1463 Local control of mammary Stat5, β-casein gene expression and milk protein synthesis in dairy cattle. J. Yang*, V. E. Baracos, and J. J. Kennelly, *University of Alberta, Canada*.

Signal transducer and activator of transcription 5 (Stat5) is involved in the signal transduction pathways of circulating hormones and growth factors, including prolactin, growth hormone and IGF-I, to regulate β-casein gene expression in the mammary cells. We hypothesized that Stat5 activity would be also subject to local control within the mammary gland. Five Holstein cows in mid-lactation were used in the study, in which half of the mammary gland (left quarters) were milked once daily (PM) and the other half (right quarters) were milked twice daily (AM and PM) for 16-18 days. The milk yield and milk protein yield of the quarters milked twice daily were significantly higher than in quarters milked once daily ($P<0.01$). In the first three days of the experiment, total milk yield was significantly reduced and milk protein concentration in the left quarters was significantly higher. However by the last three days of the study, the total milk yield was not significantly different from pre-experimental period and milk protein concentration was not different between left and right sides($P<0.05$). Mammary tissue from the front left and right quarter was obtained by biopsy just before PM milking at the last day of the study. Stat5 activity and protein abundance were significantly higher in the right quarter than that in the left quarter of mammary gland ($P<0.05$), however, the mRNA abundance for β-casein was not significantly influenced by the frequency of milking. The results indicate more frequent milking increases milk protein synthesis and milk yield in the mammary gland. The decreased Stat5 activity by once daily milking indicates the involvement of local control in milk protein synthesis and may result from inhibitors of lactation in the milk. The unchanged β-casein mRNA abundance may explain that the relatively large pool size and low turnover rate of this mRNA in the mammary gland.

1464 Protein kinase C isoforms in mammary gland and adipose tissue during lactation. R. G. Vernon*, R. A. Clegg, and E. Finley, *Hannah Research Institute, Ayr, Scotland*.

Protein kinase C comprises a family of isoforms which exhibit tissue specific distribution and roles in the hormonal regulation of metabolism, growth and differentiation. Total PKC activity appears to be elevated during pregnancy and decreases with lactation; one objective of this study was to determine the specific PKC isoforms involved. Mammary tissue was obtained from non-lactating, pregnant (110-120 d) and lactating (18 d) sheep. Proteins were separated by SDS gel electrophoresis and specific PKC isoforms determined by Western blotting and ECL. The amounts of PKCα, γ and ϵ were highest in cells from non-lactating sheep and fell during pregnancy and lactation. In contrast the amounts of the so-called atypical PKC isoforms (PKCi, PKCλ and PKC zeta, and also PKCμ were highest in cells from lactating sheep. Thus changes in the amount of specific PKC isoforms are more complex than revealed by measurement of total activity; individual PKC isoforms appear to have different functions during the lactation cycle. Adipocytes become insulin resistant during lactation. Isoforms of PKC have been implicated in insulin signalling hence the amount of specific PKC isoforms was measured in adipocytes from lactating and non-lactating sheep as described above; amounts were quantified by densitometry. Lactation had no effect on the amounts of PKCα, γ, δ and μ, while the amount of PKCϵ tended to increase. There was however, a fall ($P<0.05$) in the amount of PKC zeta (184 ± 33 and 110 ± 17 arbitrary units/mg protein for cells from non-lactating and lactating sheep, respectively, n=5), suggesting that this isoform could be involved in the insulin resistance of lactation.

Key Words: Mammary Gland, Adipose Tissue, Protein Kinase C

1465 Differences in the proliferation and morphogenesis of mammary organoids from the peripheral and medial parenchymal zones of the prepubertal bovine mammary gland. S. Ellis[1]*, S. Purup[2], K. Sejrsen[2], and R. M. Akers[1], [1]*Virginia Polytechnic Institute, Blacksburg* [2]*Danish Institute of Agricultural Sciences, Tjele, Denmark*.

Differences in the proliferation and morphogenesis of cells from the peripheral and medial parenchymal zones of the prepubertal bovine mammary gland were investigated *in vitro*. Cells from the peripheral and medial parenchymal zones were isolated by collagenase digestion of mammary tissue from a 220 kg Holstein Friesian heifer and cultured for 5 d in collagen gels. Proliferation was determined by measuring the amount of [methyl-^{3}H]-thymidine incorporated during the final 24 hr of culture. Dose response curves for IGF-I (0, 3.125, 6.25, 12.5, 25, and 50 ng/ml) and TGF-β_1 (0, 12.5, 25, 50, 100, 250, 500, 1000, and 5000 pg/ml) were determined for each cell population. Cells from the peripheral zone were more sensitive to the action of IGF-I, as shown by a growth response that was two-fold greater than the response of medial cells ($P< .05$). A biphasic response was observed for both medial and peripheral cells treated with TGF-β_1. Although the proliferation of both cell types was stimulated by TGF-β_1 treatment at concentrations from 12.5 to 500pg/ml, proliferation of the peripheral cells was greater than the proliferation of medial cells ($P< .05$). Concentrations of TGF-β_1 above 1 ng/ml inhibited cell proliferation. The morphogenesis of peripheral and medial cell populations were compared during 2 wk cultures, and by histologic examination of sections from collagen gels. The cells of the peripheral zone form multi-layered cell structures that resemble native mammary parenchyma when treated with serum or mammary gland extract. However, serum stimulation of medial parenchymal cells results in the formation of single-layered structures that do not resemble native mammary ducts. Our results demonstrate significant differences in the proliferation and morphogenesis of peripheral and medial parenchymal cell populations.

Key Words: Parenchyma, Morphogenesis, Mammary

1466 Quantitative model descriptions of amino acid, glucose and fatty acid metabolism in sows consuming varying protein and energy. J . P. McNamara*, *Washington State University, Pullman*.

The purposes were to determine milk production, body fat and body protein in sows consuming a range of energy and amino acid intakes, in order to develop new equations and parameter values in a model describing metabolism in lactating sows (Pettigrew et al., 1992, J. Anim. Sci. 70:3742-3761). Sows were either 2nd, 3rd or 4th parity. Sows consumed 6.1 kg/d DM for a 20 d lactation. Protein intake was 863 (H), 767 (M) and 678 (L) g/d with 59, 53, and 47 g/d lysine for high, medium and low protein diets at two levels of fat intake, 117 (L) and 410 (H) g/d. Litter weaning weights were 71, 74, 69, 69, 72 and 70 (SE .9, $P<.05$) for sows fed HL, ML, LL, HH, MH, and LH, respectively. The actual response in milk production using the medium protein low energy (ML) group as a control was: -412, 0, -830, -642, -232, -719 g/d. The model predicted response was: 108, 0, -778, -44, -121, -968 g/d. Body protein change observed from d 1 to d 21 of lactation was -1.35, -1.29, -2.05, 0.29, -1.50, -2.49 (SE 0.34, $P <.05$) kg. The model predicted body protein change of:-0.34, -0.80, -1.38, -0.37, -0.99, -1.92 kg only after maximal proteolysis allowance was increased 75 %. Body fat change observed was: 1.41, -2.76, -5.03, 2.79, 3.51, -2.15 (SE 1.2, $P < .05$) kg. The model predicted change in body fat of: -1.56, -1.16, -0.30, 0.37, 1.00, -0.19 kg. The model was adequate in predicting milk component response to treatments. Model behavior in response to protein intake only was adequate for body protein, however the response to differential intakes of energy and protein were inadequate. Maximal rates of proteolysis should be increased, and a better representation of the interactions between amino acids, fatty acids and glucose should be represented. Studies are required which measure proteolysis and gluconeogenesis in the lactating sow if we are to adequately predict nutrient requirements and production rates.

Key Words: Lactation, Amino Acids, Energy

1467 Inhibition of milk secretion and the extent of filling of the bovine mammary gland. K. Stelwagen, V. C. Farr, S. R. Davis*, and H. A. McFadden, *AgResearch, Hamilton, New Zealand.*

We have previously shown that mammary tight junctions (mTJ) become "leaky" after 17–18h of milk accumulation, and that this is associated with reduced milk secretion. Two experiments were conducted to study the extent of filling of the udder on inhibition of milk secretion. In exp. 1, 5 cows (cross-over design) were subjected to 24-h milk accumulation (control) or the same, but with removal of only cisternal milk after 12h. Cisternal milk was removed via drainage through previously inserted teat catheters, and following administration of adrenalin (i.v., 3 ml of 1 mg/ml). In exp. 2, 6 cows (cross-over design) were subjected to 24-h milk accumulation (control), as in exp. 1., or 24-h milk accumulation plus intramammary infusion of an isosmotic lactose/sucrose (LS) solution (pH 6.7; 300 mOsm: 146 mM lactose, 94 mM sucrose, 10 mM hepes, 2 mM $CaCl_2$) that was equivalent to 5h worth of milk secretion (i.e., 2.5 to 3 l of LS, with 45% and 55% respectively infused in front and hind quarters). Blood lactose levels were determined hourly in both experiments, and used as an indicator of mTJ permeability. In both control groups mTJ became leaky after 17h, however removal of cisternal milk (exp. 1) prevented this, but milk loss did not differ between control and drained groups (13 vs 12±2%, P<0.75). In contrast, LS infusion (exp. 2) resulted in mTJ becoming leaky much earlier, after 7h. This also resulted in a much larger milk loss compared to that in the control group (24 vs 13±1%, P<0.001). The LS solution would have diluted any chemical inhibitor(s) of milk secretion present in milk, yet losses were biggest in this group. We, therefore, postulate that increased filling of the glands results in cell shape changes, as described for other epithelia. These shape changes in turn initiate gene expression events (mechanotransduction) that culminate in inhibitory pathways being activated, involving loss of mTJ function.

Key Words: Mammary, Tight Junction, Mechanotransduction

1468 Comparative effects of epinephrine on tight junction (TJ) permeability in mammary and non-mammary epithelia. H. A. McFadden and K. Stelwagen*, *AgResearch, Hamilton, New Zealand.*

Cortisol stimulates TJ formation, yet, despite significantly elevated cortisol levels in stress-induced lactating cows mammary TJ permeabilty was not consistently decreased. Moreover, epinephrine (EPI) has been shown to increase permeability in nonmammary epithelia. The present study was to examine the effects of the stress-associated hormone EPI on TJ permeability in mammary (HC11) and kidney (MDCK) epithelia. Confluent monolayers were maintained in DMEM medium (MDCK) or RPMI 1640 supplemented with insulin (5 μg/ml), dexamethasone (1 μM), and prolactin (1 μg/ml) (HC11), on permeable Millipore PCF inserts (0.6 cm^2). TJ permeability was assessed by measuring transepithelial electrical resistance (TER) across cell monolayers. Means presented are after 48h-treatment with respectively 0, 3, 10, 20, or 50 μg/ml of EPI (MDCK, n=4; HC11, n=9 inserts per dose). TER data are expressed as a percentage of the pretreatment values, set at 100%. In MDCK cells EPI reduced the increase in TER with the two lower doses, whereas it decresed TER for the two highest doses (228^a vs 196^b vs 174^b vs 37^c vs 4^d ± 10%, ^{abcd}P<0.05). In contrast, EPI increased TER in mammary HC11 cells (141^a vs 156^{bc} vs 145^{ab} vs 164^c vs nd ± 4%, ^{abc}P < 0.05). Changes in TER were not due to changes in cell density, except for the highest dose on MDCK cells, where a decrease in TER coincided with cell loss (MDCK, 3.3^a vs 4.1^b vs 4.2^b vs 1.4^c ± 0.2 μg DNA/ml, ^{abc}P < 0.05; HC11, 18.8^a vs 19.4^{ab} vs 19.5^{ab} vs 17.5^a vs 21.1^b ± 0.8 μg DNA/ml, ^{ab}P < 0.05). Western blotting, using monoclonal antibodies against TJ-associated protein ZO-1, showed increased ZO-1 protein expression with EPI in HC11 cells, but not in MDCK. In summary, the effects of EPI on TJ are cell specific, having an adverse effect on TJ in MDCK cells, but an enhancing effect on mammary TJ. The latter may be, at least partly, mediated via TJ protein ZO-1.

Key Words: Epinephrine, Tight Junction, Mammary

1469 Influence of herd, season of calving and parity on the shape of lactation curve for milk protein and butterfat percentage as well as SCC in Holstein cows. L. T. Cziszter[1], Cs. Dorner[2], T. A. Tuan[2], A. Gáspárdy[3], and E. Szücs*[2], [1]*Banat's University of Agricultural Science and Veterinary Medicine, Timisoara (Romania),* [2]*Gödöllö University of Agricultural Science, Gödöllö (Hungary),* [3]*University of Veterinary Science, Budapest (Hungary).*

Lactation of 1515 purebred and crossbred Holstein cows were modeled by Wood's gamma function $Y_t = at^b e^{-ct}$, where Y_t is the percentage of milk protein and butterfat or SCC at time t of lactation, a is the initial value, b is the rate of incline to peak, c is the rate of decline thereafter, and e is the base of natural logarithm. For milk SCC data sets, lognormal transformation was applied prior to analysis. The effect of herd, parity (first lactation, second and subsequent lactations) and season of calving on the parameters was analyzed LSQ procedure. Seasons were winter (December, January, February), spring (March, April, May), summer (June, July, August) and fall (September, October, November). Differences between means were tested by t-test. The shape of the lactation curve was significantly altered by season of calving for all traits studied. The number of parities had a significant effect on the shape of lactation curve for milk protein percentage and SCC but not for milk protein percentage. Incomplete gamma function fitted better for milk protein percentage and SCC than for fat percentage. Parameters of function for milk protein are tabulated as folows:

	Effects	a	b	c
Seasons	Winter	5.604	−0.154	−0.00179
	Spring	4.437	−0.109	−0.00161
	Summer	4.307	−0.080	−0.00114
	Fall	6.409	−0.144	−0.00128
Parity	1st	5.678	−0.129	−0.00143
	2nd & subseq.	5.057	−0.131	−0.00164
Herd	A	5.294	−0.134	−0.00163
	B	5.262	−0.127	−0.00152

Key Words: Holstein, Lactation Curve, Milk Protein and Butterfat Percentage

1470 Effects of dietary vitamin E and different energy sources on α-tocopherol in plasma and milk. A. Baldi*, G. Savoini, L. Pinotti, C. Ferrari, and V. Dell'Orto, *Institute of Animal Nutrition, Veterinary Medicine University of Milan, Italy.*

Dietary vitamin E supplementation enhances immune response and plays an important role in prevention of mastitis in dairy cows. In peripartum period plasma vitamin E concentration decreases. Supplemental fat fed to transient cows may increase α-tocopherol concentration in plasma. In the present study we assessed if vitamin E supplementation to periparturient cows, fed isoenergetic diets that differed for energy sources (fat vs starch), in peripartum period can affect plasma and milk vitamin E concentrations. Thirty-two Friesian dairy cows were fed a basal diet formulated to provide 1000 IU of supplemental vitamin E daily. Since 10 d before anticipated calving to 21 d after, cows were fed daily one of four diets formulated to provide 1000 or 2000 IU of DL-α-tocopheril acetate and 0 or 300 g of supplemental fat (Ca salts of long chain fatty acids). The cows, which were in the 0 fat supplemented group, received an isoenergetic amount of corn. Plasma samples were collected on 3 d before and 5 d after calving and analyzed for α-tocopherol and cholesterol. Yield, composition, somatic cell count (SCC) and α-tocopherol were measured in milk 5, 10 and 20 d after calving.

Plasma α-tocopherol in 2000 IU vitamin E supplemented was higher (P<0.001) than in 1000 IU supplemented cows (before calving: 5.62 vs 3.92μ/ml; after calving: 3.86 vs 2.58μ/ml resp.), but it was not affected by dietary energy treatment. The same response was observed in milk α-tocopherol (1.16 vs 0.64 μ/ml, P<0.01). Maximum responsein milk was observed at 5 d after parturition. Milk yield and composition were not affected by treatments. Milk SCC was lower in cows which received higher supplementation of vitamin E (4.6 vs 5.1 log SCC, P<0.01). Dietary supplementation of vitamin E in periparturient dairy cows increased plasma and milk vitamin E, decreased SCC, however different energy sources did not affect any of the parameters considered.

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1471 Effect of feeding level on mammary growth in calves and prepubertal heifers. K. Sejrsen*, S. Purup, H. Martinussen, and M. Vestergaard, *Danish Institute of Agricultural Sciences, Foulum, Denmark*.

At 5 days of age 12 female calves were allocated to each of the treatment groups: MM, HM and HH. Group MM was raised on a moderate feeding level from 5 days of age to slaughter at approximately 250 kg. Groups HM and HH were offered a high feeding level to 6 weeks of age and moderate or high feeding levels, respectively, from 6 weeks to slaughter. The M and H rations contained 58 and 73 g CP/Mcal ME, respectively. ADG of the 3 groups MM, HM and HH before 6 weeks of age were 632, 883 and 895 g (SE = 28; $P < 0.001$). The corresponding ADG after 6 weeks were 758, 713 and 1204 g (SE = 29; $P < 0.001$). Average age and BW at slaughter was 270, 256 and 194 days (SE = 4.3; $P < 0.001$) and 240, 228 and 260 kg (SE = 3.8; $P < 0.001$) for groups MM, HM and HH, respectively. BW adjusted mammary gland measurements obtained by dissection for the groups MM, HM and HH, respectively, were: total gland: 929, 914 and 1971 g (SE = 110; $P < 0.001$); stroma: 554, 474 and 1726 g (SE = 106; $P < 0.001$); parenchymal tissue: 374, 440 and 245 g (SE = 46; $P < 0.05$). The corresponding unadjusted amounts of parenchymal tissue were 370, 420 and 269 g (SE = 35; $P < 0.02$). A companion trial was conducted to evaluate changes in mammary growth during the calf period. Amounts of mammary tissue in 5 group H calves at 6 weeks of age were compared with the amounts of mammary tissue of 5 group MM calves slaughtered at 12 weeks of age but at a similar BW (83 and 94 kg respectively; $P > 0.10$). The mammary glands of the group H calves contained 4.1 g (4.9 g/100 kg BW) parenchymal tissue compared with 19.6 g (20.5 g/100 kg BW) in the glands of group M (SE = 3.2 g; $P < 0.01$). However, mammary glands of 3 HM calves slaughtered subsequently at 12 weeks of age (average BW = 110 kg) contained a similar amount of parenchymal tissue 23.8 g (21.8 g/100 kg BW) as the MM calves ($P > 0.40$). Overall, these data suggest that prepubertal mammary growth until puberty is unaffected by the feeding level in the calf period and confirm a negative effect of high feeding level in the prepubertal period after 12 weeks of age.

Key Words: Mammary, Heifers, Calves

1472 The relationship between mammary gland size of sows and piglet growth rate. O. L. Nielsen and M. T. Sorensen*, *Danish Institute of Agricultural Sciences, Foulum, Denmark*.

In mammary development studies, mammary gland size has been used as an indicator of the potential for milk production. However, in the sow the relationship between mammary gland size and milk production has never been documented. The present investigation was thus carried out to study the relationship between mammary gland size and milk production. Three primiparous and 4 multiparous lactating sows and their litters were used. Litter size was 10 to 12 piglets. Sows and piglets had free access to water and sows were fed ad lib. Piglets were not creep-fed. Piglet weight gain from day 1 to weaning was used as a measure of milk production. Teat order was recorded in 5 sucklings on days 14 and 21 of lactation. The order was defined as stable if the piglet remained at the same teat for at least 4 of the 5 sucklings both days. Teat order was stable for 56 of the 73 piglets, and only these 56 teat-piglet combinations are included in the data. The sows were slaughtered after 25 to 28 days of lactation, and the udders were dissected into individual glands, weighed and analysed for DNA content. The correlations between piglet average daily weight gain and mammary gland weight or DNA content were 0.35 ($P<0.05$) and 0.41 ($P<0.03$), respectively. The correlations between piglet start weight and mammary gland weight or DNA content were 0.39 ($P<0.10$) and 0.33 ($P<0.21$), respectively. Mammary gland weight and DNA content were higher in multiparous than in primiparous sows (weight: 637 vs. 506 g, $P<0.04$; DNA content: 1496 vs. 1152 mg, $P<0.04$). DNA concentration in mammary tissue was the same for primiparous and multiparous sows (2.32 and 2.33 mg/g tissue, respectively). Average daily gain of piglets was the same in primiparous sows regardless of gland position, but in multiparous sows, piglets suckling the rear teats (5th to 7th pairs) had a lower ($P<0.05$) gain than piglets suckling the middle (3rd and 4th) and front (1st and 2nd) teat-pairs (rear: 200, middle: 243 and front: 267g/day). We conclude that milk production in lactating sows is positively correlated to mammary gland size.

Key Words: Sow, Milk Production, Mammary Gland Size

1473 Effects of retinoids on bovine mammary epithelial cell proliferation and plasminogen activator activity. F. Cheli*, A. Baldi, F. Fantuz, and V. Dell'Orto, *Institute of Animal Nutrition, Veterinary Medicine, University of Milan, Italy*.

Evidences attest that retinoids inhibit proliferation of many mammary cell lines. Bovine mammary cells produce urokinase- plasminogen activator(u-PA). A relation exists between expression of u-PA and the growth state of the cells. Aim of this study was to examine the effect of retinoids (all trans-retinoic acid, RA, and all trans-retinol, ROL)on proliferation of bovine mammary epithelial cells and plasminogen activator activity. BME-UV1 cells were used as a model system. Cells were cultured in the presence or absence of 10% FCS in DME-F12 culture medium for up to 72h. Cellular proliferation was evaluated both by incorporation of BrdU and enumeration using a hemocytometer. PA activity in the serum-free culture medium was detected using a colorimetric method that utilizes PA, present in the culture medium, to convert exogenously supplied plasminogen to active plasmin. Results showed that both retinoids markedly inhibited proliferation of BME-UV1 cells in the presence of 10% FCS after 72 h in culture. Inhibition of proliferation by RA (5μM) was 50% ($P<0.01$) and by ROL (50 nM) 30% ($P=0.05$). Cellular proliferation was inhibited more in sparse cultures than in dense ones. The effect of various concentrations of RA (0.1 to 10μM) on cell proliferation and PA activity in the culture medium in the absence of serum was evaluated. RA inhibited cell proliferation by 28% and 45% at the concentration of 0.1 and 1μM, respectively. RA at the concentration of 10μM was toxic to the cells. RA at the concentration of 1μM increased ($P<0.05$) PA activity on a per cell basis at 72 h in culture. Results indicate that retinoids are able to modulate the proliferation of bovine mammary epithelial cells and to affect plasminogen activator activity. Sensitivity of mammary epithelial cells to retinoids seems to be modulated by incubation time and cell density.

1474 Differential allele-specific transcription of κ-casein gene in Holstein cows. G. Robitaille* and D. Petitclerc, *Agriculture and Agro-Food Canada Dairy and Swine Research Centre, Lennoxville, Canada*.

It has been proposed that κ-casein (κ-CN) genetic variants A and B were expressed at different levels in the milk of heterozygous cows. We used a combination of reverse transcriptase-polymerase chain reaction and single-strand conformational polymorphism analysis to determine the relative proportion of κ-CN B allele-specific mRNA within mammary cells of heterozygous κ-CN AB cows (n=18) and to verify the differential allelic transcription of the κ-CN gene. Based on the relative proportion of κ-CN A and B specific transcripts, heterozygous κ-CN AB cows can be divided into two distinct groups. The first group of cows (10/18) included the ones for which the two κ-CN alleles were transcribed with similar efficiency (κ-CN B allele = 50.12 1 0.94%). In the second group of cows (8/18), there was more mRNA produced from allele B (56.56 1 1.29%) than from allele A, demonstrating that allele B was transcribed with greater efficiency than allele A. This suggests that one allelic gene has been mutated in the non-coding region of the gene so that the transcriptional efficiency was affected, creating two allelic subpopulations for the same genetic variant. We also determined the relative importance of the κ-CN B genetic variant in milk of cows from each group to enable comparison between the amount of transcript originating from each type of allele and the amount of genetic variant. A positive correlation (r= 0.8) was found between protein content of each genetic variant and the amount of allele-specific transcripts. The differential allelic transcription of κ-CN gene has an interesting consequence in terms of the use of κ-CN as a genetic selection marker to improve milk quality. Partially founded by Novalait Inc.

1475 Effect of different carbohydrate fermentability on metabolic status and amino acids fate in dairy cows. G. Bertoni*, E. Trevisi, F. Piccioli-Cappelli, and R. Lombardelli, *Facoltà di Agraria, Piacenza, Italy*.

We have previously reported that in two diets having a correct level of rumen escape proteins and essential amino acids, only the one with a higher content of fermentable carbohydrates determined a significant increase of protein content, particularly of the α-casein fraction, in milk of dairy cows. Since the amino acids availability was probably analogous in the two diets, this effect is possibly explained by the different amino acids partitioning among mammary gland and other body tissues. It seemed therefore interesting to study the endocrine-metabolic status of 6 late lactating dairy cows (190 DIM on average) fed with isonitrogenous (15% CP on DM with 38% of CP as RUP) diets containing 28.7% (LOW) or 36.2% (FER) of NSC on DM. The cows received each diet *ad libitum* for 4 weeks in a cross-over design. The forages (corn-silage and hay) were supplied every 12 h and the concentrates every 3 h. Feed intake and milk yield were checked daily, while blood samples were collected for endocrine-metabolic analysis every 3–4 days before morning meal and at the end of each period, with 6 collections in the period between the forage meals. GLM procedure of SAS package was used for the statistical analysis. DMI and milk yield were similar between the groups; plasma glucose contents were also similar, while higher insulin (14.2 *vs* 11.4 μU/ml, $P<0.05$) and lower GH (1.5 *vs* 1.8 ng/ml $P<0.1$) daily mean levels were observed when FER diet was fed. Moreover, the higher plasma basal content of glucagon (118 *vs* 108 pg/ml, $P<0.06$) and urea (5.3 *vs* 4.9 mmol/l, $P<0.01$) with LOW diet, seems to confirm a good availability of amino acids as well as a higher liver deamination. On the contrary, the higher level of insulin observed with FER diet probably enhanced the mammary protein synthesis from the available amino acids. Our results seem to confirm that fermentability of dietary carbohydrates can substantially modify the metabolic and endocrine status of lactating dairy cows and, consequently, affects milk composition.

Key Words: Carbohydrate Fermentability, Amino Acid, Metabolism

1476 Serum insulin-like growth factor-I and placental lactogen profiles in Holstein cows from genetic lines selected for milk yield. W. J. Weber*[1], C. R. Wallace[2], H. Chester-Jones[1], L. B. Hansen[1], and B. A. Crooker[1], [1]*University of Minnesota, St. Paul and* [2]*University of Maine, Orono*.

The relationship between endogenous concentrations of serum insulin-like growth factor-I (IGF-I) and placental lactogen (bPL) in dairy cattle is not well defined. Objectives were to determine this relationship in cows from genetic lines that differ by more than 4,000 kg milk/305 d lactation. Serum from primiparous (P) and multiparous (M) control (C, n = 18, 12) and select (S, n = 18, 18) cows was collected at − 28 ± 7, − 14 ± 3, − 7 ± 2, 1, 2, 3, 7, 14, 21, and 28 d postpartum (PP) and at 28 ± 3 d intervals until 280 d PP (non-pregnant) or throughout pregnancy. All samples were analyzed for IGF-I. Samples collected from conception through pre-calving were analyzed for bPL. Data were analyzed using PROC MIXED of SAS and results reported as least squares means which differed when $P < 0.05$. Pre-calving serum IGF-I was similar between S and C (154, 161 ng/ml), decreased at calving (74, 90 ng/ml), remained low through 7 d PP, and gradually returned to pre-calving concentrations as lactation progressed. Overall concentration of IGF-I was greater in C than S (131, 117 ng/ml) and P than M (148, 100 ng/ml). Post-calving concentrations of IGF-I in S were less than C through 84 d PP but similar through the remainder of lactation which resulted in a line by day interaction. During gestation, IGF-I and bPL profiles were nearly identical and not affected by line. Concentrations of IGF-I and bPL during gestation were stable from conception through 84 d of gestation (125, 0.07 ng/ml), increased from 84 to 250 d (181, 0.38 ng/ml), and decreased thereafter. There was a line by day interaction for both hormones during gestation. IGF-I was greater in P than M during gestation. Results indicate selection for increased milk yield prolonged the PP reduction in serum IGF-I but did not affect serum IGF-I or bPL during gestation. The strong relationship between IGF-I and bPL during gestation supports the concept that bPL may play a role in regulating serum IGF-I.

Key Words: Selection, IGF-I, bPL

1477 Casein and whey composition of milk from dairy cows milked three times daily. G. Bobe*, M. A. Faust, and G. L. Lindberg, *Iowa State University, Ames*.

To our knowledge, no data have been reported on the effect of milking time on milk protein composition. Our objective was to determine whether casein and whey composition differs among milkings. Milk samples from 12 dairy cows were obtained at 1 am,10 am, and 6 pm for three consecutive days and analyzed for composition of casein and whey proteins. The statistical model included cow, day of sampling, and time of sampling. The percentages of the four caseins and the three major whey proteins in milk did not differ significantly ($P > 0.05$) among the three milking times. We conclude that milking time does not affect casein and whey composition. Therefore, milk samples for studies of milk composition can be obtained once daily rather than several times daily.

Key Words: Casein Composition, Milking Time, Whey Composition

1478 Intravenous infusions of glucagon affect milk protein composition in lactating dairy cows. G. Bobe*, A. R. Hippen, P. She, J. W. Young, and G. L. Lindberg, *Iowa State University, Ames*.

In previous studies, intravenous infusions of glucagon into dairy cows decreased the crude protein concentration of milk. Our objective was to determine whether intravenous infusions of glucagon affect casein and whey composition of milk. In Experiment 1, multiparous, mid-lactation dairy cows (n = 5) were infused continuously for 48 h with glucagon at 5, 10, or 20 mg/d. In Experiment 2, multiparous Holstein cows (n = 14) were infused continuously for 14 d either with saline or with glucagon at 10 mg/d beginning at 21 d postpartum. In both experiments, the crude protein (CP) and true protein (TP) concentration of milk decreased significantly during glucagon infusion. In Experiment 1, the CP and the TP concentrations decreased from 3.2 % and 3.1 % to 2.6 % and 2.5 %, respectively ($P < 0.001$), and in Experiment 2, the CP and the TP concentration decreased from 3.0 % and 2.6 % to 2.7 % and 2.4 %, respectively ($P < 0.001$), suggesting decreased availability of amino acids for milk protein synthesis. Blood urea nitrogen and non-protein nitrogen in milk were not affected by glucagon, suggesting that deamination of amino acids was not influenced by glucagon. In both experiments, κ-casein as a percentage of total protein increased during glucagon infusions. In Experiment 1, κ-casein as a percentage of total protein increased from 12.2 % to 14.2 % ($P < 0.05$), and in Experiment 2, κ-casein as a percentage of total protein increased from 12.0 % to 14.2 % ($P < 0.001$). Changes of concentrations and proportions of other milk proteins were not consistently significant between experiments. Glucagon infusion changed milk protein compositions, suggesting an altered availability of individual amino acids for milk protein synthesis.

Key Words: Dairy Cows, Glucagon, Milk Proteins

1479 Cloning, analysis and SSCP characterization of the SH2 domain of the bovine gene STAT5. E. Antoniou*[1], M. Grosz[1], and C. Skidmore[2], [1]*ARS, USDA, Miles City, MT and* [2]*University of Reading, Reading, UK.*

Milk protein gene expression in mammary epithelial cells is regulated by the action of prolactin mediated through the Signal Transduction and Activator of Transcription 5 (STAT5). STAT 5 protein is phosphorylated in response to prolactin binding: this is followed by dimerization, translocation to the nucleus and binding to milk protein gene promotor sequences. The SH2 domain of the STAT proteins plays a critical role in the interaction with the kinase and in the dimerization process. We have determined the genomic sequence of the SH2 domain of the bovine STAT5 gene to investigate if mutations in this sequence might be responsible for quantitative variation in milk production. The genomic sequence is 795 bp long and contains the entire SH2 domain. After comparison with the bovine cDNA sequence (database EMBL n° Z72482), two introns of 81 and 422 bp were identified. Intron-exon boundaries are identical to those described for human STAT 1 and 2. Four differences are observed between the genomic and the cDNA sequence. When the putative protein sequences are compared, one of the differences is a silent mutation while the three others are missenses, at amino acid positions 593 (R>G), 602 (Q>L), and 617 (L>M). Further work is needed to assess if those differences are real polymorphisms, PCR artifacts or due to sequencing errors. There is a complete amino acid sequence identity between the mouse STAT5A and our STAT5 SH2 domain. Alignment of DNA sequences shows 5 silent site substitutions between ovine and bovine and 31 between murine and bovine. Coding variation between ovine and bovine (GGG for TTT) occurs in cDNA from no other species and is presumably the result of a recent mutation. We have designed PCR primers that amplify 253 bp of intron2 and 126 bp pf exon3. SSCP polymorphisms were detected in 8 families from the USDA-MARC panel. Those families contain a total of 44 offspring.

Key Words: Gene Mapping

1480 Intravenous infusions of glucagon affect κ-casein glycosylation in normal dairy cows and cows with fatty liver. G. Bobe*, A. R. Hippen, P. She, J. W. Young, and G. L. Lindberg, *Iowa State University, Ames.*

In previous studies, 14-d intravenous infusions of glucagon improved the endogenous carbohydrate status of early lactation cows, particularly cows with fatty liver. Our objective was to determine whether intravenous infusions of glucagon affect glycosylation of κ-casein. Multiparous Holstein cows (n = 15) were designated as either normal or susceptible to fatty liver and ketosis based on the ratio of liver triacylglycerol to glycogen being smaller or greater than 2.0 at d 6 postpartum. Normal cows and cows with fatty liver were infused with glucagon for 14 d at 0 or 10 mg/d beginning at 21 d postpartum. Cows with fatty liver had a greater percentage of milk proteins as glycosylated κ-casein than normal cows (6.13 % vs 4.59 %; $P < 0.01$). Glycosylated κ-casein as a percentage of total protein increased during glucagon infusions ($P < 0.01$). The increase of κ-casein as a percentage of total protein was significantly greater in cows with fatty liver than in normal cows ($P < 0.01$). Glycosylated κ-casein as a percentage of total protein increased in normal cows from 3.87 % to 5.22 %, whereas glycosylated κ-casein as a percentage of total protein increased in cows with fatty liver from 4.62 % to 6.97 %. We conclude that both intravenous infusions of glucagon and liver composition affect glycosylation of κ-casein in the mammary gland of dairy cows.

Key Words: Dairy Cows, Glucagon, Glycosylated κ-Casein

1481 Response of milk yield to increasing mammary blood flow. P. Lacasse[1]* and C. G. Prosser[2], [1]*Dairy and Swine R&D Centre, Lennoxville, Canada* [2]*AgResearch, Ruakura Research Centre, Hamilton, New Zealand.*

There is a close relationship between mammary blood flow (MBF) and milk production, but whether MBF is limiting milk yield has not been determined. Five lactating goats received close arterial (external pudic) infusion of saline or the nitric oxide donor diethylamine NONOate (0.5mg/h; NO) for 6 h, according to an incomplete latin square design. Goats were hand milked (with oxytocin) every 2 hours starting 2 hours before and ending 6 hours after the end of the infusion. In one goat a transit time flow probe was implanted around the infused and non-infused artery, whilst in the other goats a flow probe was implanted around the infused artery only. Infusion of saline did not affect MBF or milk production. As with previous results (J. Dairy Sci 79:1369), NO induced a rapid increase (up to 250% of pre-infusion level) in MBF in the infused gland only. Mammary blood flow was still 200% of the pre-infusion level at the end of the infusion period. Despite this increase in MBF, NO did not affect milk production (P>0.1). Milk yield ratio (infused/non-infused gland) averaged 1.20, 1.12 and 1.17 for the pre-infusion, infusion and post infusion periods, respectively. Similarly, protein, fat and lactose yields were not affected (P>0.1) by saline or NO infusion. These results provide no support to the contention that increasing MBF can enhance milk production.

1482 Application of in vivo stable amino acid isotope research results in a model to estimate lysine requirements of lactating sows. D. D. Koehler*[1], G. C. Shurson[2], S. M. El-Kandelgy[2], and P. J. Reeds[3], [1]*Agri-Nutrition Services, Shakopee, MN,* [2]*University of Minnesota, St. Paul,* [3]*CNRC/Baylor College of Medicine, Houston, TX.*.

Amino acid tracers have been used to quantify dietary lysine incorporation into milk proteins in six lactating multiparous sows using stable amino acid isotope tracer techniques (Koehler et al., 1996). This research found that 58.6 ± 4.8% of orally dosed lysine tracer appeared in milk within 24 hours of ingestion, indicating that the efficiency of utilization of digestible lysine for milk synthesis is approximately 59%. A model utilizing this information to calculate dietary lysine needs (in g/d and percentage of diet) of lactating sows based on observed litter growth rate and sow feed intake indicates that lactating sow lysine needs increase by 2.5 g for each 100 g increase in daily litter gain. The intercept of the model regression is 2.11, representing the maintenance lysine requirement under conditions of zero litter growth. Our model indicates lactation lysine requirements significantly higher than those commonly used in the swine industry which are based on a regression of retrospective data points taken from sow lactation studies (Pettigrew, 1993). For a sow supporting 2 kg litter growth per day our model indicates a 52.1 g/d lysine requirement versus 45.3 g/d for the same sow using the retrospective data. The retrospective regression has a slope of .026 and an intercept of −6.71. The slope indicates 2.6 grams of lysine is required for each 100 g/d incremental increase in litter growth rate. The negative intercept implies that the sow has a lysine requirement of zero at a litter growth rate of 258 grams/day. A likely explanation for the negative intercept of this model is that sows in the retrospective studies mobilized body protein to support milk production. These results suggest that current sow lactation lysine requirement estimates may fail to eliminate the mobilization of sow body protein stores and may fail to support optimal subsequent reproductive performance.

Key Words: Sow Lactation, Lysine Requirement, Stable Amino Acid Isotope