350 Effects of insulin and abomasal infusion of sodium caseinate and branched-chain amino acids on milk yield and milk protein composition in dairy cows. T. R. Mackle[1]*, D. E. Bauman[1], D. A. Dwyer[1], L. K. Ingvartsen[1], P. Y. Chouinard[1], and M. A. McGuire[2], [1]*Cornell University, Ithaca, NY and* [2]*University of Idaho, Moscow*.

Four multiparous Holstein cows (∼190 DIM) were subjected to a 4 d hyperinsulinemic-euglycemic clamp and intra-abomasal infusion of water (W) or a mixture of branched-chain amino acids (B) and sodium caseinate (C) to determine the effects of insulin and amino acids on milk yield and milk protein content and composition. Treatments were ± insulin (I) during abomasal infusion of W or 86.8 g/d B + 500 g/d C (BC). During the hyperinsulinemic clamp periods (WI and BCI), insulin was infused at 1.0 μg/kg BW/h and euglycemia was maintained by infusion of glucose. Cows were fed *ad libitum* a dry TMR, which was based on chopped alfalfa hay and concentrates, and was formulated to exceed requirements using the CNCPS. Milk yield was similar among treatments (26.5, 27.5, 28.3, 29.8 kg/d; W, BC, WI and BCI; P>.05). Milk protein content was increased (P<.01) by the WI and BCI treatments but not by BC (32.9, 33.1, 35.2 and 36.6 g/kg). By the fourth day of insulin infusion, cows receiving the BCI treatment yielded 25% more milk protein than those on W (867, 895, 995 and 1080 g/d; P<.01). Insulin but not BC increased the concentration of casein (24.9, 25.4, 26.3 and 27.3 g/kg; P<.05) and whey protein (6.5, 6.5, 7.0 and 7.4 g/kg; P<.001) in milk. Insulin treatment also caused a slight reduction in the amount of casein as a percentage of true protein (79.2, 79.7, 78.9 and 78.7%; P<0.05), due to a marginally greater increase in whey protein content relative to casein. In this study where cows were fully fed to requirements so that neither metabolizable protein nor energy were limiting, the supply of extra amino acids alone gave only modest increases in milk protein yield (+3.2%). In contrast, chronic elevation of circulating insulin caused a substantial stimulatory effect on milk protein yield, which was optimally expressed in the presence of additional amino acids (+25%).

Key Words: Milk, Insulin, Amino Acids

351 Effects of fat source on neutophil function, digestibility, and milk production. D. F. Jones*[1], W. P. Weiss[1], and T. C. Jenkins[2], [1]*The Ohio State University. Wooster,* [2]*Clemson University, Clemson, SC*.

This experiment was designed to determine the effects of fat source on blood neutrophil (PMN) function, digestibility, and production. Four diets (23% alfalfa silage, 31% corn silage, and 46% concentrate) with either 0 or 3% of the DM as tallow, fish oil (FO), or fish oil treated with ethanolamine to prevent rumen biohydrogenation were formulated. Based on milk fatty acid data protection of FO with ethanolamine was not complete. Holstein cows in late lactation were arranged in 8 blocks and fed diets for 28 d. Digestibility was determined by total collection. Viability of PMN was not different for any of the four treatments (93%). Phagocytic index (the ability of PMN to phagocytize *Staphylococcus aureus*) was significantly higher for cows fed FO (0.28) compared with cows fed the other three treatments (0.17). The survival index (*S. aureus* surviving within the PMN) was not different among treatments (1.70). The apparent digestibility of DM (67.9%), NDF (49.6%), and fatty acids (87.1%) was not different among the three sources of fat. Compared with the diet without fat, diets with fat significantly reduced milk production (29.4 and 24.0 kg/d), DMI (21 and 17.5 kg/d), milk fat yield (0.81 and 0.60 kg/d), and milk protein yield (0.95 and 0.79 kg/d). Percent milk fat (2.60%) and protein (3.30%) were not different among treatments. These results indicate FO may improve the ability of PMN to phagocytize *S. aureus*. Also, the treatment of FO with ethanolamine did not affect the digestibility of FO, however, protection was low.

352 Substrate utilization for hepatic gluconeogenesis is altered by increased glucose demand in ruminants. T. R. Overton*, J. K. Drackley, C. J. Ottemann-Abbamonte, A. D. Beaulieu, L. S. Emmert, and J. H. Clark, *University of Illinois, Urbana*.

Hepatocytes isolated from 10 Dorset wethers that were injected with 0 or 1.0 g/d of phlorizin for 72 h were used to determine whether increased glucose demand altered utilization of alanine and propionate for oxidative metabolism and gluconeogenesis. Control and phlorizin-treated wethers excreted 0 and 62.8 g/d of glucose into the urine, respectively. Phlorizin tended (P = .12) to increase conversion of [1-^{14}C]propionate and [1-^{14}C]alanine to CO_2. A phlorizin by substrate interaction (P = .05) for conversion to glucose indicated that conversion of [1-^{14}C]alanine to glucose was increased more by phlorizin than was conversion of [1-^{14}C]propionate (285% of controls vs. 166% of controls). Phlorizin did not affect estimated K_m for conversion of substrates to either CO_2 or glucose; however, phlorizin increased (P = .02) estimated V_{max} for conversion of substrates to CO_2 and tended (P = .09) to increase estimated V_{max} for conversion of substrates to glucose. Phlorizin slightly increased (P < .001) the ratio of glucose to CO_2 from [1-^{14}C]propionate and slightly decreased the ratio of glucose to CO_2 from [1-^{14}C]alanine. In vitro addition of 2.5 mM NH_4Cl decreased (P < .001) conversion of [1-^{14}C]propionate to CO_2 and glucose but had little effect on conversion of [1-^{14}C]alanine to CO_2 and glucose. Estimated K_m and V_{max} for conversion of substrates to CO_2, K_m for conversion of substrates to glucose, and V_{max} for conversion of [1-^{14}C]alanine to glucose were not affected by NH_4Cl; however, V_{max} for conversion of [1-^{14}C]propionate to glucose was decreased (P = .006) by NH_4Cl. These data indicate that utilization of propionate for gluconeogenesis is extensive, but amino acids have the potential to increase in importance as gluconeogenic substrates when glucose demand is increased substantially. Furthermore, excess ammonia (as NH_4Cl) decreases the ability of hepatocytes to utilize propionate for oxidation and gluconeogenesis.

Key Words: Gluconeogenesis, Ruminant, Amino Acids

353 Ruminally protected methionine increases peripheral methionine supply in Holstein cows during the close-up period. A. Bach[1], G. B. Huntington[2], and M. D. Stern[1], [1]*University of Minnesota, St. Paul,* [2]*North Carolina State University, Raleigh*.

Three Holstein cows (average weight 670 kg) were surgically prepared with an elevated carotid artery and indwelling catheters in the hepatic, portal, and two mesenteric veins to study the effects of methionine supplementation on amino acid metabolism during the close-up period. The study began 15 d before the expected calving date. Dietary treatments were Control (1.53 Mcal NE_L/kg, 15.6% crude protein) and Control supplemented with 60 g/d of ruminally protected methionine (MET, supplying 39 g/d of DL-methionine). Each cow received both dietary treatments in a crossover design. Cows were fed once a day. After 5 d on treatment, a blood flow marker (para-amino hippurate) was infused into a mesenteric vein, and arterial, portal, and hepatic blood samples were obtained at 0, 2, 6, 12 and 18 h after feeding. Net flux of methionine was calculated as the plasma arteriovenous difference multiplied by plasma flow. Dry matter intake (10.8 kg/d) and portal (855 L/h) and hepatic (996 L/h) plasma flows were not affected ($P > .10$) by treatment. Arterial plasma concentration of methionine was greater ($P < .05$) with MET (27.67 μM) than with Control (16.42 μM). Net portal absorption of methionine increased ($P = .10$) with MET (26.2 g/d) compared with Control (9.3 g/d). Net flux of methionine across splanchnic tissues shifted ($P < .06$) from a net uptake with Control (4.0 g/d) to a net output with MET (10.6 g/d). Therefore, MET increased by 14.7 g/d the methionine supply to the rest of the body. The net uptake of methionine by splanchnic tissues observed with Control indicates a net mobilization of methionine by peripheral tissues. Results suggest that methionine was a limiting amino acid during Control, and that MET was beneficial for periparturient cows because it increased methionine supply to peripheral tissues.

Key Words: Methionine, Plasma Flow, Nutrient Flux

354 Interactions of vitamins A and E in neonatal calves. D. C. Hammell[1], S. T. Franklin[1], and B. J. Nonnecke[2], [1]*South Dakota State University, Brookings,* [2]*USDA, ARS, NADC, Ames, Iowa.*

Calves are born with low concentrations of vitamin A and often are supplemented with high levels of vitamin A early in life as an attempt to improve vitamin A status. Little is known of effects of high supplementation of neonatal calves with vitamin A on immune parameters and vitamin E concentrations in plasma. Therefore, 53 Holstein bull calves were used to determine effects of supplementation with vitamins A and E on leukocyte populations, weight gain, plasma vitamin A and E concentrations, serum protein, and scouring. Calves were assigned to one of eight treatments at birth using a factorial design. Vitamin A was supplemented at either 0, 1700, 34000, or 68000 IU daily and vitamin E at 100 IU daily of either d-α-tocopherol or d-α-tocopheryl acetate. Calves were fed reconstituted milk replacer at 10% of body weight daily for 28 days. Amounts fed were adjusted weekly according to weight gain or loss. Results indicate no effect ($P > 0.05$) of treatment on proportions of monocytes, CD3+, CD4+, and CD8+ T-cells, null cells, or MHC class II molecules. B-Cells were higher ($P < 0.05$) in calves receiving d-α-tocopherol versus d-α-tocopheryl acetate. Serum protein, an indicator of serum Ig concentrations, was also higher in calves receiving d-α-tocopherol. By 3 wk of age, calves receiving no supplemental vitamin A had greater ($P = 0.013$) gains compared to calves receiving 68000 IU daily. By 4 wk of age, calves receiving no supplemental vitamin A had greater ($P < 0.05$) gains compared to calves receiving 34000 or 68000 IU daily. No differences ($P > 0.05$) were detected in scour scores among treatments. Supplementation of calves with vitamin A had no effect ($P > 0.05$) on vitamin A concentrations in plasma. Calves supplemented with vitamin A had decreased ($P = 0.053$) vitamin E concentrations in plasma compared to calves not receiving additional vitamin A. In summary, supplementation of calves with high amounts of vitamin A resulted in decreased growth rates and decreased plasma vitamin E concentrations. Interactions of vitamins A and E should be considered when formulating diets for calves.

Key Words: Calves, Vitamin A, Vitamin E

355 Involvement of dopamine in modulation of LH and prolactin secretion in anovulatory Holstein cows during the early postpartum period. A. Ahmadzadeh*, M. A. Barnes, R. M. Akers, and F. C. Gwazdauskas, *Virginia Polytechnic Institute and State University, Blacksburg.*

Two experiments investigated the effect of fluphenazine (FLU), a dopamine antagonist, on pituitary LH, and prolactin (PRL) secretion in anovulatory primi- and multiparous Holstein cows. In Experiment 1, 12 primiparous cows were randomly assigned to receive either saline (SAL;n=6) or .3 mg/kg BW FLU(n=6) in wk 2 postpartum. Blood samples were collected at 15 min intervals for 4 hr before and 4 hr after SAL or FLU. Immediately thereafter, all cows received 25 ug of GnRH and blood collection continued for an additional 1.5 hr. Mean serum progesterone concentration was less than 1 ng/ml. There was no difference in mean serum LH and PRL concentrations between groups prior to treatments. FLU caused a transient decrease ($P < .05$) in mean serum LH concentration (from .26 $\pm$ .02 to .14 $\pm$ .02 ng/ml) and decreased LH pulse frequency ($p < .01$;from .8 $\pm$ .1 to 0 $\pm$ .1 pulse/4h) and pulse amplitude. Mean serum PRL concentration was increased ($P < 0.01$) in FLU-treated cows (from 14.4 $\pm$ 3.3 to 118.1 $\pm$ 3.3 ng/ml). Mean serum LH and PRL remained unchanged in SAL-treated cows. In Experiment 2, 6 anovulatory multiparous cows (wk 2 postpartum) were used and all cows received FLU (.3 mg/kg BW). Experimental procedures were the same as used in primiparous cows. Mean progesterone concentration was less than 1 ng/ml. FLU decreased ($P < .05$) mean serum LH concentration (from .30 $\pm$ .01 to .20 $\pm$ .01 ng/ml) and LH pulse frequency (from 1.3 $\pm$ .1 to .5 $\pm$ .1 pulse/4h) but not LH pulse amplitude. Mean serum PRL concentrations were increased ($P < .01$; from 8.5 $\pm$ 8.9 to 75.6 $\pm$ 8.9 ng/ml) in response to FLU. Exogenous GnRH increased ($p < 0.05$) LH concentration in all cows in both experiments. These results suggest that modulation of LH secretion, at least in part, is mediated via endogenous dopamine in anovulatory cows during the early postpartum period regardless of parity. Data also confirm that, endogenous dopamine plays an inhibitory role in prolactin secretion in lactating cows.

Key Words: Dopamine Antagonist, LH, Dairy Cattle

356 Physiological regulation of Stat5 and β-casein gene expression in rat and bovine mammary gland. J. Yang*, V. E. Baracos, and J. J. Kennelly, *University of Alberta, Canada.*

Stat5 belongs to a family of molecules that acts as signal transducers and activators of transcription (Stat). It is involved in the signal transduction pathways of multiple hormones and growth factors. We hypothesized that Stat5 would play an important role in the regulation of β-casein gene expression in bovine. Stat5 will respond to prolactin, growth hormone (GH) and IGF-I and in vivo vary with milk protein synthesis in dairy cows. Stat5 DNA binding was detected in bovine mammary and positively identified by super bandshift assay. Two isoforms of Stat5 were identified in bovine mammary, as described in rat. Stat5 were observed in late pregnancy and present throughout lactation. In rat and bovine mammary explant culture, Stat5 responded to prolactin, growth hormone (GH) or IGF-I, but the β-casein mRNA abundance was not significantly altered. Without the hormone supplementation, Stat5 activity rapidly became undetectable in bovine tissue. These results provide further evidence that Stat5 is involved in the signal transduction of prolactin, GH and IGF-I. In two groups of cows with different milk protein concentration, there was a significant positive relationship between mammary Stat5 activity and milk protein concentration ($P<0.01$, n=12). The higher Stat5 activity correlated with higher milk protein concentration. Chronic infusion of GH for 2 months significantly depressed mammary Stat5 activity and protein levels even though β-casein mRNA level was significantly increased by GH treatment. The reduced Stat5 activity indicates that the signal transduction through Stat5 was down-regulated by GH infusion. In conclusion, Stat5 plays a role in the transduction of lactogenic hormone signals to the β-casein gene. Both the activity and protein level of Stat5 is physiologically regulated. Transcriptional regulation of β-casein is not evident at the level of steady-state mRNA, possibly due to the relatively large pool size or low turnover rate of this message and regulation of β-casein mRNA catabolism.

357 Milk urea nitrogen target values for dairy cows fed according to National Research Council recommendations. J. S. Jonker*, R. A. Kohn, and R. A. Erdman, *University of Maryland, College Park.*

Animal agriculture contributes significantly to non-point source N pollution of water resources. Our previously published model established a relationship between milk urea N (**MUN**) and urinary N excretion. The objectives of the current study were to develop a mathematical model to establish target MUN concentrations and to examine the effects of grouping by DIM on MUN throughout a 305-d lactation. Target MUN concentrations were established through computer simulation of predicted daily urinary N excretion from diets balanced according to NRC recommendations. Lactation curves for daily milk production, milk fat percentage, BW, and BW change were predicted for a second lactation, 600-kg Holstein cow producing 10,000-kg of 3.5% fat milk per lactation. The maximum estimated MUN concentration of 15.90 mg/dl occurred at 62 DIM. The average MUN weighted by milk production for the entire lactation was 13.51 mg/dl. A sensitivity analysis determined the effects of milk production (kg/d), milk fat percentage, BW (kg), and parity on predicted MUN concentrations. Estimated average MUN concentration was most sensitive to differences in milk production with a 2000 kg increase in milk yield per lactation resulting in a 2.85 mg/dl increase. A change in milk fat percentage of $\pm$0.5 percentage units altered estimated average lactational MUN concentration by approximately $\pm$1.71 mg/dl. Each 50-kg increase in BW resulted in an increase in average MUN concentration of 0.42 mg/dl. Changes in parity caused first lactation cows to have higher MUN concentrations than mature cows. Effects of grouping on target MUN values were examined by meeting the protein requirements for milk production of the 83rd percentile cow for a typical herd. A 1-group feeding system increased the average MUN by 9.3% to 14.77 mg/dl compared to feeding cows individually. A 3-group feeding system increased the average MUN by only 4.2% compared to feeding cows individually. Target MUN concentrations have been established for dairy farmers and will be most affected by milk production level and grouping strategies.

358 The effects of enhanced NDF digestibility of corn silage on DMI and milk yield of high producing dairy cows fed two levels of dietary NDF.

M. Oba* and M. S. Allen, *Michigan State University, East Lansing*.

Effects of NDF digestibility of corn silage and dietary NDF level on DMI and milk yield were evaluated using 8 multiparous dairy cows (70 DIM) in a duplicated 4 x 4 Latin square design. Experimental diets consisted of either brown midrib (bm3) corn silage or its isogenic normal control at two levels of dietary NDF (29% and 38%). Digestibility of NDF estimated by 30h-in vitro fermentation was higher for bm3 corn silage by 9.4 units. High NDF digestibility and low NDF treatments increased DMI and milk yield. Milk fat concentration was depressed for the high NDF digestibility treatment with the low NDF diet. Yield of FCM(3.5%) tended to be higher for high NDF digestibility treatments, while daily BW gain was higher for the cows fed low NDF diets. Both high NDF digestibility and low NDF treatments lowered ruminal pH, but daily ruminal pH variance was not affected by NDF digestibility. Feeding diets with high NDF digestibility increased the energy partitioned to milk production, possibly due to greater ruminal fermentation without increased variance of ruminal pH. The beneficial effects of enhanced NDF digestibility of corn silage on animal performance were greater when fed with a high NDF diet.

	Low NDF		High NDF		P value		
	HFD	LFD	HFD	LFD	NDF	FD	NDFxFD
DMI (kg/d)	24.7	23.9	22.9	21.5	<0.01	0.02	NS
Milk Yield (kg/d)	36.9	33.5	33.7	30.4	<0.01	<0.01	NS
3.5% FCM (kg/d)	35.6	34.3	35.8	32.6	NS	0.06	NS
Fat (%)	3.28	3.67	3.86	3.90	<0.001	0.02	0.06
Protein (%)	3.10	3.15	3.09	3.06	0.08	NS	0.15
BW Gain (kg/d)	1.10	0.79	0.00	-0.02	<0.01	NS	NS
BCS Gain (/21d)	0.17	0.22	0.10	0.04	0.07	NS	NS
Rumen pH Mean	5.62	5.78	5.73	5.90	0.06	<0.01	NS
Rumen pH Daily Variance	0.126	0.102	0.067	0.061	<0.001	NS	NS
Minimum Rumen pH	5.03	5.18	5.24	5.38	<0.001	<0.01	NS
Milk NE_L (%NE_L Intake)	66.9	64.0	72.4	68.7	<0.01	0.05	NS

HFD: High NDF Digestibility, LFD: Low NDF Digestibility

NDF: Effect of dietary NDF level, FD: Effect of NDF digestibility, NDF x FD: Interaction of dietary NDF level and NDF digestibility

359 Influence of dietary cation-anion balance on prepartum blood profiles and postpartum milk production of late gestation dairy cows grazing pasture.

K. J. Soder* and L. A. Holden, *The Pennsylvania State University, University Park*.

Twenty-four Holstein cows were utilized in a completely randomized design to characterize the effect of dietary cation-anion balance on prepartum blood profiles and postpartum milk production of late gestation dairy cows grazing pasture. Treatments began 4 wk prepartum and consisted of 1) pasture diet supplemented with a grain pellet without anionic salts (CON; +388 meq/kg) or 2) pasture diet supplemented with a grain pellet containing anionic salts (AS; +183 meq/kg). Nutrient content of pellets were similar except for anions (chloride and sulfur). Cows were rotationally grazed as a single group and individually fed pellets twice daily at a rate of 0.5% of body weight. Blood samples were collected 4 wk, 2.5 wk and 1 wk prepartum and analyzed for Ca, Mg, K, Na, and Cl. Intake was measured 3 wk and 1 wk prepartum using chromic oxide. Body weight and body condition were monitored through 100 days in milk. Cows calved on pasture, then were integrated into the regular milking herd and fed a total mixed ration. Daily milk production was recorded and weekly milk composition samples were collected through 100 days in milk. Intake was estimated 4 wk and 12 wk postpartum using chromic oxide. Using a split plot analysis with treatment and time, the least square means for milk yield, milk fat, and milk protein averaged 41.6 kg, 3.42%, and 2.96% for CON, and 41.7 kg, 3.31%, and 2.96% for AS, and were unaffected ($P > .05$) by treatment. Serum Ca, Mg, K, and Cl concentrations averaged 10.5, 2.2, 19.1, and 472.3 mg/dl for CON, and 10.3, 2.3, 19.4, and 463.2 mg/dl for AS, and were not affected ($P > .05$) by treatment. Serum Na concentrations were higher ($P < .05$) for CON cows than for AS cows (170.8 vs. 160.4 mg/dl). Prepartum pasture intakes and postpartum dry matter intakes averaged 10.4 and 23.8 kg/d for CON, and 10.9 and 23.2 kg/d for AS, and were not affected ($P > .05$) by treatment. Feeding anionic salts to late gestation dairy cows grazing pasture did not alter prepartum blood profiles or postpartum milk production or composition.

Key Words: Cation-anion, Prepartum, Grazing

360 The effects of particle size on nutrient distribution of alfalfa hay and barley silage.

P. J. Kononoff*, D. A. Christensen, A. F. Mustafa, and S. J. Pylot, *University of Saskatchewan, Canada*.

Limited studies have been conducted to determine how chemical composition changes occur with particle size distribution for different types of forages. Particle size distribution of alfalfa hay (AH) and barley silage (BS) for three different cut lengths were analyzed using the American Society of Agriculture Engineers (ASAE) Standard S424 method. The system utilizes five screens with nominal size openings of 19.0, 12.7, 6.3, 3.96, and 1.17mm with a pan to retain particles <1.17mm. AH samples were processed through the use a conventional tub grinder utilizing either a pair of 25.4mm i.d., a pair of 50.8mm i.d., or one 76.2mm i.d. and one 101.6mm i.d. sized screens. BS samples were obtained by using a theoretical length of cut of 19.0, 9.5, 4.8mm. Once sieved through the ASAE separator screen fractions were collected and analyzed in a completely randomized design for chemical composition. CP values for AH were lowest (8.8 %) for particles > 19.0mm and gradually increased to 22.8 % for particles < 1.17mm. ADF and NDF values for AH were highest (57.11 and 70.92%) for particles >19.0mm and lowest (31.09 and 37.83 %) for particles < 1.17mm. Consistent nutrient distribution according to particle size was not observed with barley silage. Lowest ADF and NDF values (21.10 and 40.61%) were observed on particles retained on the 3.96mm sized screen due to the accumulation grain. Results suggest that chemical profile according to particle size distribution is different for BS than AH.

Key Words: Particle Size, Barley Silage, Alfalfa Hay