al. (2001). In general, specifications could include carcass weight, intramuscular fat, indicators of tenderness, or other criteria as dictated by consumer demand. Many of these are included to varying degrees in certification criteria (USDA, 2002) and may have genetic components.

Discussion of optimizing genetics requires consideration of the production environment (Wilton, 1986; 1990). One major component of the production program is the crossbreeding structure being used, whether a rotational cross, terminal cross, or composite. Expressions of traits at the phenotypic level are clearly dependent on combinations of breeds as well as the genetics of individual animals. Selection objectives in genetic populations (breeds for example) depend on the use of those populations in crossbreeding. Selection objectives for populations used as either terminal or maternal populations in terminal crossing differ from each other and from those for populations used in rotational crossing.

Another major component of the production program is the nutritional regimen. Nutritional effects on carcass traits have been shown by many researchers, such as Mandell and Aalhus (2000). The combined effect of genetics and nutrition is a particularly important con-

[1]Correspondence: phone: 519-824-4120, ext. 53647; fax: 519-767-0573; E-mail: jwilton@uoguelph.ca.

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sideration in genetic improvement in situations in which the absolute level of performance of commercial progeny is important, as it is in cases of optimal carcass weight, for example.

Optimization of Genetics

Genetic improvement through selection is dependent on changing allelic frequencies. Changes in allele frequencies can be considered for both simple gene effects and for multiple, or quantitative, gene effects. Genetic improvement through intra- or interlocus allele or gene combination effects involves both measurements of interactions and optimization of heterosis. Overall genetic optimization involves matching genetics with environment, or more specifically, with management, nutrition, and marketing programs, as described by Wilton (1986).

Genomic Strategies

There are two basic strategies for improving the genetics of populations with genes of known location, either identified allele elimination or identified allele introgression. Identified allele elimination is basically the elimination of genetic defects and is important when there is complete dominance of an allele at a locus. Some examples of testing services for cattle are for protoporyphria, bovine leucocyte adhesion deficiency, citrullinaemia, complex vertebral malformation, deficiency of uridine monophosphate, and Pompe's disease (Bova-Can, 2002; ImmGen, 2002). This genomic information makes possible the use of strategies to eliminate or at least reduce heterozygotes for breeding purposes.

Identified allele introgression basically involves increasing the frequency of a desirable allele. One example is the identification of mutations in the myostatin gene (Kambadur et al., 1997) and the association of inactivated myostatin with carcass traits (Casas et al., 1998) and palatability (Wheeler et al., 2001). The strategy suggested by Wheeler et al. (2001), at least within the Piedmontese that they studied, was the use of homozygous inactivated myostatin genotypes as terminal sires to produce heterozygous progeny with improved carcass value. A comprehensive analysis of possible mating systems by Keele and Fahrenkrug (2001) indicated that the most profitable mating system depended on price sensitivity to intramuscular fat level and cost of managing dystocia.

The use of markers for simple gene effects is the same as for the use of identified genes. The RN gene in pigs (Mariani et al., 1996) is a marker for meat quality associated with glycogen metabolism and the elimination of heterozygotes can increase the rate of improvement in meat quality. In beef, a marker for intramuscular fat as associated with thyroglobulin concentrations has been reported (Genetic Solutions, 2001). The success of using this marker has yet to be determined in additional populations.

The use of markers for quantitative traits is receiving considerable attention for marker-assisted selection. A recent example is the search for quantitative trait loci for both growth and carcass composition within cattle segregating alternative forms of the myostatin gene (Casas et al., 2001). This search combines the general concept of identifying markers for economically important traits with the specific concept of identifying these markers within populations segregating for a major gene, a possibility indicated earlier by Hanset (1982). Another interesting example is provided by Echternkamp et al. (2002). Three markers on chromosomes 5 and 7 for ovulation rate and twinning have been identified and used along with measurements of ovulation rate and twinning rates in the genetic evaluation and selection of sires in a project designed to increase twinning rate.

Also, recent announcements concerning the identification of single-nucleotide polymorphisms of a draft of the bovine genome (Adam, 2002) offer possibilities for fine mapping and linking of specific genes with meat quality traits. Considerable research has been done in the area of combining markers with quantitative data to improve the accuracy of estimating breeding values, as reviewed and discussed by Van Arendonk et al. (1999) and Weller (2001). However, examples of applications of markers in genetic improvement of commercial beef cattle populations have not yet been reported.

Computing Strategies

Developments in computing have been simultaneous with developments in genomics. Computing requirements have increased for merging genomic or marker information with quantitative phenotypic information. New computing possibilities are possible and also needed to obtain more information on phenotypes. Some examples are measurement of carcass traits through video image analysis (Cannell et al., 2002) and feed intake through electronic feeding equipment (Schenkel et al., 2002). Such phenotypic data must be connected to genotypes through pedigree structures and trace-back mechanisms for commercial data. Complete data on traits such as heifer and cow fertility, survival rates of cows, and health of both cows and calves can be obtained only with expanded whole-herd data recording. More automation is still needed for sufficient data to be collected so that more traits can be genetically evaluated or for markers or candidate genes to be identified.

Major developments in computing have taken place in both database management and Internet use. Speed of accessing data and transmitting results makes new approaches in timely genetic evaluation possible. An example of the simultaneous use of extensive databases and Internet use is the development and implementation of a customized sire-selection tool described by Wilton et al. (1998). In this application, net economic values are calculated for the use of sires in a herd with a

specified production environment and a specified market. The computing algorithm requires that market prices be stated and that any variations in prices in the product according to yield, quality, or weight be considered. Sensitivity of sire rankings to variation in some of these factors has been shown by Wilton et al. (2002). Computations also require specification of the production environment in terms of crossbreeding system and feeding programs, along with appropriate prices.

Phenotypes to be used to obtain net economic values in this development are predicted from across-breed genetic evaluations (**ABC**), computed as described by Sullivan et al. (1999). Across-breed genetic evaluations for postweaning growth and ultrasonic backfat at end of test are used to predict growth rate of steers in the feedlot and time to market under specified levels of finish as a marketing criterion. Similarly, ABC for intramuscular fat at end of test are used to predict distributions of progeny for marbling score. The ABC for longissimus muscle area are used to predict retail yield of progeny. The ABC for growth rate, ultrasonically measured backfat, and feed intake (individually available through computerized feeding systems) are used to predict feed requirements of progeny in the feedlot. Similar predictions are used for female progeny in the herd, with discounted gene flows to account for expression rates and times. Across-breed evaluations for calving ease are used to predict costs associated with calving difficulties; ABC for direct and maternal weaning weight are used to predict weaning weights of progeny and ABC for growth to predict cow weight. Further refinements could be made by obtaining appropriate data and computing ABC for heifer fertility (Moyer, 2001), cow weight (Mwansa et al., 2002; Rumph et al., 2002), and survival (Snelling et al., 1995; Mwansa et al., 2002).

The endpoint of trait measurement is important in the interpretation and use of ABC in the prediction of progeny performance. For example, reranking of sires for retail yield with a change from a time-constant to a finish-constant basis has been shown by Handley et al. (1996). Fortunately the equivalence of time-constant and finish-constant endpoints as a basis for comparison was shown by Wilton and Goddard (1996) to be valid if time, weight, and finish are considered simultaneously and if production programs are optimized. Consistent time-constant ABC are used in the customized sire selection approach described by Wilton et al. (1998) and are the values used in the discussion to follow.

Net economic values for two bulls assuming two different price grids are given in Table 1, adapted from Wilton et al. (1998), as an example of the importance of customization. The first price grid is primarily based on prices relative to the carcass being a product, with little differentiation in prices for weight and no differentiation in prices for intramuscular fat, and is considered a "commodity" grid. The second price grid is based on greater differentiation of prices of the product based on weight and intramuscular fat and is considered a "quality" grid. In this example, the sire with the higher genetic evaluation for growth has the higher net economic value for the commodity grid, whereas the sire with the higher genetic evaluation for intramuscular fat has the higher net economic value for the quality grid.

The example used is based on choosing sires for specified production programs and market prices and can be repeated for a variety of programs and prices, making customization possible. As such, the example illustrates the use of computing strategies to optimize genetics by matching with the production and marketing environment. Specific levels of traits for individual herds can be obtained by linking to databases on performance measurements of animals in the herd. Choice of sires for use in those herds is made possible through linkage to databases on sires. The customization process described has been adapted for Internet use to provide timely rankings of sires (BIO, 2002).

Combining Genomic and Computing Strategies

Discovery of identifiable genes of economic importance depends on both molecular genetic techniques and measurements of phenotypes, linked by pedigree information. Databases for both genomic and phenotypic information are necessary. Computing strategies are critical for accumulation of phenotypic information on a multitude of traits as well as on pedigree information. Computing strategies are increasingly important for the incorporation of a marker or any genomic information into genetic evaluations. Genetic evaluations based on both quantitative and genomic information can be used in computing strategies to optimize genetics in terms of selection and mating systems for clearly specified products, as well as clearly defined production environments. Additional research is required to develop complete genetic improvement strategies in the beef industry.

Implications

Increases in knowledge of genomics and computing and development of strategies for their combined use can lead to improvements in optimizing the genetic component in the production of desired beef products. More efforts are required in the measurement and re-

Table 1. Net economic values and across-breed comparisons for two bulls for two price grids

	Bull 1	Bull 2
Across-breed comparison		
Postweaning gain, kg	79	13
Backfat depth, mm	0.4	1.0
Intramuscular fat, %	0.1	0.7
Net economic value		
Commodity grid	$3,642	$1,429
Quality grid	$639	$4,327

cording of an expanded range of traits, establishment of databases, improved evaluation techniques incorporating quantitative and genomic information, quantifying of genotype × environment interactions, and cost-effectiveness of the various strategies to make these improvements a reality.

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Effects of roughage source and level on intake by feedlot cattle[1]

M. L. Galyean*[2] and P. J. Defoor†

*Department of Animal and Food Sciences, Texas Tech University, Lubbock 79409 and
†New Mexico State University, Clayton Livestock Research Center, Clayton 88415

ABSTRACT: Intake by beef cattle fed high-concentrate, grain-based diets is likely controlled primarily by metabolic factors and not limited by bulk fill. Nonetheless, small increases (e.g., 5% of dry matter or less) in the concentration of bulky roughage and changing from less fibrous to more fibrous sources of roughage typically increase dry matter intake (DMI) by feedlot cattle. Reasons for increased DMI with changes in roughage level and source are not understood fully. Energy dilution effects caused by added dietary fiber might be responsible for altered DMI, but the quantity of dietary net energy for gain provided by roughage shows little relationship to changes in DMI with roughage source and level. Altered rate of ruminal acid production as a result of roughage source and level might affect DMI through various mechanisms, including increased chewing and/or rumination with increased saliva flow; altered ruminal and/or intestinal digesta kinetics; and altered site and extent of digestion. We hypothesized that much of the effect of roughage source and level on DMI by feedlot cattle could be accounted for by changes in dietary neutral detergent fiber (NDF). Data from 11 published trials involving roughage source and level effects on intake by feedlot cattle were compiled. The dataset included 48 treatment means, with roughage sources such as hays, straws, byproducts, and silages, and with roughage levels ranging from 0 to 30% of dry matter. Effects of dietary roughage level (percentage of dry matter), NDF (percentage of dietary NDF from roughage), or effective NDF (eNDF, percentage of dietary eNDF from roughage) and the random effects of trial on DMI (percentage of body weight) were evaluated using mixed-model regression procedures. Tabular values were used to obtain estimates of NDF and eNDF. Using trial-adjusted means, dietary roughage level accounted for 69.9% of the variation in DMI, whereas the percentage of dietary NDF and eNDF supplied by roughage accounted for 92.0 and 93.1%, respectively, of the variation in DMI. The relationship between dietary NDF (percentage supplied by roughage) and DMI (percentage of body weight) for trial-adjusted data was given by: DMI = 1.8562 – (0.02751 × NDF) ($P < 0.01$; root mean square error = 0.0447); intercepts differed ($P < 0.02$) among trials, but slopes did not ($P > 0.18$). Based on these results, the percentage of NDF supplied by roughage in diets can be used to predict effects of roughage source and level on DMI by feedlot cattle, and NDF supplied by roughage might be a useful method for exchanging roughage sources in finishing diets.

Key Words: Beef Cattle, Fiber, Intake, Roughage

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Introduction

Adding a low percentage of roughage to high-concentrate diets helps prevent digestive upsets and maximizes energy (NEg) intake by feedlot cattle. In a recent survey of 19 consulting nutritionists in the major cattle feeding states, Galyean and Gleghorn (2001) reported that finishing diets contained from 4.5 to 13.5% (DM basis) roughage (mean = 8.89%, mode = 9%), with alfalfa hay and corn silage being the most common sources. Both roughage level and source influence DMI, and thereby NEg intake (Defoor et al., 2002), which ultimately affects feedlot performance and carcass characteristics; however, reasons for the effects of roughage on feed intake are not fully understood. Physical and chemical characteristics of roughages, such as bulk density and concentrations of fiber (e.g., NDF) and other nutrients are likely involved (Defoor et al., 2002), and effects of roughage on DMI also seem to be associated with differences in ruminal fermentation and digesta kinetics. Nonetheless, the specific ways by which these roughage characteristics affect DMI have not been suf-

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[2]Correspondence: Box 42141 (phone: 806-742-2453; fax: 806-742-2427; E-mail: mgalyean@ttacs.ttu.edu).

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ficiently quantified to allow for prediction of differences in DMI among roughage sources and levels that occur in practice. This review will summarize the results of research in which different roughage sources and levels have been fed in high-concentrate diets for feedlot cattle. In addition, we will examine the role that the NDF supplied by roughage plays in contributing to differences in DMI among roughage sources and levels and the biological basis for the effects of roughage in feedlot diets.

Effects of Roughage Source and Level on Dry Matter Intake by Feedlot Cattle

Several studies have investigated the effects of roughage source and/or level on DMI and performance by feedlot cattle fed high-concentrate diets. Gill et al. (1981) used 240 steers in a 121-d feeding trial to evaluate five roughage levels (8, 12, 16, 20, and 24% of DM) in diets based on high-moisture corn, steam-flaked corn, or a 50:50 mixture (DM basis) of high-moisture and steam-flaked corn. Roughage was a mixture of alfalfa (⅓ on a DM basis) and corn silage (⅔ on a DM basis). Across grain type, increasing roughage level increased DMI, but effects on ADG and feed:gain (**F:G**) depended on grain type, with 8, 12, and 16% roughage being optimal for steam-flaked corn, the 50:50 mixture, and high-moisture corn grain types, respectively. Kreikemeier et al. (1990) fed steam-rolled wheat diets with 0, 5, 10, or 15% roughage (DM basis; 50:50 mixture of alfalfa hay and corn silage) to finishing beef steers. Daily DMI increased linearly ($P = 0.08$) with increasing roughage level. Similar to the results of Gill et al. (1981) with steam-flaked corn, ADG and gain:feed ratio were optimized with the 5 and 10% roughage levels.

Bartle et al. (1994) fed alfalfa and cottonseed hulls at 10, 20, or 30% of the dietary DM to finishing beef cattle. Within each roughage level, DMI was less and efficiency of gain was greater for cattle fed the alfalfa than for cattle fed the cottonseed hull diets. Across roughage level, DMI increased at a faster rate for cottonseed hull diets than for alfalfa diets (approximately 0.10 vs. 0.05 kg of DMI for each 1% increase in roughage level). Assuming that cattle fed high-concentrate diets attempted to eat to a constant energy level, the greater rate of change with cottonseed hulls might have reflected a greater rate of dietary energy dilution as the level of cottonseed hulls increased compared with alfalfa. The energy dilution with cottonseed hulls should be accounted for, in part, by its higher NDF concentration compared with alfalfa, such that a smaller percentage of cottonseed hulls would be needed to provide the same intake of NDF as a larger percentage of alfalfa. Bartle et al. (1994) reported that ADG was similar for cattle fed 10 and 20% alfalfa; however, cattle fed the 30% alfalfa diets gained less than those fed the 10 and 20% alfalfa diets, evidently because they could not consume enough DM to compensate for the energy dilution. Cattle consuming the 10% cottonseed hull diets gained at a rate similar to cattle consuming the 10 and 20% alfalfa diets. Because of the greater rate of energy dilution per unit of cottonseed hulls, however, the cattle consuming the 20 and 30% cottonseed hull diets could not consume enough DM to express their potential for ADG. We interpret these data to indicate that one step toward describing effects of roughage source and level on DMI might be to equalize the dietary percentage of NDF supplied by roughage.

Guthrie et al. (1996) fed heifers diets with alfalfa, cottonseed hulls, and sorghum sudangrass hay at either 7.5 or 15% of DM in whole shelled corn-based diets. The DMI and ADG were greater by heifers fed the cottonseed hull and sorghum sudangrass hay diets than by those fed alfalfa. Results of this experiment indicated the possibility that cattle might sometimes overcompensate for energy dilution associated with different roughage sources. Guthrie et al. (1996) compared DMI, ADG, and F:G by cattle consuming alfalfa at 10% and sorghum sudangrass hay at 5, 7.5, and 10% of dietary DM. Dry matter intake and ADG by cattle fed the three levels of sorghum sudangrass hay were greater than by those fed the alfalfa diet, but F:G did not differ among diets. Calculated dietary NDF concentration (NRC, 1996) was slightly less for the 5% sorghum sudangrass hay diet than for the 10% alfalfa diet; however, DMI per unit of BW was similar for these two treatments (1.89 and 1.87% of BW for 5% sorghum sudangrass hay and 10% alfalfa, respectively).

Theurer et al. (1999) fed alfalfa, cottonseed hulls, and wheat straw to steers as the roughage source in three finishing diets. All three diets contained a base concentration of 6% alfalfa and were formulated to supply an equal percentage of NDF from roughage by adding an additional 6% alfalfa, 2.8% cottonseed hulls, or 3.7% wheat straw. Adding 2.8% cottonseed hulls or 3.7% wheat straw was as effective for maintaining DMI and ADG as adding an additional 6% alfalfa, indicating that low-quality roughage sources generally have a higher roughage value than higher quality forages and that much of this effect can be attributed to differences in concentrations of NDF.

Shain et al. (1999) fed 224 yearling steers either dry-rolled corn-based diets with no roughage or diets balanced to provide equal percentages of NDF from alfalfa and wheat straw. Roughage sources were ground to pass through 0.95-, 7.62-, or 12.7-cm screens. The alfalfa and wheat straw contained 42.8 and 82.0% NDF and were included at 10 and 5.2% of dietary DM, respectively, to provide equal levels of NDF from roughage. Dry matter intake was least for cattle fed the all-concentrate diet, but did not differ between alfalfa and wheat straw across chop lengths. Cattle fed the alfalfa diets gained faster and were more efficient than those fed the wheat straw diets, regardless of chop length. No differences in ADG or F:G were detected between steers fed wheat straw and the all-concentrate diet, and altering roughage particle size (chop length) had no effect on ADG or F:G. Reasons for the differences noted by

Shain et al. (1999) in ADG and gain efficiency between the wheat straw and alfalfa diets are not clear; however, because the CP concentration, energy density, and DMI were similar between the diets, it is possible that differences were attributable to the effects of the roughage sources on digesta kinetics, as will be discussed in a subsequent section.

Defoor et al. (2002) used 12 medium-framed beef heifers in three simultaneous, 4 × 4 Latin square intake trials to evaluate the effects of dietary NDF supply from alfalfa, sorghum sudangrass hay, wheat straw, or cottonseed hulls fed in each Latin square at 5, 10, or 15% of the dietary DM. Within each roughage level, NDF supplied by roughage accounted for the majority of variation in NEg intake/kg of $BW^{0.75}$ among the sources. The NEg intake/kg of $BW^{0.75}$ tended ($P < 0.10$) to be greater when heifers were fed cottonseed hulls, sorghum sudangrass hay, or wheat straw than when fed alfalfa. In a second experiment, 105 heifers were used in a 140-d finishing trial to evaluate methods of dietary roughage exchange. Alfalfa at 12.5% of the dietary DM was used as a standard, and cottonseed hulls and sorghum silage were each fed at three different levels compared with alfalfa: 1) an equal percentage of DM basis; 2) an equal NDF from roughage basis; and 3) an equal NDF from roughage basis, where only NDF from particles larger than 2.36 mm (**ReNDF**) were considered to contribute to the NDF. No differences ($P > 0.10$) in DMI, ADG, gain:feed, or NEg intake/kg of $BW^{0.75}$ were detected between alfalfa and cottonseed hulls exchanged on an equal NDF basis. With sorghum silage, exchanging with alfalfa on an equal ReNDF basis resulted in no differences ($P > 0.10$) in DMI, NEg intake/kg of $BW^{0.75}$, or ADG. Defoor et al. (2002) suggested that their data provided a preliminary indication that NDF supplied by roughage and/or roughage NDF from particles larger than 2.36 mm might provide a useful index of roughage value in high-concentrate finishing diets.

Literature data make it clear that roughage source and level can have substantial effects on DMI by cattle fed high-concentrate diets. Effects of larger changes in roughage level (e.g., greater than 5% of DM) on DMI might simply reflect energy dilution, such that cattle increase DMI presumably in an attempt to maintain energy intake. It is doubtful; however, that small changes in roughage level or changes in roughage source could affect energy density enough to account for the relatively large increases or decreases often observed in DMI as a result of these changes. Occasionally, overcompensation in DMI occurs, with associated improvements in performance. Changes in the fraction of dietary NDF supplied by roughage as levels and sources change seem to be associated with effects of roughage level and source on DMI. In the next section, data from the studies reviewed above are used to evaluate the role of NDF supplied by roughage in accounting for differences in DMI by feedlot cattle.

Literature Data Analysis

To evaluate the role of NDF supplied by roughage in accounting for changes in DMI by feedlot cattle, we compiled data from the seven studies reviewed in the previous section involving 11 trials (48 treatment means) in which effects of roughage source and level on DMI by feedlot cattle were evaluated. Most data from these studies were means for pens of cattle fed for extended periods (e.g., mean values for a typical finishing period); however, data from Defoor et al. (2002) included short-term intake data from individually fed (21 d) and penned (35 d) cattle. For each data point, the average BW of cattle during the trial period was used to express DMI as a percentage of BW. Because several of the studies did not include an estimate of the NDF content of the roughage sources, tabular values from NRC (1996) were used to determine the percentage of dietary NDF supplied by roughage. Similarly, tabular effective NDF (**eNDF**) and NEg values of NRC (1996) were used to determine the percentage of dietary eNDF and megacal of NEg supplied by roughage. A summary of the data obtained from these experiments is presented in Table 1.

The dependent variable DMI (percentage of BW) was regressed on the independent variables of dietary roughage level (percentage of DM), NDF, eNDF, or NEg from roughage using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC). The basic procedures for pooling data from multiple studies described by St-Pierre (2001) were used. The dependent variable was fit to a model that included a fixed slope and intercept in addition to a random slope and intercept clustered by trial (St-Pierre, 2001). An unstructured variance–covariance matrix was assumed for the intercepts and slopes; however, the slope–intercept covariance was not significant ($P > 0.20$) for any of the dependent variables, and this term was subsequently deleted from the models. Trial-adjusted DMI data were calculated as described by St-Pierre (2001) and regressed on the independent variables using simple linear regression.

To illustrate the process and goal of the mixed-model analysis we conducted, data for DMI vs. NDF supplied by roughage for the 11 trials that comprised the data set are presented in Figure 1. Visual appraisal of the trend lines for the various trials suggests that DMI responded similarly to changes in NDF supplied by roughage across trials, but that baseline DMI values varied considerably among trials. Indeed, the change in DMI among trials (vertical differences among trend lines) is far greater than the effect of changes in NDF supplied by roughage on DMI within a given trial (vertical differences within a trial). Trial or study effects are typically important in pooled data analyses (St-Pierre, 2001), with such effects in the present analysis likely reflecting differences in cattle factors (age, type, and management), seasonal differences, differences attributable to dietary factors other than roughage, and a myriad of other unknown, random factors. The mixed-

Table 1. Summary of the literature data used for mixed model regression analyses

Reference	Trial	Roughage source[a]	DMI, % of BW	Roughage, % of DM	Roughage		
					NDF, % of DM	NEg, Mcal/kg	eNDF, g/g of NDF
Bartle et al. (1994)	1	CSH	1.89	10	88.3	0.15	0.980
	1	CSH	2.12	20	88.3	0.15	0.980
	1	CSH	2.37	30	88.3	0.15	0.980
	2	CSH	1.92	10	88.3	0.15	0.980
	2	CSH	2.16	20	88.3	0.15	0.980
	2	CSH	2.45	30	88.3	0.15	0.980
Defoor et al. (2002)	3	ALF	1.27	4.86	47.1	0.68	0.920
	3	ALF	1.29	10.01	47.1	0.68	0.920
	3	ALF	1.33	15.16	47.1	0.68	0.920
	3	SS	1.30	5.01	66	0.62	0.980
	3	SS	1.33	10.03	66	0.62	0.980
	3	SS	1.55	15.03	66	0.62	0.980
	3	CSH	1.36	5.11	88.3	0.15	0.980
	3	CSH	1.49	10.22	88.3	0.15	0.980
	3	CSH	1.69	15.31	88.3	0.15	0.980
	3	WS	1.39	5.03	78.9	0.11	0.980
	3	WS	1.42	10.1	78.9	0.11	0.980
	3	WS	1.67	15.19	78.9	0.11	0.980
	4	ALF	1.72	12.8	47.1	0.68	0.920
	4	CSH	1.58	2.61	88.3	0.15	0.980
	4	CSH	1.70	6.12	88.3	0.15	0.980
	4	CSH	1.78	12.93	88.3	0.15	0.980
	4	SSIL	1.68	3.65	60.8	0.74	0.810
	4	SSIL	1.83	8.06	60.8	0.74	0.810
	4	SSIL	1.69	14.07	60.8	0.74	0.810
Gill et al. (1981)	5	⅓ ALF:⅔ CS	2.03	8	46.37	0.89	0.847
	5	⅓ ALF:⅔ CS	2.08	12	46.37	0.89	0.847
	5	⅓ ALF:⅔ CS	2.11	16	46.37	0.89	0.847
	5	⅓ ALF:⅔ CS	2.16	20	46.37	0.89	0.847
	5	⅓ ALF:⅔ CS	2.19	24	46.37	0.89	0.847
Gutherie et al. (1996)	6	SS	1.89	4.86	66	0.62	0.980
	6	SS	2.01	7.27	66	0.62	0.980
	6	SS	1.97	9.7	66	0.62	0.980
	6	ALF	1.87	10.08	47.1	0.68	0.920
	7	ALF	2.04	10.15	47.1	0.68	0.920
	7	SS	2.18	10.16	66	0.62	0.980
Kreikemeier et al. (1990)	8	½ ALF:½ CS	2.10	0	46.55	0.84	0.865
	8	½ ALF:½ CS	2.14	5	46.55	0.84	0.865
	8	½ ALF:½ CS	2.16	10	46.55	0.84	0.865
	8	½ ALF:½ CS	2.20	15	46.55	0.84	0.865
Shain et al. (1999)	9	ALL CONC	2.49	0	0	0	0.000
	9	ALF	2.70	10	47.1	0.68	0.920
	9	WS	2.75	5.2	78.9	0.11	0.980
	10	ALF—FINE	2.52	10	47.1	0.68	0.920
	10	ALF—COARSE	2.56	10	47.1	0.68	0.920
Theurer et al. (1999)	11	ALF	1.74	12	47.1	0.68	0.920
	11	CSH–ALF mix	1.79	8.8	60.2	0.51	0.939
	11	WS–ALF mix	1.81	9.7	59.2	0.47	0.943

[a]CSH = cottonseed hulls; ALF = alfalfa hay; SS = sorghum sudangrass hay; WS = wheat straw; SSIL = sorghum silage; CS = corn silage; ALL CONC = all concentrate; ALF—FINE = alfalfa hay ground to pass a 0.95-cm screen; ALF—COARSE = alfalfa hay ground to pass a 7.6-cm screen.

model analysis allows these random effects of trial and interactions of trial with independent variables to be modeled, so that the strength of the relationship between the dependent and independent variables can be determined. Trial-adjusted data for the relationship between DMI and NDF supplied by roughage are shown

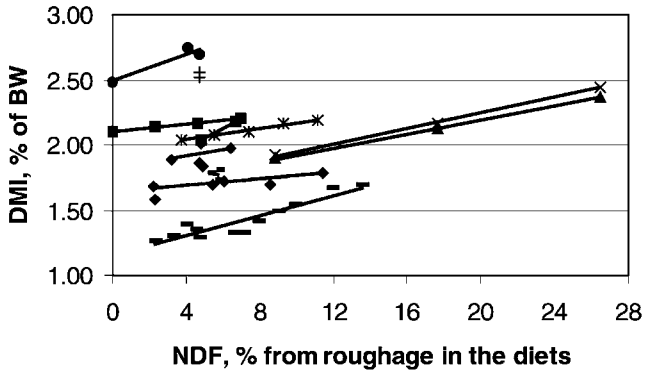

Figure 1. Plot of DMI (percentage of BW) vs. percentage of dietary NDF supplied by roughage for the 11 trials used in the data set. Individual data points are treatment means. References for the trials are shown in Table 1.

in Figure 2b. Although large random trial differences in intercepts were noted, the slope was not affected by trial. Thus, the slope of the overall line might be useful to describe the expected change in DMI per unit of BW with changes in NDF supplied by roughage. At the very least, these trial-adjusted data illustrate that there is a close relationship between DMI and dietary NDF supplied by roughage, thereby allowing us to more clearly elucidate the specific reasons (e.g., differences in NDF content) for effects of roughage source and level on DMI in these 11 trials.

Results for the regression of trial-adjusted data for DMI vs. roughage level, NDF supplied by roughage, and eNDF supplied by roughage are shown in Figures 2a, 2b, and 2c, respectively. Overall intercept and slope estimates for these three variables were highly significant ($P < 0.001$). Intercepts differed ($P < 0.02$) among trials for all three variables, but slopes did not ($P > 0.18$ for NDF and eNDF; $P > 0.27$ for roughage level). As noted previously, dietary NEg supplied by roughage also was evaluated as an independent variable, but the slope for NEg was not significant ($P > 0.28$; data not shown). This finding suggests that the relatively small differences among the data points in dietary NEg supplied by roughage were not useful for describing changes in DMI and that simply accounting for these small changes in energy dilution does not fully describe the effects of roughage source and level on DMI by feedlot cattle. Roughage level (Figure 2a) was clearly associated with changes in DMI, but the r^2 (0.699) was considerably less than for NDF supplied by roughage (0.920) and eNDF supplied by roughage (0.931). We interpret these results to suggest that most of the effect of changes in roughage level and source on DMI by feedlot cattle can be ascribed to changes in dietary NDF supplied by roughage. In these data, eNDF accounted for such a slight improvement beyond NDF that its use

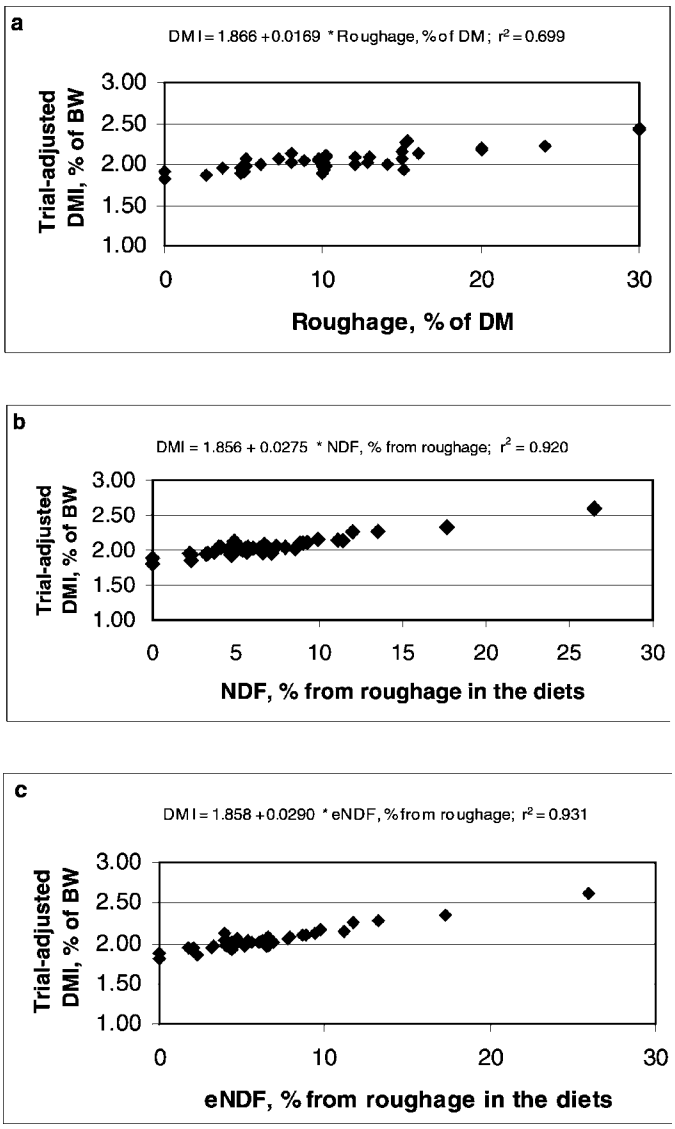

Figure 2. Plots of trial-adjusted DMI (percentage of BW) vs. a) roughage level (percentage of DM); b) NDF (percentage of dietary NDF supplied by roughage); and c) effective NDF (eNDF; percentage of dietary eNDF supplied by roughage).

would not seem worthwhile in practice. Nonetheless, eNDF might be more descriptive than NDF for certain roughage sources, as evidenced by the superior results in exchanging sorghum silage for alfalfa on the basis of NDF retained on a 2.36-mm screen compared with NDF alone in the study of Defoor et al. (2002).

If changes in DMI (and thereby intake of net energy) accurately reflect differences among roughage levels and sources, the strong relationship we observed between NDF supplied by roughage and DMI in these literature data supports the concept that NDF supplied by roughage could be used practically as a means of exchanging roughage sources in feedlot diets. As noted previously, we used NRC (1996) tabular values for NDF and eNDF in our analysis. Use of directly measured

values, or at least values selected from a local or regional historical database, should provide even greater precision in defining effects of roughage on DMI. Further research is needed to test the value of NDF supplied by roughage as a means of exchanging roughages in feedlot diets. Similarly, because our data for eNDF were derived from tabular estimates, the role of eNDF or some type of index related to the physical effectiveness of NDF in describing the effects of roughage in feedlot diets needs further study. Finally, our data included only a few commonly used roughage sources (alfalfa hay, corn silage, cottonseed hulls, sorghum sudangrass hay, sorghum silage, and wheat straw), so extension of these findings to other sources of NDF (e.g., high-fiber byproduct feeds) would require further research.

Biology of Roughage Level and Source Effects

Energy Dilution. Physical fill would rarely limit intake of high-concentrate diets, so when a high-concentrate diet is diluted by roughage, the animal typically eats more feed to maintain energy intake. Compensation via increased DMI is possible until the point that roughage (fiber) level is sufficiently high to impose restrictions on fill or perhaps eating rate. This concept is illustrated in the data of Bartle et al. (1994), in which cattle fed 10 and 20% alfalfa diets had equal ADG, but increasing the level of alfalfa to 30% of the diet decreased ADG. Similarly, cattle fed 20 and 30% cottonseed hulls, although consuming more DM, gained less than those fed 10% cottonseed hulls. Below the point of restriction, relatively small changes in fiber level in the diet resulting from either increased roughage level or a switch to a higher-fiber roughage source can stimulate DMI to the point that the total energy intake is increased (e.g., a quadratic effect of roughage level on energy intake). This concept is illustrated by the results of Guthrie et al. (1996), in which ADG was greater in cattle fed 7.5 and 10% sorghum sudangrass hay diets than in those fed a 10% alfalfa diet. As noted previously, NEg supplied by roughage was not a significant factor related to DMI in the literature data that we analyzed. This result presumably reflects the fact that for many of the data points in the literature database, the change in NEg supplied by roughage with changes in roughage source and level was relatively small. It seems likely that energy dilution per se would affect DMI of high-concentrate diets only when differences in fiber level are large (e.g., Bartle et al., 1994), whereas differences in DMI resulting from smaller, more subtle changes in fiber level (e.g., Guthrie et al., 1996) might result from factors other than energy dilution, such as changes in ruminal and/or metabolic acidity or digesta kinetics as will be discussed in subsequent sections.

Ruminal, Intestinal, and Metabolic Acidity. Alterations in the quantity of and/or temporal pattern of acid production within the gut and the subsequent metabolic acid load via absorption could account for many of the effects of NDF from roughage on DMI by feedlot cattle. Acidosis negatively affects intake by feedlot cattle (Fulton et al., 1979a,b); thus, the quadratic response in DMI often noted with small increases in NDF supplied by roughage might reflect effects on acid load. For example, if bite size were relatively constant with high-concentrate diets, the ratio of grain to NDF in each bite would decrease for a diet with a greater NDF supply from roughage compared with a lower-fiber diet. Allen (1997) noted that the balance between production of fermentation acids and secretion of salivary buffers was the primary determinant of ruminal pH. Hence, with a higher NDF intake per unit of grain, one might expect a higher, or at least more stable, ruminal pH. The resulting lower metabolic acid load also could be lower simply because the proportion of fermentable substrate per bite would be less, and the greater proportion of NDF in each bite might stimulate more chewing and saliva secretion. If the total number of bites increases until acid load becomes limiting, total energy intake might exceed what would be expected from compensation for energy dilution alone. The level of NDF from roughage required to elicit overcompensation in DMI likely differs among roughage sources and within a roughage source as NDF concentration of the source changes with maturity.

The extent to which the NDF content of the diet or NDF supplied by roughage is related to chewing time, saliva flow, and ultimately to ruminal pH, however, is open to question. In ruminally cannulated steers given ad libitum access to 90% concentrate (steam-flaked sorghum) diets (Moore et al., 1987), rumination time was greater with 10% wheat straw than with 10% of either cottonseed hulls or alfalfa (308 vs. 180 and 210 min/d, respectively). Ruminal pH was numerically greater for the diet containing wheat straw than for those containing alfalfa or cottonseed hulls (6.2 vs. 5.9 and 5.8, respectively), but did not differ among the three roughage sources. Thus, wheat straw, but not cottonseed hulls, seemed to alter chewing time and ruminal pH, even though both of these high-NDF roughages tended to increase DMI relative to alfalfa (Moore et al., 1987). Similarly, Shain et al. (1999) reported that steers fed a dry-rolled corn-based diet containing wheat straw spent more total time ruminating than steers fed a dry-rolled corn-based diet containing alfalfa; however, ruminal pH did not differ between cattle fed diets containing alfalfa or wheat straw ground to pass through a 2.54-cm screen. Pitt et al. (1996) reported a fairly strong relationship ($r^2 = 0.521$) between ruminal pH and the eNDF concentration of dairy, beef, and sheep diets. In contrast, Nocek (1997) reported that eNDF accounted for approximately 5% of the variation in ruminal pH in a dataset of mean ruminal pH values with lactating dairy cows. Allen (1997), also using a literature database, found that NDF content of the dietary DM was not related to ruminal pH in dairy cows. Nonetheless, Allen (1997) noted that forage NDF as a percentage of the DM was significantly related to ruminal

pH, which supports the concept that NDF from roughage might be related to ruminal pH, thereby accounting, at least in part, for the relationship that we observed between NDF from roughage and DMI by beef cattle fed high-concentrate diets. The statistical analyses conducted by Pitt et al. (1996), Allen (1997), and Nocek (1997) did not seem to use mixed-model methodology that would have allowed random study effects to be accounted for, which might explain some of the variation in results among these studies. In addition, animal-to-animal variation in ruminal pH and the ability to handle an acid load seems fairly substantial, even in model systems where a relatively constant acid load is applied (Brown et al., 2000). Such variation, as well as potentially large diurnal fluctuations in ruminal pH, would decrease the ability of dietary NDF or eNDF to account for a substantial proportion of the variation in mean ruminal pH.

Inherent buffering capacity has been suggested as an aspect of roughages that might account for differences in ruminal and metabolic acid loads and ultimate effects on DMI. However, Allen (1997) noted that buffering by feeds would be more likely to occur at a pH less than 5. Moreover, Allen (1997) calculated that the potential direct buffering by the diet was a small fraction of buffering by saliva. Thus, given that the maximal roughage level in feedlot diets is approximately 15%, with lower levels typically used in practice, inherent buffering capacity of roughages is probably not very important in accounting for effects of roughage source and level on DMI of high-concentrate diets by finishing beef cattle.

It is unknown whether roughage source and level affects absorption of acids from the rumen or acidity in the small and large intestines. It seems unlikely that increasing NDF supplied by roughage in a high-concentrate diet would directly affect absorption of VFA from the rumen. Similarly, direct effects of roughage on absorption of acids from the intestines seem unlikely. Allen (1997) suggested that changes in ruminal papillae surface area among diets might affect the susceptibility of cattle to acidosis, which could be related to differences resulting from dietary NDF supplied by roughage. Whether roughage source or level in feedlot finishing diets affects ruminal surface area for absorption is unknown. The NDF supplied by roughage might exert effects on digesta kinetics and associated water flux that affect digesta flow through the intestines and absorption of acid postruminally. Because DMI and water intake are positively associated (NRC, 1996), the increased DMI noted with higher dietary concentrations of NDF from roughage could be linked to a positive effect on acid load simply by an associated increase in water intake and dilution of acid. Incomplete mixing of water with ruminal contents (Allen, 1997) would tend to lessen the effects of greater water intake. In addition, increased water intake might merely shift site of acid absorption (i.e., rumen vs intestines) and thereby not greatly alter total metabolic acid load; however, the temporal pattern of acid absorption would perhaps be altered so as to spread the metabolic acid load more evenly over time. To our knowledge, effects of roughage source and level in beef cattle finishing diets on water intake have not been investigated.

Characteristics of Ruminal Digesta, Digesta Flow, and Site and Extent of Digestion. Fairly wide ranges in NDF supplied by roughage seem to affect the physical nature of ruminal contents. Moore et al. (1987) reported a tendency for cattle fed a high-concentrate diet with 10% cottonseed hulls to have a higher ruminal fill than those fed alfalfa and wheat straw diets, and a tendency for the cottonseed hull diet to have a higher percentage of ruminal DM in the fiber mat than alfalfa (2.4 vs. 0%). The lower percentage of fiber in the mat with alfalfa was probably a result of greater rate of passage of alfalfa than of cottonseed hulls and wheat straw from the rumen (Moore et al., 1990; Poore et al., 1990). The cottonseed hull diet had a lower percentage of ruminal DM in the fiber mat than wheat straw (2.4 vs. 19.9%), which was consistent with the greater time spent ruminating when animals were fed the wheat straw diet. Stratification or layering of ruminal contents has been implicated as a factor related to rumination in cattle (Van Soest, 1982; Welch, 1982; Moore et al., 1990; Poore et al., 1990). As noted previously, greater chewing during eating and rumination might result in greater saliva production, which could buffer the rumen of cattle fed high-concentrate diets (Owens et al., 1998). Conversely, greater rumination might increase mastication of grain in some diets, thereby increasing rate and extent of fermentation in the rumen (Owens et al., 1998). For example, Owens and Ferrell (1983) measured rumination time by steers fed a whole shelled corn-based diet with 5% roughage and noted a tendency for greater ADG by steers that ruminated up to 150 min/d than by those that ruminated approximately 65 min/d. This difference was most likely related to improved utilization of the whole corn as a result of greater mastication rather than increased saliva production and ruminal buffering. In contrast, Gill et al. (1981) indicated that non-ruminating steers gained faster than ruminating steers when fed diets based on steam-flaked corn, high-moisture corn, or a mixture of the two.

Changes in passage of dietary components from the rumen could be related to changes in DMI resulting from differences in roughage source and level. If NDF from roughage increases passage of the grain portion of the diet, less fermentation would occur in the rumen, resulting in a decreased acid load and potentially greater DMI. Nonetheless, grain starch seems to be used most efficiently when fermented in the rumen, and intestinal starch digestion capacity might be limited (Huntington, 1997), so passage of unfermented starch might be counterproductive to optimizing efficiency. Moore et al. (1990) fed ruminally cannulated steers 65% concentrate steam-flaked grain sorghum-based diets that contained either 35% alfalfa or 50:50 mixtures of alfalfa with cottonseed hulls or wheat straw. Replacing

half of the alfalfa with cottonseed hulls increased DMI and tended to increase the passage rate of the grain. Poore et al. (1990) measured passage rates of grain and roughage in 30, 60, and 90% concentrate diets containing 50:50 mixtures of wheat straw and alfalfa. Within each concentrate level, ruminal passage rate was greater for alfalfa than for wheat straw, decreasing by 13 and 28%, respectively, in the 90 vs. 60% concentrate diet. Passage of steam-flaked grain sorghum, however, was not influenced by diet, which does not support the hypothesis that higher roughage levels might increase passage of unfermented starch from the rumen. Similarly, Eng et al. (1964) reported that mean retention time of hay, but not of corn, was increased as concentration of corn increased from 25 to 75% in the diets of sheep. In contrast, Owens and Goetsch (1986) and Wylie et al. (1990) reported that increasing dietary roughage decreased residence time of grain in the rumen. Cole et al. (1976) reported a trend for decreased ruminal and total tract starch digestibility for diets containing 7, 14, and 21% cottonseed hulls compared with 0% cottonseed hulls in whole shelled corn-based diets, which the authors attributed to an increase in the rate of passage of grain with increasing cottonseed hulls. Other reports, however, have indicated positive effects of cottonseed hulls relative to alfalfa on total tract digestibility of starch in whole shelled corn-based diets (Teeter et al., 1981; Rust and Owens, 1982; Goetsch et al., 1986). Results of the feeding trial reported by Shain et al. (1999) suggested that compared with alfalfa, wheat straw might adversely affect the passage and utilization of dry-rolled corn, even when fed to provide the same level of NDF in the diet; however, no differences were noted in rate of ruminal starch disappearance or passage of Yb-labeled corn between the two roughage sources. Overall, currently available data suggest that effects of NDF supplied by roughage on passage of grain from the rumen, and thereby on site and extent of grain (starch) digestion, are probably not large within the normal range of roughage levels used in feedlot diets.

Summary and Conclusions

Our literature review and data analyses suggest that exchanging dietary roughage on the basis of NDF concentration instead of an equal percentage of DM basis might eliminate much of the variation in DMI and performance that often occurs when roughage sources are changed in practice. However, balancing for NDF alone might not be an entirely satisfactory means of exchange because other characteristics related to physical effectiveness of NDF sometimes affect roughage value. Formulating to a specific NDF concentration with different roughage sources probably accounts for most of the effect of roughage source and level on DMI, but it does not fully account for the aggregate of small differences in fiber sources that might affect chewing time and kinetics of digestion and passage of roughage and grain. Whether these differences in physical effectiveness of NDF are sufficiently large to warrant their consideration in formulation practices is unknown, and further research, coupled with practical evaluation of the use of NDF supplied by roughage as a means of roughage exchange, is needed.

Implications

Changes in roughage source and level affect dry matter intake by feedlot cattle. Based on analysis of published data, the percentage of neutral detergent fiber supplied by roughage in high-concentrate, feedlot diets accounts for most of the variation in dry matter intake caused by roughage source and level. Although neutral detergent fiber supplied by roughage might provide a useful basis for exchanging roughages in feedlot diets, the biological reasons for changes in dry matter intake associated with changes in roughage source and level need further study.

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Nitric oxide and the ovary[1]

C. Tamanini[2]*, G. Basini†, F. Grasselli†, and M. Tirelli†

*Dipartimento di Morfofisiologia Veterinaria e Produzioni Animali, Università di Bologna, 40064 Ozzano Emilia (BO), Italy and †Dipartimento di Produzioni Animali, Biotecnologie Veterinarie, Qualità e Sicurezza degli Alimenti-Sezione di Fisiologia Veterinaria, Università di Parma, 43100 Parma, Italy

ABSTRACT: Nitric oxide (NO) is synthesized from L-arginine by NO synthase (NOS), an enzyme with three isoforms. Two of them, neuronal and endothelial (nNOS and eNOS, respectively), are constitutive, whereas the third one, iNOS, is inducible. Nitric oxide is effective in mediating multiple biological effects, in part through the activation of soluble guanylate cyclase. Among these effects are smooth muscle cell tone, platelet aggregation and adhesion, cell growth, apoptosis, and neurotransmission. Because these mechanisms are associated with the pathophysiology of several reproductive processes, it has become clear that NO could play a key role in reproduction. Apart from its effects through the modulation of luteinizing hormone releasing hormone release, NO has been proven to act directly at the ovarian level, where it is produced by the vasculature and neurons, as well as by various cell types, including granulosa, theca, and luteal cells. Nitric oxide production is modulated by several hormones (estradiol 17β, luteinizing hormone, follicle-stimulating hormone, and human chorionic gonadotropin) and cytokines that interfere either with eNOS or iNOS expression and activity. Experiments performed with NO donors and/or NO synthase inhibitors have demonstrated that NO decreases apoptosis and inhibits both estradiol 17β and progesterone production by granulosa cells (at least in part via guanylate cyclase). Nitric oxide is possibly involved in follicle growth; it is a potent mitogen in the presence of basic fibroblast growth factor, it increases the receptors for epidermal growth factor on granulosa cells, and it regulates programmed cell death, which is an important part of folliculogenesis. Gonadotropin-stimulated eNOS and iNOS expression, as well as the inhibition of ovulation by NOS inhibitors, suggest that NO participates in the ovulatory process. After ovulation, iNOS is expressed in luteal cells, but its activity diminishes with corpus luteum development. During the luteolysis phase, NO stimulates $PGF_{2\alpha}$ synthesis while decreasing progesterone secretion. Overall data provide convincing evidence that NO plays a critical role in ovarian physiology with regard to follicle growth, ovulation, and corpus luteum function, but its clinical implications have not yet been clarified.

Key Words: Corpus Luteum, Nitric Oxide, Ovaries, Ovarian Follicles, Ovulation

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Introduction

Nitric oxide (**NO**) is an inorganic, short-lived (a few seconds) free radical gas that, due to its high solubility, freely diffuses through biological membranes. It is synthesized from L-arginine via an oxygen- and NADPH-dependent reaction that yields NO and L-citrulline (Bush et al., 1992; reviewed by Wu and Morris, 1998). Nitric oxide synthesis depends on the action of a NO synthase (**NOS**), an enzyme that exists in three isoforms that have been classified depending on tissue of origin as well as on functional properties. Two of them (neuronal [**nNOS**] and endothelial [**eNOS**]) are constitutive and seem to be responsible for the continuous basal release of NO; the third one is inducible (**iNOS**) and is expressed in response to inflammatory cytokines and lipopolysaccharides (Morris and Billiar, 1994). Both nNOS and eNOS are calcium/calmodulin dependent for their activation (Snyder, 1995), whereas iNOS is calcium independent. The three isoforms have been found in a variety of cell types, including neurons, gastric and bronchial epithelium, skeletal muscle, macrophages, cardiomyocytes, hepatocytes, and chondrocytes; the production of NO is therefore almost ubiquitous. Nitric oxide is involved in a wide range of functions: It determines vasodilation, inhibits platelet aggregation and neutrophil adhesion to endothelial

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[2]Correspondence: Facoltà di Medicina Veterinaria, Via Tolara di Sopra, 50, 40064 Ozzano Emilia (BO) (phone: +39-051-792925; fax: +39-051-792903; E-mail: tamanini@vet.unibo.it).

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cells, reduces smooth-muscle cell proliferation and migration, controls apoptosis, sustains endothelial cell barrier function (Rosselli et al., 1998), and acts as a neurotransmitter. Nitric oxide is generated by neurons, blood vessels, and cells of the immune system (which are structural and functional parts of the hypothalamus-pituitary-gonads axis), as well as by other cell types of this axis. Because NO plays an important role in the function of this system, its involvement in the mechanisms regulating the reproductive processes is quite obvious (Rosselli et al., 1998; Dixit and Parvizi, 2001).

Nitric Oxide Generation and Mechanisms of Action

Availability of L-arginine is essential for NO generation in that it is the only physiological nitrogen donor for NOS-catalyzed reactions. Competitive inhibition of arginine uptake by other naturally occurring amino acids, such as L-lysine and L-ornithine, reduces NO synthesis (Inoue et al., 1993). Nonphysiological substances derived from arginine (nitro-L-arginine-methyl esther [**L-NAME**], Ng-monomethyl-L-arginine [**L-NMMA**]) are being used to determine the effects of NO deprivation. In the same way, NO donors (sodium nitroprusside [**SNP**], *S*-nitroso-L-acetyl-penicillamine, [**SNAP**]) are also used to evaluate the involvement of NO in biological functions.

Many of the biological effects of NO may result from the alteration of multicomponent signal transduction pathways and are exerted via different mechanisms (for a review, see Schindler and Bogdan, 2001), three of which seem to be the most important. The first one involves NO binding to the heme iron of soluble guanylate cyclase, thus activating guanosine 3′,5′-cyclic monophosphate (**cGMP**), which mediates most of the effects on vessel and intravessel functions (Murad, 1994). Nitric oxide also induces the *S*-nitrosylation of thiol groups of free amino acids, peptides, and proteins (Kelly et al., 1996) and can react with other radicals, resulting in the formation of peroxynitrite, a potent oxidant effective in inducing cytotoxicity (for a review, see Droge, 2002). Taken together, the available information suggests that the effects of NO are strictly dependent on its concentration, as well as on the presence of metals, proteins, and low-molecular-weight thiols in a given cell (Davies et al., 1995).

Effects of Nitric Oxide at the Hypothalamic-Pituitary Level

Nitric oxide has been shown to modulate reproductive activity by acting at both the hypothalamic and pituitary levels (for a review, see Dixit and Parvizi, 2001). Experimental results (Bhat et al., 1995) suggest that NO is an important mediator of basal GnRH production since NO neurons are present in hypothalamic sites involved in GnRH secretion. Experiments with an NO donor (SNP) and with the NO scavenger hemoglobin demonstrated that NO stimulates GnRH release in rats; the effect may be induced both by activation of guanylate cyclase (Moretto et al., 1993) and through the activation of neuropeptide Y (Bonavera and Kalra, 1996). Nitric oxide has also been shown to promote LH secretion in the cow (Honaramooz et al., 1999); the positive effect on LH secretion is likely to be exerted via a cGMP-independent mechanism (Pinilla et al., 1998). An interaction between NO and opioids has also been proposed. The administration of naloxone (an opioid antagonist) enhances NOS activity (Bhat et al., 1996), whereas naltrexone blocks the inhibitory effect of β-endorphin on LHRH release and NOS activity in the rat (Faletti et al., 1999a). β-endorphin also blocks the positive effect of NO on PGE_2, and therefore on GnRH release. Leptin-induced LH release may be mediated via nitric oxide (Yu et al., 1997). Nitric oxide also seems to be involved in controlling the preovulatory LH surge (for a review, see Dhandapani and Brann, 2000) because NOS inhibitors have been demonstrated to reduce LH release in rats (Bonavera et al., 1994). The expression of nNOS in the preoptic area increases concomitantly with LH peak in the same species (Lamar et al., 1999). The generation of NO by hypothalamic and pituitary cells facilitates sexual behaviour in females; administration of NOS inhibitors and NO donors to conscious female rats prevents or stimulates lordosis behavior, respectively (Mani et al., 1994).

The overall data on the effects of NO in the modulation of reproductive activity at the hypothalamic-pituitary level strongly indicate that NO exerts a positive action and is effective in stimulating GnRH and LH secretion, as well as estrous behavior; nevertheless, because contrasting results have been reported in the rat (Ceccatelli et al., 1993; Chatterjee et al., 1997), further studies are required to definitely confirm the role of NO at this level.

Nitric Oxide and Ovarian Function

The involvement of NO in the modulation of ovarian function is documented by several studies aimed at demonstrating its production within the ovary and at clarifying its role in the regulation of steroidogenesis, follicle development, ovulation, luteal function, and luteal regression.

Regulation of Nitric Oxide Production within the Ovary

Nitric oxide is generated by several ovarian cells and within the ovarian vasculature; resident macrophages have also been indicated as a possible source of NO in the rat (Dave et al., 1997). Both eNOS and iNOS seem to be involved, although their expression and activity greatly depend on cell type and animal species and vary throughout the different ovarian processes (Rosselli et al., 1998). In particular, data on iNOS expression (which is usually influenced by inflammatory condi-

tions) in granulosa cells are still conflicting. Van Voorhis et al. (1994) reported that human granulosa-luteal cells express eNOS. The presence of eNOS also has been confirmed in rat mural granulosa cells (Jablonka-Shariff and Olson, 1997), in blood vessels (Van Voorhis et al., 1995), in rat stroma, thecal, and luteal cells (Zackrisson et al., 1996), and in pig granulosa cells (Ponderato et al., 2000; Takesue et al., 2001). Results from different studies indicate that rat granulosa cells from primary, secondary, and small antral follicles (Van Voorhis et al., 1995; Matsumi et al., 1998a) and rat stroma, thecal, and luteal cells (Zackrisson et al., 1996) also express iNOS. This isoform, on the other hand, has not been detected in either rat (Jablonka-Shariff and Olson, 1997) or in porcine (Ponderato et al., 2000) granulosa cells. Nitric oxide production also has been demonstrated in bovine granulosa cells (Basini et al., 1998; Basini et al., 2000); these studies, however, were not aimed at defining which isoform is responsible for NO synthesis. The expression of both iNOS and eNOS is regulated by gonadotropins (Jablonka-Shariff and Olson, 1997) since both PMSG and hCG have been shown to influence eNOS and iNOS concentrations, thus confirming that both isoforms participate in the ovarian functions. Nitric oxide production in rat ovarian cells is actively stimulated by interleukin-1, as well as by several proinflammatory cytokines (Ellman et al., 1993; Ben-Shlomo et al., 1994; Matsumi et al., 1998a); iNOS has been demonstrated to be induced in bovine thecal cells by tumor necrosis factor-α (**TNFα**), thus stimulating cGMP accumulation (Brunswig-Spickenheier and Mukhopadhyay, 1997). In addition, NO generation (measured on the basis of nitrite and nitrate concentration) increases with both estradiol levels in human follicular fluid and with follicular size (Rosselli et al., 1994; Anteby et al., 1996). These observations suggest a possible causal relationship between these characteristics, even though an inverse relationship between estradiol and nitrite concentrations has been observed in swine (Grasselli et al., 1998) and in bovine (Basini et al., 1998) follicular fluid. However, the increase of nitrite and nitrate levels in the serum of postmenopausal women subjected to E2 substitution therapy substantiates the positive effect of estrogens on NO production (Rosselli et al., 1994).

Nitric Oxide and Steroidogenesis

Nitric oxide has been shown to exert negative effects on steroidogenesis, possibly through a direct action on steroid-secreting cells rather than via an effect on local ovarian blood flow (Dave et al., 1997). The impairment of steroid production by NO has been demonstrated in different species and in different conditions (rat, Dave et al., 1997; human, Van Voorhis et al., 1994 and Rosselli et al., 1998; porcine, Masuda et al., 1997; Matsumi et al., 2000; Ponderato et al., 2000, and Grasselli et al., 2001; bovine, Basini et al., 1998 and Basini and Tamanini, 2000). The negative effect of NO on steroid production has been demonstrated by treating cultured granulosa-luteal cells with SNAP, an NO donor, or with L-NAME, an NOS inhibitor, which markedly decrease or stimulate, respectively, both estradiol and progesterone release. This effect seems to be cGMP independent (human, Van Voorhis et al., 1994; Rosselli et al., 1998; bovine, Basini et al., 2000), even though different conclusions have been drawn in other species (swine, Grasselli et al., 2001; rat, Ishimaru et al., 2001). The negative effect of NO on both basal- and gonadotropin-stimulated estradiol production in the rat may be, at least in part, exerted through an inhibition of androstenedione secretion (Dunnam et al., 1999); in addition, the cytochrome P450 aromatase, responsible for estradiol production, has been shown to be possibly inhibited (Van Voohris et al., 1994). This inhibition may be exerted through a reduction of aromatase messenger RNA (**mRNA**) levels and/or of enzyme effect (human, Snyder et al., 1996; Kagabu et al., 1999).

Nitric Oxide and Folliculogenesis

It is well known that both folliculogenesis and ovulation are regulated by a variety of factors, such as cytokines, growth factors, and locally produced substances, among which NO seems to play an important role. Nitric oxide levels have been shown to change during follicular growth (Rosselli et al., 1998). Follicular development, induced by PMSG in immature rat, is associated with an increase in eNOS (but not iNOS) expression (Van Voorhis et al., 1995; Jablonka-Shariff and Olson, 1997), whereas a subsequent stimulation with hCG induces an increase of both isoforms (Jablonka-Shariff and Olson, 1997). On the other hand, Matsumi et al. (1998b) observed a decrease in iNOS mRNA levels induced by PMSG in granulosa cells from immature rat follicles; on this basis, they suggested that NO could possibly represent a cytostatic factor. This hypothesis has been reinforced by results from a more recent study in the rat (Matsumi et al., 2000), which also show a GnRH- and endothelial growth factor (**EGF**)-induced reduction in iNOS mRNA levels. On the other hand, NO has been shown to act as an antiproliferative agent (Kuzin et al., 1996) and to inhibit mitosis (Takagi et al., 1994) in other mouse cell types. In contrast, a growth-promoting effect of NO is supported by the observation (Hattori et al., 1996) that NO increases EGF receptors in rat granulosa cells and IL-1β-stimulated NO production is effective in promoting muscle cell growth in presence of basic fibroblast growth factor (Dubey et al., 1997). However, treatment of bovine granulosa cells from different size follicles with the NO donor SNAP does not influence proliferation (Basini et al., 1998). The above findings provide evidence that the exact role played by NO in the regulation of cell growth is yet to be elucidated. It is feasible that effects of NO are strongly dependent on interactions with other growth modulatory factors acting within the ovary.

A further mechanism through which NO may be involved in the control of follicular development is its effects on apoptosis, the programmed cell death by which the majority of ovarian follicles are lost during postnatal life (Kiess and Gallaher, 1998; Li et al., 1998). High NO levels have been shown to reduce apoptosis in both swine (Ponderato et al., 2000) and bovine (Basini et al., 1998) granulosa cells, whereas an opposite effect has been induced by low NO levels in more differentiated granulosa cells (from large follicles). A protective NO effect has been also observed in rat granulosa cells from immature (Matsumi et al., 1998b; 2000) and preovulatory (Yoon et al., 2002) follicles. In addition, the IL-1β-induced antiapoptotic effects have also been reported to be NO-mediated (Chun et al., 1995). Different data have been reported by Sugino et al. (1996), whose findings in human granulosa cells do not clearly confirm NO involvement in the regulation of apoptosis. Pro- and antiapoptotic properties have also been attributed to NO in other cell types (Mannick et al., 1994; Kim et al., 1999), and possibly depend on its concentrations as well as on its possible interactions with a variety of molecules (irons, thiols, proteins, etc.) (Chung et al., 2001).

NO may also influence follicle development by mediating the effects of gonadotropins on the blood-follicle barrier, thus influencing its permeability to different substances (Powers et al., 1995).

The overall results on the effects of NO on folliculogenesis suggest that locally produced NO contributes to modulate follicle development and possibly prevents apoptosis, at least at low concentrations, whereas high levels may promote cell death via peroxynitrite formation.

Nitric Oxide and Ovulation

The ovulatory process depends on a coordinated activity of gonadotropins and steroid hormones, as well as mediators involved in inflammatory reaction, such as cytokines, prostaglandins, leukotrienes, and so forth. Results from recent studies suggest an involvement of the NOS/NO system in ovulatory mechanism(s), mainly via its effects on vasculature and prostaglandin production. Local administration of iNOS inhibitors has been reported to suppress the ovulatory process in rat, an effect reversed by sodium nitroprusside (Shukovski and Tsafriri, 1994). Similar results have been reported in hCG-treated rabbits (Hesla et al., 1997) and the systemic administration of NO blockers inhibits ovulation and suppresses the positive effect of IL-1 on LH-induced ovulation rate (Bonello et al., 1996). The role of eNOS in ovulation seems more important than that of iNOS (Mitsube et al., 1999), even though the results are still conflicting (Faletti et al., 1999b). In fact, both rat thecal and stromal compartments present high eNOS levels around ovulation (Zackrisson et al., 1996). Furthermore, eNOS deficiency in the mouse has been shown to be associated with reduced ovulatory potential after a superovulatory treatment (Hefler et al., 2002) and eNOS knockout females showed a significant reduction in hCG-induced ovulation (Jablonka-Shariff and Olson, 1998). A possible mechanism by which NO stimulates the ovulatory process involves the production of prostaglandins (which contribute to enhancing the inflammatory process in the periovulatory period) by a direct activation of cyclooxygenase (Salvemini, 1997). A cross talk between the NO and PG biosynthetic pathways, as well as a stimulatory effect of NO on $PGF_{2\alpha}$ production by large bovine follicles, has been recently reported (Basini and Tamanini, 2001). It has been suggested that NO might contribute to follicle rupture by also increasing the intrafollicular pressure (Matousek et al., 2001), either by increasing the vascular flow and the transudation of fluid to the follicular antrum or by stimulating the contractile elements of the ovarian follicle.

Nitric oxide synthesis seems to also be important for oocyte maturation because eNOS knockout mice exhibited a reduced number of oocytes in metaphase II of meiosis—a high percentage of oocytes remained in metaphase I or were atypical compared to controls (Jablonka-Shariff and Olson, 1998; 2000). Furthermore, in the same species, SNP has been demonstrated to stimulate meiotic maturation to metaphase II stages in cumulus enclosed oocytes (Sengoku et al., 2001). Conflicting data have reported a possible relationship between NO concentration in follicular fluid and oocyte quality and developmental competence (Barroso et al., 1999; Lee et al., 2000).

Nitric Oxide, Luteal Function, and Luteal Regression

Much evidence suggests that NO is involved in the regulation of corpus luteum (**CL**) function and lifespan, but opposing actions have been reported, depending on the stage of CL development. Motta et al. (2001) observed that in the midstage CL in the rat, NO stimulates both glutathione, a major antioxidant, and progesterone production, thus favoring the maintenance of CL; NO, together with PGE, seems to act through its effects on vasculature and proteolytic processes (Hurwitz et al., 1997). Recent findings indicate that iNOS-mediated NO secretion stimulates PGE synthesis, which is effective in increasing progesterone production (Hurwitz et al., 2002). Prostaglandin E has been demonstrated to enhance basal progesterone secretion also in newly formed CL from pseudopregnant rabbits (Boiti et al., 2000). A positive effect of NO on progesterone synthesis and luteal support also has been suggested in the rat by Dong et al. (1997; 1999); they speculated that NO could reduce or prevent luteolytic effects of prostaglandins, thus maintaining adequate progesterone, but the precise mechanisms by which it exerts its effects remain to be elucidated. Nitric oxide is also possibly involved in the control of luteal vascularization. In fact, NO produced by endothelial luteal cells increases blood flow by stimulating arteriolar smooth muscle relaxation and favours angiogenesis through an

increase in vascular endothelial growth factor production by capillary pericytes (Reynolds et al., 2000). The expression of eNOS, as well as total NOS activity, diminishes with CL aging (sheep, Reynolds et al., 2000; rabbit, Gobbetti et al., 1999; Boiti et al., 2002; rat, Motta and Gimeno, 1997), even though different findings have been reported in humans (Devoto et al., 2002).

As above mentioned, NO is also involved in luteolysis, which depends on an oxytocin-mediated prostaglandin release. Evidence exists that shows that oxytocin acts by enhancing NOS activity (Motta and Gimeno, 1997; Motta et al., 1997) and NO stimulates the synthesis of $PGF_{2\alpha}$ (human, Friden et al., 2000; bovine, Skarzynski et al., 2000), which in turn increases NOS activity, thus activating a positive feedback mechanism (rabbit, Boiti et al., 2000; rat, Estevez et al., 1999; Motta et al., 2001). At the same time, NO decreases progesterone production (rat, Motta et al., 1999; rabbit, Gobbetti et al., 1999; Boiti et al., 2000; bovine, Skarzynski and Okuda, 2000). Alternative mechanisms by which NO participates in luteal regression involve lowering estradiol production, resulting in the subsequent demise of the CL (Olson et al., 1996), and increasing apoptosis (Vega et al., 2000). In fact, the large amounts of NO induced by iNOS during the late stage of CL are likely to exert a proapoptotic effect.

Implications

This review provides convincing evidence that nitric oxide is involved in all the ovarian functions and plays a crucial role in reproductive processes, even though most studies have been carried out on rats and humans and very little is known about livestock. Fine-tuning of nitric oxide generation seems to be essential for ovarian physiology; however, the precise mechanisms by which it exerts its effects are not clearly understood and need further investigation. Future studies should also be aimed at verifying whether ovarian dysfunctions are associated with an altered nitric oxide production in order to clarify whether these defects can be corrected by nitric oxide.

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The use of controlled internal drug release devices for the regulation of bovine reproduction[1]

R. J. Mapletoft*[2], M. F. Martínez*, M. G. Colazo*, and J. P. Kastelic†

*Department of Large Animal Clinical Sciences, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK S7N 5B4, Canada and
†Agriculture and Agri-Food Canada, Research Centre, Lethbridge, AB T1J 4B1, Canada

ABSTRACT: Our knowledge of bovine estrous cycle physiology has expanded greatly in recent years, primarily with the advent of ultrasonography to monitor ovarian follicles. With increased knowledge, new methods of manipulating ovarian function have become available. The use of controlled internal drug release (CIDR) devices for the synchronization of estrus in cattle is now well accepted throughout the world. In fact, Canada and the United States are among the last countries in the world to have CIDR devices available for use in bovine practice. The use of CIDR devices, along with other hormones that are already on the market (e.g., gonadotropin releasing hormone) has permitted fixed-time artificial insemination with high pregnancy rates in beef cattle. New approaches, such as the use of estradiol in CIDR-based protocols, offer novel and exciting ways to manipulate the bovine estrous cycle. Recent studies suggest that steroid hormones readily available on the veterinary pharmaceutical market, such as estradiol cypionate and injectable progesterone, can be successfully used to synchronize follicular wave emergence and ovulation in CIDR-based, fixed-time artificial insemination programs. Experiments described in this report include several protocols that do not require detection of estrus, thereby permitting fixed-time artificial insemination in beef cattle. Over a 5-yr period, pregnancy rates to a single fixed-time artificial insemination have ranged from 55 to 77% in heifers and slightly less in lactating beef cows.

Key Words: Artificial Insemination, Cattle, Controlled Release, Estradiol, Gonadotropin-Releasing Hormone, Luteinizing Horomone

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Introduction

The keys to successful estrus synchronization are closely synchronized, rapid declines in circulating progestin concentrations and synchronous growth and ovulation of a viable follicle. However, $PGF_{2\alpha}$ is effective only when a fully developed corpus luteum (**CL**) is present (approximately d 7 to 18 of the cycle; Momont and Seguin, 1984) and withdrawal of exogenous progestin is effective only if either natural or induced regression of the CL has occurred. Although current techniques for estrus synchronization with $PGF_{2\alpha}$ are successful (Odde, 1990; Larson and Ball, 1992), variation in ovarian follicular wave dynamics results in poor synchrony of estrus and ovulation (i.e., the induction of luteolysis when a dominant follicle is mature will result in estrus and ovulation in 2 to 3 d, whereas the interval will be much longer if another follicle must be recruited from a new follicular wave) (Kastelic and Ginther, 1991).

Various progestins have been utilized for estrus synchronization. Progestin treatment for more than 14 d will synchronize estrus, but fertility at the induced estrus will be reduced (Wiltbank et al., 1965; Roche, 1974). Fortunately, these effects are transitory and are not apparent at the next estrus. Alternatively, shorter progestin treatment protocols (e.g., 7 to 10 d), with $PGF_{2\alpha}$ given before or at the termination of progestin treatment, have been devised to improve fertility (Odde, 1990; Macmillan and Peterson, 1993). However, these

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[2]Correspondence: 52 Campus Dr. (phone: 306-966-7149; fax: 306-966-7159; E-mail: reuben.mapletoft@usask.ca).

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protocols do not result in sufficient synchrony of estrus and ovulation for fixed-time AI. In addition, pregnancy rates were low when short-term treatments were initiated during the late luteal phase (i.e., after d 14), due to the development of a persistent follicle (Savio et al., 1993; Stock and Fortune, 1993; Custer et al., 1994; Kinder et al., 1996). Poor fertility after long-term progestin treatments or short-term treatments initiated late in the estrous cycle has been attributed to prolonged maintenance of the dominant follicle and ovulation of an aged oocyte (Ahmad et al., 1995; Revah and Butler, 1996). These results emphasize the need to synchronize follicular development to ensure the presence of a viable, growing dominant follicle at the time of progestin withdrawal and/or $PGF_{2\alpha}$ treatment.

Synchronization of Follicular Wave Emergence

Follicle Ablation. Elimination of the dominant follicle results in the emergence of a new follicular wave by removing the suppressive effect of follicular products (e.g., estradiol and inhibin) on circulating concentrations of FSH (Ko et al., 1991; Adams et al., 1993). Transvaginal ultrasound-guided follicle aspiration induced synchronous wave emergence within 2 d in heifers, and $PGF_{2\alpha}$ given 4 d later resulted in synchronous ovulation (Bergfelt et al., 1994).

Gonadotropin-Releasing Hormone. It has been shown that GnRH will induce ovulation or luteinization of a growing dominant follicle present at the time of treatment (Macmillan and Thatcher, 1991). Protocols that utilize GnRH and $PGF_{2\alpha}$ have been developed for fixed-time AI in beef and dairy cattle. The Ovsynch treatment protocol (Pursley et al., 1997) consists of an injection of GnRH followed by $PGF_{2\alpha}$ 7 d later, a second injection of GnRH 48 h after $PGF_{2\alpha}$ treatment, and fixed-time AI approximately 15 h later. Others have used a similar protocol in beef cattle with an interval of 6 d between the first GnRH treatment and $PGF_{2\alpha}$ (Roy and Twagiramungu, 1999).

Estradiol. Although treatment with progestin and estradiol has been used for several years to synchronize estrus (Wiltbank et al., 1965), it was not until recent discoveries of the effects of estradiol on follicular development that the physiological basis of these treatments was fully appreciated. In a series of experiments, estradiol treatment suppressed antral follicle growth, and suppression was more profound when estradiol was given with a progestin (Bó et al., 1994). The mechanism responsible for estrogen-induced suppression of follicular growth appeared to involve suppression of FSH through a systemic pathway (Bó et al., 2000). Thereafter, FSH surges occurred at defined times, and a new follicular wave emerged (Bó et al., 1994).

The administration of 5 mg of estradiol-17β (**E-17β**) to progestin-treated heifers (Bó et al., 1994) resulted in regression of antral follicles, followed by the emergence of a new follicular wave (on average) 4.3 d later (Bó et al., 1995), whereas the same dose of estradiol benzoate (**EB**) resulted in emergence of a new follicular wave 5.4 d later (Bó et al., 1996). More recently, Caccia and Bó (1998) showed that treatment with 1, 2.5, or 5 mg of EB (plus 50 mg of progesterone) resulted in a median interval from treatment to follicular wave emergence of 4.0 d in CIDR-treated beef cows; furthermore, this interval was significantly more synchronous in cows given 2.5 mg vs. those given 5 mg of EB. Estradiol valerate (Mapletoft et al., 1999) and estradiol cypionate (**ECP**; Thundathil et al., 1997; Colazo et al., 2002) at doses of 5 and 1 mg, respectively, resulted in longer and more variable intervals to follicular wave emergence than E-17β. The effects of lesser doses of estradiol valerate apparently have not been studied, whereas a dosage of 0.5 mg ECP appeared to be marginally efficacious (Thundathil et al., 1997).

The Controlled Internal Drug Release Device

The controlled internal drug release (**CIDR**) device has recently been approved in Canada (Bioniche Animal Health, Belleville, ON, Canada) and the United States (Pharmacia Animal Health, Kalamazoo, MI) for synchronization of estrus in beef cattle and dairy heifers. The CIDR is a T-shaped vaginal insert containing 1.9 g of progesterone (Canada) or 1.38 g of progesterone (United States) in silicon molded over a nylon spine. Although plasma concentrations of progesterone are identical between the two devices, the model marketed in the United States apparently exhausts its supply of progesterone earlier than the Canadian model (H. D. Hafs, personal communication). The CIDR is inserted into the vagina by a specialized applicator (Macmillan et al., 1991) that collapses the wings for insertion; expulsion of the CIDR causes the wings to straighten, which confers retention by pressure on the vaginal wall. A thin nylon tail attached to the end of the CIDR is exteriorized through the vagina and is used to remove the device. Label directions (for AI) indicate that the device should be in the vagina for 7 d; $PGF_{2\alpha}$ is given 24 h before device removal and estrus detection begins 48 h after device removal. Because of the short treatment period (7 d), the incidence of persistent follicles is reduced. The CIDR device is well suited to various approaches used to synchronize ovarian follicular development and ovulation.

Following CIDR insertion in ovariectomized cows, plasma progesterone concentrations increased to near luteal levels (5 to 7 ng/mL) by 24 h and then decreased to concentrations of 2 to 3 ng/mL after 2 to 3 d, where they remained until CIDR removal on d 7 (Martínez, 2002). Plasma progesterone concentrations declined to baseline by 12 h after CIDR removal. Administration of 100 mg of progesterone at CIDR insertion increased plasma progesterone concentrations by 2 ng/mL over that of a CIDR alone in ovariectomized cows (Martínez, 2002), with similar increases expected in ovary-intact cattle.

Table 1. Mean (± SEM) intervals to follicular wave emergence (WE), estrus, and ovulation, and pregnancy rates in controlled internal drug release-treated heifers given treatments to synchronize wave emergence

	Control	E-17β + progesterone	GnRH[a]	Follicular ablation
Intervals, d				
Treatment to WE	3.5 ± 0.6[b]	3.4 ± 0.1[b]	1.5 ± 0.3[c]	1.0 ± 0.1[c]
Range	(–2 to 8)[x]	(3 to 4)[z]	(–1 to 4)[y]	(0 to 2)[z]
n	18	16	16	17
PGF to estrus	2.3 ± 0.2	2.2 ± 0.2	2.2 ± 0.2	2.5 ± 0.1
Range	(1.5 to 4.5)	(1.5 to 3.0)	(1.5 to 3.5)	(2.0 to 3.5)
n	17	14	13	11
PGF to ovulation	3.6 ± 0.2	3.4 ± 0.1	3.5 ± 0.1	3.8 ± 0.1[d]
Range	(2.5 to 5.5)	(3.0 to 4.5)	(2.5 to 4.5)	(3.0 to 4.5)
n	18	16	16	16
Pregnancy rates				
Number	14/18	13/16	11/16	10/16
%	78	80	69	65

[a]Although 8 of 16 heifers ovulated in response to GnRH treatment, data points are based on all 16 heifers in the group.
[b,c]Means within rows with different superscripts differ ($P < 0.05$).
[d]One outlier excluded for this data point.
[x,y,z]Variances within rows with different superscripts differ ($P < 0.05$).

Estrus Synchronization and Fixed-time Artificial Insemination

The following is a very brief summary of studies done in the authors' laboratories, showing how the CIDR device can be use in estrus synchronization programs in beef cattle; many have been published in abstract form and are referenced accordingly. The first experiment was designed to investigate synchronization of ovarian follicular wave emergence in CIDR-treated cattle for synchronization of estrus and ovulation and to determine pregnancy rate following AI at observed estrus (Martínez et al., 2000a). A CIDR was inserted at random stages of the estrous cycle in 67 crossbred beef heifers (d 0 = the first day of the experiment) that were randomly allocated to receive 1) no further treatment (Control); 2) 5 mg of E-17β plus 100 mg of progesterone (E/P group); 3) 100 μg of GnRH (GnRH group); or 4) transvaginal ultrasound-guided follicular ablation of all follicles ≥5 mm (FA group). The CIDR devices were removed on d 9, 8, 6, or 5 after insertion, in Control, E/P, GnRH, or FA groups, respectively, so the dominant follicle of the induced wave would be exposed to exogenous progesterone for similar intervals in each group. Treatment with $PGF_{2\alpha}$ was done twice, at CIDR removal and 12 h later. Heifers were inseminated approximately 12 h prior to ovulation. Results are shown in Table 1. Although the interval from treatment to follicular wave emergence was longest in the E/P and Control groups, it was the least variable in the E/P and FA groups. The proportion of heifers displaying estrus was higher in the Control vs. FA group (94 vs. 65%, respectively; $P < 0.05$) and intermediate in E/P and GnRH groups (87 and 75%, respectively). Pregnancy rates were not significantly different among groups. Results supported the hypothesis that synchronous follicular wave emergence results in synchronous follicle development and, following CIDR removal, synchronous estrus and ovulation with high pregnancy rates to AI. The synchrony of estrus and ovulation in the E/P, GnRH, and FA groups suggested that these treatments, in combination with a CIDR, could be adapted to fixed-time AI programs.

The Use of Estradiol. Estradiol has been used to synchronize follicular wave emergence (Bó et al., 1991; 1993) and several studies have investigated the use of different estradiol preparations in progestin-based synchronization programs (Lammoglia et al., 1998; Burke et al., 1999; Martínez et al. 2000b). In addition, EB has been used to induce estrus in $PGF_{2\alpha}$-treated cattle (Welch et al., 1975; Peters et al., 1977; Dailey et al., 1983). In CIDR-treated cattle, the administration of 0.38 (heifers) or 1.0 mg (cows) of EB 24 to 30 h after CIDR removal resulted in estrus in 86 and 100% of the cattle, respectively (Lammoglia et al., 1998). Furthermore, in endocrine studies, EB treatment resulted in an LH surge between 16 and 20 h later, with significantly higher pregnancy rates than in untreated cattle (Lammoglia et al., 1998).

An experiment was designed to compare the effects of E-17β and EB on the interval to emergence of a new follicular wave in CIDR-treated heifers and on the induction of ovulation following CIDR removal (Martínez et al., 2002a). Thirty-two pubertal beef heifers received a CIDR device on random days of the estrous cycle (d 0), and were assigned to four groups in a 2 × 2 factorial design; half of the heifers received 5 mg of E-17β plus 100 mg of progesterone and the other half received 1 mg of EB plus 100 mg of progesterone by intramuscular injection. After CIDR removal and $PGF_{2\alpha}$ treatment on d 7, each group was randomly subdivided to receive an injection 24 h later (d 8) of either

Table 2. Mean (± SEM) intervals from treatment with estradiol-17β (E-17β) or estradiol benzoate (EB) to follicular wave emergence and from PGF treatment (and controlled internal drug release [CIDR] removal) to ovulation in CIDR-treated beef heifers

	Treatments			
	E-17β/E-17β	E-17β/EB	EB/E-17β	EB/EB
Number of heifers	8	8	8	8
Interval from estradiol treatment to				
Wave emergence, d	3.9 ± 0.2	3.9 ± 0.3	4.0 ± 0.3	4.5 ± 0.3
Range, d	3 to 5	3 to 5	3 to 5	3 to 6
Interval from CIDR removal to				
Ovulation, h	79.5 ± 6.0	73.5 ± 1.5	72.0 ± 0.0	81.0 ± 3.0
Range, h	72 to 120	72 to 84	72 to 72	72 to 96

1 mg of E-17β or 1 mg of EB to induce LH release and ovulation. Heifers were examined ultrasonographically to monitor follicular dynamics and to detect ovulation. There was no effect of estradiol treatment on the mean intervals to wave emergence or ovulation (Table 2).

In a second experiment with the same design, the efficacy of the two different estradiol preparations was tested in a CIDR-based fixed-time AI program in 84 lactating beef cows at random stages of the estrous cycle (Martínez et al., 2000b). All cattle were inseminated 30 h after the second injection of estradiol (i.e., 54 h after CIDR removal). Among the four treatment groups, there were no differences in the proportion of animals that displayed behavioral estrus (16/21, 19/21, 17/21, 17/21) or that became pregnant to fixed-time AI (14/21, 67%; 13/21, 62%; 11/21, 52%; and 15/21, 71%), for the E-17β/E-17β, E-17β/EB, EB/E-17β, and EB/EB groups, respectively). Results suggest that E-17β and EB can be used interchangeably in the synchronization of follicular wave emergence and ovulation for fixed-time AI in CIDR-treated cattle.

Gonadotropin-Releasing Hormone-Based Protocols. The Ovsynch protocol is much more efficacious in lactating dairy cows than in heifers (Seguin, 1997). Although the cause of this discrepancy is not known, ovulation following the first injection of GnRH occurred in 85% of cows and only 54% of heifers (Pursley et al., 1995). In addition, 19% of heifers showed behavioral estrus before the injection of $PGF_{2\alpha}$, dramatically reducing fertility to fixed-time AI (Wiltbank, 1997). In an experiment designed to confirm these results, GnRH treatment during the growing, early static, or regressing phases of development of the dominant follicle of the first follicular wave induced ovulation in 56% of beef heifers, and wave emergence occurred only when ovulation was induced; therefore, GnRH does not consistently induce emergence of a new follicular wave in beef heifers (Martínez et al., 1999).

Several experiments were conducted to determine the benefits of using a CIDR device in a GnRH-based, Ovsynch-type, fixed-time-AI program in beef cattle (Martínez et al., 2002b). In the first experiment, Simmental × cows (n = 148) and heifers (n = 48) were treated in a 7-d Cosynch program and randomly assigned to receive no further treatment (Group 1) or a CIDR device concurrent with the first GnRH treatment (d 0; Group 2). Pregnancy rates were not different (P = 0.79) in cows (Group 1, 45%; n = 71 vs. Group 2, 43%; n = 77). However, pregnancy rates were higher ($P < 0.05$) in CIDR-treated heifers (68%; n = 25) than in Cosynch controls (39%; n = 23). Data suggest that although there was no apparent benefit in lactating beef cows, the use of a CIDR device may make Ovsynch-type programs feasible in heifers.

A second experiment was designed to determine whether a CIDR would improve pregnancy rates to a single fixed-time insemination in an Ovsynch-type, estrus synchronization program in 49 beef heifers in which porcine luteinizing hormone (**pLH**) was used in place of GnRH (Martínez et al., 2002b). Heifers were randomly assigned to three treatment groups; the first group received 12.5 mg of pLH on d 0, $PGF_{2\alpha}$ on d 7, and 12.5 mg of pLH on d 9 with AI 12 h later (pLH/Ovsynch), while the second group (pLH/CIDR) was similarly treated, with the addition of a CIDR device from d 0 to 7. Heifers in the third group (EB/CIDR) received an injection of 1 mg of EB and 100 mg of progesterone on d 0 and a CIDR device from d 0 to 7. Heifers were given $PGF_{2\alpha}$ on d 7 (at the time of CIDR removal) and 1 mg i.m. of EB on d 8, with AI on d 9 (52 h after $PGF_{2\alpha}$). The proportion of heifers in estrus was significantly greater in the EB/CIDR (94%) and pLH/CIDR (71%) groups than in the pLH/Ovsynch group (41%), whereas pregnancy rates were significantly higher in the EB/CIDR group (75%) than in the pLH/Ovsynch group (38%), with the pLH/CIDR group (65%) intermediate ($P < 0.05$). Overall, in a Cosynch fixed-time breeding program in lactating beef cows, the use of a CIDR device did not influence pregnancy rates. However, the use of a CIDR device in a 7-d Cosynch program utilizing GnRH or a 7-d Ovsynch program utilizing pLH significantly improved pregnancy rates in heifers.

It has also been shown that the use of a CIDR device in Cosynch protocols applied at different herd locations increased overall pregnancy rates in beef cows in good body condition (58%), compared to Control cows treated only with Cosynch (48%; Lamb et al., 2001). It is noteworthy that CIDR devices increased pregnancy rates

in anestrous cows in that study (Lamb et al., 2001). In another study replicated over multiple sites, Lucy et al. (2001) showed that CIDR devices increased the synchrony of estrus and pregnancy rates in noncycling cattle. However, noncycling cattle had a lower pregnancy rate than their cycling herd-mates. Therefore, reproductive status can affect pregnancy rates in cattle given CIDR devices.

Combined Treatment Protocols. It was hypothesized that combinations of these treatments would be more efficacious than traditional approaches for synchronizing estrus and ovulation for fixed-time AI. Three experiments were conducted to evaluate methods of synchronization of estrus and ovulation in cattle for fixed-time AI (Martínez et al., 2000b). In the first experiment, a 7-d EB/CIDR treatment protocol was compared to a 7-d GnRH/CIDR treatment protocol or a simple 7-d CIDR protocol with the administration of $PGF_{2\alpha}$ at the time of CIDR removal. Pregnancy rate in the EB/CIDR group (76%) was higher than in the GnRH/CIDR (48%) or CIDR-treated, Control (38%) groups ($P < 0.01$). In addition, the percentage of heifers that displayed behavioral estrus in the EB/CIDR (100%) and CIDR-treated, Control (83%) groups was higher than in the GnRH/CIDR group (55%; $P < 0.01$).

A larger experiment was designed to compare progestins and methods of synchronizing wave emergence and ovulation in a fixed-time AI program (Martínez et al., 2002c). Angus-cross heifers (n = 503) were allocated into two synchronization groups and three treatment groups (2 × 3 factorial design) at random stages of the estrous cycle (d 0). At that time, heifers either received CIDR devices (n = 257) or were started on 0.5 mg·animal^{-1}·d^{-1} of melengestrol acetate (**MGA**; n = 246) and given injections of 2 mg of EB plus 50 mg of progesterone, 100 μg of GnRH or 12.5 mg of pLH. The last feeding of MGA was given the morning of d 6, and on d 7, CIDR devices were removed and all heifers received $PGF_{2\alpha}$. Consistent with their treatment on d 0, heifers were given either 1 mg EB 24 h after $PGF_{2\alpha}$ and inseminated 28 h later or 100 μg GnRH or 12.5 mg pLH 48 h after $PGF_{2\alpha}$ and concurrently inseminated. Heifers were exposed to bulls for 17 d, starting approximately 20 d after fixed-time AI. Although estrus rates differed ($P < 0.01$), there was no difference in pregnancy rates among groups ($P > 0.3$; Table 3). Overall, results suggest that the oral progestin (MGA) and the progesterone-releasing intravaginal device (CIDR) are equally efficacious, and that in combination with GnRH, pLH or EB, either can be used effectively to synchronize estrus and ovulation for fixed-time AI.

The present study is apparently the first published report of a concurrent comparison of these six treatment protocols for fixed-time AI. As pregnancy rates to fixed-time AI were not significantly different among treatments (overall rate, 58.0%), factors other than pregnancy rate (e.g., costs and management conditions) may influence the protocol selected. For example, CIDR devices are more expensive than MGA, but they can be used in both confined cattle and those at pasture. In regard to the latter, it is often difficult to ensure uniform intake of MGA in pastured cattle. In any case, the results of this experiment provide several options for fixed-time AI.

Table 3. Pregnancy rates following a single, fixed-time insemination in controlled internal drug release (CIDR)- or melengestrol acetate (MGA)-treated beef heifers in which follicular wave emergence and ovulation were synchronized with GnRH procine LH (pLH) or estradiol benzoate (EB)

	CIDR			MGA		
	GnRH	pLH	EB	GnRH	pLH	EB
Number of heifers	103	102	52	101	97	48
Estrus rate, %	66[a]	61[a]	92[b]	36[c]	33[c]	92[b]
Conception to AI, %	65	56	62	52	56	60
Conception to bull, %[d]	67	62	70	70	63	74
Total pregnancy rate, %	88	83	88	85	84	90

[a,b,c]Percentages with different superscripts differ ($P < 0.01$).
[d]Heifers not conceiving to AI.

A final series of experiments were conducted to determine the benefit of progesterone along with EB in the synchronization of follicular wave emergence in cattle treated with a CIDR and to determine the effect of interval from the second EB treatment to AI on pregnancy rates to fixed-time AI (Whittaker et al., 2002). Previous studies (Bo et al., 1994) suggested that including progesterone with estradiol might improve efficacy in synchronizing follicular wave emergence; it was hypothesized that the greatest benefit would be in cattle without a functional CL at the time of treatment. In the first experiment, lactating beef cows (n = 175) received a CIDR device on d 0 and were concurrently injected with either 2 mg of EB or 2 mg of EB plus 100 mg of progesterone. On d 7, CIDR were removed and all cows received an injection of $PGF_{2\alpha}$. On d 8, (approximately 24 h after CIDR removal), cows received an injection of 1 mg of EB and were inseminated on d 9, starting approximately 28 h after EB treatment. Overall pregnancy rate to fixed-time AI was 67%; pregnancy rate in those treated with EB alone was 64%, whereas those treated with EB plus progesterone was 70% ($P > 0.4$). In a replicate experiment in lactating beef cows and heifers (n = 137), results were similar, but the pregnancy rates differed by only 4%. Moreover, the inclusion of progesterone did not improve pregnancy rates in cattle in proestrus (13/28, 46%) or metestrus (12/19, 63%) at the beginning of treatment ($P = 0.3$). In a third experiment (unpublished), 391 lactating beef cows were treated similarly, except that inseminations were done 23 to 33 h after the second estradiol treatment. Calving rates did not differ among groups, but numerically more cows inseminated late (from 29.5 to 33.5 h after EB treatment) calved to the fixed-time AI. This trend was confirmed in a subsequent unpublished experiment involving 226 lactating beef cows. Although there would appear to be

considerable flexibility in insemination time following CIDR removal and EB treatment, later insemination times (e.g., 34 to 38 h) should be investigated further. In addition, results do not provide convincing support for the use of progesterone along with estradiol benzoate at the time of CIDR insertion.

Effects of Cyclicity

Studies were conducted to determine the effects of reproductive status (noncycling vs. cycling) in a 7-d pLH/CIDR-based Cosynch program for fixed-time AI (Kastelic et al., 2001). Seventy-seven Hereford-cross heifers were confirmed to be cycling and 22 were confirmed to be noncycling by plasma progesterone analysis. Following CIDR removal, heifers were monitored electronically (HeatWatch) for estrus, but all were fixed-time inseminated concurrent with the second pLH treatment (d 9). There was no significant difference between cycling and noncycling heifers for rate of synchronous estrus, and pregnancy rate to fixed-time AI (58%) was not significantly affected by reproductive status (cycling vs. noncycling). Although only 78% of the heifers were puberal at the time of treatment, 97% had a functional CL 7 d after fixed-time AI. However, numerically more heifers in the cycling group became pregnant, which is consistent with the results of the study reported by Lucy et al. (2001).

Resynchronization

A great deal of the genetic potential of AI bulls is not utilized because few producers take the time to rebreed cattle that do not conceive in an estrus synchronization program; time saved in a timed-AI program would be lost by watching for return to estrus in nonpregnant cattle. It was hypothesized that the knowledge and technology developed in these experiments could make it feasible to synchronize return to estrus (and ovulation) as part of a total breeding program. Macmillan and Peterson (1993) had previously reported that the reinsertion of a used CIDR device at midcycle and subsequent removal on d 21 resulted in all repeats occurring over a 3-d period. Therefore, several experiments were conducted to determine the efficacy of progestins for resynchronization of return to estrus in heifers not pregnant to fixed-time AI.

In a preliminary experiment (unpublished), a used CIDR was placed in 79 heifers from d 13 to 20 after fixed-time AI, and the remaining 80 heifers were untreated controls. Mounting was monitored electronically (HeatWatch) for 6 d after CIDR removal, and AI was done 6 to 12 h after the onset of estrus. The mean interval from fixed-time AI to the return to estrus was 22 d (range, 4 d) in the CIDR-treated group vs. 19 d (range, 7 d) in the Control group ($P < 0.001$; variance, $P < 0.07$), but estrus rates and conception rates did not differ.

A subsequent experiment was designed to compare the use of a used CIDR and MGA and to investigate whether the addition of estradiol to a resynchronization program would increase the synchrony of estrus and pregnancy rates to a single reinsemination (Martínez et al., 2001). Fixed-time inseminated heifers (n = 651) were randomly assigned to seven groups for resynchronization (n = 93 per group). Heifers received no treatment (Control), MGA (0.5 mg·animal^{-1}·d^{-1}; three groups), or a used CIDR (three groups) for 7 d, starting 13 ± 1 d after fixed-time AI. The three treatment groups were 1) no further treatment; 2) 0.5 mg of E-17β plus 50 mg of progesterone on d 13; or 0.5 mg of E-17β plus 50 mg of progesterone on d 13 and 0.5 mg of E-17β on d 21 (48 h after the last feed of MGA or 24 h after CIDR removal). Heifers were inseminated 6 to 12 h after first detection of estrus. Variability in return to estrus was greater ($P < 0.001$) in the Control group than in progestin-treated groups. Conception and pregnancy rates in heifers given a CIDR (65 and 61%, respectively) were higher ($P < 0.01$) than those given MGA (50 and 40%), but were not different from Controls (62 and 55%). In summary, following fixed-time AI, progestins (used CIDR or MGA) and estradiol-17β can be used to resynchronize follicle waves, estrus, and ovulation, facilitating a synchronous reinsemination of nonpregnant heifers. However, used CIDR devices seemed more efficacious than MGA in this study. In a follow-up study (our unpublished results), 979 beef heifers that had been fixed-time inseminated received a used CIDR device from d 13 to 20. The overall pregnancy rate to fixed-time AI was 56%. After CIDR removal on d 20, 336 heifers were detected in estrus between d 21.5 and 25.5, with a mean and mode of 22.5 d. Ninety heifers (21% of those found to be nonpregnant by ultrasound examination on d 28) were found to be not pregnant, even though they were not detected in estrus. Of the 336 heifers that were reinseminated, 238 (71%) became pregnant, for an overall pregnancy rate of 81% to two inseminations, with 4 d of estrus detection; in the previous study, untreated (control) heifers were detected in estrus over a 10-d period.

Commercial Preparations of Steroid Hormones

Although EB and E-17β were both shown to be very efficacious for the synchronization of follicular wave emergence and ovulation for fixed-time AI in CIDR-treated cattle, neither estrogen preparation is commercially available in Canada or the United States. However, a much longer-acting ester, **ECP** (Pharmacia Animal Health, Orangeville, ON, Canada) is available to practicing veterinarians. Three experiments were conducted to investigate the use of ECP for synchronizing follicular wave emergence and ovulation in beef heifers treated with a CIDR device (Colazo et al., 2002). In the first experiment, ECP was shown to be very efficacious in inducing ovulation of the dominant follicle of an E-17β-synchronized wave; 19 of 20 ECP-treated heifers ovulated between 72 and 96 h after CIDR removal, confirming earlier studies in GnRH-treated cattle (re-

viewed in Thatcher et al., 2001). In a second experiment, follicular wave emergence was more variable ($P < 0.01$) in CIDR-treated heifers given ECP (n = 30) than in those given E-17β (n = 28; 4.0 ± 0.26 d vs. 3.3 ± 0.15 d), but there was no difference in pregnancy rates to fixed-time AI when ECP was given 24 h after CIDR removal to synchronize ovulation (overall mean, 71%; $P > 0.2$).

A larger experiment was conducted to compare ECP plus a commercial source of progesterone with GnRH in a CIDR-based, fixed-time AI program (Colazo et al., 2002). On d 0, all heifers (n = 979) received a CIDR and were randomly allocated to receive either 100 μg of GnRH (n = 491) or 1 mg of ECP plus 50 mg of progesterone (Progesterone 5%, Vétoquinol N-A Inc., Lavaltrie, QC, Canada; n = 488). The CIDR devices were removed and $PGF_{2\alpha}$ was given on d 7 or 8.5 in the GnRH and ECP groups, respectively. Heifers were further subdivided to receive 0.5 mg of ECP at CIDR removal or 24 h later (with AI 58 to 60 h after CIDR removal) or a second injection of GnRH at the time of AI (52 to 54 h after CIDR removal). There was no difference in pregnancy rates between groups treated with GnRH (276/491, 56%) or ECP (277/488, 57%) on d 0. However, pregnancy rate was higher ($P < 0.01$) in heifers receiving ECP 24 h after CIDR removal (216/331, 65%) than at CIDR removal (168/320, 52%) or GnRH at AI (169/328, 51%). Data demonstrate that commercially available steroids can be used successfully to synchronize follicular wave emergence and ovulation in a CIDR-based, fixed-time AI program in beef heifers.

Use of Controlled Internal Drug Release Devices in Superstimulation Protocols

Precise control of ovarian function is essential for successful superovulation. Although gonadotropin treatments are usually initiated on d 8 to 12 of the estrous cycle to coincide with emergence of the second follicular wave, superstimulatory response can be adversely affected if these treatments are not initiated precisely at wave emergence (Nasser et al., 1993). Superstimulatory treatments can be initiated at an optimal time by synchronization of follicular wave emergence in CIDR-treated donor cattle, eliminating the need for estrus detection and the obligatory delay of 8 to 12 d. One approach involves transvaginal ultrasound-guided follicle ablation at random stages of the estrous cycle to synchronize wave emergence, followed by FSH 1 d after ablation, and $PGF_{2\alpha}$ 48 h later (Bergfelt et al., 1997). It was found that the timing of estrus could be controlled most accurately when a progestin implant was inserted for the period of superstimulation and two injections of $PGF_{2\alpha}$ were administered on the day of implant removal. In a more recent study, ablation of the two largest follicles was shown to be as efficacious in synchronizing follicular wave emergence for superstimulation as ablating all follicles ≥5 mm (Baracaldo et al., 2000), thereby eliminating the need to identify the dominant follicle. Therefore, ultrasound-guided follicular ablation can be used (along with a CIDR) to eliminate the effects of a dominant follicle prior to initiating gonadotropin treatments.

The reported asynchrony in follicular wave emergence (from 3 d before to 5 d after treatment; Martínez et al., 1999) suggests that GnRH or pLH may not be feasible for superstimulation. Indeed, when GnRH or pLH were compared to E-17β for the synchronization of follicular wave emergence prior to superstimulation (Deyo et al., 2001), the number of ova/embryos collected was reduced in the GnRH- or pLH-treated cattle. Therefore, the use of GnRH or pLH to synchronize follicular wave emergence prior to initiating superstimulatory treatments is not recommended.

The preferred approach is to use estradiol to synchronize follicular wave emergence in CIDR-treated donor cows. On d 0 (random and unknown stages of the estrous cycle), a CIDR is inserted and an injection of 5 mg of E-17β plus 100 mg of progesterone is given to synchronize follicular wave emergence. Four days later, gonadotropin treatments are initiated and CIDR are removed 48 to 72 h later, 12 h after a first injection of $PGF_{2\alpha}$. Inseminations are done 12 and 24 h after the onset of estrus (or 60 and 72 h after the first $PGF_{2\alpha}$ injection). Data from several experiments and commercial embryo transfer records show that this approach is very practical, and superovulatory responses were at least as high as when treatments were initiated around the time of emergence of the second follicular wave (reviewed in Bó et al., 2002).

The use of estradiol esters (e.g., EB or estradiol valerate) has also been investigated. Treatment with 2.5 mg of EB and 50 mg of progesterone given at CIDR insertion resulted in synchronous emergence of a new follicular wave 3 to 4 d later (Caccia and Bó, 1998). Superstimulatory treatments initiated 4 d after the administration of 5 mg of E-17β plus 100 mg of progesterone, 2.5 mg of E-17β plus 50 mg of progesterone, or 2.5 mg of EB plus 50 mg of progesterone resulted in superovulatory responses comparable to those initiated 8 to 12 d after estrus (reviewed in Bó et al., 2002). Treatment with 5 mg of estradiol valerate plus 3 mg of norgestomet resulted in less synchronous follicular wave emergence and a lower superovulatory response than 5 mg of E-17β plus 100 mg of progesterone (Mapletoft et al., 1999). Unfortunately, lower doses of estradiol valerate have not been investigated. Collectively, these studies demonstrate that exogenous control of follicle wave emergence offers the advantage of initiating superstimulatory treatments at an optimal time for follicle recruitment, regardless of the stage of the estrous cycle. The treatment is practical, easy to follow by farm personnel, and more importantly, the need for estrus detection and waiting 8 to 12 d prior to initiating gonadotropin treatments is eliminated.

Implications

Variable responses have been one of the most frustrating limitations of estrus synchronization and super-

ovulation in cattle. However, protocols that control both ovarian follicles and luteal function have provided opportunities for fixed-time artificial insemination (without estrus detection). Inserting a controlled internal drug release device and synchronizing ovarian follicular development consistently resulted in high pregnancy rates to fixed-time artificial insemination, regardless of stage of the estrous cycle. Similarly, used controlled internal drug release devices were beneficial for resynchronization of heifers not pregnant to fixed-time artificial insemination. Although variability in response to superstimulation has not been completely eliminated, protocols involving synchronization of follicular wave emergence in controlled internal drug release-treated cattle offer the convenience of initiating treatments immediately or at a self-appointed time, without estrus detection and without adversely affecting the superovulatory response or number of transferable embryos.

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Simulating the partitioning of dietary amino acids: New directions

P. J. Moughan[1]

The Riddet Centre, College of Science, Massey University, Palmerston North, New Zealand

ABSTRACT: In developing a mathematical model to allow prediction of growth in mammals, the simulation of amino acid metabolism is of particular importance because the predicted rate of protein deposition has a disproportionate influence on predicted body mass. In reality, the absorption and metabolism of amino acids in mammals is complex and highly integrated with continuous flux within and between body cells. To model amino acid transactions, however, a simplified construct of metabolism describing discrete physiological and metabolic processes must be developed. In the construct discussed here, a distinction is made between maintenance processes and those processes associated with growth. Growth is viewed as a function of nutrient deposition and support costs directly related to nutrient deposition. Several processes are emphasized and discussed, including food and amino acid intake, amino acid absorption, amino acid losses at maintenance, net protein deposition, inevitable amino acid catabolism, gut endogenous amino acid loss correlated with food intake, the turnover of body protein associated with new protein synthesis, the synthesis of non-amino acid-, non-protein nitrogen-containing compounds and preferential amino acid catabolism. The modeling of animal growth has become mainstream over the last two decades and models are being used increasingly in research, teaching, and in commercial practice. As models become more causal and less empirical, their validity and utility will be enhanced.

Key Words: Amino Acids, Models, Pig, Protein, Simulation

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Introduction

Over the last three decades, considerable attention has been given by animal scientists to the development of causally based quantitative models describing the absorption and subsequent utilization of amino acids and other nutrients during animal growth. Such models are "representations" of the real system (and thus conceptual), and there are therefore different, arguably equally acceptable, approaches or "views." Importantly, such models allow rigorous hypothesis testing around what is a relatively complex and highly interactive system. It is by the process of refutation and the formulation of new hypotheses to replace the older, less adequate ideas that modeling advances.

In this paper, a simplified conceptual framework is given that has been found useful for describing protein and amino acid transactions in the growing pig. The framework is discussed in the light of new directions that might be taken by modelers to increase the validity or application of their models. It is important to bear in mind, however, that the minimalist approach is an inherent principle to modeling, and models should not be expanded simply for the sake of greater complexity, but rather should only be further developed if this will lead to a meaningfully enhanced causal understanding of the phenomenon.

Background to Models

Some of the simplest amino acid models developed were the early static factorial models, which summed the metabolic losses of absorbed amino acids (often bulked together into a crude estimate of a "maintenance" requirement) and the amino acids deposited in new proteinaceous tissue, and then corrected the sum to take into account the inefficiency of utilization of absorbed amino acids. Such models were particularly popular in poultry nutrition (Hurwitz and Bornstein, 1973; Smith, 1978; Hurwitz et al., 1983), and an example in pig nutrition is the early work of Whittemore and Fawcett (1974). These early models paved the way for the development of more sophisticated models that take into account not only the amino acids, but also the nonprotein dietary energy and the interaction between amino acids and ME. These biological models include parameters affected by the nutritional history of the growing animal and incorporate adaptive control processes and impose limits on physiological and biochemi-

[1]Correspondence: Email: p.j.moughan@massey.ac.nz.
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Table 1. Biological processes underlying amino acid utilization in the growing pig

1. Ingestion of dietary amino acids		
2. Amino acid absorption		
3. Maintenance[a]		
		• Turnover of body protein
		• Integumental amino acid loss
		• Gut endogenous amino acid loss
		• Synthesis of non-protein nitrogen-containing compounds
		• Urinary amino acid losses
4. Growth		
		Body protein accretion
	Support costs	Inevitable amino acid catabolism
		Gut endogenous amino acid loss
		Turnover of body protein
		Synthesis of non-protein nitrogen-containing compounds
		Preferential amino acid catabolism

[a]A distinction is made between basal or maintenance processes (i.e., those occurring in the hypothetical state whereby body tissue is neither gained nor lost) and those processes associated with the accretion of new body tissue. The rate of a process at maintenance is defined as that rate commensurate with a daily food intake under which body weight is neither gained nor lost. Rates of the processes during growth are variable. It should be noted that for most of the metabolic processes, there is actually a natural continuum between maintenance and growth and that the distinction between states is arbitrary and reliant upon definition.

cal processes. Elements of these models are often causally based or deductive, and thus give considerable insight to the complex system being modeled.

A number of models simulating the uptake, metabolism, and partitioning of dietary nutrients in the growing pig have been developed (Whittemore and Fawcett, 1976; Moughan, 1981; Phillips and MacHardy, 1982; Tess, 1983; Whittemore, 1983; Moughan and Smith, 1984; Black et al., 1986; Stombaugh and Oko, 1980; Emmans, 1986; Moughan et al., 1987; Watt et al., 1987; Burlacu et al., 1988; Pettigrew et al., 1989; Pomar et al., 1991; Bridges et al., 1992; Ferguson et al., 1994; de Lange, 1995; Larduet and Savon, 1995; Knap, 1996; van Milgen et al., 2000).

As the field of pig growth modeling has developed, there has been a tendency for models to become more causal (less empirically based) and to further differentiate among the dietary nutrients and their ultimate metabolic fates (Boisen and Verstegen, 2000; Birkett and de Lange, 2001). This requires a detailed modeling of the ingestion, digestion, absorption and metabolism of amino acids.

A Framework for the Simulation of Amino Acid Metabolism

Clearly, the absorption and metabolism of amino acids in mammals is complex and highly integrated, with continuous flux within and between body cells. It is useful, however, and inherently necessary when constructing a model of metabolism, to consider amino acid metabolism as several discrete physiological processes (Table 1) that underlie or are causative to amino acid utilization. In the scheme presented here, the classical distinction is made between the "maintenance" or "basal" processes and those associated with growth. In reality, however, these multiple sets of processes are highly interrelated. Nevertheless, it is considered useful to conceptualize and represent overall metabolism in two parts: maintenance and growth. At zero nitrogen retention, there are still costs associated with body protein metabolism, and these are the classical "basal" or "maintenance" costs. With positive nitrogen retention, there are extra costs incurred associated with maintaining the proteinaceous body tissues. These are referred to here as support costs for growth.

Much has been written recently about these various maintenance and growth processes (Moughan, 1999; Black, 2000; Whittemore 2001a; Moughan and Fuller, 2002) and the present contribution will not cover the same ground. Rather, possible new directions for modeling these components will be emphasized.

Modeling the Component Processes

Simulating Voluntary Food Intake and the Ingestion of Amino Acids

The amount of an amino acid ingested is a function of the quantity of food ingested and the amino acid composition of that food. The approaches and limitations to modeling voluntary food intake in the pig have been discussed (Emmans, 1995; Kyriazakis and Emmans, 1999; Black, 2000; Whittemore et al., 2001a).

A decision must be made by the modeler as to which of the dietary amino acids should be explicitly represented in the model. The usual approach has been to include selected dietary essential amino acids or all the traditionally considered dietary essential amino acids and the dietary nonessential amino acid component. However, it is becoming increasingly apparent (Ball et al., 1986; Fuller, 1994; Reeds and Beckett, 1996) that

under certain conditions, amino acids traditionally considered to be dietary nonessential can become rate-limiting, and the concept of conditional essentiality has been introduced. Thus, in some cases (e.g., modeling amino acid transactions in the young pig), it may be important to model the metabolism of amino acids, such as proline and arginine, explicitly. Particularly in relation to energetics, it may be useful to model the gut utilization of dietary glutamic acid, and the subsequent systemic body cell synthesis of glutamate, which is energy demanding (Reeds et al., 1998). For young animals, it may also be useful to directly model the interconversions of methionine to cysteine and phenylalanine to tyrosine. Further, in some models, the uptake and metabolism of lysine have been emphasized with other amino acids described by reference to an "ideal" amino acid balance. However, there is no single "ideal" dietary amino acid balance with the balance of amino acids associated with complete utilization by the animal being influenced by numerous diet and animal factors (Black and Davies, 1991).

Amino Acid Absorption

Most models of pig growth have relied upon the use of empirically derived amino acid digestibility coefficients for the constituent dietary ingredients to predict the uptake of dietary amino acids from the gut. This approach is static and does not allow for temporal dynamics to be simulated. Bastianelli and Sauvant (1995) and Rivest et al. (2000) have recently taken an alternative approach, where an attempt has been made to model the mechanisms known to underlie the digestive and absorptive processes. This allows the varying rate of flux of amino acids following a meal to be simulated and potentially allows description of factors (both dietary and animal) known to affect amino acid digestibility. The limitation of empirically derived amino acid digestibility coefficients in not accounting for the kinetics of amino acid absorption has been discussed by Rerat (1990), who correctly emphasizes the marked influence on efficiency of protein synthesis consequent upon synchronization of the dietary amino acid supply to the sites of protein synthesis, as well as the synchronization of the supply of amino acids and nonamino energy-supplying compounds. The degree of amino acid utilization is a function of both the extent of digestion and absorption of nutrients and the timeliness of the absorption. Thus, model components that can predict the kinetics of amino acid absorption stand to improve the accuracy of prediction of pig growth. There is an opportunity for such models to be causal, based around the physical laws governing fluid flow, rates of hydrolytic breakdown, and absorption related to gut surface area.

Whatever approach is used, it is still necessary to apply some factor, to account for food-related differences in the susceptibility to hydrolysis of molecular linkages. Usually, these factors will be based on differences in ileal amino acid digestibility (Moughan, 1995). However, compelling evidence has recently been published from experiments using stable isotopes that essential amino acids are synthesized by gut microbes and are then absorbed (Fuller and Reeds, 1998; Metges, 2000). Such synthesis may make a quantitatively important contribution to amino acid supply; if so, it needs to be modeled. Studies are urgently needed to define what net contribution may accrue from intestinal bacterial proteolysis and amino acid synthesis and catabolism. If, overall, there is either a practically significant net synthesis or catabolism of amino acids in the upper digestive tract, then either digestibility coefficients will need to be refined or a description of the metabolism of the intestinal microflora be incorporated within models of the digestive process.

A final point is that when using ileal amino acid digestibility coefficients in modeling amino acid transactions, cognizance needs to be given to how apparent, true, and real coefficients (Boisen and Moughan, 1996b) should be applied, and in the case of processed proteins, the digestibility of reactive lysine should be incorporated in the model (Moughan, 2002) rather than conventional estimates of digestibility.

Maintenance

In the hypothetical state whereby a growing pig is neither gaining nor losing net body protein, metabolic processes are occurring that lead to the loss of proteinaceous material from the body, which must in turn be replaced by the diet. These processes are: 1) losses of amino acids via skin and hair; 2) losses of nitrogen of amino acid origin in urine reflecting inefficiency in the process of body protein turnover; 3) basal gut endogenous amino acid losses (mainly mucus, bile, desquamated cells); 4) the irreversible loss of amino acids in synthesizing essential non-amino acid nitrogenous metabolites (e.g., creatinine); 5) irreversible chemical alterations of amino acids (e.g., lysine to hydroxylysine); and 6) the loss of free amino acids in the urine.

The latter three processes are considered to be quantitatively minor at maintenance. The first three processes, however, are more important (Moughan, 1999) and need to be incorporated in models. Although in total, and for a rapidly growing animal, the maintenance amino acid requirement is only a small proportion (about 10% or less) of the total daily amino acid requirement, certain dietary essential amino acids are required disproportionately (e.g., cysteine loss in skin and hair, threonine loss in gut endogenous protein are relatively high).

Growth

For a rapidly growing animal, the actual net retention of amino acids into body protein explains a large part of the dietary amino acid need. However, the numerous support costs associated with this net protein accretion are certainly not insignificant and merit close

attention in the modeling of amino acid partitioning. It is possible to model amino acid retention as the net outcome of the two fundamental processes—protein synthesis and protein degradation—and this has been attempted (Pomar et al., 1991). Such an approach also allows protein retention to be related to cellular levels of DNA and messenger RNA. Although this approach has its attractions, protein synthesis and degradation have proven difficult to measure empirically, and sound data for modeling purposes are lacking. It has been more common to determine net rates of whole body protein retention under optimal dietary and environmental conditions as estimates of biologically maximal rates of body protein retention (**Pdmax**) for the particular type of animal. The intrinsic upper limit to whole body protein retention is an important constraint on growth since the cell has a finite capacity for protein synthesis and is unable to store free amino acids for later use. If, after a meal, the uptake of balanced amino acids required for protein synthesis exceeds the animal's capacity for protein synthesis, surplus amino acids are deaminated and the carbon skeletons eventually degraded. The Pdmax is influenced by genotype (breed and strain), gender, and age, and mean values reported in the literature range from as low as 90 g/d to values exceeding 200 g/d (Whittemore, 1983; Campbell, 1985). Recently, very thorough work has been conducted to determine upper limits to lean retention in North American pig populations (Schinckel, 1999). As animals are grown over progressively wider live weight ranges, it has become necessary to model the effect of age on Pdmax (Moughan, 1999; Whittemore et al., 2001b). It is also apparent that under practical farming conditions, pigs may not achieve the Pdmax value for their strain/breed as determined under breeding station or research center conditions, presumably because of effects due to factors such as subclinical disease, thermal environment, and social conditions (Baker and Johnson, 1999; Black et al., 1999; Burrin et al., 2001). For this reason the term "operational Pdmax" has been coined (Moughan et al., 1995), and operational Pdmax values have been determined on-farm (Morel et al., 1993; Moughan et al., 1995). The support costs for net protein accretion arise from processes such as endogenous gut amino acid losses, body protein turnover, inevitable amino acid catabolism, and the use of amino acids to synthesize essential non-amino acid nitrogenous compounds or in the irreversible structural alteration of amino acids. These support processes tend to be directly related to the rate of body protein retention, with higher net retentions incurring greater costs. There is extensive literature on gut endogenous amino acid losses (Boisen and Moughan, 1996a), with the method of determination being a central issue (Hodgkinson and Moughan, 2000). The traditional protein-free approach to determining gut endogenous amino acids has been discredited, and alternative approaches have been applied to yield more meaningful data. Gut amino acid losses are related to food dry matter intake and are influenced by dietary composition, especially the amount and type of plant nonstarch polysaccharides (fiber) and antinutritional factors (e.g., tannins, lectins, trypsin inhibitors). It is now possible to model some of these effects directly. Indeed, the major (and disproportionate relative to its size) impact that the gut has on both energy and protein metabolism in growing animals suggests that gut turnover and growth may merit being modeled directly.

The study of Bikker et al. (1994) demonstrates an effect of both dietary energy and protein intakes on the proportion of whole body protein associated with either the carcass or the organs (including blood). Further, and given that the carcass component (and indeed components of the carcass) and the various organs have quite different amino acid compositions, the amino acid composition of whole body protein may vary with the level of nutrition and rate of growth, and thus there may be a case for modeling the growth of different body parts separately. It may also be useful to directly and explicitly model the degradation of amino acids in the hindgut with subsequent uptake of ammonia. Considerable quantities of ammonia are absorbed in the hindgut (confer ileal/faecal digestibility coefficient differences) and are synthesized to urea. Some of this urea is recycled into the gut, but much of the synthesized urea is excreted in the urine. These processes are important both energetically and in terms of nitrogen loss.

The use of amino acids as precursors for the synthesis of other non-amino acid (or irreversibly altered) nitrogenous compounds may also be quantitatively important in some cases (Reeds, 1988; Fuller, 1994) and should be considered for explicit representation in models. By way of example, the gut may use large amounts of cysteine to synthesize mucins and glutathione (Burrin et al., 2001), which may in turn lead to a metabolic demand for methionine. In fact, methionine is involved as a methyl donor in several different pathways. With respect to gut cysteine demand, it is interesting to note recent findings from the University of Alberta (Shoveller et al., 2000) of a 35% lower requirement of piglets for methionine under total parenteral nutrition as opposed to enteral feeding.

Body protein turnover increases with the rate of net body protein accretion (Milligan and Summers, 1986) and has an important potential impact upon nitrogen utilization since body protein turnover is unlikely to be completely efficient. The turnover of different body protein depots has been directly simulated in the recent work from Knap and Schrama (1996).

The concept of inevitable catabolism refers to the catabolism of the dietary first-limiting amino acid, which occurs during growth quite unrelated to energetic need, and is simply due to the existence of active catabolic enzyme systems in the cell. The recent work of Peter Reeds and his group has shed new light on the phenomenon of inevitable catabolism, in respect of first-pass gut metabolism. It is now clear (Fuller and Reeds, 1998; Burrin et al., 2001; Stoll et al., 1999) that the gut

tissues account for a considerable degree of the overall amino acid metabolism. Also, intriguing new data point to an important role for the enterocyte in the catabolism (oxidation) of absorbed dietary amino acids, including dietary essential amino acids and the often dietary first-limiting amino acid, lysine. The observed substantial degree of "first-pass" metabolism by the gut tissue may explain an important fraction of "inevitable catabolism." In a pivotal study by Stoll et al. (1998), approximately one third of dietary lysine intake was metabolized by the gut tissues in 28-d-old pigs, with only 18% of the first-pass metabolism being accounted for by gut protein synthesis (i.e., incorporation of lysine in mucosal tissue). This infers that there is a considerable degree of first-pass lysine catabolism and that the enterocyte may be an important site for the catabolism of lysine and other dietary essential amino acids. It may be that the enterocyte has a specific catabolic requirement for certain dietary essential amino acids. This is an important new finding. From the results of a subsequent study (van Goudoever et al., 2000), it appears that the first-pass utilization of lysine is influenced by nutritional state. For 4-wk-old pigs given a high-protein (23% crude protein) diet, there was considerable lysine metabolism by the gut, but lysine use by the portal-drained viscera was derived almost entirely from arterial input. The relatively low amount of dietary lysine used in the first-pass was almost entirely oxidized (representing one third of whole body lysine oxidation or about 5% of dietary lysine intake). When a low-protein diet (about 9% crude protein) was given, overall lysine metabolism was not affected, but now, and in contrast to the high protein finding, both dietary and arterial lysine were used by the portal-drained viscera in nearly equal amounts, and intestinal lysine oxidation was suppressed. An overview of both studies (Stoll et al., 1998; van Goudoever et al., 2000) suggests that gut tissue lysine metabolism in the pig may be quantitatively important and compartmentalized, and it may be influenced by the level of nutrition, with increased catabolism accompanying higher levels of amino acid uptake. The data suggest a high obligatory visceral need for lysine, with protein intake influencing gut lysine catabolism. The results of the two studies discussed above are not entirely consistent with each other and further confirmatory studies are needed. However, the studies do suggest an important role for the gut tissues in lysine (and other dietary essential amino acids) metabolism and catabolism. This is further evidence supporting the need to model the metabolic transactions of the gut separately.

A common approach in developing pig growth models has been to describe gut endogenous amino acid losses directly, but to lump the other support costs together into a single measure of "catabolic" losses. The magnitude of such losses can be determined experimentally (after correcting for maintenance losses) by determining the difference between the amount of the absorbed first-limiting amino acid and the amount of that amino acid deposited in tissue for animals fed protein below their maximal rate of retention and given a high-energy diet (so that nonprotein energy sources are not limiting). Sound empirical data to thus describe the efficiency of utilization of the first-limiting amino acid are lacking, and this is a major weakness in modeling amino acid partitioning. Recent studies, however, have addressed this issue and useful information is beginning to emerge (Moughan, 1999; Edwards et al., 1999).

Kees de Lange and coworkers at the University of Guelph have recently conducted a series of carefully controlled serial slaughter studies that provide useful new information. Growing pigs (mean live weight of 50 to 60 kg) were fed a highly digestible casein and cornstarch diet, wherein a prescribed dietary essential amino acid was clearly first-limiting. Animals were screened, in a preliminary nitrogen balance study, for their upper limits to body protein retention (Pdmax) to allow selection of a cohort of animals with similar Pdmax. Feeding levels were varied such that the first-limiting amino acid was supplied at set proportions of the amount of the amino acid needed to meet the estimated requirement to support Pdmax. Metabolizable energy intake exceeded the determined requirement to support Pdmax. In the first study (Möhn et al., 2000) in which lysine was the first-limiting amino acid, the marginal efficiency of using absorbed (true ileal digestible reactive lysine) lysine for protein deposition was 0.75 and was not affected by ME intake or available lysine intake. Thus, approximately 25% of the absorbed available lysine was unaccounted for, presumably largely lost to catabolism. In a further study in which threonine was the first-limiting amino acid (de Lange et al., 2001), a marginal efficiency of utilization of absorbed threonine of close to 75% was determined, and the efficiency of utilization was not affected by live weight, but there was some indication that threonine utilization was highest at the lowest threonine intake. Threonine disappearance was relatively constant at 23.7% of available threonine intake, when threonine intake varied between 70 and 100% of the threonine requirement to support Pdmax. Conversely, Reijmers et al. (2000) reported that the efficiency of utilization of available methionine plus cysteine (above maintenance), decreased with increasing methionine plus cysteine intake from a high of 90% (60% of Pdmax) to a low of 71% (100% of Pdmax).

Whittemore et al. (2001b) have attempted to provide a basis for inefficiency in the utilization of the dietary first-limiting amino acid by relating the efficiency of utilization of ideal protein to protein (amino acid) losses associated with body protein turnover, which are considered to increase with the rate of protein retention. Although such an approach undoubtedly explains part of the inefficiency of utilization of the absorbed first-limiting amino acid, losses from turnover may not be the only or the most important source of inefficiency. For example, there may be, as discussed above, quite substantial "first-pass" catabolism of dietary essential

amino acids by the gut enterocytes. There is also likely to be some degree of catabolism (inevitable) of absorbed dietary amino acids by other body cells. However, losses due to body protein turnover are likely an important contribution to overall inefficiency of utilization. As more information becomes available, it would appear possible to model the inefficiency of dietary first-limiting amino acid utilization based on a component related to inevitable catabolism plus other inevitable losses, and to a possibly more variable component related to rate of body protein turnover.

The processes leading to postabsorptive inefficiency of utilization of the absorbed first-limiting amino acid collectively account for a loss of around 20 to 25% of the absorbed first-limiting amino acid. The efficiency of utilization may vary among the different absorbed amino acids and may be affected by the amount of the absorbed amino acid relative to the amount required for maximal body protein synthesis (Moughan, 1989; Seve and Henry, 1995), though this is a contentious issue (Möhn et al., 2000; de Lange et al., 2001).

There is a well-understood nutritional interaction between dietary protein and energy (nonprotein energy supplying nutrients) stemming from the fact that protein synthesis is an energy-demanding process with amino acids being both substrates for protein synthesis and compounds capable of yielding energy. In situations where the nonprotein fraction of the diet is insufficient to yield the required ATP, then amino acids will be oxidized to supply ATP. This phenomenon needs to be modeled. The first approach is to use empirically derived functions to describe the relationship between protein retention and energy intake when overall dietary energy is limiting, and thus predict the rate of body protein retention, with lipid retention being calculated as a residual function. An alternative approach is to assume that there can be long-run zero or negative body lipid retention in support of protein accretion and to then calculate energy demands and yields based on these premises. Some researchers (Whittemore and Fawcett, 1976) have assumed that there is a minimal daily rate of body lipid retention ($Ld_{minimum}$) that must be supported, whereas others (e.g., Moughan et al., 1987) have assumed that other than under conditions of severe starvation (whereby particular adaptations occur), the body has a desired minimal lipid content, and thus have modeled a minimal level of body lipid ($Lt_{minimum}$). The concept of a minimal whole body lipid to whole body protein ratio (Lt:Pt) minimum has also been advanced by Whittemore (1995). Emmans and Kyriazakis (1997) have discussed a further approach to the problem, which also has some biological appeal. They have proposed that the net material efficiency (i.e., the slope of protein retention on protein supply above maintenance) of using ideal protein for protein retention (**ep**) is directly proportional to the ratio of metabolizable energy to digestible crude protein of the food, up to a critical value at which it attains its maximal value ep*. The value ep* is analogous to "inevitable catabolism." The values for ep and ep* are assumed (based on some experimental evidence) to be constant across genotypes and for pigs of different liveweight. These empirical and deductive approaches have been recently reviewed (Emmans and Kyriazakis, 1997; Moughan, 1999).

Conclusion

The modeling of animal growth has become mainstream over the last two decades, and important causal theories of growth have been developed and demonstrated to be useful in practice. At the same time, there has been considerable progress made in our understanding of amino acid digestion and postabsorptive metabolism. The challenge for modelers is to use this new information to further develop their models of growth to enhance validity and applicability. It seems likely that the next generation of pig growth models will model the biochemical utilization of individual nutrients (including the individual amino acids) more closely and will begin to directly model amino acid transactions in the gut and other subcomponents of total body protein (e.g., liver, connective tissue vs. muscle). The modeling of protein turnover in these subcomponents, and the description of a hierarchy of amino acid use for protein deposition will become increasingly important.

Implications

The mathematical modeling of animal growth has become an important tool in animal science research and teaching. Moreover, the use of animal growth models in commercial practice offers, among other applications, a new and situation-specific approach to nutrient requirement estimation and to the development of feeding regimens. As basic knowledge is developed on the growth and metabolism of the pig, pig growth models will become causal, thus having enhanced validity and utility.

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The Enviropig physiology, performance, and contribution to nutrient management advances in a regulated environment: The leading edge of change in the pork industry[1,2]

C. W. Forsberg*[3], J. P. Phillips*, S. P. Golovan*, M. Z. Fan*, R. G. Meidinger*, A. Ajakaiye*, D. Hilborn†, and R. R. Hacker*

*University of Guelph, Guelph, Ontario, Canada N1G 2W1 and
†Ontario Ministry of Agriculture and Food, Woodstock, Ontario, Canada N4S 7Z5

ABSTRACT: The Enviropig is a transgenic pig that synthesizes phytase in the salivary glands and secretes active enzyme in the saliva. This capability enables pigs to utilize practically all the P in cereal grains and soybean meal and to excrete fecal material usually containing 60% less P than nontransgenic pigs fed the same conventional diet lacking supplemental phosphate. By computer simulation, it was determined that 33% less land would be required to spread manure from transgenic phytase pigs, and if the diet was modified to decrease crude protein, even less land would be required. Introduction of Enviropig genetics may be perceived as leading to an expansion of the pork industry, but perhaps a more realistic view is that introduction of the transgenic phytase pig would enhance sustainability of the industry in a world with increasingly stringent soil nutrient management legislation. The transgenic phytase pig is probably on the leading edge of the production of various types of genetically modified animals that will reduce the environmental footprint of animal agriculture through enhanced metabolic capabilities. These pigs, and other transgenic animals under development elsewhere, will require safety and quality testing in the country of origin and in countries to which the product is exported to ensure that they do not have a deleterious effect on human health and the environment. Consumer surveys suggest that transgenic technology directed to issues involving environmental sustainability and food safety will receive the greatest support.

Key Words: Environment, Phosphorus, Phytase, Pigs, Poultry

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Developments in Monogastric Nutrient Management Strategies

As knowledge of the precise nutritional requirements of food animals increases and the nutritional status of feedstuffs improves, there is the continuing objective to refine the dietary needs of animals in order to increase productivity and simultaneously reduce any environmental impact by decreasing the output of nutrients in fecal and urinary excretions. Various approaches may be taken to accomplish this, including 1) formulation of rations to more precisely meet the dietary requirements of the animal (e.g., reduction of the concentration of supplemental phosphate in the ration [Shen et al., 2002] or replacement of a portion of the crude protein by essential amino acids [Lenis and Jongbloed, 1999]); 2) improvement in feed digestibility by the addition of supplemental enzymes, including phytase (Simons et al., 1990) or β-glucanase and xylanase (Bedford, 2000); 3) feeding more digestible cereal grains (e.g., low-phytate corn) developed by genetic mutations [Sands et al., 2001]; and 4) expressing genes coding for enzymes that enhance metabolic potential of food animals (Ward, 2000). The expression of genes coding for novel enzymes in food animals is a rational, albeit controversial, strategy to enhance digestive capabilities. Research on the Enviropig represents the leading edge of a revolution that will ultimately change the feed industry; such research directly tackles the elusive goal of producing animals with reduced environmental impact. The development of animals with novel characteristics unveils new opportunities, but harbors uncharted challenges.

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[3]Correspondence: phone: 519-824-4120, ext. 3433; fax: 519-837-1802; E-mail: cforsber@uoguelph.ca.

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Initial Research on the Genetic Modification of Monogastric Animals to Enhance Digestion

Hall et al. (1993) explored the expression in mice of an endoglucanase gene, endoglucanase E, from *Clostridium thermocellum* to improve the capacity of monogastric animals to digest β-glucans present in cereal grains and supplements. This transgene, which was composed of the exocrine pancreas-specific promoter of the elastase I gene linked to the endoglucanase gene, gave rise to expression of glucanase in the pancreas of the mouse with secretion into the duodenum (Hall et al., 1993). The glucanase secreted into the small intestine was stable in the presence of intestinal proteases, including elastase, trypsin, and chymotrypsin. This research was extended to expression of the xylanase gene XYLY from *C. thermocellum* in transgenic mice, and localized expression in the pancreas was again achieved (Fontes et al., 1999).

With a similar objective, but with the intent to achieve regulation of synthesis linked to energy consumption, Zhang et al. (1999) constructed an endoglucanase transgene by fusing the 2.5-kb amylase Amy 2.2 promoter and signal peptide from the mouse amylase gene to the *Bacillus subtilis* endo-(1-4)-β-glucanase gene *C6.5* (Zhang et al., 1997). The transgenic mice generated with this construct expressed glucanase activity with a high degree of specificity for the pancreas. The activity was consistent with dietary starch inclusion, although expression was at a very low level. These studies on glucanase expression in the mouse model demonstrated that organ-specific expression of microbial hydrolase genes in animals is feasible.

Glycanase and xylanase expression are appropriate target genes for poultry since these enzymes reduce the high viscosity of glycan polymers in the gastrointestinal tract that interfere with digestion. An indirect benefit is reduced soiling of eggs because the excreta are less watery and sticky. These genes, however, provide limited nutritional benefit for growing and finishing pigs and variable results for weanlings since pigs have less viscous intestinal contents (Bedford, 2000). Likewise, β-glucanases contribute little to direct energy production because animals lack the β-glucosidases necessary for cleavage of low-molecular-weight β(1,3)- and β(1,4)-linked oligosaccharides products into readily digestible glucose.

Phytase is an Important Target Gene for Expression in Pigs and Poultry

Cereal grains, such as corn and barley, and plant-based protein supplements fed to pigs and poultry contain between 33 and 80% of their P in the form of *myo*-inositol hexakis dihydrogen phosphate complexed with minerals (phytate) (Jongbloed and Kemme, 1990). Pigs do not digest phosphorus in this form; instead, it is concentrated in the feces by a factor of three- to fourfold (unpublished data). As a consequence of the poor digestibility, supplemental phosphate has been included in diets to meet the dietary requirements for optimal growth (NRC, 1998). The resulting high-P manure makes an excellent fertilizer when spread on land. When the P concentration exceeds the anion-binding capacity of the soil, however, the P can leach into normally phosphate-limited freshwater and marine systems, causing eutrophication with the death of fish and aquatic animals and impacting water quality (Jongbloed and Lenis, 1998; Diaz, 2001; Sundareshwar et al., 2003).

The amount of supplemental phosphate needed in the diet can be reduced by inclusion of the enzyme phytase, usually at 250 to 1,000 U/kg of feed, which hydrolyzes a portion of the phytate, thereby releasing readily digestible phosphate. This will lead to a 25 to 50% reduction in fecal P for growing and finishing pigs and poultry (Simons et al., 1990; Ketaren et al., 1993). With higher concentrations of phytase added, the extent of hydrolysis can be improved, albeit with diminishing returns (Kornegay, 2001). These studies demonstrate that a single discrete gene coding for a phytase enzyme is able to provide the full benefit attainable, a feature of prime importance to simplify the generation of an efficient transgenic animal.

Site for Action of Phytase

Results of postslaughter and cannulation experiments with pigs have shown that dietary phytase is predominantly active in the stomach (Kornegay, 2001). The pH of the pig stomach varies from a low of 2.3 just prior to eating to as high as 4.6 after eating (Clemens et al., 1975), whereas in the chicken, the gizzard has a similar pH of 2.5 to 2.8 (Farner, 1942). The low pH is essential for phytase action since at pH values above 4.0, phytic acid begins to precipitate with multivalent cations, and at pH 6.0, it is mainly present as inaccessible precipitates (Siener et al., 2001). Maenz et al. (1999) demonstrated that multivalent cations at neutral pH are potent inhibitors of microbial phytase due to the formation of phytate-mineral complexes.

An ideal phytase enzyme for expression in animals, therefore, needs to be expressed in a gastric or pregastric location, be active and stable at pH values between 2 to 5, and resistant to pepsin. From a survey of microbial phytases reported by Wodzinski and Ullah (1996), and more recently by Lei and Stahl (2001), the *Escherichia coli appA*-encoded phytase has desirable characteristics for expression in animals not shared by other phytases, which include a high activity (Golovan et al., 2000) exceeding that of recently cloned fungal enzymes (Lassen et al., 2001) and resistance to pepsin at low pH (Rodriguez et al., 1999; Golovan et al., 2000) (Table 1). These in vitro characteristics are borne out by animal studies showing that the AppA phytase is as effective as the commercially available *Aspergillus*

Table 1. Important characteristics of an enzyme product and a transgene for expression in animals

Protein product/enzyme	
1.	Protease resistance.
2.	Suitable pH optimal depending upon site of action.
3.	Low K_m (Michaelis constant).
4.	High V_{max}.
5.	Low molecular weight.
6.	Monomeric.
7.	Temperature optimal for high activity at 37°C.
8.	Signal sequence for export if extracellular activity is a prerequisite.
9.	Post-translational sites should not affect secretion to desired location, and activity and stability.
Transgene	
1.	Promoter with appropriate recognition and regulation.
2.	Enhancer with desired strength and tissue specificity.
3.	Strong Kozak consensus sequence.
4.	Locus control region present.
5.	Sequences necessary for correct post-translational modification of mRNA.
6.	Genomic copy of structural gene if of eukaryotic origin.
7.	Codon usage and distribution suitable for expression in a monogastric animal.
8.	Sequences affecting expression removed.

phytase (on a per unit basis) in improving the P bioavailability in diets of chicks (Leeson et al., 2000), broiler and layer poultry (Igbasan et al., 2001), and in diets of young pigs (Igbasan et al., 2001).

Promoter Selection, Secretion Signals, and Construction of the Transgene

Promoter selection and specifications for construction of a transgene are critical (Table 1) to obtain organ-specific excretion of a target protein at a regulated and effective level. In the case of phytase expressed in the salivary glands, efficient unidirectional export from acinar cells into a pregastric site of the gastrointestinal tract was essential because phytate and a number of lower inositol phosphates have important roles in intracellular metabolism including 1) signaling mobilization of Ca^{2+}; 2) regulation of membrane trafficking and cytoskeleton organization; 3) gene expression; and 4) export of messenger RNA from the nucleus (York et al., 2001). Therefore, any intracellular expression of the phytase could be lethal to the embryo.

No porcine salivary promoters were available for pregastric expression of phytase; however, two murine promoters with appropriate characteristics for salivary expression were available: the rat proline-rich-protein promoter (Tu et al., 1993) and the mouse parotid secretory protein (**PSP**) promoter (Laursen and Hjorth, 1997). The *E. coli app*A structural gene, including the signal peptide sequence, which has a codon usage profile close to that of eukaryotic genes (S. P. Golovan, unpublished data), was inserted downstream of each of these promoters (Golovan et al., 2001a). The inducible proline-rich-protein promoter-appA construct was initially introduced into mice by pronuclear microinjection (Hogan et al., 1986) to test whether induction of phytase synthesis would be deleterious. Healthy transgenic offspring were obtained using this promoter, and phytase was secreted in the saliva after induction by injection of isoproterenol, documenting that phytase expression in animals was not lethal. The constitutive parotid secretory protein promoter-phytase transgene (**PSP-APPA** transgene, Laursen and Hjorth, 1997) was then successfully introduced into mice. Two healthy transgenic offspring were produced; one of these synthesized phytase and secreted the enzyme in the saliva, which demonstrated that constitutive synthesis of salivary phytase was not deleterious and at the same time resulted in reduced fecal P.

Generation of Pigs Producing Salivary Phytase

The PSP-APPA transgene (Golovan et al., 2001a) was used for the generation of transgenic pigs by pronuclear microinjection following the procedure of Wall et al. (1985). Thirty-three independent founder (G_0) transgenic piglets were obtained. Many of the individual G_0 animals were tested periodically for salivary phytase throughout the growth phase. Figure 1 illustrates the erratic results obtained by collecting saliva from the mouth of a pig with a cotton swab. Despite the variation in sampling, the ability to conduct a convenient, rapid, and noninvasive test for salivary phytase continues to be particularly useful. The figure also shows the percentage reduction of P in fecal samples from the same transgenic pig during the weaning, growing, and finishing phases compared with nontransgenic littermates when fed a conventional diet containing supplemental P. This data demonstrated the potential of founder (G_0) pigs to digest phytate P. When weanling and growing-finishing pigs from the G_1 generation of one line (WA line) of transgenic pigs were tested for true digestibility of dietary P in soybean meal as the sole source of P using an ileal cannu-

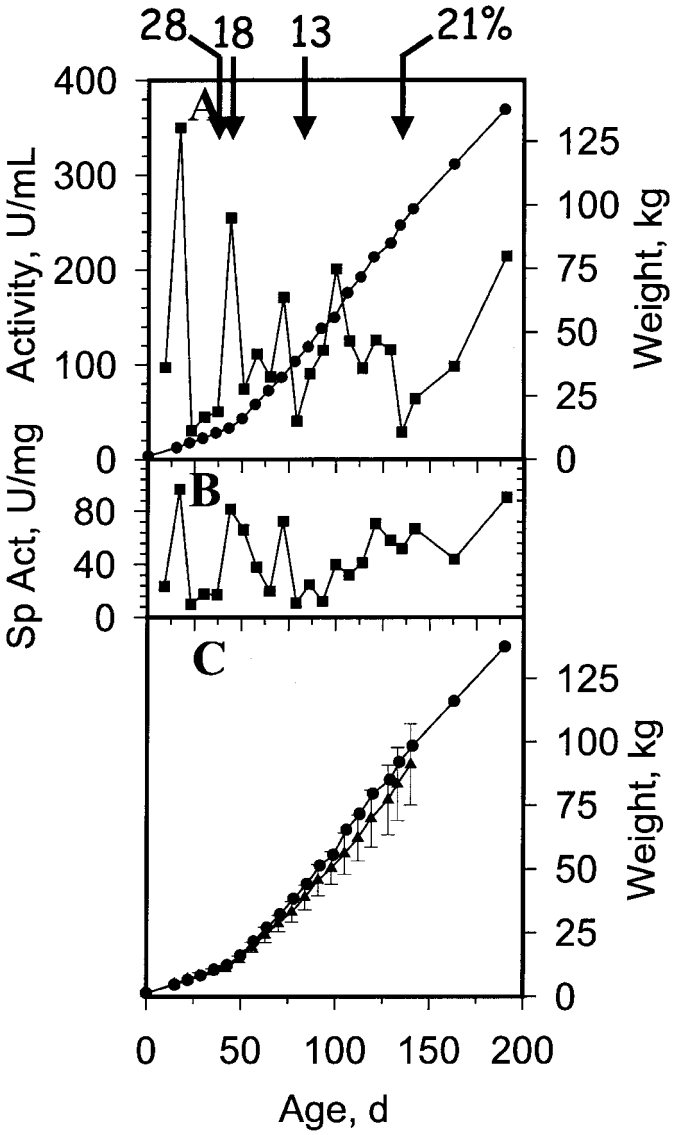

Figure 1. Salivary phytase production, growth, and fecal P reduction by the transgenic G_0 pig 421-06 compared with nontransgenic littermates receiving conventional diets with supplemental phosphate. A) Phytase activity (■) and weight gain (●); B) phytase specific activity; C) weight gain of transgenic pig (●) and nontransgenic littermates (▲, n = 4). The percentage values at the top of the figure indicate the reduction in fecal P compared with nontransgenic littermates.

lation methodology (Fan et al., 2001), they were found to digest 88 and 99% of the dietary P, respectively, compared with nontransgenic pigs that digested 49 and 52%, respectively (Golovan et al., 2001b). Fecal material from the weanling and growing-finishing phytase pigs contained a maximum of 75 and 56% less P, respectively, than that of nontransgenic pigs fed the same diet. Since the transgenic phytase pigs digested practically all of the dietary P, the P entering the terminal ileum of these pigs presumably consisted primarily of differentiated enterocytes released from the mucosa during the process of continual epithelial regeneration (Ramachandran et al., 2000).

Boars and gilts transgenic for the phytase gene that were fed a conventional cereal grain diet lacking supplemental P during the finishing phase had fecal P concentrations that were 67 and 64%, respectively, less than the corresponding nontransgenic pig in the same trial (Golovan et al., 2001b). It is worth noting that the preliminary observations on the G_0 pigs were borne out by the more comprehensive data obtained from feeding trials with G_1 and G_2weanling and growing-finishing transgenic pigs. The amount of P excreted in the urine was not determined in these studies, but more recent research on weanling, growing, and finishing pigs has shown that they excrete slightly more phosphorus in the urine than nontransgenic pigs (A. Ayodele, unpublished data). It has been reported that urinary P accounts for 6, 9, and 27% of P excreted by weanling pigs, growing pigs, and sows, respectively (Poulsen et al., 1999). The data clearly document the innate capability of the phytase pigs to extensively digest dietary phytate P.

Reduction of the Environmental Impact of Pork Production

Although P is the third most expensive nutrient fed to pigs, the cost of phosphate is not a major constraint, and overfeeding of this compound has been a common practice. In all jurisdictions, the land base for spreading of manure is a serious limitation. To assess the benefit of Enviropig genetics in terms of land area for spreading manure, we have used the NMAN 2001 manure management computer simulation program developed by the Ontario Ministry of Agriculture and Food. The simulation was for a 350-sow, farrowing-to-finishing pig operation with given defaults (Table 2). As shown in Table 3, the spreading of manure from nontransgenic pigs on low erosion soil theoretically requires 151 ha and this minimum area is necessary to avoid application of excess P. If transgenic phytase pigs were raised in place of conventional pigs, the land area required for spreading could be reduced by 33% before manure N would be applied in excess. It is generally recognized that for each 1% decrease in CP in the diet, there is an 8 to 10% reduction in manure N (Lenis and Jongbloed, 1999; Le Bellego et al., 2001). Using the NMAN program to simulate the relationship between decreasing manure N and the reduction in land required to spread manure, it can be seen that if the N content of the manure was reduced by up to 40%, the area of low erosion soil for spreading could be reduced by 60% (i.e., to 100 ha) before P would be applied in excess (Figure 2).

The benefit of competing technologies, in terms of reduced land base for spreading of manure, is not as great. For example, corn that contains 50 to 75% less phytate than unmodified strains of corn fed to monogastric food animals provide for fecal P reductions up

Table 2. Parameters for the NMAN nutrient management program for assessing the effect of the phytase pig characteristics on the land base required for the spreading of manure[a]

1. The simulation was for a 350-sow, farrowing-to-finishing farm producing 8,570,913 L of 4.2% DM manure/yr (MSTOR calculation).
2. Transgenic (Tg) phytase pigs produce manure with 60% less P. Program default values were used for the concentrations of N, P, potassium, and ammonium N (NH_4-N) in swine manure.
3. Continuous corn with an average yield of 8.13 metric tons per hectare and manure applied once a year in spring before seeding.
4. Liquid loading was not considered because it can be split into two or more applications.
5. Nitrogen from previous manure applications was taken into account. When lowering N both total N and NH_4-N were lowered by the same percentage.

[a]NMAN2001 (Feb 22, 2002 version) is a manure nutrient management computer simulation program developed by the Ontario Ministry of Agriculture and Food http://www.omafra.gov.on.ca/scripts/english/engineering/nman/default.asp.

to 50%, depending on the diet formulation (Raboy et al., 2001). Supplementation of low-phytate corn with phytase enhances the digestibility further (Sands et al., 2001). These data show that low-phytate cereals have great potential. However, to achieve a reduction in fecal P comparable to the phytase pigs, supplemental phytase is necessary. The current problems with some low-phytate cereals, as exemplified by low-phytate corn, are a lower germination rate (Lott et al., 2000) and a 4 to 23% reduction in seed weight (Raboy et al., 2000). A separate problem with low-phytate cereals may be the added cost incurred in handling them as separate commodities from conventional cereals. The reductions in fecal P as a result of feeding supplemental phytase (see above) are usually no greater than that observed for low-phytate barley. These data demonstrate the superior performance of Enviropig genetics, but this benefit is counterbalanced by the substantial investment for introduction of the new biotechnology.

Quite separate from short-term considerations, an impending problem facing the industry is the dwindling source of economically recoverable mineral P that Smil (2000) predicted would last only 80 yr at the current rate of extraction. A related problem with lower quality mineral deposits is the increasing contamination of the P by trace metals, particularly cadmium (Smil, 2000). More efficient recycling of P through a combination of phosphorus technologies will forestall this impending shortage and at the same time reduce environmental eutrophication.

Enhancing the Metabolic Capability of Food Animals

Recent advances have been made in the methodology for the generation of transgenic food animals (Wheeler and Walters, 2001; Houdebine, 2002), including poultry (Harvey et al., 2002). Even the classical pronuclear microinjection may have been improved, as was demonstrated by Chang et al. (2002). They reported that spermatozoa incubated with monoclonal antibodies recognizing a specific surface antigen will bind DNA. By surgical oviduct insemination of gilts and by artificial insemination of chickens, the gene of interest was efficiently transferred to oocytes. In all cases, up to 25% of born animals or birds were transgenic and expressed the transgene. Furthermore, problems including ectopic expression and silencing of transgenes, which are often caused by position effect (e.g., integration close to an endogenous transcriptional control element), are being solved with

Table 3. Land base necessary for spreading of manure from transgenic (Tg) and non-Tg phytase pigs given the same diet, except that supplemental phosphate was omitted from the diets of the phytase pigs

Pigs	Starting soil test	Manure applied, L/ha	Hectares for spreading manure	Reduction in land for spreading, %
Low erosion potential soil				
Non-Tg	101 ppm of P[a]	56,827.5[b]	150.8	—
Tg	101 ppm of P	85,240[c]	100.4	33
Non-Tg	10 ppm of P	No change[e]	No change	—
Tg	10 ppm of P	No change	No change	33
High erosion potential soil[f]				
Non-Tg	101 ppm of P	25,003	343	—
Tg	101 ppm of P	65,918	130	62
Non-Tg	10 ppm of P	56,828	151	—
Tg	10 ppm of P	85,238	101	33

[a]This is regarded as an excessive level of P.
[b]Application above this amount exceeds the P limit.
[c]Application above this amount exceeds the N limit.
[d]This is a medium level of P.
[e]Same as above because 154 kg over crop removal is the limiting factor if the P limit is low.
[f]Soil erodes at the rate of 36 metric tons/ha annually.

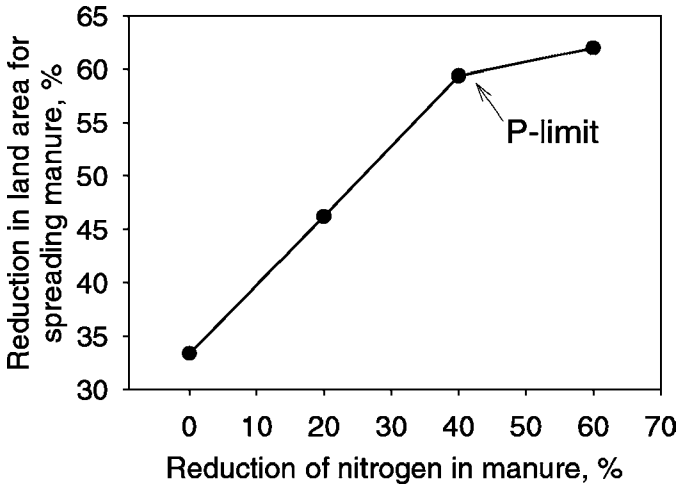

Figure 2. Effect of reducing the N in manure of transgenic phytase pigs on the land area for spreading manure. Assumes that phytase pigs excrete 60% less P and spreading is on a low erosion soil.

insulator genes (Matske et al., 2000; Bell et al., 2001) and artificial chromosomes (Huxley, 1997).

These methodologies, in addition to cloning techniques, provide the opportunity to enhance the digestive physiology, growth, fertility, and disease resistance characteristics, and milk and meat composition of domestic food animals (Wheeler and Walters, 2001; Houdebine, 2002).

Hurdles from Concept to the Meat Counter

Genetically modified food animals are subject to animal health, environmental, and food safety legislation. The criteria for a valuable transgenic food animal are good health, disease resistance, reduced impact on the environment, desired physiological characteristics, and safe consumption.

Health of the transgenic food animal is central to production because an unhealthy animal would usually be less productive. For example, transgenic pigs harboring a transgene encoding bovine GH, although exhibiting a 10 to 15% increase in daily weight gain and a 16 to 18% increase in feed efficiency, suffered serious physiological defects and were unsuitable for commercial food production (Pursel et al., 1989; Pinkert et al., 1994). To circumvent this type of problem, which in some cases may be due to recessive insertional mutations, strategies of selecting, breeding, and dissemination of transgenic livestock have been explored in detail (Van Reenen et al., 2002).

To meet environmental regulatory requirements, transgenic animals must be documented as having no deleterious effects on the environment and human health, either directly or indirectly. In Canada, they must satisfy the requirements of the Canadian Environmental Protection Act (**CEPA**), which is the joint responsibility of Environment Canada and Health Canada (www.ec.gc.ca/substances/nsb/eng/reg_e.htm) to determine that the animal is not a CEPA toxin (CEPA 99, Section 64). In the United States, the FDA administers similar requirements (www.fda.gov/cvm/biotechnology/bio_drugs.html), the environmental requirements being under 21 CFR, part 25 (www.access.gpo.gov/nara/cfr/waisidx_01/21cfr25_01.html). Transboundary movement of living genetically modified (**GM**) animals, although presently administered by individual countries, may in the future be administered through the Cartegena Protocol on Biosafety to the Convention on Biodiversity, which is a treaty under the United Nations Convention on Biological Diversity (www.biodiv.org/biosafety/protocol.asp and www.biodiv.org/convention/articles.asp), if the convention and protocol are ratified by major trading countries. The convention and protocol are, in part, designed to provide a framework within which living modified organisms (**LMO**) can be traded in a safe and responsible manner with due regard for protection of environmental biodiversity.

Recently, the NAS (2002) reported on risk issues surrounding transgenic and cloned animals and fish, including food safety, environmental safety, and animal welfare. The major food safety issue noted was the potential for allergenicity or hypersensitivity responses in some consumers. Products from somatic cell cloned cattle were not considered to be a food safety concern. Environmental concerns revolved around fitness traits, such as increased growth rate, in highly mobile species (i.e., fish carrying an up-regulated growth hormone transgene). Lesser environmental concern was expressed regarding fitness traits, such as phytase expression in less mobile animals such as the pig, although it was noted that feral pig populations do exist (Brisbin and Mayer, 2001) that in the past have caused environmental damage. Animal welfare issues were also discussed. An issue underlying scientific considerations was the need for an ethical framework since resolving and implementing science-based decisions ultimately requires public involvement.

The strategy for the safety assessment of foods derived from GM plants is well established under the Codex Alimentarius commission of the Food and Agriculture Organization (www.codexalimentarius.net/reports.asp, document ALINORM 03/34). These guidelines are very similar to the legislated assessments used in Canada (www.hc-sc.gc.ca/food-aliment/mhdm/ofb-bba/nfi-ani/e_novel_foods_and_ingredient.html) and in the United States (Chassy, 2002). Premarket food safety evaluations consider the issues listed in Table 4. Extensive studies have now been conducted to assess the safety of GM DNA for human consumption and its persistence in the environment. Studies with human volunteers show that no GM DNA survived the passage through the entire human digestive tract. Although some DNA survived

Table 4. Characteristics considered in a premarket safety assessment of a food derived from a genetically modified organism

1. Safety of the source organism and gene(s)
   (a) Safety of the inserted DNA
   (b) Safety of DNA ingestion
   (c) Safety of the antibiotic resistance marker (if used)
2. The food safety issues of the newly introduced product(s)
   (a) Potential for toxicity (protein product)
   (b) Potential for allergenicity (protein product)
   (c) Safety of any unintended effects
3. Equivalence of composition
4. Retention of nutritional value
5. The human dietary exposure

in laboratory created environments that simulated human or animal gastrointestinal tracts, the research concluded that the likelihood of functioning DNA being taken up by bacteria in the human or animal gut is extremely low (Food Standards Agency, 2002). With a reduced focus on the safety of DNA, the assessment process probably will focus strongly on the protein(s) expressed from the introduced novel genes. As GM animals are relatively new compared to GM plants or microorganisms, the various governmental agencies are still in the process of setting policies and of drafting guidance documents for the assessment of these animals. In the interim, there have been assessments on a case-by-case basis; for instance, the GM pig developed by Bresatec in Australia and the AquAdvantage salmon developed by Aqua Bounty (www.aquabounty.com/abbounty.htm) in the United States.

The GM pig developed by Bresatec (now BresaGen) is transgenic for a GH that can be switched on by addition of zinc to the diet, which allows for increased production of meat and enhanced feed efficiency (Nottle et al., 1999). Bresatec provided data to the Australian authorities to document that the GM pork was substantially equivalent to pork from nontransgenic pigs, but the Australia and New Zealand Food Authority (ANZFA) did not approve the Bresatec pork because approval was outside the Authority's charter (www.affa.gov.au/docs/operating_environment/armcanz/gene/appendix2.html).

The AquAdvantage salmon is transgenic for a salmon GH gene under control of a promoter from the ocean pout's antifreeze protein gene. The transgene is expressed in the liver and provides for year-round secretion of GH. The transgenic salmon grow at four to six times the rate of nontransgenic salmon to reach the same weight at maturity (Fletcher et al., 1999). Aqua Bounty has submitted an application to the Center for Veterinary Medicine of the FDA for the market approval of the AquAdvantage salmon, where it is under assessment as a "new animal drug" (Kleter and Kuiper, 2002).

A recent interim report by Health Canada on the preparation of guidelines for food safety assessment of transgenic livestock and fish (Health Canada, 2001) confirms the similarity of approaches for GM plants and animals; however, there will be differences to account for the different physiological characteristics of plants and animals. A key ingredient, particularly with the consideration of pigs, is the similarity to humans in terms of their nutritional (Miller and Ullrey, 1987), physiological (Higgins and Cordell, 1995; Tumbleson, 1986), and immunological (Helm et al., 2002) characteristics. Therefore, the well being of transgenic pigs would seem to be a persuasive indicator of food safety for many aspects, except perhaps for the toxicity/allergenicity of the newly expressed protein.

Allergenicity

A novel protein, such as the *E. coli* phytase present in the Enviropig, will be considered as self in the pig (Goldsby et al., 2000) and will not present an allergenic challenge to the pig. However, the phytase expressed in the pig may act as an allergen when humans consume the pork, since it has not previously been consumed as a food constituent, except through the accidental presence of *E. coli* in food consumed or because of its presence as part of the normal flora in the lower gastrointestinal tract (Tannock, 2001). A weight of evidence approach is taken to assess allergenicity of a protein, which focuses on the source of the gene, sequence homology with known allergens, physicochemical properties of the protein (such as heat stability), and digestive stability. Additional criteria include immunoreactivity of the novel protein with serum IgE from individuals with known allergies to species that are broadly related to the source of the transferred DNA and the immunogenicity of the novel protein in appropriate animal models (Taylor, 2002; www.fao.org/es/ESN/food/risk_biotech_papers_en.stm). This decision process, although well established, is likely to change as new information appears, such as the recent finding of the lack of a stringent relationship between the stability of proteins in simulated gastric fluid and allergenic potential (Dearman et al., 2002).

Salivary phytase presents an interesting case for allergenicity testing because in its native state, it is highly resistant to stomach proteases, retaining greater than 90% of its activity after exposure to a 1,000-fold excess of pepsin at pH 2.5 (Golovan et al., 2001b). However, salivary phytase is denatured by heating to 100°C, and after this treatment, is sensitive to proteases (J. P. Phillips, R. G. Meidinger, and C. W. Forsberg, unpublished data), a characteristic shared by native nonallergenic proteins (Astwood et al., 1996). It is probable that a native intact protein will have more allergenic potential than a denatured protein after digestion by pepsin. However, pork is always cooked prior to consumption because of a range of infectious microorganisms potentially present, including viruses, bacteria, fungi, and parasites (Kumar et al., 2002). Thus, rather than assessing the allergenicity of novel proteins in uncooked pork, as is done

with transgenic plant products, the cooked pork may be a more realistic product for testing.

Consumer Acceptance of Genetically Modified Foods

The ongoing debate over the human and environmental safety of GM foods is complex and multifaceted, but several recent reports show a positive view. Santerre and Machtmes (2002) surveyed 576 people, including members of community organizations, undergraduate students, graduate students, and extension educators, to see how their knowledge and attitudes toward genetically enhanced foods changed after receiving an hour of scientific instruction on food biotechnology. After this introduction, respondents were more accepting of the regulatory process; furthermore, 90% stated they would eat and serve GM foods to their families and 90% believed that their families would benefit from GM foods within the next 5 yr. The importance of providing consumers with scientific information on GM foods is borne out by a recent Ipsos-Reid study on Canadian public attitudes toward pork production. Respondents were more receptive to the future consumption of GM pork once they were aware that it would come from pigs that produced less polluting manure. Environmentally friendly applications of biotechnology were also viewed positively by U.S. consumers (Bruhn, 2002).

Implications

Transgenic technology has the potential to enhance the role that the animal industries have in world food production system. This paper describes the science, thought process, reasoning, and outcomes that evolved during the development of the Enviropig. The importance society places on solving the environmental, food safety, and food quality challenges supports the contention that this particular transgenic technology should be directed first and foremost to these issues rather than the customary economically based production area. This paper emphasizes the general understanding and value of transgenic animal technology in helping to ameliorate the environmental impact of animal production.

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Nutritional- and suckling-mediated anovulation in beef cows[1]

R. P. Wettemann[2], C. A. Lents, N. H. Ciccioli, F. J. White, and I. Rubio

Department of Animal Science, Oklahoma Agricultural Experiment Station, Stillwater 74078-0425

ABSTRACT: Nutrient intake, body energy reserves, and suckling are major regulators of reproductive performance of beef cows. Inadequate body energy reserves at parturition increase the interval to first estrus and ovulation, and postpartum nutrient intake can influence the duration of the interval in cows with thin to moderate body condition score. Suckling can increase the postpartum anestrous interval in thin cows, but has little effect on mature cows with adequate body energy reserves. The purpose of this review is to evaluate signals by which nutrient intake and body energy reserves may regulate ovarian function in postpartum beef cows. Nutritional restriction causes decreased secretion of GnRH and LH, reduces follicular growth, and decreases concentrations of estradiol in plasma. In addition to direct and indirect effects of decreased energy intake on the hypothalamus and pituitary, nutrition may influence ovarian function. Metabolic signals that communicate the adequacy of body energy reserves and nutrient intake may stimulate changes several weeks before ovulation occurs, have a permissive role, be regulated by binding proteins or receptors, or interact with stimulatory or inhibitory factors produced by adipose tissue. Metabolic signals may also have autocrine and/or paracrine effects. Adequate body energy stores and sufficient plasma concentrations of metabolic signals are prerequisites for ovulation in postpartum cows. Complex interactions between hormones, metabolic compounds, and other factors control follicular maturation, estrus, and ovulation in postpartum beef cows.

Key Words: Cow, Insulin-Like Growth Factor, Ovulation, Postpartum Period, Reproduction

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Introduction

Profitability of beef production is greatly influenced by reproductive efficiency. Beef cattle are frequently not pregnant at the end of the breeding season because of the absence of normal estrous cycles. The anestrous condition in heifers and postpartum cows is caused by reduced ovarian follicular growth and the absence of luteal activity (Wettemann, 1980). Two major factors that regulate duration of the postpartum anestrous period are suckling and nutrient intake before and after calving. If nutrient intake is inadequate and body energy reserves are depleted, the interval from calving to the first estrus is extended (Wiltbank et al., 1962; Dunn and Kaltenbach, 1980; Short et al., 1990). Suckling also inhibits the resumption of normal estrous cycles after parturition (Short et al., 1972; Edgerton, 1980; Williams, 1990).

Relationships between body energy reserves and weight loss (before and after parturition) with the duration of the postpartum anestrous period have been established (Dunn and Kaltenbach, 1980; Selk et al., 1988). The most important factor that influences pregnancy rate is body energy reserves at calving. When beef cows had a BCS (1 = emaciated, 9 = obese; Wagner et al., 1988) of five or greater at calving, the number of days from calving to first estrus and ovulation was 15 to 35% fewer than if cows calved with a BCS of less than 5 (Richards et al., 1986; Looper et al., 1997; Lents et al., 2000).

Body condition score of primiparous cows at calving influences the response to postpartum nutrient intake (Spitzer et al., 1995). When cows with a BCS of 6 were fed to gain 0.85 vs. 0.44 kg/d after calving, the percentage of cows in estrus during the first 20 d of the breeding season increased from 40 to 85%. However, when cows had a BCS of 4, the greater daily gain only increased the percentage of cows in estrus from 33 to 50%.

Energy intake and body energy stores influence concentrations of energy substrates and metabolic hormones in the blood of cattle. Chronic and acute alterations in substrates and metabolic hormones may signal the hypothalamic-pituitary-ovarian axis as to the metabolic status of the animal. However, the metabolic signals between body energy reserves and follicular maturation and ovulation have not been determined.

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[2]Correspondence: rpw@okstate.edu.

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The purpose of this review is to evaluate signals by which nutrient intake and body energy reserves may regulate ovarian function in postpartum beef cows.

Discussion

Postpartum Follicular Development

Although follicular waves are recurrent during early and mid-pregnancy (Ginther et al., 1989), they are not detectable during the last weeks of pregnancy (Ginther et al., 1996). The first dominant follicle (**DF**) occurs within 10 to 12 d after parturition in beef and dairy cows (Murphy et al., 1990; Savio et al., 1990; Stagg et al., 1995). Thus, a lack of follicular waves after parturition is not the limiting factor for the onset of estrus and ovulation.

The first postpartum DF ovulated in few (11%) beef cows (Murphy et al., 1990), whereas the first DF ovulated in most (74%) dairy cows (Savio et al., 1990). Beef cows with inadequate body energy reserves and/or suckling calves had several follicular waves before the first ovulation (Murphy et al., 1990; Stagg et al., 1995) and the number of DF before ovulation was greater with reduced postpartum nutrient intake.

Frequent pulses of LH are needed for maturation of preovulatory follicles (Roberson et al., 1989; Savio et al., 1993; Stock and Fortune, 1993). Mean concentrations of LH and frequency of pulses increase with time before the first postpartum ovulation (Stagg et al., 1998). Inadequate pulses of LH may cause recurring follicular waves and atresia of the DF. Nutritionally induced anovulation is associated with decreased secretion of LH (Wettemann and Bossis, 2000). The first DF after parturition was prolonged or ovulated when beef cows were given hourly pulses of LH (Duffy et al., 2000). Treatment of nutritionally induced anovulatory cows with one pulse of GnRH each hour will initiate ovarian luteal activity (Bishop and Wettemann, 1993; Vizcarra et al., 1997).

The ability of DF to produce estradiol is limited during the postpartum anovulatory period and increases with time after parturition (Spicer et al., 1986). Postpartum anovulatory follicles produced less estradiol than preovulatory follicles (Braden et al., 1986). Although the amount of IGF-I in follicular fluid was not influenced by time postpartum or whether a follicle was estrogen-active (Spicer et al., 1988; Rutter and Manns, 1991), amounts of IGF binding proteins in follicles could regulate the availability of IGF-I to follicular cells. Factors that increase the postpartum interval to first ovulation probably decrease steroidogenesis in follicles.

Abnormal luteal function after the first ovulation occurs frequently in beef cows. The luteal phase after the spontaneous first postpartum ovulation is usually less than 10 d (Corah et al., 1974; Werth et al., 1996; Looper et al., 1997). Similarly, a short luteal phase also occurs after early weaning (Odde et al., 1980; Copelin et al., 1987; Breuel et al., 1993) or treatment with GnRH (Kesler et al., 1980; Wettemann et al., 1982). Short-lived corpora lutea (**CL**) cannot maintain pregnancy since they regress before d 15 when maternal recognition of pregnancy occurs (Northey and French, 1980). A premature luteolytic signal causes short-lived CL (Garverick et al., 1992).

Table 1. Concentrations of LH in the serum of nutrient-restricted and control steers before (d 0) and during (d 3) restriction[a]

	Treatment				
	Control		Restricted		
Item	d 0	d 3	d 0	d 3	SE
Concentration, ng/mL	3.9	4.2	4.4	4.7	0.1
Pulse frequency, 8 h	6.3	6.8	6.0	5.8	0.8
Pulse amplitude, ng/mL	3.3	2.8	3.3	3.1	0.3

[a]Ojeda et al., 1996.

Estrous behavior usually does not occur before the first postpartum ovulation in beef (Murphy et al., 1990; Perry et al., 1991; Looper et al., 1997) and dairy cows (Graves et al., 1968; Savio et al., 1990). The duration of the luteal phase after the first estrus in beef cows is usually normal (Corah et al., 1974; Odde et al., 1980; Looper et al., 1997).

Secretion of Gonadotropin

Secretion of LH is a rate-limiting step for the initiation of follicular growth and estrus after calving. Pulsatile secretion of LH is associated with secretion of GnRH in cows (Gazal et al., 1998; Yoshioka et al., 2001) and increased frequency of exogenous GnRH pulses increased pulse frequency and mean concentrations of LH in anovulatory cows (Bishop and Wettemann, 1993; Vizcarra et al., 1997).

Nutritional effects on LH secretion in ruminants are dissimilar to monogastric animals. Short-term nutritional restriction or fasting reduces LH secretion in rats (Campbell et al., 1977) and primates (Cameron and Nosbisch, 1991), but not in cattle (Khireddine et al., 1998; Mackey et al., 2000; Amstalden et al., 2002a). To determine if products of rumen fermentation are involved in control of LH secretion, total rumen contents of fistulated steers (440 kg) were removed and either all the fluid and particulate material were replaced (control) or only 15% of the rumen contents were replaced (restricted; Ojeda et al., 1996). Restricted steers were fed 2 kg of low-quality hay each day and control steers were fed a diet of hay and soybean meal to maintain weight. Mean concentration of LH and pulse frequency and amplitude were similar in control and restricted steers before and after 3 d of feed restriction (Table 1). Although the nutritional restriction resulted in a decrease in substrate and microbes for rumen fermentation, mobilization of body fat, and a twofold increase in plasma concentration of NEFA, LH secretion was unaltered.

Secretion of estrogens and progesterone during pregnancy reduces concentrations of LH in the pituitary at parturition (Nett et al., 1987) and the concentration of LH increases in serum within a week after calving (Erb et al., 1971; Ingalls et al., 1973). The interval from calving until pulsatile secretion of LH is sufficient for maturation of the ovulatory follicle is influenced by factors such as body energy reserve, nutrient intake, and suckling. During the early postpartum period, a pulse of LH is secreted every 3 to 6 h (Walters et al., 1982; Humphrey et al., 1983; Nett et al., 1988), and the frequency increases to 1 to 2 pulses/h before the first ovulation (Peters et al., 1981; Terqui et al., 1982). Reduced pulsatile secretion of LH during the early postpartum period is probably associated with decreased GnRH secretion because the number and affinity of GnRH binding sites on the pituitary do not change during the postpartum period (Moss et al., 1985) and pulsatile treatment with GnRH causes pulsatile secretion of LH.

Concentrations of FSH increase within a week after parturition (Schallenberger et al., 1982; Peters and Lamming, 1984) and are constant until ovulation (Convey et al., 1983; Nett et al., 1988). Postpartum anestrous beef cows have adequate FSH for development of DF (Stagg et al., 1998). Similar secretion of FSH in restricted and maintenance diet heifers before the onset of nutritionally induced anovulation, and increased concentrations of FSH in serum after the onset of anovulation (Bossis et al., 1999), indicate that secretion of FSH is not limiting for follicular growth in energy-restricted cattle.

The effect of inadequate nutrition and/or minimal body fat reserves on the sensitivity of the hypothalamus to the negative feedback of estradiol has not been established. During 93 d of nutritional restriction and weight loss, heifers treated continuously with estradiol were more sensitive to the negative effects of estradiol on the number of pulses of LH and mean concentrations of LH (Imakawa et al., 1987) compared with heifers that gained body weight.

When diets of cows were restricted and they lost 1% of their body weight per week for 26 wk, ovulation ceased. Mean concentrations of LH and the number of pulses of LH were not different for intact and ovariectomized nutritionally induced anovulatory cows during the first 10 d after ovariectomy; however, amplitude of LH pulses was greater in ovariectomized than intact cows (Richards et al., 1991). Garcia-Winder et al. (1984) suggested that ovarian factors might interact with suckling intensity to inhibit secretion of GnRH from the hypothalamus of postpartum cows. Further evaluation of the role of estradiol in the regulation of hypothalamic function in postpartum anovulation is needed.

Secretion of Progesterone and Estradiol

Plasma concentrations of progesterone are minimal at parturition (Henricks et al., 1972; Smith et al., 1973) and increase after the first ovulation (Lauderdale, 1986; Perry et al., 1991; Werth et al., 1996) or luteinization (Donaldson et al., 1970; Corah et al., 1974) of a follicle. The first increase in plasma concentrations of progesterone in beef cows after calving usually persist for 3 to 9 d (Perry et al., 1991; Werth et al., 1996; Looper, 1999). This transient increase in progesterone is usually not preceded by estrus.

Plasma concentrations of estrogens decrease rapidly after parturition (see review, Wettemann, 1980). During the postpartum anovulatory period, concentrations of estradiol may increase in plasma for a short duration, but these increases may not be associated with growth and maturation of follicles (Murphy et al., 1990; Stagg et al., 1995). Concentrations of estradiol in plasma increase before the first postpartum ovulation (Echternkamp and Hansel, 1973; Perry et al., 1991; Stagg et al., 1995).

Suckling and Anovulation

Suckling prolongs postpartum anovulation, and the effect is of greatest magnitude in primiparous and thin cows (Short et al., 1990). (See Williams [1990] for a detailed review of the effect of suckling on neuroendocrine control of postpartum ovarian function.) Cows develop a bond with their calf (Silveira et al., 1993; Stevenson et al., 1997), and the effect of suckling by a cow's calf is greater than suckling by a foster calf. Although twice-daily suckling is adequate to increase the duration of the postpartum anestrous interval, twice-daily milking does not prolong anovulation (Lamb et al., 1999). Fewer cows with ad libitum suckling had ovulated by 80 d postpartum (43%) compared with cows that had calves isolated and only allowed to suckle once per day (90%; Stagg et al., 1998). However, if calves were allowed to suckle once per day and calves were adjacent to cows continuously, only 65% of the cows had ovulated by 80 d postpartum. Thus, development of the cow-calf bond prolongs the postpartum anovulatory interval even with a reduced suckling stimulus.

Body energy reserves at calving influence the effect of suckling on ovarian function. If cows had a BCS greater than or equal to 5 at calving, and calves were weaned at 35 d postpartum, all cows ovulated by 25 d after weaning (Bishop et al., 1994). In contrast, only 40% of cows with a BCS less than 5 had ovulated by 25 d after weaning. Although the interval from weaning to ovulation is greater in thin cows than in cows with greater BCS, weaning is a useful management option to increase pregnancy rates in thin cows.

Potential Metabolic Signals

Macronutrients or their metabolites can regulate gene expression and influence growth and body functions in addition to their roles as sources of energy. Glucose can influence expression of genes in cells independent of insulin (Jump, 2001), and low concentrations of cholesterol in cells increase the amount of low-

density lipoprotein receptor and synthesis of cholesterol (Brown and Goldstein, 1997). Fatty acids are also regulators of gene expression in cells, and type of fat in the diet, amount consumed, and duration of consumption can influence responses in cells by alteration of transcription factors (Jump and Clarke, 1999).

Insulin. Consumption of a meal and long-term dietary treatments have minimal effects on plasma glucose in cattle (Yelich et al., 1996; Vizcarra et al., 1998). Although plasma concentrations of glucose in cattle are extremely constant compared with monogastric animals, insulin regulates utilization of glucose by bovine cells.

There are receptors for insulin in the brain, pituitary gland (Lesniak et al., 1988), and ovarian tissue (Poretsky and Kalin, 1987). Insulin stimulates release of GnRH from hypothalamic fragments in vitro when glucose is available (Arias et al., 1992) and infusion of insulin into the cerebroventricles of nutritionally restricted ewes increased LH secretion (Daniel et al., 2000). Insulin also stimulates steroid production by bovine ovarian cells (Spicer and Echternkamp, 1995). Systemic treatment of cows with insulin may increase follicular development (Harrison and Randel, 1986) and estradiol production by large follicles (Simpson et al., 1994).

The hypothalamus, unlike other areas in the central nervous system, expresses an insulin-dependent glucose transporter (Livingstone et al., 1995). This may allow the hypothalamus to respond to increased concentrations of glucose in blood. During nutritionally induced anestrus, cows become resistant to insulin (Richards et al., 1989) and entry of glucose into hypothalamic cells may be reduced.

Early studies demonstrated that 2-deoxy-D-glucose, a glucose antagonist, induced anestrus and anovulation in cows (McClure et al., 1978), and secretion of LH was reduced by treatment of ewes (Funston et al., 1995b) and ram lambs (Bucholtz et al., 1996) with 2-deoxy-D-glucose. Phlorizin-induced hypoglycemia prevented LH and insulin secretion after early weaning of beef cows (Rutter and Manns, 1987).

Insulin-Like Growth Factor-I. Insulin-like growth factor-I is produced by the liver and has effects on many cell types to regulate carbohydrate, fat, and protein metabolism. It is also produced by other tissues and can have autocrine and paracrine effects. Concentrations of IGF-I in blood of cattle are decreased during feed restriction (Richards et al., 1991, 1995; Armstrong et al., 1993) and concentrations of GH are increased (Bossis et al., 1999). Restriction of protein and/or energy intake reduces the increase in blood IGF-I that usually occurs in response to treatment with GH (Brier et al., 1988; Ronge and Blum, 1989; Armstrong et al., 1993). The reduction in IGF-I in serum during nutrient restriction is associated with reduced binding of GH to hepatic membranes in restricted steers (Brier et al., 1988). At least six high-affinity IGFBP in biological fluids can influence the functions of IGF-I (Jones and Clemmons, 1995). Degradation of IGFBP by proteases also influences the biological activity of IGF-I (Maile and Holly, 1999).

Hypothalamic and other neural cells in rats have IGF type-I receptors (Lesniak et al., 1988; Hiney et al., 1996), and IGF-I stimulated expression of the GnRH gene in neural cells (Longo et al., 1998) and GnRH secretion (Anderson et al., 1999). Body energy reserves are related to amounts of IGFBP in hypothalami of ewes (Snyder et al., 1999).

Gene expression for type-I IGF receptors and IGFBP-5 occur in the pars tuberalis and pars distalis of the ovine pituitary and are greater than expression for IGF-II, type-2 receptor, and IGFBP-3 (Adam et al., 2000). Treatment of ovine pituitary cells with IGF-I increases LH release (Adam et al., 2000). Insulin-like growth factor-binding protein-2, -3, and -5 are present in bovine pituitary glands (Funston et al., 1995), and their activity is associated with the stage of the estrous cycle (Roberts et al., 2001).

Antral follicle development in mice requires IGF-I (Zhou et al., 1997). Follicles synthesize IGF-I, and systemic IGF-I could also influence ovarian function (Spicer and Echternkamp, 1995). Specifically, ovarian cell proliferation and steroidogenesis are stimulated by IGF-I (Spicer et al., 1993; Spicer and Chamberlain, 1998). Steroidogenesis is stimulated by IGF-I binding to type-I receptors on cells, and IGF-I is also bound to high-affinity binding proteins in extracellular fluids. Concentrations of IGF-I in follicular fluid and its receptor in granulosa cells of dominant and subordinate follicles are similar; however, dominant follicles have less IGFBP activity than subordinate follicles (Stewart et al., 1996; Yuan et al., 1998). The decrease in intrafollicular concentrations of IGFBP during terminal development of follicles (de la Sota et al., 1996; Funston et al., 1996; Stewart et al., 1996) may increase availability of IGF-I to follicular cells. Concentrations of IGFBP-4 may determine which follicle becomes dominant during selection in cattle (Mihm et al., 2000). Increased energy intake resulted in reduced concentrations of mRNA for IGFBP-2 and -4 in small follicles of heifers (Armstrong et al., 2001).

Nonesterified Fatty Acids. Adipose tissue of ruminants is metabolized and NEFA and glycerol are released and can be used as sources of energy during negative energy balance. Concentrations of NEFA in nutritionally induced anovulation of heifers are maximal during anestrus, decrease dramatically during realimentation, and then gradually increase before resumption of ovulation (Bossis et al., 1999, 2000). Plasma concentrations of NEFA do not appear to be directly involved in nutritional regulation of ovarian function in heifers. A direct effect of NEFA on the hypothalamus and/or pituitary gland has not been established in cattle and infusion of FFA did not alter LH secretion in lambs (Estienne et al., 1990).

Leptin. Concentrations of leptin in plasma are related to amounts of body fat in humans (Considine et al.,

1996; Ostlund et al., 1996) and rodents (Maffei et al., 1995; Schneider et al., 2000), but the relationship between plasma concentrations of leptin and body fat or BCS is not well established in ruminants. Nutrient intake influences amounts of messenger RNA (**mRNA**) for leptin in fat of cattle (Tsuchiya et al., 1998; Amstalden et al., 2000) and concentrations of leptin in plasma (Ehrhardt et al., 2000). Concentrations of leptin in the plasma of dairy cows are decreased by a negative energy balance (Block et al., 2001). These effects of nutrition on plasma leptin increase the difficulty of determining the effect of body fat reserves on plasma leptin. Concentrations of leptin in plasma respond in 2 d to fasting (Amstalden et al., 2000) or in 4 d to reduced nutrient intake (Ciccioli et al., 2001b). Studies have determined positive correlations between body fat and plasma leptin in calves and dairy cows (Ehrhardt et al., 2000) and ewes (Delavaud et al., 2000; Thomas et al., 2001), but minimal numbers of animals were sampled and in the studies with calves and ewes amount of body fat was confounded with dietary intake.

Since the discovery of leptin, there has been much interest as to its potential function as a signal to inform brain targets about body energy stores (Spicer, 2001; Smith et al., 2002). Receptors for leptin have been identified in the brain (Dyer et al., 1997) and pituitary (Iqbal et al., 2000) of sheep, and feed restriction increases expression of leptin receptor in hypothalamic nuclei of ewes (Dyer et al., 1997). Administration of leptin into the brain of sheep reduces feed intake and suppresses LH pulse frequency (Blache et al., 2000; Morrison et al., 2001). However, the effect of leptin on LH secretion cannot be separated from the effect of leptin on feed intake. Treatment of nonruminants with leptin increases secretion of gonadotropins (Barash et al., 1996). Fasting of cows (Tsuchiya et al., 1998) or heifers (Amstalden et al., 2000) for 48 h decreased leptin mRNA in adipose tissue and concentrations of leptin in plasma (Amstalden et al., 2000) without altering concentration or amplitude of LH pulses. Central infusion of leptin did not influence pulsatile secretion of LH in well-fed ewes (Henry et al., 1999), but did prevent the fasting-induced decrease in LH pulse frequency in wethers. Exogenous leptin prevented the fasting-induced suppression of plasma concentrations of LH in castrated rams treated with estradiol (Nagatani et al., 2000), and LH secretion in fasted ovariectomized estradiol treated cows was increased by leptin treatment (Zieba et al., 2002). Leptin has a direct inhibitory effect on the bovine ovary (Spicer and Francisco, 1997). Greater than adequate nutritional intake could result in abundant concentrations of leptin in plasma that could prevent the production of excessive amounts of estradiol by follicles (Spicer, 2001).

Nutrition and Postpartum Endocrine Function

The influence of BCS at calving and postpartum nutrient intake on endocrine and ovarian functions was evaluated in Angus × Hereford primiparous cows (Ciccioli et al., 2001a,b). During the last third of gestation, cows were fed different amounts of protein supplement to produce cows with BCS of 4 or 5 at calving. At parturition, thin (BCS = 4.4 ± 0.1) and moderate (BCS 5.5 ± 0.1) cows were allotted to diets for gains of 0.45 kg/d (**M**; n = 17) or 0.90 kg/d (**H**; n = 17) for the first 70 d after calving. Cows on the H diet weighed about 45 kg more and had greater BCS than M cows after 63 d of treatment. Ovarian and reproductive functions were not influenced by BCS at calving. Duration of ovarian cycles before and after the first postpartum estrus was not influenced by BCS at calving or postpartum nutrient intake. Eighty-eight percent of cows had short luteal phases before the first estrus and all cows had a normal luteal phase after the first estrus. The interval to first estrus and ovulation was shorter ($P < 0.01$; 100 ± 8 d) for H than M cows (120 ± 8 d). The size of the DF, determined by ultrasonography at 4 to 16 h after the onset of estrus (determined by HeatWatch), was larger ($P < 0.01$) for H (14.8 ± 0.3) than M cows (13.5 ± 0.3). Pregnancy rate from artificial insemination at 14 to 20 h after onset of first postpartum estrus was also greater ($P < 0.03$) for H (76%) than for M cows (58%).

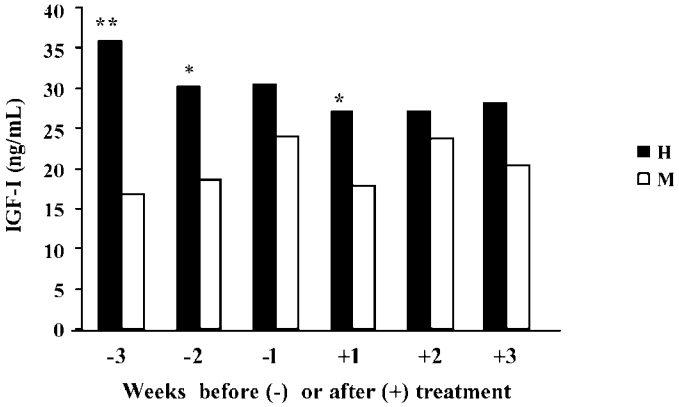

Figure 1. Concentrations of IGF-I in plasma of postpartum primiparous cows during the last 3 wk of nutritional treatment and 3 wk after treatment (adapted from Ciccioli et al., 2001b). *$P < 0.05$, **$P < 0.01$.

Concentrations of glucose and insulin in plasma during the last 3 wk of nutritional treatment (wk 8 to 10 postpartum) and the 3 wk after treatment, when all cows received the same diet, were influenced by treatment × week ($P < 0.01$). During treatment, concentrations of glucose were 5 to 10 mg/dL greater in H than M cows. However by 2 wk after treatment, concentrations of glucose in plasma of H and M cows were not significantly different. Similarly, concentrations of insulin in plasma during treatment were 40 to 50% greater in H than M cows, but were not different 1 wk after cessation of the nutritional treatment.

Concentrations of IGF-I in plasma during the last 3 wk of treatment and the first 3 wk after treatment were influenced by treatment ($P < 0.01$; Figure 1). Cows on

Table 2. Changes in hormones and metabolites in plasma of beef cattle preceding the first ovulation in postpartum cows and nutritionally induced anovulatory heifers that were realimented

	Weeks change occurred before ovulation	
Constituent	Postpartum anestrus	Nutritional anestrus[a]
Glucose	>7	>3
Insulin	>7	>3
IGF-I	>7	>3
NEFA	>7	>3
Leptin	>7	>3

[a]Bossis et al. (2000).

H treatment had greater plasma concentrations of IGF-I on wk 2 and 3 before the end of treatment and on the first week after treatment. Effect of greater nutrient intake on IGF-I in plasma decreased with time after treatment.

Leptin concentrations in plasma were 2.6-fold greater ($P < 0.01$) in H than M cows during the last 3 wk of nutritional treatment. However, within 4 d after the end of nutritional treatment, concentrations of leptin in plasma were similar for M and H cows. At the end of treatment, H cows had about a 0.75 greater BCS than M cows. This indicates that leptin is associated with feed intake and not amount of body fat.

Concentrations of insulin, IGF-I, and leptin were greater in H than M cows during nutritional treatment, but were not significantly different by 1 or 2 wk (approximately 90 d postpartum) after the end of treatment. Ovulation and estrus occurred in most cows after the end of treatment; only 32% of H cows were in estrus by 80 d postpartum. Concentrations of insulin, IGF-I and leptin during 7 wk before the first postpartum estrus were not influenced by time. Changes in concentrations of these hormones and glucose and NEFA occurred more than 7 wk before ovulation (Table 2). Similarly, when nutritionally induced anovulatory heifers were realimenated, concentrations of glucose and insulin were similar in control ovulatory and realimenated anovulatory heifers during at least 3 wk before ovulation of realimenated heifers (Bossis et al., 2000). In realimenated heifers, concentrations of IGF-I and NEFA in plasma were greater at 3 wk before ovulation than during the consumption of the restricted diet, and concentrations continued to increase until ovulation.

The stable concentrations of insulin, IGF-I, and leptin in plasma of primiparous cows during the 7 wk before the first postpartum estrus indicate that immediate changes in these constituents may not stimulate the first postpartum ovulation. These hormones could be metabolic signals by which nutrient intake and body fat stores regulate ovulation, but have a delayed effect, a permissive role, and/or the effect could be mediated by alterations in binding proteins or specific receptors, so that absolute changes in concentrations of hormones may not be necessary for the response to occur.

Nutrient Intake, Body Condition Score, and Plasma Insulin and Insulin-Like Growth Factor-I

Recently, we evaluated the roles of nutrient intake and BCS on concentrations of insulin and IGF-I in plasma of gestating cows (Lents et al., 2002). Commencing at 2 to 4 mo of gestation, cows (n = 73) were fed one of four diets for 109 d. High (**H**) cows received a 50% concentrate diet in a drylot, and moderate (**M**), low (**L**), and very low (**VL**) cows grazed native range pasture and received 2.5, 1.5 or 0.5 kg of a 42% CP supplement each day. After 109 d of treatment, all cows grazed a common pasture and received 1.5 kg of a 42% CP supplement daily. By 109 d of treatment, BCS were 6.7^{a}, 4.8^{bc}, 5.0^{b} and 4.7^{c} (means without a common superscript differ; $P < 0.05$) for H, M, L, and VL cows, respectively. On d 123, after cows were on the same diets for 14 d, BCS were 6.4^{a}, 4.8^{b}, 4.8^{b} and 4.5^{c} (means without a common superscript differ; $P < 0.05$) for H, M, L, and VL cows, respectively. Body condition scores of cows ranged from 4 to 7.5 on d 109 and 3.5 to 7.5 on d 123. The relationship between BCS and concentration of insulin in plasma on d 109, after cows had access to feed, was best fit by linear regression with $R^2 = 0.34$ ($P < 0.05$). However, concentrations of insulin in plasma after cows were fasted (no water and feed for 18 h) were not related to BCS ($R^2 = 0.01$). On day 123 of the experiment, after cows were on the same diets for 14 d, concentration of insulin in plasma was not influenced by BCS after either feeding or fasting. These results indicate that concentrations of insulin in plasma of gestating cows are influenced by nutrient intake more than by BCS.

On day 109 of gestation, the relationship between BCS and plasma concentrations of IGF-I was best fit by a quadratic equation ($P < 0.05$) with $R^2 = 0.36$ for samples collected after cows had access to feed and $R^2 = 0.27$ ($P < 0.05$) after an 18-h fast (Figure 2). After all cows were on the same diet for 14 d (d 123), BCS did not influence concentration of IGF-I in plasma samples after feeding ($R^2 = 0.01$) or after an 18-h fast ($R^2 = 0.01$) (Figure 2). Similar to the relationship between BCS and concentrations of insulin in plasma, concentrations of IGF-I in plasma of gestating cows are influenced by nutrient intake more than by BCS.

Nutritional Effect on Postpartum Follicular Growth

Mature Angus × Hereford cows were fed amounts of supplemental protein during the last 4 mo of pregnancy so they would have a BCS of 4 or 5 at calving. Follicular growth was monitored by ultrasonography to measure growth of the DF between d 27 and 33 or d 47 to 53 postpartum. When the diameter of the DF increased less than 0.75 mm in 24 h, it was aspirated using a transvaginal, ultrasound-guided needle. Concentrations of IGF-I, estradiol, and IGFBP were quantified in follicular fluid. Proestrus DF were aspirated from postpartum cows with normal estrous cycles at 48 h

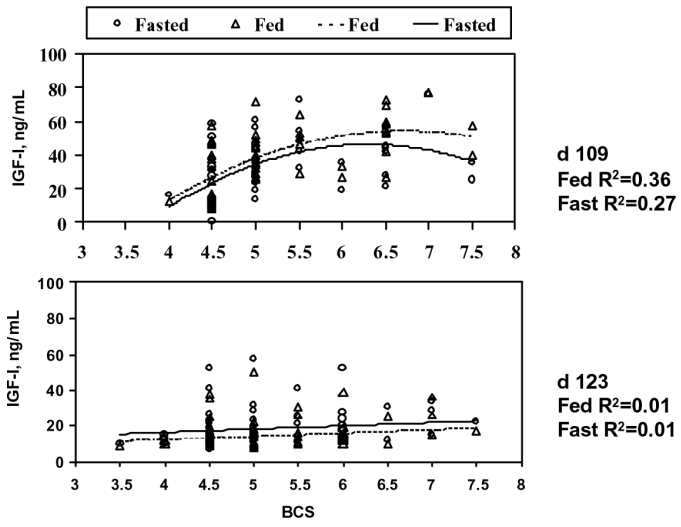

Figure 2. Relationships between BCS and concentrations of IGF-I in plasma of fed and fasted (18 h) beef cows, when cows received different diets (d 109) and after all cows received the same diet for 14 d (adapted from Lents et al., 2002).

after treatment with prostaglandin $F_{2\alpha}$. BCS at calving did not influence any of the postpartum follicular characteristics. Time of aspiration of DF was classified as either less than (short) or greater than 35 d (long) before the first estrus and ovulation. Follicles from short cows were aspirated an average of 42 d postpartum and estrus and ovulation occurred about 55 d postpartum. Follicles from long cows were aspirated about 39 d postpartum, and estrus and ovulation occurred about 85 d postpartum (Table 3). Diameters of the aspirated DF were similar (12.8 ± 0.6 mm) for short, long, and proestrus cows. Concentrations of IGF-I in follicular fluid were not influenced by estrus/ovulation classification and averaged 25.4 ± 3.6 ng/mL. Concentration of estradiol in the fluid from proestrus follicles was greater (435 ± 79 ng/mL) than the concentration in short (95 ± 56 ng/mL) or long cows (72 ± 59 ng/mL). Amounts of IGFBP-3 and -4b were greater in the DF of short than long cows. In addition, the amount of IGFBP-3 and -4b in short cows was similar to amounts in proestrus follicles. Amounts of IGFBP-2 and -4a were not different in follicles from short, long, and proestrous cows. Follicular fluid concentrations of IGF-I are not different between DF and first subordinate follicles (Stewart et al., 1996). Changes in amounts of IGFBP in follicles during several weeks before the first postpartum estrus and ovulation may result in similar total concentration of IGF-I within follicles, but may result in different biological effects on follicular growth and maturation.

Leptin Receptors in the Hypothalamus and Pituitary

We used acute nutritionally restricted anovulatory heifers to evaluate the possible role of leptin in the regulation of the onset of anestrus (White et al., 2001). When postpubertal heifers were fed 0.4× maintenance (**M**) for 14 d, 58% became anovulatory and concentrations of IGF-I were reduced ($P < 0.001$) compared with heifers fed 1.2× M. Concentration of leptin in plasma of heifers fed 0.4× M tended to be reduced ($P < 0.10$) compared with heifers fed 1.2× M. The amount of leptin receptor in the median eminence, arcuate nucleus, and anterior pituitary as determined by quantitative RT-PCR were not significantly different for 0.4× M and 1.2× M heifers. In agreement with our results, secretion of GnRH by medial basal hypothalamic explants from ovariectomized estradiol-treated cows was not influenced by leptin (Amstalden et al., 2002b). If acute nutritional restriction signals hypothalamic or pituitary function via leptin, we have no evidence that the effect is by alteration in the amount of message for synthesis of leptin receptor.

Conclusions

Body energy reserve at calving is the most important factor that influences the interval from parturition to the first estrus and ovulation in beef cows. Postpartum nutrient intake can modulate the duration of the postpartum anestrous interval; however, even if thin cows gain great amounts of weight after calving, ovulation occurs later than for cows that calve in good body condition and maintain body weight.

Decreased pulsatile secretion of GnRH is the major cause of reduced pulsatile secretion of LH and extended

Table 3. Influence of days before first postpartum (PP) estrus and ovulation on dominant follicle size and concentrations of insulin-like growth factor-I, estradiol, and insulin-like growth factor binding proteins in follicular fluid

Estrus and ovulation, d	Cows, no.	Aspiration PP, d	Follicle diameter, mm	IGF-I, ng/mL	Estradiol, ng/mL	IGFBP 3	IGFBP 4 b
<35	10	42	13.2	24.1	95[a]	42[a]	0.4[c]
≥35	9	39	12.3	22.6	72[a]	32[b]	0.1[d]
Proestrus	6	47	13.0	28.3	435[b]	40[ab]	0.5[c]
SE	—	—	0.6	3.6	63	3.9	0.1

[a,b]Means within a column that do not have a common superscript differ ($P < 0.06$).
[c,d]Means within a column that do not have a common superscript differ ($P < 0.05$).

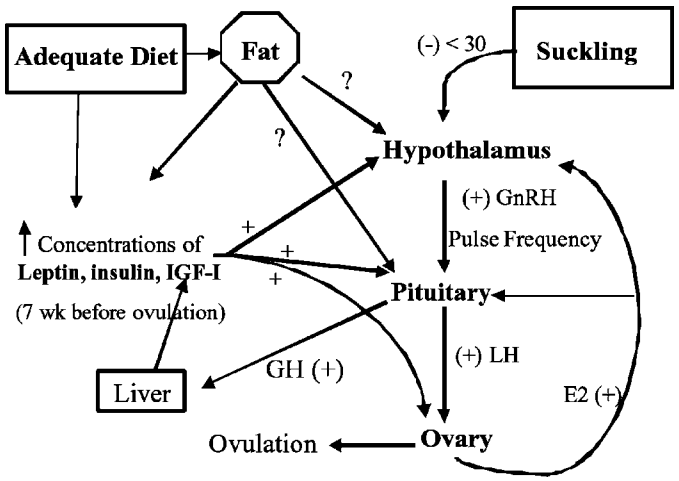

Figure 3. Control of reproductive function in postpartum beef cows.

postpartum anovulatory intervals in beef cows (Figure 3). With inadequate secretion of LH, DF do not become estrogen active and secrete insufficient estradiol to induce an ovulatory surge of LH and estrus. Adequate nutrient intake results in increased concentrations of insulin, IGF-I, and leptin in plasma and increased body fat reserves. If fat stores are sufficient (BCS greater than 5) and nutrient intake is not adequate, mobilization of fat can occur and alter plasma concentrations of insulin, IGF-I, and leptin. Secretion of growth hormone by the anterior pituitary stimulates synthesis of IGF-I by the liver except during inadequate nutrient intake when GH receptors on the liver are inadequate and the GH-IGF-I system is disconnected. Under these circumstances, tissue-specific synthesis of IGF-I may have important autocrine or paracrine effects. A stimulatory role of leptin in control of GnRH secretion in cattle is not established, however, insulin and IGF-I may enhance GnRH secretion. Leptin, IGF-I, and insulin may have direct effects on the pituitary to increase secretion of LH and on the ovary to regulate steroidogenesis.

Although concentrations of insulin, IGF-I, and leptin in plasma are relatively constant during the 7 wk before the first postpartum estrus and ovulation, this does not mean they are not metabolic signals that regulate reproduction. The hormones could a) influence early follicular or oocyte development and have delayed effects, b) have a permissive role to facilitate the effect of other hormones or factors, or c) have effects that are modulated by amounts of binding proteins or specific receptors. It is probable that unidentified compounds produced by adipose tissue have stimulatory or inhibitory effects on hypothalamic, pituitary or ovarian function since adequate body energy reserves, as well as secretion of insulin and IGF-I, are a prerequisite for postpartum ovulation.

Suckling has an inhibitory effect on pulsatile GnRH secretion during the early postpartum period, and in thin cows. If cows have adequate body energy reserves and nutrient intake, the suppressive effect of suckling on GnRH secretion is greatly diminished by 30 d postpartum.

We conclude that both body fat reserves and nutrient intake regulate anovulation in beef cows. Effects of BCS and nutrient intake on concentrations of IGFBP, as well as receptors for IGF-I and leptin, must be evaluated in the hypothalamus, pituitary, and ovary to elucidate metabolic signals that control postpartum ovulation in beef cows.

Implications

The interval from calving to conception greatly influences profitability of beef production. Inadequate body fat stores at calving and reduced postpartum nutrient intake increase the interval from calving until ovulation. Suckling suppresses ovulation during the early postpartum period in cows with moderate body fat stores, and the suppression is longer in thin cows. Restricted suckling or early weaning of calves can be used to improve reproductive efficiency in very thin cows. Insulin, insulin-like growth factor-I, and leptin may be metabolic signals or permissive cues; however, other interactive factors must be involved. Determination of metabolic signals by which body energy stores and nutrient intake regulate the interval from calving to first ovulation will allow development of management strategies to increase pregnancy rates in beef cows.

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Controlling variation in feed intake through bunk management[1]

R. H. Pritchard and K. W. Bruns

South Dakota State University, Brookings 57007

ABSTRACT: The desire to control variation in daily feed intake by feedlot cattle stems from the obvious concern that a significant aberration in grain intake can lead to clinical acidosis or death. Although less dramatic, aberrations also occur when cattle have unrestricted access to feed. A cyclic pattern of higher and lower daily DMI can cause gain efficiency to be less than that predicted from the mean DMI because responses in ADG to changes in DMI are not linear. If bunk management is a means of ameliorating either of these events, it is presumed that management ascribed to the pen is affecting variability in daily DMI by individuals within the pen. Two likely mechanisms by which bunk management may affect intake patterns are limiting availability of feed to prevent overconsumption events or affecting animal behavior so that daily intake is more consistent. Bunk management approaches that have been evaluated for their effect on production rates, and in some instances on day-to-day variability in DMI, include: 1) limiting the quantity of feed available or the amount of time feed is available each day; 2) the timing and frequency of feed deliveries; 3) linear bunk space allocation; and 4) mixed diet or segregated ingredient feeding. When bunk management approaches alter responses, it may be that the approach has a direct biological and/or behavioral effect on the animal or that the approach itself involves less variation, which is consequently favorable to the animal (or the data). The causes of variable results in bunk management research can be ambiguous. Management and feeding systems are difficult to standardize, which can cause the definitions of controls, the characterizations of treatments, and the context of responses to be inconsistent. A rudimentary limitation is that in systems where individual daily DMI is known, competition for access to feed is usually not comparable to typical pen feeding. There is evidence of favorable responses to some bunk management approaches that could be used commercially. Effects on production efficiency in these studies are of significant biological and economic importance, and underlying mechanisms must be more fully characterized to allow broad application.

Key Words: Cattle, Feed Intake, Feedlots, Management

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Introduction

Bunk management is generally thought of as the approach to feed deliveries for confined cattle. It should probably be considered a more encompassing process that includes feed batching and delivery in contexts of quality, quantity, and time. Bunk management has to be dynamic in response to type of diet, class of cattle, changes in climatic conditions, and bunk space allocations. Research on many aspects of bunk management is extremely difficult, if not impossible, to conduct without experiencing confounding or bias. These limitations bring to the forefront Wolfe's (1991) Third Law of Thermodynamics, which states "the emotion generated in scientific discussion increases proportionally with the softness of the data".

The simplistic title of this manuscript is fraught with ambiguities. Intake variation can be an issue on a pen basis or on an individual basis. It is unclear to what extent, and in what ways the two are related. The link in a cause and effect relationship to inefficiency, morbidity, or mortality exists only in extreme instances. Finally, in a feedlot setting, we are inclined to refer to feed delivered as feed intake without providing evidence that all feed offered was consumed on that day.

The overwhelming consideration of bunk management is control of clinical and subclinical metabolic disorders. In spite of our improved capabilities to weigh and mix properly proportioned diets, grain overloads do occur. Digestive disorders are a leading cause of death in feedlot cattle (USDA, 2000). Subclinical and clinical acidosis reduce gain efficiency, cause liver abscesses, and ruminitis (Owens et al., 1996). The question at hand is whether bunk management can reduce variability in feed intake and ameliorate these mala-

[1]Correspondence: Box 2170 SDSU (phone: 605-688-5165; fax: 605-688-6170; E-mail: robbi_pritchard@sdstate.edu).

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dies. To evaluate this, we will break down bunk management into component parts and examine their influence on cattle performance.

Overview

Interest in bunk management is not new. Henry and Morrison (1928) included a chapter (Counsel in the Feedlot) that represented three enduring points regarding bunk management. The first point is "Many an experienced stockman can carry steers through the fattening period without once getting them 'off feed,' but yet cannot well describe to others just why he/she is so successful." This is not necessarily a lack of communication skills. We typically describe things by contrast: more, bigger, shorter, longer. Consistent feeding is essentially unremarkable and consequently difficult to describe.

The second and third points in this chapter were in turn quoted from Mumford's Beef Production (1907). "As soon as the fattening process begins, the cattle should be fed at certain hours and in the same way. This cannot be varied 15 minutes without some detriment to the cattle. The extent of injury will depend upon the frequency and extent of irregularity. . .." We interpret this to mean that beef cattle, like dairy cows, are creatures of habit. If behavior can be modified favorably by cultivating and respecting good habits, the potential exists to improve performance. Hungate (1966) likened the rumen to a continuous culture fermentor and discussed in detail how diet, eating, rumination, and digestive tract function coordinate to achieve this steady-state condition. The consistent management steps advocated by Mumford may complement this expectation.

The final point, again attributed to Mumford (1907), is that "Scouring, the bane of the stockfeeder, should be carefully avoided since a single day's laxness may cut off a week's gain. The trouble is generally brought on by overfeeding, by unwholesome feed, or by a faulty ration. Overfeeding comes from a desire of the attendant to push cattle to better gains or from carelessness or irregularity in measuring out the feed supply. The ideal stockman has a quick discernment. . . which guides the hand in dealing out feed ample for the wants of all but not a pound in excess." Here Mumford has described weaknesses in human nature that persist as a critical element of bunk management today. It also seems that Mumford advocated a clean bunk feeding strategy, where no feed should be carried over from one feeding to the next.

Feeding vs. Eating

A dichotomy exists between the motivation behind the cattle feeder's bunk management decisions and motivation of cattle to eat. Feedlot management has to be concerned with the capability to manufacture and deliver sufficient quantities of feed in a timely fashion. Bunk volume allocation per animal may be limited. Combined, these constraints may force management to plan two to four feed deliveries per pen, per day. Inherent in multiple feedings are the advantages that feed delivery errors or weather interruptions are reduced to some degree for each multiple of feed deliveries per day.

Cattle eating behavior is driven by a loss of the satiety signals that suppress hunger. Cattle consume most feed near sunrise and especially around sunset (Stricklin and Kautz-Scanavy, 1984). Short winter days in northern latitudes promote more nighttime eating, and high effective ambient temperatures can reduce mid-day and daily DMI (Hahn, 1999). When observing eating behavior by adapted cattle in a commercial feedlot, Hicks et al. (1989) reported that 7.5 to 20% of cattle might not be observed eating in a 24-h period. It is unclear whether this was caused by digestive disorder or is an inherent eating behavior trait. Cattle not observed to be eating were not identified as morbid.

Hickman et al. (2002) noted fluctuations in day-to-day DMI with a new system for monitoring feed intake by individuals in a group-fed environment. The feed intake pattern variability was not compressed in faster-growing or more efficient steers. They did not report any episodes of inappetence in that research. Day-to-day fluctuations in DMI are not surprising when considering that the short term feed intake regulation mechanism in cattle was evolved to support relatively high roughage diets. This research does cloud the issue of how/when aberrations in DMI can lead to clinical or subclinical acidosis.

Cattle eating behavior is also driven by nonbiological signals. Like Pavlov's dogs, cattle may learn to come to the feed truck. They acquire aversions to feeds (Provenza, 1996), and they respond to changes in the weather. These intuitive and learned responses may interact with signals provided by bunk management in favorable or unfavorable ways. Ample feed, available on a consistent schedule may indeed reduce aggression at feeding time, and that may improve production. Unfortunately, data are limited on this subject.

Feed Delivery Management Approaches

Limit-fed high-concentrate diets involve a substantial restriction of feed allocation relative to expected DMI. Cattle performance can be predictable. When bias occurs, it is likely that cattle are more efficient than modeled predictions (Loerch and Fluharty, 1998). When cattle are fed at 75 to 80% of expected DMI, prehension is rapid, and cattle do not sort through feed. Amounts fed are constant from day to day, and this system would accommodate a consistent feed delivery schedule.

Programmed or restricted feeding systems involve lesser restrictions of feed deliveries, perhaps at 5 to 10% less than expected DMI. As in limit feeding, cattle can be expected to remove all feed from bunks relatively quickly, leaving bunks empty much of the day. Growth rate is predictable and gain efficiency is sometimes improved over full-fed cattle (Plegge, 1986). Feeding at

this level likely meets the criteria of ample feed for all, but with no excess. This method eliminates the human factor of the inclination to overfeed the cattle. When improved gain efficiency is observed, it is not possible to discern whether it is brought about by affecting digestibility of the diet, ruminal methane production, or reduced variability in feed deliveries (Zinn, 1995).

Clean-bunk management systems attempt to allow cattle to achieve long-term average DMI that will meet or exceed that of cattle fed ad libitum. The caveat that distinguishes clean bunk from ad libitum management is that in clean-bunk systems, it is expected that the bunks will contain no carryover feed at a specified time each day. This approach, with its many possible variations, has become a common practice in the major cattle feeding regions of the United States. This system, however, is susceptible to errors of judgment regarding feeding quantities. It may be more susceptible to time variations. By virtue of the high intake level, the effects of errors in quality or quantity of feed delivered may be more acute.

True ad libitum feeding can be achieved using feed bunks or self-feeders. The intent is to allow unrestricted access to feed at all times. Self-feeders cannot accommodate high-moisture feeds and usually can accommodate only low levels of small-particle-size roughages. Feeding schedules and variable feed deliveries presumably are not components of these systems. The animal has absolute control of daily feed consumption. It has not been proven that ad libitum access to high-grain diets results in higher DMI than that which occurs with clean-bunk management systems.

We can evaluate the bunk management systems for their application with the constraints set forth earlier. Limit feeding and restricted/programmed feeding allow management to be consistent in quantities of feed delivered and to not overfeed cattle. The cyclic feed delivery patterns described by Fulton et al. (1979) are avoided. Finite feed deliveries and competition for this feed among contemporaries dictates the upper limits of daily DMI by individuals. There is a compressed window of feed availability. Sorting is not likely under these conditions. Excess feed is not available to allow binge eating, if indeed this is the root of acidosis problems in feedlot cattle. Variability in DMI must occur at less than a critical level since growth rates are predictable and gain is efficient (Sip and Pritchard, 1991; Loerch and Fluharty, 1998). Furthermore, the literature does not make reference to an inordinate frequency of digestive disorders associated with these prescriptive feeding programs. By definition, restricted feeding has a bias that may not allow cattle to demonstrate maximal growth rates. Restricted feeding may cause reductions in carcass quality (Pritchard, 1995). By virtue of their systematic approach, these programs may cultivate favorable learned eating responses in cattle.

Ad libitum feeding provides feeds in excess. As a consequence, binge feeding cannot be curbed. In dairy systems that allow continuous access to feed, feed tossing is a common problem (Albright, 1993). This behavior is also observed in feedlots and strongly suggests that sorting is a concern since feed tossing causes diet separation. Atwood et al. (2001) showed that cattle vary in their feed preferences and that an individual's preferences may change over time. Selective eating could alter diets of individuals doing the sorting/selection, as well as those left with access to their refusals. The potential for sorted diets to affect gain efficiency is obvious when considering the large disparity of nutrients and energy density of individual ingredients included in a diet.

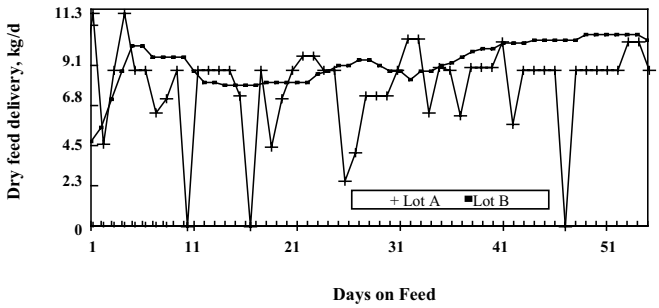

Figure 1. Feed deliveries expressed as dry matter per animal daily for representative pens. Lot A was offered ad libitum access to feed; Lot B was fed using a clean-bunk management system.

The short-term intake regulation mechanisms employed by cattle are not conducive to concentrate feeding (Preston, 1995a). The mechanism by which cattle learn to regulate grain intake on low/no roughage diets provided in self-feeders is unclear. A better understanding of this situation may aid other types of feed-delivery management. These self-fed cattle may indeed learn to reduce intake or meal sizes to avoid the discomfort of indigestion. Alternatively, they may develop an aversion to the diet. In spite of this aversion, hunger is a force strong enough to cause them to eat. Having no substitute feeds available, intake may reflect the balance of hunger and aversion. Perhaps it is a combination of these concepts that allows cattle to avoid a lethal meal size.

Fluctuating Feed Deliveries

We had occasion to feed a common set of steers either ad libitum or in a clean bunk system. Feed deliveries for a 56 d period for two representative pens are depicted in Figure 1. These two feeding programs ran concurrently, but were managed by different individuals in separate facilities. In such a comparison, the gambit of factors contributing to bunk management occur (timing, batching, etc.) and there could be facility influences on performance. For the pooled replicates of pens (n = 5), there was no effect on DMI (9.18 vs. 8.95 kg; $P > 0.10$), but ADG was reduced (0.94 vs. 1.71 kg; $P < 0.05$) and feed:gain ratio was increased (9.58 vs. 5.35; $P < 0.05$) by ad libitum feeding. It should be noted that DMI in

this study was DM delivered to the pens. Feed wastage becomes a part of the problem of lost efficiency in these data, just as it occurs in commercial feeding operations.

More controlled influences on feed delivery have been tested. Galyean et al. (1992) used programmed feeding to assure steady delivery of feed to control steers. They then introduced feed delivery fluctuations of 10% from controls either daily or weekly. Weekly fluctuations did not affect cattle performance. Daily fluctuations caused a 6.5% reduction in ADG ($P < 0.10$) and a 7% increase in feed:gain ($P < 0.10$). Most of the performance difference occurred in the initial 56 d of the 84-d study. These were heavy feeder cattle with previous bunk experience and may have been challenged to adjust to the fluctuating feed deliveries implemented at the onset of the trial.

In a similarly designed experiment, Zinn (1994) found no difference in cattle performance attributable to fluctuating deliveries. Holstein steers were on feed for 138 d and controls were program-fed to gain 1.1 kg/d. The lack of an effect of daily 20% feed fluctuations may have been because the extended time on feed could dilute out early effects (interim data were not reported). Also, the control cattle were fed to achieve a relatively low growth rate. Applying fluctuations at this level of production may not have constituted a metabolic challenge to the rumen or to the steers.

Cooper et al. (1998) applied feed delivery fluctuations of ±0.91 kg daily from control steers. Unlike the two previous examples of restricted feed deliveries, control steers in these two experiments were provided feed to appetite. During the initial 140-d trial, fluctuating deliveries caused a 1.7% increase in DMI ($P < 0.05$), with no influence on other production variables. In the second similar experiment lasting 147 d, performance was not influenced by feed deliveries.

The feed-delivery patterns depicted in graphs included by Cooper et al. (1998) bring to light how we look at variation. In those trials, the feed deliveries for the control cattle appeared to deviate as much as 20% above and below the overall mean daily DMI. The patterns for the controls may be typical, but we have yet to establish whether those deviations constitute variability that affects performance. If the typical fluctuations for controls represent sufficient variability to affect performance, the deviations imposed from the control may not cause further compromises of production efficiencies. This may be especially true in that the imposed deviations are consistent and symmetrical, unlike a cyclic intake pattern.

Bierman and Pritchard (1996) attempted to answer part of that question by allowing control treatment feed deliveries to fluctuate as we fed cattle to appetite. The managed treatment was intended to allow cattle to achieve expected maximal feed intake, but to not allow periods of higher than expected DMI. Variation in feed deliveries to control pens (Figure 2) was quite similar to that depicted in the first trial of Cooper et al. (1998). Feed delivery highs and lows were eliminated in the managed bunk treatment (Figure 2), but it appears that restricted feeding may have been imposed. Intake was reduced 12% ($P < 0.01$) by the managed bunk approach (Table 1) even though there was carryover feed present in the bunks at 0700 on 40% of the pen days. Feed carryover occurred in 69% of the pen days for cattle fed to appetite. Growth rate was not affected by bunk management. However, ADG became more variable before d 30 ($P < 0.05$) and after d 85 ($P < 0.10$) when steers were fed to appetite. Feed efficiency and marbling scores tended ($P < 0.10$) to be improved by the more restrictive bunk management. Gain efficiency was numerically higher early and late in the feeding period (Figure 3), which corresponds with periods of heterogeneity of ADG between treatments. The variable ADG was caused by a disproportionate increase in steers with very low ADG (<0.5 kg) when fed to appetite. This

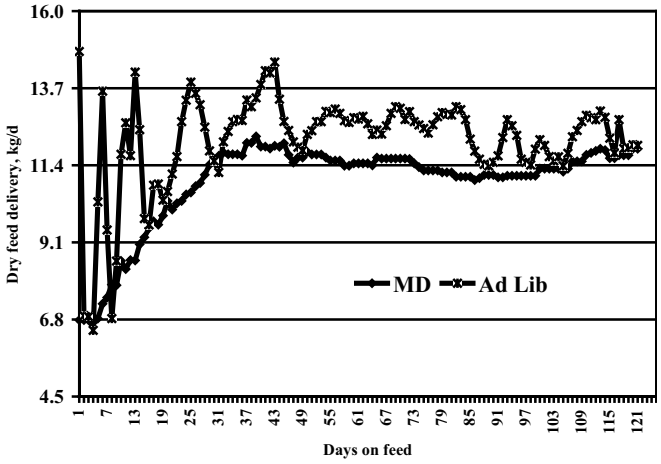

Figure 2. Feed deliveries (kg·steer^{-1}·d^{-1}) over time for ad libitum (Ad Lib) and managed delivery (MD) treatments.

Table 1. Influence of feed delivery management on steer performance and carcass traits[a]

	Treatment		
Item	Ad libitum	Managed	SEM
Initial BW, kg	392	392	3.0
Final BW, kg	604	602	5.4
ADG, kg	1.75	1.74	0.049
DMI, kg[b]	11.97	10.69	0.262
Gain:feed, g/kgc	145	162	3.6
Clean bunks, %d	40	69	—
Carcass weight, kg	373	373	3.5
Dressing, %	61.8	61.9	0.16
Fat thickness, cm	1.10	1.05	0.038
Marbling[ce]	5.31	5.67	0.103

[a]Production variables are reported on a pen basis (n = 5); carcass variables are reported on individual basis (n = 38); 121 d on feed.
[b]Means differ ($P < 0.10$).
[c]Means differ ($P < 0.05$).
[d]Percentage of pen days when no feed was present in bunks at 0700.
[e]5.0 = small^0; 6.0 = modest^0.

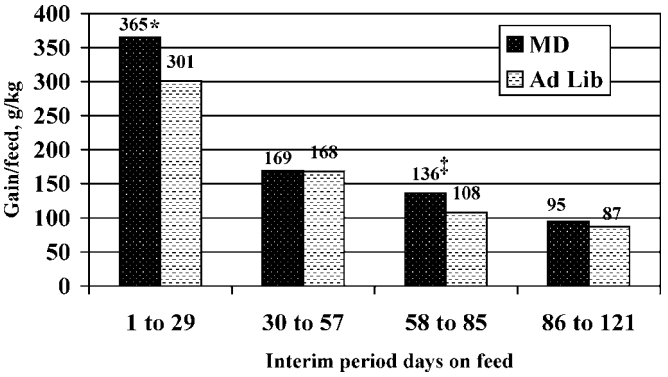

Figure 3. Interim gain:feed (g/kg) for ad libitum (Ad Lib) and managed feed deliveries (MD). *Treatment effects within interim period ($P < 0.05$). ‡Treatment effects within interim period ($P < 0.10$).

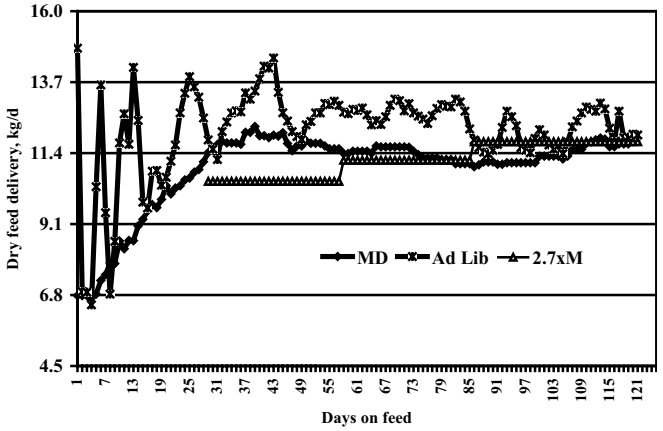

Figure 4. Feed deliveries ($kg \cdot steer^{-1} \cdot d^{-1}$) over time for ad libitum (Ad Lib), managed delivery (MD), and 2.7× maintenance DMI treatments (2.7× M).

may reflect individuals experiencing acidosis and would be consistent with responses observed for gain:feed and marbling.

It seems probable that DMI restrictions can be imposed by tightly managed feed deliveries. Production per unit of input was improved in this study. Rossi et al. (2001) demonstrated that programming growth early in the feeding period allows comparable growth rates and carcass traits to ad libitum-fed steers, but less feed is required per unit of production.

If the advantages of restricted or programmed feeding are that they prevent unacceptably high feed intakes, there may be a simpler approach. Preston (1995b) described the use of maximal intake limits on cattle during step-up and finishing phases. The principle would accommodate most classes of cattle and diets in that limits are set as multiples of maintenance energy intake. Feed deliveries are allowed to deviate as needed for the circumstances at hand, but overfeeding is avoided by the limits. The limits proposed were 2.1, 2.3, and 2.5 times maintenance DMI for step-up and 2.7 times maintenance DMI for finishing. We have calculated the 2.7 times maintenance value for the latter periods of the experiment reported by Bierman and Pritchard (1996) and imposed those levels on the daily feed delivery graph (Figure 4). Managed deliveries were coincidentally similar to the 2.7 times maintenance threshold. In contrast, when cattle were fed to appetite, feed deliveries frequently spiked well above the maximal intake limit, but did not increase ADG.

Feeding Schedules-Frequency

When limit-feeding Holsteins once daily in the summer, Reinhart and Brandt (1994) reported an 18% increase ($P < 0.05$) in ADG by feeding in evenings rather than in mornings. In a clean-bunk finishing program, Pritchard and Knutsen (1995) reported similar DMI, but higher ($P < 0.05$) ADG and lower ($P < 0.05$) feed:gain during the summer–fall seasons when cattle fed once daily received the delivery at 1630 rather than 0730. The mode(s) of action for this response has not been elucidated. Thermodynamics may be involved. How evening feeding influences variation in eating behavior has not been studied. Pritchard and Knutsen (1995) indicated that based on within-day changes in BW, diurnal fill patterns were altered by the time of feeding. Mitloehner et al. (1999) reported that evening feeding has influences on behaviors that may indirectly relate to variability in feed intake by individuals.

Hanke et al. (1981) saw no advantage to multiple daily feed deliveries. We (Pritchard and Knutsen, 1995) have observed that in some, but not all, instances, feeding twice daily results in better gain efficiency than feeding once daily in the morning. The reason behind the inconsistent response is unclear. As mentioned previously, multiple feedings may reduce the magnitude of feeding errors, and the opportunity for binge feeding may be reduced as well.

It may be that multiple feeding may better accommodate the inherent variability in an individual steer's access to feed or the accuracy of its short-term intake regulation. Soto-Navarro et al. (2000) studied the influence of once vs. twice daily feeding, with or without imposed fluctuations in feed delivery on ruminal conditions. Interactions occurred where feeding twice daily caused numerical increases in the amount of time ruminal pH was below 6.2 ($P < 0.10$) and in the rate of acetate production ($P < 0.05$). Both responses would be considered liabilities in growing-finishing cattle. However, when feed deliveries were fluctuated, ruminal conditions became more favorable for steers fed twice daily than those fed once daily. In group-feeding situations, variability in feed availability to an individual is inevitable. Consequently, multiple feed deliveries may be beneficial to the ruminal environment although that has not been specifically tested.

Implications

Feed delivery management research is fraught with ambiguities. Feed delivery management can affect production efficiency of cattle fed high-grain diets. Although variability in feed delivery, as we assess it in feeding programs, may not be documenting variability in intake by individuals within a pen, systems that achieve more consistent feed deliveries seem beneficial. Bunk management programs that prevent cyclic intake patterns and/or overconsumption for pens of steers may be most beneficial. Both the reference point from which the magnitude of feed delivery deviations is measured and the types of deviations that occur are relevant when assessing variability. To minimize variation, management must anticipate physiological and behavioral responses by cattle to their environment. More research is needed to develop management programs that minimize digestive disorders and are adaptable to different diets, cattle, climates, and feeding situations.

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Environmental stress in confined beef cattle[1]

T. L. Mader[2]

University of Nebraska, Northeast Research and Extension Center, Concord 68728

ABSTRACT: The performance, health, and well being of cattle are strongly affected by climate. Almost annually, heat waves and/or periods of severe winter weather cause significant losses in one or more regions of the United States. In the past 10 yr, economic losses in the feedlot industry alone averaged between $10 million to $20 million/year as a result of adverse climatic conditions. For each animal that dies from climatic stress, corresponding economic losses approach $5,000 due to mortality and associated live animal performance losses. Management systems are needed that incorporate information and guidelines regarding cattle responses to weather challenges. Altering the microclimate by providing protection from the environment is one of the most useful tools to help animals cope with climatic conditions. For most cattle, facilities and management programs do not need to eliminate environmental stress completely, but rather minimize the severity of the environmental challenge and aid the animal in adapting to it. Inexpensive management alternatives, such as the use of bedding in winter or sprinklers in summer, need to be considered. When designing or modifying facilities, it is important that changes made to minimize impact of the environment in one season do not result in adverse effects on animals in another season. For instance, using permanent wind barriers to minimize cold stress in the winter for feedlot cattle may require that shade or sprinklers be provided in the summer to minimize heat stress. In addition to facility changes, dietary manipulation may be beneficial for cattle challenged by environmental conditions.

Key Words: Beef Cattle, Environment, Feed Intake, Management Alternatives, Stress

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Introduction

Whereas new knowledge about animal responses to the environment continues to be developed, managing cattle to reduce the impact of climate remains a challenge (Hahn, 1995, 1999; Sprott et al., 2001). In particular, additional environmental management strategies are needed to guide managers when making decisions prior to and during periods of adverse weather (Mader, 1986; Mader et al., 1997a; Mader and Davis, 2002). In 1992, 1995, 1997, and 1999, individual feedlots lost in excess of 100 animals during severe heat episodes. The heat waves of 1995 and 1999 were particularly severe, with cattle losses in individual Midwestern states approaching 5,000 animals each year (Busby and Loy, 1996; Hahn and Mader, 1997; Hahn et al., 2001). Early snowstorms in 1992 and 1997 resulted in the loss of over 30,000 head of feedlot cattle each year in the Southern Plains of the United States. The winter of 1996/1997 also caused hardship for cattle producers because of greater than normal snowfall and wind with up to 50% of the newborn calves being lost in many areas, and over 100,000 head of cattle lost in the Northern Plains states. In addition to losses in the 1990s, in the winter of 2000/2001 (Hoelscher, 2001a,b,c), feedlot cattle efficiencies of gain and daily gain decreased approximately 5 and 10%, respectively, from previous years as a result of late-autumn and early-winter moisture combined with prolonged cold stress conditions. These recent examples suggest that rational, cost-effective management systems are needed to reduce climate-related losses in feedlot cattle. Hahn (1999) has previously provided a detailed review of how the animal responds to thermal heat loads. This report summarizes results that support improvements in environmental management of cattle cared for in feedlots during both hot and cold weather. Application of these strategies can be extended to the cow-calf and stocker cattle segments of the industry when they are managed in confined areas.

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[2]Correspondence: Haskell Agricultural Laboratory, 57905 866 Rd. (phone: 402-584-2812; fax: 402-584-2859; E-mail: tmader@unlnotes.unl.edu).

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Table 1. Effect of adding bedding (wheat straw) to feedlot pen surfaces during winter and spring[a]

	None	Bedding	SE
Initial weight, kg	293	293	2.7
Final weight, kg	653	655	4.5
Daily DMI, kg			
d 0 to 67	6.73	6.63	0.09
d 68 to 172	9.35	9.55	0.11
d 0 to 172	8.33	8.41	0.11
ADG, kg			
d 0 to 67	1.36	1.41	0.03
d 68 to 172[b]	1.36	1.49	0.01
d 0 to 172[b]	1.36	1.46	0.02
Feed:gain[c]			
d 0 to 67	4.95 (0.202)	4.70 (0.213)	0.14
d 68 to 172[b]	6.90 (0.145)	6.44 (0.155)	0.06
d 0 to 172[b]	6.14 (0.163)	5.77 (0.173)	0.09

[a]Pooled analysis of studies, weighted by the number of pens per treatment, per trial, conducted by Birkelo and Lounsbery (1992) and Stanton and Schultz (1996). Approximate harvest dates for cattle were middle to late May.
[b]Means differ ($P < 0.05$).
[c]Parenthetical numbers are gain:feed.

Management Strategies

Cold Mitigation Strategies. One of the quickest methods of minimizing cold stress is to provide insulation or shelter for the animal. If bedding is used, the added residue contributes to added waste in the pens. In addition, if the bedding constitutes a fibrous feed source, cattle will sometimes consume the bedding instead of their normal high-energy diet, thereby reducing ME intake and performance. Nevertheless, a summary of two trials conducted in South Dakota (Birkelo and Lounsbery, 1992) and Colorado (Stanton and Schultz, 1996) found that providing approximately 1 kg of straw/animal daily as bedding during the feeding period improved gains approximately 7% and efficiency of gains more than 6% (Table 1). The benefits of bedding were not observed in the early part of these studies, but rather in the last 90 to 100 d of each study, which corresponds to the late-winter and early-spring feeding period. It is during this time that cattle in these studies were heavier, and the adverse effects of wetter conditions and mud would likely be most prevalent and difficult for cattle to cope with. In these studies, the economic benefit of providing bedding averaged $11/animal after taking into account bedding cost.

Additional feedlot studies (Mader et al., 1997a), involving both heat and cold challenges, have been conducted to evaluate year-round effects of shelterbelts or tree wind breaks provided for winter wind protection. A series of feeding trials were conducted during each season of a 3-yr period in which cattle were fed in outside lots with access to shelter or shelterbelts (tree windbreak north and northwest of pens) or outside lots with no access to shelter or a windbreak. Performance of yearling animals was not improved during the winter by providing wind protection, possibly because normal to slightly better than normal winter feeding conditions existed during the years that trials were conducted (Table 2). Also, providing wind protection or shelter resulted in decreased cattle gains in the summer. However, cattle fed in the unprotected area did have greater fat thickness in the winter and greater intramuscular fat in the winter and autumn than cattle fed in protected areas. In a follow-up study, performance of heavier steers fed during a 2- to 3-mo feeding period was severely impaired when protection was not provided in the winter (Table 3). Data from these studies indicate that benefits of feeding cattle in sheltered or protected areas in the winter can be offset by lower performance experienced by cattle fed in those same areas in the summer. However, as cattle approach slaughter weights, the benefits of providing protection from cold challenge are greatly increased. In addition, fat deposition was enhanced in cattle exposed to moderate cold stress and maintained by cattle exposed to more severe cold stress even though performance was reduced.

Other studies (Mader et al., 2001) were conducted at the University of Nebraska to evaluate the effect of diet energy level and/or energy level adjustments on finishing steer performance. In winter trials, two levels of alfalfa hay (7.5%, Low and 15%, High) along with two diet switch feeding regimens (7.5 to 15%, Low-High and 15 to 7.5% alfalfa hay, High-Low), with the switch occurring under cold stress conditions, were fed in two facilities (with and without wind protection). The common feedlot practice of switching from low- to high-roughage diets was not found to be beneficial. For cattle exposed to the greatest cold stress (fed in facilities without wind protection provided), the opposite was found in that switching from high to low roughage diets appeared to be the most beneficial. The extra ME from starch would appear to be more beneficial than the extra heat increment derived from fiber.

Proper feedlot pen layout and design are also crucial for minimizing effects of adverse climates. Mounds need to be built into feedlot pens, especially in the Northern Plains and Western Cornbelt of the United States, to minimize mud problems during wet periods and enhance airflow during hot periods. Proper design and strategic use of windbreaks is also warranted. Mader et al. (1997a) found that feedlot cattle do not necessarily need wind protection in moderate winters; however, if wind protection is provided, it is best to place it outside the pen to prevent excessive drifting of snow into the pens. Windbreaks will provide protection downwind to a distance of 5 to 10 times their height. Tree shelterbelts should be a minimum of 25 m from fence lines, whereas other forms of protection, mainly temporary, can be set closer. If a windbreak is located very near pens, it should have 10 to 20% open space to allow some air movement through the windbreak to prevent excessive drifting in front of the shelter, which adds to snow buildup and moisture in pens. If possible, having any wind protection near cattle in the summer should be avoided.

Table 2. Effect of facility and season on feedlot steers (3-yr summary)

	Facility[a]				
Variable	OS	SP	NP	SEM	Season mean
ADG, kg					
Winter	1.40	1.44	1.47	0.03	1.42 ± 0.02[b]
Spring	1.51	1.50	1.47	0.02	1.50 ± 0.02[c]
Summer	1.37[f]	1.34[f]	1.48[g]	0.04	1.40 ± 0.02[b]
Autumn	1.40	1.42	1.42	0.04	1.44 ± 0.02[bc]
Facility mean	1.43	1.43	1.46	0.02	
Daily DMI, kg					
Winter	9.68	9.50	9.77	0.10	9.68 ± 0.11[c]
Spring	9.02	8.88	8.94	0.10	8.97 ± 0.09[b]
Summer	10.16	10.00	10.38	0.15	10.15 ± 0.10[d]
Autumn	10.53	10.60	10.48	0.18	10.65 ± 0.11[e]
Facility mean	9.88	9.78	9.93	0.09	
DMI, percentage of BW					
Winter	2.22	2.17	2.22	0.02	2.21 ± 0.02[b]
Spring	2.21	2.18	2.20	0.02	2.19 ± 0.02[b]
Summer	2.20	2.14	2.21	0.03	2.18 ± 0.02[b]
Autumn	2.33	2.33	2.33	0.04	2.35 ± 0.02[c]
Facility mean	2.24	2.21	2.24	0.02	
Feed:gain					
Winter	6.97	6.66	6.77	0.13	6.90 ± 0.12[c]
Spring	5.99	5.93	6.10	0.09	6.01 ± 0.10[b]
Summer	7.43	7.52	7.04	0.19	7.32 ± 0.10[d]
Autumn	7.58	7.52	7.39	0.24	7.45 ± 0.12[d]
Facility mean	6.99	6.92	6.85	0.10	
Fat thickness, cm					
Winter	1.38[h]	1.37[h]	1.62[i]	0.06	1.43 ± 0.04[d]
Spring	1.40	1.44	1.50	0.04	1.45 ± 0.03[d]
Summer	1.09	1.03	1.09	0.05	1.07 ± 0.03[b]
Autumn	1.29	1.15	1.24	0.06	1.23 ± 0.04[c]
Facility mean	1.29[hi]	1.24[h]	1.35[i]	0.03	
Marbling score[j]					
Winter	5.54[h]	5.45[h]	5.82[i]	0.08	5.59 ± 0.07[d]
Spring	5.41	5.37	5.38	0.07	5.36 ± 0.05[b]
Summer	5.35	5.30	5.55	0.09	5.39 ± 0.06[bc]
Autumn	5.41[f]	5.41[f]	5.67[g]	0.09	5.54 ± 0.07[cd]
Facility mean	5.42[h]	5.38[h]	5.60[i]	0.05	
Quality grade[k]					
Winter	7.20[hi]	7.13[h]	7.30[i]	0.04	7.20 ± 0.03[c]
Spring	7.13	7.12	7.11	0.03	7.11 ± 0.02[b]
Summer	7.10[fg]	7.06[f]	7.18[g]	0.04	7.11 ± 0.02[b]
Autumn	7.08[h]	7.06[h]	7.24[i]	0.04	7.16 ± 0.03[bc]
Facility mean	7.13[h]	7.09[h]	7.21[i]	0.02	

[a]OP = overhead shelter enclosed on the north side; SP = shelterbelt to north and northwest; NP = no wind protection.
[b,c,d,e]Seasonal means within a column bearing different superscripts differ ($P < 0.05$).
[f,g]Facility means within a row bearing different superscripts differ ($P < 0.10$).
[h,i]Facility means within a row bearing different superscripts differ ($P < 0.05$).
[j]4.5 = average slight; 5.5 = average small.
[k]6.5 = average select; 7.5 = average choice.

Heat Mitigation Strategies. In restricted-feeding studies, Mader, et al. (1999b) housed feedlot steers under thermoneutral or hot environmental conditions. Steers were offered a 6% roughage finishing diet ad libitum (**HE)**, offered the same diet restricted to 85 to 90% of ad libitum DMI levels (**RE**), or offered a 28% roughage diet ad libitum (**HR**). Steers fed the HR diet tended to have lower respiratory rates and significantly lower body temperatures under hot conditions than HE- and RE-fed steers, whereas RE-fed steers had significantly lower body temperature than HE-fed steers (Figure 1). The lower body temperature of the HR- and RE-fed steers would indicate that ME intake prior to exposure to excessive heat load influences the ability of cattle to cope with the challenge of hot environments and that lowering ME intake can lower body temperature.

In regard to the use of restricted or managed feeding programs, Galyean (1999) provided an excellent review of concepts and research. Benefits of using restricted-feeding programs under hot conditions have been re-

Table 3. Effect of winter weather stress and harvest date on short-fed feedlot steers[a]

	Windbreak (WB)			No wind protection (NWB)			
Item	Heavy	Light	Mean	Heavy	Light	Mean	SE
Initial weight, kg[b]	475	432	454	480	441	460	2
Final weight, kg[cd]	533	542	537	515	528	521	5
Days on feed	51	86	69	51	86	69	
ADG, kg[cd]	1.12	1.28	1.20	0.69	1.01	0.85	0.07
Daily DMI, kg[b]	10.39	9.54	9.97	9.98	9.45	9.72	0.14
Feed:gain[cde]	9.30 (0.108)	7.44 (0.134)	8.37 (0.120)	14.76 (0.68)	9.41 (0.106)	12.09 (0.085)	0.73
Fat thickness, cm	0.97	0.97	0.97	0.94	0.99	0.97	0.04
Quality grade[bf]	7.02	7.31	7.16	7.08	7.25	7.17	0.03
Yield grade	2.3	2.2	2.3	2.3	2.3	2.3	0.1

[a]Harvest dates were January 28 and March 3 for the heavy and light groups, respectively. Windbreak and no wind protection = feedlot location; heavy, light, mean = weight group.
[b]Heavy vs. light group ($P < 0.10$).
[c]WB vs. NWB ($P < 0.10$).
[d]Determined from hot carcass weight divided by 0.62.
[e]Parenthetical numbers represent gain:feed.
[f]6.5 = average select; 7.5 = average choice.

ported by Mader et al. (2002). In addition, Reinhardt and Brandt (1994) found the use of restricted feeding programs to be particularly effective when cattle were fed in the late afternoon or evening vs. morning. Implementing a bunk management regimen, whereby bunks are kept empty for 4 to 6 h during the daytime hours could be used to minimize peak metabolic heat load occurring simultaneously to peak climatic heat load. Even though this forces the cattle to eat in the evening, it does not appear to increase night-time body temperature (Davis et al., 2002) provided bunks are kept empty a few hours prior to feeding. Although slight feed intake reductions can occur with bunk management programs, particularly when they are first implemented, benefits of both bunk management and restricted-feeding programs are observed for several days after cattle are moved to a normal feeding program (Figure 2).

In addition to altering feeding regimen, sprinkling can be effective in minimizing heat stress. Benefits of sprinkling tend to be enhanced if sprinkling is started in the morning, prior to cattle getting hot (Figure 3; Davis et al., 2002). These data also show significant benefits to sprinkling or wetting pen surfaces. Sprinkling of pen surfaces may be as much or more beneficial than sprinkling the cattle. Kelly et al. (1950) reported feedlot ground surface temperatures in excess of 65°C by 1400 in Southern California. Cooling the surface would appear to provide a heat sink for cattle to dissipate body heat, thus allowing cattle to better adapt to environmental conditions vs. adapting to being wetted. Wetting or sprinkling can have adverse effects, particularly when the cattle get acclimated to being wet and failed or incomplete sprinkling occurs during subsequent hot days (Davis, 2001).

Shade also has been found to be beneficial for feedlot cattle exposed to hot climatic conditions; however, in research conducted in northeast Nebraska (Mader et al., 1999a), positive benefits occurred only in the early portion of the feeding period and only in cattle with wind barriers provided. In this study, three summertime trials were conducted over consecutive years. Shaded and unshaded cattle were fed in pens with or without wind protection provided. Performance was similar for shaded and unshaded cattle fed in the facility without wind barriers provided; some benefit to shade was found in facilities that had wind barriers provided. In general, the response to shade occurred within the first 56 d of the feeding period, even though shade use tended to increase with time cattle were on feed. This suggests that cattle must adapt to shade or social order around and under shade before optimal shade use occurs. Although no heat-related cattle deaths occurred in this study (Mader et al., 1999a), these results suggest that shade improves performance in the summer when cattle are fed in facilities that restrict airflow and for cattle that have not become acclimated to hot conditions. Once cattle are acclimated or hot conditions subside, compensation by unshaded cattle offsets much of the benefits of providing shade.

Benefits of using shade would most likely be found in areas with greater temperature and/or solar radiation (Figure 4; Hahn et al., 2001). More consistent benefits of using shade would likely occur the further south cattle are in the United States. Mitlöhner et al. (2001) found excellent results to providing shade for cattle fed near Lubbock, TX. The overall economic benefit of using shade depends not only on location, but also on cost of structures and maintenance. Also, heat stress is dependent not only on temperature and solar radiation, but also on humidity and wind speed. Adjustments for humidity can be made by using the temperature-humidity index (NOAA, 1976; Hubbard et al., 1999), which has been adapted for use in the livestock safety index (Livestock Conservation Institute, 1970). Adjustments for solar radiation and wind speed have also been developed and need to be considered for when predicting heat stress (Davis, 2001).

In contrast to hot environment results, when cattle were exposed to cold conditions at or below thermoneutral levels, feeding higher-energy diets tended to enhance cattle performance when compared with feeding higher-roughage (lower energy) diets (Mader et al., 2001). An elevated metabolic rate is indicative of metabolic adaptation to cold stress. Thus, a need exists for greater ME intake in the winter to minimize cold stress, whereas in a hot environment, animals must dissipate metabolic heat when there is a reduced thermal gradi-

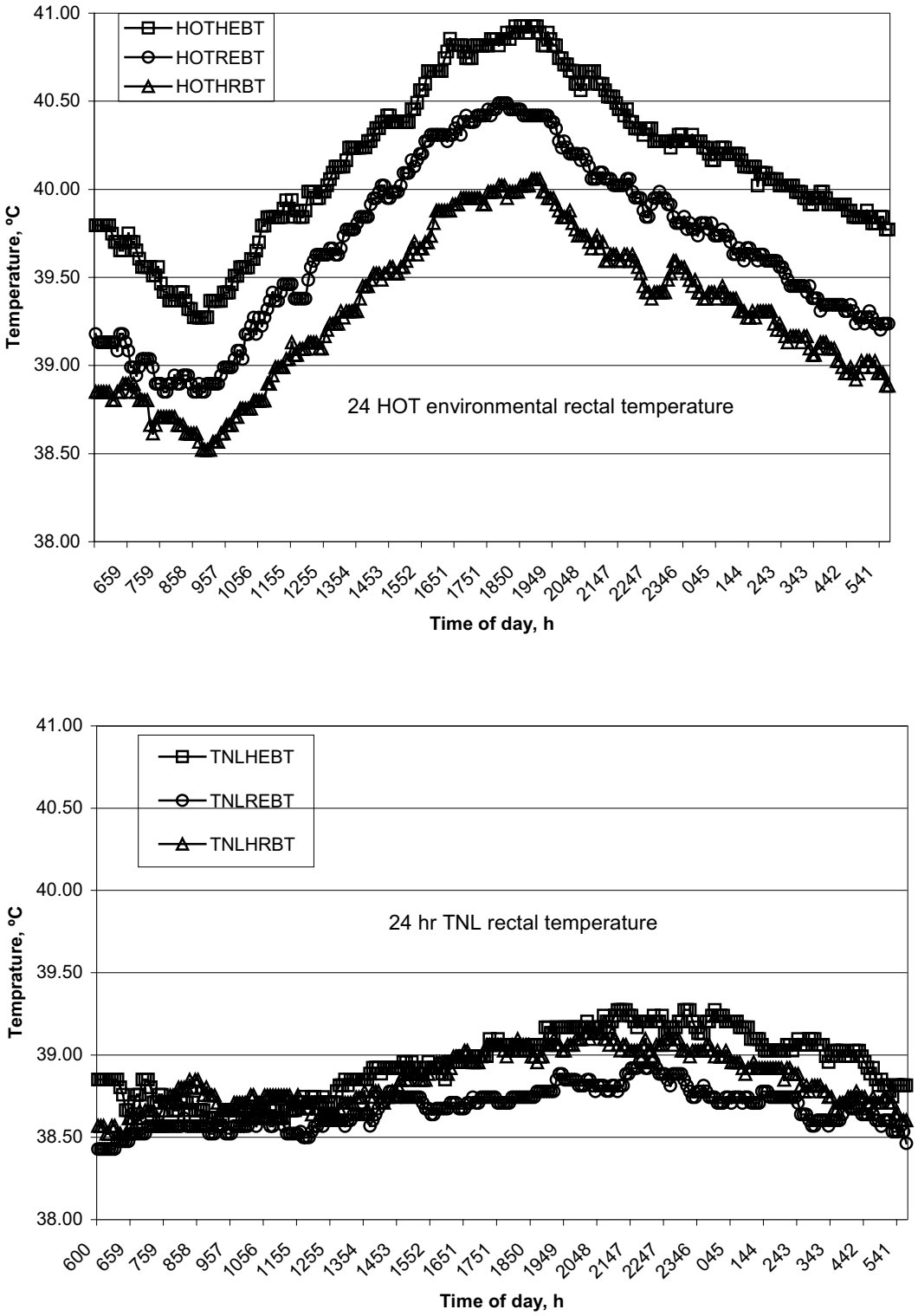

Figure 1. Body temperature (BT) of cattle exposed to thermoneutral (TNL) or hot (HOT) environments and fed a 6% roughage, high-energy diet ad libitum (HE) or restricted to 90% of ad libitum (RE) or fed ad libitum a 28% roughage diet (HR). Standard error = 0.1. Figure was derived from Mader et al. (1999b).

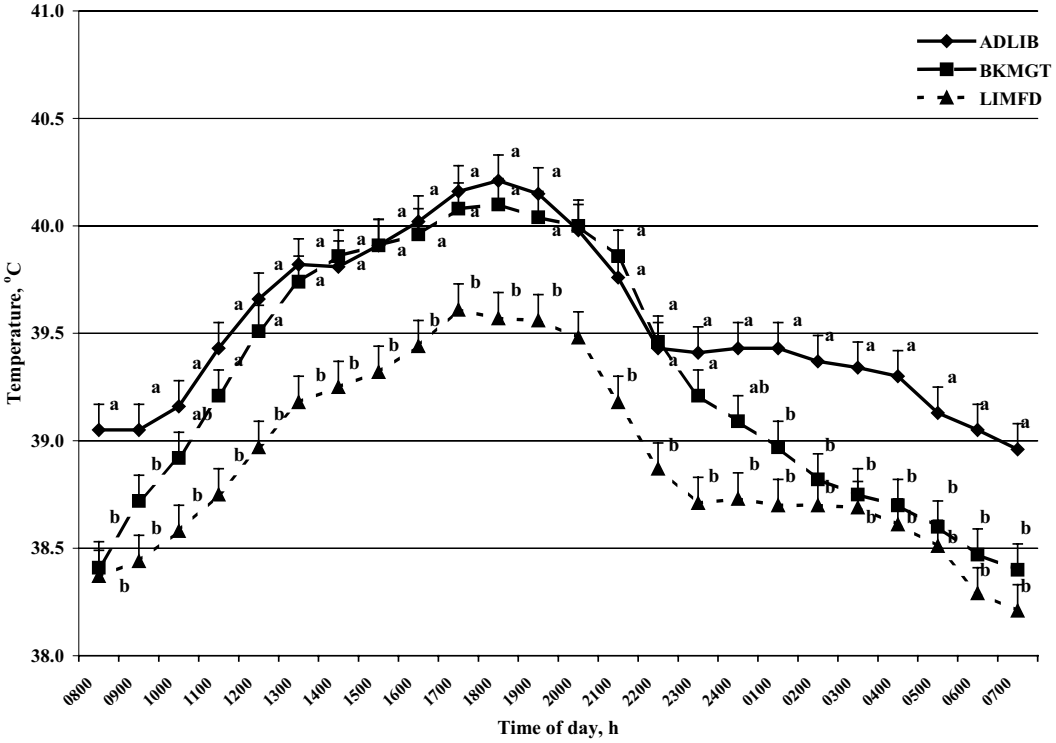

Figure 2. Carryover of previous nutritional regimen on tympanic temperature (TT) of steers during severe heat stress conditions (mean daily temperature-humidity index > 77). At the time these TT were obtained, all steers had been fed ad libitum. Treatments had been imposed for a 23-d period, which had ended approximately one week prior to taking TT. The treatments were as follows: ADLIB steers had been fed ad libitum at 0800; bunk management (BKMGT) steers had been fed at 1600 with bunks empty at 0800; and limit-fed (LIMFD) steers had been fed 85% of predicted ad libitum intake at 1600. [a,b]Means within a time with unlike superscripts differ ($P < 0.05$).

ent between the body core and the environment. The higher-producing animals, which consume more feed, thereby creating more metabolic heat, would appear to be more susceptible to heat stress. In addition, evaporation of moisture from the skin surface (sweating) or respiratory tract (panting) is the primary mechanism used by the animals to lose excess body heat in a hot environment. Under these conditions, waterer space available and water intake per animal becomes very important. During heat episodes, Mader et al. (1997b) found that as much as three times the normal waterer space (7.5 vs. 2.5 cm of linear space per animal) may be needed to allow for sufficient room for all animals to access and benefit from available water.

Intake and Net Energy for Maintenance Requirement Considerations

Although management strategies can be implemented to buffer the animal against adverse environmental conditions, the primary factors limiting the precision of predicting performance is our ability to predict DMI (Hicks, et al., 1990). Additionally, a key component of performance is our ability to predict NE_m requirements of cattle, particularly when they are exposed to adverse climatic conditions.

The effects of ambient temperature (**T**) on DMI, as described in the NRC (1996), are based on incremental change in T with adjustments ranging from a 16% increase for T between –15°C and –5°C to –35% for T > 35°C and no night cooling taking place. Although large variation exists among cattle relative to the effect of T on DMI, the general relationships can be determined. Separate hot- and cold-condition DMI curves can be defined from existing databases (Table 4). In addition, a polynomial equation can be determined that fits a full range of T (Table 4, Figure 5). However, the influence of no nighttime cooling on DMI is not completely accounted for in this equation. Frank et al. (2001) derived an algorithm that assumes the average effects of T on DMI at T > 24°C were in between those observed with and without night cooling. The percentage change in DMI was equal to

$$(1 - \{(T - 24) \times [0.01 + 0.0015 \times (T - 24)]\}) \times 100$$

At an average T of 40°C, this equation would predict DMI to be approximately 50% of normal in feedlot cattle, which is a very likely scenario; however, it may not be the case for all cattle in general.

To better account for effects of no nighttime cooling mentioned in NRC (1996), the negative effects of rela-

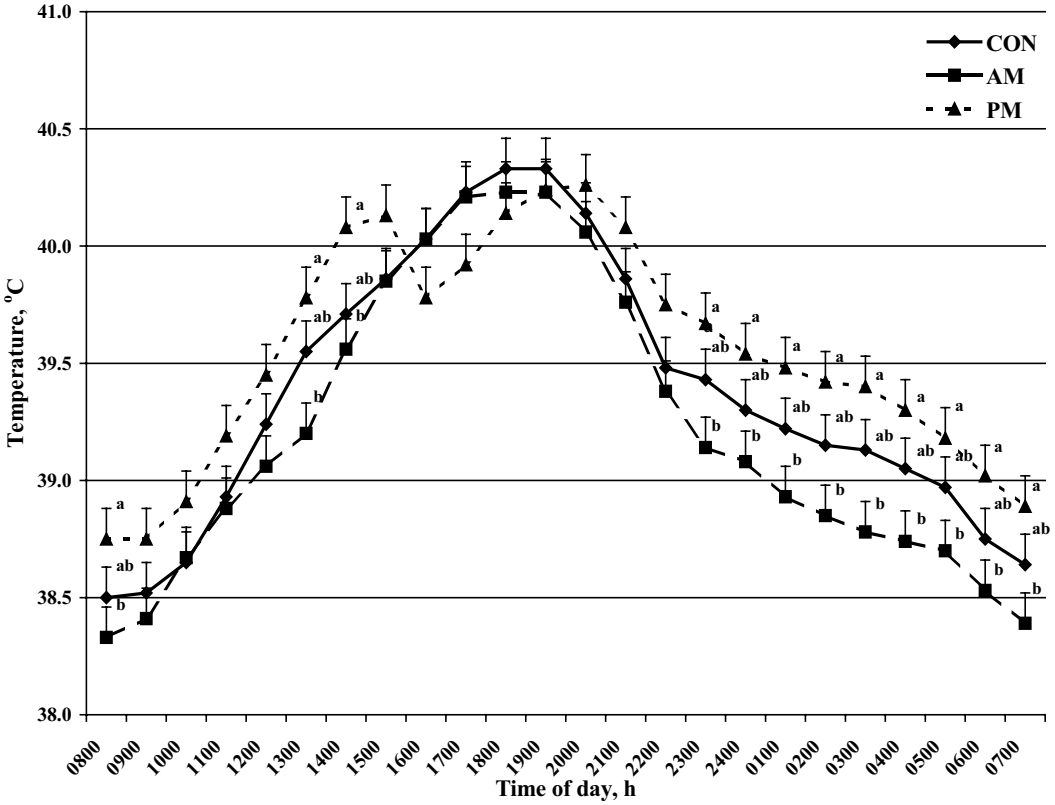

Figure 3. Tympanic temperatures of steers during severe heat stress (mean daily temperature-humidity index > 77). No water was applied to control (CON) mounds, versus mounds were sprinkled between 1000 and 1200 (AM) and 1400 and 1600 (PM), respectively. [a,b]Means within a time with unlike superscripts differ ($P < 0.05$).

tive humidity (**RH**) on the evaporative cooling process need to be considered. The ability of cattle to lose body heat (cool down) at night is dependent not only on T, but also on atmospheric moisture levels, or more specifically, RH, at night. Generally, RH is lower during daytime hours, but reach maxima when nighttime temperatures are typically the lowest, between 0400 and 0800 (Davis, 2001). Cattle feeding areas in the Southern Plains (AZ, NM, Western TX) often have high T during the day, but can cool more and quicker at night due to the low RH, whereas cattle fed in the Western Cornbelt can be subjected to more heat stress as a result of high RH even though actual average temperatures may be less than those found in the southern Plains.

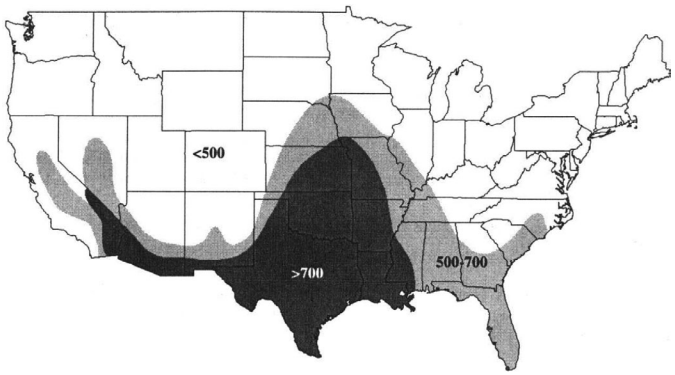

Figure 4. Areas of the mainland United States having selected categories of yearly hours above 85°F (Hahn et al., 2001). Areas >700 h would likely benefit the most from shade.

The temperature-humidity index (**THI**) was developed to adjust effects of T for RH. Under hot conditions, assuming thermoneutral conditions range between 15 and 25°C (NRC, 1996), a separate equation (Table 4) can be used to describe effects of THI on DMI. Using the THI equation more effectively accounts for the nighttime cooling effects on DMI. In addition, an increase in NE_m requirements is found in cattle exposed to hot conditions. The NE_m increase is largely dependent on the level and intensity of panting (NRC, 1981; 1996). However, NE_m requirements under hot conditions are also dependent on body condition. Cattle with greater body condition begin displaying signs of heat stress sooner than those with less body condition. By combining data reported in NRC (1981) and Davis (2001), an adjustment for body condition score can be incorporated into a NE_m requirement (Table 4), based on THI. In this analysis, it is assumed that the BCS of cattle in previously reported studies (NRC, 1981) averaged 5 (scale of 1 to 9).

In contrast to developed algorithms shown in Table 4 and Figure 5, DMI data shown in Table 2 indicates

Table 4. Regression of temperature (T), temperature humidity index (THI), and body condition score (BCS) on percentage change in feedlot cattle DMI and NE_m requirement[a]

Response variable and independent variable	Regression coefficient (SE)	R^2
DMI (T < 16°C)		
Intercept	5.1 (0.127)**	0.999
T	–0.15 (0.016)*	
T × T	–0.0019 (0.00077)	
T × T × T	–0.00054 (0.000048)*	
DMI (T > 24°C)		
Intercept	–33.0 (27.38)	0.997
T	3.65 (1.759)	
T × T	–0.0948 (0.0276)	
DMI (Full T Range)		0.998
Intercept	4.65 (0.233)**	
T	–0.080 (0.0187)**	
T × T	0.00551 (0.000835)**	
T × T × T	–0.00047 (0.000035)**	
T × T × T × T	–0.0000043 (0.0000006)**	
T × T × T × T × T	–0.000000059 (0.00000002)**	
DMI (THI > 70)		
Intercept	–229.74 (105.472)	0.997
THI	7.2125 (2.63341)	
THI × THI	–0.0561 (0.016341)	
NEm (THI > 65 and BCS > 5)		
Intercept	–64.94 (3.673)**	0.994
THI	0.905 (0.034)**	
BCS	1.21 (0.41)**	

[a]THI = (0.8 × T) + [(% relative humidity/100) × (T – 14.3)] + 46.4.
*$P < 0.10$.
**$P < 0.05$.

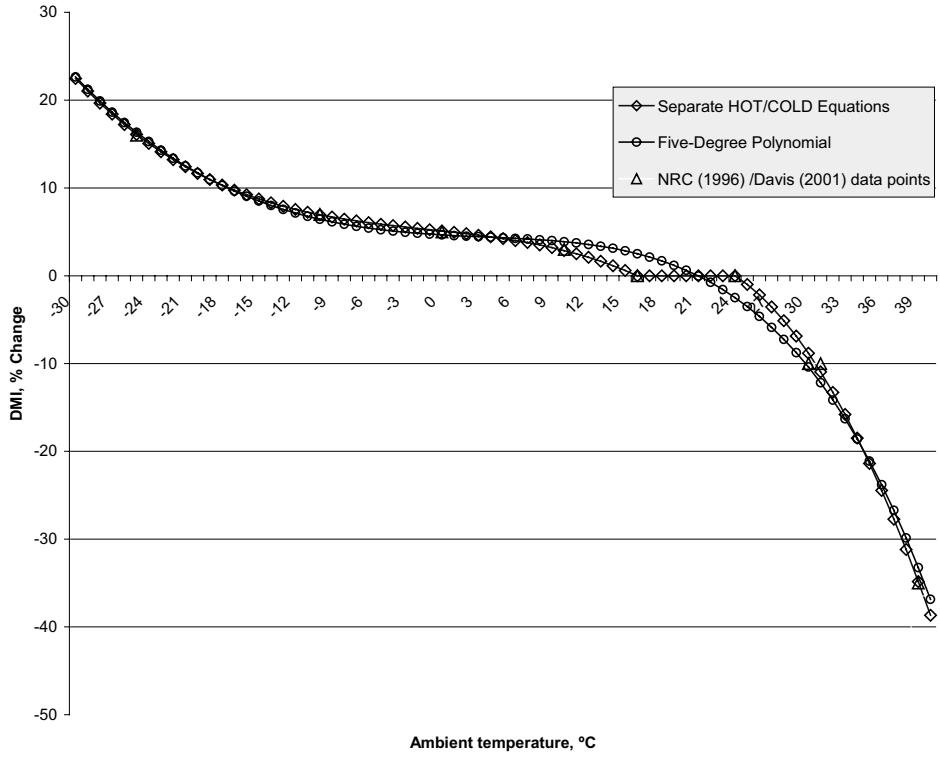

Figure 5. Graphical representation of the effects of temperature (T) on DMI, based on equations defining separate curves for 16°C > T > 24°C (HOT/COLD), a best fit 5° polynomial curve, and base value data points.

nonexistent differential intakes between summer- vs. winter-fed cattle. However, Kreikemeier and Mader (2002) reported over 20% greater DMI in winter vs. summer feedlot feeding studies. As indicated previously, large variation in DMI can exist in feedlot cattle. Seasonal patterns are likely dependent on normal vs. abnormal environmental conditions, as well as variations in these conditions. Short-term, sharp declines in DMI may be observed more often in the winter than in the summer due to the effects of winter storms that often accompany changing ambient temperatures (NRC, 1987). Lower DMI in the winter could be attributed to decreases in effective pen or bunk space due to pen conditions and/or negative social interactions among cattle. In addition, energy-dense diets provided to feedlot cattle and associated acidic end-products of fermentation are also factors limiting DMI (NRC, 1987). Increases in DMI brought on by cold stress, for instance, may be limited unless diet soluble starch content is reduced.

Implications

Beef cattle are traditionally managed outdoors with exposure to natural and variable environmental conditions. Cattle are particularly vulnerable not only to extreme environmental conditions, but also to rapid changes in these conditions. Management alternatives, such as the strategic use of wind protection and bedding in the winter or sprinklers and shade in the summer, need to be considered to help cattle cope with adverse conditions. In addition to these changes, manipulation of diet energy density and intake may also be beneficial for cattle challenged by environmental conditions. Algorithms designed to predict effects of environmental conditions on dry matter intake and maintenance energy requirements can be used with currently accepted prediction equations to better define and predict impact of the environment on beef cattle.

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A review of methods to synchronize estrus in replacement beef heifers and postpartum cows[1]

D. J. Patterson[2], F. N. Kojima, and M. F. Smith

Department of Animal Science, University of Missouri, Columbia 65211

ABSTRACT: This review considers methods currently available to control estrous cycles of postpartum beef cows and replacement beef heifers. Development of methods to control the estrous cycle of the cow has occurred in six distinct phases. The physiological basis for estrus synchronization followed the discovery that progesterone inhibited preovulatory follicular maturation and ovulation. Regulation of estrous cycles was believed to be associated with control of the corpus luteum, the life span and secretory activity of which are regulated by trophic and lytic mechanisms. Phase I (Progesterone Phase) included efforts to prolong the luteal phase of the estrous cycle or to establish an artificial luteal phase by administering exogenous progestins. Later, progestational agents were combined with estrogens or gonadotropins in Phase II (Progesterone-Estrogen Phase), whereas Phase III (PG Phase) involved prostaglandin $F_{2\alpha}$ (PG) and its analogs as luteolytic agents. Treatments that combined progestational agents with PG characterized Phase IV (Progestogen-PG Phase). Precise monitoring of ovarian follicles and corpora lutea over time by transrectal ultrasonography expanded our understanding of the bovine estrous cycle and particularly the change that occurs during a follicular wave. We now know (Phase V, GnRH-PG Phase) that precise control of estrous cycles requires the manipulation of both follicular waves and luteal lifespan. This review includes specific discussion of progestins, PG, GnRH, and various combinations of these hormones or their analogs used to more precisely control the interval and timing of estrus following treatment (Phase VI, Progestogen-GnRH-PG Phase). The review also addresses the potential benefits of these treatments in eliciting a response from peripubertal heifers and anestrous cows, and points to the flexibility in matching specific protocols with the particular beef management system involved. Recent advances in the development of methods of artificially inseminating beef cows and heifers at a fixed time with high fertility are discussed, which should potentially result in a dramatic increase in the adoption of AI in beef herds.

Key Words: Artificial Insemination, Beef Cattle, Estrous Cycle, Synchronization

 J. Anim. Sci. 81(E. Suppl. 2):E166–E177

Introduction

The percentage of beef cattle inseminated artificially is predicted to increase substantially with the advent of sexed semen (Seidel, 1998). Currently, however, surveys indicate that fewer than 5% of the beef cows in the United States are bred by AI, and only half of the cattlemen that practice AI use any form of estrus synchronization to facilitate their AI programs (Corah and Kiracofe, 1989; NAHMS, 1994). The inability to predict time of estrus for individual females in a group often makes it impractical to use AI because of the labor required for estrus detection (Britt, 1987). The development of methods of artificially inseminating beef cows and heifers at a fixed time with high fertility should result in a dramatic increase in the adoption of AI in beef herds.

Expanded use of AI and/or adoption of emerging reproductive technologies for beef cows and heifers requires precise methods of estrous cycle control. Effective control of the estrous cycle requires the synchronization of both luteal and follicular functions. Efforts to develop more effective estrus synchronization protocols have focused recently on synchronizing follicular waves by injecting GnRH, followed 7 d later by injection of PG (Ovsynch, CO-Synch, and Select Synch). A factor contributing to reduced synchronized pregnancy rates in beef cows treated with the preceding protocols is that 5 to 15% of estrous cycling cows show estrus on or before PG injection (Kojima et al., 2000). New protocols for inducing and synchronizing a fertile estrus in postpartum beef cows and replacement heifers in which the

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[2]Correspondence: S132 Animal Science Research Center (phone: 573-882-7519; fax: 573-882-4798; E-mail: pattersonD@missouri.edu).

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GnRH-PG protocol is preceded by either short- or long-term progestin treatment offer significant potential to enhance response to estrus synchronization, increase pregnancy rate to AI during the synchronized period, and facilitate insemination at a fixed time (Kojima et al., 2000; Wood et al., 2001; Perry et al., 2002).

Definitions, Protocols, and Terms

The following definitions, protocols, and terms referred to throughout this manuscript are defined below.

Protocols

PG: Prostaglandin $F_{2\alpha}$ (Lutalyse Sterile Solution, Pharmacia Animal Health, Kalamazoo, MI; Estrumate, Bayer Corp., Shawnee Mission, KS; ProstaMate, Phoenix Scientific, Inc., St. Joseph, MO; In Synch, Agri Laboratories, Ltd., St. Joseph, MO). MGA-PG: Melengestrol acetate (MGA; 0.5 mg/animal per day) is fed for a period of 14 d with PG administered 17 or 19 d after MGA withdrawal. GnRH-PG, Select Synch: Gonadotropin-releasing hormone injection (Cystorelin, Merial Ltd., Iselin, NJ; Factrel, Fort Dodge Animal Health, Overland Park, KS; Fertagyl, Intervet, Inc., Millsboro, DE) followed after 7 d with an injection of PG. MGA Select: MGA is fed for 14 d, GnRH is administered 10 or 12 d after MGA withdrawal, and PG is administered 7 d after GnRH. 7-11 Synch: MGA is fed for 7 d, PG is administered on the last day MGA is fed, GnRH is administered 4 d after the cessation of MGA, and a second injection of PG is administered 11 d after MGA withdrawal.

Terms

Estrous response: The number of females that exhibit estrus during a synchronized period. Synchronized period: The period of time during which estrus is expressed after treatment. Synchronized conception rate: The proportion of females that become pregnant of those exhibiting estrus and inseminated during the synchronized period. Synchronized pregnancy rate: The proportion of females that become pregnant of the total number treated.

Development of Methods to Synchronize Estrus

The development of methods to control the estrous cycle of the cow has occurred in six distinct phases. The physiological basis for estrus synchronization followed the discovery that progesterone inhibited ovulation (Ulberg et al., 1951) and preovulatory follicular maturation (Nellor and Cole, 1956; Hansel et al., 1961; Lamond, 1964). Regulation of estrous cycles was believed to be associated with control of the corpus luteum, the life span and secretory activity of which are regulated by trophic and lytic mechanisms (Thimonier et al., 1975). The Progesterone Phase included efforts to prolong the luteal phase of the estrous cycle or to establish an artificial luteal phase by administering exogenous progesterone. Later, progestational agents were combined with estrogens or gonadotropins in the Progesterone-Estrogen Phase. Prostaglandin $F_{2\alpha}$ and its analogs were reported in 1972 to be luteolytic in the bovine (Lauderdale, 1972; Liehr et al., 1972; Rowson et al., 1972; Lauderdale et al., 1974) and ushered in the PG Phase. Treatments that combined progestational agents with PG characterized the Progestogen-PG Phase. All of these protocols addressed control of the luteal phase of the estrous cycle since follicular waves were not recognized at the time.

Precise monitoring of ovarian follicles and corpora lutea over time by transrectal ultrasonography expanded our understanding of the bovine estrous cycle and particularly the change that occurs during a follicular wave (Fortune et al., 1988). Growth of follicles in cattle occurs in distinct wave-like patterns, with new follicular waves occurring approximately every 10 d (6 to 15 d range). We now know that precise control of estrous cycles requires the manipulation of both follicular waves and luteal lifespan (GnRH-PG Phase).

A single injection of GnRH to cows at random stages of their estrous cycles causes release of LH, which leads to synchronized ovulation or luteinization of most large, dominant follicles (≥10 mm; Garverick et al., 1980; Bao and Garverick, 1998; Sartori et al., 2001). Consequently, a new follicular wave is initiated in all cows within 2 to 3 d of GnRH administration. Luteal tissue that forms after GnRH administration is capable of undergoing PG-induced luteolysis 6 or 7 d later (Twagiramungu et al., 1995). The GnRH-PG protocol increased estrus synchronization rate in beef (Twagiramungu et al., 1992a,b) and dairy (Thatcher et al., 1993) cattle. A drawback of this method, however, is that approximately 5 to 15% of the cows are detected in estrus on or before the day of PG injection, thus reducing the proportion of females that are detected in estrus and inseminated during the synchronized period (Kojima et al., 2000). This information stimulated research in the Progestogen-GnRH-PG Phase.

Synchronization of Estrus and Ovulation with the GnRH-PG-GnRH Protocol

Administration of PG alone is commonly utilized to synchronize an ovulatory estrus in estrous cycling cows. However, this method is ineffective in anestrous females, and variation among animals in the stage of the follicular wave at the time of PG injection directly contributes to the variation in onset of estrus during the synchronized period (Macmillan and Henderson, 1984; Sirois and Fortune, 1988). Consequently, the GnRH-PG-GnRH protocol was developed to synchronize follicular waves and timing of ovulation. The GnRH-PG-GnRH protocol (Figure 1) for fixed-time AI results in the development of a preovulatory follicle that ovulates in response to a second GnRH-induced

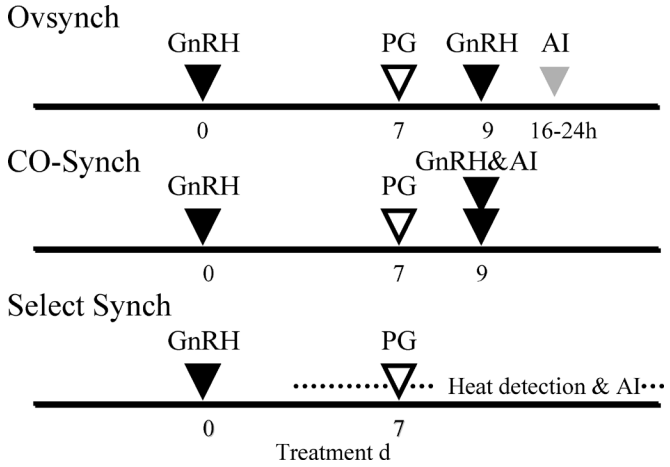

Figure 1. Methods currently being used to synchronize ovulation in postpartum beef cows: Ovsynch, CO-Synch, and Select Synch.

LH surge 48 h after PG injection (Ovsynch; Pursely et al., 1995). Ovsynch was validated recently as a reliable means of synchronizing ovulation for fixed-time AI in lactating dairy cows (Pursley et al., 1995; Burke et al., 1996; Schmitt et al., 1996; Pursley et al., 1997a,b). Time of ovulation with Ovsynch occurs between 24 to 32 h after the second GnRH injection and is synchronized in 87 to 100% of lactating dairy cows (Pursley et al., 1997a). Pregnancy rates among cows that were inseminated at a fixed time following Ovsynch ranged from 32 to 45% (Pursley et al., 1997b; 1998). The Ovsynch protocol, however, did not effectively synchronize estrus and ovulation in dairy heifers (35% pregnancy rate compared with 74% in PG controls; Pursley et al., 1997b).

Protocols for fixed-time insemination were recently tested in postpartum beef cows. Pregnancy rates for Ovsynch treated beef cows were compared with those of cows synchronized and inseminated at a fixed time following treatment with Syncro-Mate-B (Geary et al., 1998a). Calves in both treatment groups were removed from their dams for a period of 48 h beginning either at the time of implant removal (Syncro-Mate-B) or at the time PG was administered (Ovsynch). Pregnancy rates following fixed-time AI after Ovsynch (54%) were higher than those for Syncro-Mate-B-treated cows (42%). One should note that on the day following timed insemination, cows were exposed to fertile bulls of the same breed; no attempt was made to determine progeny paternity. Additionally, we do not know the incidence of short cycles among cows that were anestrous prior to treatment and that perhaps returned to estrus prematurely and became pregnant to natural service.

Recently, variations of the Ovsynch protocol (CO-Synch and Select Synch) were tested in postpartum beef cows (Figure 1). It is important to understand that treatment variations of Ovsynch currently being used in postpartum beef cows have not undergone the same validation process that Ovsynch underwent in lactating dairy cows. At this point, we do not know whether response in postpartum beef cows to the protocols outlined in Figure 1 is the same or different from lactating dairy cows due to potential differences in follicular wave patterns. Differences in specific response variables may include: a) the relative length of time to ovulation from the second GnRH injection; b) the anticipated range in timing of ovulation; and c) the degree of ovulation synchrony that occurs.

Two variations from Ovsynch being used most extensively in postpartum beef cows are currently referred to as CO-Synch and Select Synch. CO-Synch (Geary et al., 1998b) is similar to Ovsynch in that timing and sequence of injections are the same and all cows are inseminated at a fixed time. CO-Synch differs from Ovsynch, however, in that cows are inseminated when the second GnRH injection is administered, compared to the recommended 16 h after GnRH for Ovsynch treated cows. Select Synch (Geary et al., 2000) differs, too, in that cows do not receive the second injection of GnRH and are not inseminated at a fixed time. Cows synchronized with this protocol are inseminated 12 h after detected estrus. It is currently recommended that for Select Synch-treated cows, detection of estrus begin as early as 4 d after GnRH injection and continue through 6 d after PG (Kojima et al., 2000). Select Synch, similar to Ovsynch, was less effective than the MGA-PG protocol in synchronizing estrus in beef heifers (Stevenson et al., 1999).

Synchronization of Estrus with the MGA-PG Protocol

Melengestrol acetate, as a progestogen, was shown to be effective for estrus synchronization of beef cows and heifers (Zimbelman et al., 1970). Estrus synchronization programs designed for heifers and postpartum beef cows should be evaluated in relation to their effect on conception (Patterson et al., 1989; Folman et al., 1990). Until recently, there was little published evidence comparing methods of estrous cycle control that utilize PG alone to methods that utilize progesterone or progestogens in conjunction with PG. Feeding MGA for 14 d followed by PG injection 17 d after MGA feeding (MGA-PG protocol) is an effective method of estrous cycle control in heifers (Brown et al., 1988; Patterson and Corah, 1992). More recently, an increase in estrous response, synchronized conception and pregnancy rates, and fecundity in the postpartum cow was reported among cows treated with the MGA-PG protocol when compared with PG alone (Figure 2A and B; Patterson et al., 1995).

We know from work with both dairy (Britt et al., 1972) and beef cows (Zimbelman et al., 1970; Patterson et al., 1995) that the second synchronized estrus after MGA, whether spontaneous or induced with PG, may be inherently more fertile. Reported differences in conception rate for beef cows are shown in Figure 2B (Patterson et al., 1995). Treated cows in that study each received 0.5 mg of MGA or carrier without MGA for 14

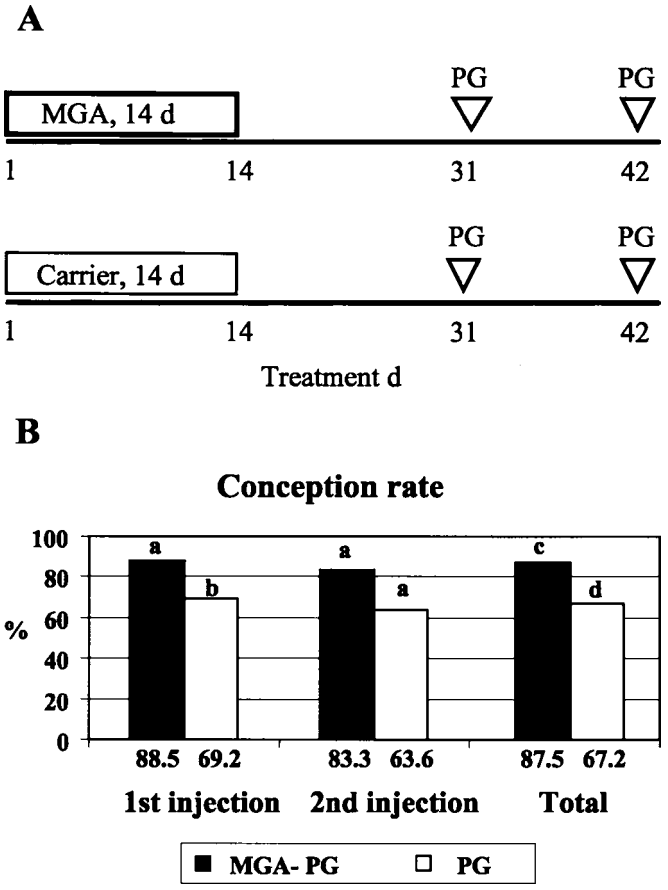

Figure 2. A) Melengestrol acteate (MGA)-treated cows each received 0.5 mg of MGA/d for 14 d or carrier only, with prostaglandin $F_{2\alpha}$ (PG) administered 17 d after MGA or carrier withdrawal. Cows that failed to exhibit estrus within 6 d after PG were reinjected with PG a second time 11 d later (Patterson et al., 1995). B) Synchronized conception rates of cows exhibiting estrus after each of two PG injections. Cows pretreated with MGA before PG experienced a 20% improvement in synchronized conception and pregnancy rate, as well as a 15% twinning rate (adapted from Patterson et al., 1995).

d. All cows received PG 17 d after the last feeding day of MGA or carrier without MGA. Control and treated cows that failed to exhibit estrus within 6 d after the first injection of PG were reinjected with PG 11 d later (Figure 2A). Many of the cows that failed to respond to PG on d 17 after withdrawal of MGA were cows that were anestrus prior to MGA treatment. Fralix et al. (1996) reported that up to 20% of anestrous cows experience short cycles prior to PG administered on d 17 after MGA withdrawal (Figure 3). To remedy this problem, unresponsive cows may be reinjected with PG 11 d later, or on d 42 from the beginning of treatment (Figure 2).

The administration of MGA at the recommended daily rate of 0.5 mg prevents the expression of behavioral estrus, blocks the preovulatory surge of LH, and ovulation (Zimbelman and Smith, 1966; Zimbelman et al., 1970; Imwalle et al., 2002). Until recently, however,

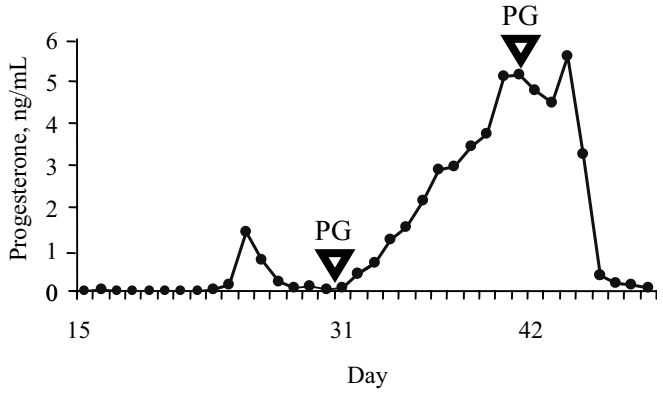

Figure 3. Progesterone profile depicting a short luteal phase subsequent to melengestrol actetate (MGA) withdrawal. This cow would not respond to prostaglandin $F_{2\alpha}$ (PG) administered 17 d after MGA withdrawal, but would be expected to exhibit estrus when reinjected with PG 11 d later (adapted from Fralix et al., 1996).

there was no evidence to suggest that MGA would induce cyclicity in peripubertal heifers (Patterson et al., 1990; Imwalle et al., 1998) or improve conception and increase ovulation rate in postpartum beef cows (Patterson et al., 1995; Fralix et al., 1996; Patterson et al., 1997).

The disadvantages of the MGA-PG system include: 1) anestrous cows that experience a short luteal phase after the period of MGA feeding, which in some cases necessitates a second PG injection (Fralix et al., 1996); 2) although twinning was not detected in an extensive study of MGA in beef cattle estrus synchronization (Zimbelman et al., 1970), the potential for an increased incidence of twinning (Patterson et al., 1995) was reported, which is undesirable in many beef production systems from a management viewpoint; 3) the overall length of the treatment period; and 4) the difficulty in some management situations of ensuring adequate intake of MGA on a daily basis.

Advantages of MGA for synchronization of estrus are ease of administration and cost. Furthermore, MGA recently received clearance from the FDA (Federal Register, 1997) for use in reproductive classes of beef cows and heifers and dairy heifers; therefore, research of methods for use in estrous cycle control involving MGA bear increased significance and marked relevance to current industry needs. These are important considerations for widespread use of any successful estrus synchronization treatment and are essential to expanded application of AI in beef cattle. The MGA-PG protocol avoids problems with reduced conception and offers advantages compared with untreated controls (Brown et al., 1988; Patterson and Corah, 1992).

Effect of the MGA-GnRH-PG Protocol on Estrus Synchronization

Twagiramungu et al. (1995) reported an increase in estrous response in postpartum beef cows over PG-

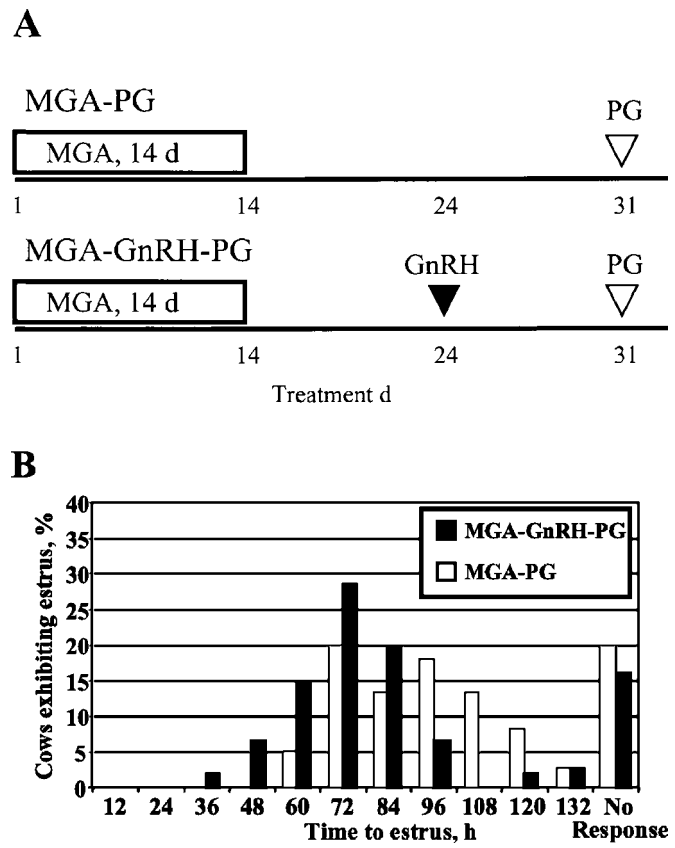

Figure 4. A) Cows were fed melengestrol acetate (MGA) for 14 d. Gonadotropin releasing hormone (GnRH) was administered to half the cows 10 d after MGA withdrawal, and all cows were injected with prostaglandin $F_{2\alpha}$ (PG) 7 d later (Patterson et al., 1999). B) Estrous response for MGA-PG- or MGA-GnRH-PG-treated cows (Patterson et al., 1999).

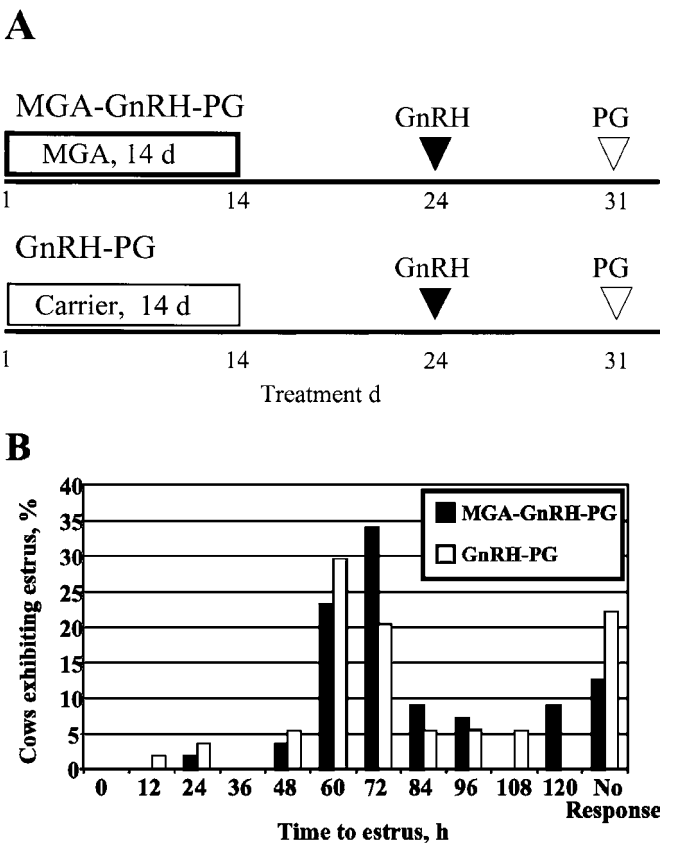

Figure 5. A) Cows were fed melengestrol acetate (MGA) or carrier without MGA for 14 d. Gonadotropin-releasing hormone (GnRH) was administered to all cows 10 d after MGA and carrier withdrawal, and all cows were injected with prostaglandin $F_{2\alpha}$ (PG) 7 d later (Patterson et al., 2000a). B) Estrous response for MGA-GnRH-PG- or GnRH-PG-treated cows (Patterson et al., 2000a).

treated controls when cows received an injection of GnRH 7 d before PG. Advantages of the GnRH-PG system include simplicity of administration and short duration of treatment. The major disadvantage of the GnRH-PG protocol is the percentage of cows that exhibit estrus after GnRH and before PG, which in some cases may be as high as 15% of the total number of cows treated (Kojima et al., 2000). In order to inseminate all cows that respond during the treatment period, estrus detection is required for a period of 10 d, beginning 4 d before and 6 d after PG administration.

There have been few studies designed to evaluate progestin treatment prior to administration of the GnRH-PG protocol. The addition of MGA to the GnRH-PG protocol was compared with the standard MGA-PG protocol in postpartum beef cows (Patterson et al., 1999). The design and results from that study are shown in Figures 4A and 4B. None of the cows in the MGA-GnRH-PG group exhibited estrus before PG. Synchrony of estrus was improved among MGA-GnRH-PG treated cows, with about 80% of the cows assigned to that treatment exhibiting estrus 48 to 96 h after PG (Figure 4B). Additionally, there was no difference between MGA-GnRH-PG and MGA-PG protocols in synchronized conception (78 and 83%, respectively) or pregnancy rate (65 and 67%, respectively) during the synchronized period.

The MGA-GnRH-PG protocol was also compared to the GnRH-PG protocol in postpartum beef cows. The design and results from that study are shown in Figures 5A and 5B (Patterson et al., 2000a). Synchrony of estrus was improved among MGA-GnRH-PG-treated cows compared to cows that did not receive MGA (GnRH-PG) and high pregnancy rates to AI were maintained (70 and 59%, respectively). The distribution of estrus among MGA-GnRH-PG-treated cows was similar to the distribution illustrated in Figure 4B, demonstrating the repeatability of response following this treatment.

Using MGA to Synchronize Estrus in Heifers

A Modified MGA Protocol for Heifers

Recent studies with heifers show that both synchrony of estrus and total estrous response improved when PG

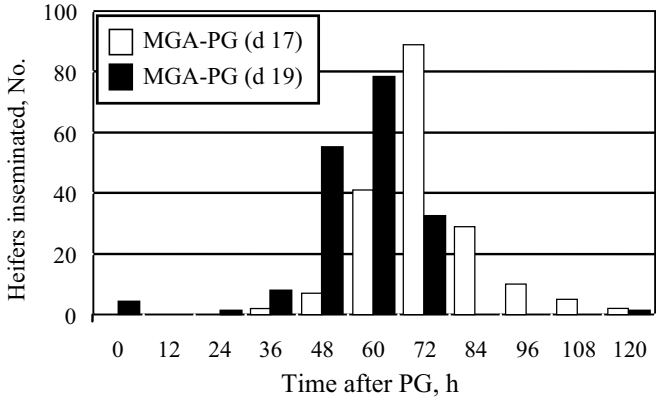

Figure 6. Insemination times for heifers synchronized with 14- to 17- or 14- to 19-d melengestrol acetate-prostaglandin $F_{2\alpha}$ protocols (adapted from Lamb et al., 2000).

was administered 19 d after MGA withdrawal compared with heifers that were injected on d 17 after MGA withdrawal (Figure 6; Nix et al., 1998; Deutscher, 2000; Lamb et al., 2000). No difference in fertility between treatments was reported.

We evaluated a modified MGA-PG protocol for inducing and synchronizing a fertile estrus in beef heifers (Figure 7; Wood et al., 2001). The first modification changed the day of PG injection from d 31 to d 33 of treatment. The second modification was the addition of a GnRH injection on d 26 of treatment. Wood et al. (2001) found that injection of GnRH on d 26 of the MGA-PG protocol induced luteal tissue formation and initiated a new follicular wave on approximately d 28 in cycling beef heifers (Figure 8B). The proportion of heifers with synchronized follicular waves on d 33 was increased significantly compared to heifers that did not receive GnRH (Figure 8A and B; Wood et al., 2001).

Wood (2000) also reported differences in estrous response and synchrony of estrus during the synchronized period among heifers assigned to the treatments illustrated in Figure 7. This difference in estrous response and degree of synchrony was based on the percentage of heifers that were pubertal at the time treatment with MGA began. Figure 9A and B illustrate these differences (Wood, 2000).

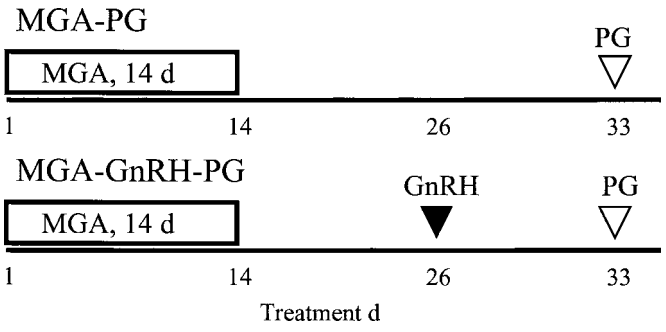

Figure 7. A modified long-term melengestrol acetate (MGA) protocol. Heifers were fed MGA for 14 d; 19 d after MGA withdrawal, prostaglandin $F_{2\alpha}$ (PG) was administered to all heifers. Half the heifers were administered GnRH 7 d before PG (adapted from Wood et al., 2001).

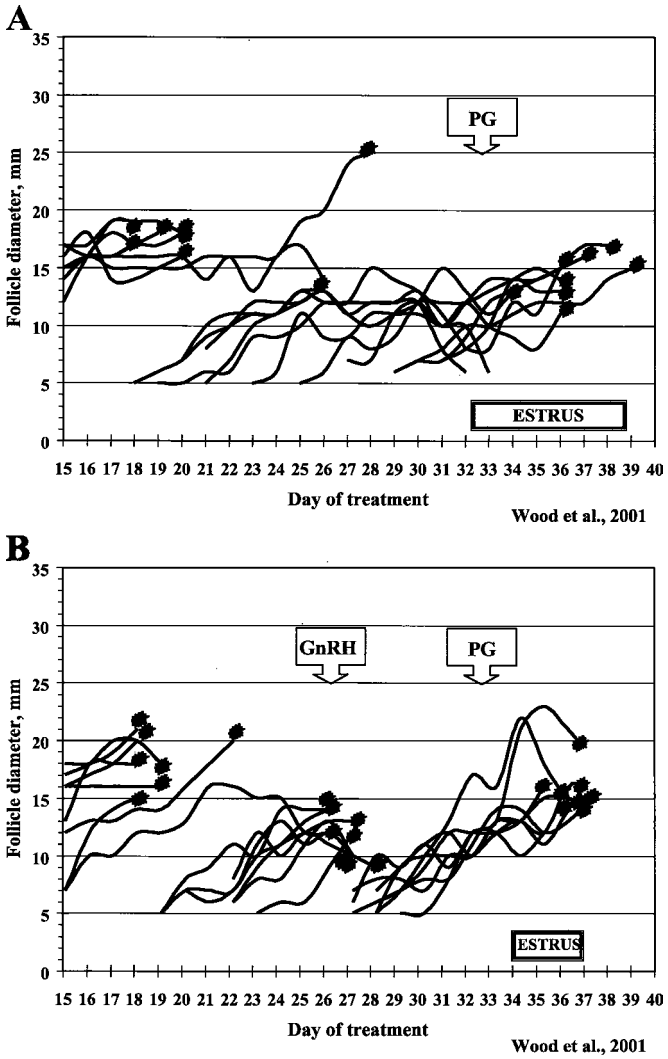

Figure 8. Patterns of dominant follicle development in (A) melengestrol acetate (MGA)-prostaglandin $F_{2\alpha}$ (PG)- and GnRH-treated (B; MGA-GnRH-PG) heifers. Administration of GnRH (B) caused the synchronized development of a dominant follicle before PG injection. Follicular development in MGA-PG-treated heifers (A) was poorly synchronized (adapted from Wood et al., 2001).

Figure 9A shows the distribution of estrus where only 30% of the heifers were pubertal at the time treatment with MGA began, whereas Figure 9B illustrates the distribution of estrus for heifers where 56% of the heifers were pubertal at the same time. The increased cyclicity of heifers shown in Figure 9B was associated with a reduced variance in the interval to estrus among MGA-GnRH-PG-treated heifers. AI pregnancy rates remained high for both MGA-GnRH-PG- and MGA-PG-treated heifers and were not different (67 and 60%,

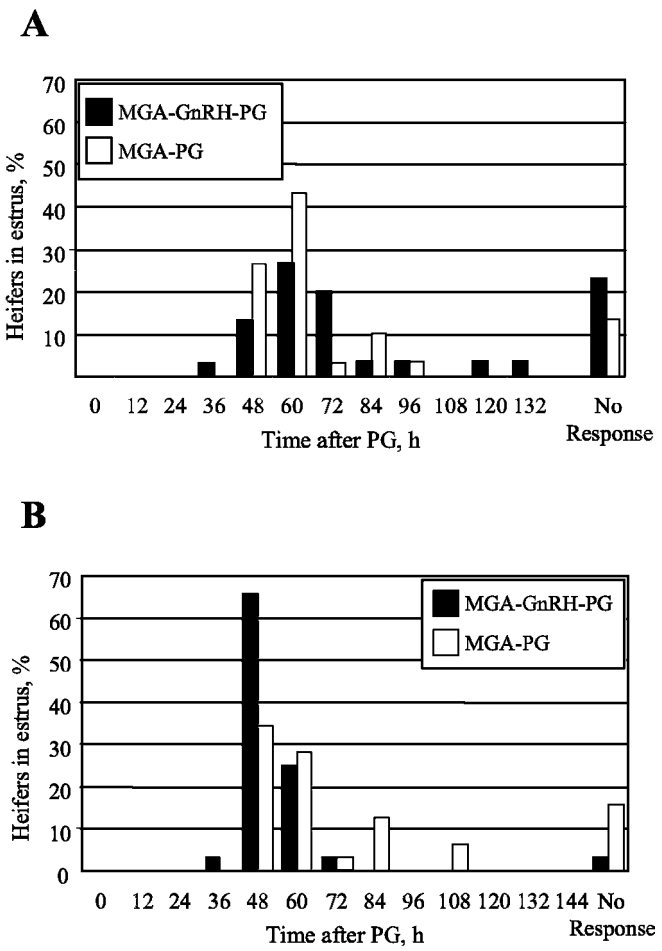

Figure 9. Percentage of heifers observed in estrus for melengestrol acetate (MGA)-prostaglandin $F_{2\alpha}$ (PG)- and MGA-GnRH-PG-treated heifers. Cyclicity rates were 30 and 56% for heifers at Location 1 (A) and 2 (B), respectively, at the time treatment with MGA began (adapted from Wood, 2000).

respectively [Location 1] and 75 and 72%, respectively [Location 2]).

Collectively, results from several studies indicate that the decision to add GnRH to a 14- to 19-d MGA-PG protocol for heifers should involve careful consideration of age, weight, and pubertal status of heifers at the time treatment with MGA-PG is initiated (Wood, 2000; Kojima et al., 2001). In situations where heifers are scheduled to begin an estrus synchronization treatment with MGA, we recommend that reproductive tract scores (**RTS**; Anderson et al., 1991; Patterson et al., 2000b) be performed within 2 wk prior to the initiation of treatment. We further recommend that heifers are ready to begin treatment with MGA if 50% of the heifers within a group are assigned RTS of 4 or 5 (Patterson et al., 2000b). This indicates that these heifers have reached puberty and are estrous cycling. Based on the age and weight of prepubertal or peripubertal contemporaries, up to 70% of these heifers can be expected to exhibit estrus and ovulate after MGA withdrawal, so

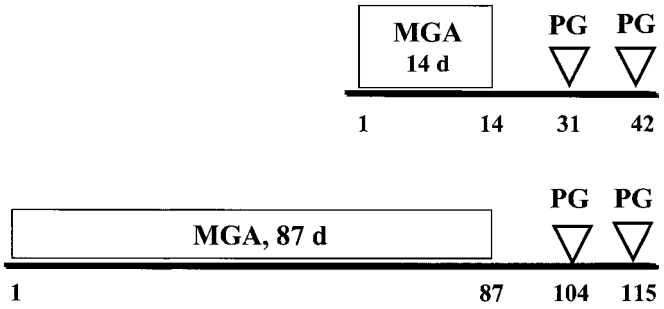

Figure 10. Treatment schedule for long-term and short-term feeding of melengestrol acetate (MGA; adapted from Patterson et al., 1993).

the potential estrous response during the synchronized period is up to 80%. Estrous response among heifers that were assigned scores of 2 or 3 was lower than for those assigned scores of 4 or 5. However, as RTS increased, estrous response improved (Patterson et al., 2000b; Funston et al., 2002).

Considerations Related to Long-Term Feeding of MGA to Heifers

Long-term feeding of MGA to beef heifers and associated effects on fertility may be a concern in specific production systems. It is not uncommon for heifers to be placed on MGA for extended periods of time and subsequently exposed for breeding after placement in backgrounding programs that necessitate long-term MGA administration. Zimbelman et al. (1970) reported no negative effect of either long-term or repeated intervals of feeding MGA to beef cows and heifers other than the expected reduced conception rate when cattle were bred at the synchronized estrus 3 to 7 d after the last day of MGA feeding. Patterson et al. (1993) designed a study (Figure 10) to compare estrous response and fertility during synchronized estrous periods among beef heifers that were fed MGA for 87 d (long-term, **LT**) or 14 d (short-term, **ST**) prior to PG. Heifers were stratified by age and weight to LT- or ST-MGA treatments (Table 1), and received 0.5 mg of MGA per animal, per day for 87 or 14 d. Heifers in each group were administered PG 17 d after MGA withdrawal. Heifers in both groups that failed to exhibit estrus within 6 d after the first injection of PG, were administered a second injection of PG 11 d after the first injection (Figure 10). Transrectal ultrasonography was used to examine

Table 1. Ages and weights of heifers at the time prostaglandin $F_{2\alpha}$ (PG) was administered[a]

Treatment	No. of heifers	Age, d	Weight, kg
Short-term, 14 d	31	427	393
Long-term, 87 d	30	423	387

[a]Adapted from Patterson et al. (1993).

Table 2. Estrous response and fertility of heifers treated long-term or short-term with melengestrol acetate (MGA)

	Short-term MGA, 14 d			Long-term MGA, 87 d		
Response variable	1st PG[a]	2nd PG[a]	Total	1st PG[a]	2nd PG[a]	Total
Estrous response	24/31 (77%[b])	4/7 (57%)	28/31 (90%)	16/30 (53%[c])	10/14 (71%)	26/30 (87%)
Synchronized conception	15/21 (63%)	3/4 (75%)	18/28 (64%)	12/16 (75%)	6/10 (60%)	18/26 (69%)
Synchronized pregnancy	—	—	18/31 (58%)	—	—	18/30 (60%)
Final pregnancy	—	—	28/31 (90%)	—	—	27/30 (90%)

[a]1st PG refers to animals that responded to prostaglandin $F_{2\alpha}$ (PG) administered 17 d after MGA withdrawal; 2nd PG refers to animals that failed to respond to the first injection of PG that were reinjected 11 d later.
[b,c]Percentages within a row and between treatments with different superscripts differ ($P < 0.05$; adapted from Patterson et al., 1993).

ovaries of all heifers at the end of treatment with MGA and at the time PG was administered. Heifers that failed to exhibit estrus after the first injection of PG were reexamined prior to the second PG injection. All heifers were exposed for natural-service for an additional 45 d after the AI period.

More ST-treated heifers exhibited estrus after the first injection of PG than LT-treated heifers (Table 2; $P < 0.05$). Total response after the two injections of PG, however, did not differ between treatments. Furthermore, there were no significant differences between treatments in synchronized conception and pregnancy rates, or pregnancy rates at the end of the breeding period (Table 2). A higher incidence of luteinized follicular cysts (Table 3) was observed among heifers in the LT-treatment compared with heifers in the ST-treatment (LT, 11/30 [37%]; ST, 0/31 [0%]). This observation may explain differences in estrous response between treatments following the first injection of PG.

These data indicate that long-term feeding of MGA may result in a higher than normal incidence of luteinized follicular cysts and an associated reduction in estrous response after PG. The data indicate, however, that reinjection with PG resulted in satisfactory breeding performance among heifers that were fed MGA for extended periods of time.

Using MGA to Synchronize Estrus in Postpartum Beef Cows

Development of the MGA Select Protocol for Postpartum Cows

Patterson et al. (2002) compared the 14- to 19-d MGA-PG protocol in postpartum suckled beef cows with or without the addition of GnRH on d 12 after MGA withdrawal and 7 d before PG as described by Wood et al. (2001; Figure 5). Table 4 provides a summary of the number of cows within age group by treatment, the mean number of days postpartum on the first day of MGA feeding, the BCS of cows on the day GnRH was administered, and the percentage of cows that were estrous cycling prior to initiation of MGA treatment. Cyclicity rates of cows at the onset of MGA feeding were lower among cows ≥5 yr of age than cows ≤4 yr of age. Mean intervals to estrus differed between treatments ($P < 0.06$), with longer intervals observed among cows assigned to the MGA-PG protocol. Mean intervals to estrus for MGA-GnRH-PG- and MGA-PG-treated cows were 74.4 ± 1.8 and 81.1 ± 2.3 h, respectively. Table 5 provides a summary of estrous response and the synchronized conception and pregnancy rates of cows assigned to the two treatments. Estrous response was higher ($P < 0.05$) among MGA-GnRH-PG-treated cows than MGA-PG-treated cows. These data demonstrate an improvement in estrous response among postpartum beef cows in which anestrous rates were high.

There was no difference between treatments in conception rate of cows during the synchronized period. Synchronized pregnancy rate (number of cows pregnant of the total number treated), however, was higher among cows ≥5 yr of age assigned to the MGA-GnRH-PG treatment compared with MGA-PG-treated cows of the same age. Final pregnancy rate of cows at the end of the breeding season was the same for cows assigned

Table 3. Ovarian morphology of heifers treated long-term or short-term with melengestrol acetate (MGA)

Treatment	Normal	Abnormal[a]
Short-term	31/31 (100%)	0/31 (0%)
Long-term	19/30 (63%)	11/30 (37%)

[a]Abnormal = presence of luteinized follicular cysts, 20 to 45 mm in diameter (adapted from Patterson et al., 1993).

Table 4. Number of cows, age, days postpartum, body condition score, and cyclicity status for cows in each treatment (mean ± SE)[a]

Treatment	Age, yr	No. of cows	Days postpartum[b]	BCS[c]	Cyclicity, %
MGA-PG	2, 3, 4	52	47 ± 2	5.2 ± .08	35
	≥5	48	40 ± 2	5.2 ± .09	15
MGA-GnRH-PG	2, 3, 4	53	47 ± 2	5.3 ± .10	38
	≥5	48	39 ± 2	5.3 ± .10	13

[a]Adapted from Patterson et al. (2002). MGA = melengestrol acetate; PG = prostaglandin $F_{2\alpha}$
[b]Means reflect days postpartum for cows in each treatment on the first day of MGA feeding.
[c]Body condition scores were assigned on the day GnRH was administered.

Table 5. Estrous response, synchronized conception and pregnancy rates for cows assigned to MGA-PG or MGA-GnRH-PG treatments[a]

Treatment	Age, yr	Estrous response no., %	Synchronized conception no., %	Synchronized pregnancy no., %
MGA-PG	2, 3, 4	44/52 (85)	36/44 (82)	36/52 (69)
	≥5	32/48 (67[b])	22/32 (69)	22/48 (46[b])
	Total	76/100 (76[b])	58/76 (76)	58/100 (58)
MGA-GnRH-PG	2, 3, 4	46/53 (87)	33/46 (72)	33/53 (62)
	≥5	42/48 (88[c])	34/42 (81)	34/48 (71[c])
	Total	88/101 (87[c])	67/88 (76)	67/101 (66)

[a]Adapted from Patterson et al. (2002). MGA = melengestrol acetate; PG = prostaglandin $F_{2\alpha}$.
[b,c]Values with different superscripts within columns differ ($P < 0.05$).

to the respective two treatments (97% for both MGA-GnRH-PG- and MGA-PG-treated cows).

Perry et al. (2002) conducted an experiment to determine whether pretreatment with MGA prior to a GnRH-PG-GnRH (control) protocol would improve pregnancy rates resulting from fixed-time AI. Cows were assigned by age and days postpartum to one of two treatments. Control and MGA-treated (Figure 11) cows were fed a supplement carrier with or without MGA for 14 d. Gonadotropin-releasing growth hormone was administered to all cows 12 d after MGA or carrier withdrawal and 7 d prior to PG. All cows were administered GnRH and artificially inseminated 72 h after PG. Pregnancy rates to fixed-time AI are reported in Table 6. There was no difference between treatments at location 1 (MGA = 58% [26/45]; Control = 51% [23/45]). However, there was a difference ($P < 0.03$) in pregnancy rate to fixed-time AI between treatments at location 2 (MGA = 63% [44/70]; Control = 45% [30/67]). Furthermore, when results from both locations were combined, the overall difference remained significant (MGA = 70/115 [61%]; Control = 53/112 [47%]; $P < 0.05$). These results indicate that pregnancy rates resulting from fixed-time insemination are improved significantly when treatment with MGA precedes the GnRH-PG-GnRH protocol. This approach to estrus synchronization that involves a 14-d feeding period of MGA followed 12 d (d 26) later by an injection of GnRH, and PG on d 19 after MGA withdrawal (d 33) was recently named MGA Select (Figure 12).

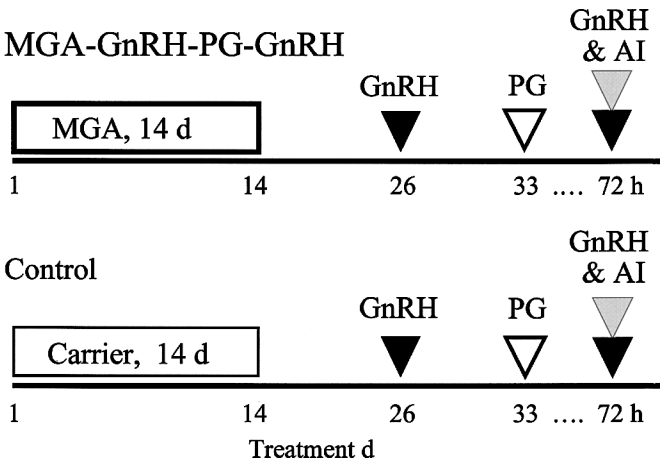

Figure 11. Treatment schedules and timing of fixed-time insemination for melengestrol acetate (MGA)-treated and Control (modified CO-Synch) cows (adapted from Perry et al., 2002).

Development of Methods to Shorten the Length of Treatment, 7-11 Synch

Kojima et al. (2000) developed an estrus synchronization protocol for beef cattle that was designed to shorten the feeding period of MGA without compromising fertility and to improve synchrony of estrus by synchronizing development and ovulation of follicles from the first wave of development (Figure 13A). This new treatment, 7-11 Synch, was compared with the GnRH-PG protocol. Synchrony of estrus during the 24-h peak response period (42 to 66 h) was significantly higher ($P < 0.01$) among 7-11 Synch treated cows. Furthermore, the distribution of estrus was reduced ($P < 0.05$) from 144 h for GnRH-PG-treated cows to 60 h for cows assigned to the 7-11 Synch treatment (Figure 13B; Kojima et al., 2000). The 7-11 Synch protocol resulted in a higher degree of estrus synchrony (91%) and greater AI pregnancy rate (68%) during a 24-h peak response period compared to the GnRH-PG protocol (69 and 47%, respectively).

The 7-11 Synch protocol has also shown significant potential for use in conjunction with fixed-time AI programs (Hixon et al., 2001; Kojima et al., 2002). These studies report high pregnancy rates resulting from fixed-time insemination of cows that were synchronized with the 7-11 Synch protocol, thereby eliminating the need to detect estrus. Further research is needed to more precisely determine the appropriate time of AI following treatment with 7-11 Synch and the necessity of administering GnRH at AI (Kojima et al., 2002).

Summary and Conclusions

Estrus synchronization and AI remain the most important and widely applicable reproductive biotechnolo-

Table 6. Fixed-time AI and final pregnancy rates of MGA-treated and Control cows[a]

Item	Location 1 no., %	Location 2 no., %	Total no., %
Pregnancy rate to fixed-time AI			
MGA-treated	26/45 (58%)	44/70 (63%[b])	70/115 (61%[b])
Control	23/45 (51%)	30/67 (45%[c])	53/112 (47%[c])
Final pregnancy rate			
MGA-treated	38/45 (84%)	64/70 (91%)	102/115 (89%)
Control	38/45 (84%)	59/67 (88%)	97/112 (87%)

[a]Adapted from Perry et al. (2002). MGA = melengestrol acetate.
[b,c]Percentages within a column and category with different superscripts differ ($P < 0.05$).

gies available for cattle (Seidel, 1995). Although hormone treatment of heifers and cows to group estrous periods has been a commercial reality now for over 30 yr, producers have been slow to adopt this management practice. Perhaps this is because of past failures, which resulted when females that were placed on estrus synchronization treatments failed to reach puberty or to resume normal estrous cycles following calving, and the reality that early estrus synchronization programs failed to manage follicular waves, resulting in more days in the synchronized period and precluded timed insemination with acceptable pregnancy rates. Patterson et al. (1999) proposed the general hypothesis that progestin treatment prior to the GnRH-PG estrus synchronization protocol would successfully: 1) induce ovulation in anestrous postpartum beef cows and peripubertal beef heifers; 2) reduce the incidence of a short luteal phase among anestrous cows induced to ovulate; 3) increase estrous response, synchronized conception and pregnancy rates; and 4) increase the likelihood of successful fixed-time insemination. New methods of synchronizing estrus in beef cattle outlined in this review present the opportunity to enhance results from AI and thereby reduce the period of time required to detect estrus or eliminate the need entirely. Further research is needed to more precisely determine the appropriate timing of fixed-time insemination following administration of these protocols and their extended application to the beef cattle industry.

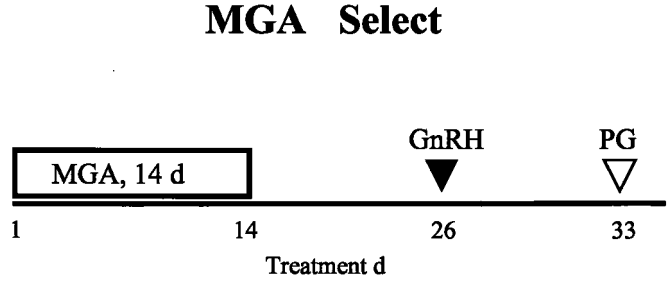

Figure 12. The MGA Select protocol. This treatment protocol involves a 14-d feeding period of melengestrol acetate (MGA), followed by the administration of GnRH on d 12 after MGA withdrawal and prostaglandin $F_{2\alpha}$ (PG) 7 d later.

Implications

New methods of inducing and synchronizing estrus for postpartum beef cows and replacement beef heifers in which the gonadotropin-releasing hormone-prostaglandin $F_{2\alpha}$ protocol is preceded by a progestin offer significant potential to more effectively synchronize estrus with resulting high fertility. These new protocols may provide significant opportunity to enhance results from fixed-time artificial insemination and offer the beef cattle industry the prospect of expanding the use of artificial insemination.

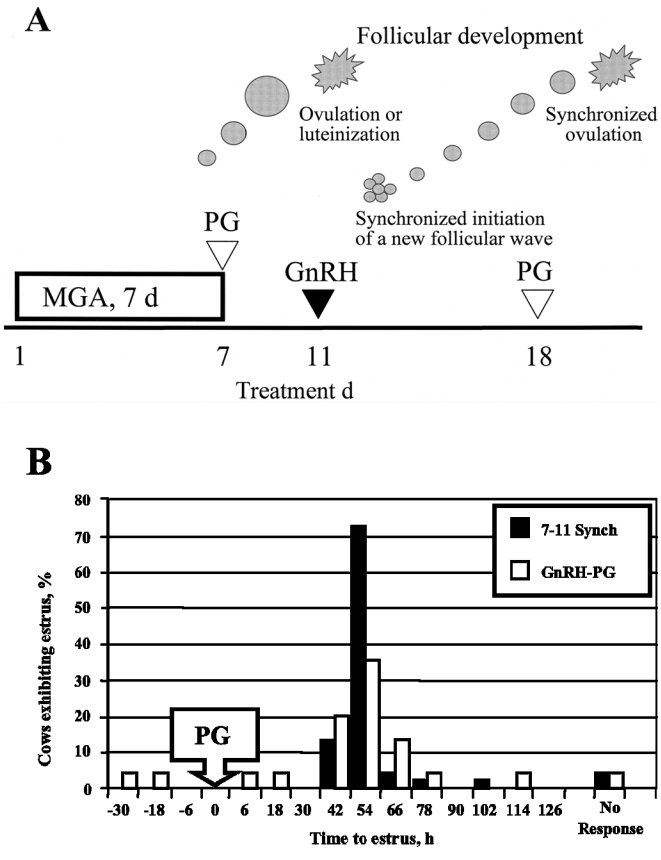

Figure 13. A) Illustration of the treatment schedule and events associated with the 7-11 Synch protocol (adapted from Kojima et al., 2000). B) Estrous response of cows treated with the 7-11 Synch or GnRH-prostaglandin $F_{2\alpha}$ (PG) protocols (adapted from Kojima et al., 2000).

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Bacterial direct-fed microbials in ruminant diets: Performance response and mode of action[1,2]

C. R. Krehbiel[3]*, S. R. Rust†, G. Zhang*, and S. E. Gilliland*

*Department of Animal Science, Oklahoma State University, Stillwater 74078 and
†Department of Animal Science, Michigan State University, East Lansing 48824

ABSTRACT: Direct-fed microbials (DFM) have been shown to increase daily gain and feed efficiency in feedlot cattle, enhance milk production in dairy cows, and improve health and performance of young calves. However, their effects on performance have been mixed, and the mode of action remains unclear. Bacteria used as DFM have been defined as single or mixed cultures of live organisms, which, when fed to animals, beneficially affect the host. The original concept of feeding DFM to man and livestock was based primarily on the potential for beneficial intestinal effects, including the establishment of a desirable gut microflora and/or prevention of the establishment of pathogenic organisms. More recently, however, there has been some indication that certain bacterial DFM might have beneficial effects in the rumen, such as decreasing the potential for ruminal acidosis. In several experiments, supplementing feedlot cattle with lactate-utilizing and/or lactate-producing bacteria has been shown to improve feed efficiency and daily gain (approximately 2.5%), with little change in DMI. In addition, increased milk yield (0.75 to 2.0 kg/d) has been reported in studies using dairy cows fed DFM, with little change in milk composition. Few attempts have been made to determine the mechanisms responsible for the beneficial effects of DFM, but the potential for a decrease in subacute acidosis has been evaluated. Responses to bacterial DFM have included a decrease in the area below subacute ruminal pH, increases in ruminal propionate concentrations, increased protozoal numbers, and changes in viable bacterial counts. Effects on some blood variables (lower CO_2 and LDH) also suggest a reduced risk of metabolic acidosis. Recent research has shown that DFM decreased fecal shedding of *Escherichia coli* O157:H7 from infected calves. Therefore, a possible application for DFM might be to reduce shedding of this pathogen from cattle. Overall, data indicate that DFM have the potential to decrease ruminal acidosis in feedlot cattle and dairy cows, and improve immune response in stressed calves. More research is needed to describe the mode of action, and thereby improve the efficiency of DFM use.

Key Words: Feed Additives, Probiotics, Ruminants, Rumen Fermentation

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Introduction

The term "probiotic" has been defined as "a live microbial feed supplement, which beneficially affects the host animal by improving its intestinal microbial balance" (Fuller, 1989) and has been used to describe viable microbial cultures, culture extracts, enzyme preparations, or various combinations of the above (Yoon and Stern, 1995). Therefore, the U.S. FDA has required feed manufacturers to use the term "direct-fed microbial" (**DFM**) instead of probiotic (Miles and Bootwalla, 1989) and has narrowed the definition to "a source of live, naturally occurring microorganisms" (Yoon and Stern, 1995). Microorganisms used as DFM for ruminants include viable cultures of fungi and bacteria.

Concern regarding the use of antibiotics and other growth stimulants in the animal feed industry has increased in recent years. There has been increasing emphasis placed on disease prevention as a means of reducing the use of antibiotics and also public concern about pathogens in meat and meat products. As result, interest in the effects of DFM on animal health and performance has increased. For ruminants, microbial cultures have been used to potentially replace or reduce the use of antibiotics in neonatal and stressed calves, to enhance milk production in dairy cows, and to im-

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[3]Correspondence: 208 Anim. Sci. Bldg. (phone: 405-744-8857; fax: 405-744-7390; E-mail: kclinto@okstate.edu).

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prove feed efficiency and daily gain in beef cattle. Most recently, cultures of *Lactobacillus acidophilus* have been shown to reduce fecal shedding of *Escherichia coli* O157:H7 by feedlot cattle. Although responses to DFM have been positive in many experiments, basic mechanisms are not well defined and are not clearly understood. Enhancing our understanding of the mode of action of DFM would improve our ability to select and apply appropriate DFM to ruminant diets. This review summarizes the literature pertaining to bacterial DFM and their influence on health and performance of ruminant animals. Moreover, information on underlying mechanisms is discussed.

History

Historical information pertaining to the use of bacterial DFM has been reviewed (Stern and Storrs, 1975; Newman and Jacques, 1995; Yoon and Stern, 1995). In his book, *The Prolongation of Life*, Metchnikoff (1908) first proposed that consuming lactobacilli capable of living in the intestinal tract was desirable (Yoon and Stern, 1995). He suggested that longevity of the Bulgarians was partly due to their consumption of a fermented milk product and that lactobacilli present in the fermented product prevented disease caused by enteropathogens. Metchnikoff's (1908) postulation led to several studies on the efficacy of the *Lactobacillus* species during the 1920's (Stern and Storrs, 1975). Stern and Stoors (1975) reported that the early popularity of *L. acidophilus* therapy in the United States reached its peak by about the mid-1930s, and then faded. Following World War II, antibiotics came into use and were often so efficient that they destroyed all the intestinal bacteria (Mannheim, 1951). The net effect was an increase in the incidence of "antibiotic diarrhea" and related side effects, and interest in acidophilus therapy for restoration of normal intestinal microorganisms began to be renewed. Since then (mid-1950s), there has been a slow but steady increase in the study of bacterial DFM for humans and animals. However, production responses of growing and lactating ruminants and interest in the corresponding mode of action of bacterial DFM have not occurred until more recently (Yoon and Stern, 1995).

Bacterial Direct-Fed Microbials in Dairy Production

Preruminant Calves. In terms of ruminant production systems, the efficacy of bacterial DFM has been studied most extensively in the neonatal dairy calf. Bacterial DFM, such as species of *Lactobacillus*, *Enterococcus*, *Streptococcus*, and *Bifidobacterium*, have been studied in young calves, and the data have been reviewed (Newman and Jacques, 1995). In general, the importance of bacterial DFM (primarily *Lactobacillus* species) fed to young and/or stressed calves has been to establish and maintain "normal" intestinal microorganisms rather than as a production (i.e., gain and efficiency) stimulant. For dairy calves, rapid adaptation to solid feed by accelerating the establishment of ruminal and intestinal microorganisms and avoiding the establishment of enteropathogens, which often results in diarrhea, is the primary goal. In the neonate and in stressed calves, the microbial population is in transition and extremely sensitive; abrupt changes in diet or the environment can cause alterations in microbial populations in the gastrointestinal tract (**GIT**; Savage, 1977). For example, Tannock (1983) reported that stress often leads to an increased incidence of diarrhea in neonates, which is associated with decreases in the population of *Lactobacillus* in the gut. Moreover, Sandine (1979) reported that fecal counts of lactobacilli normally are higher than coliforms in healthy animals and reversed in those suffering from diarrhea.

Feeding calves viable cultures of species of *Lactobacillus* and *Streptococcus* has been reported to decrease the incidence of diarrhea (Bechman et al., 1977; Maeng et al., 1987; Fox, 1988). In a more recent experiment by Abu-Tarboush et al. (1996), calves fed *L. acidophilus* 27SC had a significantly lower scour index during wk 5, 7, and 8 compared with calves fed the control diet, which confirmed the beneficial effect of lactobacilli in reducing the incidence of diarrhea in dairy calves suggested by earlier research. The decreased incidence of diarrhea might be associated with a consistently increased shedding of *Lactobacillus* (Gilliland et al., 1980; Jenny et al., 1991; Abu-Tarboush et al., 1996) and an inconsistent decreased shedding of coliforms (Bruce et al., 1979) in feces in response to supplements of *Lactobacillus*. Previous researchers (Ellinger et al., 1980; Gilliland et al., 1980; Abu-Tarboush et al., 1996) have suggested that animals experiencing normal stools are less likely to be shedding coliforms in feces. Fecal shedding of coliforms has generally not increased when calves were not experiencing diarrhea (Ellinger et al., 1980; Gilliland et al., 1980; Abu-Tarboush et al., 1996), and authors have suggested that this could be related to the fact that animals were not experiencing intestinal disorders (e.g., diarrhea). Interestingly, in experiments where there has been no advantage to feeding bacterial DFM (Morrill et al., 1977; Jenny et al., 1991), calves were generally experiencing no health problems.

Rapid adaptation to solid feed by neonatal calves also depends on the development of the ruminal epithelium and ruminal capacity. In one experiment (Nakanishi et al., 1993), lactic acid bacteria added to starter diets were suggested to affect ruminal function in the young animal. Holstein calves supplemented with yogurt containing *L. acidophilus* tended to ruminate more at 30 d than untreated calves, indicating that *L. acidophilus* may promote ruminal development. There were no performance benefits associated with the treated calves in this experiment and any possible microbial changes were not determined (Nakanishi et al., 1993).

Performance results for neonatal calves consuming bacterial DFM have been variable. Morrill et al. (1977), Ellinger et al. (1978), and Abu-Tarboush (1996) reported no improvement in daily gain as a result of feed-

ing lactobacilli. In contrast, Bechman et al. (1977) reported improved (17%) rates of gain when 2.5×10^{11} cfu/d of *L. acidophilus* species was added to milk or milk replacer. Feed efficiency is generally not altered by feeding DFM to young calves (Jenny et al., 1991; Abu-Tarboush et al., 1996). Beeman (1985) used 52 Holstein male calves that had a history of diarrhea and antibiotic therapy to evaluate the effects of feeding a culture of *Lactobacillus* on weight gain of calves convalescing from neonatal diarrhea. All animals were treated with antibiotics for 3 d before the study was initiated. At the 2-wk evaluation, calves treated with lactobacilli gained an average of 8.0 kg, whereas control calves gained an average of 3.5 kg. By d 56 of the experiment, average BW gains were 47.3 and 37.8 kg for treated and control groups, respectively. These benefits were hypothesized to result from improvement of intestinal conditions because of lower fecal scores (i.e., less scouring) in calves fed DFM.

Performance response is likely not important early in the preruminant's life when enteric disease is most prevalent. Improved health and reduction in the incidence or severity of diarrhea, though difficult to measure for statistical analysis, is most likely a more important response. As suggested by Newman and Jacques (1995), more experiments that include detailed information about the microbial supplement, and fecal culture data from scouring experimental animals are needed to determine the usefulness of microbial supplements in neonatal calves.

Milk Yield and Composition in Dairy Cows. Limited research has evaluated the efficacy of bacterial DFM for lactating dairy cows. Table 1 summarizes five experiments in which bacterial DFM, or combinations of bacterial and fungal DFM, have been fed to lactating cows. It should be cautioned that all experiments were published as abstracts, and therefore information was limited and not peer reviewed. In general, increased milk yield has been a consistent response, whereas changes in milk composition have been variable. Jaquette et al. (1988) and Ware et al. (1988a) reported that milk yield was 1.8 kg/d greater for cows fed a diet containing 2.0×10^9 cfu of *L. acidophilus* (BT1386) per day compared with those fed a control diet. Dry matter intake and milk fat and milk protein percentage were not affected by *L. acidophilus* (Table 1). In a more recent experiment, Gomez-Basauri et al. (2001) evaluated the effect of a supplement containing *L. acidophilus*, *L. casei*, *Enterococcus* (*Streptococcus*) *faecium* (total lactic bacteria = 10^9 cfu/g) and mannanoligosaccharide on DMI, milk yield, and milk component concentration. Cows fed lactic acid bacteria and mannanoligosaccharide consumed 0.42 kg less DM and produced 0.73 kg/d more milk. The authors reported that milk yields increased over time for DFM- and mannanoligosaccharide-fed cows, whereas control cows maintained constant milk yields.

Other experiments have been conducted with combinations of fungal cultures and lactic-acid bacteria (Ko-

Table 1. Effects of bacterial direct-fed microbials on dry matter intake, milk yield, and composition in lactating dairy cows

Treatment	n	DMI, kg/d	Milk			Diet	Reference
			Yield, kg/d	Fat, %	Protein, %		
Control	16	—	29.1[a]	3.81	3.34	Corn silage, alfalfa, pelleted grain	Jaquette et al. (1988)
L. acidophilus (BT1386)	16	—	30.9[b]	3.75	3.36		
Control	550	21.2	31.8[a]	3.64	—	Alfalfa hay, silage, whole cottonseed, grain concentrate, protein	Ware et al. (1988)
L. acidophilus (BT1386), 2×10^9 cfu/d	550	21.4	33.6[b]	3.63	—		
Control	6	—	8.20[a]	3.30[a]	3.09	Tropical feeding conditions	Komari et al. (1999)
S. cerevisae (yeast culture)	6	—	9.34[b]	3.96[b]	3.15		
S. cerevisae and *L. acidophilus*	6	—	9.28[b]	3.57[b]	3.13		
Control	32	24.6	48.2[c]	—	3.01[a]	—	Block et al. (2000)
5×10^9 cfu of yeast plus 5×10^9 cfu of *L. plantarum/E. faecium*	32	25.1	49.1[d]	—	3.27[b]		
Control	100	25.0[a]	38.8[c]	4.24[c]	3.02	Corn silage, alfalfa/grass hay, crop silage, whey, commercial feed blend	Gomez-Basauri et al. (2001)
L. acidophilus, *L. casei*, *E. faecium* (10^9 cfu/g) and mannanoligosccharide	100	24.6[b]	39.6[d]	4.34[d]	3.04		

[ab]Means in a column with different superscripts differ ($P < 0.05$).
[cd]Means in a column with different superscripts differ ($P < 0.10$).

mari et al., 1999; Block et al., 2000). Milk yields were increased by 1.08 and 0.90 kg/d, respectively, when lactating cows were fed *S. cerevisae* in combination with *L. acidophilus* or 5×10^9 cfu of yeast in combination with 5×10^9 cfu of *L. plantarum*/*E. faecium*.

In contrast to feeding bacterial DFM directly, Colenbrander et al. (1988) found that treatment of alfalfa silage with *L. acidophilus* did not improve DMI, milk yield, or milk composition in dairy cows, but efficiency (kg of fat-corrected milk/kg of feed) of milk production was improved by 7.1%. The *L. acidophilus* may not have survived in the silage; thus few, if any, viable cells would have been consumed.

These studies suggest that bacterial DFM fed alone or in combination with fungal cultures might be efficacious for increasing milk production by lactating dairy cows. However, studies have been minimal, and more research is needed before recommendations to dairy producers should be made.

Bacterial Direct-Fed Microbials in Beef Production

Stressed Calves. Newly received beef calves entering the feedlot undergo a variety of stresses, such as recent weaning, transport, fasting, assembly, vaccination, castration, and dehorning. Such stresses can alter microorganisms in the rumen and lower gut (Williams and Mahoney, 1984), resulting in decreased performance and increased morbidity and death loss. Administration of bacterial DFM to repopulate the gut might reduce these changes in the microbial population. In the early to mid-1980s, several research trials (Crawford et al., 1980; Hutcheson et al., 1980; Kiesling and Lofgreen, 1981; Davis, 1982; Kiesling et al., 1982; Hicks et al., 1986) were conducted at different locations to evaluate the efficacy of a combination bacterial DFM containing live cultures of *L. acidophilus*, *L. plantarum*, *L. casei*, and *S. faecium*. Averaged across all trials, feeding the DFM at processing, throughout the receiving period (average = 30 d), or both resulted in a 13.2% increase in daily gain, 2.5% increase in feed consumption, and a 6.3% improvement in feed:gain (Fox, 1988). The greatest performance response to the bacterial DFM generally occurred within the first 14 d of the receiving period (Crawford et al., 1980; Hutcheson et al., 1980). Morbidity was reduced by 27.7% in cattle receiving the bacterial DFM compared with control cattle. However, it should be pointed out that morbidity was generally low. Similarly, Gill et al. (1987) fed a bacterial DFM during a 28-d receiving period and reported a 9.3% increase in daily gain, 9.5% improvement in feed efficiency, and a 10.9% reduction in morbidity. In contrast, other research has shown no performance response to feeding bacterial DFM to newly weaned (Dew and Thomas, 1981; Kercher et al., 1985; 1986) or newly received (Kiesling and Lofgreen, 1981; Krehbiel et al., 2001) feedlot calves.

In a more recent experiment (Krehbiel et al., 2001), 466 newly received calves from southern Oklahoma and northern Texas auction barns were received and used to study the effects of administering 5×10^9 cfu lactic acid-producing bacteria (*E. faecium*, *L. acidophilus*, *Bifidobacterium thermophilum*, and *B. longum*) on health and performance. Daily gain did not differ among calves receiving DFM vs. no DFM. However, calves treated with DFM during their first antimicrobial treatment were less likely to be treated a second time within 96 h. In addition, the number of calves treated twice tended to be lower for calves administered DFM compared with calves not receiving DFM. These data suggested that DFM might improve recovery of morbid newly received feedlot calves.

Dose titration studies for bacterial DFM fed to newly received calves are limited and more are needed. Orr et al. (1988) showed a quadratic relationship for daily gain when lightweight (185 kg) steer calves were fed 0, 2.2×10^6, 2.2×10^8, or 2.2×10^{10} cfu of *L. acidophilus* daily. Daily gain was significantly greater for calves fed 2.2×10^6 or 2.2×10^8 cfu of *L. acidophilus* than when control or 2.2×10^{10} cfu was fed. Feed intake and feed efficiency did not differ among treatments (Orr et al., 1988). In contrast, Lee and Botts (1988) showed a similar improvement in performance over control animals when 2.2×10^8, 2.2×10^9, or 2.2×10^{10} cfu of *S. faecium* was fed.

Although studies are limited, these results suggest that the addition of bacterial DFM to the diet can improve health and performance of stressed stocker calves. Similar to the neonatal calf, response to bacterial DFM might be greater when newly weaned and/or received beef calves are more prone to health problems. However, Gill et al. (1987) suggested that extremely healthy calves and extremely sick calves might be less likely to respond to DFM treatment.

Feedlot Cattle. Supplementing diets on a daily basis with lactate-producing and/or lactate-utilizing bacteria has recently been shown to improve feed efficiency and daily gain of feedlot cattle (Swinney-Floyd et al., 1999; Galyean et al., 2000; Rust et al., 2000a,b). Ware et al. (1988b) was one of the first to report that *L. acidophilus* BT1386 increased daily gain and improved feed efficiency in yearling steers fed a high-concentrate diet compared with controls. However, *L. acidophilus* did not affect DMI, USDA yield grade, USDA quality grade, dressing percentage, marbling score, or incidence of liver abscesses. More recent experiments have evaluated the efficacy of *Propionibacteria* species fed alone or in combination with *Lactobacillus* species (Swinney-Floyd et al., 1999; Galyean et al., 2000; Rust et al., 2000a,b). Swinney-Floyd et al. (1999) showed improvements in feed efficiency when feedlot steers were supplemented with a combination of *L. acidophilus* 53545 and *P. freudenreichii* P-63. During the first 10 d of high-concentrate feeding, daily gains were 0.93, 1.11, and 1.63 kg/d, and feed efficiencies were 5.17, 5.32, and 4.50 kg daily DMI/kg ADG for control, *P. freudenreichii*

Table 2. Distribution of treatment groups by location

State		Treatments[a]						
		CON	TRT2	TRT3	TRT4	TRT5	TRT6	TRT7
CO	No. of pens	8	8	—	—	—	—	—
	No. of animals/pen	9	9	—	—	—	—	—
IA	No. of pens	8	—	8	8	—	—	—
	No. of animals/pen	6	—	6	6	—	—	—
MI 2000	No. of pens	10	10	10	10	—	—	—
	No. of animals/pen	7	7	7	7	—	—	—
MI 1999	No. of pens	10	—	10	—	10	10	—
	No. of animals/pen	7	—	7	—	7	7	—
MI 1998	No. of pens	10	10	10	—	—	—	10
	No. of animals/pen	8	8	8	—	—	—	8
TX	No. of pens	12	12	12	12	—	—	—
	No. of animals/pen	5	5	5	5	—	—	—
Total	No. of pens	58	40	50	30	10	10	10
	No. of animals/pen	400	282	328	178	70	70	80

[a]Treatments are as follows: CON = control; TRT 2 = 10^9 *P. freudenreichii* (PF24) + 10^6 *L. acidophilus* (LA45) cfu·animal^{-1}·d^{-1}; TRT 3 = 10^9 PF24 + 10^6 LA45 + 10^6 LA51 cfu·animal^{-1}·d^{-1}; TRT 4 = 10^9 PF24 + 10^4 LA45 + 10^4 LA51 cfu·animal^{-1}·d^{-1}; TRT 5 = 10^9 PF24 + 2×10^6 LA 51 cfu·animal^{-1}·d^{-1}; TRT 6 = 10^8 PF24 + 10^6 LA45 + 10^6 LA51 cfu·animal^{-1}·d^{-1}; and TRT 7 = 10^9 PF24 + 10^8 LA45 + 10^8 LA51 cfu·animal^{-1}·d^{-1}.

alone, and the combination of *P. freudenreichii* and *L. acidophilus*, respectively. Feed efficiencies for the 120-d experiment were 5.17, 5.32, and 4.97 kg daily DMI/kg ADG, and liver abscesses at harvest were 8, 8, and 0% for the respective treatments.

Data from six research trials (n = 1,249; 184 pens) conducted in four states (CO, IA, MI, and TX) were assembled to summarize the effects of varying concentrations and strains of *L. acidophilus* (**LA45** and LA51) and *P. freudenreichii* (**PF24**) on feedlot performance and carcass characteristics of feedlot steers (McPeake et al., 2002). Treatments represented and their distribution across locations are shown in Table 2. Data were analyzed using the mixed model procedure for repeated measures (SAS Inst., Inc., Cary, NC). Because of unequal replication of treatments at each location, the year and location were compressed to allow for data analysis across experiments. Feedlot data were analyzed using initial weight as a covariate to account for location differences in starting weight. Least squares means were separated using the Tukey adjustment factor for selected treatments. Orthogonal contrasts included control (**CON**) vs. all DFM treatments; CON vs. 10^9 PF24, 10^6 LA45, and 10^6 LA51 cfu·animal^{-1}·d^{-1} (**TRT3**); 10^9 PF24 and 10^6 LA45 cfu·animal^{-1}·d^{-1} (**TRT2**) vs. TRT3; and the linear relationship between 10^9 PF24, 10^4 LA45, and 10^4 LA51 cfu·animal^{-1}·d^{-1} (**TRT4**), TRT3, and 10^9 PF24, 10^8 LA45, and 10^8 LA51 cfu·animal^{-1}·d^{-1} (**TRT7**), respectively.

From d 0 to 28, cattle fed TRT2 had greater ($P < 0.05$) DMI than cattle fed TRT3, TRT4, or 10^9 PF24 and 2×10^6 LA51 cfu·animal^{-1}·d^{-1} (**TRT5**; Table 3). Dry matter intakes were greater ($P < 0.05$) for steers fed TRT5 or 10^8 PF24, 10^6 LA45 and 10^6 LA51 cfu·animal^{-1}·d^{-1} (**TRT6**) vs. CON, TRT2, TRT3, or TRT4 from d 57 through 84. Cattle fed treatments CON through TRT6 were more efficient than cattle consuming TRT7 from d 57 through 84. Dressing percentage ($P = 0.62$), quality grade ($P = 0.59$), and percentage USDA Choice ($P = 0.73$) were not influenced by bacterial DFM (data not shown). However, hot carcass weight (**HCW**, kg) ($P = 0.04$) and carcass ADG (kg/d) ($P = 0.12$) were 346, 349, 350, 350, 349, 344, and 350, and 1.58, 1.61, 1.63, 1.62, 1.62, 1.62, 1.56, and 1.62 for CON through TRT7, respectively.

Contrasts performed to estimate differences between CON steers and steers receiving diets inoculated with DFM revealed greater ($P = 0.007$) final live weight, overall ADG ($P = 0.02$), overall DMI ($P = 0.07$), HCW ($P = 0.02$), and carcass ADG ($P = 0.05$) for treated steers (Table 4). In addition, contrasts for steers receiving TRT3 compared with CON revealed greater ($P = 0.007$) final live weight, overall ADG ($P = 0.02$), HCW ($P = 0.008$), and carcass ADG ($P = 0.01$) for steers inoculated with the DFM. Calculated feed energy values (NE_m, $P = 0.12$; NE_g, $P = 0.07$; ME, $P = 0.08$) tended to be greater for diets containing DFM. There were no effects ($P > 0.10$) of feeding LA45 compared with LA45 and LA51. Interestingly, a positive linear effect ($P = 0.05$) was observed for DMI with increasing *L. acidophilus*. However, this resulted in a trend ($P = 0.12$) for a linear increase in DMI:ADG. Diet NE_g ($P = 0.14$) and ME ($P = 0.16$) tended to increase with increasing *L. acidophilus*. Results of these analyses suggest that feeding combinations of lactic acid- and propionic acid-producing bacteria in diets of growing/finishing cattle might improve growth rate (2.6%) and carcass weight (6 kg) in feedlot steers.

Huck et al. (2000) studied the effects of phase feeding of bacterial DFM on growth performance and carcass characteristics of finishing heifers. *Lactobacillus acidophilus* BG2FO4 and *P. freudenreichii* P-63 were fed

Table 3. Least squares means and standard errors for the effects of bacterial direct-fed microbials on feedlot performance of crossbred feedlot steers

Item	Treatments[a]						
	CON	TRT 2	TRT 3	TRT 4	TRT 5	TRT 6	TRT 7
Final wt., kg	568 ± 2.99	574 ± 3.05	575 ± 3.01	573 ± 3.31	575 ± 5.19	572 ± 5.51	574 ± 4.61
DMI, kg/d							
d 0 to 28	7.99 ± 0.14[bc]	8.41 ± 0.16[b]	7.67 ± 0.15[c]	7.63 ± 0.17[c]	7.39 ± 0.27[c]	7.74 ± 0.25[bc]	8.01 ± 0.26[bc]
d 29 to 56	9.09 ± 0.14	9.38 ± 0.16	9.05 ± 0.15	8.94 ± 0.17	8.61 ± 0.27	8.84 ± 0.25	9.15 ± 0.26
d 57 to 84	9.86 ± 0.14[c]	9.83 ± 0.16[c]	10.19 ± 0.15[c]	9.82 ± 0.17[c]	11.40 ± 0.27[b]	11.43 ± 0.25[b]	10.32 ± 0.26[bc]
d 85 to harvest	9.66 ± 0.14	9.60 ± 0.16	10.08 ± 0.15	10.11 ± 0.17	9.54 ± 0.27	9.47 ± 0.25	10.63 ± 0.26
d 0 to harvest	9.22 ± 0.06	9.38 ± 0.08	9.32 ± 0.07	9.21 ± 0.10	9.30 ± 0.18	9.43 ± 0.18	9.59 ± 0.16
ADG, kg							
d 0 to 28	1.90 ± 0.04	2.03 ± 0.05	1.90 ± 0.05	1.81 ± 0.07	1.98 ± 0.07	1.79 ± 0.06	1.99 ± 0.09
d 29 to 56	1.79 ± 0.04	1.80 ± 0.05	1.87 ± 0.05	1.86 ± 0.07	1.81 ± 0.07	1.94 ± 0.06	1.84 ± 0.09
d 57 to 84	1.62 ± 0.04	1.54 ± 0.05	1.62 ± 0.05	1.71 ± 0.07	1.68 ± 0.07	1.66 ± 0.06	1.38 ± 0.09
d 85 to harvest	1.21 ± 0.04	1.30 ± 0.05	1.32 ± 0.05	1.32 ± 0.07	1.31 ± 0.07	1.25 ± 0.06	1.47 ± 0.09
d 0 to harvest	1.56 ± 0.02	1.59 ± 0.02	1.61 ± 0.06	1.58 ± 0.06	1.61 ± 0.06	1.60 ± 0.06	1.60 ± 0.06
Feed:gain							
d 0 to 28	4.32 ± 0.17	4.26 ± 0.19	4.11 ± 0.18	4.47 ± 0.24	3.75 ± 0.37	4.40 ± 0.38	3.83 ± 0.35
d 29 to 56	5.17 ± 0.17	5.26 ± 0.19	4.97 ± 0.18	5.04 ± 0.24	4.83 ± 0.37	4.56 ± 0.38	4.84 ± 0.35
d 57 to 84	6.45 ± 0.17[b]	6.76 ± 0.19[b]	6.48 ± 0.18[b]	5.90 ± 0.24[b]	7.07 ± 0.37[b]	7.01 ± 0.38[b]	8.29 ± 0.35[c]
d 85 to harvest	8.18 ± 0.17	7.53 ± 0.19	7.98 ± 0.18	8.02 ± 0.24	7.75 ± 0.37	7.98 ± 0.38	7.37 ± 0.35
d 0 to harvest	6.02 ± 0.04	6.01 ± 0.05	5.91 ± 0.05	5.89 ± 0.07	5.87 ± 0.12	5.98 ± 0.12	6.09 ± 0.11
Feed NE_m, Mcal/kg[d]	2.39 ± 0.02	2.40 ± 0.03	2.45 ± 0.03	2.44 ± 0.04	2.47 ± 0.06	2.39 ± 0.06	2.35 ± 0.06
Feed NE_g, Mcal/kg[d]	1.52 ± 0.01	1.52 ± 0.01	1.54 ± 0.01	1.54 ± 0.01	1.55 ± 0.02	1.52 ± 0.02	1.50 ± 0.02
Feed ME, Mcal/kg[d]	3.38 ± 0.02	3.39 ± 0.02	3.42 ± 0.02	3.41 ± 0.03	3.44 ± 0.05	3.38 ± 0.05	3.34 ± 0.04

[a]Treatments are as follows: CON = control; TRT 2 = 10^9 *P. freudenreichii* (PF24) + 10^6 *L. acidophilus* (LA45) cfu·animal^{-1}·d^{-1}; TRT 3 = 10^9 PF24 + 10^6 LA45 + 10^6 LA51 cfu·animal^{-1}·d^{-1}; TRT 4 = 10^9 PF24 + 10^4 LA45 + 10^4 LA51 cfu·animal^{-1}·d^{-1}; TRT 5 = 10^9 PF24 + 2×10^6 LA 51 cfu·animal^{-1}·d^{-1}; TRT 6 = 10^8 PF24 + 10^6 LA45 + 10^6 LA51 cfu·animal^{-1}·d^{-1}; and TRT 7 = 10^9 PF24 + 10^8 LA45 + 10^8 LA51 cfu·animal^{-1}·d^{-1}.

[b,c]Means in a row with different superscripts differ ($P < 0.05$).

[d]Values for feed NE_m, NE_g, and ME (DM basis) were calculated from performance data.

alone or in sequence across a 126-d finishing experiment. Treatments included: 1) no bacterial DFM; 2) 5×10^8 cfu·animal^{-1}·d^{-1} of *L. acidophilus* fed during the entire experiment; 3) 1×10^9 cfu·animal^{-1}·d^{-1} of *P. freudenreichii* fed during the entire experiment; 4) *L. acidophilus* fed for 28 d, then *P. freudenreichii* for the remainder; or 5) *P. freudenreichii* fed for 28 d, then *L. acidophilus* for the remainder. Feeding either *L. acidophilus* BG2FO4 or *P. freudenreichii* P-63 throughout the entire experiment did not affect daily gain, DMI, or feed efficiency. Feeding *P. freudenreichii* for 28 d followed by *L. acidophilus* improved daily gain, but not feed efficiency, compared with controls. Heifers fed *L. acidophilus* for 28 d followed by *P. freudenreichii* had greater gain (5.0%) and improved feed efficiency (5.1%) compared with controls. These authors suggested that growth performance of finishing cattle could be improved by targeting the appropriate DFM to a particular phase of production.

In summary, these results suggest that feeding bacterial DFM to feedlot cattle results in a 2.5 to 5% increase in daily gain and an approximately 2% improvement in feed efficiency, whereas DMI is inconsistent. In studies reviewed, carcass weight was generally increased by 6 to 7 kg.

Reduction of E. coli O157:H7 in Feedlot Cattle. Feedlot cattle have been recognized as a host for *E. coli* O157:H7. This organism appears to be confined to the GIT and is shed in feces. Ohya et al. (2000) developed and studied the effect of a DFM containing lactic-acid producing *Streptococcus bovis* LCB6 and *L. gallinarum* LCB 12 isolated from adult cattle on the elimination of *E. coli* O157:H7 from experimentally infected Holstein calves. An increase in VFA, especially acetate, correlated with the diminution of *E. coli* O157:H7. These authors suggested the possible application of bacterial DFM to reduce fecal shedding of *E. coli* O157:H7 from cattle. Similarly, results by Zhao et al. (1998) suggest that bacteria inhibitory to *E. coli* O157:H7 can be isolated from feces and intestinal tissue samples of cattle, grown in culture, and fed to reduce the carriage of *E. coli* O157:H7 in feedlot cattle. In a recent experiment (M. Brashears and M. Galyean, personal communication), supplementing feed with certain strains of *L. acidophilus* (NPC 747 and NPC 750) was shown to decrease the incidence of *E. coli* O157:H7 in the feces of finishing beef cattle. On d 14, 28, and 42 of the experiment, steers consuming both cultures (NPC 747 and NPC 750) had significant reductions in the incidence of cattle shedding *E. coli* compared with controls. At slaughter, strain NPC 747 was the most effective at decreasing the incidence of shedding of *E. coli* O157:H7. Based on these results, supplementing feed for cattle with certain DFM might decrease the incidence of *E. coli* O157:H7 in feedlot cattle. However, the incidence of *E. coli* shedding at slaughter was small (only 10% of control animals were positive) in the latter study, and more data are needed to validate the results.

Table 4. Contrast means and probability levels (parenthesis) for differences in overall feedlot performance and carcass characteristics by steers fed or not fed (CON) bacterial direct-fed microbials (DFM)

	Treatment contrasts[a]			Linear dose response[b]
Item	CON vs. DFM	CON vs. 10^6 LA45 +LA51	LA45 vs. LA45 + LA51	10^4 10^6 10^8
Final BW, kg	568 574 ($P = 0.01$)	568 575 ($P = 0.01$)	574 575 ($P = 0.75$)	573 575 574 ($P = 0.88$)
DMI, kg/d	9.22 9.37 ($P = 0.07$)	9.22 9.32 ($P = 0.28$)	9.38 9.32 ($P = 0.61$)	9.21 9.32 9.59 ($P = 0.05$)
ADG, kg	1.56 1.60 ($P = 0.02$)	1.56 1.61 ($P = 0.02$)	1.59 1.61 ($P = 0.59$)	1.58 1.61 1.60 ($P = 0.57$)
Feed:gain	6.02 5.92 ($P = 0.29$)	6.02 5.92 ($P = 0.10$)	6.01 5.92 ($P = 0.21$)	5.89 5.92 6.09 ($P = 0.12$)
Hot carcass weight, kg	345.6 348.9 ($P = 0.02$)	345.6 350.0 ($P = 0.01$)	349.2 350.0 ($P = 0.67$)	350.0 350.4 350.3 ($P = 0.98$)
Dressing percentage	61.49 61.34 ($P = 0.32$)	61.49 61.49 ($P = 0.99$)	61.34 61.49 ($P = 0.44$)	61.49 61.53 61.52 ($P = 0.99$)
Quality grade[c]	18.64 18.72 ($P = 0.61$)	18.64 18.75 ($P = 0.43$)	18.64 18.75 ($P = 0.50$)	18.74 18.83 18.81 ($P = 0.91$)
Choice, %	59.92 61.94 ($P = 0.56$)	59.92 62.45 ($P = 0.53$)	59.25 62.45 ($P = 0.49$)	63.42 62.45 65.83 ($P = 0.77$)
Carcass ADG, kg	1.58 1.61 ($P = 0.05$)	1.58 1.63 ($P = 0.01$)	1.61 1.63 ($P = 0.46$)	1.62 1.63 1.62 ($P = 0.94$)
Feed NE_m, Mcal/kg[d]	2.39 2.42 ($P = 0.39$)	2.39 2.45 ($P = 0.12$)	2.41 2.45 ($P = 0.32$)	2.44 2.45 2.35 ($P = 0.21$)
Feed NE_g, Mcal/kg[d]	1.52 1.53 ($P = 0.28$)	1.52 1.54 ($P = 0.07$)	1.52 1.54 ($P = 0.26$)	1.54 1.54 1.50 ($P = 0.14$)
Feed ME, Mcal/kg[d]	3.38 3.40 ($P = 0.33$)	3.38 3.42 ($P = 0.08$)	3.39 3.42 ($P = 0.27$)	3.41 3.42 3.34 ($P = 0.16$)

[a]Treatment contrasts were CON (control) vs. all DFM treatments; CON vs. 10^9 *P. freudenreichii* (PF24), 10^6 *L. acidophilus* (LA45) LA45, and 10^6 LA51 cfu·animal^{-1}·d^{-1} (TRT3); 10^9 PF24 and 10^6 LA45 cfu·animal^{-1}·d^{-1} vs TRT3, respectively.

[b]Linear relationship between 10^9 PF24, 10^4 LA45, and 10^4 LA51 cfu·animal^{-1}·d^{-1}, TRT3, and 10^9 PF24, 10^8 LA45, and 10^8 LA51 cfu·animal^{-1}·d^{-1}, respectively.

[d]23 = Prime; 20 = Choice; 17 = Select; 14 = No roll.

[c]Values for feed NE_m, NE_g, and ME (DM basis) were calculated from performance data.

Mode of Action

Variable response to feeding bacterial DFM in ruminant production systems emphasizes the need for greater understanding of underlying mechanisms. Research conducted to determine the potential mode of action of bacterial DFM has most often used the human or rodent model. Holzapfel et al. (1998) reviewed the literature and outlined several general microbiological criteria as keys for DFM to be efficacious. These included nonpathogenicity (i.e., safety), survival through regions of the gut (saliva, gastric, and bile), specificity to the host, and genetic stability. Upon reviewing the literature, it is apparent that in many animal studies, the use of nonhost-specific species and/or strains might be the reason why there was no response to bacterial DFM. Assuming criteria are met, bacterial DFM have been reported to modify the balance of intestinal microorganisms, adhere to intestinal mucosa and prevent pathogen adherence or activation, influence gut permeability, and modulate immune function (Salimen et al., 1996; Holzapfel et al., 1998). It has also been observed that certain lactic acid bacteria showed adjuvant properties by stimulation of a specific antibody response after injection with pathogenic microorganisms. Data supporting the occurrence of these mechanisms of DFM fed to livestock species are discussed.

Bacterial Direct-Fed Microbials and the Gut

Competitive Attachment. Early research (Jones and Rutter, 1972) suggested that attachment to the intestinal wall was important for enterotoxin-producing strains of *E. coli* to induce diarrhea. Therefore, it seems logical that bacterial DFM could compete with pathogens for sites of adherence on the intestinal surface. Attachment is believed to support proliferation and reduce peristaltic removal of organisms (Salimen et al., 1996). In support, Muralidhara et al. (1977) found that homogenates of washed intestinal tissue collected from piglets dosed with *L. lactis* had markedly higher numbers of attached *Lactobacilli* and lower *E. coli* counts than scouring or normal control pigs. Similarly, in the study of Abu-Tarboush et al. (1996), the adherence of *L. acidophilus* 27SC to the GIT was confirmed in young calves; the organisms used were apparently compatible with the GIT.

Adhesion is thought to be mediated either nonspecifically by physicochemical factors, or specifically by

adhesive bacterial surface molecules and epithelial receptor molecules (Holzapfel et al., 1998). Nonspecifically, the ability of bacteria to adhere to epithelial cells appears to depend on the interaction between an acidic mucopolysaccharide forming the outer layer of the bacterial cell wall and the similar mucopolysaccharide layer on the intestinal cells (Fuller and Brooker, 1974). Fibrils are often found on adhering bacteria and might reinforce attachment (Fuller and Brooker, 1980).

Antibacterial Effect. Many species of lactobacilli have demonstrated inhibitory activity against pathogens. *Lactobacillus acidophilus* has been shown to be antagonistic toward enteropathogenic *E. coli*, *Salmonella typhimurium*, *Staphylococcus aureus*, and *Clostridium perfringens* (Gilliland and Speck, 1977). Mann et al. (1980) showed that a strain of *E. coli*, which causes illness and death when it is the sole microbial species in young lambs, could be tolerated in the presence of lactobacilli. Lactic acid has been shown to decrease counts of coliforms throughout the GIT of piglets (Ratcliffe et al., 1986). This might result from a reduction in pH, which can prevent growth of many pathogens (Fuller, 1977).

Hydrogen peroxide produced by lactobacilli appears to be partially responsible for the antagonistic interaction (Gilliland and Speck, 1977). Hydrogen peroxide has been demonstrated to have bacteriocidal activity in vitro (Reiter et al., 1980); however, it might not have much involvement in the gut since oxygen is necessary for its formation by lactobacilli. A number of reports suggest that antimicrobial proteins and/or bacteriocins either mediate or facilitate antagonism by *L. acidophilus* (Hamdan and Mikolajcik, 1974; Gilliland and Speck, 1977; Barefoot and Klaenhammer, 1983). However, because of the presence of proteolytic enzymes, their importance might be limited.

Immune Response. Modulation of host immunity may represent another mechanism by which DFM promote intestinal health and overall well-being of the host (Erickson and Hubbard, 2000; Isolauri et al., 2001). The animal host immune system is capable of mounting both nonspecific (innate) and specific (adaptive) immune responses against a variety of pathogens when encountered. In addition to its role in the digestion and absorption of nutrients, the GIT provides its host a protective defense against a constant presence of antigens from food and microorganisms in the gut lumen. Besides epithelial cells, immune cells in the GIT consist of natural killer cells, macrophages, neutrophils, dendritic cells, and T and B lymphocytes that are aggregated in Peyer's patches, lamina propria, and intraepithelial regions. Upon infection by an antigen via the oral route, immune cells are rapidly activated, leading to enhanced phagocytosis as well as the production of a vast array of humoral mediators (Zhang and Ghosh, 2001). Interleukin (**IL**)-1, IL-6, tumor necrosis factor-α (**TNF-α**), interferons (**IFN**), reactive oxygen/nitrogen intermediates, and antimicrobial peptides are among the first humoral mediators produced in response to pathogenic bacteria, and they collectively either provide immediate protection for the host or help induce the development of specific immune responses. Cytokines produced later during microbial infection direct responses toward either cell-mediated T-helper type-1 (**Th1**) or humoral Th type-2 (**Th2**) immunity. Interleukin-2 and IL-12 promote development of Th1 cells from naïve T cells, whereas IL-4, IL-10, and transforming growth factor-β (**TGF-β**) inhibit the production of TNF-α, IL-1, IL-6, IL-12, and IFN-γ, and thus enhance Th2 immune responses (Infante-Duarte and Kamradt, 1999).

Bacterial DFM have been shown to affect the innate, humoral, and cellular arms of the immune system. Oral administration of lactobacilli generally resulted in an augmentation of innate immune responses (i.e., enhanced phagocytosis and natural killer cell activity), as well as an elevated production of immunoglobulin (**Ig**) A and a decreased IgE production in both humans and animals (Erickson and Hubbard, 2000; Isolauri et al., 2001). However, influence of DFM on cytokine production and T and B cell responses show mixed results depending on the strain, dose, and duration of feeding DFM, as well as the type of tissues and cells analyzed. *Lactobacillus rhamnosus* and *L. bulgaricus* strongly induced production of IL-2, IL-6, IL-10, TNF-α, and Th1-promoting cytokines (IL-12, IL-18, and IFN-γ), but not Th2-promoting cytokine, IL-4, in peripheral blood mononuclear cells (Miettinen et al., 1998). In contrast, *L. acidophilus*, *L. bulgaricus*, *L. casei*, and *S. thermophilus* did not alter gene expression of IL-6, TNF-α, and IFN-γ in Peyer's patches, spleen, or lymph nodes of mice after 14 d of oral exposure (Tejada-Simon et al., 1999). *Lactobacillus johnsonii* had a very low potential to induce proinflammatory responses, but rather favored the induction of TGF-β in an intestinal epithelial cell line (Haller et al., 2000b). Furthermore, some species of probiotics appear to be capable of altering the immunomodulatory effects exerted by other species. For example, *L. reuteri* DSM12246 was shown to potentially suppress *L. casei*-induced production of IL-6, IL-12, and TNF-α in dendritic cells (Christensen et al., 2002), suggesting that the composition of bacterial DFM administered should be considered. Increased populations of helper (CD4+) and activated (CD25+) T cells were observed in the blood of elderly people after 3-wk consumptions of *B. lactis* HN019 (Gill et al., 2001), but this did not happen in mice fed daily with *L. acidophilus*, *L. rhamnosus*, or *B. lactis* for 4 wk (Gill et al., 2000) or in human peripheral blood mononuclear cells stimulated in vitro for 3 to 5 d with *L. johnsonii* or *L. sakei* (Haller et al., 2000a).

These data provide evidence that bacterial DFM have the potential to protect animals and humans against pathogenic organisms. Several mechanisms are likely involved, but an ability to adhere to and colonize the GIT is most likely important. Bacterial DFM also show promise as immune modulators, although more re-

search is needed to determine the underlying mechanisms.

Bacterial Direct-Fed Microbials and Ruminal Fermentation

The original concept of feeding bacterial DFM to livestock was based primarily on potential beneficial postruminal effects; however, there has been some indication that certain bacterial DFM also might have beneficial effects in the rumen, in particular, helping to prevent ruminal acidosis. Ruminal acidosis can be characterized by low ruminal pH (below 5.6) and high ruminal concentrations of total VFA (subacute) or lactic acid (acute). Lactate-producing bacteria (*Lactobacillus* and *Enterococcus*) might help prevent ruminal acidosis in dairy cows (Nocek et al., 2002), potentially because the presence of these bacteria cause the ruminal microorganisms to adapt to the presence of lactic acid in the rumen (Yoon and Stern, 1995). Inoculation of in vitro fermentation with lactate-utilizing bacterium *Megasphaera elsdenii* has been shown to prevent lactate accumulation when a highly fermentable substrate was used (Kung and Hession, 1995).

Megasphaera elsdenii. Megasphaera elsdenii inoculation has modified ruminal fermentation and prevented the accumulation of lactate during the transition from low- to high- concentrate diets in both in vitro and in vivo studies (Greening et al., 1991; Kung and Hession, 1995). In the study of Kung and Hession (1995), the pH of cultures treated with *M. elsdenii* (8.7×10^6 cfu/mL of culture fluid) was decreased below 5.5 at 4 h and remained at approximately 5.3 (24-h culture), whereas the control was decreased to 4.8. Lactate concentration peaked at more than 40 m*M* in control after 8 h and remained fairly constant thereafter, but in the *M. elsdenii* treatment, it was less than 5 m*M* through incubation. Total VFA concentration of cultures treated with *M. elsdenii* was more than twice that of control (131.4 vs. 63.3 m*M*). Acetate concentration was not significantly different after 2 h. The concentration of propionate, butyrate, valerate, isobutyrate, and isovalerate for control and *M. elsdenii* inoculation at 6 h were 38:47, 2:35, 1:15, 1:11, and 1:2 (m*M*, control:*M. elsdenii*), respectively. Therefore, most differences in VFA concentration between treatments resulted from increased butyrate, valerate, and branched-chain fatty acids.

Greening et al. (1991) reported that inoculation with *M. elsdenii* significantly decreased minimal pH and lactate concentration in acidosis induced beef cattle. Minimal pH for control, inoculation prior to acidosis induction, and 0 h, or 2 h after acidosis induction were 4.65, 4.73, 5.51 and 5.26 and maximal lactate concentrations were 124, 121, 49.9, and 45.9 m*M*, respectively. Accumulated total VFA were 472, 507, 910, and 870 m*M*·h for respective treatments. Robinson et al. (1992) reported the effects of inoculation with *M. elsdenii* on feed intake, ruminal pH, osmolarity, lactate, and VFA concentration in acute acidosis-induced steers fed a 90% concentrate diet. In that study, the interaction between inoculation and day of diet switch moderated pH, lactate, VFA, and feed intake significantly. Steers inoculated with *M. elsdenii* ate 24% more DM.

Propionibacteria. Kung and Hession (1995) discussed the choice of *M. elsdenii* for inoculating ruminal fermentations that have not been adapted to readily degradable carbohydrates. During the feeding of readily degradable and soluble carbohydrates, *M. elsdenii* seems to be the major ruminal lactate utilizer (Counotte et al., 1981) because *S. ruminantium* undergoes catabolite repression (Russell and Baldwin, 1978) and is relatively acid-intolerant (Mackie and Gilchrist, 1979). Furthermore, *M. elsdenii* simultaneously uses lactate, glucose, and maltose (Russell and Baldwin, 1978) and would compete with lactate-producing organisms for substrate. Although *Propionibacterium* is a lactate utilizer, it has been focused on propionate production rather than lactate fermentation for use as a DFM. Propionate is quantitatively the most important single precursor of glucose synthesis among VFA, and therefore has a major impact on hormonal release and tissue distribution of nutrients (Nagaraja et al., 1997). For growing ruminants and lactating cows, propionate has been estimated to account for 61 (Reynolds et al., 1994) to 67% (Huntington, 2000) of glucose release. Propionate spares glucogenic amino acids in gluconeogenesis, and consequently reduces the maintenance cost of metabolizable protein and possible heat increment (Van Soest, 1994). Nutrient intake lags nutrient demand during early lactation, especially in dairy cows, and therefore ruminal supply of propionate might not be sufficient (Overton et al., 1999). Also, decreased acetate:propionate has been accompanied with a decrease in methane production according to the stoichiometric laws of chemical balance and its equation (Van Soest, 1994). When acetate:propionate decreases, CH_4 production declines, and energy retention by cattle would theoretically increase (Wolin, 1960).

Volatile fatty acid proportion depends on species of microorganisms and culture conditions. Propionate production by *Propionibacterium* is usually accompanied by the formation of acetate and CO_2. This occurs for stoichiometric reasons and to maintain hydrogen and redox balance. Also, product ratios are controlled for thermodynamic reasons, such as ATP production and entropy generation (Lewis et al., 1996). However, *Propionibacterium* seems to produce propionate more efficiently compared with *M. elsdenii*. Although the culture condition was very different, *P. shermanii* fermented 1.3 m*M* of glucose resulting in final concentrations of 0.8 m*M* acetate and 2.3 m*M* propionate, whereas incubations with 6 m*M* lactate resulted in final concentrations of 1.72 m*M* acetate and 3.38 m*M* propionate during a 14-d incubation (Johns, 1951). The highest rate of decarboxylation of succinate occurred at about pH 5.0. *Megasphaera elsdenii* fermented 8 m*M* of glucose to 6.8 m*M* acetate and 4.3 m*M* butyrate, and fermented 40 m*M* of lactate to 13.5 m*M* acetate, 8.4 m*M* propio-

nate, and 8.5 m*M* butyrate during a 12-h incubation (Hino et al., 1994). Mackie et al. (1978) and Mackie and Gilchrist (1979) reported that in the rumen of sheep, during stepwise adaptation to a high-concentrate diet, *Propionibacterium* accounted for 40 to 50% of the lactate utilizers on occasion; however, the population of *Propionibacterium* usually seemed very low. Therefore, the concept of daily or periodic supplementation of *Propionibacterium* may be on the basis of the increment of propionate production when cattle are fed a high-concentrate diet.

The effect of increasing dosage levels (none, 10^7, 10^8, 10^9, and 10^{10} cfu) of *P. acidipropionici* on ruminal fermentation in steers fed a high-concentrate diet was recently studied (Kim et al., 2000). When supplemented with *P. acidipropionici*, all dosage levels and the post-test period had lower levels of acetate, but only the 10^7 and post-test period were significantly greater than the pretest period. Propionate levels were greater for all dosage levels. Numerically, propionate increased as the dosage level increased and tended to decrease in the post-test period. Consequently, acetate:propionate decreased at all dosages except 10^8. It would appear the *P. acidipropionici* altered ruminal metabolism toward less acetate and more propionate. Butyrate concentration decreased as the dose of *P. acidipropionici* increased. When *P. acidipropionici* was removed, butyrate concentration returned to near pretest levels. This suggests that *P. acidipropionici* did effectively reduce butyrate concentration in the rumen. There was no effect on pH, lactate, or branched-chain fatty acids with supplementation of *P. acidipropionici* (Kim et al., 2000). In contrast, Ghorbani et al. (2002) fed *Propionibacterium* or *Propionibacterium* and *E. faecium* and found no effect on ruminal concentrations of L-lactate, total VFA, propionate, isobutyrate, and isovalerate, or the ratio of actetate:propionate. Acetate concentration in ruminal fluid was greater for steers receiving *Propionibacterium* and *E. faecium* than for steers receiving *Propionibacterium* alone or control. In contrast to Kim et al. (2000), steers fed *Propionibacterium* alone had greater concentrations of ruminal butyrate (Ghorbani et al., 2002). Other researchers (Slyter et al., 1992; Kung and Hession, 1995) have reported accumulation of butyrate when *M. elsdenii* is grown in pure culture.

Ghorbani et al. (2002) found no effect of *Propionibacterium* P15 or a combination of *Propionibacterium* P15 and *E. faecium* EF212 on ruminal pH. Mean ruminal pH of steers fed steam-rolled barley was 5.71. In contrast, results from studies with *Lactobacillus* species have shown lower area under the pH curve (Huffman et al., 1992; Nocek et al., 2000), suggesting reduced risk of subacute ruminal acidosis. Similar to ruminal pH, blood pH was not affected by bacterial DFM supplementation in the study of Ghorbani et al. (2002). However, steers fed *Propionibacterium* and *E. faecium* tended to have lower concentrations of blood CO_2 and had lower concentrations of LDH than control steers. The authors suggested that lower blood CO_2 and LDH indicated that feeding a lactate-producing bacteria along with a lactate-utilizing bacteria reduced the risk of metabolic acidosis.

Aviles (1999) conducted an experiment with six ruminally-cannulated steers in an acidosis challenge study to evaluate the effects of a lactate utilizer, *P. acidipropionici*, strain DH42, on ruminal acidosis. In that study, *P. acidipropionici* significantly lowered ruminal and blood pH 2 h after feed engorgement. However, ruminal VFA and lactate levels were unaffected by treatment.

Huffman et al. (1992) suggested that *L. acidophilus* might modify subacute ruminal acidosis. Ruminally fistulated steers were fed a 50% concentrate diet for 12 d. On d 13, steers were dosed with a 100% concentrate diet via a ruminal cannula to induce subacute acidosis. Feeding *L. acidophilus* (5×10 cfu/d) reduced the amount of time that ruminal pH was below 6.0 compared with control. Recently, Van Koevering et al. (1994) reported that ruminal concentrations of D-lactate and total lactate were lower in steers fed *L. acidophilus*. These data suggested that *L. acidophilus* alone might decrease the severity of subacute acidosis.

Feeding *Propionibacterium* increased protozoa (especially *Entodinium*) and decreased amylolytic bacteria in the rumen of feedlot steers (Ghorbani et al., 2002). Similarly, Van Koevering et al. (1994) reported that including cultures of lactobacilli in the diet prolonged retention of protozoa. The mechanism by which bacterial DFM stimulate protozoa remains unclear (Ghorbani et al., 2002).

In summary, DFM might reduce the risk for subacute acidosis by reducing the time ruminal pH remains below 5.6. Lower blood CO_2 and LDH also suggest a lower risk for metabolic acidosis. However, these responses seem to depend on the species of DFM fed. The concept of supplementation of a combination of *Propionibacterium* and *Lactobacillus* might be developed with the aforementioned characteristics of microorganisms with the ruminal ecosystem in mind. In particular, the inhibition of methane production with lactate production by *Lactobacillus* may promote propionate production by *Propionibacterium* and improve the energy efficiency in the rumen, and consequently animal performance.

Implications

Bacterial direct-fed microbials fed to ruminant livestock have been shown to decrease scours in neonatal calves, increase milk yield in dairy cows, decrease morbidity in newly weaned calves and/or calves newly received in the feedlot, and increase daily gain and carcass weight in feedlot cattle. Moreover, strains of *Lactobacillus acidophilus* were shown to reduce fecal shedding of *Escherichia coli* O157:H7 by feedlot cattle at harvest. Although the mode of action is not fully understood, it seems that adhesion, colonization, inhibitory action, and stimulation of immune function are all important for direct-fed microbials to improve health. Some bacterial direct-fed microbials also seem able to

function in the rumen. Depending on the species or combination of species, they can increase ruminal propionate concentration and decrease area below subacute ruminal pH, suggesting the potential for more efficient energy utilization and a reduction in acidosis exists.

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Tannins for suppression of internal parasites

B. R. Min[1] and S. P. Hart[2]

E (Kika) de la Garza Institute for Goat Research, Langston University, Langston, OK 73050

ABSTRACT: It is increasingly evident that gastrointestinal parasite (GIP) control programs based on dewormers are failing because of increased dewormer resistance; thus, alternative GIP control strategies are necessary. Condensed tannins (CT) have biological effects that may aid in the control of GIP. The CT bind proteins and other molecules tightly at near-neutral pH, such as occurs in the rumen, with dissociation in the acidic pH of the abomasum, freeing them for digestion. Plant CT may have direct or indirect effects on GIP. Direct effects might be mediated through CT-nematode interactions, thereby affecting physiological functioning of GIP. Condensed tannins extracted from various forages can markedly decrease the viability of the larval stages of several nematodes in sheep and goats. Condensed tannins also may react directly by interfering with parasite egg hatching and development to infective stage larvae. Indirectly, CT can improve protein nutrition by binding to plant proteins in the rumen and preventing microbial degradation, thereby increasing amino acid flow to the duodenum. Several sheep studies have shown that improved protein nutrition decreases parasite infestation. This is assumed to be mediated by enhanced host immunity, which may be especially important with selection for immunity to GIP. Therefore, CT might counteract parasites by one or more of the aforementioned mechanisms, and mechanisms involved might differ between CT from different forage species. In conclusion, CT in forages have the potential to aid in the control of GIP.

Key Words: Digestive Tract, Forage, Nutrition, Parasites, Ruminants, Tannins

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Introduction

Gastrointestinal parasites (**GIP**) cause marked production losses to livestock throughout the world (Sykes, 1994). Control of ruminant GIP over the past decades has been achieved by the use of anthelmintic drugs, but control of GIP is becoming more difficult due to the increased resistance of parasites to common anthelmintics, which has been reported in goats, sheep, and cattle (Prichard, 1994; Waller, 1994; Pomroy et al., 2002). Alternative parasite management strategies using forages containing condensed tannins (**CT**) have recently been suggested (Niezen et al., 1995; Barry et al., 2001; Min et al., 2002b). It seems possible that consumption of forage CT may reduce GIP numbers and improve animal performance through direct and indirect mechanisms. Alternative, nondrug GIP control strategies that are practical and realistic for introduction into farm production systems are required. This review, based on published literature, summarizes data on CT and GIP and on the nutritional consequences of CT-containing grazed forages.

Forages and Condensed Tannins

Tannins are usually classified either hydrolyzable tannins (**HT**) or CT (proanthocyanidins) based on their molecular structure. Hydrolyzable tannin molecules contain a carbohydrate (generally D-glucose) as a central core. The hydroxyl groups of these carbohydrates are esterified with phenolic groups, such as ellagic acid or gallic acid (Haslem, 1989). Hydrolyzable tannins can be further metabolized to compounds such as pyrogallol (Murdiati et al., 1992), which are potentially toxic to ruminants (Dollahite et al., 1962). Some rumen bacteria involved in this degradative pathways include *Eubacterium oxidoreducens, Streptococcus bovis, Syntrophococcus sucromutans,* and *Coprococcus* spp. (Tsai et al., 1976; Krumholz and Bryant 1986a,b). Plants that are considered to be toxic due to HT include *Clidemia hirta* (harendog; Murdiati et al., 1991), *Quercus ilex* (oak; Camp et al., 1967), *Terminalia oblongata* (yellow wood; Doig et al., 1990) and *Ventilago viminolis* (supplejack; Pryor et al., 1972).

The CT are the most common type of tannin found in forage legumes, trees, and shrubs (Barry and McNabb,

[1]Current address: 11708 Highway 70 South, TAMU, P.O. Box 1658, Vernon, TX 76386.

[2]Correspondence: P.O. Box 730 (phone: 405-466-3836; fax 405-466-3138; E-mail: shart@luresext.edu).

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Table 1. Characteristics of condensed tannins (CT) in different forage species[a]

Forage	CT, g/kg of DM	MW range	CT structure[b]
Temperate legumes			
Lotus corniculatus (birdfoot trefoil)	48	1,800 to 2,100	67:30
Lotus pedunculatus (big trefoil)	77	2,200	19:64
Onobrychis viciifolia (sainfoin)	29[2]	2,040 to 3,060	81:19
O. arenaria	29–38	1,560 to 3,300	75:25
O. vaginalis	—	1,980 to 2,700	80:20
O. antasiatica	—	1,650 to 2,490	76:24
Hedysarum cornarium (sulla)	51–84	—	—
Medicago sativa (lucerne)	0.5	—	Trace D
Tropical legumes			
Lespedeza cuneata (*Sericea lespedeza*)	46[c]	14,000 to 20,000	Mainly PD
Leucanea diversifolia	96	—	PC/PD
Desmodium ovalifolium 13089	232	—	—
Grass			
Lolium perenne (perennial ryegrass)	1.8	—	Trace D, C
Herbs			
Chicorium intybus (chicory)	3.1	—	—

[a]References: McLeod, (1974); Terrill et al. (1992); Koupai-Abyazani et al. (1993); Jackson et al. (1996); Foo et al. (1996; 1997); Min et al. (1997; 2001a), Bermingham et al. (2001).
[b]PC:PD ratio = procyanidin:prodelphinidin; D = delphinidin; C = cyanidin.
[c]Extractable CT.

1999). Structurally, CT are complexes of oligomers and polymers of flavanoid units linked by carbon-carbon bonds (Hagerman and Butler, 1981; Foo et al., 1986). The CT exist as oligomers of flavan-3-ols (catechin) or flavan-3,4-diols (epicatechin), and those occurring in temperate forages have a relative molecular mass of 2,000 to 4,000 comprising 10 to 12 oligomers of CT (Foo et al., 1986). Together, these differences can produce an infinite variety of chemical structures, which in turn affect the physical and biological properties of the CT. Condensed tannins accumulate in the vacuoles of cells in various tissues of many forage species. Structure and range of molecular weights (**MW**) for forage CT are summarized in Table 1. This review paper deals mainly with CT.

Nutritional Effects of Condensed Tannins

Condensed tannins can complex with numerous types of molecules including proteins, polysaccharides, nucleic acids, and minerals (Spencer et al., 1988; Haslem, 1989). Condensed tannin complexes are mainly by hydrophobic/hydrogen interactions (Hagerman and Butler, 1981; Haslem, 1989). Formation of the CT-protein complex is influenced by many factors, such as pH, composition, and MW of both the CT and the proteins (Asquith and Butter, 1986). Although CT interact with carbohydrates, particularly starch, their affinity for carbohydrates seems to be much less than for proteins (Haslem, 1989). Moderate levels of CT (20 to 40 g of CT/kg of DM) bind to protein by hydrogen bonding at near neutral pH (pH 6.0 to 7.0) in the rumen to form CT-protein complexes, but dissociate and release bound protein at pH less than 3.5 in the abomasum (Barry et al., 2001). Thus, CT-containing plants can protect dietary protein against degradation in the rumen and increase AA supply to the abomasum and small intestine, resulting in a improved nutritional status of the animal.

At similar CT concentrations (0.25 to 1.75 mg of CT/mg of total soluble plant protein), *Lotus pedunculatus* CT was more effective at protecting the plant protein from degradation by rumen microorganisms than *Lotus corniculatus* CT (Aerts et al., 1999). This effect of CT on plant protein degradation may be due to differences in the chemical structure influencing the reactivity of the CT (Table 1). The average MW of CT in *L. corniculatus* is 1,900, whereas that in *L. pedunculatus* is 2,200. In addition, the CT from *L. pedunculatus* contains a predominance of prodelphinidin (**PD**)-type subunits. Conversely, the CT from *L. corniculatus* is comprised predominantly of procyanidin (**PC**) subunits with a dominance of epicatechin (67%; Foo et al., 1996; 1997). The large number of free hydroxyl groups enables hydrogen bonding with proteins and other molecules, but the extent of the association appears to be affected by the size of the polymer, the predominance of prodelphinidin relative to procyanidin units, the types of terminal groups (2,3-*cis* or 2,3-*trans*), and the structure of potential binding sites (C4/C8 or C4/C6 interflavanoid linkages that effect the shape of the CT polymer chain; Hagerman and Butler, 1981; Foo et al., 1996; 1997). Therefore, the chemical structure of CT, as well as its concentration, needs to be considered in studies involving protein degradation and GIP control.

Barry and Forss (1983) defined CT associated with plant protein after mastication as bound CT, and the CT remaining in the supernatant after high-speed centrifugation as free CT. It has been suggested that high concentrations of free CT in the rumen can react with other sources of protein after chewing by animals, such as enzymes secreted by rumen bacteria, and so inhibit

rumen carbohydrate fermentation (Barry and Manley, 1986). Therefore, high CT concentrations such as those in *L. pedunculatus* (63 to 106 g of CT/kg of DM) substantially depressed feed intake, digestibility, and animal production in sheep (Barry and Duncan, 1984; Waghorn et al., 1994). Other forages with high concentrations of CT (over 50 g of CT/kg of DM) that have resulted in antinutritional effects when consumed include *Lespedeza cuneata* (sericea lespedeza; Windham et al., 1990), *Acacia aneura* (mulga; Pritchard et al., 1988), and *Eucalyptus melliodora* (eucalyptus; Foley and Hume, 1987). However, not all of the antinutritional effects of CT can be attributed to their high concentration in the diet. *Onobrychis viciifolia* (Sainfoin; 50 to 80 g of CT/kg of DM), *Hedysarium cornarium* (Sulla), and *L. cuneata* (52 g of CT/kg of DM), when fed to sheep and goats, had a higher nutritive value than similar forages without CT (Ulyatt et al., 1976; Niezen et al., 1995; Min et al., 2003a).

When ruminants are fed on high-quality fresh forages containing high concentrations of N (25 to 35 g of N/kg of DM), carbohydrate digestion in the rumen is efficient; however, degradation of forage N is excessive, resulting in surplus levels of ammonia (20 to 35%) in the rumen and absorption of that ammonia from the rumen, which is ultimately excreted as urea in the urine (Ulyatt et al., 1975). Therefore, a reduction of protein degradation in the rumen will increase the quantity of protein digested in the small intestine, potentially increasing animal production. It has been reported that CT in forages markedly reduces protein solubilization and degradation in the rumen and reduces ruminal proteolytic activity, but may inhibit extracellular microbial enzymes (proteinases, cellulases, and hemicellulases; Chung et al., 1998a,b; Min et al., 2001a; 2002a).

Figure 1 shows the reduction in rumen ammonia concentration as related to CT concentration compared with polyethylene glycol (**PEG**) supplementation or nonCT-containing forages. The specific effects of CT can also be assessed using PEG, which binds and inactivates CT. Figure 1 implies that CT in forages reduced rumen degradation of forage protein to ammonia (McNabb et al., 1996; Min et al., 2000). In situ and in vitro experiments have shown that this is due to the action of CT in *L. pedunculatus* and *L. corniculatus* slowing the rates of both solubilization and degradation of forage proteins by rumen microorganisms (McNabb et al., 1996; Min et al., 2000). In the absence of CT, 27% of the total N in *Trifolium repens* (white clover) forage was immediately solubilized, with the rate of rumen solubilization of the insoluble component being 15%/h (Min et al., 2000). These values show that the total N in *T. repens* forage was rapidly solubilized compared to the CT-containing forage *L. corniculatus* (23% total N immediately solubilized and 8%/h for the rate of rumen solubilization, respectively). In ruminants fed fresh forages, most proteins are rapidly solubilized, releasing 56 to 65% of the N concentration in the rumen as soluble N during mastication; consequently, a large

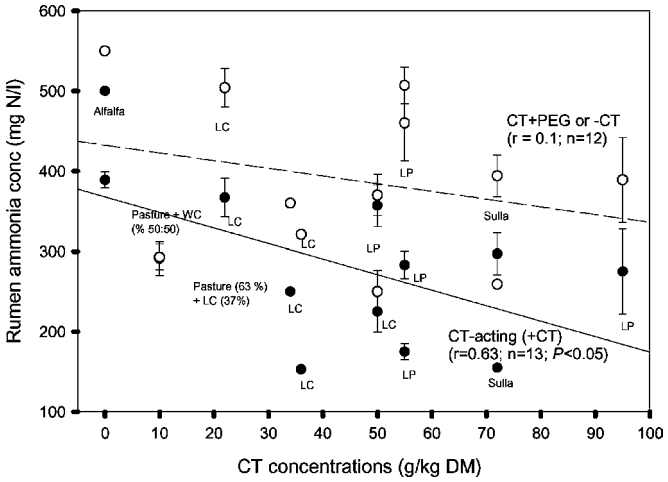

Figure 1. The relationship between condensed tannins (CT) concentration in fresh forage species and ruminal ammonia concentration in sheep. Animals were offered either a nonCT containing-forage, forage + polyethylene glycol (PEG) supplementation (○; nonCT), or CT-containing pasture without PEG (●; CT containing). WC = white clover, LC = *Lotus corniculatus*, LP = *L. pedunculatus*. The bars indicate the SEM when available. References: Barry and Manley (1984; 1986); Waghorn et al. (1987a; 1994); Terrill et al. (1992); Waghorn and Shelton (1992); Stienezen et al. (1996); Wang et al. (1996a). Min et al. (1998); and Douglas et al. (1999)

amount of soluble N is degraded by rumen microorganisms and N is lost as ammonia absorbed from the rumen (Ulyatt et al., 1975). The minimal concentration of CT (g/g of protein) needed to reduce proteolysis in laboratory studies is 1:10 (wt/wt; Tanner et al., 1994) or 1:12 (wt/wt; Jones and Mangan, 1977), with 5 mg of CT/kg of DM or greater being required to prevent bloat in cattle (Li et al., 1996).

Responses of rumen ammonia concentrations in sheep grazing CT-containing forages to PEG supplementation were due to the effect of PEG in preventing binding of CT to protein (Figure 1). Twice-daily oral PEG supplementation to sheep was sufficient to observe responses, but it is still not known if the response was maximal. However, sheep and goats exhibited different levels of tolerance to the effects of CT (Narjisse et al., 1995). Narjisse et al. (1995) reported that rumen ammonia in sheep was depressed by tannins extracted from *Quercus ilex* leaves (oak; 1 g of tannins/kg of BW), but was not affected in goats. The absence of a CT effect noted in goats should be considered with caution. The lack of effect of tannins in goats might result from the greater ability of their rumen microbial (*Streptococcus caprinus*) population to degrade tannins and their higher urea recycling and salivary secretion capabilities (Cocimano and Leng, 1967; Brooker et al., 1994). However, the diversity of tannin-tolerant rumen microorganisms is probably poorly represented by the few ruminal isolates that have currently been described.

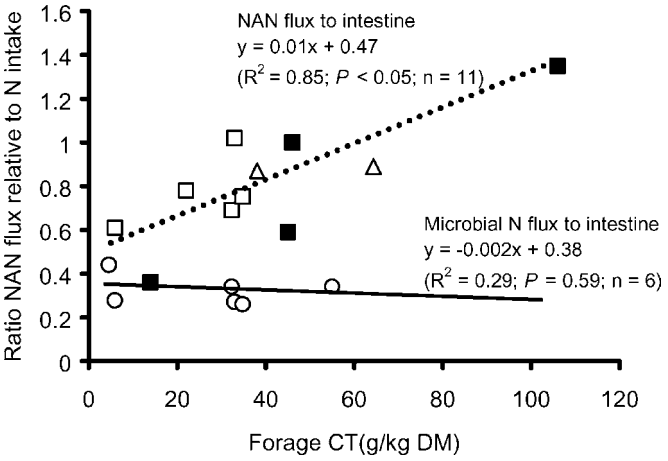

Figure 2. Effect of forage condensed tannins (CT) on fraction of intake nitrogen reaching the small intestine. Figure shows the relationship between CT concentration (DM basis) in forage species DM (x-axis) and the ratio of nonammonia N (NAN) flowing at the abomasum or duodenum (□, *Lotus corniculatus*; ■, *L. pedunculatus*; △, Sainfoin; △, Sulla) and microbial N (○, *L. corniculatus* and *L. pedunculatus*) per unit of N consumed by sheep. References: Barry and Manley (1984; 1986); Waghorn et al. (1987a,b; 1994); McNabb et al. (1993); Wang et al. (1996b); Bermingham et al. (2001); and Min et al. (2001a).

Tolerance of protozoa, fungi, and bacteria to CT needs investigation, as does the role of tolerant microorganisms in the degradation of tanniferous forages. Goats produce more protein-rich saliva during eating than sheep (Dominigue et al., 1991). Gilboa (1995) also found that the parotid saliva of goats was relatively rich in proline (6.5%), glutamine (16.5%), and glycine (6.1%), which are known to enhance the affinity of proteins to CT (Mehansho et al., 1987). Furthermore, the concentration of protein in parotid saliva was significantly higher in goats (550 μg/mL) fed *Ceratonia siliqua* (carob; high in CT) than in goats (212 μg/mL) fed wheat straw (no CT), suggesting that exposure of goats to CT enhanced the secretion of proteins in parotid saliva (Gilboa, 1995). Hence, data on the effect of tannins on N digestion obtained with sheep and cattle may or may not be directly applicable to goats; more research is needed on the effects of forage CT in goats.

To further understand the effect of CT concentration on N digestion in the rumen, duodenal (abomasal) nonammonia N (**NAN**) outflow per unit of N consumed has been plotted against CT concentration in fresh forages for sheep (Figure 2). Total NAN flux progressively increased with increasing CT concentration, whereas rumen microbial N outflow was little affected. This indicates that increasing CT concentration increased the amount of undegraded and total protein flowing to the small intestine.

Moderate levels of CT (20 to 40 g of CT/kg of DM) in forages (*L. corniculatus*) fed to sheep increased absorption of essential AA from the small intestine by 62% (Waghorn et al., 1987b). Increased milk and wool production in sheep (Barry and McNabb, 1999; Min et al., 1999) and increased milk production in dairy cows (Woodward et al., 1999) were also observed when dietary forages contained CT (20 to 40 g of CT/kg of DM). Production of milk protein in dairy cows and sheep was increased by 40% with CT-containing forages. However, forages with high concentrations of CT (55 to 106 g of CT/kg of DM) may reduce productivity (Barry and Manley, 1984).

Condensed Tannins and Gastrointestinal Nematodes

In most experiments, the effects of CT are determined by comparing CT-containing legumes to commonly grown forages that do not contain CT, such as *Medicago sativa* (alfalfa), *T. repens* (white clover), and *Lolium perenne* (perennial ryegrass). Alternatively, since PEG complexes with CT, thereby inactivating it, the effects of CT can be quantified by comparing nonPEG-treated animals (CT-active group) with animals given PEG (CT-inactive; control). However, Niezen et al. (1998b) reported that the use of PEG does not seem appropriate in parasitology studies. Total nematode burdens in lambs did not differ between ryegrass and *L. pedunculatus*, but were greatest in lambs fed ryegrass and given PEG, suggesting that PEG may have some unidentified effect on GIP numbers.

Parasitism of the abomasum and small intestine causes extensive protein losses in the digestive tract of sheep (Kimambo et al., 1998). Alternative, nondrug parasite-control strategies have recently been suggested based on using forages that contain CT (Table 2; Niezen et al., 1995; Barry et al., 2001). Condensed tannins may have direct effects on internal parasites themselves or may indirectly control the parasites by increasing the resistance and resilience of animals to

Table 2. The effect of grazing condensed tannins (CT)-containing forage (*Hedysarum coronarium*; sulla) on the growth and parasite status of anthelmintic-drenched (parasite free) and nondrenched (parasitised) lambs. *Medicago sativa* (lucerne) also was grazed as a control legume[a]

Item	*Medicago sativa*	*Hedysarum cornarium*	*P*-value[b]
Total condensed tannins, g/kg of DM	1.5	110	0.001
Live weight, g/d			
Anthelmintic drenched	230	247	NS
Non-drenched	37	207	0.001
Fecal egg counts, eggs/g			
Nondrenched	2,220	1,320	0.01
Total worm burden			
Nondrenched	18,676	10,553	0.01

[a]Niezen et al. (1995; 1998a,b).
[b]NS = not significant.

GIP infections through improved protein nutrition. Possible direct effects could be mediated through CT-nematode interactions, which reduce nematode viability. It has been reported (Table 2; Niezen et al., 1995) that direct effects of CT on GIP may account for reduced fecal egg counts (**FEC**) and nematode burdens in lambs that grazed *Hedysarum coronarium* compared to *M. sativa* in New Zealand. Evidence in support of the direct affect of CT was provided by Molan et al. (2000) who demonstrated that the CT extracted from *L. pedunculatus, L. corniculatus, H. coronarium*, and *O. viciifolia* forages reduced the rate of larval development (eggs to L_3 larvae) by 91%, reduced the number of eggs hatching by 34%, and decreased the mobility of L_3 larvae by 30%.

Indirect effects on resistance and resilience could be mediated by changes in the supply of digested protein. Protein supplementation appears to be effective in enhancing specific immune responses for intestinal parasite infection (Bown et al., 1991). It has been shown that animals fed high planes of nutrition are better able to resist infection and disease, whereas disease is more severe in animals with low protein intakes (Bown et al., 1991). Protein supplementation has been shown to increase the resistance of sheep to *Haemonchus contortus* (Wallace et al., 1996). Hence, dietary CT may benefit parasitised ruminants by improved protein nutrition, which in turn may enhance animals' immune response to the parasite infection (Niezen et al., 2002; Min et al., 2003a). This offers exciting possibilities for the future. The effect of CT in *L. cuneata* (52 g of CT/kg of DM) on the immune response was compared with a control forage low in CT (crabgrass/tall fescue; 2.0 g of CT/kg of DM) in grazing Angora does (Min et al., 2003a). Immune response was greater ($P > 0.01$) for *L. cuneata* than for the control at 12 and 24 h after injection of 250 μg of phytohemagglutinin. Furthermore, grazing lambs on CT containing forage (*H. coronarium*) was associated with higher antibody titers of secretory-excretory antigens against adult worms (Niezen et al., 2002).

The FEC and parasite burdens at slaughter (Table 2) were considerably lower for lambs grazing *H. coronarium* (CT-containing forage) than for lambs grazing *M. sativa* (Niezen et al., 1995; 1998a,b). Dewormed lambs grew at similar rates when grazing *H. coronarium* or *M. sativa*. However, nondewormed lambs grew much better on the *H. coronarium*, indicating a reduced need for anthelmintic drugs to control GIP in grazing lambs. Recently, Min et al. (2003b) showed that GIP were controlled when Angora does were grazed (81 d) on *L. cuneata* (52 g of CT/kg of DM) in spring and summer, but not when goats were grazed on control forages (crabgrass/tall fescue; 2.0 g of CT/kg of DM). Tracer goats that grazed *L. cuneata* had a 76% reduction in total adult worm burdens compared with the control. The *L. cuneata* diet was also associated with a reduction in the numbers of *Haemonchus* (94%) and *Teladorsagia* spp. (100%) in the abomasum and *Trichostrogylus* (45%) in the small intestine.

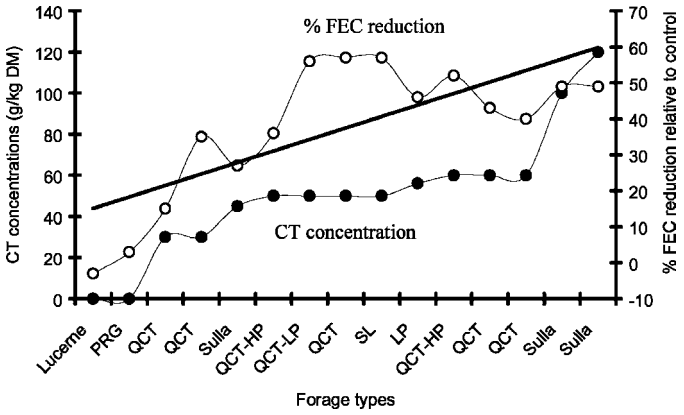

Figure 3. The effect of forage condensed tannins (CT) concentration (●) on percentage fecal egg count (FEC) reduction (○) relative to control. Calculated as (CT active – CT inactive group) ×100/CT inactive group. PRG = perennial ryegrass/white clover, QCT = Quebracho CT, QCT-HP = QCT-high protein, QCT-LP = QCT-low protein, SL = Serricea lespedeza; LP = *Lotus pedunculatus*. References: Terrill et al. (1992); Niezen et al. (1995; 1998a,b); Jackson et al. (1996); Athanasiadou et al. (2000a,b; 2001), Butter et al. (2000); and Min et al. (2002b).

Fecal egg count reductions (percentage) from the action of CT have been plotted against CT concentrations for sheep and goats fed either CT-containing forages or drenched with CT extracts from plants (Figure 3). The results show that FEC were reduced by 50% with CT-containing forages (45 to 55 g of CT/kg of DM) relative to nonCT-containing forages. When CT concentration increased above 55 g of CT/kg of DM, the responses became variable, as shown in Figure 3. However, when CT concentration decreased below 45 g of CT/kg of DM, the FEC response was inconsistent. Therefore, beneficial effects of CT in plants for FEC occur in the range 45 to 55 g of CT/kg of DM. It is not yet fully understood how CT affects GIP, and this area merits further study.

The effect of CT in *L. cuneata* (46 g of CT/kg of DM) on FEC in goats has been studied using a crossover design with ryegrass/crabgrass (0.6 g of CT/kg of DM; Min et al., 2002b). Goats that consumed *L. cuneata* had significantly lower FEC (1,162 eggs/g) than goats that grazed on nonCT-containing control forage (2,722 eggs/g). This research (Min et al., 2002b) showed a 57% reduction in FEC and a 61% reduction in total fecal egg output in goats that consumed forage *L. cuneata* (66×10^4 eggs/d) compared with control forage (168×10^4 eggs/d). These results suggest that forage containing 5% extractable CT may substantially reduce the contamination of pastures with infective larvae and result in reduced need for anthelmintic drenches.

More controlled studies have demonstrated an effect of CT (0 to 60 g of CT/kg of pelleted food) from Quebracho extract (from the bark of the tropical dicotyledon *Schinopsis* spp.) on nematode egg production (Athanasiadou et al., 2000b; 2001). Per capita fecundity of worms

(FEC [eggs/g] divided by the total number of female worms recovered) of *Trichostrongylus colubriformis* was reduced in sheep drenched with 16% Quebracho CT (wt/wt) of food intake compared to worm fecundity in control sheep (0.055 vs. 0.181 eggs/female worm per day; Athanasiadou et al., 2000b; 2001). However, the effect of CT on worm fecundity could not be conclusively attributed to CT. Athanasiadou et al. (2000a) reported that utero fecundity of worms was not significantly different amongst sheep given different levels of Quebracho CT treatments. Niezen et al. (1998a) also showed that different forage types, including CT-containing forages, had different effects on the fecundity of worms (utero eggs per female nematode) among different major gastrointestinal nematodes. Therefore, the effect of CT in many other CT-containing forages on fecundity of worm needs to be assessed.

CT may enhance resistance of GIP infection through increases in protein supply, which are prioritized for tissue repair and immune response (Barry et al., 2001; Niezen et al., 2002). The CT could complex with nutrients and inhibit nutrient availability for larval growth or decrease GIP metabolism directly through inhibition of oxidative phosphorylation (Scalbert, 1991), causing larval death (Athanasiadou et al., 2001). Inhibition of the electron transport system by CT was observed with *Photobacterium phosphoreum* (Scalbert, 1991). Molan et al. (2000; 2002) have shown that CT extracted from several forages can disrupt the life cycle of nematodes by preventing their eggs from hatching and by preventing larval development to the infective stages. Molan et al. (2000; 2002) suggested that the extracted CT from forages (400 μg of CT/mL) are more potent inhibitors of egg hatching and larval development (87 to 100%) than of larval motility (21 to 39% inhibited). Furthermore, in vitro larval development was evaluated under conditions similar to those observed in field conditions, with CT in fecal material from digested forage showing that CT reduced larval development by 69% (Min et al., 2003b). Therefore, it seems that CT may counteract parasites by one or more of the above mechanisms, and that the mechanism involved may differ between different forage species. If CT binding were due to their physical characteristics, greater precipitation would be expected with higher MW CT molecules (Scalbert, 1991). Scalbert (1991) also pointed out that if the binding mechanisms of CT and flavanols are related, we might deduce that PD will be more inhibitory than PC. The CT from *L. cuneata* (Table 1) is predominantly of PD type of subunits. Therefore, chemical composition and molecular size of CT, as well as concentration, may be factors in GIP control.

Implications

Condensed tannin-containing forages have the potential to help control anthelmintic-resistant gastrointestinal parasites. They have been shown to decrease fecal egg counts in sheep and goats and may decrease hatch rate and larval development in feces. This reduces pasture contamination and ingestion of infective larvae and by itself might provide adequate control of gastrointestinal parasites.

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Does supplemental dietary microbial phytase improve amino acid utilization? A perspective that it does not

O. Adeola[1] and J. S. Sands

Department of Animal Sciences, Purdue University, West Lafayette, IN 47907

ABSTRACT: Environmental concerns regarding the excretion of large quantities of P in effluents from intensive animal production operations have led to the current routine use of microbial phytase. Following extensive investigations, microbial phytase supplementation of plant-based diets has been shown to consistently improve the utilization of plant phytin-bound P, and a plethora of data is available in the literature to support this. The release of P from plant phytin during the digestion process is theorized to release other nutrients that may be bound in the phytin complex. Furthermore, hydrolysis of phytin is hypothesized to attenuate the inhibitory effect of phytin on digestive enzymes and consequently to ameliorate the depression of nutrient absorption. Although a limited pool of data exists on small increases in apparent amino acid digestibility in swine and poultry literature, these increases have seldom translated into improved growth performance when the effect of enhanced phytin P utilization is factored out. Conversely, there are also data on a lack of response in amino acid utilization (both pre- and postabsorptive) to microbial phytase supplementation. Several factors might play important roles in amino acid utilization response to dietary microbial phytase supplementation. Identification of such factors and quantification of their effects on the magnitude of response to phytase would be important in ascribing a meaningful "amino acid response factor" (or amino acid equivalency value) to supplemental microbial phytase in plant-based diets and in moving the swine and poultry nutrition industry ahead.

Key Words: Amino Acids, Digestibility, Phytase, Phytin, Pigs, Poultry

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Introduction

A significant proportion of the P in mature cereal grains and oilseeds is present as phytin P. These cereals and oilseeds in plant-based diets fed to livestock contain substantial quantities of phytin, which is poorly digested by swine and poultry, as seen in the excretion of considerable quantities of P in manure from intensive operations. The accumulation of P in soils may eventually result in run-off and, along with N, lead to eutrophication of surface waters, a condition that is detrimental to aquatic animals. The poor digestive utilization of phytin-bound P by monogastric animals and its consequences on diet cost, environment, and digestibility of minerals and proteins have lead to extensive research efforts directed toward understanding the process of phytic acid digestion. Supplementation of diets with microbial phytase has proven to be an effective and realistic method for enhancing the digestibility of phytic acid in monogastric animals. However, supplementation of diets with microbial phytase does not consistently enhance the digestibility of nutrients other than P that may be bound to phytic acid.

The nutritional (Cheryan, 1980; Ravindran et al., 1995; Selle et al., 2000) and environmental (Jongbloed and Lenis, 1998) consequences of phytin, as well as the application, structure, and kinetic properties of phytase (Dvorakova, 1998; Liu et al., 1998; Maenz, 2001), have been the topics of a number of excellent reviews. The intent of this paper is not another extensive review, but rather to provide a perspective that microbial phytase supplementation of diets does not improve amino acid utilization. This brief overview looks at factors that may play roles in amino acid utilization response to dietary microbial phytase supplementation. Identification of such factors and quantification of their effects on the magnitude of response to phytase would be important in ascribing a meaningful "amino acid response factor" (otherwise referred to as amino acid equivalency value) to dietary supplemental microbial phytase.

Structure and Occurrence of Phytic Acid

Phytic acid is chemically described as myo-inositol 1,2,3,4,5,6-hexakis dihydrogen phosphate (Maga,

[1]Correspondence: phone: 765-494-4848; fax: 765-494-9346; E-mail: ladeola@purdue.edu.

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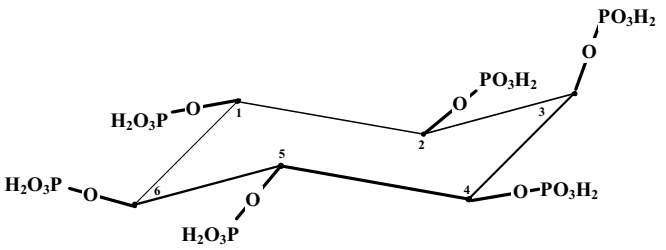

Figure 1. Structure of fully protonated phytic acid (myo-inositol 1,2,3,4,5,6-hexakis phosphate). Adapted from Graf (1986).

1982). The fully phosphorylated myo-inositol ring exists in a chair conformation (Figure 1) in dilute solution (Maenz, 2001). There are 12 proton dissociation sites on the phytic acid molecule—six of which are strongly acidic—with an approximate pKa value of 1.5; three sites are weakly acidic with pKa values between 5.7 and 7.6; and the remaining three sites are very weakly acidic, with pKa values greater than 10 (Maenz, 2001). Dissociation of the protons leaves the molecule with several negative charges, which may attract positively charged molecules, and thus confers on phytic acid a high chelation capacity for multivalent cations and proteins when the pH is conducive. Chelates thus formed can exist either as soluble or insoluble complexes that precipitate out of solution (Cheryan et al., 1983; Champagne et al., 1990; Maenz et al., 1999), and this is both pH and concentration dependent.

Phytin constitutes between 0.7 and 2% of most cereal grains and oilseeds and serves as the storage form of P, representing 50 to 85% of the total P, or even higher in selected varieties (Cheryan, 1980). Concentrations of phytic acid, phytic acid P, and total P, as quantified by the classical iron precipitation method, were measured by Eeckhout and De Paepe (1994) and by a recently developed modified HPLC method (Kasim and Edwards Jr., 1998). The HPLC method allows the various proportions of inositol phosphates to be determined. Phytic acid associates with K^+, Mg^{2+}, and, to a lesser extent, Ca^{2+} to form phytin in plants. The size of globoid phytin crystals depends, to a large extent, on the ratio of divalent cations to K^+; ratios with higher divalent cations favor the formation of large insoluble crystals (Maenz, 2001). The site of phytin in the seed fraction varies between different grains, grains and legumes, and between legumes (Maga, 1982). The variable location of phytin in the seed fractions has further implications for processing of ingredients for use in animal feeds. The site of phytin in several important cereal grains and oilseeds has been reviewed (Cheryan, 1980; Maga, 1982). Phytin is concentrated mainly in the bran (aleurone layer, testa, and pericarp) of cereal grains. In wheat and rice, most of the phytin is found in the outer layers—the pericarp and aleurone—with the endosperm almost devoid of phytin. More than 80% of the phytin in rice is concentrated in the bran fraction. In the case of corn, over 90% of the phytin is distributed in the endosperm and concentrated in the germ (O'Dell et al., 1972). In dicotyledonous seeds, including oilseeds and other grain legume seeds, phytin accumulates in globoid crystals that are evenly dispersed within protein bodies (Erdman, 1979). Phytin in peanuts, cottonseed, and sunflower are concentrated within globoids, which may serve as storage sites. It is interesting to note that phytin in soybean meal, as in other oilseeds, is closely associated with protein bodies, but is unique in that there appears to be no specific site of localization as it is distributed evenly in the seed. The structure, form, and site of phytin in grains and legume seeds may determine the extent of interactions with other nutrients, and thus could be important factors in digestive utilization of phytate by monogastric animals.

Factors Involved in Dietary Effects of Phytic Acid

Maenz (2001) summarized that phytin and protein can form binary complexes through electrostatic links of its charged phosphate groups with either the free amino group on arginine or lysine residues present within protein or with the terminal amino group on proteins. These binary phytin-protein complexes may be formed at acidic pH de novo in the gut from the protein bodies of oilseeds and in the protein-rich aleurone layers of cereal grains (Selle et al., 2000).

Furthermore, de novo formation in the gastrointestinal tract of loose electrostatic associations of phytin and proteins occur when optimal pH conditions exist (Maenz, 2001). At low pH, a deionized phytin-protein complex is formed as a result of charge effects, with the protein acting as the cation and the acid providing the anion. At low pH, the protein possesses a net positive charge and phytin is negatively charged, which results in a strong electrostatic phytin-protein interaction. Okubo et al. (1976) studied the pH range at which the glycinin component of soy proteins binds to phytin. No binding was observed above the isoelectric point (pH 4.9), with the extent of binding increasing with decreasing pH. The maximal binding of 424 equivalents of phytin per mole of glycinin was observed at pH 2.5. This value was found to correlate well with the total number of positively charged amino acid residues of glycinin at pH 2.5, which includes lysine, histidine, and arginine residues in addition to amino-terminal groups. These findings were supported by Omosaiye and Cheryan (1979), who observed that there was little or no removal of phytin by repeated ultra-filtration at pH 2 and that the phytin:protein ratio changed very little as compared to ultrafiltration at neutral pH. From this study, it can be hypothesized that the extent to which protein digestion is inhibited by phytin-protein interactions will vary between proteins due to differences in the total number of cationic groups available to participate in binding with phytate. The pH range in the stomach of pigs and the gizzard-proventriculus of the chicken

would facilitate the formation of phytin-protein complexes. The interaction between proteins and phytin may influence the enzymatic digestion of proteins in the stomach of pigs and in the gizzard-proventriculus of the poultry. It is also noteworthy that the stomach in pigs (Jongbloed et al., 1992; Yi and Kornegay, 1996) and the gizzard-proventriculus in chickens (Liebert et al., 1993) have been identified as the main sites of phytin hydrolysis. Thus, the formation of phytin-protein complexes may influence the rate and extent of phytin hydrolysis and, as a consequence, influence the hydrolysis of nutrients that may be complexed with phytin.

Under the prevailing pH conditions in the small intestine (>6.0), ternary complexes of phytate, Ca^{2+} and protein may be formed (Cheryan, 1980). These ternary complexes are formed only in the presence of divalent cations, especially, Ca^{2+}. It does seem that the cationic bridge formed by multivalent mineral facilitates the association of the negatively charged phosphate group on the phytin with the free carboxyl group of aspartic or glutamic acid residues within proteins, or the terminal carboxyl group of proteins, or the imidazole group of histidine (Selle et al., 2000). The stability of the ternary complex increases with pH up to pH 10, at which point the complex dissociates and the phytin becomes insoluble, whereas the protein remains in solution. Okubo et al. (1975) showed that filtration at pH 8.5 resulted in almost no removal of phytin. Reducing the pH to 7.1 increased the removal of phytin to some extent, but was still less than expected. When the pH was lowered to 5.0, there was a significant increase in phytin removal, indicating the absence of phytin-protein interactions at this pH. The dialyzable phytate in defatted soy flour was observed to be about 40% at pH 7.5 (de Rahm and Jost, 1979). Further, increasing the Ca^{2+} concentration reduced the dialyzable phytate, which suggested that Ca^{2+} was important in the formation of nondialyzable complexes. Okubo et al. (1975) and Gifford and Clydesdale (1990) also observed that multivalent cations are required in the formation of phytin-protein complexes. At intermediate pH, the formation of ternary phytin-Ca^{2+}-protein complexes may influence the enzymatic digestion of proteins in the small intestines of pigs and poultry. The extent to which such complexes influence protein digestion would be affected by the concentration of cations in the diet. Furthermore, since interactions between protein and phytin are mediated by cations, the addition of an organic acid, such as citric acid, may reduce the formation of the ternary complex by chelating free cations. Taken together, the form and extent of de novo formation of binary and ternary complexes of phytin and protein are likely to be important variables that influence the effectiveness of nutrient hydrolysis in plant-based diets.

The dietary effects of phytin may be mediated by its association with minerals. Phytic acid readily forms complexes with multivalent cations, with Zn^{2+} forming the most stable complex, followed by Cu^{2+}, Co^{2+}, Mn^{2+}, Ca^{2+}, and Fe^{2+} in decreasing order of stability (Maenz et al., 1999). Association of phytic acid with cations could result in the formation of either soluble complexes or insoluble chelates that precipitate out of solution. The degree of solubility of phytin-mineral complexes depends on the concentrations of phytic acid and cations and the pH of the solution (Cheryan, 1980). Complexes with monovalent cations, such as K^+ and Na^+, are soluble over the full pH spectrum, and most chelates with divalent cations are soluble at a pH less than 3.5 (Selle et el., 2000), implying that phosphate groups on the phytin molecule have a higher affinity for protons than do cations. This partial protonation of phytin will diminish the net involvement of cations with the molecule and therefore prevent the formation of insoluble complexes (Maenz, 2001). When concentration of divalent cations exceeds the concentration of phytin, insoluble chelates of phytin and mineral that precipitate out of solution are formed at neutral and basic pH. Again, this chelation process is likely to have profound influence on the efficiency of digestive utilization of nutrients.

Protein and Amino Acid Utilization Response to Microbial Phytase

In broad terms, phytases are classified as 3- and 6-phytase on the basis of the site on the phytic acid molecule of initial dephosphorylation. Generally, 3-phytases are of microbial origin (E.C. 3.1.3.8) and commence hydrolysis at the C3 atom of the inositol ring, whereas 6-phytases are of plant origin (E.C. 3.1.3.26) and commence phosphate cleavage at the C6 atom of the inositol ring (Dvorakova, 1998). There is no single enzyme that is capable of fully dephosphorylating phytic acid; therefore, a combination of phytase and nonspecific phosphatases are involved in the process (Maenz, 2001). Fungal species are the most widely used microorganisms for the expression of phytases (Liu et al., 1998). The phytase produced by *Aspergillus niger* is the most extensively studied and possesses two separate pH optima, one at 2.5 and one at 5.5, with a temperature optima of approximately 60°C. All of the agronomic species of cereals, legumes, and oilseeds possess some phytase activity, but only cereals such as barley, wheat, rye, and triticale possess appreciable amounts of phytase activity (Eeckhout and De Paepe, 1994).

The efficacy of phytase in dephosphorylating phytin in plant-derived ingredients and thereby improving its availability for pigs and poultry is established. However, the same cannot be said of protein and amino acid utilization responses to microbial phytase due to a number of conflicting reports. A model of phytase enhancement of protein and amino acid utilization or amino acid digestibility is therefore not consistently supported by available data. Selle et al. (2000) and Kies et al. (2001) provided an extensive review on current knowledge of the influence of phytin and phytase on protein utilization in pigs and poultry and proposed four possible phytin-protein complexes that can result in lower protein digestion. These include complexes

present in feedstuffs, de novo formation of protein-phytin complexes during intestinal transit in the animal, de novo formation of phytin-free amino acid complexes during gastrointestinal passage in the animal, and complexes involving phytin and proteolytic enzymes. Conceptually, protein and/or amino acids that are complexed with phytin may be less accessible to proteolytic enzymes during intestinal transit, and ternary complexes of phytin, cations, and protein formed during intestinal passage could potentially weaken the activity of proteases. This process, among others, may involve mineral chelation, and thus the removal of cofactors required for optimal proteolytic enzyme activity. Selle et al. (2000), however, concluded that the rationale for the protein responses to microbial phytase remains largely speculative, and several modes of action are probably involved. In one of the early experiments designed to scrutinize the possible adverse nutritional effects of phytic acid, Thompson and Serraino (1985) investigated the apparent total-tract amino acid digestibility response of rats to diets containing dephytinized or normal rapeseed flour. Results of the study did not support the theory of phytic acid reduction of amino acid digestibility since there were no differences in apparent or true total amino acid digestibility between dephytinized and normal rapeseed flours.

Whereas some studies show protein and amino acid digestibility response to microbial phytase supplementation, there are other studies in which dietary supplementation with microbial phytase had no effect on protein or amino acid utilization. The reader is referred to Selle et al. (2000), Kies et al. (2001), Ravindran et al. (2000; 2001), and Rutherford et al, (2002) for other reviews and studies that emphasize protein, amino acid, and energy responses to microbial phytase supplementation. In this section, studies that failed to show protein and amino acid utilization response to microbial phytase supplementation are emphasized.

Studies with Pigs

There are conflicting and inconsistent reports as to the efficacy of phytase for improving N or amino acid digestibility and retention in pigs. Phytase has been reported to increase digestibility (Mroz et al., 1994; Kemme et al., 1998) and retention (Keteran et al., 1993; Mroz et al., 1994) of protein and or amino acids in pigs. Interestingly, Keteran et al. (1993) observed changes in protein retention, despite the fact that the apparent digestibility of protein was not improved. Bruce and Sundstol (1995) also reported that phytase had no effect on the protein digestibility of pigs, and the study reported by Traylor et al. (2001) showed that phytase did not improve ileal digestibility of amino acids in soybean meal for pigs. Officer and Batterham (1992) fed diets containing 40% linola meal to 40-kg pigs and found that the addition of phytase increased the ileal digestibility of lysine and histidine, but produced nonsignificant increases for other amino acids. When diets consisting of corn, tapioca, soybean meal, barley, and pea as the main ingredients were fed to 45- to 110-kg pigs, an overall positive effect on ileal digestibility of amino acids was observed (Mroz et al., 1994). Kemme et al. (1999) studied the effects of phytase, lactic acid, and sodium phytate on apparent ileal digestibility of amino acids in 37-kg pigs. They concluded that, in general, phytase stimulated the apparent ileal digestibility of N and amino acids. Mroz et al. (1994) evaluated the effects of microbial phytase supplementation on nutrient digestibility in pigs that were surgically fitted with postvalve T-cannulae. Apparent total-tract digestibility, apparent ileal digestibility, and retention of nutrients in pigs fed a corn-, tapioca-, and soybean meal-based diet with no added inorganic P were determined. According to Mroz et al. (1994), microbial phytase enhanced the apparent total-tract digestibility of all amino acids except cysteine and proline and the apparent ileal digestibility of methionine and arginine.

Table 1 summarizes the results of three studies in which microbial phytase supplementation of P-adequate pig diets did not affect apparent total-tract digestibility or retention of nitrogen in pigs. Sands (2002) observed that microbial phytase supplementation of protein-adequate or -deficient diets did not affect N retention in pigs raised from 10 to 20 kg of BW. In a subsequent study, microbial phytase supplementation of low- or high-phytin diets had no effect on N digestibility or retention (Sands, 2002). Similarly, one of the data sets reported by Ketaren et al. (1993) indicated that

Table 1. The effects of microbial phytase on apparent total tract digestibility (%) and retention (%) in pigs

Study/diet	Phosphorus digestibility[a]	Nitrogen digestibility	Nitrogen retention
Sands (2002)[b]			
1. Adequate-protein diet	49.9	89.5	66.8
2. Diet 1 + phytase	63.8	88.3	63.4
3. Deficient-protein diet	48.4	85.6	55.9
4. Diet 3 + phytase	62.1	85.6	64.0
Sands (2002)[c]			
1. Low-phytin diet	23.7	77.3	—
2. Diet 1 + phytase	38.9	75.8	—
3. High-phytin diet	21.2	75.4	—
4. Diet 3 + phytase	43.9	73.7	—
Ketaren et al. (1993)[d]			
1. Soybean meal diet	—	—	42.8
2. Diet 1 + phytase	—	—	44.0

[a]Phytase effect, $P < 0.05$.
[b]A 20% CP corn-soybean meal diet containing 7.1% Ca and 4.0% P = adequate protein diet; 16% CP corn-soybean meal diet containing 6.9% Ca and 3.6% P = a deficient-protein diet for 10-kg pigs. Phytase was added to Diets 2 and 4 at 1,200 units/kg.
[c]Used a low-phytin diet containing 60% corn, 18% soybean meal, and 5% soy hulls containing 14% CP, 6.2% Ca, and 2.2% phytin or a high-phytin diet containing 60% corn, 8% soybean meal, 13.5% canola meal, and 5% rice bran containing 14% CP, 8.4% Ca, and 3.9% phytin for 30-kg pigs. Phytase was added to Diets 6 and 8 at 1,200 units/kg.
[d]Used a 60% sucrose, 37.3% soybean meal diet containing 16.9% CP, 0.6% Ca, and 0.25% P for 40-kg pigs. Phytase was added to Diet 10 at 1,000 units/kg.

Table 2. The effects of microbial phytase (1,200 units/kg) on apparent ileal digestibilities (%) of P and amino acids in pigs

Study/diet	Phosphorus[a]	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val
Traylor et al. (2001)[b]											
1. Diet	49.8	93.2	89.4	87.9	86.4	89.9	90.2	82.5	80.5	89.3	85.8
2. Diet 1 + phytase	66.9	93.1	89.8	88.8	87.6	88.8	89.0	80.9	78.7	87.7	85.1
Rice (2002)[c]											
1. Diet	39.0	86.3	82.1	76.2	79.0	78.7	77.6	80.1	70.8	85.1	74.9
2. Diet 1 + phytase	53.9	86.8	82.1	75.1	80.0	79.8	79.3	81.0	72.5	85.1	75.1
Sands (2002)[d]											
1. Low-phytin diet	16.3	88.0	86.6	76.5	81.9	76.2	72.9	81.0	73.1	90.8	76.7
2. Diet 1 + phytase	30.5	87.8	86.6	76.6	81.3	75.6	72.9	80.7	71.7	91.7	77.2
3. High-phytin diet	10.6	88.0	87.2	77.0	81.0	77.4	77.6	79.7	75.0	91.5	78.9
4. Diet 3 + phytase	36.1	87.0	86.1	75.1	80.7	75.1	77.3	79.2	74.2	92.3	77.3

[a]Phytase effect, $P < 0.05$.
[b]Used a 44.8% corn starch, 20% sucrose, 30.5% soybean meal diet containing 14.6% CP, 0.5% Ca, and 0.4% P (1% dicalcium phosphate) for 25-kg pigs.
[c]Used a 11% CP, 0.44% Ca, and 0.38% P diet for 60-kg pigs.
[d]Used a low-phytin diet containing 60% corn, 18% soybean meal, 5% soyhulls containing 14% CP, 6.2% Ca, and 2.2% phytin, or a high-phytin diet containing 60% corn, 8% soybean meal, 13.5% canola meal, 5% rice bran containing 14% CP, 8.4% Ca, and 3.9% phytin for 30-kg pigs.

microbial phytase supplementation of P-adequate diets did not affect N retention in 40-kg pigs (Table 1). Data from four studies in which phytase had no effect on ileal digestibility of amino acids in pigs are presented in Table 2. Phytase supplementation of a 14.5% protein diet (soybean meal as the sole protein source) failed to improve ileal digestibility of amino acids in pigs fitted with simple T-cannula (Traylor et al., 2001). In pigs fitted with steered ileo-cecal valve cannula that allowed for a complete collection of digesta, microbial phytase supplementation of an 11% (low) protein diet had no effect on ileal digestibility of amino acids (Rice, 2002). The data of Sands (2002) also showed that microbial phytase supplementation of low- or high-phytin diets did not improve ileal digestibility of amino acids in pigs fitted with simple T-cannula.

Studies with Poultry

Supplementing a variety of cereals, oilseed meals, and cereal byproducts fed to 5-wk-old broilers with 1,200 phytase units/kg of diet improved the ileal digestibility of all amino acids (Ravindran et al., 1999). The results of this study also revealed significant negative correlations between dietary phytin concentration and CP digestibility and mean amino acid digestibility of the ingredients evaluated, as well as a significant negative correlation between inherent amino acid digestibility and phytase response. This finding led to the suggestion that the solubility of phytin and proteins influencing the degree of phytin-protein complexes in different ingredients may be more relevant than total phytin concentration. Ravindran et al. (2000), using broiler chicks, also observed that apparent ileal digestibility of essential amino acids was negatively influenced by dietary phytin and that these negative effects were alleviated by the addition of microbial phytase. Furthermore, the digestibility of amino acids in broilers was improved by microbial phytase supplementation of a lysine-deficient diet (Ravindran et al., 2001). In turkey experiments, Yi et al. (1996) observed that adding phytase to a low-P diet improved N retention, and phytase supplementation of a low-protein diet increased ileal digestibility of amino acids.

Results of studies relating to the effects of microbial phytase on protein utilization in broiler chicks are summarized in Table 3. In growth assays wherein soybean meal was fed as the sole source of protein to provide 5, 10, or 15% protein, or corn gluten meal was fed as the

Table 3. The effects of microbial phytase on apparent nitrogen retention (%) and protein efficiency ratio in poultry (grams of weight gain/gram of protein intake)

Study/diet	Nitrogen retention	Protein efficiency ratio
Peter and Baker (2001)[a]		
1. 5% CP from soybean meal	25.1	4.22
2. Diet 1 + phytase	19.3	4.25
3. 10% CP from soybean meal	40.9	3.69
4. Diet 3 + phytase	45.5	3.83
5. 15% CP from soybean meal	46.3	3.34
6. Diet 5 + phytase	47.4	3.33
7. 10% CP from corn gluten meal	—	1.47
8. Diet 7 + phytase	—	1.47
Ledoux and Firman (2001)[b]		
1. 90% of ideal protein	70.7	3.74
2. Diet 1 + phytase	68.8	3.76
3. 100% of ideal protein	65.7	3.54
4. Diet 3 + phytase	66.5	3.52

[a]Used diets containing 44 to 60% corn starch, 29.81% dextrose, 5% soybean oil, 2.09% dicalcium phosphate, and 1.3% limestone for chicks during the period 8 to 21 d posthatching. Phytase was added to Diets 2, 4, 6, and 8 at 1,200 units/kg.
[b]Used diets containing 57 to 64% corn, 23 to 34% soybean meal, 3 to 4% soybean oil, 1.8% dicalcium phosphate, and 1.2% limestone for chicks during the period 1 to 21 d posthatching. Phytase was added to Diets 2 and 4 at 1,000 units/kg.

Table 4. The effects of microbial phytase on apparent ileal digestibilities (%) of P and amino acids in poultry

Study/diet	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val
Biehl and Baker (1997)[a]										
1. Diet	93.4	95.7	96.4	95.1	93.3	78.2	81.2	90.8	—	95.1
2. Diet 1 + phytase	95.9	97.3	97.8	96.6	96.8	79.8	81.6	91.7	—	96.6
Sebastian et al. (1997)[b]										
1. Diet	91.6	85.2	82.5	89.5	90.2	91.5	86.9	79.0	—	85.6
2. Diet 1 + phytase	90.1	82.7	76.4	86.4	86.0	89.5	82.5	75.2	—	81.6
Namkung and Leeson (1999)[c]										
1. Diet	86.2	88.3	78.7	82.2	84.2	91.4	81.0	74.1	78.0	77.7
2. Diet 1 + phytase	82.6	84.3	82.8[e]	84.1	85.9	91.3	83.3	74.7	79.7	81.2[e]
Zhang et al. (1999)[d]										
1. Diet	91.8	89.6	87.4	90.0	88.2	90.4	89.6	81.4	—	86.7
2. Diet 1 + phytase	91.8	89.8	87.9	90.3	88.8	90.4	89.9	82.2	—	87.1

[a]True digestibility using cecectomized roosters fed 30 g of soybean meal containing 46.5% CP. Phytase was added to Diet 2 at 1,200 units/kg.
[b]Apparent ileal digestibility in chicks during the period from 1 to 21 d posthatching fed a 22.4% CP corn-soybean meal diet containing 0.58% Ca and 0.44% P. Phytase was added to Diet 2 at 600 units/kg.
[c]Apparent ileal digestibility in chicks during the period from 1 to 21 d posthatching fed a 23% CP corn-soybean meal diet containing 0.8% Ca and 0.6% P. Phytase was added to Diet 2 at 1,150 units/kg.
[d]Apparent ileal digestibility in chicks during the period from 1 to 21 d posthatching fed a corn-soybean meal diet containing 0.8% Ca and 0.6% P. Phytase was added to Diet 2 at 600 units/kg.
[e]Phytase effect, $P < 0.05$.

sole source of dietary protein to provide 10% protein, sulfur amino acids or lysine was first limiting, respectively. Microbial phytase supplementation failed to improve utilization of the first-limiting amino acids in these diets since neither N retention nor protein efficiency ratio was affected by phytase addition (Peter and Baker, 2001). In a similar vein, phytase addition to a diet with a full complement of amino acids (100% of ideal protein) or to a diet deficient in amino acids for proper growth (90% of ideal protein) had no effect on N retention or protein efficiency ratio (Ledoux and Firman, 2001). Table 4 summarizes the results of five studies in which microbial phytase supplementation of broiler diets did not improve apparent ileal digestibility of amino acids (Sebastian et al., 1997; Zhang et al., 1999) or true amino acid digestibility in soybean meal intubated into cecectomized roosters (Biehl and Baker, 1997). The diets fed to determine the ileal digestibility of amino acids were mostly corn-soybean meal-based and contained approximately 23% protein. Microbial phytase supplementation at between 600 and 1,200 units/kg had no effect on the digestibility of amino acids.

Conclusions

The evidence is incontrovertible that microbial phytase is effective for improving digestive utilization of plant-derived phytin P. However, literature-derived information presented herein point out, in several instances, a lack of microbial phytase-induced improvement in protein and amino acid utilization; therefore, the totality of research on phytase-induced effects on protein and amino acid utilization in pigs and poultry epitomizes a conflicting base of information. The challenge is to identify and quantify the factors involved in the inconsistencies in protein and amino acid utilization response to microbial phytase. There are three broad categories of factors that have the potential to affect an animal's response to dietary microbial phytase supplementation: feed factors, issues relating to experimental protocols, and animal factors. In the feed factor category, the concentration and source of phytin, protein quality, and concentrations of divalent cations, vitamin D, and mineral chelators in the diet are likely to affect protein and amino acid response to microbial phytase. Processing of diet and the site and method of sampling (especially in pigs with respect to ileal cannulation or slaughter methods) have the potential to affect response to phytase. Animal factors, including species, genetics, and sex, are likely to impact response as they relate to gastrointestinal transit time and pH, as well as brush-border phytase activity regulation. Until several of these factors are adequately quantified, essential information relating to when and how much response can be expected will be elusive.

Implications

The cost effectiveness of microbial phytase addition to swine and poultry diets would be considerably improved if it were established that the enzyme consistently improved protein and amino acid utilization. A lack of consistency in amino acid utilization response to phytase supplementation calls for caution in the use of any overly simplistic guidelines that ascribe an "amino acid response factor" to microbial phytase supplementation. Clearly, interactions among phytin, minerals, proteins, phytase, and protein-hydrolyzing enzymes and their effects on protein and amino acid utili-

zation by the animal are a multifaceted subject affected by several factors that merit further research. The opportunity exists to identify these factors, quantify the magnitude of impact of the factors, measure actual response for specific feed ingredients and diets, and use the information generated for a more accurate diet formulation that matches animal amino acid needs with dietary supply.

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Partitioning of energy intake to heat, protein, and fat in growing pigs

J. van Milgen[1] and J. Noblet

Unité Mixte de Recherches sur le Veau et le Porc, Institut National de la Recherche Agronomique, 35590 Saint-Gilles, France

ABSTRACT: Modeling aspects of energy metabolism in growing pigs involves establishing "rules" on the partitioning of dietary energy between protein deposition (PD), lipid deposition (LD), and heat production (HP) at a given point in time, as well as the changes that occur during growth. Growing pigs rarely retain more than 50% of their ME intake; the remainder is lost as heat. Part of the heat loss is due to the heat increment, which includes the transformation of dietary nutrients to PD and LD, and to the associated energy (ATP) cost. Consequently, different nutrients are used with different efficiencies and, due to the ATP cost associated with protein synthesis and turnover, PD is energetically less efficient than LD. Different modeling approaches have been adopted to represent partitioning of energy between PD and LD (e.g., by assuming minimal ratios of LD:PD, marginal LD:PD, and lipid:protein mass or the existence of an upper limit to PD). Most of the HP is associated with biophysical processes (e.g., "maintenance," physical activity) requiring ATP, which are not directly related to PD and LD. Since it is virtually impossible to obtain direct estimates of these ATP requirements, indirect methods must be used. For example, the cost of maintenance may be estimated by measuring the fasting HP. Estimates of the fasting HP typically range from 700 to 800 kJ/(kg of $BW^{0.60}$·d), which corresponds to 50 to 60% of the total HP. Also, HP associated with physical activity is an important component of HP (15%), but can be variable between individual animals. Feed intake in nonproducing, mature mammals theoretically equals maintenance energy requirements. This implies that, while maturing, maintenance will become an increasingly important component of energy intake. In addition, while maturing, a decreasing fraction of the energy intake above maintenance is used for PD. The result is that PD typically reaches a maximum at 60 to 80 kg in growing pigs and decreases thereafter. In contrast, with aging, an increasing fraction of the available energy is used for LD, and maximal LD may not be reached before slaughter (110 to 130 kg). In modeling, this has been represented by assuming that the aforementioned energy partitioning rules (e.g., minimal LD:PD ratio, upper limit to PD) change with BW and/or age.

Key Words: Energy, Pigs, Heat Production, Lipids, Models, Pigs, Protein

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Introduction

Growing pigs rarely retain more than 50% of the GE given. Although for most diets 80 to 90% of GE is digested, not all this energy is available for metabolism as energy will be lost in the urine and as methane. The ME content of a diet is the difference between DE and these 'material' energy losses.

All ME not retained by the animal is lost as heat. The retained energy (primarily protein and lipid) can be measured directly by the comparative slaughter technique. Although it requires simple equipment, it requires considerable labor and gives an estimate of the average energy retention over a longer period of time. Alternatively, partitioning of ME can be determined by measuring heat production. Most commonly, indirect calorimetry is used, which is based on the measurement of gas exchanges between the animal and its environment. When nutrients are oxidized, animals consume oxygen and produce carbon dioxide, whereas methane is produced by gut microbes during fermentation. These gas exchanges and the nitrogen excretion from protein catabolism combined with the stoichiometry of nutrient oxidation allow calculation of heat production (Brouwer, 1965). Calorimetry has the advantage over the serial slaughter technique in that it can be used to measure energy balance over successive short periods of time, even within days. We have further refined this technique to obtain estimates of different components of heat production (van Milgen et al., 1997; van Milgen and Noblet, 2000). Both techniques provide an estimate of the total energy balance of the animal,

[1]Correspondence: phone: +33 2 23 48 56 44; fax: +33 2 23 48 50 80; E-mail: jaap.vanmilgen@rennes.inra.fr.

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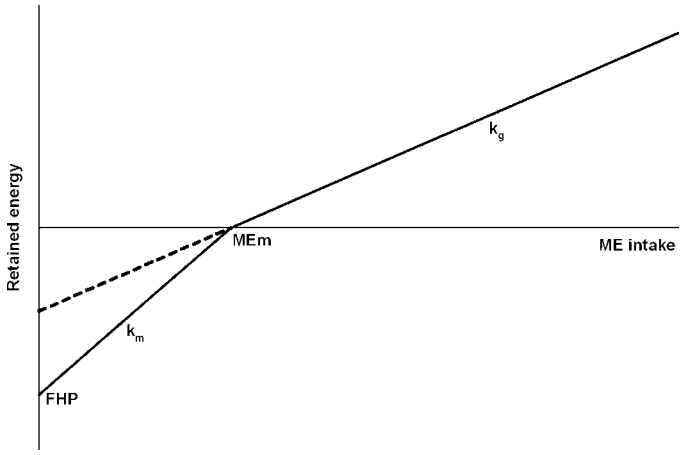

Figure 1. Relation between energy retention and ME intake. FHP = fasting heat production; ME_m = ME intake for maintenance; k_g = energy efficiency for growth; and k_m = relative efficiency of using dietary ME for maintenance compared to using ME from body reserves.

which, in combination with the nitrogen balance, allows calculation of lipid retention. The calorimetry technique typically gives higher estimates for energy and protein retention than does the comparative slaughter technique (Quiniou et al., 1995; Birkett and de Lange, 2001b). Although heat production may be affected by ambient temperature and health status, these aspects will not be considered here.

Early Energy Models

Early models addressing the response of an animal to a changing energy supply date back almost a century ago (for reviews, see Blaxter, 1962, and Emmans, 1995). Energy retention was seen as a two-stage process (Figure 1), with a breakpoint at the ME supply resulting in zero energy retention ($\mathbf{ME_m}$). The slopes of the line segments represent the energy efficiencies below maintenance ($\mathbf{k_m}$) and for growth ($\mathbf{k_g}$). At zero energy intake, animals will mobilize body reserves in order to cover the energy requirements for maintenance. It is important to realize that with increasing ME intake, dietary energy will progressively replace energy from body reserves to cover maintenance up to the point where the dietary ME supply equals ME_m. The k_m is therefore the efficiency of using dietary energy relative to using energy from body reserves to cover the maintenance energy requirement. The fact that it is a relative efficiency is the main reason that k_m is greater than k_g. If body reserves are used less efficiently than dietary energy for maintenance purposes, then k_m will exceed unity (see also the section on "partitioning of heat production–maintenance").

The assumption of constant energy efficiency above maintenance is difficult to justify. If the composition of the gain changes with ME intake, and if the efficiencies of protein and lipid deposition are different, k_g cannot be constant. To account for this, Kielanowki (1965) proposed that the ME intake of animal could be seen as the sum of the maintenance energy requirement and the energy required for protein and lipid deposition:

$$ME = ME_m + PD/k_p + LD/k_f$$

where PD and LD are the protein and lipid deposition (kJ/d), respectively, and k_p and k_f are the corresponding energy efficiencies. Although it is acknowledged that there is considerable variation in reported energy efficiencies, k_p is typically much smaller than k_f (0.60 and 0.80, respectively; Noblet et al., 1999). Consequently, more energy is required to deposit 1 kJ of energy as protein than as lipid. Due to the greater energy density of lipid, approximately 50 kJ of ME (39.8/0.80) is needed to deposit 1 g of lipid, whereas approximately 40 kJ of ME (23.8/0.60) is needed to deposit 1 g of protein. The efficiency of protein deposition is not to be confused with efficiency of depositing lean tissue, which consists primarily of water and protein. The result of the association of water and protein is that less feed energy is required to deposit 1 g of lean tissue than 1 g of adipose tissue. Kielanowski's approach has been criticized (Bernier et al., 1987; Emmans, 1995; Birkett and de Lange, 2001b) for various reasons including reversion of the controlled variable (ME intake) and the observed effect (changes in PD and LD) and multicollinearity or intercorrelation between the predictor variables resulting in inconsistent parameter estimates. In addition, the equation only considers "animal" aspects of energy metabolism, and differences in energy efficiencies between nutrients are not considered.

Partitioning of Heat Production

Maintenance

The concept of a maintenance energy requirement has been widely adopted by animal nutritionists, even though it may be difficult to define and/or measure it unambiguously (Knap, 2000; van Milgen et al., 2000). The idea behind this concept is to separate production costs from the maintenance cost by assuming additivity of the two processes. The ARC (1981) defined maintenance as "the requirement of nutrients for the continuity of vital processes within the body so that the net gain or loss of nutrients by the animal as a whole is zero." Feeding pigs at maintenance energy level does not imply that a constant body weight is maintained. During short periods of time, pigs can deposit protein at the expense of body lipid while maintaining zero energy retention and while gaining body weight (Le Dividich et al., 1980). It is unlikely that body lipids are used for protein deposition when growing pigs are fed to maintain body weight for prolonged periods of time (Lister and McCance, 1967). However, such experi-

ments may have severe consequences on the normal physiology of the animal. Despite the criticism and problems of measurement, the concept of maintenance has been widely adopted in animal nutrition.

The metabolic rate (or heat production per unit of time) has in the past been expressed relative to the body surface area. The surface areas of two bodies of similar shape and density but of different size are in proportion to the two-third power of their weights (Kleiber, 1975). Consequently, metabolic rate would be proportional to $BW^{0.67}$. Differences in BW should not be seen as the ultimate cause of changes in maintenance energy expenditure or metabolic rate, but rather as a convenient way to scale these (see Kleiber, 1975, for a critique on surface law theory). In many textbooks on energy metabolism, ME_m is assumed to be proportional to the three-quarter power of BW. This value originates from the comparison of fasting heat production (**FHP**) between different species of mature animals (Kleiber, 1975). When the maintenance energy expenditure is compared for animals of different BW within a species, the power is typically lower than 0.75 and, for growing pigs, a value close to 0.60 is often found (Brown and Mount, 1982; Noblet et al., 1999). The mode of expression has an important impact on estimated ME_m requirements at different body weights, and thus on predicted energy retention. For example, suppose that one has obtained a reliable estimate of ME_m at 60 kg of BW. If maintenance is constant per kg of $BW^{0.60}$, it would result in a 18% higher maintenance requirement at 20 kg of BW compared to assuming a constant maintenance requirement per kg of $BW^{0.75}$. However, at greater BW, the ranking is reversed so that at 120 kg of BW, ME_m is 10% lower when using the power 0.60 compared to using 0.75. Moreover, the choice of the power not only affects the maintenance energy requirement, but also the estimated energy efficiencies of protein and lipid deposition (Bernier et al., 1987; Noblet et al., 1999).

The maintenance energy requirement is essentially an ATP requirement. For a given ATP requirement, and with the knowledge that the efficiency of ATP synthesis differs between nutrients, the ME requirement for maintenance will be diet-dependent. This problem is partially circumvented in net energy systems, which use the FHP as an estimate of the maintenance energy requirement (Noblet et al., 1994). Although the FHP can be measured during reasonably standardized conditions, it varies with the length of fasting period and feeding level prior to fasting (Koong et al., 1982; de Lange et al., 2002). During fasting, energy from body reserves is mobilized in order to generate ATP for essential functions. However, normally fed growing animals will seldom mobilize body reserves (other than glycogen) in order to supply energy for essential functions. The direct utilization of measured FHP as an estimate of maintenance requirements is therefore, from a physiological point of view, incorrect. The extrapolated FHP obtained through regression (Figure 1) does not suffer from this drawback since nutrients for maintenance are (statistically) supplied by the diet, and the efficiency of ATP synthesis from the diet is accounted for. Birkett and de Lange (2001b) argued that the extrapolated FHP is independent of the diet, but depends on the relative contribution of protein and lipid to energy gain or loss. In a recent study on the energetic efficiencies of different nutrients (van Milgen et al., 2001), we estimated that the extrapolated FHP was 62% of the measured FHP in growing pigs. It was hypothesized that when growing animals are fasted for a short period of time, visceral organs will diminish rapidly in size, and nutrients from these organs (primarily proteins) will be catabolized to provide energy for maintenance functions. The efficiency of using energy from body reserves for these functions was greater than when using dietary protein (52%), but smaller than when using starch or lipid (84 and 88%, respectively), even though the latter are accompanied by an additional energy cost for ingestion, digestion, and absorption. Corresponding k_m values would be 84 (i.e., 52/62), 135, and 142% for protein, starch, and lipid, respectively.

The genotype (or leanness) also appears to have an important impact on FHP, with lower estimates for obese Meishan barrows and higher estimates for lean Piétrain boars (van Milgen et al., 1998). Exploiting data on the body composition of these pigs, it was concluded that viscera had a greater contribution to total FHP that did total muscle mass. This hypothesis is consistent with the aforementioned observation that the previous plane of nutrients affects FHP (Koong et al., 1982; de Lange et al., 2002). Some nutritional models directly or indirectly account for this by assuming that maintenance is a function of protein mass, protein turnover, or growth rate (Whittemore and Fawcett, 1976; Moughan and Smith, 1984; Knap and Schrama, 1996). Measured values for activity-free FHP range from 700 to 800 kJ/(kg $BW^{0.60}$·d) in growing pigs offered feed close to ad libitum (Le Bellego et al., 2001; van Milgen et al., 2001; Le Goff et al., 2002). Taking into account k_m and the energy cost of physical activity (see next section), current estimates for ME_m typically range from 850 to 1,000 kJ/(kg $BW^{0.60}$·d) (Noblet et al., 1999).

Physical Activity

Heat production due to physical activity is an important source of variation between different animals and may be affected by housing conditions. Energy expenditure per hour of standing appears at least fourfold greater in pigs than in other domestic species (Noblet et al., 1993). Different techniques exist to measure physical activity, including measurement of standing duration, motion detection, or force detection (e.g., Schrama et al., 1996; van Milgen et al., 1997). Heat production due to physical activity is estimated from statistical relations between the variation in heat production and variation in recorded physical activity. Consequently, the definition of "physical activity" has

an important impact on the heat production with which it will be associated. For example, in our laboratory, we measure activity as the standing duration (through interruption of infrared beams) and by continuous recording of vertical forces the pig exerts on its cage. It appears that approximately 60% of the total force is recorded when animals are lying (approximately 21 h/d) and the rest while standing (Le Goff et al., 2002). Changing our notion of activity (i.e., force detection rather than standing duration) has since doubled the heat production that we attribute to physical activity. Our current estimate is approximately 200 kJ/(kg $BW^{0.60}$·d) (Quiniou et al., 2001; van Milgen et al., 2001), which corresponds to 3 h of standing per day. This estimate is of similar magnitude as values determined by motion detection for group-housed pigs kept in a respiration chamber (Schrama et al., 1996). Physical activity appears rather variable between individual animals and can be affected by feeding level, type of diet, and genotype (Susenbeth and Menke, 1991; Schrama et al., 1996; Le Goff et al., 2002). Because of its contribution to heat production and thus to energy retention, it is important to obtain reasonable indicators of physical activity.

Thermic Effect of Feeding

The previous two components are mainly (but not exclusively) determined by the animal. The thermic effect of feeding (**TEF** or heat increment) is defined as the difference between the total heat production minus FHP and heat production due to physical activity (van Milgen and Noblet, 2000). In our quantification of components of heat production, we further distinguish a component that has a distinguishable dynamic relation to patterns of feed intake (the short-term TEF) and one that does not (long-term TEF). The long-term TEF is calculated by difference between the basal heat production in the fed state and the FHP. Processes such as hindgut fermentation and intermediary metabolism are thought to contribute to the long-term TEF. Heat production due to feed intake, digestion, and absorption are assumed to be part of the short-term TEF. The distinction between these two phenomena is, of course, arbitrary, but is required to estimate the different components of heat production. In most situations, it will be more practical to study the effect of diet variation on total TEF.

Different biophysical and biochemical processes contribute to the TEF. For instance, although lipid is quantitatively the most important form of energy storage in the body, dietary energy is mainly supplied as starch. The biochemical efficiency of the conversion of starch to lipid is 84% (Baldwin, 1995), so that for this conversion, at least 16% of the dietary energy is lost as heat. Theoretical efficiencies of using nutrients for lipid deposition decrease in the following order: lipid, starch, and protein. Experimentally determined values were 0.88, 0.84, and 0.52 for lipid, starch, and protein, respectively (van Milgen et al., 2001)–values that are similar to those used in the NE system (0.90, 0.82, 0.58; Noblet et al., 1994). In addition to the cost of nutrient transformation, the ATP utilization associated with metabolism has an important impact on TEF. Synthesis of a peptide bond from amino acids requires at least 5 ATP and, based on the efficiency of ATP synthesis, the maximal efficiency of protein deposition ranges from 85 to 90%. However, experimentally observed k_p values (approximately 60%; Noblet et al., 1999) suggest that considerably more ATP is required, and protein turnover (i.e., the repeated synthesis and breakdown of peptide bonds) may be one of the reasons for this difference (Reeds et al., 1981).

As indicated above, dietary protein is an inefficient energy source when used for purposes other than protein deposition. Apart from the material energy loss, four ATP are required to synthesize 1 mol of urea (2 ATP/mol N). This explains part, but far from all, of the energy loss associated with protein-rich diets. Le Bellego et al. (2001) observed that replacing 1 g of protein (given in excess of PD) with 1 g of starch lowered the heat production of pigs by 7 kJ. A considerable part of this heat production could be due to a diet-induced protein turnover (Reeds et al., 1981; Roth et al., 1999). The metabolic efficiencies of using dietary protein for protein deposition or for other energetic purposes (lipid deposition or ATP synthesis) were found to be of similar magnitude (van Milgen et al., 2001). The low energetic efficiency of using dietary protein implies that sufficient amino acids should be supplied in order to exploit the animals' potential to deposit lean tissue, but excess supply should be avoided (both from an energetic and environmental perspective). This favors diets with a balanced supply of amino acids. It has been shown that diets with CP levels as low as 12.3% can be used in growing pigs without affecting growth performance (Le Bellego et al., 2001; Noblet et al., 2001).

Partitioning of Energy Retention Between Protein and Lipid Deposition

Protein deposition in growing pigs not only depends on the supply of available amino acids, but also on the supply of energy. Although different mechanisms have been proposed to represent the relationship between PD and energy intake (e.g., ARC, 1981), the linear-plateau model has been most widely adopted. It assumes that PD increases linearly with energy intake up to a point where other factors start limiting protein deposition. The PD_{max} is the upper limit of PD, whereas the slope of the relation is determined by the partitioning of ME intake between PD and LD. Whittemore and Fawcett (1976) assumed that growing pigs maintain a minimal ratio between LD and PD, which thus determines the slope of the relation between PD and energy intake. Kyriazakis and Emmans (1992a,b) suggested that the efficiency of using ideal protein was a linear-plateau function of the dietary energy:protein

ratio. However, as this function intersects the origin, the approach is essentially similar to a linear-plateau function between PD and energy intake (by multiplication of both sides of the equation by the dietary protein supply). De Greef and Verstegen (1995) argued that if there are linear relations between PD and LD on the one hand and energy intake on the other hand, the LD:PD ratio will vary with energy intake due to the existence of intercepts at zero energy intake. They proposed existence of a minimal marginal LD:PD ratio (i.e., the ratio between the slopes of the linear line segments). De Lange (1995) assumed that the partitioning of energy retention was governed by the body lipid:protein mass ratio, rather than a constant LD:PD ratio or marginal LD:PD ratio. Koong (1977) and van Milgen and Noblet (1999) reversed Kielanowski's equation by specifying two related equations for PD and LD:

$$PD = k_pX(ME - ME_m)$$
$$LD = k_f(1 - X)(ME - ME_m)$$

In these equations, the energy supply above maintenance ($ME - ME_m$) is partitioned into a fraction designated toward protein deposition (X), whereas the complement ($1 - X$) is designated toward lipid deposition. These equations are functionally the same as Kielanowski's (resulting in similar values for k_p and k_f as in the original equation), but require a specific definition of energy partitioning (here specified as "X"). The partitioning of energy above maintenance will be affected by factors such as feeding level and body weight. Both approaches presumed that reducing feed intake increases X and thus results in leaner animals. Koong (1977) assumed that X varied with energy intake according to a Michaelis-Menten equation, whereas van Milgen et al. (2000) assumed that X was a linearly declining function of energy intake, up to the point where PD_{max} starts limiting PD. The latter results in a curvilinear plateau model for the relation between PD and energy intake. The approach described above indicates that both PD and LD are equal to zero when ME intake equals ME_m. As indicated earlier, there is experimental evidence that at low energy intakes, growing animals can mobilize body lipid while depositing protein (Le Dividich et al., 1980). However, such a physiological scenario is difficult to reconcile with the concept of maintenance (van Milgen et al., 2000). Nevertheless, a positive PD and negative LD remain possible if X is allowed to be greater than one.

Apart from choosing a mechanism of energy partitioning (e.g., PD_{max}, minimal LD to PD, minimal marginal LD to PD, minimal L to P, X), the parameters that describe these mechanisms will most likely change during the course of growth. Moreover, they depend on genotype, sex, and environmental conditions. Using animals fed close to ad libitum, van Milgen and Noblet (1999) observed that extremely lean boars maintained a constant partitioning of energy within the observed BW range (20 to 100 kg). For five other types of animals (including Large White females and barrows), the fraction of energy designated toward PD declined linearly with increasing BW. For the latter groups, this resulted in increased fatness as body weight increased. Black et al. (1976), using the linear-plateau model, assumed that the slope of the relationship between nitrogen retention and ME intake declined exponentially with increasing body weight. The NRC (1998) used a similar approach based on DE.

The choice of an appropriate function to describe variation in PD_{max} has been the subject of a rather intense debate. One of the problems is that ad libitum feed intake is not necessarily sufficient to attain PD_{max}; PD at ad libitum feed intake is therefore not to be confused with PD_{max}. Using different levels of feed intake at each BW, Möhn and De Lange (1998) observed little difference in PD_{max} at 25, 40, and 70 kg of BW. Black et al. (1986) described PD at ad libitum feed intake by an equation containing components of the logistic function, whereas the NRC (1998) used a polynomial function. Whittemore et al. (2001) described PD at ad libitum feed intake as a Gompertz function of present weight and mature BW. With this approach, PD reached a maximum at 60 to 80 kg of BW and approached zero as animals reached maturity. Van Milgen et al (2000) also used a Gompertz function (for PD_{max}), but described it as a function of age rather that mature BW. Although the choice between these functions (or any functions) will only marginally affect the predicted growth in pigs allowing ad libitum access to feed, it does affect results under specific growth conditions, such as compensatory growth. The underlying difference between these approaches is the question of whether growing pigs have a notion of "state" (i.e., current and mature body weight, protein, and/or lipid mass) vs. a notion of "age." Biological reality probably involves both.

In addition to these rather empirical descriptions of PD_{max}, some models are based on the separation of protein deposition in processes of synthesis and degradation. Pomar et al. (1991) assumed that protein synthesis was driven by a protein precursor pool, the kinetics of which varied according to a variant of the logistic function. Also, Lovatto and Sauvant (2003) described protein synthesis and degradation separately, each as an exponentially declining function of age (i.e., protein mass is described by a variant of the Gompertz function). These approaches undoubtedly represent biological reality better than empirical models of protein deposition. However, the difficulties that one will encounter during parameterization of these models will, in our opinion, preclude widespread practical utilization. Moreover, the types of functions that are used to pilot these models are very similar to models that consider protein deposition at a more aggregate level.

The hypothesis that energy partitioning at and below PD_{max} changes during growth results in relatively complicated PD response curves (e.g., Figure 9 in Black et

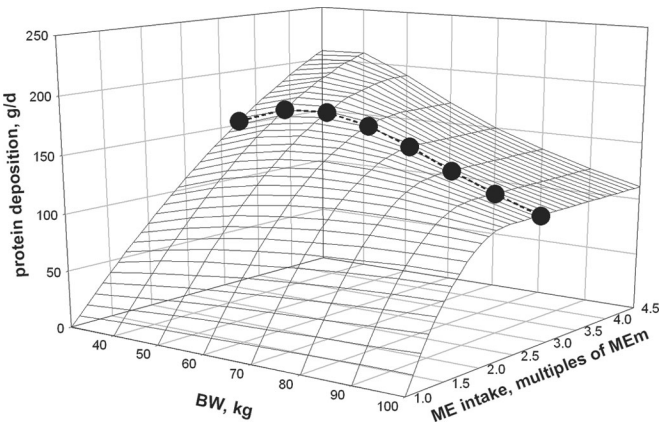

Figure 2. The response surface of protein deposition as a function of ME intake and body weight. For a given body weight, protein deposition is described by a curvilinear-plateau function of ME intake. The connected points indicate the feed intake capacity of a Large White barrow and the corresponding protein deposition. ME_m = ME intake for maintenance.

al., 1986 or Figures 3 and 4 in NRC, 1998). Figure 2 illustrates the PD response surface to ME intake during the growing and finishing period of pigs using the curvilinear-plateau model (van Milgen et al., 2000). The ad libitum feed intake is indicated by the dotted line. Light animals have a very high PD_{max}, which appears beyond the feed intake capacity, resulting in a PD that is much lower than PD_{max}. For heavier animals, PD_{max} is "within reach of appetite," but its value is considerably lower. The result is that total protein deposition does not necessarily change much during the growing phase (25 to 100 kg). However, the sensitivity to a changing energy supply is much greater in lighter vs. heavier animals. A reduction in feed intake will therefore result in a reduction of both PD and LD in light animals, whereas it will mainly affect LD in heavier animals.

Future Development of Energy Models

In the past, there have been different efforts to model metabolism, growth, and lactation (e.g., Pettigrew et al., 1992) based on biochemical pathways. This trend is currently continuing (Boisen, 2000; Chudy, 2000; Birkett and de Lange, 2001a), which is to be applauded because nutritional biochemistry contributes significantly to the overall energetic efficiency of growth. The consequence of this approach is that energy requirements are no longer expressed as DE, ME, or NE requirements, but rather as the nutrient (e.g., ATP) requirement for a specific process. Although such an expression may bear a close resemblance to physiological reality, we have to be careful in extrapolating this to the whole-animal level. If an animal requires ATP for a maintenance process (e.g., a sodium pump), it will convert a nutrient to ATP and then use this ATP for this process. Both the conversion of the nutrient to ATP and the actual ATP utilization will result in heat production. One may argue that the latter is diet-independent, whereas the former is not. However, there is currently considerable doubt concerning the stoichiometry of ATP synthesis from different nutrients. In older biochemistry textbooks, it is assumed that 1 mol of glucose yields 38 mol of ATP. Uncertainties concerning the coupling of ATP synthesis to mitochondrial oxygen consumption have resulted in estimates that are much lower: 31 ATP/glucose (e.g., van Milgen, 2002). This "new" finding would require that ATP-based energy systems would have to revise the efficiencies of ATP synthesis from nutrients as well as the whole-animal ATP requirements, even though the heat production of the overall process has not been changed. A more mechanistic approach to whole-animal energy metabolism should definitely not be discouraged. It is an intellectual effort that will prove its usefulness in the future as these approaches allow for a better quantification of the (relative) contribution of different processes involved in energy metabolism. Nevertheless, it is essential that these approaches are able to predict traditional indicators of energy metabolism with which end-users are accustomed.

The separation of nutrient requirements into a component for maintenance and one for growth has been (and continues to be) the basis for many energy systems. The maintenance energy requirement for growing animals remains a fuzzy concept, for which there is currently no suitable alternative. Although there are many uncertainties concerning the factors that affect maintenance, there is at least one certainty: the ad libitum ME intake in a mature, nonproducing animal equals the maintenance energy requirement, and both PD and LD equal zero (within very narrow margins). The argument that Whittemore et al. (2001) make in favor of the Gompertz function to describe PD_{max} (i.e., it equals zero for mature animals) can also be made for the ad libitum feed intake capacity above maintenance. Consequently, total feed intake consumption above maintenance during the course of life should be in close relationship with mature BW and body composition. This is a phenomenon that is little exploited in most models since feed intake is seen as some function of BW without a specific relation to maturity. Alternatively, a reverse approach has been proposed (e.g., Kyriazakis and Emmans, 1999) where feed intake is predicted relative to the growth potential. These approaches differ fundamentally in the way growth and feed intake are interpreted in terms of cause and effect. In the first approach, feed intake (while ignoring the force that drives it) results in growth, whereas in the latter approach, the animal has the desire to attain maturity and feed intake is a consequence of this. The very short life span of growing pigs (relative to maturity) probably makes the first approach easier to implement in pig growth models. However, if recovery mechanisms, such as compensatory feed intake and gain (e.g., after a period of heat

stress or after disease), are to be included in growth models, a more mechanistic description of feed intake regulation will be required.

It would not be realistic to suggest that current models (or those developed in the near future) are able to predict growth. Most models use both feed intake and the upper limit of protein deposition as user inputs. Rather than predicting growth, these models analyze growth by indicating the (nutritional) factors that are potentially limiting performance. Through its relation with body water, calibration of protein deposition is essential in order to "predict" growth. On the other hand, lipid deposition is calculated as the energy retention after protein deposition and its associated cost, and maintenance is accounted for. Although lipid deposition has less influence on performance than has protein deposition, it has an important impact on carcass quality. Errors in establishing the maintenance energy requirement will therefore have the greatest impact on lipid deposition. An alternative approach would be to use lipid deposition capacity or lipid mass as a user input (e.g., based on backfat thickness measurements) and consider maintenance, rather than lipid deposition as the residual phenomenon. Such an approach does not necessarily change the PD and LD response curves to energy, but it will shift our focus of attention from trying to predict the response at the maintenance feeding level to predicting the response at the ad libitum feeding level. From both a biological and experimental point of view, such an approach may prove to be more fruitful.

Implications

Feed intake capacity and the partitioning of metabolizable energy between protein deposition, lipid deposition, and heat production in pigs change considerably during the growing and finishing periods. Although this review only addresses the energy aspects during the course of life, these changes also affect other aspects of nutrition. For example, the partitioning of energy between protein deposition and lipid deposition determines amino acid requirements. As this partitioning changes during growth, so will the optimal ratio between amino acids and energy. Traditionally, nutrient requirements originated from feed recommendation tables, and feeds were formulated to meet or exceed these requirements. As systems of energy and amino acid utilization become more refined, the classical notion of "requirement" and "feed value" becomes less clear. The use of mathematical models then becomes essential to quantify the growth response of an animal to changes in nutrient supply.

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Heterogeneity of protein expression within muscle fibers[1]

B. W. C. Rosser* and E. Bandman†[2]

*University of Saskatchewan, College of Medicine, Department of Anatomy and Cell Biology, Saskatoon, Saskatchewan, S7N 5E5, Canada and University of California, Department of Food Sciences and Technology, Davis 95616

ABSTRACT: Skeletal muscle fibers are elongated multinucleated cells. Along its length, an individual fiber may contain thousands of myonuclei, each controlling protein synthesis within its surrounding cytoplasm. Therefore, a fiber can be considered to be a series of myonuclear domains, each responding to distinct localized signaling mechanisms that may result in differential gene expression within the fiber. This brief review examines phenomena that produce distinct subsets of proteins within different regions of a muscle fiber. These include changes in protein expression associated with activity-induced fiber-type transformation, muscle development, and denervation. Myosin heavy-chain (MyHC) proteins are fundamental structural and functional components of the muscle fiber. They are represented by different isoforms, each of which is the product of a separate gene that may be differentially expressed during the development of distinct muscle fiber types. We have found that in mature chicken and pigeon pectoralis muscle, the tapered ends of fibers contain the neonatal MyHC isoform in addition to the adult isoform found throughout the lengths of the fibers. Examination of serial sections along the length of muscle fibers of chicken pectoralis at different stages of development illustrates that repression of neonatal MyHC isoform expression proceeds as a gradient from the centrally located motor endplate toward the ends of a fiber. In denervated mature fibers, myonuclei furthest from the endplate are the first to reexpress neonatal myosin. We hypothesize that trophic factor(s) emanating from the vicinity of the motor endplate represent a potential localized signaling pathway that may differentially modulate MyHC gene expression along the length of the muscle fiber. Muscle fibers grow in length by the addition of new sarcomeres to their tapered tips, and growing fibers have smaller myonuclear domains (less cytoplasm per nucleus). Additional experiments using chicken pectoralis demonstrated that myonuclear domains are significantly smaller in those areas of the fibers expressing predominantly neonatal myosin. In maturing muscle, the volume of cytoplasm per nucleus is less within the ends of the fibers. Thus, when an increase in the expression of one or more gene products is required within a specific region of the muscle fiber, transcriptional output may be enhanced by the concentration of myonuclei within that region.

Key Words: Denervation, Development, Muscle, Muscle Fibers, Myosins

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Introduction

Vertebrate skeletal muscle cells caudal to the brachial arches are derived from myotomes of the somites (Wigmore and Evans, 2002). In early embryonic development, myogenic regulatory factors are responsible for transformation of these mesodermal cells into myoblasts (Perry and Rudnicki, 2000; Stockdale, 2000). Initially, each myoblast is a small spindle-shaped cell with a single central nucleus (Swatland, 1994). There are embryonic, fetal, and adult waves of myoblasts (Stockdale, 1997). Embryonic and fetal myoblasts divide repeatedly by mitosis and are highly mobile, aggregating into clusters. Subsequently, these myoblasts align with their long axes parallel to one another and fuse to form small, multinucleate cells called myotubes (Leiber, 1992). During growth and maturation, additional myoblasts are incorporated into each myotube. The resultant cells are elongate, fusiform-shaped multinucleate cells termed muscle fibers (McComas, 1996).

Muscle fibers, along with a few other select animal cell types, such as osteoclasts and cytotrophoblasts, are

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[2]Correspondence: phone: 530-752-2490; fax: 530-752-4759; E-mail: ebandman@ucdavis.edu.

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exceptional in that they are multinucleate (Allen et al., 1999). A typical fiber may contain hundreds or thousands of myonuclei (Cullen and Landon, 1994; Tseng et al., 1994). Each myonucleus regulates gene expression within the surrounding portion of the fiber (Hall and Ralston, 1989; Pavlath et al., 1989; Ono et al., 1994).

It has long been held that, with the exception of certain proteins associated with the motor endplate, expression of most proteins is relatively uniform along the lengths of skeletal muscle fibers. Certainly, this may be the case for some proteins (Pette et al., 1980). However, accruing evidence indicates that there is pronounced regional variation in the expression of many genes (Newlands et al., 1998; Apel et al., 2000; Rossi et al., 2000). It is the purpose of this review to highlight some of the research showing heterogeneity of gene/protein expression within individual muscle fibers.

Protein Subsets within Specialized Regions of the Muscle Fiber

The neuromuscular junction (**NMJ**) is the site at which a motor neuron communicates with a muscle fiber, and is comprised of highly specialized portions of three cells: motor neuron, Schwann cell, and muscle fiber. Due to its accessibility, the NMJ has been widely used by neurobiologists as a model for the study of synaptic development. Consequently, the NMJ has been one of the most studied regions of the muscle fiber. Numerous proteins involved in complex signaling pathways are localized at the NMJ. As a complete description of NMJ gene expression is well beyond the scope of this paper, for a comprehensive overview the reader is referred to review articles (Meier and Wallace, 1998; Sanes and Lichtman, 1999).

Acetylcholine receptors (**AChR**) are initially distributed evenly and at low densities within the plasmalemma along the length of developing muscle fibers. Two trophic factors, agrin and neuregulin, pass from the developing nerve terminal to the muscle fiber. Each of these factors act collectively to concentrate AChR beneath the nerve terminal. Agrin cooperates with muscle-specific tyrosine kinase to coordinate AChR clustering by the AChR-associated protein rapsyn. Neuregulin interacts with erbB kinases to stimulate selective expression of AChR subunit genes by synaptic myonuclei. Calcitonin gene-related peptide is another nerve-derived trophic factor that stimulates AChR synthesis, although gene knockout studies indicate that it is not as crucial as agrin and neuregulin (Meier and Wallace, 1998). The neurotransmitter acetylcholine indirectly represses AChR subunit gene expression by extrasynaptic myonuclei (Sanes and Lichtman, 1999). After development, although the NMJ comprises less than 0.1% of the muscle fiber surface, it contains over 95% of the AChR (Tsim and Barnard, 2001). Myonuclei at the NMJ are clustered and are larger and rounder than extrasynaptic myonuclei. Also, unlike extrasynaptic myonuclei, there is upregulation of synaptic genes (Apel et al., 2000).

The subsarcolemmal cytoskeleton of muscle fibers is organized into distinct compartments, including the NMJ, costameres (at each Z-line), and the myotendinous junction (**MTJ**). At these locations, multiple proteins form elaborate complexes that anchor actin filaments to the sarcolemma. Fibronectin, laminin, vinculin, talin, and other proteins are especially concentrated at the MTJ (Tidball, 1991; Berthier and Blaineau, 1997). Again, since a thorough explanation of these regions is beyond the scope of our paper, the reader is referred to a review (Berthier and Blaineau, 1997).

Fiber ends display localized expression of additional proteins. Acetylcholinesterase is concentrated at the tips of some fibers, although its purpose there is not fully understood (Trotter, 1993; Rosser et al., 1995; Paul and Rosenthal, 2002). Myostatin is a negative regulator of muscle growth. It is normally present at the ends of muscle fibers of the mature rat and is elevated by muscle unloading (Wehling et al., 2000). In addition, the expression of certain myogenic regulatory factors (MyoD and myogenin) have been reported to be greater at the ends of fibers in mature rat muscle. The expression of these factors along the lengths of the fibers is increased by stretch-induced hypertrophy (Zador et al., 1999).

Heterogeneity of Myosin Heavy-Chain Expression within Fibers

Myosin is a fundamental structural and functional component of all skeletal muscles. In skeletal muscle, the myosin molecule is a hexamer consisting of two heavy (**MyHC**) and four light chains (Lowey, 1994). A diverse multigene family encodes MyHC (Bandman et al., 1994; Weiss and Leinwand, 1996; Shrager et al., 2000). There are many biochemically distinct but related MyHC isoforms that are expressed during various stages of development (Tidyman et al., 1997; McKoy et al., 1998) and in a functional muscle fiber type-specific manner (Bottinelli et al., 1994; Reiser et al., 1996).

There is a sequential expression of MyHC isoforms within developing and maturing muscle fibers. This is true of both fast- and slow-contacting fibers (Bandman and Rosser, 2000). Because this differential expression is usually gradual, developing and maturing fibers normally coexpress more than one MyHC isoform (Gordon and Lowey, 1992; Gauthier and Orfanos, 1993; Baldwin and Haddad, 2001).

It was formerly held that each mature skeletal muscle fiber type expressed a characteristic MyHC isoform, and that those mature fibers observed expressing multiple isoforms were undergoing a fiber type transition in response to altered functional demands (Pette and Staron, 1997). Whereas increased neuromuscular activity, mechanic loading, or hypothyroidism induced a change from fast to slow MyHC isoforms, the opposite

initiated a transition from slow to fast MyHC. This dynamic process yielded transitional fibers, each containing a mix of MyHC isoforms (Pette and Staron, 2000; Baldwin and Haddad, 2001).

It is now appreciated that large numbers of mature fibers in normal muscle may typically express more than a single MyHC isoform (Peuker and Pette, 1997; Wu et al., 2000; Lefaucheur, et al., 2002). It is thought that these fibers are stable hybrids and not transitional fiber types (Stephenson, 1999: Lutz and Lieber, 2000). However, it is not generally known that these fibers may exhibit pronounced heterogeneity of MyHC expression along their lengths.

We have shown that in addition to the mature (adult) MyHC isoform normally expressed throughout the length of a fiber, the ends or terminal tips of fast-contracting fibers of mature chicken (*Gallus gallus*; Rosser et al., 1995) and pigeon (*Columba livia*; Bartnik et al., 1999) pectoralis muscle express an isoform characteristic of earlier development. Heterogeneity of MyHC expression along the lengths of individual fast fibers was also observed in studies of adult rabbit hind-limb muscles (Peuker and Pette, 1997). Similarly, multiple myosin isoforms were found to be synthesized and localized differentially along the lengths of certain extraocular fiber types of mature mammals (Lucas and Hoh, 1997; Rubinstein and Hoh, 2000; Porter, 2002). Variation in MyHC isoform expression was also observed along the lengths of intrafusal muscle fibers of mature rat (Kucera et al., 1992), human (Liu et al., 2002), and chicken (Maier, 1994). MyHC composition was reported to vary significantly between consecutive 1-mm segments along the length of fibers from mature frog hind-limb muscles (Lutz et al., 2001). In experimentally injured leg muscles of chicken, a MyHC isoform characteristic of early development was reexpressed within the damaged parts of the muscle fibers (Zhang and Dhoot, 1998).

Speed or velocity of contraction has been directly correlated with MyHC isoform content in single fibers from chicken (Reiser et al., 1996) and mammals (Bottinelli et al., 1994; Hilber et al., 1999; Bottinelli and Reggiani, 2000). It has been postulated that variation of MyHC content along a fiber's length should yield correspondingly different contractile properties within the fiber (Rubinstein and Hoh, 2000; Lutz et al., 2001). Similarly, it has been suggested that variation in myosin content along fiber lengths might be responsible for the differences observed in contractile properties of adjacent segments of single fibers from mature humans (Wilkins et al., 2001).

Development and Influence of Innervation

Each embryonic muscle fiber initially receives input from many motor neurons. In both birds and mammals, this polyneuronal innervation is lost from twitch fibers by the early postnatal stages (Navarette and Vrbova, 1993; Bennett, 1999). Subsequently, each twitch fiber is normally innervated by one motor neuron at a single motor endplate located on the central one-third of the fiber's length (Trotter et al., 1992; Engel, 1994).

Innervation is essential for the typical myosin expression seen during development (Washabaugh et al., 1998; Pette and Staron, 2001). Innervation regulates MyHC isoform expression in both fast and slow fibers of developing quail (*Coturnix coturnix*; Lefeuvre et al., 1996). Not only is proper motor innervation required for normal maturation of a fiber, it also represses traits representative of earlier development (Grinnell, 1994).

Myosin Heavy-Chain Expression at the Ends of Developing and Denervated Muscle Fibers

The avian pectoralis muscle is, in most birds, substantially larger than any other muscle of the body. In chickens and other birds, this muscle consists of serially arranged fibers that overlap one another to a considerable extent (Gaunt and Gans, 1993; Sokoloff and Goslow, 1998). A transverse section through the belly of such a muscle reveals populations of very small diameter fiber profiles that are, in fact, the tapered ends of larger fiber profiles seen in more distant sections (Swatland, 1983; Rosser et al., 2000).

In the chicken, the pectoralis consists almost exclusively of fast-twitch glycolytic (type IIB) fibers (see Rosser et al., 1996; Rushbrook et al., 1998). Within most of these fibers, at least six MyHC are expressed during development: ventricular, embryonic 1, embryonic 2, embryonic 3, neonatal, and adult (Hofmann et al., 1988; Tidyman et al., 1997; Rushbrook et al., 1998). Embryonic MyHC protein isoforms are largely supplanted by the neonatal MyHC isoform approximately 10 to 20 d after hatching (Bandman, 1985). Whereas the neonatal isoform first appears around hatching, an adult MyHC isoform appears approximately 20 d after hatching. The adult isoform almost totally replaces the neonatal isoform approximately 90 d after hatching (Bandman and Rosser, 2000). Cell culture experiments with chicken pectoralis show uneven acquisition of neonatal isoform along the lengths of the fibers (Cerny and Bandman, 1987a). Innervation is necessary for the usual embryonic to neonatal to adult MyHC isoform transformations observed in developing chicken pectoralis, and denervation experiments demonstrate that innervation also represses neonatal and embryonic MyHC isoforms in the mature muscle (Bandman et al., 1990).

There is a centrifugal gradient in MyHC transition within developing muscle fibers. We revealed this by following profiles of individual fibers in numerous serial cross-sections of chicken pectoralis (Figure 1; Rosser et al., 2000). During posthatch maturation, neonatal myosin was first lost from the largest fiber profiles. By locating motor endplates, we deduced that the neonatal-to-adult MyHC isoform change was initiated near the centrally located motor endplate of each muscle fiber (Rosser et al., 2000). Thereafter, during develop-

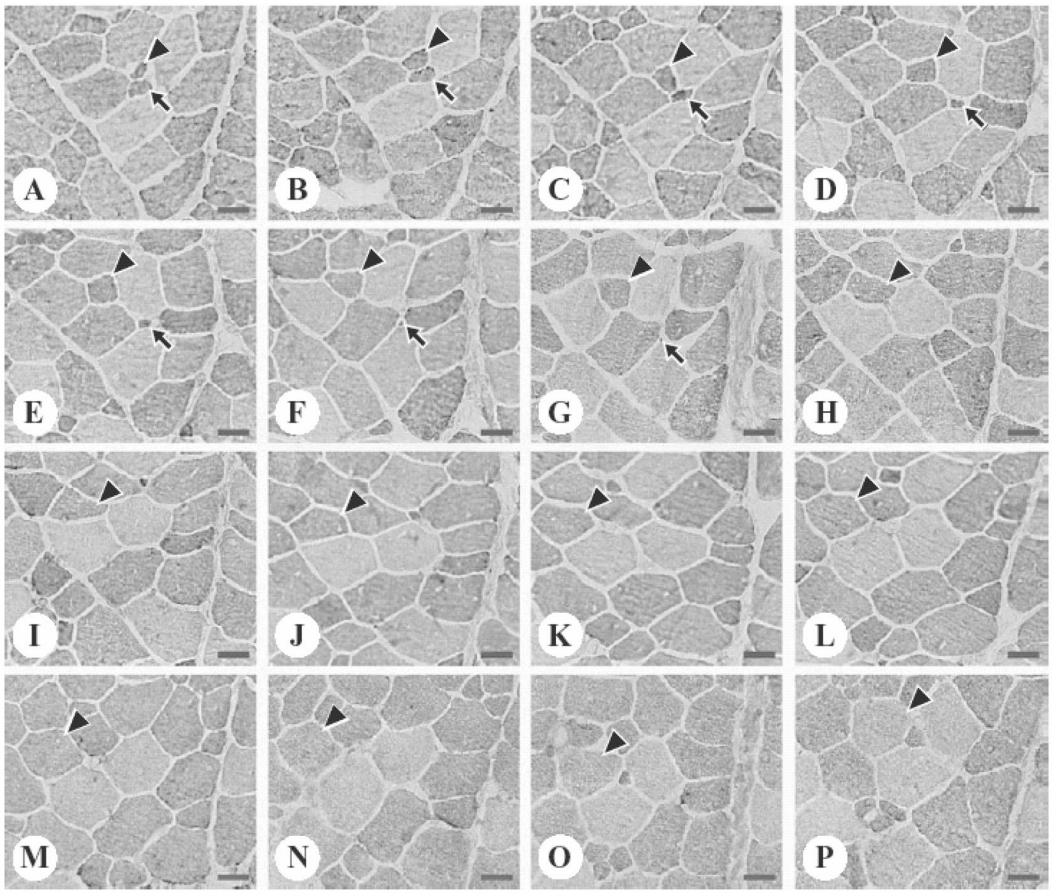

Figure 1. Immunocytochemical labelling of neonatal MyHC by 2E9 antibody, in serial sections of pectoralis muscle from a 41-d-old chicken. The distance of each section, in microns, from that in panel A is 360 (B), 680 (C), 1,180 (D), 1,300 (E), 1,600 (F), 1,760 (G), 2,220 (H), 2,540 (I), 2,720 (J), 3,240 (K), 3,560 (L), 3,820 (M), 4,220 (N), 4,400 (O), and 4,900 (P). Bar = 20 μm. The large arrowhead throughout the series, panels A to P, identifies sections (or fiber profiles) along the length of the same single fiber. The small arrow in panels A to G identifies serial sections through another fiber. Initially, in panel A, these two fiber profiles are of comparable diameter and 2E9 labeling intensity. The fiber profile indicated by the arrow diminishes in diameter from panel A until it is lost in panel H. The fiber profile indicated by the arrowhead increases in diameter but decreases in staining intensity as one proceeds through the sections from panel A. Small, darkly labeled fiber profiles are the tapered ends of larger more lightly labeled fiber profiles. (Figure reprinted with the permission of Wiley-Liss Inc., a subsidiary of John Wiley & Sons Inc., from Rosser et al., 2000.)

ment, this change progressed toward the fiber ends (Figure 2).

Denervation of chicken pectoralis for 8 wk results in reexpression of some neonatal MyHC isoform within all fibers (Cerny and Bandman, 1987b). We severed the nerves to the left pectoralis of mature chickens for 3, 7, 15, and 21 d and demonstrated that this reexpression progressed from the tapered fiber ends toward the central regions of the fiber (Figure 3; Rosser et al., 2000). The right pectoralis served as the control.

Our results are consistent with trophic factor(s) emanating from the motor endplate, which play a major role in modulating myosin expression. If membrane depolarization and/or twitch contractile activity were solely responsible for regulating myosin expression, one would expect uniform expression of any given myosin isoform throughout the length of the fiber. However, the regional distribution of neonatal myosin seen during both development and denervation indicates that an additional factor must be involved in regulating myosin expression.

Our data may also be consistent with a signal originating from the tapered ends of the fibers and dissipating near the center. This hypothesis has some support since a number of regulatory proteins are localized at fiber ends (see "Protein Subsets within Specialized Regions of the Muscle Fiber"). Nonetheless, our experiments do clearly show that denervation—which directly impacts the centrally located motor endplate—reverses the centrifugal gradient of neonatal MyHC repression normally seen during development.

Our observations have led others to propose that a centrifugal gradient in MyHC transition within developing muscle fibers may explain the presence of small fibers of different MyHC phenotype in mammalian muscles (Gojo et al., 2002). However, additional work

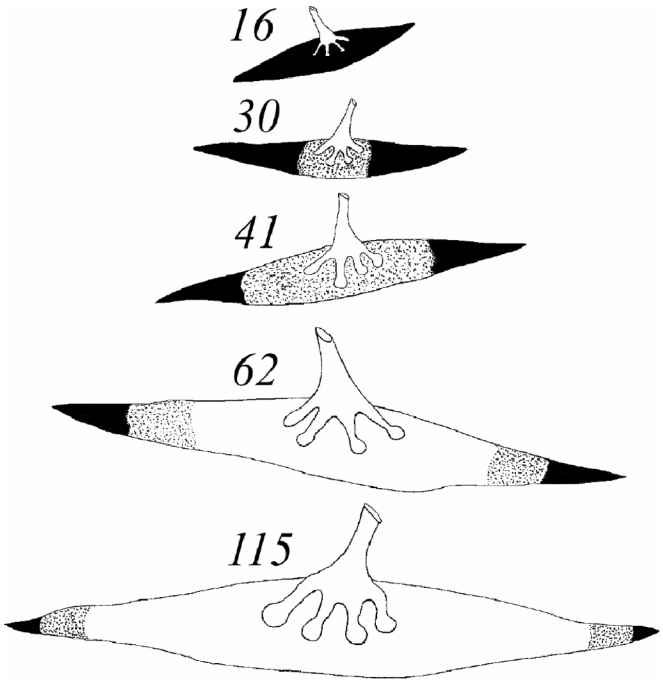

Figure 2. Schema of motor endplate and muscle fiber at different ages. The number indicates days after hatching. The darker the stippling, the greater the amount of neonatal myosin heavy-chain isoform. During development, repression of neonatal myosin heavy-chain isoform radiates from the motor endplate toward the fiber ends.

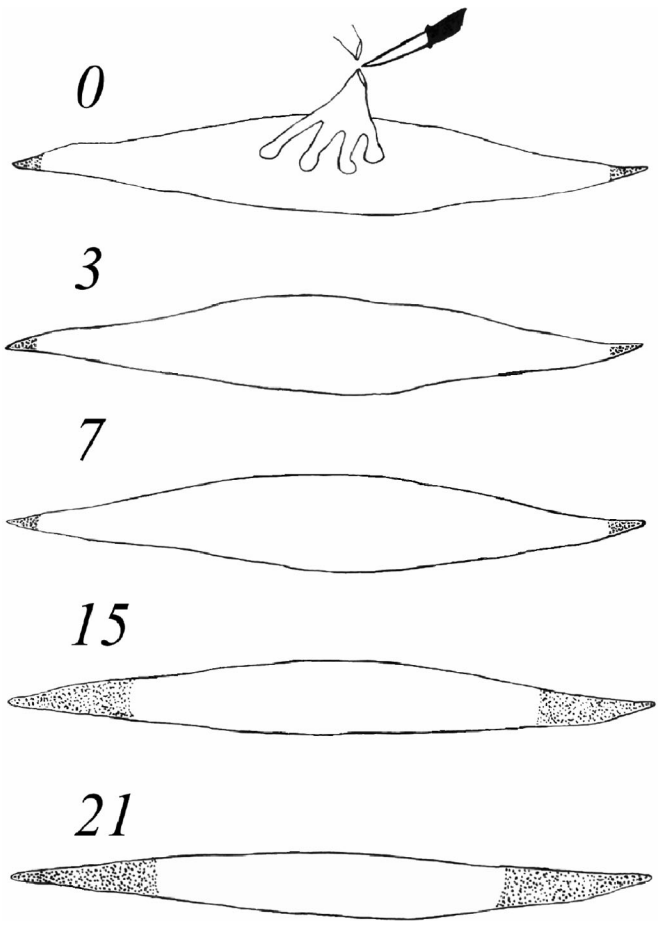

Figure 3. Schema of muscle fiber of chicken pectoralis, illustrating the effect of denervation on distribution of neonatal myosin. The number indicates days after surgical denervation. Stippled regions indicate the presence of neonatal myosin heavy chain isoform. Denervation for 2 and 3 wk resulted in an overall decrease in diameter and reexpression of neonatal myosin from the ends toward the central regions of the fibers.

is required to extend our observations and theories to mammalian muscle. There is no shortage of experimental models available for study since many muscles of large mammals (rabbit, goat, horse, cattle, pig), and certain muscles of small mammals (mouse, rat, cat) and humans, have muscle fibers arranged in series (Trotter et al., 1992; Paul and Rosenthal, 2002). Invariably, serial sectioning of these muscles should demonstrate that smaller fiber profiles seen in cross-section are the tapered ends of larger fiber profiles (see Swatland, 1994).

Variation of Myonuclear Domains within Developing and Mature Muscle Fibers

Each myonucleus is responsible for gene expression in its surrounding cytoplasm. The region of cytoplasm associated with an individual myonucleus is termed the "myonuclear domain" (Landing et al., 1974; Allen et al., 1999) or DNA unit (Cheek, et al., 1971; Mozdziak et al., 1997). Myonuclear domain size is correlated with muscle fiber type and MyHC expression. Cytoplasmic volume per myonucleus is smaller in fibers expressing slow as compared to fast MyHC (Allen et al., 1999; Schmalbruch and Lewis, 2000). It has been hypothesized that fibers highly active in protein synthesis have smaller domains (Edgerton and Roy, 1991). There was an inverse correlation between myonuclear domain size and rate of growth in chicken pectoralis (Knizetova et al., 1972). In developing turkey (*Melleagris gallopavo*) pectoralis, younger, smaller fibers were shown to have smaller myonuclear domains (Mozdziak et al., 1994). Similar results have been reported from a study of rat muscle (Ohira et al., 2001).

We have shown that in chicken pectoralis myonuclear domains expressing neonatal MyHC within the tapered end regions of maturing muscle fibers are smaller than domains in other regions of the fibers (Rosser et al., 2002). Myonuclei were counted and formulas used to calculate mean myonuclear domain sizes. Fiber profiles were classified as neonatal, transforming (between neonatal and adult in neonatal MyHC content), or adult. Volume of cytoplasm/myonucleus (Figure 4) was different for adult, transforming, and neonatal (mean = 16,132, 12,899, and 8,130 μm^3/myonucleus, respectively). Transforming and adult profiles had significantly ($P < 0.001$) larger myonuclear domains than did

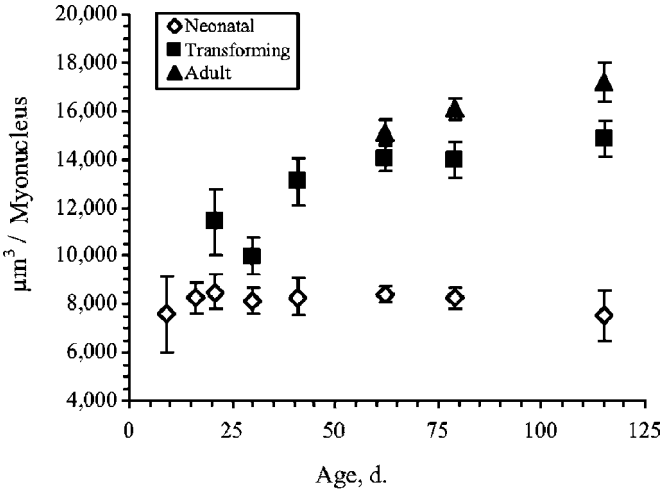

Figure 4. Myonuclear domain (volume of cytoplasm per myonucleus) in maturing chicken pectoralis. Values are expressed as mean ± SD. The overall pattern of myonuclear domain size is adult or transforming > neonatal. Those fiber profiles classified as neonatal from 21 d onward are predominately the ends of the muscle fibers. (Figure reprinted with permission of University of Basque Country Press, Rosser et al., 2002.)

neonatal profiles. Since neonatal MyHC is located at the ends of the fibers, our work demonstrated smaller domains at the terminal tips of maturing muscle fibers. Whereas there may have been a localized concentration of myonuclei in the immediate vicinity of the motor endplate of each fiber, as has been reported by researchers studying other experimental models (see "Protein Subsets within Specialized Regions of the Muscle Fiber"), we did not specifically quantify myonuclear numbers within this tiny region of the fiber.

Increased transcriptional output in muscle fibers appears to be enhanced by regional concentration of myonuclei. The ends of muscle fibers are the sites of longitudinal growth (see Swatland, 1994), and myonuclear domains are thought to be smaller in growing muscle (Winchester and Gonyea, 1992). We integrated these earlier observations by showing that fiber ends contain smaller myonuclear domains. A number of other sources provide indirect support for the hypothesis that increased transcriptional output is enhanced by the regional concentration of myonuclei. Rossi et al. (2000), in studying acetylcholinesterase expression along the lengths of individual fibers, suggested that the regulation of transcription could be an on/off event at individual myonuclei. Newlands et al. (1998) studied the expression of a variety of genes within individual fibers and determined that myonuclei were independently regulated and that not all myonuclei were transcriptionally active. Newlands et al. (1998) proposed that the number of myonuclei simultaneously expressing the same gene is an important mechanism regulating transcriptional output of a fiber. Hypotheses pertaining to the effects of myonuclear recruitment and/or concentration on transcriptional activity currently wait further testing.

Other Proteins

Most research to date on protein heterogeneity within muscle fibers has been focused on MyHC. However, most other myofibrillar proteins in skeletal muscle also exist as a number of isoforms (Schiaffino and Reggiani, 1996). It is possible that other proteins, such as troponin, titin, and C protein, could also modulate the functional properties of a muscle fiber (Bottinelli, 2001) and exhibit variation in isoform expression along the fiber length. Certainly, myosin light-chain isoforms have been directly correlated with contractile properties (Reiser et al., 1996) and show regional variation in isoform expression (Lutz et al., 2001). Formerly, researchers correlated subtle variations in energy-generating enzyme activities with fine differences in contractile properties (Nemeth et al., 1991), although it was held that metabolic capacity did not vary along a fiber (Pette et al., 1980). The MyHC hegemony in studies of muscle fiber typing and function is being questioned (Botinnelli, 2001). It is probable that future studies will show a greater array of genes exhibiting regional expression within muscle fibers.

Implications

Earlier studies established that differences in contractile speeds among skeletal muscle fibers are correlated with myosin heavy-chain content. Thus, our work showing that fiber ends have a myosin heavy-chain content different from that found along the rest of the fiber length suggests that there are variations in intracellular contractile properties. Our findings show a centrifugal gradient in repression of developmental myosin heavy-chain proteins within developing fibers and the centripetal reexpression of this isoform in denervated fibers, indicating regulation by trophic factor(s) emanating from the motor endplate. However, the causative factors have yet to be determined. Previous work demonstrated that fiber ends are the site of longitudinal growth and that myonuclear domains are smaller in growing muscle. We integrated these findings by showing that fiber ends contain smaller domains. Thus, increased transcriptional output in muscle fibers seems to be enhanced by regional concentration of myonuclei.

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The multifactorial nature of food intake control[1]

J. M. Forbes

University of Leeds, Leeds LS2 9JT, U.K.

ABSTRACT: Three approaches to predicting and understanding food intake by beef cattle are discussed and compared. Although many physiological factors are known to be involved in intake control, these are not sufficiently quantifiable to form an adequate basis for intake prediction. Prediction equations derived from observed effects of animal and feed factors on intake are useful within the range of conditions under which the data were collected, but they do not predict adequately outside this range. The concept of an intermediate approach to intake prediction and understanding is presented, suggesting that proportional deviations of resource supplies from the feed (e.g., energy, protein, fiber) from the animal's optimal supply ("requirement") generate discomforts. These, when squared and added, yield a total discomfort signal, which the animal minimizes. The fact that feed intakes by individual animals fluctuate considerably from day to day provides a means whereby animals can assess whether an intake somewhat higher or lower than their current average intake will improve their well-being. Examination of intake data from beef cattle fed grass silage suggests that intake is controlled over a period of several days. It is concluded that different levels of understanding of the control of voluntary intake are needed for different reasons, and that no single approach will serve all purposes.

Key Words: Beef Cattle, Physiology, Prediction, Voluntary Intake

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Introduction

Beef cattle are almost always fed ad libitum to maximize growth rate and profitability. Intake control involves energy because changes in energy requirements of the animal and/or in the digestible or metabolizable energy content of the diet cause intake to change in the appropriate direction. For example, results summarized by Baumgardt (1970) show that beef cattle decrease their DMI as the concentration of DE increases (Figure 1a). It appears as if DE intake is being held constant (Figure 1b), but closer examination shows that DE intake systematically decreases by a small amount as DE concentration increases (Figure 1c) (see also Grovum, 1987). It seems highly unlikely that animals eat in order to achieve constancy in the supply of only one of the many resources provided by feed.

With forage-based diets, beef cattle, as other ruminant animals, increase their DM intake as the rate and extent of digestion increases. This has been attributed to a physical limit to intake and, because of its slow rate of digestion and correlation with forage intake in sheep, NDF has been used to account for the bulkiness of feeds. However, there is large variability in NDF intake of forages (Ketelaars and Tolkamp, 1992), which strongly suggests that bulk is not the only factor affecting forage intake.

We can conclude that intake is controlled and that energy- and bulk-sensing mechanisms are involved. Many other factors are also implicated in the control of intake, including nutrients (such as amino acids, minerals and vitamins), disease, and environmental conditions and social pressures in a multifactorial manner. In order to use information on these factors, it is necessary to construct a conceptual framework as to how intake might be controlled. The rest of this review will examine three approaches: physiological, empirical, and teleological.

Physiological Approach

Numerous metabolites, hormones, and nervous pathways have been proposed to act as signals in providing the central nervous system (**CNS**) with information about nutrient status (Forbes, 1995). Many of these have first been proposed for simple-stomached animals and then adopted for ruminants (e.g., distension of the digestive tract, cholecystokinin [**CCK**], leptin). Other signals are not effective in ruminants (e.g., glucose), whereas yet others are peculiar to ruminants (e.g., VFA, rumen-degradable protein [**RDP**]). As each new candidate

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[2]Correspondence: Centre for Animal Sciences (phone +44-0-113-3433066; fax: +44-0-113-3433144; E-mail: j.m.forbes@leeds.ac.uk).

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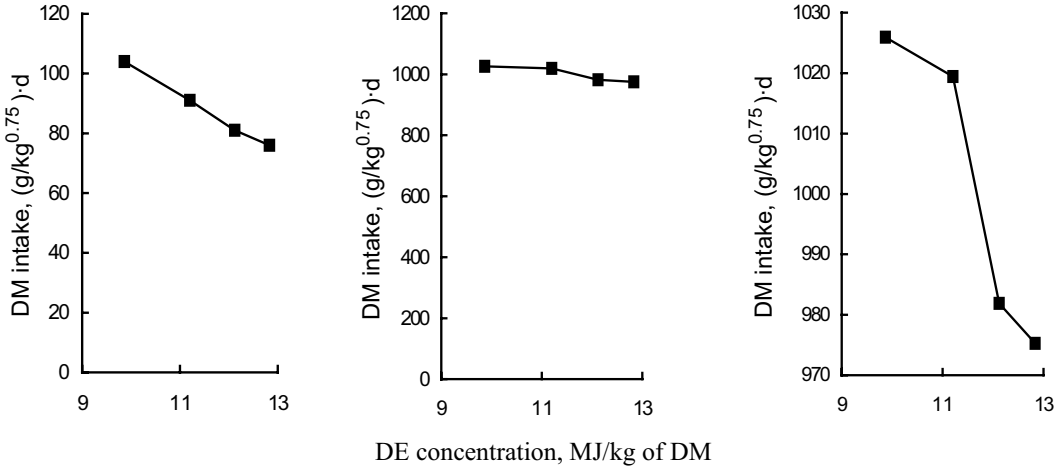

Figure 1. Intake of pelleted feeds of different DE concentrations by growing beef cattle (Montgomery and Baumgardt, 1965): a) DMI; b) DE intake; c) DE intake on an extended scale.

comes along, previous favorites tend to be forgotten. However, a role for CCK is no less likely now than it was in 1973 when its effect on intake was first reported. It is likely, therefore, that the physiological mechanisms controlling intake are complex and truly multifactorial. Diagrammatic representation of the factors thought to be important is probably the best way of integrating them into a scheme of intake control (e.g., Blundell, 1991) since attempts to quantify models of such complexity are likely to end in unstable solutions (France and Thornley, 1984). In many cases, there are no adequate experimentally derived equations with which to build quantitative models based on physiological factors, such as those outlined above. Thus, whereas studies of the detailed physiology of the control of food intake will continue to reflect aims to prevent obesity in human beings, they are unlikely in the short term to provide practical solutions for the formulation of animal feeds.

Empirical Approach

Given the limitations in ability to account for the many factors that control appetite, empirical equations have been developed to predict intake with different types of beef cattle in different types of production situations (CSIRO, 1990; AFRC, 1991; NRC, 1987; 2000). These equations are developed by collecting and analyzing data to produce equations that can be used for prediction of DMI in formulating diets, predicting performance, etc. Information is typically used that is measurable in production situations.

The NRC (1987) published comprehensive compilations of equations on prediction of feed intake in beef cattle. The graphs presented there are plots of various equations that have been proposed to predict DMI in beef cattle, and in some cases, show considerable discrepancy between the predictions of equations from different sources. No validation is presented, in common with most published prediction equations (Pittroff and Kothmann, 2001), and, as always with this type of approach, predictions of intake are limited to the range in which the observations on which the equations are based were made. However, NRC (2000) gives some validation of the use of the equation for growing yearlings:

$$\text{DMI} = \{[\text{SBW}0.75 \times (0.2435\text{NE}_\text{m} - 0.0466\text{NE}_\text{m}^{\,2} - 0.1128)]/\text{NE}_\text{m}\}$$

where DMI is measured as kilograms per day, SBW is starting body weight in kilograms, and NE_m is given in Mcal/kg of DM. In one comparison with experimental results from cattle fed high-energy diets, this equation accounted for 76% of the variation, with an overall prediction bias of 0.16% and standard error of Y of 0.34kg. Other data sets were less well fitted, but adjustment factors for body fat, breed, feed additive, environmental temperature, and mud were calculated to adapt the basic equation to different management situations.

Looking at the numerous factors with which intake is correlated, and which presumably affect intake, there can be no doubt that the control of voluntary food intake is multifactorial. In the NRC (1987; 2000) approach, such factors as the sex of the animal and the use (or not) of anabolic agents are applied to the predictions based on live weight and dietary energy concentration as multipliers. There are interactions, however. For example, the reduction in the DE concentration of a medium-quality diet for an animal with low nutrient requirement results in it eating more since it is not at the "physical limit," whereas for an animal with a high nutrient requirement, a reduction in the DE concentration causes it to eat less since it is already eating to its physical capacity. Another example: The intake of a small, fat animal is likely to be reduced more by high environmental temperatures than that of a large lean animal of the same body weight.

Whereas prediction of intake by regression analysis is very suitable when the situation for which prediction is

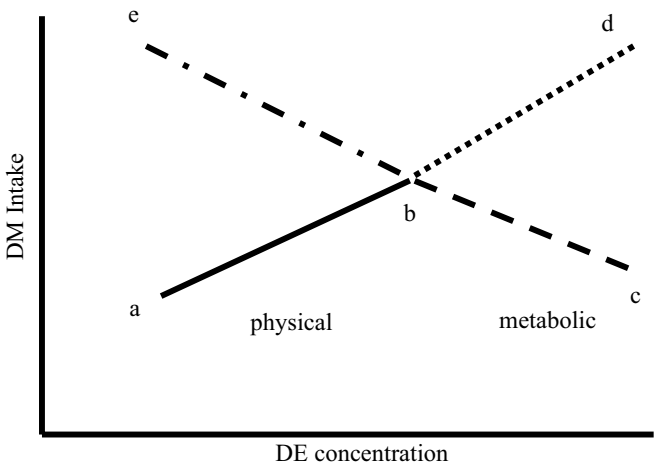

Figure 2. Notional relationship between food intake and diet digestible energy (DE) concentration (see text for explanation).

required is within the range of observations used in the equations, it can give misleading results outside this range. Therefore, these equations are of limited use in exploring underlying features of intake control and predicting intake in novel situations (e.g., intake of novel genetically modified [**GM**] food by an novel GM animal).

Teleological Approach

Teleology is defined as "the doctrine of adaptation to purpose" (Webster, 1915). In this case, the "purpose" is survival and the transmission of genes to the next generation. For the purposes of this paper, the concept is emphasized rather than the mechanism.

Simple "rules" can be proposed, such as "animals eat for calories," but whereas this approximates in some situations for simple-stomached animals, it is not valid if we wish to encompass forage-based diets for ruminants. The next step is to propose that "animals eat to satisfy nutrient (energy) requirements unless prevented by a limiting factor," and examples of this approach can be found (Conrad et al., 1964; Forbes, 1977; 1980). A more sophisticated example was proposed by Poppi et al. (1994), who used six limiting factors, intake being predicted as that resulting from the application of the most limiting factor.

However, it seems most unlikely that the animal ignores one limiting factor just because another factor is even more limiting. For example, consider an animal being fed a series of diets with ever increasing DE concentration (Figure 2). As DE increases (*a* to *b*) from a low level, intake increases as rate and extent of digestion increase and release rumen capacity more quickly. Eventually, there comes a point at which the animal is eating sufficiently to meet its requirements for energy, and from that point upwards (*b* to *c*), intake decreases. It is difficult to imagine that below the threshold, there is no input to the intake-controlling systems of the body from sensors sensitive to energy-related signals (*b* to *e*). Equally, can we envisage that once above the threshold, there is no input whatever from stretch receptors in the rumen wall and elsewhere (*b* to *d*)? The convergence of pathways carrying signals from the periphery to the CNS (Forbes, 1996) seems to deny the possibility that intake is limited solely by whichever factor happens to be most limiting at a particular time.

An alternative concept is required, therefore, that more readily acknowledges the multifactorial nature of feed intake control. The following is an outline of such an approach (Forbes, 1999; Forbes and Provenza, 2000), which is offered as a stimulus to further thought and experimentation.

There is feedback from many sensors providing the CNS with information on such factors as energy status of the liver, extent of repletion of adipocytes (leptin), degree of stretch of various viscera, and external factors, such as weather and the behavior of other animals, both fellow-herbivores and predators. It is proposed that the strength of the signal from each of these sensors to the CNS is proportional to the deviation from optimal (e.g., if the optimal intake of ME by a beef steer is 150 MJ/d, and it is eating 10 kg of feed with an ME concentration of 12 MJ/kg, then the absolute deviation is 150 – 120 = 30 MJ and the proportional deviation is 30/150 = 0.20.

The word "discomfort" is proposed to describe this deviation because animals will expend effort in order to reduce it. Discomfort increases with an excess or deficiency of a nutrient, with physical distension of stomach or intestines, above a certain daily grazing time, with social pressures, and with numerous other things. Some factors only cause discomfort when above a threshold (gut fill, eating time), whereas others do so when provided in greater or lesser amounts than are optimal. The optimal intake of a nutrient is that which, in the absence of other constraints, allows the animal to achieve its potential to grow, fatten, lactate, etc. It is not always something that can be measured, but estimates usually can be made from observations of responses to controlled changes in nutrient supply. The argument is always likely to be circular, however, as maximal rate of production can only be achieved with animals fed ad libitum.

It is further proposed that discomfort increases in a nonlinear manner, with increases in deviation giving ever-increasing effects on discomfort. Evidence on which to base the shape of the response curve to different degrees of stimulation of abdominal receptors is surprisingly sparse. Increasing the volume of a balloon in the reticulum of goats from 800 to 1,200 mL resulted in a greater increase in the number of impulses per second in the afferent nerve fibers than did the increase from 400 to 800 mL, whereas a further increase from 1,200 to 1,400 mL gave an even steeper increase (Iggo, 1955); this type of dose-response information is, however, lacking for chemical stimulants and the small number of "doses" used prevents a proper description of the shape of the dose-response curve. It would be circular to base the implementation of this concept on intake responses to different doses of stimulus, hence the difficulty of pro-

viding sufficient evidence on which to base a fully quantitative model based on the concept.

For simplicity, squaring has been chosen to provide the exaggeration of greater deviations, but could easily be replaced by an exponential or sigmoid response curve; an advantage of squaring is that it converts negative deviations into positive signals. However, this symmetry of the response curve is not based on data and again is illustrative rather than definitive. Again, there are few data to provide the basis for a more sophisticated treatment of this feature of the concept.

Weighting factors were used in previous expositions of the concept. These are not justified, however, with our current level of knowledge and have been omitted from the present description. One of the intentions of this concept is to allow various discomforts to be expressed in a common currency; weighting would be an admission that this is not possible, but it must be accepted that considerable experimentation will be required to provide data on which to base differential weighting of the various discomforts.

Once the discomforts have been calculated, they are added together to provide a signal of total discomfort; treatments imposed experimentally have additive effects (Forbes, 1996). According to our definition of discomfort, animals seek to minimize the total by adjusting their intake and/or choice, continuing in the direction that results in a reduction in discomfort, and learning as they go.

The next consideration is the properties of the food and environment that should be included in a semi-quantitative model based on the concept of minimal total discomfort. Whereas a large number of factors could be included, many of them are of little importance in many situations, especially where the food(s) on offer provide adequate amounts of such components as minerals and vitamins. Clearly, the supply of energy, the content of fiber, and the availability of protein in the diet are of central interest in most cases and can be described by the concentrations of ME, NDF, and CP in the food. Whereas this is a considerable simplification of reality (it ignores the ratio of VFA, the degradability of fiber, RDP, rumen-undegradable protein, and individual amino acids, to mention a few), it provides a starting point from which to develop a more comprehensive treatment. There is little point in including the time per day beyond which the animal would prefer not to eat, for example, if it is clear from the outset that this limit will not be reached. The decision as to which factors to include depends on the situation and the interests of the individual. Any attempt to proscribe the factors to be included would imply greater confidence in the concept than is currently warranted.

Interactions between the effects of different controlling factors are not explicitly included in the present discussion. However, in response to such a question as: "Is there really a 'protein optimum' that is independent of energy intake?" the answer is that there is a combination of energy and protein that is optimal, and it is this which the animal is trying to achieve. Failure to achieve it means that, although the animal might have achieved minimal total discomfort for the present circumstances, this is not zero discomfort.

In biology, it is generally assumed that the behavioral programs that can be observed in animals today evolved because they contributed to animals' fitness (survival and reproduction). Is minimization of discomfort identical to fitness maximization? If not, how could such programs have been selected in the evolutionary process? There is a difference between long-term, selectable traits and short-term responses by individual animals to the current situation. It's possible to envisage how animals evolved to cope with fibrous forages, for example, by developing the capacity to store digesta long enough for microbial action to yield nutrients. Having been provided with that anatomy and physiology, the individual then has to live in the real world and be flexible in dealing with different food conditions, including ones in which fiber digestion is not the primary limiting factor to intake. What is selected for is not just a single solution for a specific ecological niche, but an adaptable framework for responding to a changing environment, both in space and time.

A Simple Example

In this simple example, animals' requirements are, for illustrative purposes: ME, 150 MJ/d; CP, 1.00 kg/d; NDF, 6.0 kg/d (no discomfort is generated by a "deficiency" of NDF); and the feed has a composition of 11.0 MJ of ME/kg; 0.14 kg of CP/kg; and 0.70 kg of NDF/kg. An approximate intake is proposed, and the deviation of actual supplies of nutrients resulting from this intake from that "required" are squared and added. Intake is changed up or down and the calculations repeated, iterating until the intake that produces minimal total discomfort is reached. Figure 3 shows the discomfort associated with ME, CP, and NDF at different levels of intake. Total discomfort is also shown, and this is minimized, for this combination of animal requirements and feed composition, at 8.7 kg DM/d.

In its present form, the model is a semi-quantitative working hypothesis. If it is to have potential for prediction, there are a number of questions to be addressed, not least the weightings to be applied to each discomfort and which factors to include for any particular practical situation. However, an advantage of the approach is that it can incorporate almost any factor that influences feed intake (e.g., rate of grazing of sparse pasture via a discomfort generated by time spent grazing above a threshold; Forbes, 2001). Although there is no opportunity in this paper to discuss the use of the minimal total discomfort theory in relation to diet selection, it should be noted that prediction of the animal's behavior towards a single food is a special case of the more general situation of choice feeding, which the model can easily be extended to deal with, as is supplementation with a fixed daily amount of another feed.

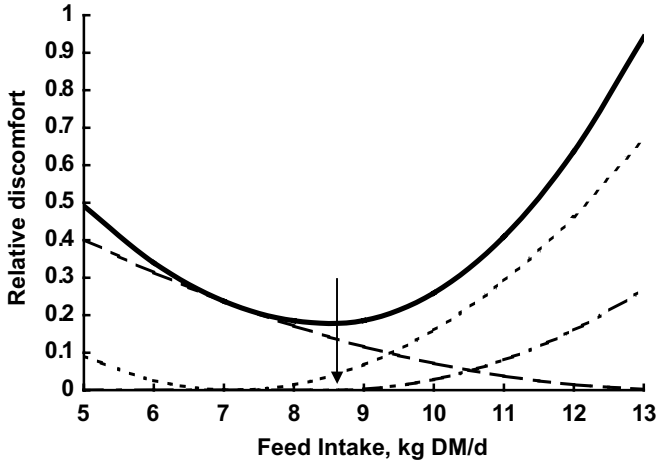

Figure 3. Postulated relative discomfort due to ME (—), CP (···), NDF (– · –), and total discomfort (▬), generated in a beef animal by different daily rates of food intake. Assumed animal "requirements" were: 150 MJ of ME/d, 1.00 kg of CP/d, 6.0 kg of NDF/d. Food composition: 11.0 MJ of ME/kg, 0.14 kg of CP/kg, and 0.70 kg of NDF/kg. The vertical arrow indicates the intake at which total discomfort is calculated to be minimal.

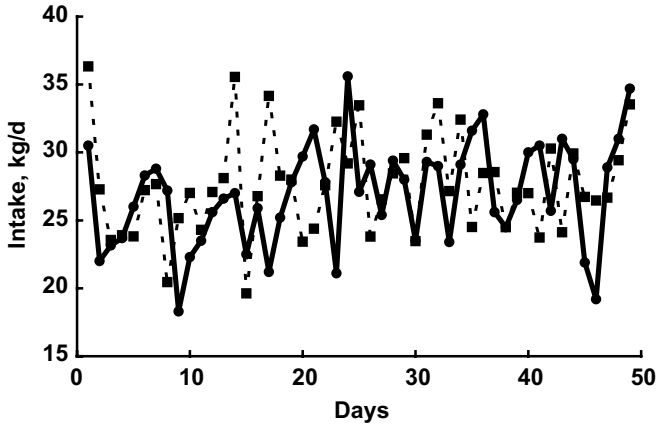

Figure 4. Daily intakes of grass silage by a beef animal. Solid line = observed intakes (R. Kirkwood, unpublished data); dashed line = random numbers with the same mean and SD as the observed intakes.

Day-to-Day Variation in Intake

An animal can only know whether it should be eating more (or less) than at present in order to minimize its discomfort if intake varies from time to time. Even when it has arrived at the optimal state, it can only know whether it should maintain the same level of intake if it tries out the effects of different rates of intake. The concept that animals find an optimal intake by "experimenting" with a range of intakes, which implies that learning is involved, is supported by clear demonstrations that ruminants, like other animals, learn to associate the sensory properties of the food with the consequences of eating that food (Forbes and Provenza, 2000).

Examination of the weights eaten by individual animals on a series of consecutive days confirms the experience of anyone who has studied food intake, that there are considerable daily fluctuations. Examples have been given elsewhere for dairy cows (Forbes, 2001; Forbes and Provenza, 2000) and sheep (Forbes and Provenza, 2000), and Figure 4 shows daily intakes of grass silage by a growing beef animal also given 3 kg of concentrate feed per day (R. Kirkwood, unpublished results). Examination of the records for many more individuals shows similar fluctuations, and these are only partly related to variation in the mean intake of the group of animals of which the individuals are members. Although an optimist might see patterns in such data, plotting randomly generated data with the same means and SD as the observed intakes produces similar fluctuations and pseudo-patterns (Figure 4). However, autocorrelation of the observed data gives positive correlations approaching statistical significance between intakes on consecutive days, and negative correlations, significant in some cases, between intakes separated by 3 or 4 d, whereas randomly generated data do not show such correlations. This suggests that intake might be organized over periods of 3 to 4 d, and further analysis is warranted.

Any suggestion that these fluctuations are animals' responses to changing DM concentration of the silage in order to maintain a constant DMI is refuted by the fact that daily DMI is positively related to DM concentration, whereas there is no significant relationship between the intake of fresh matter and DM concentration (A. Jolaosha, personal communication). Therefore, until we have further evidence, we must conclude that the daily fluctuations in intake by individual animals are only weakly organized in relation to time. They could, nevertheless, still allow the animal to average its intake over several days in order to minimize total discomfort.

This leads us to the hypothesis that these beef cattle fed on grass silage are controlling their forage intake over a period of several days; a high intake one day is followed by lower intakes subsequently to give a more constant level of intake over periods longer than a few days. Yeates et al. (2002) analyzed the patterns of meals taken by dairy cows offered a free choice of two feeds with different protein contents and concluded that they did not balance their protein and energy intakes over a sequence of meals, up to a whole day, even though there was considerable evidence of balancing of diet over periods of many days or weeks. Kyriazakis et al. (1999) stated: "There is little evidence that animals modify their diet selection in response to short-term systemic fluctuations of their internal environment. On the other hand, long-term changes in the internal state of the animal lead to consequent long-term changes in diet selection." They proposed that the extent to which the animal's internal state deviates from optimal is a more important determinant of diet selection than "what time period

matters to the animal," and what applies to diet selection should also apply to feed intake.

Conclusion

Approaches used to predict food intake or to describe intake control mechanisms depend on the needs of the user. On the one hand, those who seek to manipulate feeding pharmacologically require information on a limited part of the complex network of physiological processes underlying many of the activities of the animal's body. It is likely to be a very long time before a model to predict feed intake can be developed in which all of the factors controlling appetite are accurately accounted for in feedlot situations. On the other hand, prediction of intake based on observations made under similar conditions offers an empirical solution, but one that will not allow such questions as how to best feed (GM?) animals with novel requirements and physiology, kept in a novel environment, with (GM?) plant materials of novel composition and structure.

This paper has therefore concentrated on a concept in which it is argued that animals experiment in order to learn to minimize total discomfort. Such a framework is hypothetical, but it takes into account sound principles, such as responses to discomfort, integration of feedbacks, learning, and day-to-day variation in intake. It is unlikely that such a hypothesis can be proved or disproved by any single experiment. Rather, its acceptance will depend on whether more credible hypotheses arise and whether it is judged to have potential to be developed into a tool of practical use and/or improved understanding.

Implications

Beef cattle do not control their feed intake in order to achieve a constant intake of a single resource (energy, protein, fiber). This implies that a more sophisticated approach must be developed for formulation of optimal diets and feeding programs than those based on a few independent factors. However, attempts to invoke detailed physiological pathways immediately come up against lack of quantitative relationships and lack of understanding of how numerous hormonal and neural pathways interact. Therefore, the approach adopted here, in which factors of importance in the situation being considered are incorporated into models of total discomfort, may provide a conceptual framework for future developments that account for more of the variation in factors that control intake.

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Modeling chemical and physical body composition of the growing pig

C. F. M. de Lange*[1], P. C. H. Morel†, and S. H. Birkett*

*Department of Animal and Poultry Science, University of Guelph, Guelph, ON, Canada N1G 2W1 and
†Institute of Food, Nutrition and Human Health, Massey University, Palmerston North, New Zealand

ABSTRACT: In pig growth models, masses of body lipid (L) and body protein (P) are key state variables that can be related quantitatively to chemical and physical body composition for predicting growth response and carcass characteristics. The main chemical constituents in the empty body weight (EBW) are water (Wa), L, P, and ash. Within pig genotypes, Wa is independent of L and closely related to P (e.g., $Wa = a \times P^b$). The scaling parameter (b) is remarkably constant across pig types, at about 0.855, and represents changes in distribution of P with increasing EBW and differences in Wa-to-P ratios among body pools. The parameter "a" ranges between 4.90 and 5.62 and seems to vary with pig genotype. The ash-to-P ratio, about 0.20, has little significance on estimates of EBW. Gut fill, the difference between live body weight (LBW) and EBW, ranges between 0.03 and 0.10 of LBW; it varies with LBW, feeding level, diet characteristics, and time off-feed. The distribution of P and L over the main physical body components (dissectible muscle and fat, viscera, blood, bone, integument) varies considerably among groups of pigs and appears influenced by EBW, pig genotype, feeding level, diet characteristics, and possibly thermal environment and health status. Except for extreme pig genotypes, the distribution of lean over the main carcass cuts is relatively constant; however, little is known about the observed variation in the distribution of L over body fat depots. Representing dynamic effects of animal and external factors on sizes of physical body components is an apparent weakness in pig growth models, further complicated by inconsistencies in defining some of the physical body components, and dissectible lean tissue in particular. Improved accuracy in representing physical body composition will provide more insight on manipulation of carcass value and efficiencies of converting diet nutrients into pork products.

Key Words: Body Composition, Chemical Composition, Growth Models, Physical Properties, Pigs

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Introduction

The essence of pork production is the efficient conversion of nutrients supplied by a range of feedstuffs into high-quality pork products (Pond and Maner, 1984). This conversion encompasses relationships between nutrient intake and chemical body composition, and between chemical and physical body composition of growing pigs (Walstra, 1980). The amount of muscle tissue and its distribution are the main determinants of the amount and quality of pork that can be derived from the pig's carcass. Largely because of concerns about human health and sensory evaluation of fresh pork products, the distribution and fatty acid composition of fat tissue in the pig's body should be considered as well. Moreover, because visceral organs contribute to the inefficiency of converting dietary nutrients into pork products (de Lange et al., 2001), careful consideration should be given to nutrient needs of visceral organs in the pig.

The relationships between nutrient intake and chemical and physical body composition are affected by a range of factors associated with nutrition, pig genotype, environment, and stage of maturity. In order to identify practical means to manipulate pork quality and production efficiencies, an understanding of these relationships is required. This can be achieved by representing them causally and mathematically using a pig growth model.

Body protein mass (**P**) and body lipid mass (**L**) are key state variables in pig growth models. For predicting growth responses and carcass characteristics, P and L must be related quantitatively to chemical and physical body composition.

In this review, the mathematical representation of chemical and physical body composition of the growing pig is addressed. Main concepts are discussed and areas where further information is required are identified. Potential impacts of the pig's social and thermal

[1]Correspondence: phone: 519-824-4120 ext. 56477; fax: 519-836-9873; E-mail: cdelange@uoguelph.ca.

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Table 1. Chemical composition (%) of the empty body of pigs at various BW[a]

Component	Birth	7 kg of BW	25 kg of BW	Market weight (approx. 110 kg of BW; extremes)	
				Fat pigs	Lean pigs
Water	77	66	69	48	64
Protein	18	16	16	14	18
Lipid	2	15	12	35	15
Ash	3	3	3	3	3

[a]Derived from McMeekan (1940), Richmond and Berg (1971a,b), de Greef et al. (1994), Whittemore (1993), Bikker et al. (1995; 1996a,b), and Coudenys (1998).

environment and disease status on body composition are not addressed.

Chemical Body Composition: Prediction of Body Water and Ash Content

The four main chemical constituents in the pigs' empty body weight (**EBW**) are water (**Wa**), P, L, and ash (Table 1). The pig's body contains only minor amounts of carbohydrates (small stores of glycogen stores in the liver and muscle). For estimates of the whole-body mineral composition, the reader is referred to ARC (1981), Rymarz (1986), Hendriks and Moughan (1993), and Mahan and Shields (1998).

Given the high Wa content in the pig's body, an accurate prediction of Wa from P and L is critical for an accurate prediction of EBW. Water is closely associated with protein present in lean tissue and visceral organs. Given this association, Wa can be predicted from P with a reasonable accuracy, using allometric relationships that require only two parameters: Wa (kg) = a × P (kg)^b (Figure 1). Since the parameters a and b are determined statistically, care should be taken when estimating these parameters: if data covering only a limited range in Wa and P values are used, then different combinations of the two parameters may yield similar statistical fits (e.g., Emmans and Kyriazakis, 1995; Schinckel and de Lange, 1996). The scaling parameter (b) is remarkably constant across studies and pig types, at about 0.855 (ARC, 1981; de Greef, 1995; Emmans and Kyriazakis, 1995; Coudenys, 1998; Weis, 2001), and represents changes in distribution of P over various body pools with increasing EBW and differences in Wa to P ratios among these pools. Moreover, the observation that the ratio of Wa to $P^{0.855}$ is not influenced by BW and energy intake level, or by feed intake-induced variation in L to P ratios, provides further support to this rather simple and empirical approach to estimating Wa (de Greef, 1995; Weis, 2001). Maintaining the scaling parameter constant allows for a more robust and routine estimation of the parameter a, which varies between about 4.90 and 5.62 for different pig genotypes (ARC, 1981; de Greef, 1995; Emmans and Kyriazakis, 1995; Coudenys, 1998; Weis, 2001), and seems positively related to P at maturity (Emmans and Kyriazakis, 1995) or the genetically determined maximum body protein deposition rate ($\mathbf{PD_{max}}$).

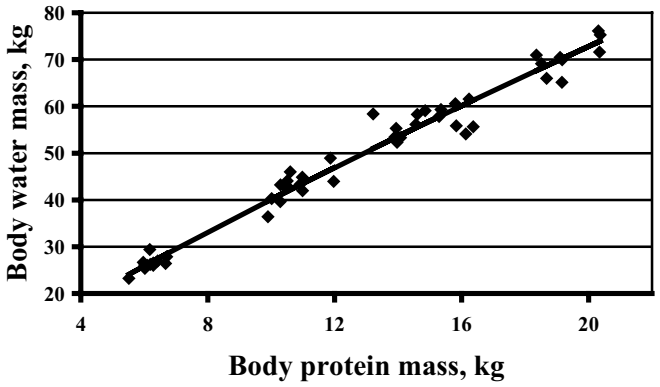

Figure 1. Relationship between body water mass and body protein mass in intact male Yorkshire pigs (Weis, 2001). Pigs varied in BW between 50 and 125 kg and were exposed to four energy intake levels ($Y = 5.5513\ X^{0.8592}$; $n = 50$, $r^2 = 0.8912$).

A more mechanistic approach to estimating Wa is undertaken via a dynamic representation of the distribution of P over the various body tissues and the Wa-to-P ratios in these body tissues. For example, in castrated male purebred Yorkshire pigs, Coudenys (1998) observed a Wa-to-P ratio of 4.52 in visceral organs, whereas BW did not influence this ratio. In that study, a gradual decline in Wa-to-P ratio was observed in the dissected loin, from 4.45 at 25 kg BW to 3.43 at 125 kg BW. Both the gradual decrease in the contribution of visceral organs to BW and the reduced Wa-to-P ratio in dissected lean contribute to the reduction in whole-body Wa-to-P ratio with increasing BW. This mechanistic approach to representing Wa requires, however, an accurate representation of sizes of each of the body tissues and of the Wa-to-P relationship in each tissue in different groups of pigs.

Ash can be predicted from P with reasonable accuracy: Ash (kg) = c × P (kg), with c varying between 0.186 and 0.210 (ARC, 1981; Moughan et al., 1990; Hendriks and Moughan, 1993; de Greef, 1995; Whittemore, 1983), reflecting the close association between bone tissue, which contains most of the body ash, and lean tissue. The observed variability in this parameter has little impact on prediction of BW and carcass characteristics.

Given the associations between P, Wa, and ash, most of the variation in chemical body composition between different groups of pigs can be attributed to variation in L content (Table 1).

Physical Body Composition

Gut fill (**GF**) represents the difference between EBW and live body weight (**LBW**) and is often assumed to

Table 2. Effect of diet ingredient composition and feeding level on gut fill, % of live body weight (LBW), in pigs at 110 kg of LBW[a]

Diet ingredient composition	Gut fill, % of LBW
Cornstarch and casein	
Fed ad libitum	2.42
Corn and soybean meal	
Fed ad libitum	3.00
Barley and canola meal	
Fed ad libitum (3.5 kg/d)	4.67
Fed restricted at 2 kg/d	3.26

[a]Derived from McNeilage (1999); feeding strategies were implemented 12 d before slaughter; 12 h pre-slaughter feed withdrawal.

represent a constant 5% of LBW (ARC, 1981; Stranks et al., 1988). However, findings such as those reported by Whittemore et al. (1988) and Lorschy et al. (1997) indicate that GF represents close to 9% and 4.5% of LBW in pigs at 20 kg and 100 kg LBW, respectively. Based on these two studies, GF can be predicted from LBW: GF (kg) = 0.277 × LBW (kg)$^{0.612}$. Other factors that influence GF are feeding level, diet characteristics (Table 2) and time off-feed (Stranks et al., 1988). Intake of fiber is likely to mediate feed intake and diet composition effects on GF (Whittemore, 1993).

The main tissues in the EBW of growing pigs are muscle (edible lean tissue), fat, visceral organs, bones, blood, and skin. As indicated in Table 3, physical body composition varies considerably between pig genotypes. The other tissues, including nervous, lymphatic, and vascular tissues, contribute less than 10% to empty body weight in growing pigs.

In market weight pigs, between 45 and 60% of the whole-body P is present in lean tissue, whereas approximately 15% of P is present in visceral organs (Rook et al., 1987; de Greef et al., 1994; Bikker et al., 1995, 1996a,b; Coudenys, 1998). Body fat tissues can be divided into three main categories: fat associated with muscles (intra- and intermuscular fat; intramuscular fat is also referred to as *marbling*), subcutaneous fat, and abdominal fat. Subcutaneous, abdominal, and most of the intermuscular fat constitute dissectible fat, in which approximately 70% of whole-body L is present (Rook et al., 1987; de Greef et al., 1994; Bikker et al., 1995, 1996a,b; de Greef, 1995).

Table 3. Physical composition (%) of the empty body of growing pigs of different genotype[a]

	Tissue				
Sex/Genotype	Muscle	Visceral organs[b]	Fat tissue	Bone	Skin
Male					
Synthetic Line	51.1	15.4	12.4	8.7	3.4
Pietrain	54.2	12.8	14.0	7.5	3.0
Large White	43.8	17.6	15.5	8.7	4.2
Female					
Large White	45.2	16.0	17.1	8.2	3.6
Castrates					
Large White	43.3	16.4	17.9	8.4	3.6
Meishan	27.8	16.7	28.1	7.2	7.4

[a]Adjusted to a mean empty body weight of 47.2 kg; derived from Quiniou and Noblet (1995).
[b]Includes hair and blood.

For a detailed discussion about the structure and significance of the other tissues in swine, including bone, skin, and hair; nervous, lymphatic, and vascular tissue; and blood and reproductive organs, the reader is referred elsewhere (Swatland, 1994; de Lange et al., 2001). Obviously, these tissues have important functions in the pig's body. However, these tissues represent only a small proportion of LBW. As a result, growth of these tissues has only minor impact on the prediction of physical body composition.

Prediction of Carcass Weight and Carcass Lean Content

Carcass generally constitutes LBW minus the gut fill, blood, visceral organs (including reproductive organs), hair, and the outer skin layer. Depending on the processing method, carcass may or may not include head, feet, tail, skin, kidneys, and leaf fat (Rook et al., 1987; NPPC, 1991; Swatland, 1994; OPCAP, 1996; Hicks et al., 1998). According to Whittemore and Fawcett (1974) and Whittemore (1993), the carcass weight-to-EBW ratio, also referred to as *carcass dressing percentage* (**%Carc**), increases with body lipid content: %Carc (%) = 66 + 0.09 × LBW (kg) + 0.12 × P2 back fat thickness (mm). This relationship may be valid in a large population of pigs but is not consistent with the observed inverse relationship between energy intake and carcass dressing percentage within populations of pigs (Bikker et al., 1996 a,b; Coudenys, 1998). Moreover, variability in visceral organ mass is not considered in this mathematical equation.

Within carcass processing methods, variability in %Carc can be attributed largely to variation in visceral organ mass and GF. Some of the factors that are known to influence visceral organ size are BW, feeding level, diet composition, and pig genotype (Koong et al., 1983; Bikker et al., 1996a; Quiniou and Noblet, 1995; Coudenys, 1998; Nyachoti, 1998). As illustrated in Figure 2 visceral organ mass, as a fraction of BW, is inversely related to BW. The scaling parameter of the allometric relationship between visceral organ mass and BW (approximately 0.45) appears to reflect both changes in feed intake and maintenance energy requirements with increasing BW (van Milgen and Noblet, 2003). Not only does feed intake stimulate visceral organ growth (Figure 2), it also alters the distribution of whole-body P between visceral organs and dissected lean tissue and the composition of dissected lean (Table 4). Among the visceral organs, mass of the gastrointestinal tract is most sensitive to diet characteristics, and to diet fiber levels in particular

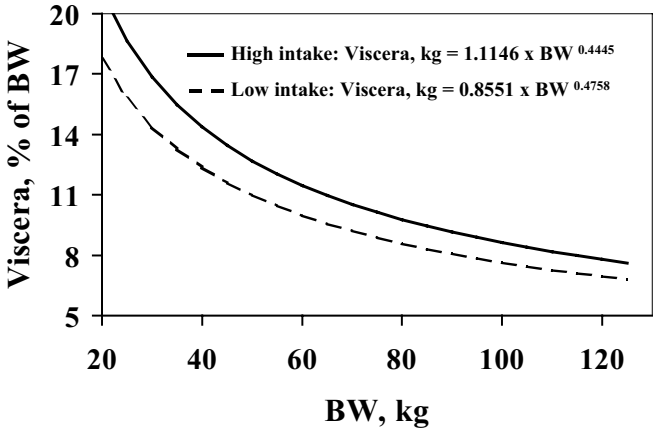

Figure 2. Relationship between visceral organ mass (Viscera) and BW in castrated male Yorkshire pigs exposed to two energy intake levels (Coudenys, 1998). Pigs varied in BW between 25 and 125 kg and were exposed to close to ad libitum (high intake) or high intake minus 35% (low intake) feeding levels (n = 32, r^2 = 0.793).

(Table 5). The aspects of dietary fiber that mediate these responses must still be clarified (Jørgensen et al., 1985; de Lange and Fuller, 2000). Given the pig genotype effects on visceral organ mass (Table 3), it is difficult to establish robust mathematical relationships to represent the effects of BW, feeding level, diet characteristics, pig genotype, and possibly health status on visceral organ mass in the growing pig.

The definition of lean tissue and methods used to estimate body lean tissue content might differ between packing plants, pig breeding organizations, and research institutions (Rook et al., 1987; NPPC, 1991; Swatland, 1994; Bikker et al., 1996a,b; OPCAP, 1996; Hicks et al., 1998). The latter should be considered carefully when interpreting physical body composition data and when predicting carcass lean tissue content. Carcass lean tissue content (**%Lean**) can be predicted

Table 4. Distribution of total body protein in lean tissue and visceral organs in pigs at approximately 85 kg body weight and fed at two levels of energy intake[a]

	Feeding level, × maintenance	
Distribution	2.2	3.7
Percentage of empty body weight present in:		
Lean tissue	51.9	44.4
Visceral organs	14.4	17.0
Percentage of whole body protein present in:		
Lean tissue	57.6	52.4
Visceral organs	12.5	16.0
Composition of dissected lean tissue, %		
Protein	20.2	19.1
Lipid	7.9	10.3

[a]Derived from Bikker et al. (1996a,b).

Table 5. Effect of additional dietary fiber intake on gastrointestinal tract mass (g/kg)

	Diet		
Reference	Control	+ Fiber	% Change
Pond et al. (1988)[a]	20.9	26.6	+27
Anugwa et al. (1989)[b]	25.5	29.9	+14
Jørgenson et al. (1996)[c]	31.7	43.6	+38
Nyachoti (1998)[d]	40.0	51.4	+28
Pluske et al. (1998)[e]	17.9	23.1	+29
McNeilage (1999)[f]	33.2	37.1	+12

[a]Diet contained 80% additional alfalfa meal.
[b]Diet contained 40% additional alfalfa meal.
[c]Diet contained 5.9 vs 26.8% total fiber; added fiber from barley, peas, and pectin.
[d]Diet contained casein-cornstarch vs barley-canola meal; excluding stomach in 25-kg pigs.
[e]Diet contained 5% added guar gum and 7% added resistant starch; weight of hindgut only.
[f]Diet contained casein-cornstarch vs barley-canola meal; implemented 12 d before slaughter.

from EBW and L: %Lean = 100 × (0.711 − L / EBW) (TMV, 1994). However, this equation does not reflect variability in the distribution of body tissues, P, and L between the carcass and viscera, nor does it reflect variation in the distribution of P and L within in the carcass (Tables 3, 4, and 5). A more accurate approach is first to predict carcass weight and to then predict %Lean in the carcass from carcass weight and carcass L and P content (Quiniou and Noblet, 1995). This approach will, however, not eliminate some the pig genotype biases in the prediction of %Lean (Wagner, 1992; Gu et al., 1992a; Hicks et al., 1998).

Even though the amount of lean tissue in the carcass of market weight pigs varies considerably across groups of pigs, the distribution of lean tissue among the primal cuts is rather constant (Richmond and Berg, 1971a,b; Gu et al., 1992a,b; OPCAP, 1996; Coudenys, 1998). Generally, approximately 25, 29, 14, 11, and 13% of lean tissue in the pig's body is present in the loin, ham, Boston butt, picnic, and belly, respectively (Gu et al., 1992b). The remainder represents muscles that are present in the neck, head, and lower parts of the legs. Only in Pietrain pigs does the lean tissue distribution differ substantially from that in other main pig genotypes. Due to double muscling in the loin and hind limb, a larger proportion of total body lean tissue mass is present in the loin and ham primal cuts in this pig genotype (Swatland, 1994).

In carcass grading schemes, %Lean is generally estimated from physical measures of body fatness, such as P2 backfat thickness. This is largely because %Lean is inversely related to body fatness, body fatness is more variable than body leanness, and body fatness can be estimated easily from back fat measurements (e.g., Rook et al., 1987; Whittemore, 1993). Even when measures of body fatness are combined with measures of carcass lean content, such as backfat depth and loin eye area, the estimation of carcass lean content is

Table 6. Distribution of body lipid and fat tissue content in selected line of pigs[a]

Item	Pig type/line[b]		Pig type/line[c]
	Control	Selected	LW
Body protein mass, kg	13.30		14.3
Body lipid mass, kg	18.9	16.2 (–14%)	28.2
Body fat tissue mass, kg			
Total	18.2	15.8 (–13%)	
Subcutaneous	12.1		17.08
Intermuscular	3.81	3.62 (–5%)	9.74
P2 backfat thickness, mm	19	14 (–26%)	22
P2/L ratio	1.01	0.86 (–15%)	0.78

[a]Rook et al. (1987).
[b]Large white entire male pigs (control) were compared to pigs that were selected against back fat thickness (selected); values in brackets represent change due to genetic selection.
[c]Large White × Landrace pigs (male, female, and castrated male).

generally more sensitive to the measure of body fatness (Rook et al., 1987; NPPC, 1991; OPCAP, 1996; Schinckel et al., 1996). Substantial genotype and sex biases can exist when estimating %Lean from simple carcass measurements (Rook et al., 1987; Wagner, 1992; Wagner et al., 1999; OPCAP, 1996; Hicks et al., 1998). In the practical application of pig growth models, it is thus more important to predict accurately the estimated %Lean than the actual %Lean. This may be achieved by modeling the physical body measurements that are used to estimate %Lean and then using the prediction equations to estimate %Lean according to the various carcass-grading schemes. This implies that body fat distribution should be considered carefully for the prediction of %Lean.

Both the body fat tissue distribution (Jones et al., 1980; Rook et al., 1987; OPCAP, 1996) and total body fat tissue content vary considerably between groups of pigs (de Greef et al., 1994; Bikker et al., 1995, 1996a,b; Gu et al., 1992b; Thomke et al., 1995). For example, there are clear differences between pig genotypes in the intramuscular fat content—it is generally higher in Duroc pigs than in Landrace or Yorkshire pigs—even though differences in subcutaneous and abdominal fat content between pigs of different genotypes may be small (OPCAP, 1996). According to Rook et al. (1987), the distribution of fat tissues in the pig's body can be altered rather easily through genetic selection (Table 6). These data suggest that by selecting against backfat thickness, L and fat tissue content and, in particular, P2 backfat thickness can be reduced, altering the relationship between L and P2 backfat. It is of interest to note that the total amount of subcutaneous fat tissue is not altered significantly in this study, suggesting a substantial redistribution of subcutaneous fat tissue due to selection against backfat thickness. Variation in energy intake is an important determinant of variation in body fat distribution, as well total content, within pig genotypes (Campbell et al., 1985; Campbell and Taverner, 1988; de Greef et al., 1994; Bikker et al., 1995, 1996a,b; Quiniou et al., 1995, 1996a,b; Coudenys, 1998). Given the large variability in body fat tissue distribution between groups of pigs and the many influencing factors, it is difficult to predict this distribution accurately for individual groups of pigs. To evaluate body fat tissue distribution and to model %Lean, it is suggested that the relationships between chemical body composition, %Lean and physical body measurements—those that are used to estimate %Lean in carcass grading systems—should be established in a representative subsample of pigs for each of the main pig genotypes.

Implications

In pig growth models, body composition is generally predicted from masses of body lipid and protein. Close attention should be paid to the relationship between body water mass and protein, which is remarkably constant across body weights and feeding regimens, but varies with pig genotype. The distribution of protein and lipid over the main physical body components (dissectible muscle and fat tissue, viscera, blood, bone, integument) varies considerably among groups of pigs and seems influenced by BW, pig genotype, feeding regimens, thermal environment, and health status. The dynamic representation of physical body components is an apparent weakness in pig growth models. This is further complicated by inconsistencies in defining physical body components, and dissectible lean tissue in particular. Improved accuracy in representing physical body composition will provide more insight on manipulation of carcass value and efficiencies of converting diet nutrients into pork products.

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Some recollections of early swine research with selenium and vitamin E[1]

J. E. Oldfield

Oregon State University, Corvallis 97331-6702

ABSTRACT: Despite the much-publicized finding of protective effects of selenium against myopathies in young ruminants (white muscle disease), the earliest discovery of the health benefits of selenium was made with swine in 1957. Because the body fat of pigs more closely resembles the fat in their diets than does that of ruminants, swine were useful subject animals for the investigation of dietary antioxidants, and much has been learned from them concerning the metabolic functions of both selenium and vitamin E. Swine research also played an important role in establishing nutrient essentiality status for selenium and in gaining approval from regulatory agencies (FDA) for its supplementary addition to livestock diets. These findings added significantly to the developing knowledge of the role of selenium in animal nutrition and subsequently to the acceptance of selenium supplementation as a production practice with various species of farm animals worldwide. This paper will examine some steps in the assembly of information concerning dietary antioxidants, including, more recently, implications for human nutrition and disease control.

Key Words: Pigs, Selenium, Vitamin E

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Introduction

The lively interest that has arisen over the last few years concerning the potential health benefits of antioxidant substances in the human diet makes it timely to review our knowledge of selenium and vitamin E, two important members of the antioxidant family. Swine have played an important part in this research, partly to improve their health and production efficiency, but also as a suitable animal model for the development of knowledge applicable to humans. In this paper, some of the early research findings related to selenium and vitamin E gained through studies with swine will be discussed.

Selenium

Much of the early interest in selenium was directed toward its protective effect against myopathies, such as the one commonly known as "white muscle disease." However, it is interesting to recall that the earliest published reference to selenium's role as an essential nutrient for domestic animals was generated with swine as the subject animal (Eggert et al., 1957). The predominant lesion indicative of selenium deficiency in swine was hepatosis dietetica, often accompanied by mulberry heart (Pellegrini, 1958).

To begin with, it was difficult to change from regarding selenium as a toxin or even as a carcinogen, to now thinking of it as an essential micronutrient. Kubota et al. (1967) and Ku et al. (1972) published useful surveys of selenium status in the United States and established the direct relationship between dietary and animal tissue selenium. Areas of selenium toxicity have been mapped around the world, as have, more recently, those of selenium deficiency (Oldfield, 1999), and it is significant that the latter are much more extensive than the former.

Because selenium and vitamin E often appeared to work together, it was questioned early on whether selenium was in fact an essential nutrient in its own right or merely an adjuvant to vitamin E. Swine research contributed to the evidence that selenium was an essential nutrient when it was shown that high levels of vitamin E did not eliminate the need for selenium (Ewan et al., 1969). This was confirmed with reference to reproduction when a single, subcutaneous 2.5-mg dose of Se as selenite given to sows just before mating resulted in a significant increase in farrowing percentages in Australian piggeries (Levander, 1986). The diet fed these sows was considered adequate in vitamin E.

One of the perplexing things that delayed the practical application of selenium supplementation in the United States was the FDA's refusal to authorize its use because of fears that it might be a carcinogen. When this concern was removed, research, again conducted

[1]Correspondence: phone: 541-737-1894; fax: 541-737-4174; E-mail: James.E.Oldfield@orst.edu.
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with swine (Meyer et al., 1981), allowed the FDA to recognize selenium as an essential nutrient, and it approved the addition of up to 0.3 ppm of Se to prestarter and starter diets for pigs (Ullrey, 1992) in 1982.

Reilly (1996), who has chronicled the selenium scene for many years, has estimated that selenium has generated over 100,000 papers in the scientific literature. In spite of the tremendous amount of research that selenium has attracted, investigations with it continue to generate new understandings—many of them involving swine. For example, research at Ohio State University has shown that low-Se diets fed to boars caused abnormal mitochondria to occur in their sperm, along with a 25% lower ATP concentration (Marin-Guzman et al., 2000), and the authors suggested that selenium might enhance the maturation of sperm in the epididymis. There was no benefit, however, from adding selenium to semen extenders in terms of sperm motility.

The benefits conveyed by selenium to animal health and productivity stimulated a research effort into the most effective form of selenium to use. Much of the attention was focused on comparisons of organic vs. inorganic sources; the organic form was frequently supplied by selenized yeast and the inorganic forms were the sodium salts. Apparently, different forms of selenium perform differently at varying levels in the diet. Cary et al. (1973) and Mahan and Moxon (1978) found no differences in the effects of organic or inorganic selenium in pigs when given at levels below 0.1 ppm in the diet using tissue retention as the criterion. In contrast, when the selenium content of the feed exceeded 0.1 ppm, organic forms of selenium were clearly better utilized. Interestingly, the availability of inorganic selenium proved higher than that of organic forms for the seleno-enzyme, glutathione peroxidase (Goehring et al., 1984). In Finland, inorganic selenium (sodium selenate) has been added to fertilizers and converted to organic forms by crops that are then fed to livestock, thereby raising the selenium content of meats and benefiting human consumers (Hartikainen and Ekholm, 2001).

Vitamin E

Although the fat-soluble dietary factor that was to become known as vitamin E was discovered in 1922, deficiency symptoms in swine were not described until more than a quarter-century later (Adamstone et al., 1949). Vitamin E has proven to be a multistructured compound, and during the four decades since its original discovery, eight structurally similar, naturally occurring compounds have shared its vitamin status. When selenium's status as an essential micronutrient was being established, it became evident that the two substances, vitamin E and selenium, acted synergistically. Hoekstra (1975) proposed that their synergism related to the process of antioxidation, wherein tocopherols tended to prevent oxidative damage to polyunsaturated fats in cell membranes, whereas selenium, as part of seleno-enzyme glutathione peroxidase, catalyzed the destruction of lipid hydroperoxides. This explains how these two nutrients play separate but interrelated roles in the cellular defense system against oxidative damage.

Vitamin E's antioxidant capabilities quickly found a practical application in commercial swine production. Tocopherols in the pigs' diets become a part of their tissue fats and inhibit the formation of oxidation products, which tend to reduce the acceptability of pork products in the human diet (Najman et al., 1976). Unlike ruminant animals, swine store a body fat that closely resembles the fat in their diet, and this may well have flavors and odors that are objectionable to consumers of their meat. Such flavor and odor compounds are most often the products of oxidation; if so, they will respond positively to the supplementary use of vitamin E and selenium, which improve the shelf life and quality of pork cuts.

Another of the persistent problems in swine husbandry has been that of iron deficiency anemia in baby pigs, for which a common treatment is giving iron, often as iron-dextran, by injection. It was noticed in Swedish studies that baby pigs that were deficient in vitamin E and selenium had a low tolerance to these iron-dextrose injections (Lannek et al., 1962). Pretreatment with vitamin E, selenium, or the commercially produced antioxidant, ethoxyquin, helped to prevent this intolerance. The literature has a number of reports linking baby pig anemia to vitamin E deficiency, but the relationship may not be causal (e.g., Nafstad, 1965). Canadian workers (Fontaine et al., 1977) concluded that vitamin E did not significantly influence erythropoiesis in growing pigs.

Ullrey (1981) has noted that not long ago, most nutritionists would have considered a vitamin E deficiency in swine to be unlikely, and recalled that the 1968 revision of the NRC's *Nutrient Requirements of Swine* stated that, "it is unlikely that practical swine diets would be deficient in vitamin E unless the diet contained excessive amounts of highly unsaturated fatty acids." With pigs raised in confinement, however, the commonly used corn-soybean diets of the Midwestern states could quite likely be deficient in both selenium and vitamin E. Beyond this, stress factors may act to increase pigs' requirements for both selenium and vitamin E. Common stresses are imposed by a cold, damp environment (Naftalin and Howie, 1969) and by infectious diseases (Keahy and Whitehair, 1966). Therefore, it appears that deficiencies of vitamin E and selenium can occur in commonly fed swine rations in this country. Indeed, Ohio studies (Mahan and Moxon, 1978) have reported losses and necropsy signs characteristic of vitamin E/selenium deficiency in pigs fed diets containing 22 IU of vitamin E and 0.1 mg of supplemental Se/kg.

Vitamin E functions, as does dietary selenium, to improve animals' immune response. Ellis and Vorhies (1976) have reported that increasing the vitamin E level of the swine diet over that generally considered adequate led to increased titers of serum antibodies to

Escherichia coli bacteria. Addition of 100,000 IU of vitamin E/t resulted in antibody titers 2 to 3 times higher than those of the pigs on the control diet.

The issue of vitamin E requirements is complicated by the fact that both the animals' needs and the nature of the diet must be considered. Swedish investigators recorded that, whereas a combination of 5 IU of vitamin E and 0.008 mg of Se/kg prevented deficiency signs in weanling pigs (Hakkarainen et al., 1978), the supplements were inadequate when given separately. Beyond the influence of unsaturated fats already mentioned, interactions may occur between vitamin E and some of the trace elements, including iron, copper, and zinc (Lannek et al., 1962). Ullrey (1981) recognized these interrelationships when he wrote, ". . . when Se supplements are restricted to 0.1 mg/kg, vitamin E supplements for corn-soybean diets should be at least 10 to 20 IU/kg. For problem herds, higher levels of vitamin E may be helpful, with the greatest benefit to be expected in the breeding herd and among young pigs. Supplements of 30 IU of vitamin E should be adequate under most circumstances. When diets contain considerable amounts of oxidized fat or the pigs are stressed by infection, even higher levels of vitamin E may be necessary."

What's Next?

Both selenium and vitamin E have been clearly established as essential nutrients, and there are many examples of a synergism of action between them. The two belong to a group of substances, along with vitamins A and C, that exert antioxidant powers that protect animals and humans from peroxide damage, and although this may be their major function, it is not the only one. Whereas much of the research reported herein has been directed toward improving the health and productivity of food-producing animals, recent evidence suggests that they may, particularly in the case of selenium, also provide benefits to human health. Their areas of activity include two of the most dreaded human ailments: cardiovascular diseases and cancer. Neve (1996) suggested that selenium protects against cardiovascular disease through the action of glutathione peroxidase against oxidation of lipids and subsequent reduced platelet aggregation. In men with cardiovascular disease, platelet aggregability is inversely related to selenium status (Neve, 1996). Peplowski et al. (1981) observed that supplemental selenium and vitamin E had a positive and additive effect in enhancing immune responses in weanling swine. The most widely cited study of selenium's protection against cancer is the one headed by Clark (1996) at the Arizona Cancer Center, in which 1,312 individuals with a history of nonmelanoma skin cancer were subjected to either dietary selenium supplementation (200 μg/day, as selenized yeast) or a placebo. The skin cancer did not respond, but there were striking effects on other cancers, including a 46% reduction in lung cancer, 58% reduction in colon cancer, and 63% fewer cancers of the prostate (Clark et al., 1996). An excellent review of the human health implications of selenium has been provided by Rayman (2000). The U.S. National Cancer Institute is now funding a 12-yr study in which 32,000 men will be given selenium and vitamin E to determine whether they are protective against prostate cancer. When one adds to this mounting evidence of health protection and the finding by Beck et al. (1994) that selenium will inhibit the pathogenicity of certain viruses, the potential usefulness of these substances becomes very great, indeed. And to the members of the American Society of Animal Science, it is especially noteworthy that much of the background research has been done with swine.

Implications

The antioxidants selenium and vitamin E are critical for both animal and human health and well being. Swine have been instrumental in the development of that understanding. Current research activity suggests that the understanding of the total contribution of these nutrients to health and well being will continue to grow.

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Effect of bunk management on feeding behavior, ruminal acidosis and performance of feedlot cattle: A review

K. S. Schwartzkopf-Genswein[1]*, K. A. Beauchemin*, D. J. Gibb*, D. H. Crews, Jr.*, D. D. Hickman*, M. Streeter†, and T. A. McAllister*

*Agriculture and Agri-Food Canada, Lethbridge, AB T1J 4B1 Canada;
†Alpharma Inc., Fort Lee, NJ 07024

ABSTRACT: Nutritionists and feedlot managers commonly attribute metabolic digestive disturbances such as subclinical acidosis to large daily shifts in feeding behavior and erratic feed intake by cattle. This perception is based on the fact that following a period of feed deprivation, free choice access to a high-concentrate diet can result in acidosis. Whether daily variability in voluntary intake of a high-grain diet by full-fed cattle compromises health or alters maintenance of ruminal pH at levels high enough for optimal ruminal function (i.e., pH <5.8) is less clear. Periodic abundance of available starch allows amylolytic bacteria (e.g., *Ruminobacter amylophilus*, *Streptococcus bovis*, *Lactobacillus* spp.) to proliferate and produce excessive quantities of fermentation acids. Presumably heightened volatile fatty acid production stimulates satiety receptors in cattle, which in turn results in the commonly observed "off-feed" or low intake syndrome. Despite this well-accepted relationship, comparatively few studies have actually demonstrated that voluntary variability in ad libitum feed intake impairs growth performance of cattle. Ruminal pH profiles differ substantially among cattle, even among those fed identical diets in equal amounts at the same time. It seems, therefore that factors other than meal size, and feeding regimen determine an animal's susceptibility to subclinical acidosis and ultimately influence growth performance. Feedlot management practices developed to regulate feeding behavior, and decrease variations in feed intake by penned cattle include programmed feeding, multiple feed deliveries per day, and consistent timing of feed delivery. However, the efficacy of these practices in reducing animal-to-animal variability is assessed largely on the basis of intake per pen, with little or no appreciation for the variation in feed intake among individuals. Further characterization of this variability in feeding behaviors among penmates could provide the foundation for further refinement of present feeding practices.

Key Words: Acidosis, Cattle, Feed Intake, Feeding Behavior, Management

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Introduction

The relationship between feeding management, feed intake, animal performance, and the incidence of metabolic disorders such as ruminal acidosis remains unclear. Nutritionists and feedlot managers attribute subclinical acidosis and reduced performance to erratic feeding behavior and intake by cattle, which is believed to result in losses of as much as $15 to 20 per animal in lost efficiency. Although several studies have concluded that large variations in intake by cattle fed high-concentrate diets may cause digestive disturbances (Fulton et al., 1979; Britton and Stock, 1987) few studies have confirmed that variability in ad libitum feed intake reduces growth performance of cattle.

The goal of bunk management practices such as programmed feeding, multiple feed deliveries per day, and consistent timing of feed delivery is to reduce variability in intake. The effectiveness of these practices is based on pen average intakes even though there can be significant differences in the ruminal profiles, and feeding behavior among individuals within a pen. The ability to monitor the feeding patterns, and ruminal profiles of individual feedlot cattle will aid in identifying factors, other than feeding regime and meal size, that increase an animal's susceptibility to subclinical acidosis, and reduce performance. This paper will review past work as well as introduce new approaches including radio frequency technologies to gain a better understanding of the complex relationship between feed intake, feeding behavior, acidosis, and performance.

[1]Correspondence: Lethbridge Research Centre, 5403- 1 Avenue South, P.O. Box 3000, Lethbridge, Alberta Canada, T1J 4B1 (phone: +1-403-3815841; fax: +1-403-3824526; E-mail: gensweink@agr.gc.ca).
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Effect of Intake on Rumen Ecology

The impact of intake on ruminal ecology probably is most profound during the transition from a forage-based to a grain-based diet. During this transition, fibrolytic bacteria become less prevalent, and amylolytic bacteria increase (Goad et al., 1998; Tajima et al., 2001). Once transition is complete, the size of various carbohydrate-utilizing microbial populations is remarkably constant in ruminants fed forage or concentrate diets at moderate levels of intake (Leedle et al., 1982). For example, Mackie and Gilchrist (1978) found that numbers of cellulolytic bacteria in the rumen remained unchanged once adaptation from roughage to a 70% corn diet was complete. Similarly, Klieve et al. (2002; unpublished data) found that ruminal *Streptococcus bovis* populations remained relatively constant after steers were adapted from a forage-to a concentrate-based diet. Numbers of cellulolytic bacteria typically decrease, and amylolytic bacteria increase when intake of nonstructural carbohydrates results in a ruminal pH of less than 5.5 (Slyter et al., 1970; Allison et al., 1975).

Earlier reports indicated that ruminal protozoa were virtually absent (Eadie et al., 1970; Lyle et al., 1981) or dramatically reduced (Slyter et al., 1970; Vance et al., 1972) in cattle fed a high-grain diet. However, researchers have more recently shown that whereas protozoal diversity is reduced, numbers of some protozoal genera (e.g., *Entodinium* spp.) remain high in cattle fed wheat- (Kreikemeier et al., 1990), barley- (Hristov et al., 2001) corn-, and sorghum-based diets (Towne et al., 1990a, 1990b; Franzolin and Dehority, 1996). *Entodinium* spp. may reduce the risk of subclinical, and clinical acidosis through their ability to utilize lactate (Newbold et al., 1987) and to moderate the rate of starch digestion by ingestion of starch granules (Williams and Coleman, 1992).

The development of subclinical, and clinical acidosis in feedlot cattle involves a complex interaction among intake, diet composition, ruminal microorganisms, and the animal (Figure 1). Establishment of a stable microflora during transition from a forage to a concentrate diet is not immediate. Introduction of highly fermentable starch into the diet increases the availability of free glucose, and stimulates the growth of most ruminal bacteria, thereby increasing production of VFA, and decreasing ruminal pH (Owens et al., 1998). Competition for substrate normally moderates the growth rate of lactic acid-producing bacteria (e.g., *S. bovis*, *Lactobacillus* spp.), and the number of these microorganisms seldom exceeds 10^7 cells / mL of ruminal fluid. Accumulation of lactic acid is curtailed by an increase in the number of lactic acid-utilizing bacteria (i.e., *Selenomonas* spp, *Anaerovibrio* spp., *Megasphaera elsdenii* and *Propionibacterium* spp.), and protozoa (i.e., *Entodinium* spp.) in the rumen. Thus, a balance between production and utilization of lactic acid is maintained and concentrations of lactic acid in ruminal fluid from animals perceived to have subclinical acidosis seldom exceeds

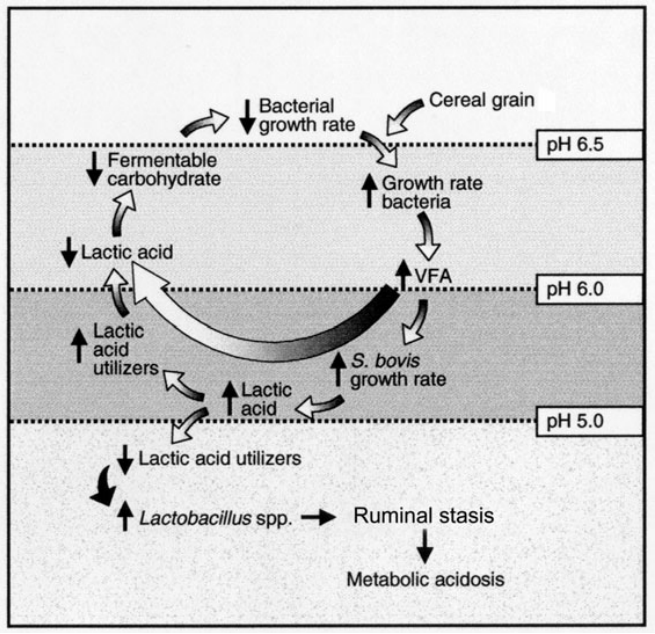

Figure 1. Metabolic consequences of feed intake in finishing feedlot cattle on ruminal pH and microbial populations of the rumen. Note that in the majority of animals ruminal pH decreases below 6.0 without a significant increase in ruminal lactic acid concentration or in numbers of *Streptococcus bovis* in the rumen.

10 m*M* (Harmon et al., 1985; Burrin and Britton, 1986; Goad et al., 1998; Hristov et al., 2001; Ghorbani et al., 2002). However, in a small subset of cattle the microbial population may become unstable, resulting in clinical acidosis. In these individuals, competition for substrates does not restrict growth of *S. bovis*, and with a doubling time of 20 min, this bacterium may soon reach populations of 10^9 cells / mL of ruminal fluid (Allison et al., 1975; Klieve et al., 2002, unpublished data). The resulting abundance of glycolytic intermediates (e.g., pyruvate, fructose-1,6-diphosphate) promotes a shift in the metabolism of *S. bovis* from production of acetate, and formate to production of lactate (Russell and Hino, 1985). Lactic acid ($pK_a = 3.1$) is over 10 times as strong an acid as the VFA normally produced in the rumen (average $pK_a = 4.8$), thus production of this alternative metabolite exacerbates the decline in pH. Cellulolytic bacteria, and protozoa are inhibited at pH below 6.0 (Stewart, 1977; Williams and Coleman, 1992), and as the pH decreases below 5.2 in select individuals, the normally diverse microflora of the rumen is largely replaced by acid-tolerant *S. bovis* and *Lactobacillus* spp.. With a continuous drop in pH, growth of *S. bovis* is inhibited (Therion et al., 1982). At pH below 4.7, a virtual monoculture of acid-tolerant Lactobacilli develops (Allison et al., 1975). Identification of the factors that contribute to the susceptibility of these few individuals to subclinical or clinical acidosis may provide the key to developing feeding practices that further reduce the risk of acidosis.

Subclinical Ruminal Acidosis

In North America, diets consumed by feedlot cattle typically contain mostly grain, and provide little fiber from forages. These high quality diets are rapidly digested in the rumen, which leads to high concentrations of VFA in ruminal fluid (> 100 m*M*), and relatively low ruminal pH (Beauchemin et al., 2001).

Clinical acidosis is often precipitated by an abrupt dietary change that destabilizes the ruminal microbial populations. Numerous in vitro, in sacco, and in vivo studies have shown that the ability of the major cellulolytic bacteria to degrade cellulose is negatively affected when pH is below 6.0 (Mould et al., 1983, 1984; Russell and Wilson, 1996). Various pH threshold values have been set arbitrarily to define subclinical acidosis: 5.5 (Hibbard et al., 1995; Reinhardt et al., 1997), 5.6 (Cooper et al., 1999), 5.8 (Beauchemin et al., 2001; Ghorbani et al., 2002; Koenig et al., 2002), and 6.0 (Bauer et al., 1995; Krehbiel et al., 1995). However, mean pH may not be a good indicator of this condition. In many cases, mean pH is calculated by averaging several spot samples taken before, and after feeding. This approach can be misleading because it does not reflect the diurnal fluctuations in pH. For example, cattle with a mean ruminal pH of 6.0 can experience long periods each day during which the pH is below 5.5. Recently, indwelling electrodes have been used to measure diurnal fluctuations in ruminal pH over extended periods of time (Dado and Allen, 1993; Krause et al., 1998; Cooper et al., 1999). For the purposes of this discussion, subclinical acidosis will be considered to exist when ruminal pH falls below 5.8 for more than 12 h /d.

Ruminal pH Profiles of Feedlot Cattle

Mean ruminal pH of feedlot cattle fed high-grain diets is usually between 5.6 and 6.2. The lower values are typically observed when more fermentable diets are fed (Krause et al., 1998; Cooper et al., 1999; Beauchemin et al., 2001; Ghorbani et al., 2001; Koenig et al., 2002). Under typical commercial feeding conditions, ruminal pH varies significantly over the course of the day. Most feedlot cattle experience low ruminal pH at least a portion of each day. The pH is highest just before the morning feeding, as cattle tend to ruminate at night, and eat during the day. After feeding, the pH drops as fermentation of dietary carbohydrate commences. Nadir or lowest pH typically occurs 11 to 13 h after feeding (Figure 2). Mean nadir pH in feedlot cattle usually varies from 5.0 to 6.5 (Bauer et al., 1995; Goad et al., 1998; Beauchemin et al., 2001, Koenig et al., 2002) and is influenced primarily by the rate of ruminal feed digestion. Thus, factors influencing this rate, such as forage-to-concentrate ratio, source of grain, and extent of grain processing as well as size, number, and frequency of meals can also have large effects on nadir pH.

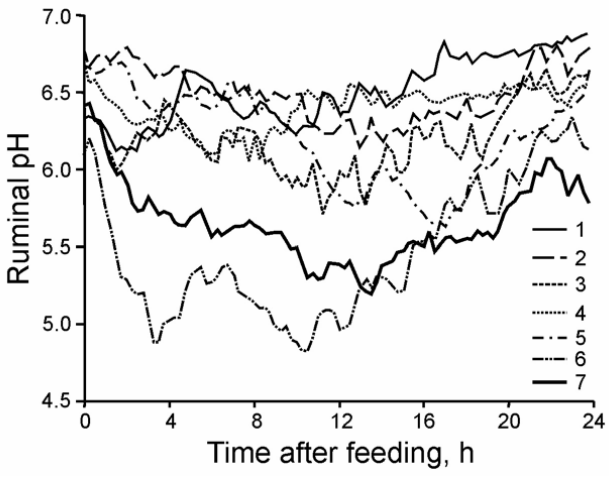

Figure 2. Changes in ruminal pH following feeding of a high-grain finishing diet (115% of ad libitum intake, 0900 h daily) to feedlot steers fitted with indwelling ruminal pH electrodes. Animals were individually penned and each line represents an individual steer. Values shown are 15-min averages of values recorded at 60-s intervals. Variation in ruminal pH of individual steers was not correlated with DMI (Krause et al., 1998).

Adaptive Responses

Individual cattle vary in their ability to cope with the metabolic challenges posed by extensive and rapid fermentation of high-grain diets. It is well recognized that feedlot cattle require an adaptation period of 10 to 14 d to make the transition from high-forage to high-grain diets. In fact, clinical and subclinical acidosis can be induced experimentally by eliminating this adaptation phase, and abruptly changing diet composition (Goad et al., 1998; Coe et al., 1999). Moreover, cattle can vary markedly in their ability to cope with the dietary factors that predispose them to acidosis after adaptation to high-grain diets (Dougherty et al., 1975; Brown et al., 2000). Thus, it is not surprising that under typical commercial feedlot conditions patterns of ruminal pH vary tremendously among animals receiving the same diet (Figure 2).

The reasons that some animals experience subclinical acidosis while others are metabolically capable of coping with this challenge are not clear. Differences among cattle may be related in part to the stability of microbial populations as discussed previously. Additionally, the ability or inability to maintain high ruminal pH may also be related to their feed preference, and selectivity at the bunk or the rate at which particular animals consume feed. Cattle that consume grain selectively may consume insufficient fiber for stimulating adequate chewing and salivary secretion to balance the acids produced during fermentation. Intake of forage fiber is known to stimulate rumination activity during which time salivary secretion increases (Beauchemin, 1991). Cattle consuming high-grain diets secrete only

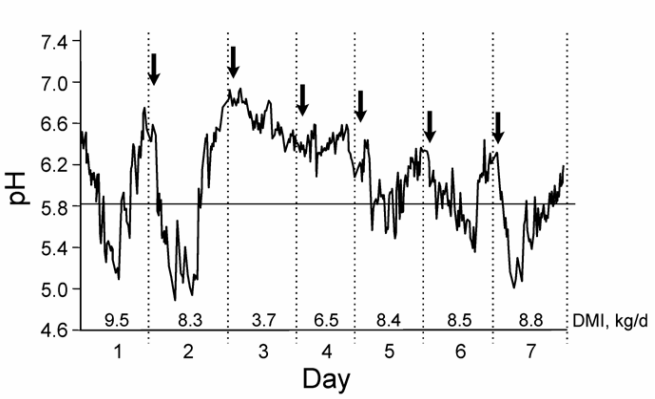

Figure 3. Ruminal pH and DMI of a single steer given continual access to a barley-based finishing (fully adapted) diet (14.6% CP) over a 7-d period. Arrows indicate feed delivery times.

60 to 70% as much saliva as cattle fed similar amounts of forage, and saliva secretion among animals receiving the same diet can vary by as much as 25% (Bailey, 1961). However, the relationship between the variation in salivary output by individual animals, and the incidence of subclinical acidosis has not been established. Subclinical acidosis can reduce feed intake, and thereby impede growth of feedlot cattle. Low ruminal pH can cause erratic intake patterns (Bauer et al., 1995; Fulton et al., 1979; Stock et al., 1995). Brown et al. (2000) observed a high correlation for feedlot cattle between feed intake and lowest daily ruminal pH on the previous day indicating that animals may adjust their subsequent intake if pH is low. Similarly, we observed that when ruminal pH is low, some animals decrease intake presumably in an attempt to limit the production of fermentation acids and restore pH conditions to a "comfortable" level (Figure 3, K. A. Beauchemin, unpublished data). Once the pH is restored, the cattle resume a high level of feed intake that leads once again to excessive production of acids, causing the cycle to repeat. This effect was monitored using an indwelling pH probe in the rumen of a feedlot steer fed a diet containing 92% barley-based concentrate (14.6% CP), on a DM basis.

Intake and Acid Utilization

Acid Absorption

Differences in the susceptibility of feedlot cattle to subclinical acidosis are not easily explained by acid absorption or metabolism. Fatty acids are readily absorbed from the rumen, (Bergman, 1990; Sharp et al., 1982) at rates enhanced by lower pH (Masson and Philipson, 1951) despite the fact that only a low proportion of VFA exist in the undissociated form at physiological pH ranges. Assuming an average pK_a of 4.8 for VFA, the proportions of VFA that are in the free acid form are approximately 0.6%, 6%, and 39% at ruminal pH of 7, 6, and 5, respectively (Mathews and van Holde, 1990). Hydration of CO_2 at the rumen wall forms HCO_3^- and supplies a proton for converting dissociated VFA to the more readily absorbed free acid form (Bugaut, 1987).

Even if VFA absorption in the rumen is reduced due to parakeratosis or other tissue damage, the omasum, abomasum, and large intestine efficiently absorb VFA (Stevens, 1973a,b; Stevens et al., 1980). The low pH of the abomasum likely ensures that all ruminal VFA is absorbed prior to the duodenum (Peters et al., 1990).

Acid Metabolism

The gut plus its contents uses a disproportionate amount of energy (approximately 25% of total oxygen consumption) for the size of the tissue (approximately 6% of BW), with essentially all of this energy being derived from VFA (Britton and Krehbiel, 1993). Absorption rates for the primary ruminal VFA are butyrate > propionate > acetate. However, quantities appearing in venous effluent are in the reverse order, due to preferential metabolism of butyrate, and propionate (Bergman, 1990).

Fatty acids are utilized through intermediary metabolism following formation of the respective CoA metabolites. Differences between species may exist in the rate of formation of these metabolites in specific tissues. For example, propionyl-CoA synthetase activity is approximately equal in ruminal epithelium and liver in sheep, whereas it is three to four times higher in liver than epithelium tissue of cattle (Elliot, 1980).

The majority of propionate and butyrate entering portal blood is metabolized in the liver. Consequently, acetate accounts for over 90% of the VFA in arterial blood (Bergman, 1990). Providing that the required metabolic cofactors (e.g., biotin, vitamin B_{12}) are available, it is unlikely that VFA metabolism accounts for the variability in susceptibility of feedlot cattle to subclinical acidosis.

Intake and Feeding Behavior

Animal scientists traditionally have focused largely on the nutritional and physiological aspects of metabolic disorders and performance. As a result, a plethora of research studies have evaluated diet formulations, feed processing techniques, and feeding management aimed at improving intake, and performance, and reducing the occurrence of metabolic disorders. However, factors other than these may determine an animal's susceptibility to subclinical acidosis and consequently its growth performance. Feeding behavior, dominance, temperament, and motivation may play as large a part in subclinical acidosis as the type, and amount of feed an animal ingests (Zinn, 1994; Voisenet et al., 1997;

Owens et al., 1998; Grant and Albright, 2001). Cattle fed commercially typically are housed in large groups where social status and learning may affect eating patterns (Galyean and Eng, 1998).

Recent studies indicate that intake patterns differ markedly among individuals within a pen (Gibb et al., 1998; Hickman et al., 2002; Schwartzkopf-Genswein et al., 2002). Large pen trials comparing individual feeding patterns with ruminal pH and performance are nonexistent because continuous monitoring of ruminal pH on individual animals is difficult at best. The development of new technologies that overcome such data acquisition problems will aid in unraveling the complex relationship between intake, metabolic disorders, and performance.

Feeding Management

One goal of feeding management has been to moderate feeding behavior, and reduce daily variation in intake among penned feedlot cattle (Bauer et al., 1995; Gibb et al., 1998; Galyean, 2001). Feeding regimes may act in harmony with feeding behavior or they may be disruptive. For example, digestive upsets may be minimized due to a more uniform intake of fermentable carbohydrates if the feeding regime leads to smaller, more frequent eating episodes. In contrast, intermittent binge feeding is believed to contribute to metabolic disturbances (Pritchard and Knutsen, 1995). At this time, however, the extent to which feeding can be manipulated to maximize DMI, and minimize digestive upsets in group-fed cattle is largely unknown.

Ad libitum vs Restricted Feeding.

Feedlot cattle usually are given continual access to feed in an attempt to maximize feed intake. This strategy is known as ad libitum or free-choice feeding. Bunk management strategies in this scenario focus primarily on maintaining bunk hygiene by adjusting the amount of feed provided so that residual feed (weighbacks) does not exceed a small percentage of what was delivered (Galyean, 1999; Schwartzkopf-Genswein and Gibb, 2000).

Restricted feed delivery limits intake relative to actual or anticipated ad libitum intake (Galyean, 1999). The goal of restricted feed delivery is to improve performance by reducing digestive problems from overconsumption of feed. Generally, feed is restricted by 0.5 or 1 kg per meal. Whereas ad libitum feeding attempts to maximize intake on a daily basis, restricted feeding strives to maximize mean intake over the course of the feeding period (Galyean, 1999). However, restricting feed access may cause subclinical acidosis and, an overall reduction in mean intake. This is because restricted feeding typically results in animals becoming meal eaters (consuming a few large meals); although the variation in their total daily feed intake is reduced (Zinn, 1995), variability in the ruminal environment within a day may be increased. Studies investigating the effects of bunk management have produced conflicting results. Fanning et al. (1999) reported that as a consequence of changes in eating patterns, cattle fed on a restricted protocol exhibited lower, and more variable ruminal pH than those provided ad libitum access to feed. Larger meals and a faster rate of eating resulted in a greater pH decline in the restrictively-fed cattle. In contrast, Gibb et al. (1998) reported that individual cattle exhibited less day-to-day variation in time spent at the bunk when they were limit-fed (95% of ad libitum intake) than when they were on full feed.

The assumption that positive animal responses to bunk management (if present) are a direct result of reduced acidosis is unsubstantiated. The findings of Fanning et al. (1999) contradict these assumptions, but the practice of slickbunk management to improve performance is not necessarily discredited. Increased eating rates and less frequent meals are commonly observed among cattle given limited access to feed (Gibb et al., 1998; Prawl et al., 1998; Fanning et al., 1999; Schwartzkopf-Genswein et al., 2002). Increased eating rates have been associated with improved performance in sheep (Church et al., 1980) and with increased intake and performance by cattle (Frisch and Vercoe, 1969; Prawl et al., 1997). Streeter et al. (1999) identified an apparent negative correlation between time spent at the feed bunk and ADG, which suggests that cattle with higher growth rates likely also have higher eating rates. Similar results were found by Schwartzkopf-Genswein et al. (unpublished) for cattle receiving an 85% barley grain finishing diet. In contrast, cattle that spent the most time at the bunk had the highest ADG when the diet consisted of 60% barley silage. These data must be interpreted carefully, however, because the relationships between average time duration at the bunk, attendance, and intake are poor (Gibb et al., 1998; Schwartzkopf-Genswein et al., 2002).

Variation in Feed Intake

It is commonly assumed that fluctuations in intake can cause acidosis and reduce mean DMI (Britton and Stock, 1987). This belief, held by many cattle feeders, is supported by a study of Galyean et al. (1992) in which deliberate 10% fluctuations in feed delivery to cattle reduced gain by 6% and feed efficiency by 7%, compared to cattle receiving feed according to a constant programmed feeding schedule based on BW. In that study, impaired performance was attributed to subclinical acidosis arising from intake variation, even though ruminal pH was not measured. This theory remains prevalent despite a mounting body of research that has contradicted these findings (Zinn, 1994; Stock et al. 1995; Cooper et al., 1998a; Owens et al., 1998; Soto-Navarro et al., 2000; Hickman et al., 2002). Monitoring ruminal pH in a metabolism trial, and animal performance in a finishing trial that included deliberate fluctuations in intake, Cooper et al. (1998b) observed that ruminal

pH was lower in limit-fed cattle with day-to-day intakes varying by 1.4 kg, than in those receiving constant amounts of feed. Ruminal pH did not differ among cattle consuming a fluctuating amount of feed and there were also no differences in pH among ad libitum fed cattle. During the study, an equipment malfunction delayed feeding for 4 h at which time it was observed that delayed feeding (such as could arise in a commercial lot, with equipment breakdown or inconsistent timing of feed delivery) can have a greater effect on ruminal pH than fluctuating quantities of feed. In a finishing trial by Cooper et al. (1998a), fluctuating the daily amount fed by 1.8 kg/d numerically increased intake but did not affect ADG or feed conversion. Similar results were obtained by Schwartzkopf-Genswein et al. (2002) in a finishing trial comparing cattle fed at a constant level (ad libitum intake) with cattle fed amounts of feed fluctuating by 10% above, and below ad libitum intake for three consecutive days. Constant- and fluctuating-fed animals exhibited similar intake, ADG, feed efficiency, and time spent at the bunk. However, mean ruminal pH was 0.10 units lower in fluctuating-fed compared to constant-fed animals, and remained lower for a greater portion of the day in a smaller metabolism trial in which the same feeding strategies were applied (Figure 4; Schwartzkopf-Genswein et al., 2002). This study suggested that mean ruminal pH may increase when cattle are introduced to high-grain diets, and their day-to-day intake is more likely to fluctuate after a period of adaptation; however, mean, and minimum pH became similar between feeding regimes, as did animal performance. These data imply that cattle consuming high-concentrate diets can adjust metabolically to inconsistencies in feed delivery, and intake after an adaptation period of at least 28 d.

A recent study conducted by Hickman et al. (2002) evaluated the relationship between eating patterns and performance in feedlot cattle by electronically tracking individual visits, and feed consumption. Daily variation in intake (defined as difference in total amount of feed consumed between consecutive days) by individual animals was compared among animals grouped according to high, average or low DMI, ADG, or feed efficiency. High ADG steers (n = 9) had daily variation in intake that was on average 0.36 kg higher, consumed 2.1 kg more feed, and spent 3.7 min / d less time at the bunk than did low ADG steers (n = 13). Similarly, steers with the highest feed efficiency (feed:gain) had higher daily variation in intake (0.38 kg), and lower intake (1.1 kg) than steers with low feed efficiency. It was concluded that the best-performing (ADG and feed efficiency) cattle have the most variable feeding patterns which is contrary to industry belief. This study is valuable as it illustrates the variation in intake by individual animals that may be lost when performance, and intake parameters are assessed on a pen basis. Patterns of intake by individual cattle categorized into high or low ADG groups are presented in Figure 5. Feed intake by individuals in the low ADG group was consistently below the pen average, whereas those in the high ADG group consistently consumed more (Figure 5). The need for further investigation, and refinement of feeding practices to include the evaluation of individual feeding behavior is emphasized. Daily variation in feed intake may not impact ruminal pH as much as the rate at which feed is consumed.

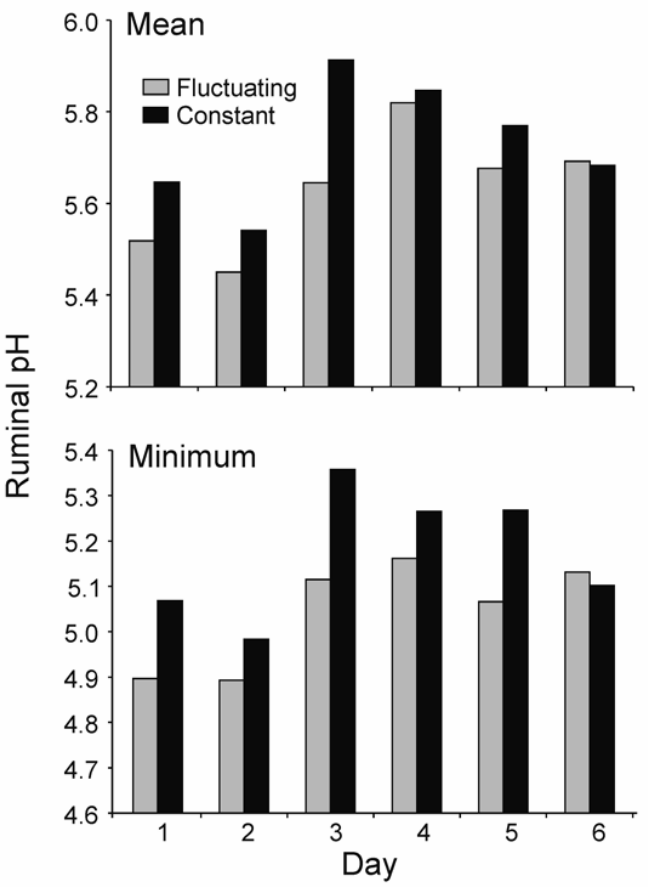

Figure 4. Mean (upper graph) and average minimum (lower graph) ruminal pH recorded in feedlot steers, (n = 6) fed a high-grain finishing diet offered once daily. Steers received feed delivered in a constant amount equal to their previously determined ad libitum intake (black bars) or at 110% of ad libitum for d 1 to 3, followed by 90% of ad libitum intake for d 4 to 6 (grey bars). Mean pH values are averages of values recorded at 15-min intervals using indwelling pH probes.

Finally, cattle may have an ability to alter or regulate their intake patterns such that variation in feed delivery has less impact than expected. Evaluation of the effects of early or late feed delivery (0800 or 2100 h) on performance, and feeding behavior of cattle revealed no changes in diurnal feeding patterns in response to feeding times (Figure 6; Schwartzkopf-Genswein et al., 2000). Even restricting feed delivery to 85% of ad libitum intake was not sufficient to change the normal diurnal feeding patterns of those cattle. This study indicates that certain behaviors are inherent, and not easily

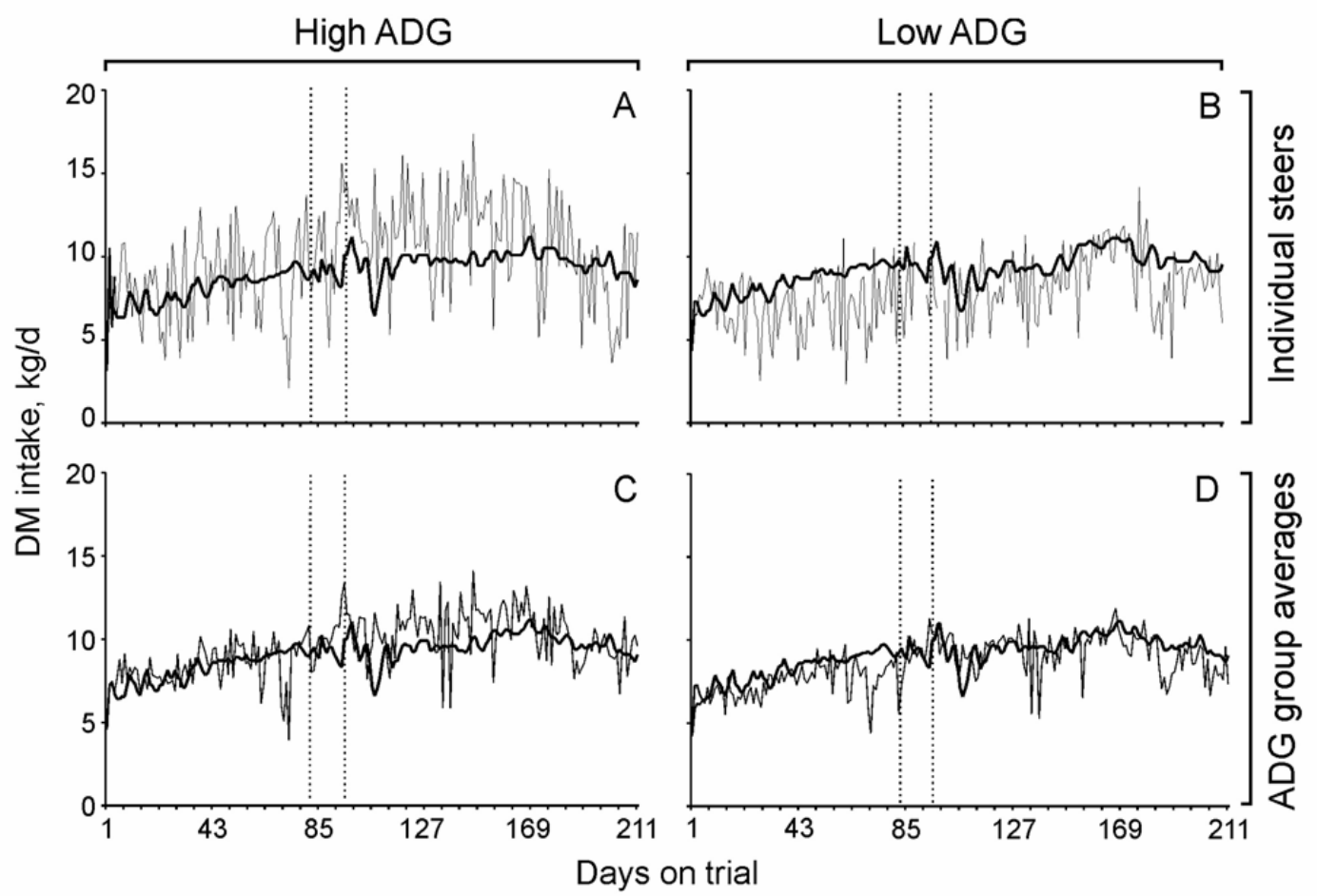

Figure 5. Daily variation in dry matter intake by feedlot steers receiving barley silage/barley grain-based diets (growing diet at 80% silage and 20 % grain; finishing diet at 20% silage and 80 % grain on an as-fed basis) ad libitum in a 211-d feeding trial during which individual intake was monitored by radio frequency transmission, and after which steers were categorized as having high, average, or low ADG (over mean + 1 SD, within mean ± 1 SD, or below mean – 1 SD). Panels A and C contain data collected on an individual steer (A;), and the entire group (n = 9) of (C) high ADG steers; panels B and D contain data collected on an individual steer (B), and the entire group (n = 13) of (D) low ADG steers. Bold lines indicate amounts of feed (kg DM/steer) delivered to each steer's pen (A, B) or mean of deliveries to all pens (C, D). Dashed vertical lines indicate the transition period (separates growing and finishing periods) where grain was gradually increased from 20% to 80% (as-fed basis). Mean (± SD) start and finish weights for steers were 311.7 ± 50.6 and 577.9 ± 53.7 kg, respectively.

altered. Thus, the tendency of individuals to adapt their feeding patterns to any change in feed delivery may also play an important role in modulating metabolic disorders.

Potential for the Genetic Improvement of Feeding Behavior

Several studies, involving both beef and dairy cattle, suggest that various measures of temperament, and other behavioral characteristics are slightly to moderately heritable (Hohenboken, 1986; Le Neindre et al., 1995). However, studies of the inheritance of feeding behavior have been limited in scope because of the difficulty in collecting sufficient data for parameter estimation. Voisenet et al. (1997) showed a favorable correlation between docility, and performance. With further study, the potential may exist to select for improvements in the behavioral aspects of feed intake. However, indirect selection may have been practiced, as Schutz and Pajor (2001) suggest, for more optimal feeding behavior as a correlated response to improved growth rate. Since intake variation does not appear to have a drastic effect on performance, selection for feed efficiency may still be the most useful criterion to employ in animal breeding programs.

Implications

The majority of pen-fed cattle can and do tolerate fluctuations in feed delivery and consumption. Recent

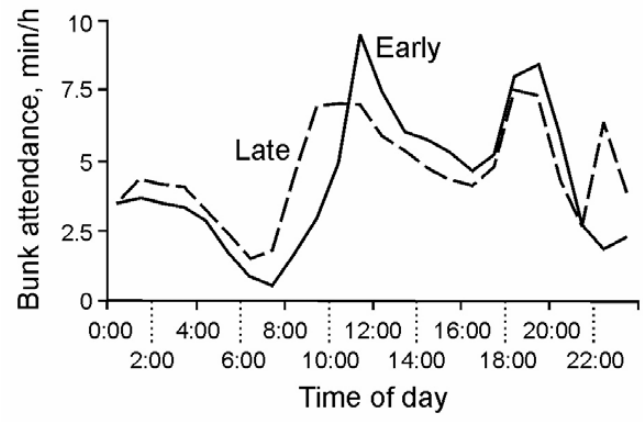

Figure 6. Effect of timing of once-daily feed delivery on bunk attendance patterns of feedlot cattle (n = 240) given 24-h, free-choice access to a barley-based diet over a 210-d period (December- July). Early: feed delivered at 0800 daily; late: feed delivered at 2100 daily. Values plotted are means of hourly totals recorded over the course of the study. Mean ADG and feed efficiency for early and late fed animals were (1.22 ± 0.10 and 1.25 ± 0.10 kg/d) and (6.25 ± 0.2 and 6.37 ± 0.2), respectively.

data have shown marked individual variability in feed intake, feeding behavior, and ruminal pH profiles. The role of management in subclinical acidosis may remain unclear if pen average data continue to be the evaluation benchmark. It is imperative to monitor individual animal feeding behavior (using technology such as radio frequency) so that the variables related to metabolic disorders can be identified. A growing body of data demonstrates that feedlot cattle on finishing diets can readily adapt (physiologically and behaviorally), such that day-to-day intake variability does not negatively affect performance; however, cattle may be less able to adapt to changes during the transition period. Future studies should include evaluations of intake variation and the incidence of subclinical acidosis during the transition period when instability in ruminal microflora and pH are highest.

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Modeling stochasticity: Dealing with populations rather than individual pigs

C. Pomar*,[2], I. Kyriazakis†, G. C. Emmans†, and P. W. Knap‡

*Dairy and Swine Research and Development Centre, Agriculture and Agri-Food Canada, Lennoxville, Quebec, Canada J1M 1Z3; †Animal Nutrition and Health Department, Scottish Agricultural College, West Mains Road, Edinburgh, EH9 3JG, Scotland, UK; and ‡PIC International Group, Schleswig, Germany

ABSTRACT: Pig production efficiency is the result of the responses of individual animals. However, experimental results are usually interpreted on the basis of mean animal responses with little emphasis given to variation around the means. Animals with different genetic potentials may respond differently to treatments, which makes it difficult to translate average population responses into either individual animal responses or across populations having different variation between animals. Nutritional theories that form the basis of current pig models are all at the level of individual animals. The problem of how to integrate across individuals to obtain population predictions is rarely addressed. To illustrate the effect of between-animal variation on population responses to dietary treatments, a pig growth model that predicts voluntary feed intake from parameters that predict the potential rates of protein and lipid retention was used. Feed intake is limited only by the ability of pigs to lose heat. Maximum heat production was set to always be that of a pig growing to its potential on a standard, balanced diet. An individual animal is defined by assigning values to three genetic parameters: protein weight at maturity, the ratio of body lipid-to-protein at maturity and a rate parameter. The model was made stochastic by assigning variation to these parameters. Population responses to increasing levels of available protein intake indicated that the linear-plateau model used to represent protein responses for an individual pig is compatible with the curvilinear response observed in experiments on populations. Increasing the time over which individual animal responses were measured also increases the curvilinearity of the response. Variation between animals has little effect on population feed intake, but it decreased population protein deposition rate, daily gain, and feed conversion ratio. It is concluded that mathematical models designed to simulate populations responses to treatments need to integrate the effect of population variation on growth and performance.

Key Words: Genetic Variation, Growth, Protein, Simulation Models

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Introduction

The efficiency of pig production systems results from the efficiency of individual animals (Knap, 1995). However, reported research results give only rudimentary information about the variation observed between animals. Results are mainly interpreted as average responses to treatments with little emphasis given to the variation around means. This variation may be essential in the understanding of the biological mechanisms implicated in the response of populations to treatments. Examples of this are the impact of variation on population protein efficiencies and growth performance (Pomar, 1995; Ferguson et al., 1997), on thermoregulatory maintenance requirements (Knap, 1999, 2000a) and nutrient requirements (Pomar, 1995; Leclercq and Beaumont, 2000). Several mathematical models have been developed for a variety of applications and species, such as growing pigs (Whittemore and Fawcett, 1976; Black et al., 1986; Moughan et al., 1987; Pomar et al., 1991a; Ferguson et al., 1994; Knap, 2000a) among others. Model development evolves from empirical models to mechanistic ones in which biological principles are represented (France and Thornley, 1984). However, these principles are often derived from results obtained on populations of individuals that show phenotypic variation. Population means are then applied to single-animal models. Subsequently, such single animal models, perhaps representing some notional "average" animal, are used to model group-animal responses by assuming that all pigs have equal growth potentials and

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[2]Correspondence: P.O. Box 90, 2000, Route 108 East (phone: 819-565-9171; fax: 819-564-5507; E-mail: pomarc@agr.gc.ca).

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are at the same stage of growth. However, population responses may differ from individual responses as a result of between-animal variation (Curnow, 1973; Fisher et al., 1973; Pomar, 1995). The objective of this study was to use a pig growth model that predicts voluntary feed intake, performance, and protein and lipid deposition to deal with variation. The aim was to show the impact of between-animal variation on the interpretation of the biological principles represented within the model and the response of populations to various protein intakes.

Model Description

The model predicts voluntary feed intake, body weight, and composition of growing-finishing pigs during the growth period. Diet composition and pig genotype are inputs to the model. In addition, different assumptions about variation are studied. Animals are assumed to have free and continuous access to a single homogenous feed at any one time. The feed contains no toxins and is such that the rate of intake is not limited by the digestive capacity of the pig. The environment is thermally neutral at all times. Under these conditions, the pig is able to eat the amount of a balanced diet that is sufficient to satisfy its nutrient requirements. When the feed is unbalanced in terms of its protein-to-energy ratio, the amount of food eaten is limited by the amount of heat that the pig can lose. Maximum heat loss is set to what the pig would produce were it to be fed on a balanced standard diet. State model variables are actual body masses of protein (**Pt**) and lipid (**Lt**). The energy system used was that of Emmans (1994). Euler's integration method was used to solve the differential equations with an integration step (**dt**) of 1 d. Rate variables are expressed in daily basis, energy is in megajoules, mass in kilograms, and concentrations in kilogram basis when not explicitly specified in the text.

Feeds are defined in terms of their contents of digestible energy (**DEc**), crude protein, apparent ileal digestibility, chemical score (**ChSc**) estimated as the amount of digestible amino acids of the diet that are in an ideal balance, digestible fat (**DFc**), and total indigestible organic matter content (**IOMc**). The digestible protein content of a diet (**DPc**) is calculated as the product of the crude protein content and its apparent ileal digestibility. Balanced digestible protein content (**BPc**) is calculated as the product of DPc and ChSc. Effective energy content (**EEc**) of the diet is the difference between DEc of the diet and losses resulting from the eaten diet (Emmans, 1994). In this energy system, energy losses from fermentation are considered negligible. The EEc of the feed is calculated from equations relating DEc and EEc to the chemical composition of the feed (see below). Although the net and effective energy systems are fundamentally different, both systems can be used successfully in mathematical models predicting the metabolic utilization of feed energy (Rivest et al., 1996). The model predicts, on a daily basis, body composition, protein and lipid deposition, and feed intake at any age and body condition. The model is based on that used by Knap (1999, 2000a), which includes some elements of the model of Moughan and Smith (1984), and uses the rules proposed by Emmans (1988, 1994, 1997) to predict feed intake, as well as the partitioning rules and other elements suggested by Kyriazakis and Emmans (1995, 1999). The basic rules driving protein and lipid deposition, simulating energy utilization, and predicting feed intake are similar to those used by Wellock et al. (2003).

A pig is genetically characterized by three parameters, which are the mature total body protein weight (**Pm**), its inherent fatness represented by the lipid-to-protein ratio at maturity (**LPRm**), and the maturing rate constant (**B**). Emmans (1988, 1997) and Ferguson et al. (1994) have presented the detailed theoretical description of this model. In this model, Pt and Lt define the actual state of the animal. Total body water and ash are quantified following Emmans and Kyriazakis (1999). Total body weight is the sum of the fill (gut and bladder) and the chemical constituents of the empty body, which are the protein, lipid, ash, and water masses.

The intrinsic potential for protein deposition rate (**dPd**) is assumed to follow the derivative of a Gompertz growth function (Emmans and Kyriazakis, 1999, 2001) as follows.

$$dPd = B \times Pt \times \log_e(Pm/Pt)$$

The potential rate of protein deposition is estimated from two parameters (B and Pm) that are assumed specific to each pig and from its current protein weight. An implication is that compensatory protein growth is not allowed. Any delay in protein retention alone will simply decrease the retention of the other body constituents (namely, lipid, ash, and water) and will delay the attainment of body's mature size. The above framework is similar to the one used by Ferguson et al. (1994, 1997), Knap (2000a), and Wellock et al. (2003).

Associated with Pt is body lipid mass (Lt), which is predicted from LPRm and Pm. The value of the allometric coefficient b1 (Emmans, 1988) is calculated from LPRm only. The coefficient b1 and Lt are represented as follows.

$$b1 = 1.46 \times LPRm^{0.23}$$

$$Lt = LPRm \times Pm \times (Pt/Pm)^{b1}$$

To minimize integration errors, the desired lipid deposition rate (**dLd**) is then calculated by difference between Lt weights at times t + dt and t. The dPd and dLd are combined with estimations of protein and energy requirements to predict the unconstrained desired feed intake in a thermally neutral environment. Thus, under nonlimiting conditions, pigs will eat daily an unbal-

anced feed, until the requirement for the more-limiting nutrient is met. All nutrients other than the more-limiting one will be consumed at least to adequacy. The daily effective energy ($\mathbf{EE_{req}}$) and ideal protein requirements ($\mathbf{P_{req}}$) are calculated as follows.

$$EE_{req} = EE_{maint} + (50 \times dPd) + (56 \times dLd)$$

$$P_{req} = P_{maint} + (dPd/e_p)$$

where 50 and 56 are the effective energy costs (MJ/kg) of protein and lipid retention, respectively, and $\mathbf{e_p}$ is the efficiency of using ideally balanced protein for protein deposition. Here, EE_{maint} is the energy, and P_{maint} the ideal protein, requirements for maintenance calculated as suggested by Emmans (1994). Thus, when energy is the first-limiting nutrient, the desired feed intake (dFIe) is estimated as follows.

$$dFIe = EE_{req}/EEc$$

Similarly, when protein (or any essential amino acid) is the first limiting nutrient, dFIp will be that needed to meet its requirement:

$$dFIp = P_{req}/BPc$$

A feed is balanced, by definition, when dFIe = dFIp. Otherwise, the desired feed intake (**dFI**) is the maximal value between dFIe and dFIp. Pigs are thus expected to eat extra protein on low-energy feeds and extra energy when feed protein content is the limiting factor. In the first case, pigs are assumed to deaminate any excess protein and to lose its nitrogen and part of its energy in the urine (Emmans, 1994). On low-protein feeds, the energy eaten above the requirement leads to additional lipid retention (Ferguson et al., 1994). On imbalanced feeds, the pig may be either fatter or leaner than it seeks to be. It is assumed that the pig will attempt to return instantly (i.e., within a day) to its normal fatness by reducing or increasing lipid deposition rate. Its desired rate of lipid deposition will then be either below or above the value calculated from its genetic potential (Kyriazakis and Emmans 1999). However, the ability of the pig to achieve its desired normal fatness will depend on the composition of the feed and its capacity to lose heat (see below). This compensatory lipid growth is in agreement with previous experimental results (Tullis et al., 1986; Kyriazakis et al., 1991).

The small number of parameters (Pm, LPRm, and B) used to characterize the animal's genetic potentials for growth of protein and lipid, and the ability to predict voluntary feed intake, are attractive when the relationship between growth potentials and feed intake is of interest. The approach, also used in another form by Black et al. (1986), differs from those used in many other simulation models in which feed intake is either predicted from simple relationships or treated as input. In these latter models, the pig's appetite needs to be characterized jointly with the genetic potential for protein deposition.

It is possible that the dFI needed to satisfy the requirement for the first-limiting nutrient (energy or the first-limiting amino acid in this model) cannot be achieved. This is due to constraints arising either from the feed that is offered or from the environment in which the animal is kept. In the first case, the feed constraint may be represented by its volume in terms of dry or organic matter intake (Black, et al., 1986; Ferguson et al., 1994; Pomar and Matte, 1995; Whittemore et al., 2001a) or feed bulkiness as measured by its water-holding capacity (Kyriazakis and Emmans, 1995; Whittemore et al., 2001c,d). For the purpose of present study, which is to investigate the effect of between-animal variation on pig population responses, the effect of feed bulkiness is avoided and no direct feed constraint is allowed.

In the model, pigs are assumed to be in thermoneutral conditions at all times and no thermoregulation mechanisms were incorporated into the model. The capacity to lose heat when unbalanced diets are offered is set to that predicted on a balanced feed. A balanced diet contains 12.6 MJ/kg of effective energy, a protein-to-energy ratio that meets the requirement and a protein balance and chemical composition similar to the one suggested by NRC (1998) for 20- to 50-kg BW pigs. The contents of IOMc and DFc are 0.12 and 0.03 kg/kg, respectively. The digestible energy content is 14.2 MJ/kg. The ratio between EE_{req} and P_{req} is the optimal ratio of the reference diet, from which optimal balanced protein content can be estimated as follows.

$$DPc = 12.6 \times P_{req}/EE_{req}$$

The relationship between DEc and EEc is

$$DEc = EEc + (3.8 \times IOMc) + (10.3 \times DPc) - (0.9 \times 12 \times DFc)$$

where 10.3 = 5.63 + 4.67. The value of 5.63 is the energy contained in the nitrogenous compounds excreted in the urine. The values of 3.8 and 4.67 are the heat productions associated with organic matter defecation and protein excretion, respectively (Emmans, 1994). It was assumed that 90% of DFc can be retained in the pig's body as body lipid and 12 is the adjustment to account for the difference in heat increment of forming lipid from lipid and nonlipid dietary sources (Emmans, 1994). The ME dietary content (**MEc**), corrected to zero protein deposition, can then be estimated as

$$MEc = DEc - (5.63 \times DPc)$$

The amount of heat produced (**H**, MJ/d) by the reference pig, which is also the maximal amount of heat that any simulated pig can produce, is estimated as

$$H = (FI \times MEc) - [(hp - 5.63) \times dPd] - (hl \times dLd)$$

where FI is the actual feed intake and hp and hl are the heat of combustion of the retained protein and lipid, respectively.

Heat loss will limit the voluntary feed intake of pigs on an unbalanced diet, or where body lipid needs to be increased following a previous energy deprivation. Heat loss is independent of the composition of the feed given and of the body fatness of the pig.

Generating Populations

As outlined above, a pig is described in this model in terms of only Pm, LPRm, and B; Gompertz functions are used to determine dPd and dLd. Between animals of the same population, there is likely to be a negative correlation between the values of B and Pm (Emmans 1988; Knap 2000b). Therefore, B and Pm cannot be simulated as being independent and their covariation needs to be estimated. To avoid this, Emmans and Fisher (1986) scaled the parameter B according to Taylor's rule (Taylor, 1968, 1980) as follows.

$$B^* = B \times Pm^{0.27}$$

where **B*** is the scaled value of B. The values of B*, Pm, and LPRm are then uncorrelated (Ferguson et al., 1997). Two genetic lines are used in the following simulations. The first, probably a traditional pig line, was that characterized by Ferguson and Gous (1993) and Ferguson et al. (1997). Average mean genetic parameters of this line are μPm = 38 kg, μLPRm = 2.5 kg/kg and μB* = 0.0294/d. The second line, which may represent a modern genotype, was characterized by Knap (2000b) from data of van Lunen (1994) collected from 60 female pigs of a synthetic sire line. This synthetic line was identified by Knap (2000b) as the most advanced meat-type pig genotype available in the early 1990s, which may represent pigs used today in well-managed piggeries. Mean genetic parameter values defining this line are μPm = 32 kg, μLPRm = 1.2 kg/kg, and μB* = 0.04079/d. Because data to estimate these parameters are scarce and it is difficult to determine their variability by experimentation, these authors estimated parameter variation by simulation as outlined by Knap (2000b) and Knap et al. (2002). Their results indicated that the genetic coefficients of variation for B*, Pm, and LPRm were 0.02, 0.10, and 0.15, respectively for the traditional line and 0.03, 0.07, and 0.15, respectively for the modern line. For each simulated pig within a population, values for B*, Pm, and LPRm are drawn at random, uncorrelated, and normally distributed (Emmans and Fisher, 1986; Ferguson et al., 1997; Knap, 2000a). The values that characterize the animals are drawn before each simulation and maintained throughout their simulated life. When comparing populations with different between-animal genetic variation, five populations are generated having 0, 0.5, 1, 1.5, and 2 times the estimated genetic variation of the above reference populations. The multiplier is 0 in the case of a single animal. Because the nutritional and the simplified thermal environment affects the expression of the genetic potential of the pig, generating genetic variation on B*, Pm, and LPRm simulates phenotypic variation. Therefore, pigs of the same genetic population but reared in different environments may show different growth curves and variances. Population values were obtained by simulating 2,500 pigs, ensuring that variances were stable between runs.

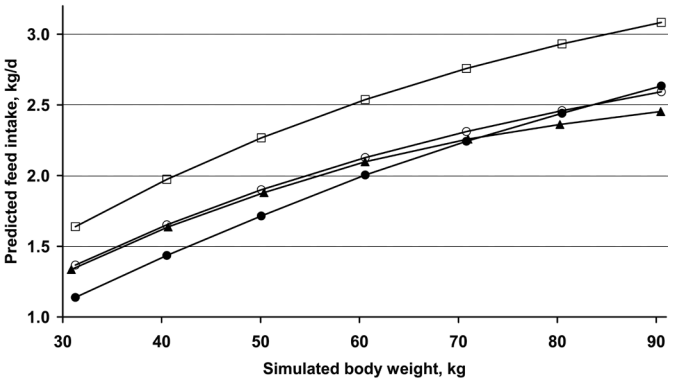

Figure 1. Predicted voluntary feed intake over the 30- to 90-kg BW of the traditional (—●—) and modern (—▲—) simulated pig genetic lines and according to Patterson and Walker (1989) (–□–) and NRC (1998) (–○–) prediction equations.

Model Results and Discussion

The model's mathematical and logical properties were checked for consistency throughout development. The mathematical stability of the model was evaluated at different integration steps to ensure stability. Because the relationships driving protein and lipid accretion and predicting feed intake are similar to those used by Ferguson et al. (1994, 1997), Knap (2000a), Knap and Jorgensen (2000), and Kyriazakis and Emmans (1999), the model did not need to be validated in that respect. The main difference from previous models is the mechanism used to constrain feed intake when pigs are fed with unbalanced diets through setting a maximum to heat loss.

Voluntary Feed Intake

The proposed model predicts voluntary feed intake based on the animal's energy and protein requirements and the composition of the offered diet. Depending on the pig's fatness and the composition of the diet, feed intake may be limited by the amount of heat that the pig is able to dissipate into the environment.

Model predictions of feed intake were evaluated by comparing them to the predictions of several published relationships between feed intake and live body weight. Feed intake predictions based on the NRC (1998) and Patterson and Walker (1989) relationships are shown graphically (Figure 1) because most of the other pub-

lished predictions lie between them (Whittemore et al. 2001a). The NRC (1998) relationship is proposed for a combination of barrows and gilts, which can be adjusted upward for castrates and downward for intact males and females. The model was set up to predict the voluntary feed intake of the traditional and the modern genetic lines, both fed successively on three feeds containing 14.23 MJ DEc/kg and having all other nutrients in excess, including lysine and protein. Feeds were formulated according to the NRC (1998) recommendations for the 30- to 90-kg BW interval. Model predictions were adjusted to account for 5% feed wastage.

Mean values for the traditional line were as follows: an ADG of 868 g/d, an average daily feed intake (ADFI) of 2.01 kg/d, and daily rates of deposition of protein (**ADPG**) and lipid (**ADLG**) of 141 and 207 g/d, respectively. For the modern line, the equivalent values were 991, 2.11, 179, and 175, respectively. In the 30- to 90-kg BW interval, the modern genotype ate 7.17% less feed than the traditional one but 28.6% less than the amount predicted by the NRC (1998) and 2% more that the amount eaten by the Patterson and Walker (1989) pigs. Nonetheless, both simulated genetic lines tended to eat less food than others pigs, the feed intake of which has been described in the literature (Whittemore et al. 2001a). This low feed intake in the simulated genetic lines is the result of their low amount of body fat. In fact, increasing Pm and LPRm genetic parameters increases fat deposition, and therefore, average ADFI, while decreasing B increases the slope of the predicted ADFI with weight. Differences in the slope between the two simulated lines resulted mainly from differences in the B parameter value.

When predicting ADFI, most of the proposed relationships use weight or protein masses to predict the pig's daily intake. Therefore, these equations need to be tailored to each genotype and periodically adjusted to account for genetic changes. However, the modeling framework proposed here is much more flexible than these empirical relationships because it can predict ADFI of pigs according to their genetic growth potentials and actual growth conditions.

The Effect of Variation Between Pigs on Protein Deposition

The amount of protein (amino acids) required to satisfy the total needs of a growing pig is the sum of the requirements for maintenance and retention, the latter corrected by the efficiency of utilization of the absorbed protein (or amino acids). The efficiency (e_p) with which the absorbed protein above maintenance is used for protein deposition is defined as the proportion of the additional ingested ideal protein, which is retained when protein is limiting and when energy is not limiting. Practically, e_p can be estimated by measuring the increase in protein deposition in pigs fed with increasing levels of protein in conditions under which this nutrient is always supplied below requirements. The relationship between protein retention and supply has been represented by a constant efficiency (Zhang et al., 1984), as two-phase linear (Taylor et al., 1979; Batterham et al., 1990), as curvilinear (ARC, 1981; Moughan, 1989; Fuller and Garthwaite, 1993), or by a linear plateau (Campbell et al., 1984). Furthermore, Bikker (1994) indicated that the goodness of fit of these models was quite similar, and, although the linear-plateau model tended to show a better fit, no firm conclusion could be drawn about the degree of curvature of the transition between the linear and plateau phases. Analogous linear-plateau models are also proposed to represent the responses between protein retention and energy supply (Black et al., 1986; NRC, 1998; Whittemore et al., 2001b).

The linear-plateau is frequently the preferred model (Whittemore et al. 2001b). This model was suggested by Black and Griffiths (1977) in sheep and Campbell et al. (1984) in pigs and applied to pigs by Whittemore and Fawcett (1976) and many other authors. However, other authors have suggested that e_p is not constant but decreases gradually as protein or energy intakes increase (ARC, 1981). However, the adequacy of the linear-plateau model is not always supported by experimental results and some concerns have been raised in relation to its appropriateness when representing pig populations (Baker, 1986; Moughan, 1999). Curnow (1973), Fisher et al. (1973), Fuller and Garthwaitte (1993), and Pomar (1995) suggested that a curvilinear response of protein deposition to protein intake, at the level of the population, might result from variation in individual animal responses. In this section, the effect that between-animal genetic variation may have on the evaluation of e_p when protein intake increases for populations with different degrees of heterogeneity was studied.

Populations of 50-kg BW modern pigs were fed for 1 d with 11 diets, all having 0.20 kg/kg dietary crude protein and the same ileal digestibility. The chemical score (ChSc) (i.e., protein quality) varied from 0.68 to 0.93. Varying ChSc allowed the pigs to eat the same amount of DPc, but amounts of ideal protein ranged from 212 to 290 g/d. Increases in ChSc may be obtained by adding lysine and other crystalline essential amino acids to give proportions closer to those of an ideal protein. In this exercise, pigs were restrictedly fed at 1.9 kg/d to facilitate the interpretation of the results. For a single 50-kg BW pig fed with the highest ChSc diet, ADG was of 997 g/d and ADPG and ADLG were 181 and 159 g/d, respectively. The pig growth model was constructed assuming that the response to limiting protein follows the linear-plateau model. The response of the population with zero variation is the direct outcome of this assumption (Figure 2). Maximal protein deposition rate is obtained for ChSc > 0.75. The slope of the response represents the marginal efficiency for protein utilization (e_p), whereas the intercept represents the protein requirements for maintenance.

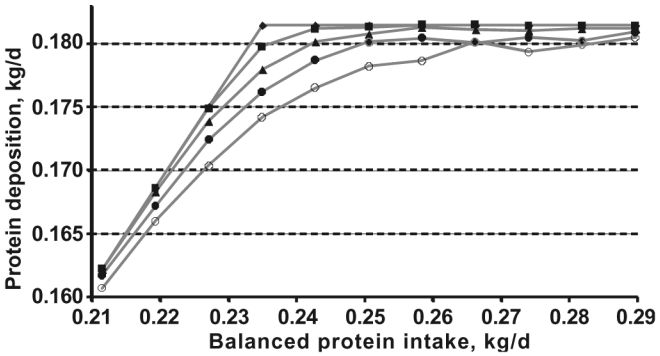

Figure 2. Effect of between-animal variation (0 [–◆–], 0.5 [–■–], 1 [–▲–], 1.5 [–●–], and 2 [–○–] times the standard coefficient of variation of the population) and balanced protein intake on average daily protein deposition rate of 50-kg pig populations.

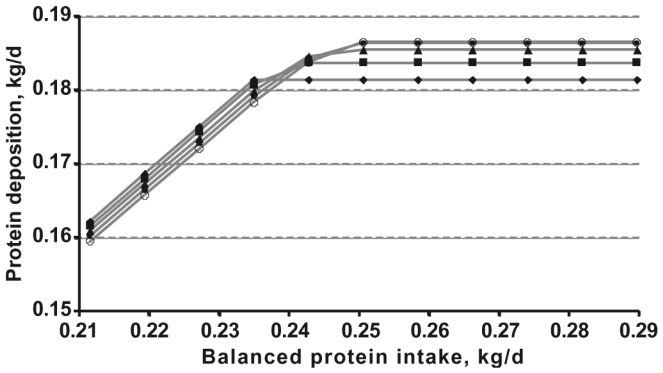

Figure 3. Effect of data collection length (1 [–◆–], 7 [–■–], 14 [–▲–], 21 [–●–], and 28 [–○–] d of collection) and balanced protein intake on average daily protein deposition rate on 50-kg BW pigs.

The simulation results shown in Figure 2 suggest that the curvilinear response of ADPG to increases in protein intake observed by several authors (e.g., Bikker, 1994) can be explained by the differences in animal responses, in this case, generated as between-animal variation. The length and degree of curvature of the transition zone between the two linear phases increase with the population variability. In the transition zone, one part of the population is underfed, whereas the rest is overfed in protein. The proportion of pigs overfed increases as ChSc increases. Similarly, for a fixed ChSc, the proportion of pigs underfed in the transition zone increases with population variation. This explains the lower protein deposition and greater degree of curvature of the response in populations with larger variation. Finally, although it cannot be ascertained whether or not the linear or curvilinear model is the best model for representing an individual response, the results of this study indicate that the linear-plateau model for individual pigs is compatible with the observed experimental curvilinear responses seen in populations.

The Effect of the Duration of Data Collection Periods on Measurements of Protein Efficiency

Experimental error is a measure of the variation that exists among observations on experimental units treated alike. This variation comes from the inherent variability that exists in the experimental material to which treatments are applied and from the variation resulting from deficiencies in the experimental procedures used (Steel et al., 1997). In previous sections, the problem relating to the inherent variability of the experimental material (i.e., animals) was addressed. In this section, another source of variation that results from the experimental procedures used when measuring protein efficiency is considered. A fact often neglected is that an animal's response to nutrient intake may change over the interval during which data are collected. In particular, when measuring protein deposition during a specific interval length, it should be noted that this variable might be changing over time.

The effect of length of the collection period can be illustrated by simulating the response of a single pig to increases in balanced protein intake. In this case, the modern genotype was fed with the 11 diets used in the previous section and data were collected for 1, 7, 14, 21 or 28 d. Pigs were restrictedly fed at 1.9 kg/d to simplify the interpretation of the results.

As in the previous section, maximal protein deposition was obtained for ChSc = 0.75. However, when the length of the period of data collection was increased from 1 to 28 d, the point at which protein deposition was maximal was greater, and a curvilinear transition zone appeared between the two linear phases with constant e_p (i.e., $e_p = 0.82$ and $e_p = 0$) (Figure 3). Values shown in this figure are average values for these collection periods. Because pigs are still increasing ADPG during these periods, maximal protein deposition also increases with the length of the collection period. Similarly, protein requirements for maintenance increases with time, and thus with the length of the collection period, which explains the fact that pigs fed with equal amounts of protein will decrease ADPG as the collection period increases. However, e_p remains unchanged as indicated by the fact that the initial slopes of the responses are similar. But what should be noted is that for the single simulated pig in this exercise, the transition zone between the two linear phases increases with the duration of the collection period.

These results are different in magnitude from those obtained by Pomar (1995), but, in both cases, increasing the length of the data collection period increased the degree of curvilinearity of the transition zone. The results again do not prove that the linear-plateau model is the most appropriate one to represent an individual response at a time, but they suggest that, for individuals, the linear-plateau model is compatible with the observed curvilinear response of populations. When planning experiments designed to measure variables

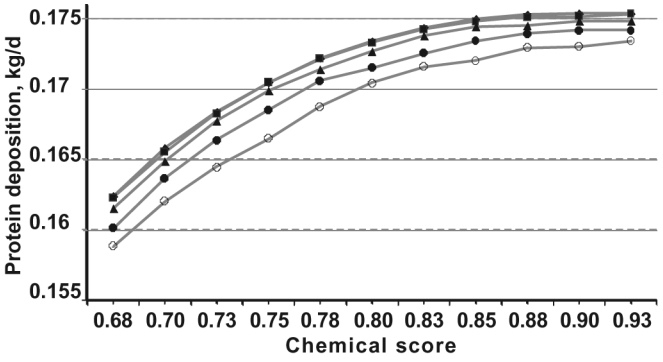

Figure 4. Effect of between-animal variation (0 [–◆–], 0.5 [–■–], 1 [–▲–], 1.5 [–●–], and 2 [–○–] times the standard coefficient of variation of the population) on average daily protein deposition rate of pigs (25- to 90-kg BW) fed with dietary protein of different chemical scores.

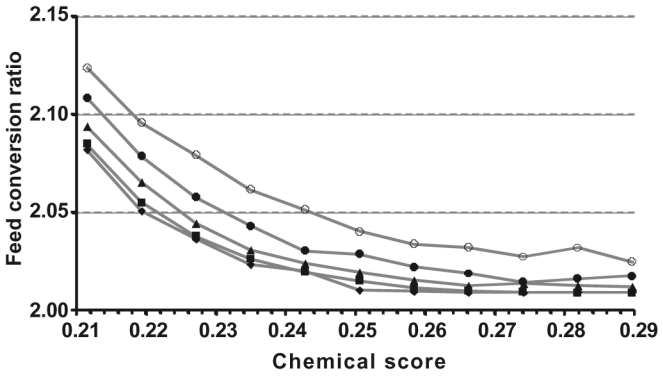

Figure 5. Effect of between-animal variation (0 [–◆–], 0.5 [–■–], 1 [–▲–], 1.5 [–●–], and 2 [–○–] times the standard coefficient of variation of the population) on average feed conversion ratio of pigs (25 to 90 kg BW) fed with dietary protein of different chemical scores.

that change over time, as shown for protein deposition in this simulation exercise, the length of the collection period should be considered. For instance, the information gathered from experiments with large data collection periods, within which the variable of interest shows important variation, has little relevance to understanding underlying biological mechanisms implicated in animal responses.

The Effect of Variation Between Animals on Growth Responses

From results presented in the previous sections, it should be expected that population responses to increases in ideal protein supply would be affected both by their genetic variation and by the length of the growing interval. To study the effect of between-animal variation on growth performance and intake, the 11 diets and the five modern pig populations used in the previous sections were fed ad libitum from 25 to 90 kg BW.

Average results across dietary treatments ranged from 1.94 to 1.89 for ADFI, from 965 to 906 g/d for ADG, from 175 to 159 g/d for ADPG, and from 171 to 156 g/d for ADLG. Dietary treatments had little effect on standard deviations (**STD**) for these variables, but they increased with the genetic population variation. The maximum simulated STD values for populations having genetic variations of 0.5, 1, 1.5, and 2 times the estimated mean between-animals genetic variation were estimated. They were as follows: 46, 91, 136, and 182 g/d for ADFI; 28, 56, 86, and 117 g/d for ADG; 4.6, 9.2, 13.9, and 19.6 g/d for ADPG; 9.3, 18.5, 28.4, and 37.0 g/d for ADLG. For most of these variables, maximal STD was obtained at the higher protein intakes.

On average, ADFI is little affected by dietary treatments although minimum values are obtained at low ChSc (results not shown). However, the effect of varying ChSc on ADPG and feed conversion ratio is more striking (Figures 4 and 5). Pigs were fed with a single feed throughout the growth period. For most of the pigs, this feed did not provide enough protein during the initial phase of growth but protein was oversupplied afterward. The extent of each of these two growth phases is determined by the genetic characteristics of the pig. For lean pigs, which have higher nutrient requirements, the initial growth phase is longer than for fatter pigs and increasing ChSc will reduce the length of this initial phase and increase the length of the latter. It is unusual and profitless to feed pigs to satisfy the maximal nutrient requirements of the overall growth period (Jean dit Bailleul et al., 2000), and, for most of the pigs of a herd, these two phases should be expected.

Increasing population genetic variation decreased ADPG (Figure 4) and ADG (results not shown). In fact, underfeeding pigs in protein reduced pig performance, whereas little change in performance is observed when pigs are overfed in that nutrient. Thus, the detrimental effect of protein deficiency is higher in populations with higher variation. For instance, simulated ADPG ranged from 162 ± 0 to 159 ± 15.6 g/d at the lowest ChSc and from 175 ± 0 to 173 ± 19.6 g/d at the highest ChSc diet. Similar figures are obtained for ADG, the values of which ranged from 918 ± 0 to 906 ± 105 g/d at the lowest ChSc and from 965 ± 0 to 951 ± 118 g/d at the highest ChSc diet. Similar conclusions can be drawn when studying the effect of population heterogeneity on feed conversion (Figure 5).

The simulated phenotypic variation resulted from the interaction between the generated genetic variation and the nutritional and the simplified thermal environment of the pig. The responses of real populations to a given diet and environment are not only the result of variations in the genetic characteristics of the pig, but also the result of variations in nutrient composition, the environmental conditions in which animals are raised, and others. These other nongenetic sources of variation are difficult to simulate mechanistically in a proper way and have been avoided in this and previous stochastic

models (Pomar et al., 1991b; Pomar, 1995; Ferguson et al., 1997; Knap, 2000a).

Implications

Results indicate that the linear-plateau model used to represent the response to protein supply in growing pigs is consistent with the curvilinear response that may be observed in experimental conditions. The curvilinear response may result from the variation between experimental animals and from limitations on the experimental procedures used. Models designed to simulate population responses need to integrate the effect of population variation on growth performance. The results show that the response of a population to treatments will differ in magnitude and shape from that assumed for an individual animal. Stochastic models that properly represent populations are therefore needed. Allowing for population variation is essential when models are to be used to predict nutrient requirements or to economically optimize swine production systems. For these applications, recommendations are particularly sensitive to the magnitude and shape of the model response as was found here.

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Characterization of pig genotypes for growth modeling

P. W. Knap*[1], R. Roehe†, K. Kolstad‡, C. Pomar§, and P. Luiting*

*PIC International Group, D-24837 Schleswig, Germany; †Christian-Albrechts University, D-24118 Kiel, Germany; ‡Akvaforsk and Agricultural University, N-1430 Ås, Norway; and §Agriculture and Agri-Food Canada, Lennoxville, Canada J1M 1Z3

ABSTRACT: The models dealt with herein are driven by descriptors of pig growth potential and environment, predicting growth from their interaction. Growth potential parameters relate to resource intake and partitioning to maintenance, protein (P) deposition (PD), and lipid (L) deposition (LD); these parameters quantify genotype (breed, etc.). Simulation of a pig's growth requires characterization of its potential in terms of the associated model parameters. This requires a set of parameters that fully describe the potential, measurement of resource input, and partitioning in a genotype, and using these measurements to quantify those parameters for that genotype. Resource partitioning is commonly covered by potential PD, required LD, and ME_m. Description of the first two features commonly requires three parameters. The ME_m here is restricted to a neutral environment without functions for coping with stressors, which would require extra parameters. Nutrient intake is best modeled as resulting from nutrient requirements and from constraints to physical uptake, be they external or genetic. Intake and partitioning observations must reflect potential; environmental load must be minimized. Repeatedly measuring whole-body P and L and ad libitum ME intake over a sufficiently wide maturity range (for example from 10 to 175 kg of BW) requires serial slaughter trials with chemical analysis or in vivo techniques such as ultrasound. The latter allow for the description of individual growth patterns and for quantification of variation in addition to mean levels. Parameters can be estimated in three ways. First, P and L observations can be fitted to P and L growth functions. Then, ME_m comes out as the remainder of the ME budget, given valid assumptions about PD and LD efficiency. Second, observed feed intake, growth, and body composition can be fitted to their simulations (parameter calibration, inverted modeling) to avoid P or L measurement. This requires serial data and iteration to match resource requirements to allowance. Third, differential nutrient restriction techniques can be used.

Key Words: Genotypes, Growth, Models, Pigs

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Introduction

Characterization of pig genotypes as it is dealt with here should produce input parameters for a specific group of growth models. The genotype's potential for protein (**P**) and lipid (**L**) deposition drives these "potential deposition" models: Deposition is predicted from this (fixed) potential and quantification of environmental load. The latter term signifies the suppressive effect of external factors (nutrition, climate, etc.) on the potential's expression. Genotype characterization is difficult here because it is unclear if pigs are fully expressing their potential. Other models ("operational accretion" models, not covered here) do not assume a fixed potential, and must be parameterized by measuring animals in the environment to be simulated. This type of genotype characterization has been dealt with by Schinckel and De Lange (1996). The difficulty is that it is unclear if the environment has been representative for the target setting.

The genotype is broadly defined here as a type of pig that differs genetically from others. Depending on required detail, this could be a breed (e.g., Duroc vs. Pietrain), a strain within a breed (e.g., PIC's PB427 vs. Belgian herdbook Pietrain), or an individual within a strain. For many deterministic applications, genotype characterization aims at a breed or strain in some stage of its development, and the simulated pig is its typical representative (e.g., the average PB427). Characterization is done by specifying the population means of genotype-specific parameters; together, these represent the relevant part of the population potential. Stochastic simulation deals with individual pigs, and characterization must specify not only population means but also

[1]Correspondence—phone: +49-4621-54359; fax: +49-4621-54336; E-mail: pieter.knap@pic.com.

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(co)variances to generate replicates. Either action requires a concise set of model parameters that fully describe the potential, real-life measurement of resource input and partitioning in a genotype, and using those measurements to quantify those parameters for that genotype.

Resource Intake

When modeling is used to support the understanding of the growing pig as a biological input-output system, it is not very useful to deal with nutrient intake as an input parameter or as a characteristic of an external rationing scheme. Instead, voluntary intake can be modeled as an output parameter. It can be driven by nutrient requirements due to genotype-specific deposition processes (related to resource partitioning, as discussed in the next section) and body maintenance. Such drives are described by Black et al. (1986) and Emmans (1997). Additionally, intake can be constrained by environmental factors such as feed composition (bulkiness, as discussed later in this section; nutrient density), climate (particularly by hot conditions as in Black et al., 1986; Knap, 1999; and Wellock et al., 2003), health (reviewed by Knap and Bishop, 1999; tentatively modeled for a specific disease by Black et al., 1999) and social conditions (such as group size with limited feeder capacity), and/or by animal-intrinsic factors related to effective gut size and feed intake capacity. The latter would require a genotype-specific model parameter, interacting with current body size and characteristics of the feed, such as "bulkiness." This was described by Ferguson et al. (1994) as a function of organic matter digestibility, and by Whittemore et al. (2003a) as a function of water-holding capacity. The associated genotype-specific parameter (the capability of the pig to deal with feeds with high bulk content) can be measured in real life as described by Whittemore et al. (2003b). When required in the simulation, voluntary intake can be overridden by any kind of rationing scheme after it has been calculated.

Resource Partitioning: Deposition

Resource partitioning in growing pigs is commonly modeled in terms of body maintenance requirements (**ME_m**), potential protein deposition (**PD**), required (alternatively called minimal/essential/inevitable/desired) lipid deposition (**LD**), and surplus lipid deposition, roughly in that order of precedence. Although published "potential deposition" models at first glance seem to differ widely in the way they describe potential PD and required LD, most (such as Whittemore and Fawcett, 1974; Moughan and Smith, 1984; Black et al., 1986; Emmans, 1988; De Greef, 1992; Walker and Young, 1993; Kyriazakis et al., 1994; Quiniou et al., 1996; Möhn and De Lange, 1998; Van Milgen et al., 2000) use essentially the same basic algorithm with two to four genotype-specific parameters. Any energy left after these two processes and body maintenance have been covered is commonly used for surplus lipid deposition. Differences between genotypes in resource partitioning are then due, in the first place, to variation in the parameters of the potential protein and required lipid deposition rules.

The focus here is on the rules for both processes described by Emmans (1988), not because these are necessarily superior to alternative rules but because they have attractive mathematical features that work well in examples such as the following ones. Emmans modeled potential protein deposition by describing potential protein mass as a Gompertz function of age:

$$P = P_\infty \times e^{-e^{-b \times (age - t^*_P)}} \quad [1]$$

where P is body protein mass as the genotype "desires" it to be at a particular age, P_∞ is potential mature protein mass, t^*_P is the x-coordinate of the sigmoid's point of inflexion, and b is the specific growth rate (dy/dt)/y in that point of inflexion. The derivative of this function gives the potential rate of protein deposition (PD, in kg/d), which, in the point of inflexion (at $P = 0.368 \times P_\infty$ for the Gompertz function) reaches its maximal value of $PD_{max} = b \times P_\infty/e$ (e is the natural logarithm base).

According to Emmans's rules, body lipid follows a similar pattern. Assuming full allometry between potential protein and desired lipid (not between actual protein and actual lipid: surplus LD is not dealt with here, nor is a lower LD than desired in the case of nutrient restriction) leads to a common rate parameter b for both fractions, and desired body lipid mass is modeled as:

$$L = L_\infty \times e^{-e^{-b \times (age - t^*_L)}} \quad [2]$$

The points of inflexion t* cancel out of the derivatives of [1] and [2]. This leaves three parameters for this model to describe the genotype's intrinsic drive for protein and lipid deposition (not necessarily its operational levels): b, P_∞, and L_∞.

It must be stressed again that Eq. [1] and [2] are not sufficient to predict actual protein or lipid deposition in pigs under practical conditions; that requires a set of nutrient partitioning rules to deal with the effects of unbalanced or insufficient diets and of environmental load. Emmans's assumption of full allometry between potential protein and desired lipid is difficult to verify. Knap (2000b) analyzed serial slaughter data from the literature to obtain estimates of b, P_∞, and L_∞ for a wide range of pig genotypes (see the "Observations" section that follows), checked for differences between separate b_P and b_L estimates, found these to be nonsignificant ($0.53 \le P \le 0.97$), and concluded that those data presented no reason to abandon the assumption. One of the analyses in the "Serial Measurements" section below shows similar trends, although to a lesser degree. Abandoning the assumption of full allometry would mean

that Eq. [1] and [2] need to be solved for four parameters (b_P, b_L, P_∞, L_∞) rather than three. This illustrates a crucial point of systems modeling: Reduction of the number of parameters to be estimated may lead to the introduction of strong assumptions. Finding the right balance between the two is often a matter of subjective judgement and always a matter of debate.

Resource Partitioning: Maintenance

The energy requirements for body maintenance (ME_m) vary within animal populations, with a phenotypic CV of approximately 0.1 and a heritability of about 0.3 (reviewed by Knap, 2000c). They are commonly modeled as a simple function of body weight (e.g., $ME_m = \alpha \times BW^{\beta}$) or body protein mass, which implies maintenance of a body in environmentally neutral, unchallenging conditions without the need to switch on additional coping functions (Whittemore, 1983; Cleveland et al., 1983; Baldwin and Hanigan, 1990). Those functions would include the following: 1) service functions (Gill and Oldham, 1993), such as circulation, coordination, respiration, and excretion, 2) protein turnover and active transport of molecules across cell membranes, and 3) physical activity at its basic level. Differences between genotypes can be conveniently modeled in terms of variation in the above parameter α, which then becomes the fourth genotype-specific model parameter (where necessary in conjunction with β) to describe resource partitioning. The ME requirements for protein turnover and membrane transport are related to body composition, but not to such an extent that they have important consequences for the between-animal variation in ME_m (Knap, 2000a). Assuming little variation in service functions, much of the ME_m variation must then be due to variation in basic activity levels.

Additional maintenance functions include activity above the basic level and functions to cope with climatic, immunological, and social stressors. When such functions are switched on, the parameters α and/or β in the above equation for ME_m can become strongly inflated and difficult to handle. Such cases are more usefully dealt with by dedicated routines that model the additional function as such (see the references for constraining factors in "Resource Intake") and leave neutral ME_m unchanged. This may require one or more additional genotype-specific parameters, such as the one for immunocompetence in Figure 1.

Observations

When observations on resource intake and partitioning are to properly reflect the animal's growth potential, they must be collected under minimal environmental load so that additional maintenance functions are not triggered and do not act as a resource sink. Hot climatic conditions, infectious conditions, and overcrowding can suppress energy intake so that the requirements of potential deposition cannot be fulfilled.

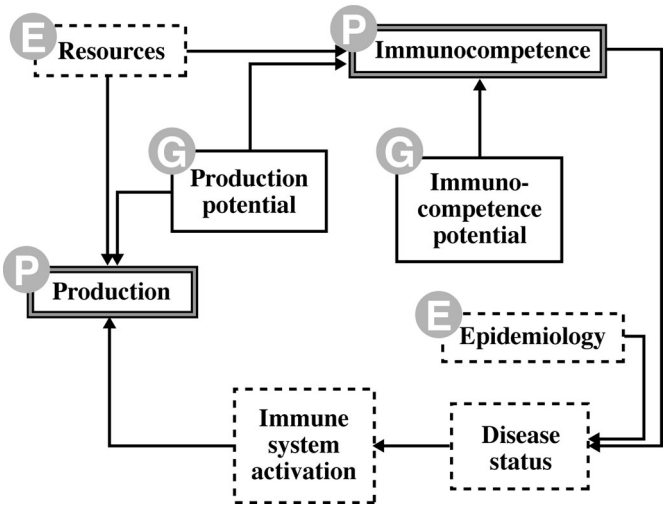

Figure 1. Genetic (G), environmental (E), and phenotypic (E) entities involved in the relation between livestock production and infectious disease. After Knap and Bishop (1999).

On the other hand, when energy intake is increased (such as in cold climatic conditions), neutral ME_m will be overestimated if the environmental load is not properly measured and adjusted for (which is difficult).

Fitting the three genotype-intrinsic parameters (b, P_∞, and L_∞) that drive potential protein and lipid deposition (Eq. [1] and [2]) to observed data requires measurement of whole-body protein and lipid mass and ad libitum ME intake at various stages of developmental maturity, with a wide enough maturity range to allow for a meaningful fit of the sigmoid curve. An example is in Knap (2000b), where serial slaughter data from the literature were analyzed to obtain genotype by sex-specific estimates of the above parameters for a wide range of pig genotypes. The nature of such data (serial slaughter and chemical analysis of body composition, with a single data point per animal) leads to estimates of the b, P_∞ and L_∞ population means only (the animals referred to here had not been raised in conditions that allow for direct comparison of those estimates across genotypes).

Prediction of the within-population variation requires longitudinal analysis as described in the next section, with serial in vivo measurements per animal—for example, with ultrasound, electrical conductivity, x-ray, or isotope dilution techniques (Forrest et al., 1989; Allen, 1990). The prediction of such variation is of foremost interest in the context of animal breeding, where differences between individual animals are the central theme. Kinghorn (1998) and Knap (2000c) provide further discussion, whereas Pomar et al. (2002) show why the modeling of variation may be of interest outside an animal breeding context.

Serial Measurements

To illustrate the concept of pig genotype characterization based on serial within-animal measurements (also

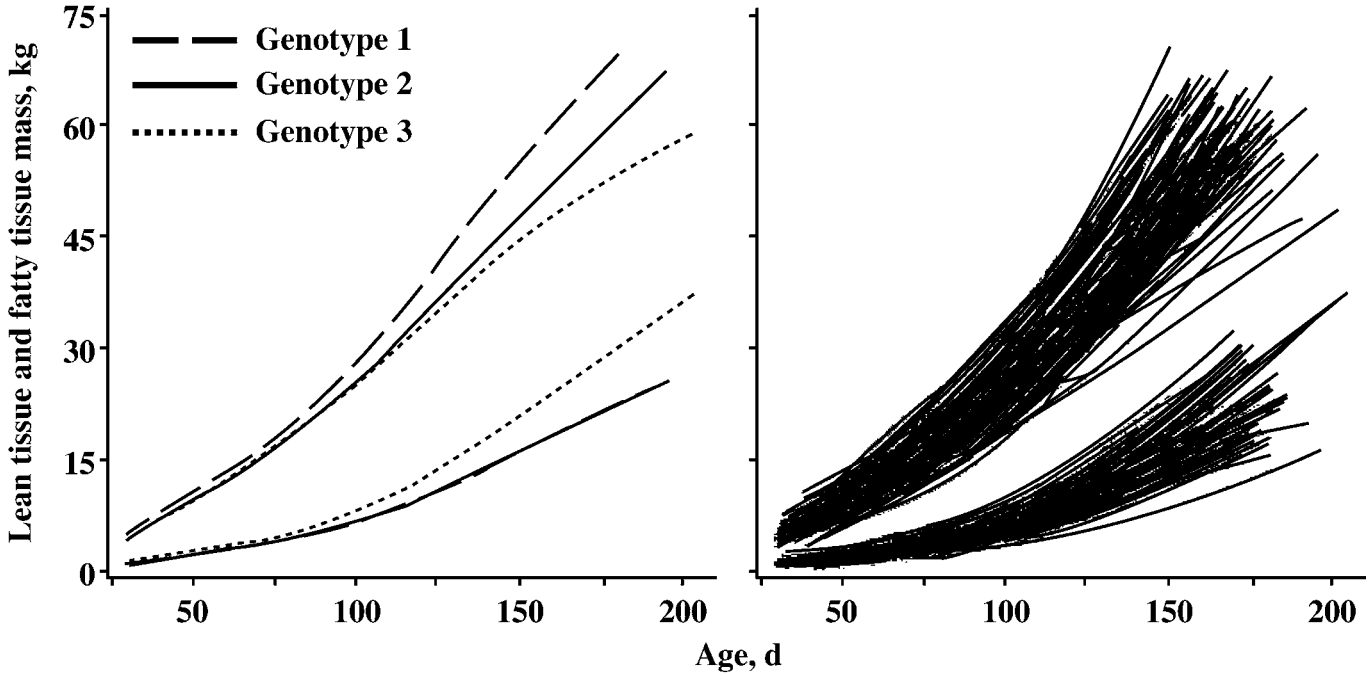

Figure 2. Lean (muscle plus viscera) tissue (top) and fatty tissue mass (bottom) measured by x-ray computer tomography in 141 pigs of three genotypes in relation to age. Lines are spline interpolation plots; data from Kolstad (2001). Left: genotype means; right: individual pigs. Genotype 1: modern Norwegian Landrace (NoL); Genotype 2: modern Norwegian Duroc; Genotype 3: cross of Genotype 1 with 1975 NoL.

referred to as longitudinal measurements) of body composition, data from two experiments are used, as described by Kolstad (2001) and Landgraf et al. (2002).

The data of Kolstad (2001) were collected with a different aim than carrying out the current analysis. These data comprise computer tomography x-ray observations of body muscle mass, lean viscera mass, and fatty tissue mass in 141 pigs of three genotypes and two sexes. Computer tomography scans were obtained at five body weights in the range of 10 to 105 kg. The development of observed total lean tissue mass (muscle plus viscera) and fatty tissue mass in relation to age is shown in Figure 2.

These graphs show that at this end weight of 105 kg, most pigs had barely reached the point of inflexion of their growth curves with the associated PD_{max} and LD_{max} levels; this may indicate that nutritional conditions had been limiting the expression of potential deposition rates. As a consequence, attempts to fit sigmoid equations to the individual lean and fatty tissue data failed, and the parameters of Eq. [1] and [2] could not be estimated.

Instead, for each pig that reached an end weight over 70 kg, second- or third-degree polynomials of lean and fatty tissue mass were fitted as a function of age, and the derivatives of these were used to determine maximal tissue growth rate within the range of observations; an important disadvantage of this method is that it is difficult to quantify the accuracy (standard error) of the predictions. The PD_{max} and LD_{max} can then be predicted assuming that lean tissue contains 19% protein and fatty tissue contains 75% lipid at the stage of maximal protein deposition, following the results obtained by Wagner et al. (1999) from carcass dissection of pigs in the same body weight and body fatness range as Kolstad (2001). These results, which underestimate the true potential levels because many of these pigs had not attained their maximal growth yet, show effects ($P < 0.01$) of sex and genotype on PD_{max} and LD_{max}. The genotype by sex means and between-animal standard deviations of these traits are in Table 1.

The specific growth rates (b in Eq. [1] and [2]) were calculated (and likely overestimated) by dividing PD_{max} and LD_{max} by their estimated contemporary P and L mass. Table 1 shows that b_P and b_L differ between genotypes ($P < 0.02$) but not between sexes ($P > 0.4$). All of the b_P predictions are somewhat lower than their associated b_L values ($P < 0.0001$). This means that realized LD was not allometric to realized PD, which suggests again that these pigs had been fed an unbalanced diet, and potential deposition was not expressed.

All these traits show CV well over 10% (in seven out of 24 cases over 20%), much higher than Knap's (2000a) suggested CV for b (3%) and PD_{max} (6%). The P_∞ values that can be (under)estimated from the PD_{max} and b_P values in Table 1 ($P_\infty = PD_{max} \times e/b_P$) range from 17 to 30 kg; the lowest values (for genotype 3) are indeed unrealistically low. The corresponding L_∞ values are 1.4, 1.9, and 3.1 times higher (for genotypes 1, 2 and 3, averaged over sexes), which is fully in the range found by Knap (2000b).

Table 1. Statistics of maximum protein and lipid deposition (PD_{max}, LD_{max}) in growing pigs[a] and of the associated specific growth rate parameters (b_P, b_L)[b]

			PD_{max}, kg/d		LD_{max}, kg/d		b_P, kg·d^{-1}·kg^{-1}		b_L, kg·d^{-1}·kg^{-1}	
Genotype	Gender[c]	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	m	20	0.133	0.0185	0.193	0.0408	0.0120	0.00140	0.0148	0.00225
1	f	22	0.121	0.0215	0.244	0.0451	0.0135	0.00286	0.0159	0.00227
2	m	22	0.102	0.0168	0.212	0.0635	0.0114	0.00228	0.0136	0.00178
2	f	28	0.096	0.0118	0.243	0.0463	0.0118	0.00237	0.0142	0.00207
3	m	8	0.100	0.0181	0.282	0.0674	0.0138	0.00343	0.0141	0.00214
3	f	8	0.079	0.0088	0.286	0.0440	0.0126	0.00293	0.0134	0.00096

[a]Approximated in a subset of data from Kolstad (2001). Genotype 1 = modern Norwegian Landrace (NoL); Genotype 2 = modern Norwegian Duroc; Genotype 3 = cross of Genotype 1 with 1975 NoL.
[b]b_P quantifies the growth rate of body protein in the point of inflexion of its sigmoid growth curve (where it attains its maximal level, PD_{max}) as a proportion of body protein mass at that time: $b_y = (dy/dt)/y$.
[c]m = castrated males, f = females.

Landgraf et al. (2002) reported on magnetic resonance measurements of body muscle mass and fatty tissue mass in eight pigs of a commercial three-way cross; in the course of the same experiment, 442 female and castrated male pigs were subjected to measurement of deuterium (D_2O) dilution to estimate total body water mass (and from that, lipid and protein mass; Susenbeth, 1984) at six body weights in the range of 15 to 140 kg. This higher end weight had been expressly chosen to avoid the problems with fitting sigmoid functions to the data that were encountered with the previous dataset, expecting that the pigs would have reached a reasonable proportion of their mature weight at 140 kg.

Presented here are the results of a preliminary analysis of the D_2O dilution measurements in 14 of these pigs. Eq. [1] and [2] were fitted to the data of each individual pig using the MODEL procedure of SAS (SAS Inst., Inc., Cary, NC) as in Knap (2000b). Iterations did not converge in one of these 14 cases. Six other cases produced very high standard errors for the estimates of P_∞ (five times), L_∞/P_∞ (five times), b (three times), and PD_{max} (once), but most estimates fell within the range of the results from the remaining seven cases, which produced estimates for P_∞ ranging from 27.7 to 40.7 kg, for L_∞/P_∞ ranging from 1.95 to 3.49 kg/kg, for b ranging from 0.0089 to 0.0128 kg·d^{-1}·kg^{-1}, and for PD_{max} ranging from 0.118 to 0.137 kg/d. Overall, the estimates of the 13 "converging" pigs amount to mean values ± standard deviations of $P_\infty = 34.4 \pm 5.12$ kg, $L_\infty/P_\infty = 2.83 \pm 0.76$ kg/kg, $b = 0.0104 \pm 0.0015$ kg·d^{-1}·kg^{-1}, and $PD_{max} = 129 \pm 10.5$ kg/d, rather fat and slow-growing compared with the sire lines analyzed in a similar way by Knap (2000a) but within the realistic range. The CV are 15, 27, 14, and 8%, respectively, again higher than suggested by Knap (2000b). The observations and fitted curves are in Figure 3. Applying Eq. [1] to body weight resulted in a mean estimate of the asymptote at $BW_\infty = 240$ kg.

Taken together, the results of these analyses of the Kolstad and Landgraf data suggest that there is substantial between-animal variation in the traits that Emmans (1988) chose to drive his growth model. Measurement of these traits on the individual animal level is feasible with the use of x-ray or isotope dilution techniques but requires data collection over a wide body weight range; based on the latter data set, 140 kg seems just sufficient to allow for a satisfactory fit of sigmoid functions in pigs with an asymptotic weight of 240 kg, and a sigmoid describes protein deposition better than lipid deposition. Based on the former one, 105 kg is certainly insufficient.

The predicted P_∞, L_∞/P_∞, and b values from these two datasets show much more between-animal variation than suggested by Knap (2000a). In that study, the output of a stochastic growth simulation model (mean values and between-replicates variation of growth rate, feed intake, and body protein content) was compared to literature findings, and the CV (input variables to the model) of P_∞, L_∞/P_∞, and b were altered until a satisfactory match was achieved. Those input parameters were assumed in the simulation model to be independently and normally distributed with uniform distribution characteristics both across replicates and over time. By contrast, the variation found in the present analyses was estimated directly from real data (even though the estimation is a double prediction: protein and lipid mass were predicted from x-ray or D_2O dilution readings), the results were fitted to polynomial or sigmoid functions, and the parameters of these were further analyzed separately. As such, the variance of these predicted values includes the true residual variance, variance due to inaccuracy in the prediction equations for protein and lipid mass, variance due to lack of fit (particularly the six cases with high standard errors in the Landgraf data), variance due to unstable convergence and small numbers of observations, and bias (particularly the cases with end weights considerably below the points of inflexion in the Kolstad data). Clearly, both approaches can be improved. First, by fitting the stochastic model to consistent data (like the Kolstad and Landgraf data) rather than to a conglomerate of literature values. Second, by analyzing the data with multivariate random regression techniques to predict the parameters of interest and to estimate their

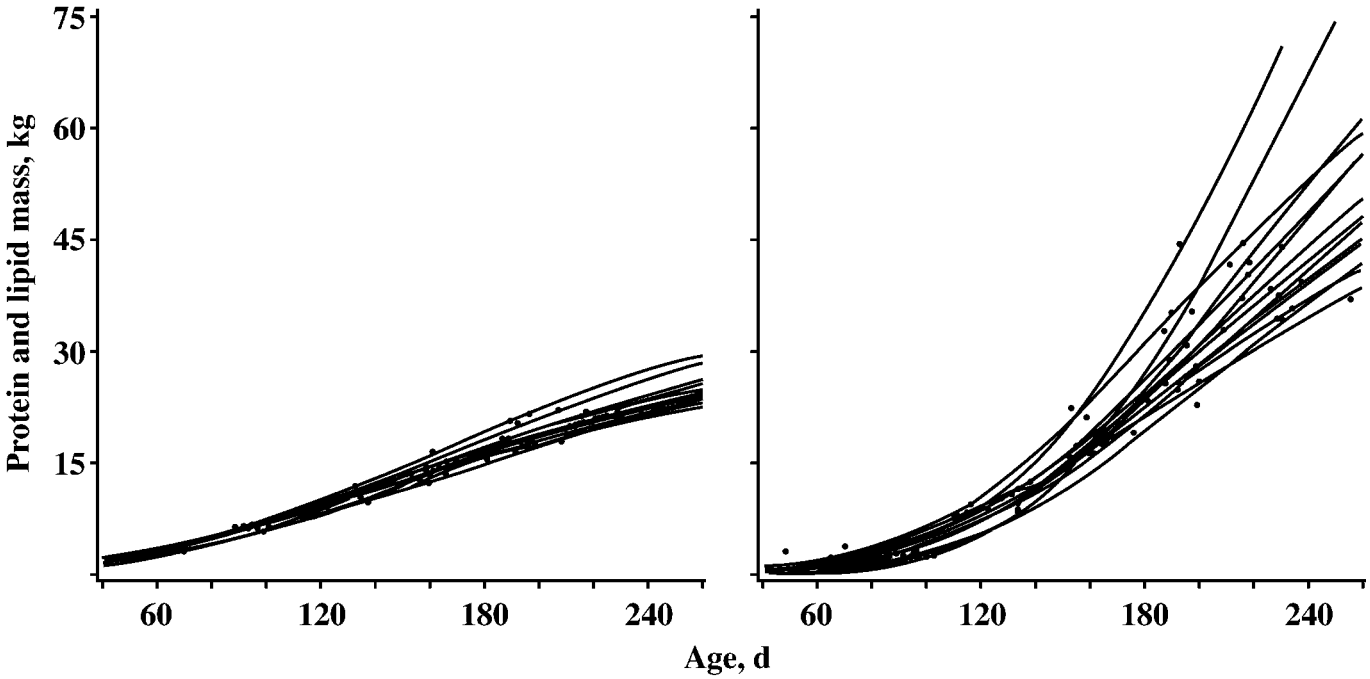

Figure 3. Protein (left) and lipid mass (right) measured by D_2O dilution in 14 pigs in relation to age. Dots are observations, lines are Gompertz curves fitted according to Eq. [1] and [2]; data from Landgraf et al. (2002).

(co)variation simultaneously, taking proper account of their interdependence.

Not surprisingly, fitting population-level sigmoid equations to pooled observations of P and L as determined in serial slaughter trials (as in Knap, 2000b) is much less demanding than fitting individual animals. Another issue is whether it is possible, in practice, to feed and house a growing pig in such a way that its growth potential is fully expressed. Both the Kolstad (2001) and the Landgraf (2002) results show deviations from the allometric double Gompertz model implied in Eq. [1] and [2], which suggests that imbalanced diets have suppressed potential protein deposition in these pigs or that this model is not the appropriate descriptor of the potential, or possibly both. See Knap (2000a,b) for more discussion on the same issue.

Inverted Modeling

An alternative to the direct measurement of model parameter-related traits is "inverted modeling," or "reverse simulation" as it was called by Bourdon (1998). In animal science, this notion goes back to Baldwin (1976), who suggested that estimates of model parameters could be obtained as follows: "assign initial values to unknown parameters in the model; compute, using these values and diet input data, … body energy change estimates; compare these computed estimates with experimental data for each diet input and compute from this comparison, an error of estimate; and allow a computer routine to systematically adjust parameter values in … iterative solutions until differences … between computed and real data are minimized." This approach is an iterative one. The model is "inverted" in the sense that phenotypic observations on traits that conventionally would be model output are now used as input to obtain prediction errors that are iteratively minimized by changing the value of some model parameters, and the final values of these parameters are the output of this iterative process. The procedure is similar to "fitting of equations to data."

A more elegant way of obtaining the same would be to algebraically rework the model equations, to end up with the fundamental parameters (rather than the observations) on the left-hand side, and coding this inverted model as a new computer program. The most important advantage of this analytical approach is that the resulting program directly produces a unique solution without any need for iteration. As a consequence, analytical model inversion has attracted much attention from a wide variety of scientific and engineering disciplines. The problem with this approach is that many differential equation systems, which is what growth models essentially are, cannot be inverted analytically without serious difficulties. Many of those problems are "ill-posed," having no solution, a series of nonunique solutions, or solutions that are unstable relative to the delivered input (Tikhonov and Arsenin, 1977).

If an analytically inverted pig growth simulation model could be provided with input in terms of final body weight and backfat depth and cumulative feed intake, and with a description of the average nutritional and climatic conditions during the growth period, that

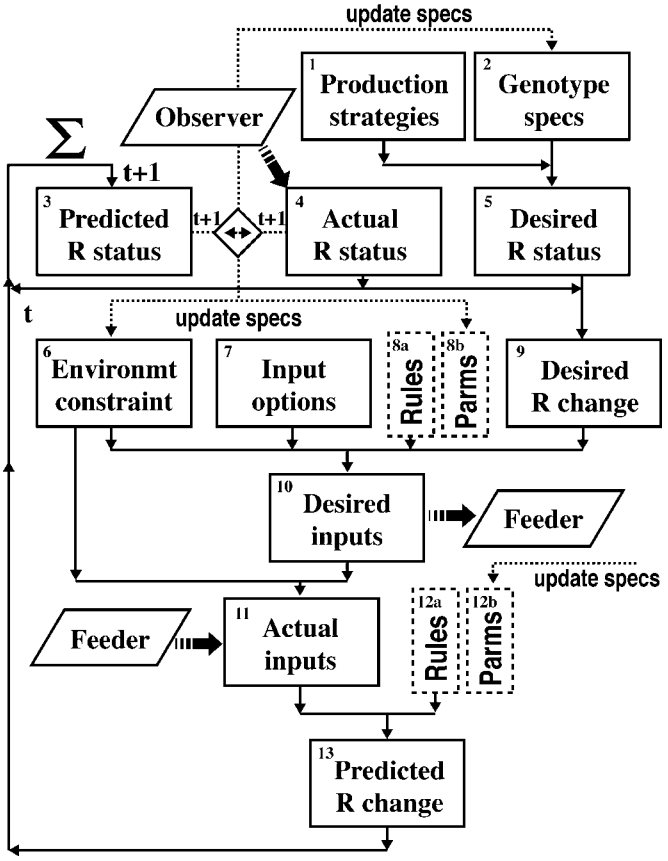

Figure 4. Iterative resource (R) status diagnosis and diet prescription.

model would likely produce a range of possible genotype characterizations that match those phenotypic and environmental specifications. All those genotypes would eventually realize the specified phenotypic performance, but they may differ in the way they arrive there. An iterative (rather than analytical) inversion approach would yield a flat optimum in this case, with the same inconclusive results. What is required in such a case is a more exhaustive description of the phenotype (and of the associated environmental conditions) to make the matching process in the inverted model more powerful. This would require serial measurement of phenotype and environment along the growth trajectory, providing the system with more animal-intrinsic information to help it focus on animal-intrinsic model parameters.

This process is illustrated in Figure 4, where the symbol "R" is used to denote resources; the general principles have been described by Wathes et al. (2001) and Whittemore et al. (2001). The model itself comprises rules and parameters, as in entities 8ab and 12ab in Figure 4. It is assumed that the animal's genetic make-up (specified in Entity 2) results in a metabolic drive to reach a certain resource status (body mass and composition, heat production) at any point in its development. That is, an animal-intrinsic potential development pattern over time is assumed, which leads to a genetically desired resource status (Entity 5). This potential pattern will only be achieved in a nonlimiting environment (in terms of stress and nutrient supply). In most cases, the actual resource status (Entity 4) will deviate from the desired situation; the animal is then assumed to adapt its nutrient input (or its interactions with stressors) to reduce this deviation. In addition to the genetically desired status, the pig producer's production strategies (specified in Entity 1) may have specific desires with regard to the pig's developmental pattern, often contradictory to genetic desires.

The system must be able to observe, at any given time, the pig's actual resource status, determine its desired resource status, compare the two to estimate the pig's desired change in resource status (Entity 9), and work out the required resource input, taking into account the available options for nutrient intake (Entity 7) and the limiting effect of current environmental factors (specified in Entity 6). This required resource input must then be translated into a specification of dietary requirements (Entity 10), which is output to the feeder. An important part of this translation is the relationship between pig appetite, required performance, and diet nutrient density. The required nutrient input may be further constrained by limiting environmental factors (stressors, bulkiness of the feed; specified in Entity 6). The model must therefore receive information from the feeder about the pig's actual nutrient consumption (Entity 11) and predict from it the realized change in resource status (Entity 13). The predicted realized change in resource status can then be added to the actual resource status that was measured in the current time step, which gives the predicted resource status (Entity 3) for the coming time step. The loop of the system is closed during that time step when this prediction is compared to its realized (observed) value.

This comparison allows for "iterative status diagnosis." When predicted values deviate from realized ones, some of the information that made the system arrive at that prediction must have been wrong. Apart from measurement errors by the observer and the feeder, there may be errors in the parameters used in the model calculations (entities 8b and 12b), the specification of the pig's genetic make-up (Entity 2), or in the specification of limiting environmental factors (Entity 6). These three entities can then be updated (as by "update specs" instructions in Figure 4) by simultaneous examination of factors potentially affecting the observation—expectation difference. This would result in estimates of model parameters, genetic specifications and environmental specifications that become gradually more accurate as the pig grows and its accumulating information becomes available for retrospective analysis.

Pig growth models assume different relations between PD and feed protein intake, between LD and feed energy intake, and between PD and energy intake. This situation can be exploited for growth parameter estimation: Targeted over- or undersupply of feed energy and/or protein can be used to bring the animal in a specific

stage within those relationships, allowing for uncomplicated measurement of parameters such as ME_m, PD_{max}, genetically desired LD, and the regression of PD and LD on energy intake. These differential nutrient restriction techniques can be implemented through Entity 7 in Figure 4.

Implications

Simulation of the growth of pigs of a particular genotype with "potential deposition" models requires specification of the model parameters that drive body maintenance and potential growth of body protein and lipid. In stress-free nonlimiting environments this requires four to six parameters: maintenance metabolizable energy requirements, two to four parameters to describe potential protein and lipid growth, and one to describe effective feed intake capacity. These can be estimated by fitting observed body protein and lipid mass to growth functions or by inverted modeling, fitting observed feed intake, growth rate, and body composition to their simulated values by calibrating model parameters. In a stressful or limiting environment, more parameters will be needed to describe the coping strategies of a particular genotype. A model requires functions to deal with such parameters to allow for a proper fit of data measured in such environments and avoid biased estimates of the potential parameters.

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Consumer attitudes toward biotechnology: Lessons for animal-related applications

C. M. Bruhn[1]

Center for Consumer Research, University of California, Davis, CA 95616

ABSTRACT: Newer techniques of biotechnology, such as recombinant DNA, offer scientists a range of tools to enhance the quality and environmental sensitivity of agricultural production. This article briefly summarizes consumer attitudes toward biotechnology in the United States and Europe. Few U.S. consumers have read or heard a lot about biotechnology, and concern about biotechnology was low on the list of concerns of consumers in the United States. When asked to volunteer food-related concerns, only 2% expressed concerns about the safety of foods modified by biotechnology. People supported applications that benefit the environment, with modifications that provided direct consumer benefits, such as increased nutritional value or better taste, endorsed by slightly fewer people. Most consumer research has focused on plant applications of biotechnology; modification of animals is likely to be more emotionally charged because the majority of U.S. consumers believe that animals have rights that people should not violate. Few European consumers considered themselves knowledgeable about biotechnology. Knowledge of basic biology seemed to be lacking, putting people at risk for misinformation. Fifty-eight percent or more of Europeans believed that genetically modified plants were fundamentally different from traditional plants and believed their own genetic material would change if they consumed genetically modified food. Communication programs in Europe are challenging because government and industry sources were trusted by few consumers. Experience in the United States indicates that communication can change attitudes. Frequent and effective communication that highlights potential benefits and addresses public concerns is a prerequisite for increasing public acceptance.

Key Words: Biotechnology, Consumer Attitudes, Genetic Engineering

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Introduction

Modern biotechnology can be applied in a broad range of areas, offering advantages to producers, the environment, and human health (Institute of Food Technologists, 2000). Farmers can benefit from increased yields on the same acreage, decreased production costs, less exposure to pesticides and herbicides, and the potential to practice reduced till production (Phipps and Park, 2002; Fawcett and Towry, 2002). Plant breeders have used rDNA technology to develop corn that is nutritionally more dense and easier for animals to digest (Mazur et al., 1999). Consumers could benefit from foods with improved nutritional characteristics, lower levels of natural toxins and increased quality (Gura, 1999; Dowd et al., 1999). In the future, people with allergies may find the proteins that trigger allergic reactions have been removed, allowing consumption of previously prohibited foods (Institute of Food Technologists, 2000). Scientists are also developing feed with lower levels of phytate: this has environmental ramifications because it will reduce phosphorus, nitrogen, and odor from animal waste (Mazur et al., 1999). Additionally, scientists have been able to modify pig saliva to more thoroughly digest nutrients (Golovan et al., 2001). Plants have been developed that remove high levels of salt from the land, thus opening the potential for bioremediation (Zhang and Blumwald, 2001). Because of these potential benefits, producers are planting crops modified by biotechnology and researchers in the corporate and university settings continue to develop new applications. As with any change, however, negative consequences may occur. Palumbi (2001) points out that technology may affect evolutionary change at a cost to society of $30 to $50 billion per year. Consumers may view the changes from agricultural applications of biotechnology in food production in a positive or negative light. This paper briefly reviews U.S. and European consumer attitudes toward applications of biotechnology.

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[1]Correspondence: Food Science and Technology (phone: 530-752-2774; fax: 530-752-3975; E-mail: cmbruhn@ucdavis.edu).

Attitudes Among Consumers in the United States

Scientifically designed consumer telephone surveys conducted for industry, education, and public interest organizations by professional research groups and universities provide a consistent picture of U.S. consumer's knowledge and attitudes toward this technology. In one survey, only 9% of consumers indicated that they have read or heard a lot about biotechnology, with an additional 30% indicating that they have read or heard some and a further 33% indicating they have read or heard a little about biotechnology (Cogent Research, 2002). Only 35% of consumers recognized that foods modified by biotechnology are currently in the supermarket.

Most U.S. consumers have a positive attitude toward biotechnology when they hear the benefits this technology can provide. In telephone interviews conducted with a nationally representative sample of 1,000 adults, only 37% considered genetically engineered food acceptable; however, when the purpose of genetic modification was included, such as raising crops that are resistant to pests or less costly to grow, acceptance increased to between 60% and 70%, respectively (Princeton Survey Research Associates, 2002). Similarly, 71% of consumers indicated they would purchase produce modified by biotechnology to reduce pesticide use, and 54% said they would purchase products modified for better taste in another 1,000-person nationwide telephone survey (Cogent Research, 2002). Furthermore, 61% believed biotech will provide benefits for themselves and their family within the next 5 yr (Cogent Research, 2002).

Modifications by biotechnology were seen as risky by few U.S. consumers. When asked in an opened-ended question about food safety concerns, only 2% volunteered concerns about genetically modified food (Cogent Research, 2002). In contrast, concerns about foodborne disease and safe handling were mentioned by 41% and 42%, respectively. Others found that the public considered specific environmental risks from genetic modification important. When asked whether a series of risks were very, somewhat, or not at all important, the potential for genetic material introduced through this newer method to contaminate traditional plants was considered a very important risk by 64% of consumers (Pew Initiative, 2002). Other potential risks and the percentage of consumers considering the risk very important included the potential to create superweeds (57%), to develop pesticide-resistant insects (57%), to reduce genetic diversity (49%) and the potential that modified plants could harm others (48%).

The percentage of consumers holding a positive view toward biotechnology decreased in the 5 yr preceding the writing of this article. In 2002, 61% of consumers responded that they expect to receive personal benefits from biotechnology, whereas, in 1997, 78% held that view (Cogent Research, 2002). Similarly, 71% of consumers in 2002 indicated they would purchase products modified to reduce pesticide use, but, in 1997, 77% said they would buy these products.

This attitude change could have resulted from media coverage of biotechnology that focused on unanticipated and uncontrolled potential risks. The Center for Media and Public Affairs analyzed coverage of biotechnology in national television news programs, newspapers, news wires, magazines, local television news, and talk shows in 1997, 1999, and 2001. Coverage increased from less than 1% of articles in 1997 to 6% in 1999 and 12% in 2001. A content analysis of articles in 1999 found claims of harm were described in 70% of the articles, whereas discussions of benefits were only in 30% of the stories (International Food Information Center, 2000). Discussion of harm focused on environmental or human health, whereas benefits were generally limited to increased production. This is not likely to be an important benefit to consumers where food is available in abundance. In 2001, articles on Starlink corn represented 73% of articles on biotechnology, while the ability to detect biotechnology components was the focus of 11% of the articles and labeling of biotechnology foods was discussed in 10% of the articles (International Food Information Center, 2002). Negative comments exceeded benefits by an 8:1 ratio. Although Starlink corn led to no known human illness, the potential for an allergic response was frequently mentioned. Furthermore, the presence of Starlink in human food illustrated that modified plant products approved for animal but not human use could be in a wide variety of human foods.

Many consumers valued potential benefits made possible by biotechnology. When specific benefits were identified, 74% ranked cleaning toxic pollutants as very important (Pew Initiative, 2002). Other potential benefits and the percentage of consumers considering the benefit very important included reducing soil erosion, 73%; using less fertilizer, 72%; developing drought-resistant plants, 68%; developing disease-resistant trees, 67%; and using less pesticide, 61%.

Few consumer studies have focused on animal applications of biotechnology. Early work found that 80% of consumers believed animals have rights that people should not violate (Hoban and Kendall, 1993). Unfortunately, the researchers did not explore the nature of these rights. Consumers may have little knowledge as to how animals are currently produced and handled. When specifically asked, fewer than 40% of consumers indicated support for traditional crossbreeding practices. In contrast, when asked about the techniques of biotechnology in conjunction with specific benefits, over 40% expressed support for using biotechnology to produce leaner meat, and over 50% supported the use of biotechnology to enhance animal disease resistance.

Sociologist Thomas Hoban has speculated that a number of factors will make consumer response to animal applications of biotechnology more sensitive than response to plant applications (personal communication, January 2003, slide set). The emotional bonds peo-

ple have with companion animals and the popularity of animal cartoon characters has led people to anthropomorphize animals. Furthermore, although plants are not mobile—yet pollen is airborne—modified animals may accidentally escape from captivity and modify wild species. People may also be concerned that once scientists were able to modify animals, humans will be next. These considerations suggest that public acceptance of animal modifications will be strongly influenced by the importance of the benefits from modification and the ability to control and contain the introduced trait.

People who strive to influence government policy have called for mandatory labeling of foods or ingredients that result from genetic modification (Nestle, 1998). Some advocate labeling so that consumers can choose or avoid products produced by this technology; others argue that labeling is essential for consumers to know what they are selecting. Focus groups held by the United States Food and Drug Administration found that some consumers are concerned that they have unknowingly consumed genetically modified foods, whereas consumers in other focus groups said the widespread use of modified corn and soy products with no known ill effect was comforting (Levey and Derby, 2001; Teisl et al., 2002). Although the mandatory labeling of any food or ingredient modified by biotechnology was an important consideration among some consumers in focus groups (Levey and Derby, 2001; Teils et al., 2002), focus group findings represent the attitudes of participants and not necessarily the attitudes of the population (Krueger, 1994). Survey findings from a statistically representative sample of U.S. consumers indicated that mandatory labeling was not a high priority among U.S. consumers. When asked whether there was information not currently on a food label they would like to see, 76% responded "No." Of those who wanted additional information, most cited nutritional information, with only 1% asking for information on genetic engineering (Cogent Research, 2002). When asked on another survey to select one item from a list of potential label additions, 17% chose labeling concerning whether the product was genetically altered; 33%, concerning whether pesticides were used in production; and 8%, whether the product was imported; 16% responded that they needed no additional information, and 15% said they did not know (Bruskin Research, 2001).

Attitude studies indicate that mandatory labeling may mislead consumers into thinking there is a significant difference between the biotechnologically modified and traditional food. In a survey sponsored by the Center for Science in the Public Interest, consumer perception of the safety of food was affected by including a term related to gene or biotechnology. If a label on a loaf of bread included the statement "contains genes from wheat," 15% of consumers considered the bread not as safe as bread without such a statement and 35% did not know about the bread safety (Bruskin Research, 2001). If the loaf of bread included the statement "contains genetically engineered wheat," 31% considered the bread not as safe as one without this statement and 28% did not know. Response to the terms *biotechnology* and *genetic engineered* was comparable; however, the terms had been used interchangeably throughout the survey. When a benefit for modification was provided, "contains genetically engineered wheat—reduces pesticide use," 21% considered the engineered product safer than a product without such a label, 28% considered it not as safe, and 22% indicated they did not know. Few consumers indicated they would be willing to pay more for labeling of genetically modified food, with 44% saying they would pay nothing more.

Most consumers (59%) support the U.S. Food and Drug Administration position that mandates labeling if an allergen is introduced into the food or if the food changes in nutritional value, composition, or safety (Cogent Research, 2002). A proposition requiring mandatory labeling on food and ingredients modified by genetic engineering was rejected by 73% of Oregon voters (Monsanto, 2002). That this labeling would provide no new useful information to consumers and would be costly to implement were believed to be major factors contributing to the defeat of the measure.

Attitudes Among Consumers in Europe

Attitudes among European consumers have been polled periodically by the Directorate-General for Education and Cultures' "Citizens' Center" as requested by the European Commission's Directorate-General for Research. The study was carried out in every country of the European Union between November and December of the year prior to the publication date. Few Europeans (11%) considered themselves informed about biotechnology (INRA, 2000). Questions related to facts of biology indicate that basic knowledge was lacking among the general population. In 1999, only 34% of European consumers correctly responded that genetically modified animals are not always larger than conventional animals. Only 35% responded that the following statement is false: "ordinary tomatoes do not contain genes while genetically modified tomatoes do" (INRA, 2000). Furthermore, only 42% recognized that eating genetically modified fruit does not change your personal genes, with 24% believing human genes would be changed, and the remaining uncertain. For comparison, 46% of U.S. consumers recognized that ordinary tomatoes also contain genes and 62% knew that eating genetically engineered fruit did not change a person's genes (Hoban, 1998). European consumer response to these questions in 1996 and 1999 was similar, except for the question on change in personal genetic codes, where more consumers responded correctly in 1996 (48%) compared to 1999 (42%) (INRA, 2000). When basic knowledge is lacking and the belief persists that the consumption of modified fruit can change human genetic material, it is no wonder that European consumers are concerned about potential risks associated with eating genetically modified food.

Only about half of European consumers were aware of biotechnology's many applications (INRA, 2000). Slightly over half, 56%, were aware that genetic modification could be used to make plants resistant to insect attack. About half were aware that these tools could be used to detect diseases or prepare human or animal medications. Only 28% knew that biotechnology could be used to clean toxic spills.

When asked to rate on a scale of 1 to 4 whether an application was useful, risky, or should be encouraged, the applications considered most useful were cleaning toxic spills, rated 3.24; using genetic material to detect disease, 3.4; and preparing human medicines 3.27 (INRA, 2000). The applications considered most risky were food production, 3.0, and the cloning of animals to produce medicines and vaccines, 2.92. Those applications European consumers felt should be encouraged were cleaning of toxic spills, 3.17; cloning animals whose milk can be used to produce medicines, 3.01; and detecting hereditary diseases, 3.01. Production of foods received a rating of 2.19.

Although some may interpret the relatively low score for food applications as meaning Europeans are not supportive of food applications, an examination of the question consumers were asked indicates that another interpretation is possible. Benefits of the genetic modification of food were described as "to give them a higher protein content, to keep them longer, or to change the taste." These benefits may not be very appealing to European consumers. Food applications that offer more compelling benefits may be better received.

Both food and nonfood applications of biotechnology were viewed as having some degree of risk. Even an application perceived as risky, such as cloning animals, received a high rating for "should be encouraged," 3.01 out of 4, because of the potential benefit. This suggests that Europeans may accept changes from biotechnology if the benefits were viewed as important.

When asked who is doing a good job in the area of genetic modification, most European consumers supported the work of consumer organizations, 70%; followed by newspapers, 59%; and environmental organizations, 58% (INRA, 2000). The same study showed that the government and the food industry were considered to be doing a good job by only 45% and 30%, respectively. Similarly, few European consumers, 3% to 4%, indicated that they trusted information from international or national public authorities, respectively. Consumers expressed the greatest trust in information from consumer organizations, 26%, and medical professionals, 24%.

Attitude Change

People can change their attitudes in response to information from a credible source. Trusted information sources are described as knowledgeable, concerned with public welfare, truthful, and with a "good track record." Less-credible sources are characterized by exaggeration, distortion, and vested interest (Frewer, et al., 1996). Consumers in the United States considered health authorities, such as the American Medical Association or the American Dietetic Association, as the most credible, followed by university scientists and regulatory groups like the FDA (Hoban, 1994).

Many groups are involved in education in the United States. An industry organization has funded television advertisements that address the benefits of this technology. Professional societies, such as the American Dietetic Association and the Institute of Food Technologists, have prepared material for their members, the media, regulators, and the public. Universities and colleges also have outreach programs to keep the public informed about new developments.

Consumer surveys taken following outreach programs show increased recognition that biotechnology can be used to reduce pesticides, produce healthier foods, produce hardier corps, and develop new medicines. For example, a brief video describing the potential risks and benefits of biotechnology followed by an open discussion presented to community organizations found an increased recognition that biotechnology offers society both risks and benefits (Bruhn and Mason, 2002). The majority of those participating in the program felt that society would benefit from the applications that this technology could provide.

The biotechnology industry's monitoring of consumer response to television advertisements that describe some of the beneficial applications of this technology found that 1 in 10 consumers could describe a food- or crop-related benefit. Tracking attitudes from March 2000 to July 2002 found that the consumer awareness that biotechnology could be used for various applications increased. Agreement that biotechnology allowed farmers to grow more food to feed the world's population increased from 61% to 70% (Council for Biotechnology, 2001). Agreement that biotechnology could be used to develop hardier crops that are able to grow in poor conditions, such as drought, increased from 58% to 65%. Similarly, recognition that biotechnology could be used to develop healthier foods, such as foods that are lower in fats or higher in nutrients increased from 45% to 54%. Recognition that biotechnology could be used to reduce the need for chemical pesticides increased from 42% to 48% (Council for Biotechnology, 2001).

Implications

The key to consumer acceptance of modifications by biotechnology directed toward plant or animal application is perceived risk and benefit. These studies indicate that consumers expect human, animal, and environmental safety to be protected. The studies discussed indicate that consumer attitudes are more likely to be positive when people understand why animals or plants are modified, they view the potential benefit as important, and neither the animal nor the environment is harmed. Although consumers in the United States

have positive attitudes, few are aware of all of the potential applications under development. European consumers are less aware, and many do not trust regulators or the food industry. Discussions with community leaders provide an opportunity to respond to consumer concerns and address both risks and benefits. Advertisements can increase public awareness of potential benefits. Messages that highlight potential benefits, address consumer concern, and are delivered by trusted, knowledgeable sources are critical to long-term acceptance.

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