1476 Finishing system (feedlot or pasture) and copper supplementation affect conjugated linoleic acid in beef muscle. T.E. Engle*[1] and J.W. Spears[2], [1]*Colorado State University, Fort Collins,* [2]*North Carolina State University, Raleigh.*

Sixty Angus steers (413 kg initial wt) were used to determine the effect of copper (Cu), corn type, and finishing system (confinement vs pasture) on performance and fatty acid composition of beef longissimus muscle. Steers in confinement were fed high concentrate diets, containing either control or high oil corn, individually, using Calan gate feeders. Steers grazing pasture (tall fescue) were maintained in 4 replicates with each replicate consisting of 5 steers. Salt was used to limit concentrate intake in pasture steers to approximately 67% of that observed in confinement steers. One half of the steers in each treatment received a CuO needle bolus at the initiation of the study while the remaining steers received no supplemental Cu. Equal number of steers per treatment were slaughtered after 91, 112 or 133 days on feed. Gain was lower ($P < 0.01$; 1.22 vs 1.57 kg/d) for pasture steers compared with those fed in confinement. Copper increased ($P < 0.05$) ADG in steers fed control corn (1.75 vs 1.50 kg/d) and pasture (1.29 vs 1.14 kg/d) but not in steers fed high oil corn diets (1.51 vs 1.54 kg/d). Gain, DM intake and gain:feed did not differ between steers fed control or high oil corn. Longissimus muscle C18:1 ($P < 0.06$), C18:3 ($P < 0.07$) and C18-conjugated dienes ($P < 0.001$) were higher in pasture-finished steers relative to the control and high oil corn finished steers. The C18:1 trans isomer ($P < 0.06$), C18:0 ($P < 0.06$), and total saturated fatty acids ($P < 0.06$) were lower in longissimus muscle of steers that received a Cu bolus. C18-conjugated dienes were higher in longissimus muscle of steers receiving a Cu bolus finished on pasture ($P < 0.06$) and in steers receiving a Cu bolus finished on the control diet ($P < 0.07$) relative to the non-Cu bolus supplemented steers. These results indicate that finishing cattle on pasture with limited grain increases C18-conjugated dienes in longissimus muscle and that Cu supplementation may alter lipid metabolism.

Key Words: Copper, Fatty Acid, Steer

1477 Interaction of steam reduction and tempering on the feeding value of steam-flaked corn for feedlot cattle. R. A. Ware*, S. A. Rodriguez, and R. A. Zinn, *University of California, Davis.*

Two trials were conducted to evaluate the interaction of steam reduction (419 vs 335 kPa pressure along a 6.35 cm diam steam line, measured 90 cm proximal to steam chest splitter valves) and tempering (60 min exposure to 40 g/kg water plus .275 mg/kg of SarTemp®; SarTec, Anoka, MN) on the feeding value of steam-flaked corn (SFC) for feedlot cattle. The SFC was prepared by steaming for 30 min before flaking to a density of .31 kg/L. The SFC was then allowed to air-dry before inclusion into experimental diets. Experimental diets contained 76% SFC (DM basis). Ninety-six crossbred steer calves (347 kg) were used in a 56-d trial to evaluate treatment effects on performance and dietary NE value. There were no treatment effects ($P > .20$) on ADG (1.24 kg/d), DM conversion (5.60 kg DM/kg ADG), and dietary NE_m and NE_g (2.24 and 1.55 Mcal/kg, respectively). Expected (NRC, 1984) dietary NE values for maintenance and gain were 2.23 and 1.55 Mcal/kg. Close agreement between observed and expected dietary NE indicates that across treatments the NE values for SFC were consistent with tabular values (2.38 and 1.68 Mcal/kg, respectively). Four Holstein steers (223 kg) with ruminal and duodenal cannulas were used in a 4×4 Latin square design to evaluate treatment effects on digestive function. Intake of DM was limited to 2% of BW. The percentage of corn starch that was reactive to amylglucosidase (a measure of starch solubility) was similar across SFC treatments, averaging 22%. Ruminal digestion of OM averaged 65% and was not affected ($P > .20$) by treatments. Tempering increased (5%, $P < .05$) ruminal starch digestion. However, ruminal starch digestion was not influenced by steam reduction (81 vs 80%, $P > .20$). Total tract digestion of OM and starch also were not affected ($P > .20$) by treatments, averaging 68 and 98%, respectively. We conclude that if the lower quarter of the steam chest is maintained at temperatures greater than 100° C, reducing steam application by as much as 20% will not detrimentally affect the feeding value of SFC. Due to the absence of a main effect of steam reduction on the feeding value, the interaction of tempering at lower level of steam application was not fully addressed in this study.

Key Words: Steam, Corn, Cattle

1478 Alternate equation forms for heat production estimation in ruminant growth and composition models. J.W. Oltjen* and R.D. Sainz, *University of California, Davis.*

As an alternative to the static representation of heat production (HP) as the sum of that used for maintenance and that used for gain in feeding systems or dynamic growth models, we have investigated a multiple regression on visceral (V, kg) and non-visceral (M, kg) protein and accretion of V and M. Either HP representation can be used in our recently developed dynamic model of the two protein and fat pools. That is, the fit of the model to data, using either traditional concepts of net energy and maintenance, or more general functions for heat production, can be compared to choose the best functional description. In the model, net energy for gain, the difference between metabolizable energy intake and heat production, drives the growth of V and M. Our objective was to estimate parameters and fit of heat production equations for both 72 growing lambs (Ferrell et al., 1986, Brit. J. Nutr. 56:595) and 144 steers (Sainz et al., 1995, J. Anim. Sci. 73:2971) undergoing various trajectories of compensatory and normal growth. The best equation for rams (r^2=.987) was HP, mCal/d = .243 (±.080) M + 2.52 (±.81) V + 14.6 (±3.3) dM/dt + 67.6 (±21.9) dV/dt where figures in parenthesis are SE of regression coefficients. For steers the experimental design provided fewer growth trajectories and no weight loss periods; the equation HP, mCal/d = .0657 (±.0696) M + 1.63 (±.60) V + 16.7 (±3.4) (dM/dt+dV/dt) fit steers (r^2=.965). In the regression, heat production per V is generally much greater than that for M. In the growth model, V responds faster than M to changing energy intake by the animal; this makes maintenance requirement a dynamic variable depending on nutritional history as well as current energy intake. Thus, the static form of maintenance function used in traditional feeding systems is likely to be inappropriate, especially in dynamic situations.

Key Words: Energy Metabolism, Body Composition, Growth Models

1479 Effects of moisture, roller setting and saponin-based surfactant on growth performance of feedlot steers. Y. Wang*[1], T. A. McAllister[1], and D. Greer[2], [1]*Agriculture and Agri-Food Canada Research Centre, Lethbridge, AB,* [2]*AgriChem, Inc., Anoka, MN.*

The effects of roller setting, moisture content, and a saponin-based surfactant (GrainPrep®, AgriChem, Inc.) on performance of feedlot cattle were studied using 138 individually fed British cross steer calves ($n = 23$) and six barley silage/rolled barley grain diets for backgrounding (Bk) and finishing (Fn). Barley grain was rolled dry (D), tempered to 20% moisture (M), or tempered (20% moisture) and treated with 60 ppm of surfactant (MS); and with rollers set to produce optimal particle size from dry barley (+D) or set to produce optimal particle size from MS barley (+MS). The resulting diets were fed daily as total mixed rations, with a 4-wk transition from Bk to Fn. Dry matter intake (DMI) was higher ($P < 0.01$) during Bk, Fn and overall when barley was rolled at +D, than at +MS. With barley rolled at +D, average daily gain (ADG) was higher ($P < 0.01$) during Bk, but was unaffected ($P > 0.05$) during Fn, and tended to be higher ($P = 0.084$) overall. Feed efficiency (FE) tended ($P = 0.106$) to be improved during Bk, but was poorer during Fn ($P < 0.001$) and overall ($P < 0.011$), when rollers were set at +D than at +MS. Tempering the barley did not affect DMI, ADG or FE during Bk ($P > 0.05$), but greatly increased DMI and ADG ($P < 0.001$) and improved FE ($P < 0.001$) during Fn and overall. Enhancement of growth rate by tempering was greater ($P < 0.05$) if barley had been rolled at +D than at +MS. Tempering with GrainPrep® (MS diets) increased ($P < 0.05$) ADG, relative to M diets, when rollers were set at +MS. Roller setting did not affect ($P > 0.05$) carcass characteristics, but M and MS diets increased ($P < 0.05$) warm carcass weight and saleable meat yield and tended to increase ribeye area ($P = 0.072$), relative to D diets. Tempering barley grain prior to rolling improved growth performance relative to rolling dry grain. The positive effects of GrainPrep® on performance were enhanced when barley was rolled to 72% of original bulk density (+MS) as compared to 78% (+D).

Key Words: Saponin, Barley grain, Feedlot cattle

1480 Effects of high oil corn and shade on respiration rates and acid-base balance of Angus and Bonsmara x Beefmaster feedlot steers. T. C. Bramble[1], C. R. Richardson*[1], J. D. Thiebaud[2], F. N. Owens[3], and G. R. Chapman[4], [1]*Texas Tech University, Lubbock,* [2]*Texas Tech Howard Hughes Medical Institute, Lubbock,* [3]*Du Pont Specialty Grains, Des Moines, IA,* [4]*Amarillo, TX.*

This experiment was designed to determine if high oil corn (HO) or supplemental shade affects respiratory rates (RR) and acid-base balance (ABB) of feedlot steers in partially slotted floor outdoor feedlot pens. Steers, Angus (A) (n=59; BW = 322 ± 2.2 kg) and Bonsmara x Beefmaster (B) (n = 56; BW = 294 ± 2.1 kg) breeding were fed for 150 d (July 17 to December 13, 2000). Breed was nested within each of the 24 pens and steers were blocked into pens by weight. Treatments were: NST (no shade, typical corn), NSHO (no shade, high oil corn), ST (shade, typical corn), and SHO (shade, high oil corn). The HO replaced T on a weight basis, and diets were formulated to meet or exceed NRC (1996) requirements. Shade structures, black, 80% light-occluding polypropylene cloth fixed 3 m above pens, covered 67% of the pen area (9.8 m^2 of shade/steer). On 25 d throughout the study, RR were measured for one A and one B steer in each pen (n = 48) at 0730 and 1600. Blood gas measurements [(pH, pCO_2, pO_2, HCO_2, TCO_2, and base excess (BE)] to assess the ABB of steers were based on jugular vein blood samples (n = 24, 1 steer/pen, 12 A, 12 B) taken on d 0, 28, 56, 85, 115, and 150. The RR was 22% faster (P < .01) for NS than S steers and 80% faster (P < .01) for A than for B cattle in the afternoon (1600). Shades decreased RR of A cattle by 26% (P < .02) and of B cattle by 15% (P < .01). Cattle fed HO had greater (P = .02) RR than cattle fed T. On d 28 and 85, BE values were greater (P = .07) for shaded steers. Bicarbonate values were higher for S cattle on d 28 (P = .07), and blood was more (P < .10) basic in pH on day 85 for cattle fed HO. Shades may increase the alkalinity of venous blood even though they reduce RR. This suggests that respiration rate alone is inadequate as a predictor of blood acid-base balance of growing steers given ad libitum access to feed.

Key Words: High oil corn, Acid-base balance, Respiration rate

1481 An evaluation of breed and diet on plasma leptin concentration in beef steers. K.A. Johnson*[1], P.S. Mir[2], P.S. Kuber[1], Z. Mir[2], D.H. Keisler[3], C.T. Gaskins[1], J.J. Michal[1], J.R. Busboom[1], and J.J. Reeves[1], [1]*Washington State University, Pullman, WA,* [2]*Agri-Food Canada, Lethbridge, Alberta,* [3]*University of Missouri, Columbia, MO.*

To examine the impact of breed and diet on plasma leptin concentration 36 steers of Limousin (L n=12), Wagyu (W n=12) and Limousin x Wagyu F1 cross genetics (LW n=12) were randomly assigned to one of two dietary treatments (0% and 6% sunflower oil). Blood samples were collected at the initiation and end of the feeding trial and plasma leptin determined by radioimmunoassay. At the same time, measurements of body weight, ultrasound measurement of ribeye area (REA) and fat thickness (FT) were made. Predictions of intramuscular lipid were made from the ultrasound images. After the backgrounding phase (112d), steers were fasted and plasma collected for leptin analysis. At harvest (d 212) carcass weight, FT, REA, yield grade (YG) and marbling measurements were collected. There was no effect of diet on leptin at any time. Initial leptin was not different among breed groups (L 2.04 ± 0.29 ng/ml; LW 3.12 ± 0.61 ng/ml; W 2.97 ± 0.62 ng/ml). Leptin at fasting did not differ (P<.13) by breed (L 1.35 ± 0.31 ng/ml); LW 1.90 ± 0.32 ng/ml; W 2.25 ± 0.40 ng/ml). Wagyu had greater (P<.02) final leptin (13.2 ± 1.43 ng/ml) than L (9.53 ± 0.97 ng/ml) or LW (9.76 ± 0.93 ng/ml). Carcass weight, REA, and YG differed by breed at harvest (P<.01). Marbling scores and quality grade were different (P<.0001) among breed types with W (866.7; prime -) being highest, followed by WL (583.3; choice -) and L (470.8; select +). Correlation analysis indicated no significant relationship between plasma leptin collected at any time and carcass traits. While plasma leptin was not useful as a predictive tool in determining carcass fatness, the final leptin concentration did reflect final quality grade.

Key Words: Leptin, Beef Cattle, Wagyu

1482 Corn processing method in finishing diets containing wet corn gluten feed. T.L. Scott*, C.T. Milton, T.J. Klopfenstein, and R.A. Stock, *University of Nebraska-Lincoln.*

Four finishing trials were conducted to determine the effects of corn processing method on performance of steers fed diets containing wet corn gluten feed (WCGF). In Trial 1, 300 calves were fed five factorially arranged treatments: 1-4) dry-rolled (DRC) or steam-flaked corn (SFC) with or without 32% WCGF; and 5) SFC+DRC without 32% WCGF. Intake was similar when feeding corn grain and increased (P<0.10) by different magnitudes (1.2 and 0.7 kg/d) when feeding DRC and SFC with WCGF. Feeding WCGF improved (P<0.10) ADG and reduced (P<0.10) efficiency compared to feeding corn grain alone. Intake and performance were similar when WCGF or DRC replaced SFC. Daily gain was similar and efficiency improved (P<0.10) by feeding SFC versus DRC. In Trial 2, the DRC/WCGF and SFC/WCGF from Trial 1 were used as control treatments and another 180 calves were fed three treatments: 1) high-moisture (HMC), 2) finely-ground, and 3) whole corn all with 32% WCGF. When feeding WCGF, whole corn increased (P<0.10) intake compared to SFC, HMC, or finely-ground corn with DRC being intermediate. Processing method did not affect ADG. Efficiency was reduced (P<0.10) by feeding whole corn compared with other treatments. Feeding SFC, HMC, or finely-ground corn improved (P<0.10) efficiency compared to DRC. Efficiency was improved (P<.10) by feeding SFC compared to finely-ground corn. In Trial 3, 192 yearlings were fed four factorially arranged treatments: 1-4) DRC or SFC with or without 22% WCGF. Intake was similar in steers fed corn grain and increased (P<0.10) by different magnitudes (1.1 and 0.5 kg/d) when feeding DRC and SFC with WCGF. Feeding WCGF improved (P<0.10) ADG while feed efficiency was similar. Both ADG and efficiency were improved (P<0.10) by feeding SFC compared to DRC. In Trial 4, the DRC/WCGF and SFC/WCGF from Trial 3 were used as control treatments and an additional 96 yearlings were fed two treatments: 1) HMC and 2) finely-rolled corn both with 22% WCGF. When feeding WCGF, intake was similar; however, SFC improved (P<0.10) both ADG and efficiency compared with other treatments. Performance was similar among steers fed DRC or HMC. Feeding HMC improved (P<0.10) efficiency compared to finely-rolled corn. In diets containing WCGF, feed efficiency tended to be improved by more intensive processing methods.

Key Words: Beef Cattle, Grain Processing, Byproduct Feeds

1483 Sub-clinical ruminal acidosis in feedlot cattle fed a barley-based diet. G. R. Ghorbani*[1,2], K. A. Beauchemin[1], and D. P. Morgavi[1], [1]*Agriculture and Agri-Food Canada, Lehtbridge, AB, T1J 4B1, Canada,* [2]*Isfahan University of Technology, Isfahan, Iran.*

Sub-clinical acidosis can reduce feed efficiency and growth rate in cattle, thereby causing significant economic losses to the feedlot cattle industry. A study was conducted to determine the prevalence of subclinical ruminal acidosis in feedlot cattle fed for maximum growth rate. Measurements of rumen pH, blood pH and DMI were made using six ruminally cannulated steers over a 9-week period. On three occasions during the experiment, ruminal pH was measured every 15 min for 6 d using indwelling electrodes. DMI was measured for each animal daily and blood pH was measured before feeding three times during the experiment. Steers were provided ad libitum access to a diet containing steam-rolled barley, barley silage, and a protein-mineral supplement; 87, 9, and 4% (DM basis), respectively. Mean values (SD) were: DMI, 9.29 (1.87) kg/d; blood pH, 7.37 (0.028); ruminal pH, 5.70 (0.41); maximum pH, 6.43 (0.25); minimum pH, 5.17 (0.43); hours pH < 5.8, 12.43 (8.09); area below 5.8 and the curve, 6.59 (5.8) pH x h/d; hours pH < 6.2, 17.8 (5.91), and area below 6.2 and the curve, 12.43 (6.2) pH x h/d. There was a significant effect ($P < 0.05$) of day on all rumen pH variables, except for maximum pH. Furthermore, there was no correlation ($P > 0.05$) between pH variables (mean pH, hours pH < 5.8, or < 6.2) and blood pH or DMI. Using the DMI and forage eNDF (measured as the proportion of the DM retained on the Penn State Particle Separator) obtained in this experiment, the NRC (1996) model predicted a mean rumen pH of 5.81, 0.11 units higher than observed. The eNDF needed to be reduced in the model from 34% to 18% to accurately predict the observed pH. In conclusion, the incidence of ruminal subclinical acidosis in feedlot cattle fed barley-based diets is prevalent, and higher than would be predicted based on the NRC (1996) model.

Key Words: Sub-clinical acidosis, Feedlot diet, Rumen pH

1484 Adaptation of the Cornell Net Carbohydrate and Protein System to sheep: validation of feed digestibility. A. Cannas*[1], D.G. Fox[2], A.N. Pell[2], and P.J. Van Soest[2], [1] *University of Sassari, Sassari, Italy,* [2] *Cornell University, Ithaca, NY.*

A new feeding system for sheep, based on the structure of the Cornell Net Carbohydrate and Protein System (CNCPS) for cattle, was developed. The goal was to overcome some of the limitations of currently available feeding systems for sheep, with special regard to the estimation of feed values at different levels of intake. This system, called sheep-CNCPS, integrated current knowledge on sheep requirements into the structure of the CNCPS for cattle. The equations used to estimate energy and protein requirements were obtained by incorporating those published in the literature. Except for the predictions of feed and liquid passage rates, which were based on new equations developed for this system, the submodels used to estimate the supply of nutrients were those of the CNCPS for cattle. The sheep-CNCPS predictions of feed digestibility were validated by using 14 published in vivo total tract digestibility experiments, in which 56 different diets were tested. The sheep-CNCPS slightly underestimated DM digestibility (%) (mean bias -2.07, model bias -3.3, RMSPE 4.17; n=41) and OM digestibility (%) (mean bias -1.17, model bias -1.6, RMSPE 3.97; n=36). NDF digestibility (%) was predicted with lower accuracy than OM and DM (mean bias -2.17, model bias -5.1, RMSPE 9.90; n=56). When two distinct outliers were discarded, the prediction of NDF digestibility was substantially improved (mean bias -1.01, model bias -2.9, RMSPE 7.58). Dietary CP digestibility (%) was grossly underestimated (mean bias -5.58, RMSPE 15.31; n=42), with three feeds having negative digestibility, probably because of the large overestimation of fecal metabolic nitrogen. When they were discarded, the prediction was markedly improved (mean bias -2.53, model bias -2.0, RMSPE 9.45). Overall, the sheep-CNCPS predicted feed DM, OM, and NDF digestibility quite accurately, but it was less reliable in the case of CP digestibility.

Key Words: Sheep, Feeding system, Digestibility prediction

1485 Effect of moisture heat damage on ruminal degradation of cottonseed dry matter and crude protein using nylon bag technique in sheep. A. Estrada* and R. Barajas, *Universidad Autonoma de Sinaloa (Mexico).*

To determine the effect of moisture heat damage on ruminal degradation of cottonseed dry matter and crude protein a study using the nylon bag technique was conducted. Four sheep (Pelibuey; BW = 34 kg) were fitted with T canula in rumen. The animals were fed a diet containing 12% corn straw, 10% cottonseed, 10% moisture heat damaged cottonseed, 57% sorghum grain, 8% sugar cane molasses, 0.9% urea, and 2.1 mineral premix (15% CP and 3.55 Mcal DE/kg). Moisture heat damaged cottonseed was processed by adding 30% water and bulk-stacking for 7 d. Pairs of nylon bags (12 x 18 cm) containing five grams of ground undamaged cottonseed (UCS) or moisture heat damaged cottonseed (DCS) were placed in the rumen, and incubated for 3, 6, 9, 12, 24, 48, and 72 h. After removal from rumen, residual DM and CP content were determined. Water addition and stack processing increased the cottonseed temperature from 34 to 65 °C in 5 d (7.7 °C/day; $R^2 = 0.99$; $P<0.001$). After that time temperature was constant. DM solubility of DCS was 33% lower ($P<0.01$) than UCS-DM. At 12 h of incubation DCS-DM was 18% lower ($P<0.01$) than UCS-DM. The degradation rate (c) of DCS-DM was lowered by 33% (0.09 vs. 0.06%/h). The crude protein solubility was diminished ($P<0.03$) by moisture heat damage (40 vs. 31%). Rumen degradation rate of CP from DCS was 32% lower than UCS-CP (0.028 vs. 0.019%/h). The true ruminally degraded CP of UCS was calculated to be 79.4% and the corresponding value of DCS was 70.6%. Consequently the rumen undegradable crude protein of cottonseed was increased in 42% (20.6 vs. 29.5%) due to moisture heat damage. It is concluded that the water addition to cottonseed and its further bulk-stacking for 5d is able to increase its temperature enough to reduce the solubility and degradability of cottonseed crude protein in rumen, and to increase significantly its rumen undegradable crude protein content.

Key Words: Cottonseed, Heat damage, Crude protein

1486 Effect of close-up protein supplementation on milk, fat and protein yields of late gestation primiparous Holstein dairy cows. P. H. Robinson*[1], J. M. Moorby[2], and M. Arana[3], [1] *University of California, Davis, CA,* [2] *IGER, Aberystwyth, UK,* [3] *UCCE, Stockton, CA.*

To define the impact of supplementation of CP in the late (close-up) gestation period of primiparous dairy cows, on yield of milk and its components, a diet of corn silage (17% of DM), alfalfa hay cubes (24%), oat hay (25%), barley (16%) and corn grains (16%) was limit fed at 12.1 kg DM/d. A supplement of canola meal (60%), dried distillers grains (20%), blood meal (10%), feather meal (5%), and corn gluten meal (5%) was not fed (11.7% CP; DM), or fed at 1.1 kg/d (14.4% CP) or at 2.3 kg/d (16.6% CP). Milk, protein and fat yields of 154 primiparous Holstein heifers, that were close-up for 1 to 18 d and offered one of the diets (n = 51, 53 and 50 for diets 11.7, 14.4 and 16.6% CP), were measured monthly for the first 150 d of lactation. Following calving, all cows received the same diet containing 17.7% CP and 32% NDF (DM). For statistical analysis of treatment means, cows were allocated to one of four groups based upon time close-up (1-4, 5-8, 9-12 and 13-18 d). Data were analysed by multiple regression with diet CP, its square, days close-up, its square and cube, and their interactions, with progressive removal of non-significant terms. Milk yield was highest for cows offered the 14.4% and 16.6% CP diets for more than 8 d. Protein yields were similar for all diets, but tended to be highest for the 14.4% CP diet fed for 9-12 d. Fat yields were not significantly described by the parameters of the regression model. The amount of supplement fed close-up, and the length of time that animals were close-up and received it, both influenced milk and milk protein yield. Overall, the optimal combination was 14.4% CP for 9-12 d.

	Days close-up			
Diet CP %	1-4	5-8	9-12	13-18
Milk, kg/d				
11.7	32.6	32.1	33.0	32.3
14.4	29.0	33.8	34.4	33.4
16.6	33.9	31.3	32.0	34.5
Protein, kg/d				
11.7	1.06	1.04	1.07	1.04
14.4	0.94	1.06	1.08	1.05
16.6	1.14	1.02	1.01	1.07
Fat, kg/d				
11.7	1.12	1.07	1.22	1.11
14.4	1.04	1.18	1.18	1.10
16.6	1.21	1.14	1.11	1.21

Regressions: Milk R^2=86.2; s.e. obs.=0.571; $P<0.05$. Protein R^2=86.5; s.e. obs.=0.027; $P<0.05$. Fat R^2=74.1; s.e. obs.=0.029; P=0.20.

Key Words: Close-up, Transition, Protein

1487 Effect of close-up dry period protein supplementation on milk, fat and protein yields of multiparous Holstein dairy cows. J. M. Moorby[1], P. H. Robinson*[2], and M. Arana[3], [1] *IGER, Aberystwyth, UK,* [2] *University of California, Davis, CA,* [3] *UCCE, Stockton, CA.*

To define the impact of supplementation of CP in the late (close-up) dry period of multiparous dairy cows, on yield of milk and its components, a diet of corn silage (17% of DM), alfalfa hay cubes (24%), oat hay (25%), barley (16%) and corn grains (16%) was limit fed at 12.1 kg DM/d. A supplement of canola meal (60%), dried distillers grains (20%), blood meal (10%), feather meal (5%), and corn gluten meal (5%) was not fed (11.7% CP; DM), or fed at 1.1 kg/d (14.4% CP) or at 2.3 kg/d (16.6% CP). Milk, protein and fat yields of 177 multiparous Holstein cows, that were close-up for 1 to 18 d and offered one of the diets (n = 46, 68 and 63 for diets 11.7, 14.4 and 16.6% CP), were measured monthly for the first 150 d of lactation. Following calving, all cows received the same diet containing 17.7% CP and 32% NDF (DM). For statistical analysis of treatment means, cows were allocated to one of four groups based upon time close-up (1-4, 5-8, 9-12 and 13-18 d). Data were analysed by multiple regression with diet CP, its square, days close-up, its square and cube, and their interactions, with progressive removal of non-significant terms. Milk yield was highest for cows offered the 14.4% CP diet for 9-12 d, but declined on all diets fed longer. Protein yields were highest for the 16.6% CP diet fed for up to 12 d, lowest on the 11.7% CP diet fed for 1-4 d, and declined with all diets fed for 13-18 d. Fat yields were highest for the 16.6% CP diet for 1-4 d, but declined on all diets fed

for 13-18 d. The amount of supplement fed close-up, and the length of time that animals were close-up and received it, both influenced milk and milk component production. Overall, the optimal combination was 14.4% CP for 9-12 d.

	Days close-up			
Diet CP %	1-4	5-8	9-12	13-18
Milk, kg/d				
11.7	42.1	40.5	42.1	40.2
14.4	41.2	41.6	42.9	40.0
16.6	42.0	41.9	41.8	39.6
Protein, kg/d				
11.7	1.31	1.28	1.37	1.29
14.4	1.36	1.33	1.36	1.28
16.6	1.36	1.34	1.33	1.28
Fat, kg/d				
11.7	1.43	1.38	1.36	1.28
14.4	1.35	1.32	1.37	1.39
16.6	1.42	1.44	1.35	1.27

Regressions: Milk R^2=99.2; s.e. obs.=0.088; P<0.01. Protein R^2=99.9; s.e. obs.=0.001; P<0.01. Fat R^2=89.9; s.e. obs.=0.017; P=0.03.

Key Words: Close-up, Transition, Protein

1488 Simulation of the effect of N excretion on environmental pollution arising from dairy cows using a dynamic model. E. Kebreab*[1], J. France[1], J.A.N. Mills[1], R. Allison[2], and J. Dijkstra[3], [1]*The University of Reading,* [2]*ADAS Bridgets,* [3]*Wageningen Institute of Animal Sciences*.

Agriculture is one of the major sources of nitrogen (N) pollution. To increase animal products, cattle and especially dairy cows, are offered increasingly higher amounts of N but the efficiency with which N is converted to animal product is low leading to excess N to be excreted in feces and urine. From an environmental perspective, losses of N as urine is less desirable due to its greater tendency to leaching and volatilization as ammonia, the major source of which is urea from urine. A dynamic model was developed to predict the amount and source of N in excreta and to assess the impact of manipulating diet to reduce N pollution by improving N utilization. Five N balance experiments conducted at the Centre for Dairy Research (The University of Reading) were used to parameterize the model and an independent data from experiments conducted at ADAS Bridgets were used to evaluate the model. The model showed that energy and protein content of the diet affect N utilization. The model predicted that cows fed a low degradable starch supplement (corn) gave up to 65% lower N excretion and a higher protein concentration in milk. In addition, feeding cows corn-based diets reduced urinary N excretion by almost 30% compared with barley-based concentrates. As a result, feeding corn-based diets has a potential to reduce ammonia emissions by up to 26%. It was also shown that it is possible to improve N utilization in dairy cows by decreasing protein intake on balanced diets. Reducing the protein content from 190 to 160 g/kg DM substantially reduced N in urine without compromising lactational performance which could potentially reduce ammonia emissions from dairy cows by 21% and nitrous oxide by 15%. Diets with low degradable protein sources also reduced N output in urine with little change in milk production. Inclusion of a lower degradable protein in the diet will potentially contribute to a reduction of ammonia emissions by 20% and nitrous oxide by 8%.

Key Words: N utilization, Environmental pollution, Dairy cows

1489 Should residual plots use Y or Yhat? N.R. St-Pierre*, *The Ohio State University*.

Evaluation and validation of models (empirical or mechanical) require analyses of error structure in the form of residual plots. The appearance of numerous residual plots in recent literature where the residuals are plotted against observed (**Y**) values to assess a model's bias raises the question whether residuals should be plotted against **Y** or against predicted values (**Yhat**). This requires knowing the expected relationship under the assumption of an unbiased model. The objectives of this research were to derive the expected relationship between residuals and **Y** and to determine whether **Y** or **Yhat** should be used for the assessment of bias. Assume a true model of the form $\mathbf{Y} = \mathbf{X}\beta + \epsilon$. This model is estimated by $\mathbf{Y} = \mathbf{Xb} + \mathbf{e}$, and $\mathbf{Yhat} = \mathbf{Xb}$. Least-squares estimates of **b** are unbiased and have minimum variance among all linear estimators of β. The correlation between the residual vector **e** and the vector of observations **Y** is calculated as follows. The numerator of the correlation coefficient is shown to be equal to **e'e**, the residual sum of squares. The denominator of this correlation is equal to the square root of **e'e** multiplied by the total sums of squares. Algebraic simplifications show that the correlation between **e** and **Y** is equal to the square root of (1-R^2). That is, under the assumption of an unbiased model, the residuals are correlated with the observed values and the slope of **e** regressed on **Y** is equal to (1-R^2). Thus, a graph of **e** versus **Y** will show a positive slope between **e** and **Y** unless the model is a perfect predictor (i.e., the R^2 is equal to 1.0). Significant slopes linking **e** to **Y** have been erroneously interpreted as evidence of biased models. The correlation between **e** and **Yhat** is easily shown to be equal to zero. Thus, the slope of **e** regressed on **Yhat** is expected to be zero under the assumption of an unbiased model. These results clearly prove that plots of residuals on observed values are incorrect for assessing a model's bias. Residual plots should be done on **Yhat**.

Key Words: Residual plot, Model assessment, Bias

1490 Short-term mammary blood flow responses to changes in circulating metabolite concentrations. S.R.L. Cieslar*[1], D.R. Trout[1], T.G. Madsen[2], N.G. Purdie[3], and J.P. Cant[1], [1]*University of Guelph, Ontario,* [2]*The Royal Veterinary and Agricultural University, Frederiksberg C, Denmark,* [3]*University of Queensland, St. Lucia, Australia*.

Mammary blood flow (MBF) is locally regulated to match nutrient delivery with demands for milk synthesis. The objective of this experiment was to determine which of the major milk precursors would elicit a MBF response when its concentration was elevated in the arterial supply, and to define the time-course of such a response. Four multiparous cows fed a TMR (CP 14.6%, ADF 27%, NDF 36%, NE_L 1.54 Mcal/kg) twice daily, ad lib or restricted to 70% of ad lib, were infused via an external iliac arterial catheter for 20 min with saline (0.09 g/min), glucose (2.2 g/min), amino acids (complete milk protein profile @2.0 g/min), triglycerides (Intralipid^{TM} @2.0 g/min) or insulin (20 μg/min). Base line blood flow was determined by venous dilution of arterial PAH 10, 5 and 0.5 min prior to infusion. Following the start of infusion, blood samples were collected continuously via peristaltic pump every 30 sec for the first 2 min and every 2 min for the next 28 min. Iliac plasma flow dropped **15 to 31%** within the first minute of infusion of each milk precursor. During glucose and amino acid infusions, plasma flow returned to its baseline value by 20 min, but at the cessation of infusion a reactive hyperemia of **5 to 17%** was observed for approximately 4 min. The triglyceride infusion continued to depress plasma flow **(40 to 53%)** until at least 10 min after the infusion had stopped. Insulin, on the other hand, increased iliac plasma flow by **20 to 101%** during and after infusion. There were no differences in response to infusions between ad-lib and restricted feeding of the diet. The results indicate that the mammary glands modify their blood flow rate within seconds of being exposed to a new concentration of milk precursors.

Key Words: Arterial Infusion, Blood Flow, Mammary

1491 True intestinal digestibility of nitrogen, lysine and methionine estimated with sheep on intragastric infusion and by mobile bag technique. T. Hvelplund*[1], L. Misciattelli[1], F.D.DeB Hovell[2], and M.R. Weisbjerg[1], [1]*Danish Institute of Agricultural Sciences, Denmark,* [2]*University of Aberdeen, UK*.

Lysine (Lys) and Methionine (Met) are often considered to be the most limiting amino acids in diets for high producing dairy cows. Methods for estimation of their availability in rumen undegraded protein is therefore of great importance. The true intestinal digestibility (dig) of nitrogen (N), Lys and Met was investigated for blood meal (100°C, 90h) and soybean meal (SBM) (0.8% HCHO) and for 16 hour rumen incubated residues of: SBM (120°C, 40min), SBM (160°C, 120 min), rapeseed meal, fishmeal, meat and bone meal, and peas (120°C, 40min). Intestinal digestibility was measured in lambs (n=4), fitted with a rumen cannula, an abomasal catheter and a cannula in the terminal ileum, in 4∗4 latin square experiments. Cr-mordanted cellulose powder was used as flow marker. Corrections for endogenous protein secretions was based on values obtained from infusing only cellulose. The lambs received ruminal VFA infusion and abomasal protein infusions at energy intakes of 1.5∗maintenance. Intestinal disappearance (dis) of N, Lys and Met

from mobile bags was determined by inserting polyamide bags (11 μm pores size), into the duodenum and recovering the bags from the faeces, using three cannulated dry cows. Significant correlations between disappearance from mobile bags and in vivo true intestinal digestibility in sheep (n=8) was observed. Blood meal gave very low bag dis, probably due to clogging of the bag pores and results are not included. Lys values for rapeseed meal are missing for analytical reasons. The regression equations were: N-dig = 0.02 + 0.97∗N-dis (P<0.0001, R^2 =0.96, n=7) Lys-dig = -0.19 + 1.18∗Lys-dis (P=0.003, R^2 =0.91, n=6) Met-dig = 0.26 + 0.76∗Met-dis (P<0.0001, R^2 =0.96, n=7) Although based on few observations the results show that the mobile bag technique can predict intestinal digestibility of crude protein and of Lys and Met.

Key Words: Amino acids, Digestibility

1492 Effects of diet on milk allantoin and its relationship with milk production in dairy goats. B.R. Min*[1], R. Puchala[1], and S.P. Hart[1], [1]*E (Kika) de la Garza Institute for Goat Research, Langston, OK, 73050.*

Forty-four Alpine goats (56±11 kg BW) were used to study the relationship between milk production and milk allantoin concentration with pastured dairy goats receiving different levels of concentrate supplementation. Multiparous dairy goats were divided into four groups and were supplemented with 0.66 (groups A and B), 0.33 (group C), and 0 kg concentrate (group D) per kg of milk over 1.5 kg/d. Mixed vegetative forages were rotationally grazed by the goats except for group A (confined and fed alfalfa hay). Milk production was recorded daily and milk samples were collected twice monthly from March to September, 2000 and analyzed for allantoin by HPLC. Milk allatoin (mg/d) was positively correlated (R^2 = 0.63; y = 22.2x-20.7; P < 0.001) with daily milk yield (kg/d) over the four groups. Allantoin output in milk has significantly correlated with milk production range from R^2 = 0.58 (y = 31.2x-45; P < 0.001), 0.62 (y = 23.5x-29; P < 0.001), 0.53 (y = 19.1x-12.6; P < 0.01) and 0.44 (15x-4.9; P < 0.03) in groups A, B, C and D, respectively. Increased excretion of allantoin in milk was correlated (R^2 = 0.44; y = 23.5x+22.7; P < 0.001) to concentrate DM intake suggesting that concentrate provided additional energy for increased microbial protein production with conventional mixed forage diets. Milk allantoin was positively correlated (R^2 = 0.48; y = 0.37x+23.8; P < 0.001) with milk urea (mg/d), but was not correlated with blood plasma urea concentrations. Milk allantoin has the potential to be used as an indicator for microbial protein synthesis in dairy goats.

Key Words: Allantoin, Microbial protein synthesis, Milk and plasma urea

1493 Correction for microbial contamination does not alter estimates of intestinal digestibility of rumen undegraded protein. Y. G. Goh*[1] and G. A. Broderick[2], [1]*Kangwon National University, Chunchon, South Korea,* [2]*U.S. Dairy Forage Research Center, Madison, WI.*

Intestinal digestibility was estimated for the putative RUP from 4 protein sources: solvent soybean meal (SSBM), expeller soybean meal (ESBM), blood meal (BM) and corn gluten meal (CGM). RUP were prepared with in situ and in vitro pre-incubations. Proteins were added to dacron bags; bags were soaked in buffer then inserted into the rumens of two cannulated cows. After 16 h of in situ incubation, bags were removed, washed and dried (24 h, 60 C). Residues were harvested and ground. Rumen contents were collected from the same cannulated cows and filtered through 8 layers cheesecloth; this fluid was mixed with an equal volume of McDougall's buffer and incubated with proteins in vitro at 39 C for 16 h under CO_2. Residues were collected by filtering onto 2 layers of cheesecloth, then washed, dried (24 h, 60 C) and ground. Mixed rumen microbes also were isolated by differential centrifugation during in situ and in vitro incubations. Residues and mixed microbes were analyzed for total N and purines. Intestinal N digestibility was estimated by pepsin-pancreatin digestion. Samples (8 mg N) of residues or mixed microbes were incubated with 7.5 ml 0.01% (w/v) pepsin in 0.01 N HCl for 3 h at 39 C. Then 3.75 ml 0.2 N NaOH and 3.75 ml of 0.053% (w/v) pancreatin (0.1 M phosphate buffer, pH 8) were added and incubated for 6 h at 39 C. Incubations were stopped with trichloroacetic acid (TCA); TCA supernatants (30,000 x g) were analyzed for total soluble N. Apparent and true intestinal digestibility of RUP was computed from the total N recovered as TCA-soluble N, before and after correcting for the digestibility and amount of microbial N based on total purines. Apparent and true pepsin-pancreatin digestibility were not different (P = 0.49). Overall RUP digestibility was different (P < 0.01) for protein, averaging 74.3, 71.9, 81.1 and 49.7% for, respectively, SSBM, ESBM, BM and CGM. RUP digestibility for in vitro residues (mean = 71.6%) was greater (P < 0.01) than for in situ residues (mean = 67.0%); however, there was a significant pre-incubation by protein interaction (P < 0.01).

Key Words: RUP, Pepsin-pancreatin, Intestinal digestibility

1494 In vitro effects of feed oils, ionophores, tannic acid, saponin-containing plant extracts and other bioactive agents on ruminal fermentation and protozoal activity. A. N. Hristov*[1], M. Ivan[2], and T. A. McAllister[2], [1]*Department of Animal and Veterinary Sci., University of Idaho, Moscow, ID 83844-2330,* [2]*Agriculture and Agri-Food Canada, Lethbridge Research Centre, Lethbridge, AB T1J 4B1.*

The effects of various feed oils and bioactive agents on ruminal fermentation and protozoal activity were evaluated in duplicate 4-h in vitro incubations. Rumen inoculum was obtained from two heifers fed 90% rolled barley grain. ^{15}N-casein was included in the incubation media as a N tracer. Linseed, canola, coconut, soybean and palm oils, herring meal and lecithin at 0.5, 1.0 and 2.0%, monensin (MON) at 2.5, 5 and 10 ppm and salinomycin (SAL) at 1.25, 2.5 and 5 ppm, Yucca powder (YP), Quillaja extract (QE), tannic acid (TA) and bentonite (BEN) at 0.1, 0.2 and 0.4%, and Tween 80 (T80) at 0.05, 0.1 and 0.2% were studied (n = 4). Blanks (0% or 0 ppm) were prepared for each additive. Compared to the Blank, ammonia concentration was decreased (P < 0.05) by MON, SAL, YP, TA and BEN. TA, MON, and SAL increased (P < 0.05) and BEN and YP decreased (P < 0.05) the total free amino acids concentration. TA reduced (P < 0.05) total VFA concentration and acetate:propionate ratio. BEN, TA, palm oil, QE, and SAL reduced (P < 0.05) carboxymethylcellulase, xylanase, and amylase activities, TA, lecithin, and QE decreased (P < 0.05) protozoal numbers (84% *Entodinium* spp.), and fish meal, TA, MON, and SAL reduced (P < 0.05) the rate of protozoal engulfment of bacteria. All levels of fish meal and SAL and the higher levels of QE and T80 depressed (P < 0.05) the incorporation of ^{15}N from casein into bacterial cells. Lecithin was the only additive that effectively reduced protozoal numbers (by 27%) without impeding the growth of rumen bacteria.

Key Words: Rumen Protozoa, Oils, Bioactive Agents

1495 In vitro rates of bacterial incorporation of nitrogen fractions from ^{15}N-labeled whole-crop barley ensiled at two dry matter contents. A. N. Hristov*[1] and T. A. McAllister[2], [1]*Department of Animal and Veterinary Sci., University of Idaho, Moscow, ID 83844-2330,* [2]*Agriculture and Agri-Food Canada, Lethbridge Research Centre, Lethbridge, AB T1J 4B1.*

Greenhouse-grown barley (*Hordeum vulgare* L) was ensiled with and without wilting and used to determine the effect of DM content at ensiling on the in vitro rate of bacterial incorporation of N fractions of whole-crop barley silage. The barley was grown in N-free soil and fertilized with ^{15}N-ammonium sulfate. It was harvested at early-dough and ensiled in 3-L laboratory silos, either directly (at 18.2% DM) or after wilting to 30.8% DM. Average ^{15}N-enrichment of barley silage total N was 3.743 at % exc. Freeze-dried silage samples were fractionated into soluble protein N (SP), rapidly degradable protein N (RDP), and slowly degradable protein N (SDP) corresponding to protein fractions B1, B2, and B3 in the Cornell Net Carbohydrate and Protein System. ^{15}N-enrichment of bacterial N was measured at 18 time-points during duplicate 48-h incubations of the three N fractions in buffered ruminal fluid (n = 4). ^{15}N-casein was used as a control N source. As determined by the 95% confidence intervals, fractions RDP and SDP did not differ (P > 0.05) in the rate of incorporation of their N into bacterial protein: 0.0434 and 0.0350 (RDP) and 0.0347 and 0.0436 h^{-1} (SDP), unwilted and wilted silage, respectively. Nitrogen from SP was incorporated into bacterial N at a higher (P < 0.05) rate (0.2601 and 0.1573 h^{-1}, wilted and unwilted silage, respectively) than N from RDP or SDP with no effect (P > 0.05) of silage DM content; the highest (P < 0.05) rate of incorporation was observed with casein-N (0.8360 h^{-1}). In the absence of heating or spoilage, DM content of the barley silage did not alter the utilization of SP, RDP or SDP by rumen bacteria. Levels of

soluble protein in the silage may have a major impact on the rate of N incorporation and subsequent bacterial protein synthesis.

Key Words: Barley Silage, Wilting, Nitrogen Fractions

1496 Effect of barley variety and amylopectin content on bacterial utilization of ammonia-N in vitro.

A. N. Hristov*, J. K. Ropp, and C. W. Hunt, *Department of Animal and Veterinary Sci., University of Idaho, Moscow, ID 83844-2330.*.

The objective of this study was to determine the effect of barley variety and amylopectin content on ammonia utilization by mixed ruminal bacteria in vitro. Three consecutive, 8-hour incubations (n = 3) were conducted with barley and corn grain as substrates (10 g/L incubation media). Ruminal inoculum was obtained from a lactating dairy cow fed a 45% forage:55% concentrate diet. The ammonia-N pool was labeled with ^{15}N-ammonium sulfate. Samples were taken at 0, 2, 4, 6, and 8 h. Three barley varieties (WestBred Gustoe, WBG; Nebula; Baronesse) and their waxy counterparts were tested in this study. The control grain was corn. All barley varieties were tested at three substitution levels: 25, 50, and 75% with the remaining being corn. Ammonia concentration was progressively reduced ($P < 0.001$) with incubation time but was not affected ($P > 0.05$) by grain source or barley amylopectin content. Across sampling points, the waxy variety of WBG effected higher ($P < 0.05$) bacterial incorporation of ammonia N as compared to corn (by 7.6 and 11.9%, 25 and 75% substitution levels, respectively). Across varieties, barley treatments had numerically higher ($P > 0.05$) ^{15}N-enrichment of the bacterial protein compared to corn (by 4.9, 5.0, and 5.4%, 25, 50, and 75% substitution levels, respectively). At the 75% substitution level the waxy barley lines resulted in 8.1% higher ($P < 0.01$) ^{15}N-enrichment of the bacterial protein than the non-waxy parent lines. At the end-point of the incubation, barley treatments tended ($P < 0.1$) to have higher ^{15}N-enrichment of the bacterial protein as compared to corn, and the waxy barley lines had higher ($P < 0.05$) ^{15}N-enrichment than their non-waxy parents (by 3.3, 3.6, and 5.3%, 25, 50, and 75% substitution levels, respectively). These results demonstrated that barley, as compared to corn, tended to stimulate ammonia utilization in the rumen and higher amylopectin content of barley was associated with more intensive ammonia incorporation by ruminal bacteria.

Key Words: Barley, Rumen Ammonia , Bacterial Utilization

1497 Fractionation of ammonia nitrogen isotopes by ruminal bacteria in vitro.

A. N. Hristov*, *Department of Animal and Veterinary Sci., University of Idaho, Moscow, ID 83844-2330.*.

The heavier of the two stable isotopes of nitrogen, ^{15}N, is often used as an ammonia tracer in nutritional studies with ruminants assuming identical metabolism of the ^{14}N/^{15}N isotopes in the rumen. There is evidence that, at natural abundance level, ruminal microorganisms discriminate against ^{15}N from ammonia, and as a result, ruminal microbial protein is depleted in ^{15}N. However, ^{15}N tracer studies are usually conducted at enrichment levels considerably higher than the natural abundance of ^{15}N. It is not clear if at these levels of enrichment the magnitude of isotope fractionation is large enough to effect differences in ^{15}N-ammonia metabolism by ruminal bacteria. To determine this, two incubations with ruminal inoculum obtained from a lactating dairy cow fed a 45% forage:55% concentrate diet were conducted (n = 4). The incubation media contained 5.3 mg/ml carbohydrates (glucose:sucrose:starch, 5:1:2). The ammonia N pool was labeled by addition of ^{15}N-ammonium sulfate enriched at 10.7 (N10) or 20.5 (N20) at % exc. Incubations were carried out for 6 h and samples were taken at 0, 1, 2, 4, and 6 h. Bacterial pellets were isolated and analyzed for ^{15}N-enrichment. We hypothesized that if there were a significant discrimination against ^{15}N by ruminal bacteria, the ^{15}N-enrichment of the bacterial N from N20 would be less than 91.6% higher than that from N10. Total VFA concentration was increasing ($P < 0.001$) with incubation time and was not different ($P > 0.05$) between the two treatments. Individual VFA and acetate to propionate ratio were not influenced ($P > 0.05$) by treatment. The ^{15}N-enrichment of bacterial N was increasing ($P < 0.001$) with incubation time and did not differ ($P = 0.266$) between the two treatments (1.659 and 1.704 at % exc., N10 and N20, respectively). Source of N by incubation or incubation time interactions were not significant ($P > 0.05$). The results from this study indicate that, at ^{15}N-enrichment levels considerably higher than natural abundance, isotope fractionation by mixed ruminal bacteria is insignificant.

Key Words: Nitrogen Isotopes , Fractionation , Ruminal Bacteria

1498 Effect of Jackbean urease immunization on nitrogen recycling in mature sheep.

J.C. Marini*, K.W. Simpson, A. Gerold, and M.E. Van Amburgh, *Cornell University.*

Previous experiments have shown that immunization with Jackbean urease (JBU) reduces urea hydrolysis in the gastrointestinal tract of numerous species. A consequence for farm animals is a lower energy requirement to detoxify ammonia and more energy was geared towards production. We sought to study the effects of JBU immunization on nitrogen (N)-metabolism in mature non-gravid, dry ewes. Sheep were immunized subcutaneously either with Freund's adjuvant (C, n=8), saline and 5,000 IU JBU (S, n=8) or Freund's adjuvant and 5,000 IU JBU (F, n=8) on days 1 and 22. Nitrogen balance and urea kinetic studies were performed on d32-35 and 60-63. A pelleted diet containing 16.4 %CP and 2.6 Mcal/kg ME was fed at maintenance level of intake. Immunization with JBU resulted in an increase ($P < 0.05$) in circulating anti-JBU IgM and IgG. Group F produced a greater IgG response than group S, but a similar IgM response. Serum from groups S and F inhibited JBU activity in vitro ($P < 0.01$). The results for N balance and urea kinetics were similar within a group ($P>0.05$) at days 32 and 60 and were pooled for further analysis. There was no effect of treatment on N balance or N excretion ($P > 0.05$). Urea kinetics, measured by continuous infusion of [^{15}N^{15}N]urea did not differ among treatments ($P > 0.05$). Urea production (UER), urinary urea nitrogen (UUN), urea recycled to the gastrointestinal tract (GER) and back to the ornithine cycle (ROC) averaged 25.3, 12.8, 12.5 and 5.8 g N/d, respectively. The results indicate that immunization against JBU produce a strong, and effective humoral immune response in sheep. However, immunization against JBU did not alter N-metabolism in mature, non-gravid sheep.

Item	Control	+Saline	+Freund's	sem	P<
Number of animals	8	8	8		
Body weight, kg	71.6	66.7	69.3	2.43	0.36
DMI, kg/d	1.2	1.1	1.1	0.07	0.24
N intake, g/d	31.1	29.2	29.7	0.67	0.14
Urine N, g/d	15.1	13.5	15.2	0.63	0.11
Fecal N, g/d	8.5	8.0	8.8	0.65	0.66
N balance, g/d	7.4	7.7	5.6	1.04	0.34
UER, g/d	26.4	24.1	25.5	1.30	0.44
UUN, g/d	13.2	11.8	13.3	0.55	0.11
GER, g/d	13.2	12.3	12.1	1.18	0.77
ROC, g/d	6.1	5.4	5.8	0.59	0.68
PUN, mg/dl	16.4	18.2	15.9	0.91	0.20

Key Words: Urea kinetics, N15, Urease

1499 Incorporation of nitrogen from ammonia, amino acids, peptides, or protein by mixed ruminal bacteria in vivo.

A. N. Hristov*[1], J. K. Ropp[1], R. J. Wallace[2], and T. A. McAllister[3], [1]*Department of Animal and Veterinary Sci., University of Idaho, Moscow, ID 83844-2330,* [2]*Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB,* [3]*Agriculture and Agri-Food Canada, Lethbridge Research Centre, Lethbridge, AB T1J 4B1.*

To compare bacterial uptake of different forms of N in the rumen, four sources of ^{15}N were given as intraruminal pulse doses to dairy cows in a 4 × 4 Latin square experiment. Three ^{15}N sources were derived from ^{15}N-casein: protein (PRO, unprocessed casein), amino acids (AA, acid hydrolysis) and peptides (PEP, enzymatic hydrolysis); ^{15}N-labeled ammonium sulfate (NH3) was used as a control. The cows (DIM 205 17.9) fed a 43% forage:57% concentrate diet were given equal amounts of ^{15}N from the four N sources. Representative samples (12) of the fluid- and loosely associated to the feed particles ruminal bacteria were collected for a period of 24 h and analyzed for ^{15}N-enrichment. The areas under the ^{15}N-enrichment curves (AUC) of bacterial N were not different ($P > 0.05$) between treatments. The shortest time ($P < 0.05$) to peak ^{15}N-enrichment of the bacterial protein was associated with the PEP treatment, 1.65 h, followed by the PR and NH3 treatments (2.0 and 2.5 h, respectively). The AA treatment had the longest ($P < 0.05$) time to peak enrichment (3.1 h). The rate of incorporation of the tracer was the highest ($P < .05$ vs AA and NH3 and $P < 0.1$ vs PR) with the PEP treatment (3.58 h^{-1}) and was similar among the other treatments,

varying from 2.25 (NH3) to 2.71 (PR) h^{-1}. The peak ^{15}N concentration in the bacterial protein was higher ($P < 0.05$) with the NH3 treatment (0.0151 at % exc.) as compared to the AA and PEP treatments (0.0099 and 0.0125 at % exc., respectively). No differences ($P > 0.05$) in the rate of tracer disappearance (average of 0.099 h^{-1}) or tracer half-life in the bacterial N pool were detected among treatments. Under the conditions of this trial, ruminal bacteria showed no sizable differences in the extent of incorporation of N from NH3, AA, PEP, or PR but incorporated N from PEP at a higher rate.

Key Words: Dairy Cows, Nitrogen Source , Ruminal Bacteria

1500 Influence of methionine and/or lysine deficiencies, formulated at three different protein levels, on protein nitrogen metabolism when fed to lactating dairy cows. D. C. Weakley*[1], M. D. Hanigan[1], L. F. Reutzel[1], J. A. Besancenez[1], K. B. Cunningham[1], H. C. Puch[1], and B. K. Sloan[2], [1]*Purina Mills, Inc., St. Louis, MO,* [2]*Aventis Animal Nutrition, Alpharetta, GA.*

Twelve lactating dairy cows (575 kg, 77 DIM) were assigned by parity to 6 treatments in two periods of feeding in a randomized block design, preceded by a common covariate period. Three of the treatments comprised diets formulated to be deficient in methionine (met) and/or lysine (lys), according to CPM Dairy ver. 1.0, at either 15.5, 17.5 or 19.5% dietary CP. To confirm that these diets were indeed deficient, the remaining three treatments comprised the previous diets supplemented with Smartamine M (M) and/or ML (ML) at levels necessary to establish calculated levels of 2.20 and 6.82% of metabolisable protein, as met and lys, respectively. Diets contained (DM basis) 47% corn silage and 13% alfalfa hay. Cows were allowed to adjust to their diets for a period of two weeks, followed by a 5 day total collection of milk, urine and feces. Neither DM intake (18.0±2.38 kg), milk production (32.5±4.32 kg), milk fat percent (3.36±0.281) or yield (1.070.130 kg) or milk lactose yield (1.54±0.204 kg) were significantly influenced by treatment. However, there was a significant main effect by amino acid supplementation on milk total protein (2.98 vs. 3.10%; P≤0.03), milk true protein (2.56 vs. 2.69%; P≤0.06), and milk lactose (4.82 vs. 4.76%; P≤0.09) for the deficient and supplemented diets, respectively. A greater loss of urinary N with the supplemented 15.5% CP diet, coupled with a loss of retained N but no loss in milk N, suggested a mobilization of amino acid N from body stores. Within the higher protein diets, M and/or ML resulted in a depression in urinary N loss and an improvement in N retention. Met and/or lys supplementation of deficient diets improved milk N across all protein levels.

Protein, % M and/ or ML	15.5 none	15.5 M	17.5 none	17.5 M + ML	19.5 none	19.5 ML	Effect (*P*)
N intake, g/d	451^{a}	479^{ab}	474^{ab}	493^{abc}	589^{c}	553^{bc}	A (.01)
N in feces, g/d	148	156	148	152	156	141	
N in urine, % of intake	27.9^{a}	36.5^{abc}	39.1^{bc}	32.3^{ab}	44.7^{c}	38.5^{abc}	A(.06) C(.10)
N in milk, % of intake	30.6^{ab}	33.0^{b}	29.6^{ab}	31.0^{ab}	27.4^{a}	30.1^{ab}	B (.11)
N retained, % of intake	9.4^{b}	-2.1^{a}	0.04^{ab}	6.1^{ab}	1.5^{ab}	6.1^{ab}	C (.03)

Effects: A=Protein, B=M/ML, C=Protein x M/ML. Means in the same row not followed by a common letter differ (LSD, P<0.05)

Key Words: Lactating cows, methionine, lysine

1501 Effect of level of cracked Pima cottonseed in the diet of lactating dairy cows on milk yield and plasma gossypol. J. Prieto*[1], E. DePeters[1], P. Robinson[1], J. Santos[1], J. Pareas[1], S. Taylor[1], M. Calhoun[2], B. Baldwin[2], and S. Kuhlmann[2], [1]*University of California, Davis, CA,* [2]*Texas Agricultural Experiment Station, San Angelo, TX.*

Lactating Holstein cows were fed one of four diets containing cracked Pima cottonseed to determine its effects on milk yield and plasma gossypol concentration. All diets contained 43% concentrate, 12% cottonseed, and 45% chopped alfalfa hay. The proportion of whole Upland cottonseed to cracked Pima cottonseed varied from 100:0, 66:34, 34:66, and 0:100 (U:P). Primiparous cows (4) were fed the diets in a 4x4 Latin square design. Multiparous cows (3) were fed the diets in a Youden square with five periods. Periods were 35d. Milk yield (kg/d), milk fat (%), and DM intake (kg/d) were not affected by the inclusion of cracked Pima cottonseed into the diet of either primiparous or multiparous cows. For primiparous cows, milk yield, milk fat, and DM intake were 36.9, 4.2, 28.3; 39.0, 3.9, 28.2; 40.3, 3.7, 29.1; and 38.2, 4.1, 27.8 for the 100:0, 66:34, 66:34, and 0:100 U:P diets, respectively. Similar data for multiparous cows were 41.9, 3.7, 33.4; 41.5, 3.7, 30.7; 41.5, 3.7, 31.9; and 38.4, 3.9, 29.3, respectively. Upland and cracked Pima cottonseed contained (% of meats DM) 1.3% and 1.7% free gossypol and 0.52% and 0.85% minus (-) isomer, respectively. Gossypol is a natural defense compound in the plant that protects it against pests and diseases, but it can have anti-nutritional quality affects when consumed by animals. Total plasma gossypol concentration significantly increased linearly with increasing proportion of cracked Pima in the diet for primiparous (4.1, 5.8, 6.9, and 8.7 ug/ml; P<0.01) and multiparous (4.1, 7.1, 9.5, and 11.7 ug/ml; P<0.01) cows. Cracked Pima cottonseed, fed at levels up to 4 kg/d, had no effect on production performance even though plasma gossypol concentrations increased sharply.

Key Words: Cottonseed, Gossypol, Cows

1502 Effects of intake and lactation on absorption and metabolism of leucine and phenylalanine by splanchnic tissues of dairy cows. C. K. Reynolds*[1], B. J. Bequette[2], J. S. Caton[3], D. J. Humphries[1], P. C. Aikman[1], B. Lupoli[1], and J. D. Sutton[1], [1]*University of Reading, Reading, UK,* [2]*Rowett Research Institute, Aberdeen, UK,* [3]*North Dakota State University, Fargo, USA.*

The objective was to measure effects of intake and lactation on endogenous (END) duodenal (DUO) and ileal Leu flow and absorption and kinetic transfer of Leu and Phe across portal-drained viscera (PDV) and liver of 3 multiparous, catheterized, cannulated Holstein x Friesian cows (648 kg BW). A TMR was fed at 2 intakes (Lo and Hi) during 2 stages, late lactation (LAC) and after dry-off, giving 4 levels of DMI (Table). Measurements (mmol/h) of DUO and ileal flow and PDV blood flow and Leu and Phe sequestration (use) and transfer were made during week 4 of 5 wk intake periods using ^{13}C-Phe and ^{13}C-Leu (jugular vein) and ^{2}H-Phe and ^{2}H-Leu (DUO) infusions. ^{13}C-Leu infusion continued for measurement of END Leu flow on d 6 and 7 of infusion. Milk yield was 15.4 ± 2.9 kg/d. Body flux of Leu (51.5, 63.7, 73.6 and 95.1) and Phe (20.5, 25.5, 28.7 and 33.8) increased (P < .02) with DMI. Fractional END Leu flow in the DUO (8 ± 1 %) or ileum (15 ± 2 %) was not affected. DMI and LAC increased DUO flow and thus absorption of Leu and Phe. Within stage, absorptive use of Leu and Phe was greater for Hi than Lo. PDV use of arterial Leu, but not Phe, was increased by DMI. Absorption and arterial use of Leu by PDV were greater than for Phe, while rates of absorptive use of Leu and Phe were more similar. We conclude that intake relative to requirement alters PDV sequestration and absorptive recovery of Phe and Leu.

	Dry-Lo	Dry-Hi	LAC-Lo	LAC-Hi	SEM	P-DMI	P-Stage
DMI, kg/d	8.0	12.0	14.9	19.4	.4		
DUO Leu flow	35.4	46.6	82.0	106.9	3.3	.01	.03
Ileal Leu flow	9.8	15.2	21.1	26.2	1.1	.01	.31
Absorptive Leu use	-.6	6.4	-6.8	12.9	5.4	.01	.02
PDV arterial Leu use	15.2	21.8	28.9	31.6	6.1	.07	.56
DUO Phe flow	16.8	22.7	39.1	51.0	1.4	.01	.01
Ileal Phe flow	4.5	7.0	10.0	12.7	.7	.01	.26
Absorptive Phe use	2.5	6.3	-1.9	7.0	2.0	.03	.02
PDV arterial Phe use	3.7	3.7	8.4	10.5	2.2	.44	.26

Key Words: Absorption, Endogenous , Amino acids

1503 Peptide amino acid net flux in ruminal vein of dairy cow. D. Remond*[1], C. L. Girard[2], and B. Chauveau[1], [1]*INRA, Clermont Fd-Theix/France,* [2]*AAC , Lennoxville/Canada.*

The objective of the study was to determine whether luminal peptides can cross the ruminal epithelium in vivo. Four lactating dairy cows were fitted with a ruminal cannula, catheters in the right ruminal vein and in a mesenteric artery, and a blood flow probe around the right ruminal artery. Each cow received a ruminal injection of a solution containing 320 g of peptone from casein and 24 g of glycylglycine. Blood samples were collected at −20, 0, 20, 40, and 60 min (0 was just before the injection). Plasma was deproteinized with sulfosalicylic acid. Supernate was filtered through a 3,000 Mr cut-off filter. Part of the filtrate was hydrolyzed in 6 N HCl at 110°C for 24h. The difference in AA concentration before and after hydrolysis was attributed to peptide (PAA). Control values were obtained averaging sampling times before the injection. The effect of the injection was tested using ANOVA for repeated measurements. Ruminal blood flow (68 ± 7 L/h) and arterial free amino acid (FAA) concentration (1.89 ± .07 mM) were not significantly affected by the injection. Total FAA net flux in the ruminal vein (-10.4 ± .9 mmole/h) was not affected by the injection. Total arterial PAA concentration linearly increased ($P < .05$) from .15 ± .01 before the injection to .21 ± .02 mM 60 min latter. Arterial peptide Glu, Gly, and Ala increased linearly ($P < .10$), whereas peptide Pro responded in a cubic manner ($P < .05$). During the control period, total PAA net flux in the ruminal vein was not different from zero. It was not significantly affected by the injection. However, peptide Pro net flux sharply increased ($P < .01$) after the injection (from 0 to 11 μmole/min 20 min after the injection), and responded in a quadratic manner with time. The increase in arterial PAA concentration suggested absorption of peptides from the digestive tract. The rumen contribution to this absorption appeared limited, but when high peptide concentrations are generated in the rumen, small peptides containing Pro may cross the ruminal wall.

Key Words: Rumen, Peptide, Absorption

1504 Effects of abomasal casein or essential amino acid infusions on splanchnic leucine and phenylalanine metabolism in lactating dairy cows. J. S. Caton*[1], C. K. Reynolds[2], B. J. Bequette[3], B. Lupoli[1], P. C. Aikman[1], and D. J. Humphries[1], [1]*North Dakota State University, Fargo, USA,* [2]*University of Reading, Reading, UK,* [3]*Rowett Research Institute, Aberdeen, UK.*

The objective was to measure the effects of abomasal casein (CAA) or essential amino acid (EAA) infusion on Leu and Phe absorption and kinetics (mmol/h) in 3 multiparous, late lactation (8.9 kg milk/d), catheterized, cannulated Holstein x Friesian cows (673 kg BW). A TMR of 30 % dried lucerne, 20 % grass silage, and 50 % concentrates (DM basis) was fed hourly at 12.6 kg DM/d. Small intestinal absorption was measured on d 5 and 7 of 7-d abomasal infusions (18 L/d) of water followed by 7-d infusions of CAA or EAA equal to 800 g casein protein/d in a single-reversal design with a 3 wk interval. Portal-drained viscera (PDV) and liver (LIV) kinetic flux was measured using infused ^{13}C-Phe and ^{13}C-Leu (jugular) and ^{2}H-Phe and ^{2}H-Leu (duodenum) on d-6 of abomasal infusions. Both infusions increased ($P < .01$) body flux and true absorption of Leu (62.5 vs. 87.8 and 37.6 vs 57.9, respectively) and Phe (24.9 vs 30.1 and 18.4 vs 26.3, respectively). Infusion of EAA increased ($P < .09$) sequestration (use) of Leu (-4.9 vs 10.9) and Phe (3.5 vs 11.5) during absorption, but CAA had no affect (0.4 vs 0.2 and 6.9 vs 8.3, respectively). Use of arterial Leu by PDV was increased ($P < .06$) by infusion of EAA (21.0 vs 36.2), but not CAA (26.0 vs 28.2). Of total (absorptive + arterial) PDV use, 6 % of Leu and 66 % of Phe was from absorptive supply. Both infusions increased ($P < .03$) total PDV release of Leu (36.8 vs 56.9) and Phe (12.9 vs 22.6). Total LIV use (17.9) and release (23.0) of Leu was not affected, but infusions increased ($P < .01$) net LIV removal of Phe (8.4 vs 15.8) due to changes in total use (20.8 vs 26.2) and release (12.4 vs 10.4). Data suggest either the form (free vs protein) or composition (EAA vs CAA) of abomasal supply affect PDV utilization of absorbed Leu and Phe.

Key Words: Absorption, Gut metabolism, Amino acids

1505 Effect of type of cottonseed and gossypol intake on plasma gossypol and performance of lactating Holstein dairy cows. J.E.P. Santos*[1], M. Villasenor[1], D. Ringen[1], E.J. DePeters[1], P.H. Robinson[1], M.C. Calhoun[2], B. Baldwin[2], and J.P. Reynolds[1], [1]*University of California - Davis,* [2]*Texas A&M University.*

Objectives were to evaluate the effects of type of cottonseed on lactational performance and plasma total gossypol (TG) concentration of cows. Holstein dairy cows, 856, on 3 dairy farms in central California were assigned at calving to one of two treatment diets (428/treatment) based on lactation number, calving date and previous lactation 305-d mature equivalent milk yield in a randomized complete block design. Cows were assigned 3 to 20 d after calving and remained on diets for 170 d. Cottonseed represented 10% of the diet DM, and treatments consisted of replacing whole Upland cottonseed (WUP) with a blend of WUP and cracked Pima (BUPCP) cottonseed (33:67). Whole Upland and cracked Pima cottonseed contained (% of meats DM) 1.33% and 1.66% free gossypol and 0.60% and 0.89% minus (-) isomer, respectively. Only the 809 cows that remained on the study for more than 60 days in milk (DIM) were included in the analysis. Blood samples were collected from all cows at 60 and 90 DIM for determination of plasma gossypol, but results for statistical analysis were available for 430 cows. Group DM intake did not differ between BUPCP and WUP (24.3 vs 24.4). Estimated free gossypol intake was 26.2 and 17.9 g/d for BUPCP and WUP, respectively. Plasma TG (ug/ml) at 60 and 90 DIM was higher for cows fed BUPCP than WUP (6.80 vs 2.89, and 8.82 vs 3.37; $P < 0.001$). Proportion of plasma TG as (-) isomer was greater at 60 and 90 DIM for cows fed BUPCP vs those fed WUP ($P < 0.001$). Cows fed BUPCP had a greater increase in plasma TG from 60 to 90 DIM than those fed WUP (+2.47 vs +0.49 ug/ml; $P < 0.001$). In BUCPC and WUP fed cows, yields of milk (39.8 vs 39.6 kg/d), 3.5% fat corrected milk (40.0 vs 39.6 kg/d), and milk fat (1.40 vs 1.39 kg/d), as well as concentrations of milk fat (3.54% vs 3.51%) did not differ. However, true protein content (2.94% vs 2.90%; $P< 0.003$) increased and milk true protein yield (1.17 vs 1.15 kg/d; $P = 0.11$) tended to increase for cows fed BUPCP vs those fed WUP. Replacement of whole Upland cottonseed with a blend of whole Upland and cracked Pima cottonseed increased plasma TG, as well as the proportion of plasma TG as (-) isomer. Yields of milk and milk components were not affected, but milk protein content was improved.

Key Words: Cottonseed, Plasma gossypol, Dairy cows

1506 Use of an inhibitor *in vitro* method to determine protein degradability coefficients in the NRC (2001) protein evaluation system. J.R. Newbold*[1], B. De Wannemaeker[1], and P. Gerardy[1], [1]*Provimi Research and Technology Centre.*

NRC(2001, Nutrient Requirements of Dairy Cattle, Seventh Revised Edition) recommends the *in situ* (IS) technique to measure rumen degradable protein (RDP, % of CP), calculated from immediately-degraded N (A), slowly-degraded N (B), undegradable N (C) and rate of degradation of B (Kd). Our objective was to predict these values using an inhibitor *in vitro* (IIV) method. Foods were: solvent-extracted soybean (n=2), sunflower (n=2) and canola meals, corn gluten feed, corn gluten meal, palm kernel expeller meal (PK), potato protein (PP) and a commercial bypass protein ('Aminolac', Provimi BV, Rotterdam). Soy, canola and sunflower meals were solvent extracted. Foods in polyester bags were incubated in rumens of two non-lactating cows for 0, 3, 6, 24, 48 and 210h. In IIV tests, intact foods and a fraction soluble in phosphate buffer were incubated with rumen fluid and inhibitors of deamination and microbial growth for 4h. TCA-soluble N at 0h gave a measure of fraction A. Calculations assumed passage rate=6%/h. Fraction A was higher (P=0.025) for IS than IIV (19.6% and 12.3%, se=2.76). This was marked for PP (A IS=31.2%, A IIV=2.2%), indicating loss from bags of N in small particles. Excluding PP data, RDP IS was not different from RDP IIV (means=48.8% and 51.5%%, se=3.48, P>0.05). RDP IS = 9.2 + 0.77(RDP IIV), r^2=0.75. Fit of the model describing IS data was poorer for PK than other foods. After exclusion of PK, RDP IS = 13.3 + 0.74(RDP IIV), r^2=0.87. RDP IIV of soluble N <100% for soybean, sunflower and canola meals, suggesting that the IS method overestimates RDP for these foods. The IIV method can be used to measure RDP as defined by NRC(2001). The IIV method has advantages where particle size is small or where part of fraction B is soluble in rumen fluid.

Key Words: Protein, Degradability, Bypass

1507 Intake and production by Holstein cows fed different amounts and sources of supplemental protein prepartum and postpartum. J.P. Underwood*, J.K. Drackley, and J.H. Clark, *University of Illinois, Urbana, IL* .

Improving metabolizable protein supply in late-gestation cows might benefit health and subsequent milk production. Sixty pregnant nonlactating cows were blocked by expected parturition date and assigned to one of three prepartum diets: 12% CP, soybean meal (SBM) supplement (LSB); 15% CP, SBM supplement (HSB); and 15%CP, animal-marine protein (AMP) supplement. Diets were formulated to supply an estimated 927, 981, and 1100 g/d of metabolizable protein, respectively. Cows were fed diets from 21 d prepartum to parturition. After parturition, cows were assigned to one of two diets containing 18% CP (SBM supplement or AMP supplement); thus, treatments were in a 3 x 2 factorial arrangement. Dry matter intake (DMI) and milk production were recorded for 63 d postpartum. Prepartum DMI did not differ among LSB,HSB, and AMP (10.9, 11.8, and 11.7 kg/d, respectively). Postpartum DMI was similar between groups fed SBM or AMP (19.9 and 18.9 kg/d). Cows fed AMP postpartum produced 36.4 kg/d of milk vs. 34.4 kg/d for cows fed SBM (P=0.21). Milk fat and protein percentages were not affected by prepartum or postpartum diets. Cows fed AMP tended (P=0.10) to produce more 4%fat-corrected milk (FCM) than SBM supplemented cows (33.8 kg/d vs. 31.8 kg/d). An interaction of protein source (at 15% CP)and postpartum diet was detected for FCM (P<0.05); means were 30.8, 34.7, 33.8, and 32.0 kg/d for cows fed HSB prepartum, SBM postpartum; HSB prepartum, AMP postpartum; AMP prepartum, SBM postpartum; and AMP prepartum, AMP postpartum, respectively. Efficiency (FCM/DMI) was greater (P<0.05) for cows fed AMP postpartum (1.88 vs. 1.70). An interaction of postpartum diet by week (P<0.05) indicated that cows fed AMP postpartum lost more body weight than cows fed SBM. Body condition score (BCS) was not affected by diets. Increasing the amount or source of protein fed to nonlactating cows during the last 21 d prepartum did not affect DMI, milk production, or BCS.

Key Words: Protein, Dry Period, Transition Cow

1508 Effect of barley and rapeseed meal supplementation on amino acid profile of microbial fractions and postruminal amino acid supply in lactating dairy cows fed grass-red clover silage. M. Korhonen*, S. Ahvenjrvi, A. Vanhatalo, and P. Huhtanen, *MTT, Agrifood Research Finland*.

Four ruminally cannulated dairy cows were used to study AA composition of omasal canal digesta, bacterial, and protozoal fractions as well as omasal canal AA flow as affected by diet. Cows were given grass-red clover silage alone (S), supplemented with 6 kg of barley (B), with 2.1 kg of rapeseed meal (R), or with 6 kg of barley and 2.1 kg of rapeseed meal (BR) according to 4x4 Latin Square design with 2x2 factorial arrangement and 21 d periods. During last week of each period silage intake was restricted to 95% of ad libitum intake. Omasal canal DM flow was measured using triple marker (Co, Yb, INDF) method. Amino acid flow entering the omasal canal was calculated based on DM flow and AA composition of reconstituted digesta. Liquid- (LAB) and particle- (PAB) associated bacteria were isolated from reticular digesta and protozoa from omasal canal digesta. Microbial protein flow was determined by using 15N as a microbial marker. Omasal canal AA flows were 1452, 1756, 1754, and 2008 g/d (SEM 92.7) on diets S, B, R, and BR, respectively. On diet R, increase in AA flow was of nonmicrobial, whereas on diet B it was mainly of microbial origin. This changed slightly AA profile of digesta, and resulted in a better marginal utilization of AA for milk protein synthesis (55 vs 44%) on diet R than on diet B. Flows of LAB- and protozoa-NAN were 100, 159, 123, and 173 g/d (SEM 12.4) and 9, 25, 9, and 20 g/d (SEM 4.4) on diets S, B, R, and BR, respectively. Diet had only minor effect on AA composition of microbial fractions, but differences were observed between LAB and PAB for 10 out of 19 AA studied, and also between bacteria and protozoa for 15 out of 17 AA studied. Differences in AA composition and flows of various microbial fractions had, however, only small effect on AA profile of microbial protein. Consequently, AA composition of postruminal digesta seemed to be affected mainly by proportions of microbial and nonmicrobial proteins.

Key Words: Amino Acids, Bacteria, Protozoa

1509 Effect of type of dietary protein on mRNA expression for urea cycle enzymes in lactating dairy cows. J.R. Townsend*, S.M. Crowder, J.C. Velez, and S.S. Donkin, *Purdue University, West Lafayette, IN* .

Liver metabolism adapts to changes in dietary protein level and amino acid profiles. The objective of this study was to determine the response in liver of lactating dairy cows to changes in the type of protein in the diet. Thirty-six Holstein dairy cows were fed a diet containing 18.5% crude protein (CP) and 1.76 Mcal/kg NEL and either 12.6, 11.7, or 10.9% rumen degradable protein (RDP). The quantity of RDP and rumen undegradable protein (RUP) in the diet was manipulated by altering the amounts of soybean meal and heat-treated soybean meal used in the ration. Diets were fed for 11 weeks, milk production and feed intake were measured daily, and milk composition was determined weekly. Liver biopsies and blood samples were obtained from a subset of 16 cows during weeks 2, 5, 8, and 11. Argininosuccinate synthetase (AS) and carbamoyl phosphate synthetase I (CPS-I) mRNA were measured in liver biopsy samples and blood was used for plasma urea nitrogen (PUN) analysis. Milk production was 34.8, 38.2, 37.1 1.8 kg/day, dry matter intake was 23.8, 25.8, 25.4 .82kg/day for 12.6, 11.7, and 10.9% RDP respectively. Protein intake exceeded requirements for CP, RDP, and RUP. Feeding 10.9% RDP decreased (P<.05) MUN and PUN compared to the other two diets. A time on experiment effect (P<.05) was observed for AS and CPS-I mRNA and PUN. There was a tendency (P<.10) for correlation between PUN and dry matter intake(r=.22), crude protein (CPI) (r=.22), and RDP (r=.32) intake. Abundance of CPS-I and AS mRNA were correlated (P<.05, r=.76) as were MUN and PUN (r=.56), and MUN and DMI (r=.50). The intake of RDP was correlated (P<0.05) with MUN(r=.62), and CPI was correlated (P<.05) with MUN (r=.50). The data indicate that when dietary protein is overfed, mRNA for urea cycle enzymes are closely related to other measures of protein utilization such as MUN and PUN. Overfeeding protein in this experiment may have masked any effects of RUP or RDP on mRNA for urea cycle enzymes.

Key Words: Liver, Gene expression, Protein

1510 Responses of dairy cows fed grass silage-cereal diet to increased supply of histidine provided either by abomasal infusion of histidine or dietary inclusion of rape seed meal. A. Vanhatalo*, P. Huhtanen, M. Korhonen, and T. Varvikko, *MTT Agrifood Research Finland, Jokioinen, Finland*.

A 4x4 Latin square was conducted with four cows to investigate responses to supplementation of grass silage-cereal diet with His supplied either with abomasal infusion of 6.5 g/d His (H), 1.5 kg/d of rape seed meal (RSM) and 3.25 g/d of His (HR) or 3 kg/d of RSM (R). Each treatment was designed to provide an additional His supply of 6.5 g/d. The design was aimed to evaluate how much of the response to RSM could be explained by the response to His alone. The control diet (C) consisted of grass silage (18.9% CP) ad libitum and 9 kg/d of a cereal based concentrate (12.6% CP). Glucose was infused abomasally at a rate of 250 g/d on each treatment. Treatments had no significant effects on silage or total DMI, rumen fermentation, microbial protein production, or OM digestibility of diets (P>0.05). Milk yields for the treatments C, H, HR and R were 32.5, 31.8, 33.4, and 33.2 kg/d (SEM 0.23), protein yields 1080, 1068, 1126 and 1132 g/d (SEM 16.3), and lactose yields 1643, 1611, 1692, and 1680 g/d (SEM 11.2), respectively. Increases of these parameters were linear or quadratic (P≤0.03) with increasing amount of RSM. Treatments did not affect milk composition except for milk urea, which increased with increasing amount of RSM (P<0.05). Arterial plasma concentration of urea, and essential and branched chain amino acids (AA) increased linearly (P<0.03) with increasing amount of RSM. Arterial glucose was not affected by the treatments, but concentrations of BHBA and insulin increased (P<0.05), and NEFA decreased due to His supplementation. The production potential of cows was realized exceptionally well with the basal diet because production responses to RSM were less than half of those usually derived with RSM on grass silage diets. Also, opposite to earlier findings, arterial concentration of His on the basal diet (>42 μM) was higher than expected suggesting that some other nutrient or AA but His limited milk production in the present study.

Key Words: Grass silage, Histidine, Dairy cow

1511 A comparison of different methods to measure milk urea nitrogen. R.A. Kohn*, K.R. French, and E. Russek-Cohen, *University of Maryland, College Park*.

Milk urea nitrogen (MUN) is measured routinely on dairy farms to evaluate protein nutrition. The objective of this study was to compare methods that are currently used by Dairy Herd Improvement Association (DHIA) labs for analysis of MUN. Two replicate samples from each bulk tank on 10 different dairy farms were sent to 14 DHIA labs throughout the U.S. for MUN analysis. The mean MUN of all samples was 14.0 mg/dl. The lowest farm averaged 6.8 mg/dl and the highest averaged 19.1 mg/dl across labs. For the Foss 6000, Bently Chemspec, Skalar and CL 10 methods, greater than 98% of the variation in measured MUN was attributed to farm to farm differences. This result was desired because it indicates that labs can consistently quantify the variation that occurs among farms. For the Foss 4000 system, less than 60% of the variation in MUN was attributed to farm to farm differences. The remaining variation was attributed to lab differences (33.8%), lab by farm interaction (5.7%), and random error (1%). The results for each method of MUN analysis were compared to reference values determined by CL 10. The difference from the CL 10 method was represented as the root mean square prediction error (RMSPE) where a smaller number indicates greater accuracy. The RMSPE for the Foss 4000, Foss 6000, Bently, and Skalar methods were: 2.48, .63, .65 and .86 mg/dl respectively. The error of the Foss 4000 relative to the CL 10 reference was further partitioned to mean bias (0.7%, $P>.1$), slope bias (51.7%, $P<.01$), lab bias (13.7%, $P<.01$), lab by slope interaction (19.6%, $P<.01$) and residual error (14.3%). The Foss 4000 system underestimated the higher values while overestimating the lower values, and it was not consistent across labs. Thus, this method should not be used to estimate urinary N or protein adequacy. For the other methods, differences from the CL 10 were slight and would not have affected predictions of urinary N or expected MUN.

Key Words: Milk urea nitrogen, Analysis methods

1512 A role for rumen degraded protein in regulating intake rate of digested fiber. W. C. Ellis*[1], J.H. Matis[1], Dennis Herd[1], H. Lippke[1], F.M. Rouquette[1], D. P. Poppi[2], and R. J. Wallace[3], [1]*Texas A & M University,* [2]*University of Queensland,* [3]*Rowett Research Institute*.

It was hypothesized that insufficient flux of rumen degraded CP, RDP, successively limits growth rate of NDF digesting bacteria, rate of digestion of potentially digestible NDF (PDF) and ruminal turnover and intake rate of digested PDF (DF). Intake rate of DF should then be positively related to ruminal flux proportions of RDP/DF required to optimize turnover of rumen load of DF by digestion and by escape. An experiment was conducted to measure these hypothesized responses in dynamics of intake and digestion of PDF. Ten lambs were fed diets providing ratios of either 0.11 or 0.24 of RDP/DF achieved by use of mineral supplemented cottonseed hulls alone or supplemented with cottonseed meal. The DF and voluntary intake was measured for each of the five lambs per diet. Rumen load of indigestible NDF (IF) was then measured at slaughter two h after an AM feeding. Ruminal turnover of PDF was assumed equal to turnover of IF computed as mean intake rate of IF preceding slaughter divided by rumen load at slaughter. Lambs receiving the 0.11 vs. the 0.24 RDP/DF diet exhibited greater live weight gains and plasma concentrations of amino acids and reduced levels of plasma 3-methylhistidine; responses reflecting greater flux of metabolizable amino acids and deposition rate. Lambs receiving diets of 0.24 vs. 0.11 RDP/DF had greater ($P < 0.05$) rumen load and turnover rates of IF and greater ($P < 0.05$) rates of digestion of PDF and voluntary intake of DF. Buoyancy of feed fragments derived from rate of digestion of PDF was postulated to be the metabolically related force constraining turnover rate of PDF (assuming turnover of PDF equals turnover rate of IF). Assuming 70% ruminal degradation of dietary CP, the results were compared to recomputed literature data from four other related experiments. Results of these five experiments conform to expectations that products of RDP nutritionally limit growth rate of PDF digesting bacteria and, consequently, intake rate of DF over the range of RDP/DF observed.

Key Words: Rumen, Protein, Intake

1513 The prediction of microbial protein supply to growing lambs fed raw and dry roasted legume seeds as protein supplements from the urinary excretion of purine derivatives. P. Yu*[1], L. Boon-ek[2], A.R. Egan[2], and B.J. Leury[2], [1]*Department of Animal and Poultry Science, University of Saskatchewan, Canada,* [2]*Institute of Land and Food Resources, University of Melbourne, Australia*.

Urinary excretion of purine derivatives was used to estimate the microbial N supply to growing lambs in the experiment designed to examine the effect of supplementation and dry roasting whole lupin (*lupinus angustifolius*) seeds (WLS) and whole faba (*vicia faba*) beans (WFB) as protein supplements on rumen microbial N supply to the host animal. Lambs were fed a fixed quantity of oat straw and alfalfa hay plus a daily supplement of either of the following: no legume seeds (CTRL), raw WLS, roasted WLS, raw WFB, or roasted WFB. Legume seeds were dry roasted at 150C for 45 min (as the desirable conditions in the previous study). All diets were isonitrogenous (15.9% CP). In the supplemented diets, about 55% of protein was supplied by WLS or WFB protein. The amount of legume seeds per metabolic liveweight (WLS: 20 g/$LW^{0.75}$; WFB: 25 g/$LW^{0.75}$) was kept the same throughout. At the end of feeding experiment, the excretions of the purine derivatives, allantonin, uric acid plus hypoxanthine and xanthine, were measured after total urine collection during N balance trial to estimate microbial N supply to the duodenum. The results show that lambs fed the five dietary treatments with similar total DM and N intakes, averaging 1158 and 30 g/d across treatments, but different BCP (= rumen bypass feed protein), DVE (= truly digested protein in the small intestine) and OEB (= degraded protein balance) values did not differ in urinary excretion of purine derivatives (averaging 13.0 mmol/d) (allantoin, averaging 10.5 mmol/d; uric acid plus hypoxanthine plus xanthine, averaging 2.5 mmol/d), total purine derivatives absorption (averaging 15.4 mmol/d), microbial N supply (averaging 11.2 g N/d) to the duodenum and efficiency of microbial synthesis (averaging 23.7 g of N/ kg DOMR (digestible OM fermented in the rumen) to growing lambs. No relationships were detected between the estimated protein values of BCP, DVE and OEB and microbial N supply. These results indicated that dry roasting and supplementation had no significant effects on the flow of microbial N into the duodenum.

Key Words: Purine derivatives, Microbial N supply, Lamb

1514 A Role for Ruminally Degraded Protein in Determining Yield and Efficiency of Rumen Efflux Microbial Protein. W.C. Ellis*[1], Dennis Herd[1], J.H. Matis[1], H. Lippke[1], F.M Rouquette[1], D.P. Poppi[2], and R. J. Wallace[3], [1]*Texas A & M University,* [2]*University of Queensland,* [3]*Rowett Research Institute*.

Evidence at the microbial level suggests that optimal growth rate and efficiency of the rumen microbial ecosystem requires specific carbon structures derived from ruminal degradation of dietary protein. However, such a requirement at the dietary level remains obscure. The present objective was to evaluate relationships between flux of ruminal degraded entities upon rumen microbial protein efflux, MPE. Data were computed from literature sources reporting MPE and rumen flux of CP (RDP), NDF (RDF) and of non-structural carbohydrates (RNSC), the latter being computed as OM - [(NDF+lipids+CP)0.8]. Ruminal flux of degraded carbohydrates, RDCHO, was computed as the sum RDF+ RNSC and energetic efficiency of MPE computed as MPE/RDCHO. The database consisted of 154 treatment means from 36 experiments with data from both non-lactating and lactating cattle and mixed and all forage diets. In order for data with sheep to be expressed, all variables were expressed as daily flux per kg BW and relations between MPE as the dependent variable and daily flux of ruminally degraded nutrients as the independent variables were examined. Variations in MPE were equally (R square= 0.79 to 0.89) associated with ruminal flux of truly digested OM, RDNSC and RDP. Variations in efficiency of MPE/RDCHO were primarily associated with flux proportions of RDP/RDF (R square= 0.74). Variations in MPE and MPE/RDCHO were most correlated with flux of RDP and RDF. Thus MPE/RDF was logically and statistically highly correlated with RDP/RDF in animals receiving either forage or mixed diets (R square=0.89 and 0.9, respectively). These associations suggest that variations in MPE were related to the product of flux of RDCHO and energetic efficiency MPE was determined by flux proportions of RDP/RDF. A dietary essential role for deaminated products of amino acids is implicated specifically to enhance growth rate of RDF

digesting bacteria. Variations in RDP/RDCHO were only of a sufficiently range in the forage data to develop reliable prediction equations for MPE: Daily MPE, g/kg BW = daily RDCHO g/kg BW (0.04+0.62 (RDP/RDCHO)); R square=0.83

Key Words: Rumen, Microbial , Protein

1515 Effect of type of cottonseed and gossypol intake on reproduction and health of lactating Holstein dairy cows. J.E.P. Santos*[1], M. Villasenor[1], C.H. Holmberg[1], D. Ringen[1], E.J. DePeters[1], P.H. Robinson[1], B. Bretz[1], and P.W. Jardon[2], [1]*University of California - Davis,* [2]*Visalia, CA.*

Objectives were to evaluate the effects of type of cottonseed on reproductive performance and health of dairy cows. Holstein dairy cows, 856, on 3 dairy farms in central California were assigned at calving to one of two treatment diets (428/treatment) based on parity, calving date and previous lactation 305-d mature equivalent milk yield in a randomized complete block design. Cows were assigned 3 to 20 days in milk (DIM) and remained on diets for 170 d. Cottonseed represented 10% of the diet DM, and treatments consisted of replacing whole Upland cottonseed (WUP) with a blend of WUP and cracked Pima (BUPCP) cottonseed (33:67). Estimated free gossypol intake was 26.2 and 17.9 g/d for BUPCP and WUP, respectively. Estrous was synchronized with two injections of PGF2a (Lutalyse) 14 d apart. Cows were inseminated after the second PGF2a injection (> 44 DIM). Within each dairy, the same technician artificially inseminated all the cows with semen from sires randomly distributed across the two treatments. Pregnancy diagnosis was performed 35 to 45 d after AI, and pregnant cows were reconfirmed at 170 DIM. A postmortem diagnosis was performed in cows that died. Treatment did not affect DIM at first AI and it averaged 58.7 d. Estrus detection after the second PGF2a injection tended to be greater for BUPCP vs WUP (56.9 vs 50.9%; $P < 0.09$). In BUPCP and WUP fed cows, first AI conception rate (29.4 vs 28.2%), percentage of pregnant cows at 170 DIM (74.8 vs 79.4%), and incidence of cystic ovarian disease (8.1 vs 6.1%) did not differ. Average days open for pregnant cows was similar between BUPCP and WUP (91.0 vs 89.8). However, incidence of abortions was greater for cows fed BUPCP vs WUP (8.5 vs 4.1%; $P< 0.05$). Percentage of cows dead or sold was similar, and DIM when cows left the study (dead or sold) did not differ between treatment groups. Nevertheless, cows fed BUPCP tended to have a greater incidence of lameness (17.6 vs 13.1%; $P< 0.08$), but a lower incidence of mastitis (10.6 vs 18.6%; $P < 0.003$) than those fed WUP. No dead cow showed any lesions compatible with gossypol toxicity. Replacement of whole Upland cottonseed with a blend of whole Upland and cracked Pima cottonseed did not affect reproduction and health of lactating Holstein dairy cows.

Key Words: Cottonseed, Gossypol, Dairy cows

1516 Bloodmeal and fishmeal addition to receiving diets. J. W. Lehmkuhler*[1], E.E.D. Felton[1], C.J. Fu[1], and M. S. Kerley[1], [1]*University of Missouri.*

Two experiments were conducted to evaluate the response of a 1:1 bloodmeal and fishmeal combination (BMFM) in receiving cattle. In experiment one, Simmental calves (six heifers and 30 steers) were randomly assigned to nine pens. Cattle were fed a corn and corn silage based diet. Treatments included a soybean meal (SBM) control, low, and high BMFM. Dietary CP was calculated to be 13%, 13%, and 16%, respectively. Intake was restricted during the first 21 d to model low intake responses of newly received cattle to ruminally undegradable protein (RUP) and was increased the following 25 d. Initial, mid-term, and final weights were similar (P>.1) among treatments averaging 347, 351, and 380 kg, respectively. Similarly, no treatment differences were observed for ADG or GF during the 21d restriction period, step-up period, or for the entire receiving period. In experiment two, sixty Angus-sired calves were randomly assigned to twelve pens in a 2X2 factorial design of treatments. Treatments investigated were level of crude protein (low-13%CP vs. high-16%CP) and form of protein (SBM vs. BMFM). Diets consisted of corn, late-bloom alfalfa hay, and the pelleted supplemental treatments. Diets were fed at similar levels for all treatments for 21 d. No treatment differences (P>.1) were observed for initial and ending weights (305 and 330 kg, respectively). Form or level of protein had no effect (P>.1) on ADG. Average daily gain for treatments were 1.1, 1.2, 1.3, and 1.2 kg/d for the low SBM, low BMFM, high BMFM, and high SBM, respectively. Due to the similar intakes and lack of response in ADG, treatment differences were not significant for GF and averaged 0.16. Though not statistically significant, a numerical difference in the interaction for FG was observed. Feed conversions were 9.1, 6.9, 6.9, and 7.7 for the low SBM, low BMFM, high BMFM, and high SBM, respectively. We inferred that addition of RUP and level of protein may alter performance and efficiency of receiving cattle. Further studies are warranted to investigate the relationship between level and form of protein in relationship to energy intake of newly arrived cattle.

Key Words: feedlot, receiving, ruminant

AMSA Graduate Student Research Posters (M.S. and Ph.D. Divisions) and AMSA General Abstracts

1517 Oxymyoglobin and lipid oxidation in α-tocopherol supplemented pork liver microsomes. S Lee*, A L Phillips, and C Faustman, *University of Connecticut, Storrs, CT.*

The biological antioxidant, α-tocopherol, has been used endogenously or exogenously to delay oxymyoglobin (OxyMb) and lipid oxidation in meat. α-Tocopherol quenches free radicals originating from lipid oxidation and this, in turn, appears to protect OxyMb against oxidation. In muscle membranes, α-tocopherol is located close to membrane-bound enzymes that generate free radicals, and acts to protect membrane lipids by scavenging free radicals. An OxyMb porcine microsome model was used to study the effects of α-tocopherol on OxyMb or lipid oxidation *in vitro*. Pork liver microsomes were isolated from pigs fed either a control or vitamin E-supplemented diet (Phillips et al., 2001, Meat Sci., In press), and combined with horse heart OxyMb prepared by hydrosulfite-mediated reduction. OxyMb (0.15 mM) was incubated with microsomes (1mg/ml) at 25 and 37 °C, pH 5.6. During incubation, OxyMb oxidation was measured spectrophotometrically by use of a diffuse-integrating sphere, and percent metmyoglobin (MetMb) was calculated. Lipid oxidation was measured by a thiobarbituric acid reactive substances (TBARS) method. MetMb formation increased with increasing temperature, and was greater at 37 °C than at 25 °C ($P<0.05$). At 37 °C, MetMb reached 50% within 2 hours incubation, whereas 8 hours was required at 25 °C. There was no significant effect of α-tocopherol on delaying OxyMb oxidation either at 25 or 37 °C. Lower TBARS were observed in microsomes from vitamin E-supplemented than control pork livers ($P<0.05$). These results differ from those observed with beef muscle microsomes where both OxyMb and lipid oxidation were delayed with elevated α-tocopherol levels.

Key Words: Oxymyoglobin, Lipid oxidation, α-Tocopherol

1518 Effect of high oil corn and vitaimin E supplementation on beef steak case-life properties. M.S. Eibs*[1], B.J. Johnson[1], D.M. Wulf[1], B.C. Shanks[1], and T.A. Wittig[1], [1]*South Dakota State University.*

The objective of this experiment was to investigate the effects of high oil corn and vitamin E supplementation on steak case-life properties. Steers (n = 84) were fed a high concentrate diet consisting of either typical corn (**C**: 79.5% of ration) or high oil corn (**HOC**: 79.5% of ration) for 112 days with (**+E**) or without (**-E**) vitamin E supplementation during the last 50 d (1,000 IU/hd/d). Steaks (2.54 cm thick) were removed 24 h postmortem from the 12th rib and utilized in retail display panel (**RDP**), thiobarbituric reactive substances determination (**TBARS**), and tocopherol analysis. Two storage treatments were used prior to RDP: 1) domestic chilled (**DC**), chilled storage for 13 d postmortem; and 2) export chilled (**EC**) chilled storage for 34 d postmortem. Steaks were appraised for 9 d (d 0 to 8) under simulated retail meat display conditions by a 5-member panel and color was measured with a Minolta colorimeter. On d 8, TBARS of RDP samples were determined. HOC grain contained more ($P <0.05$) α- and γ-tocopherol than C grain (14.62 and 84.90 vs 8.01 and 41.68 ppm). Ribeye steaks +E contained higher ($P <0.05$) levels of α-tocopherol than ribeye steaks -E. Steaks from HOC had higher concentrations of γ-tocopherol than C steaks (P