T289 Rumen fluid inhibits proliferation and stimulates expression of cyclin-dependent kinase inhibitors 1A and 2A in bovine rumen epithelial cells. A. Wang* and H. Jiang, *Virginia Polytechnic Institute and State University*, *Blacksburg*.

It has been known for decades that microbial fermentation within the rumen is critical to rumen development and maturation in young ruminants, but the underlying mechanism is largely unknown. In this study, we determined the effect of rumen fluid, which should contain all products from rumen fermentation, on growth of rumen epithelial cells in vitro. Rumen epithelial cells were isolated from newborn Holstein calves using the serial tryptic digestion procedure and were cultured in minimum essential medium supplemented with 10% fetal bovine medium. After 3 d of initial culturing, the cells were treated with 1% or 10% (v/v) rumen fluid from lactating Holstein cows or phosphate buffered saline (PBS). Addition of 10% rumen fluid to the culture medium for 72 h decreased the cell number by 36% ($P < 0.05$, n = 4), while that of 1% rumen fluid had no effect compared with addition of PBS. As revealed by DNA fragmentation analyses, 10% rumen fluid did not induce apoptosis in the cultured rumen epithelial cells (n = 4). Flow cytometric assays showed that 10% rumen fluid inhibited the transition of rumen epithelial cells from the G1 phase to the S phase during the cell cycle ($P < 0.05$, n = 3). Real-time RT-PCR analyses of mRNA for key cell cycle regulators indicated that 10% rumen fluid did not change the expression of cyclin D1, D2, D3, E1, or E2 mRNA or that of cyclin-dependent kinase inhibitor 1B or 2B mRNA ($P > 0.1$, n = 3), but caused nearly 3-fold increase in the expression of cyclin-dependent kinase inhibitors 1A and 2A mRNA in rumen epithelial cells ($P < 0.05$, n = 3). These mRNA data support the possibility that rumen fluid inhibits proliferation of rumen epithelial cells in vitro by increasing the expression of cyclin-dependent kinase inhibitors 1A and 2A. The result that rumen fluid inhibits proliferation of rumen epithelial cells in vitro suggests that the stimulatory effect of rumen fermentation on rumen development in vivo is mediated by indirect mechanisms.

Key Words: cattle, rumen fluid, rumen epithelial cells

T290 Short-term postpartum supplementation on hepatic gene expression in primiparous spring-calved beef cows on grazing conditions. I. Whole rice middlings. A. L. Astessiano*, C. López-Mazz, A. C. Espasandín, P. Soca, R. Pérez-Clariget, and M. Carriquiry, *School of Agronomy, UdelaR*, *Uruguay*.

The aim of this work was to evaluate the effects of short-term supplementation of beef cows on blood glucose and insulin concentrations and hepatic gene expression. The experiment was carried at the Experimental Station Bernardo Rosengurtt (Cerro Largo, 32°35′S, 54°15′W). Primiparous suckled crossbred cows (Hereford/Angus), blocked by calving date and body condition score (BCS) at calving, were at 64 ± 14 d postpartum, randomly assigned to 2 treatments: control (grazing of native pastures, 20 kg DM/animal/d; 8.5% CP, 63%NDF; n = 8; CON) and supplementation (2 kg DM/animal/d of whole rice middlings, 10%CP, 14%NDF, 9%EE; n = 8; SUP) for a 21 d. Glucose and insulin concentrations were measured at −2, 7, and 22 d of initiation of the nutritional treatment. Liver biopsies were collected at the end of the nutrition treatment (d 21). The amount of mRNA for growth hormone receptor (GHR), insulin-like growth factor-I (IGFI), IGF binding proteins-2 (BP2),-3 (BP3), insulin receptor (IR) and hypoxanthine-phosphoribosyltransferase (HPRT, endogenous control) were measured by SYBR Green real time RT-PCR. Means from mixed analyses were considered to differ when $P < 0.05$. Cow body weight and BCS did not differ between treatments. Insulin and glucose concentrations were not affected by nutritional treatment, but there was a trend ($P = 0.08$) for interaction between treatment and sampling day in glucose concentrations as glucose tended to increase from d 7 to 22 only in SUP cows. Expression of HPRT was similar between treatments. There were no differences in hepatic GHR, IGFI, and BP3 mRNA due to nutritional treatment. However, BP2 mRNA and BP2/BP3 mRNA ratio tended ($P < 0.10$) to be less in SUP than CON cows. The GHR mRNA was positively correlated with IGFI (r = 0.62, $P = 0.01$) and BP3 (r = 0.51; $P = 0.05$). Results could indicate that short-term postpartum supplementation with rice middlings could improve metabolic status of spring-calved cows at the initiation of the breeding period.

Key Words: liver, mRNA, somatotropic axis

T291 Short-term postpartum supplementation on hepatic gene expression in primiparous spring-calved beef cows on grazing conditions. 2. *Lotus subbliflorus* cv. Rincon. A. L. Astessiano*[1], R. Perez-Clariget[1], G. Quintans[2], P. Soca[1], and M. Carriquiry[1], [1]*School of Agronomy, UdelaR*, *Uruguay*, [2]*Instituto Nacional de Investigación Agropecuaria*, *Treinta y Tres, Uruguay*.

Primiparous suckled crossbred cows (Hereford/Angus) were used in a randomized block design to evaluate the effect of short-term supplementation on blood glucose and insulin concentrations and hepatic gene expression. The experiment was carried in INIA Treinta y Tres (Uruguay, 33°15′S, 54°28′W). Cows, blocked by calving date and body condition score (BCS) at calving, were at 48 ± 10 d postpartum, randomly assigned to 2 treatments: control (grazing of native pastures, 57 kg DM/ha/d, 12.8%CP, 55.9%NDF; n = 30; CON) and supplementation (123.8 kg DM/ha/d of grazing native pastures improved with *Lotus subbiflorous* cv. Rincon, 13.2%CP, 52.9%NDF; n = 30; SUP) for 23 d. Glucose and insulin concentrations were measured at −2, 12, and 26 d of initiation of the supplementation. Liver biopsies were collected at the end of the nutrition treatment (d 23). The amount of mRNA of growth hormone receptor (GHR), insulin-like growth factor I (IGFI), IGF binding proteins-2 (BP2),-3 (BP3), insulin receptor (IR) and hypoxanthine phosphoribosyltransferase (HPRT, endogenous control) were measured by SYBR Green real time RT-PCR. Means from mixed analyses were considered to differ when $P < 0.05$. Cow body weight (BW) and BCS were increased ($P < 0.05$) in SUP cows after 7 or 21 d of initiated the nutritional treatment, respectively. Calf BW increased at d 21 for SUP cows. Glucose concentrations along the period evaluated did not differ between treatments but insulin concentrations were greater ($P < 0.01$) in CON cows. Expression of HPRT mRNA was similar between treatments. Although GHR, IGFI, and BP2 mRNA were not affected by nutritional treatment, abundance of BP3 mRNA was greater ($P = 0.04$) and IR mRNA tended ($P = 0.07$) to be greater for CON than SUP cows. Short-term supplementation with improved pastures of suckled cows during the early postpartum did not improve hepatic expression of somatotropic axis genes.

Key Words: liver, mRNA, somatotropic axis

T292 Effects of glucose on suckling aggressiveness in newborn Holstein and Brown Swiss calves. M. D. DenBeste* and H. D. Tyler, *Iowa State University*, *Ames*.

To determine potential associations between suckling aggressiveness and glucose concentrations in newborn Brown Swiss (B) and Holstein (H)

calves, glucose concentrations were altered via intramuscular injection of insulin (1 mL) or an oral dose of glucose (25 mg) to 19 calves within 5.35 ± 2.72 min after umbilical cord rupture. Calves born from 19 H cows and heifers (9 carrying B embryos) were assigned to treatment randomly by alternating treatments based on birth order within breed. Initial blood samples were collected from calves within 3.25 ± 1.52 min and glucose and insulin treatments were administered within 5.35 ± 2.72 min after umbilical cord rupture. A second blood sample was obtained 57.85 ± 3.17 min after treatments were administered. Samples were analyzed to determine glucose, non-esterified fatty acids (NEFA), leptin, ghrelin, and glutamate (GLU) concentrations. Glucose, NEFA, leptin, ghrelin, and GLU were analyzed using the General Linear Model procedures of SAS. Calves were fed 2 quarts of colostrum replacer and suckling aggressiveness scores were given 1 – weakly, 2 – moderately, and 3 – aggressively. The FREQ procedure (CHISQ option) was used to determine the frequency of suckling aggressiveness scores within each breed. B calves suckled weakly ($P < 0.05$) when compared with H calves. NEFA, leptin, and ghrelin concentrations did not differ significantly ($P > 0.05$) between breeds, treatments, or suckling aggressiveness scores either at birth or post-treatment. Glucose concentrations only differed significantly ($P < 0.05$) between treatments post-treatment. Prior to treatment, B calves had lower concentrations of GLU ($P < 0.05$) than H calves and calves that subsequently suckled weakly had higher concentrations of GLU ($P < 0.10$) than calves that subsequently suckled more aggressively. Breed differences were still apparent in post-treatment samples. In conclusion, B calves suckled weakly when compared with H calves and altering glucose concentrations at birth had no effect on suckling aggressiveness. However, calves that suckled weakly had higher concentrations of GLU than calves that suckled aggressively.

Key Words: glucose, suckling aggressiveness, newborn calves

T293 Butyrate stimulates the cAMP/protein kinase A signaling pathway. A. Wang*, H. Si, D. Liu, and H. Jiang, *Virginia Polytechnic Institute and State University*, *Blacksburg*.

Butyrate is one of the major short chain fatty acids formed by microbial fermentation in the gastrointestinal tract. Butyrate is known as not only an important source of energy for ruminants, but also a signaling molecule, as it stimulates rumen development and inhibits growth of various types of cells in vitro. However, the signaling pathway induced by butyrate in cells is unknown. Here, we report that butyrate can activate the cAMP/protein kinase A (PKA) signaling pathway in the human epithelial colorectal adenocarcinoma cell line Caco-2 cells. The Caco-2 cells were cultured in minimum essential medium supplemented with 20% fetal bovine serum and were treated with 0.01, 0.1, 1, or 10 m*M* of butyrate or phosphate buffered saline (PBS) as a control for 30 min (for cAMP, ATP, protein phosphorylation, and PKA, phosphodiesterase, and adenylate cyclase activity assays) or 16 h (for luciferase assay). All experiments were repeated 3 or 4 times. Compared with PBS, 0.1 m*M* or higher concentrations of butyrate increased intracellular cAMP accumulation ($P < 0.05$, n = 4) and PKA activity ($P < 0.01$, n = 3). The same concentrations of butyrate also induced phosphorylation of the cAMP response element (CRE)-binding protein (CREB) ($P < 0.05$, n = 3) without changing its expression, and increased CRE-driven luciferase reporter gene expression ($P < 0.01$, n = 4). Moreover, butyrate at 0.1 m*M* or higher concentrations increased intracellular ATP levels ($P < 0.05$, n = 4), whereas it had no effect on the activity of phosphodiesterase or that of adenylate cyclase. Taken together, these data indicate that butyrate stimulates the cAMP/PKA signaling pathway in Caco-2 cells and that this stimulation may be due to increased ATP accumulation. This result suggests the possibility that butyrate affects rumen development or cell growth through the cAMP/PKA signaling pathway.

Key Words: butyrate, cAMP, protein kinase A

T294 The effect of forage availability on the somatotropic axis in free-ranging alaskan moose (*Alces alces*). A. A. Parillo*[1], J. P. Richmond[1], K. S. White[2], J. Crouse[3], B. W. Dale[4], and S. A. Zinn[1], [1]*University of Connecticut*, *Storrs*, [2]*Alaska Department of Fish and Game*, *Juneau*, [3]*Alaska Department of Fish and Game*, *Soldotna*, [4]*Alaska Department of Fish and Game*, *Palmer*.

To determine if components of the somatotropic axis reflect the availability of forage or nutritional status in adult moose (*Alces alces*), 3 distinct Alaskan populations of free-ranging moose [Gustavus (n = 42), Skwentna (n = 24), and Nelchina (n = 11)] were used. Forage availability, low (Gustavus), medium (med; Nelchina) or high (Skwentna) varied with population. Moose from each population were captured once in fall (October–November; high forage availability) and once the following winter (March; low forage availability). At capture, blood samples were collected via venipuncture to determine if forage availability influenced the somatotropic axis, and rump fat was measured to assess body condition. Concentrations of GH and IGF-1 were quantified by RIA using bovine and human antisera, respectively. Western ligand blots were used to quantify IGFBP-2 and −3. Rump fat was greater in fall than winter (2.4 ± 0.26 vs. 1.1 ± 0.24 cm; $P \leq 0.01$), but was similar across the 3 populations [1.68 ± 0.16 (low), 1.57 ± 0.27 (med) and 2.1 ± 0.18 (high) cm; $P \geq 0.17$]. Concentrations of GH averaged 3.4 ± 1.2 ng/mL and were not different between populations ($P \geq 0.15$) or season ($P \geq 0.12$). Average IGF-1 concentrations were greater ($P \leq 0.03$) in high than low (135.4 ± 17.6 vs 89.8 ± 17.1 ng/mL) forage availability and greater ($P \leq 0.01$) in fall [117 ± 20.01 (low), 138 ± 25.1 (med), and 220 ± 16.4 (high) ng/mL] than in winter [62.5 ± 14.2 (low), 42.2 ± 30.6 (med), and 50.6 ± 18.7 (high) ng/mL]. Conversely, IGFBP-3 was greater ($P \leq 0.01$) in low compared with high (72.1 ± 6.0 vs 43.4 ± 7.4 AU) forage availability, and greater ($P \leq 0.01$) in fall compared with winter (69.2 ± 4.4 vs 34.4 ± 4.6 AU). Similarly, IGFBP-2 was greater ($P \leq 0.01$) in low than high (47.1 ± 3.1 vs 24.4 ± 4.0 AU) forage availability, and was greater ($P \leq 0.01$) in winter than fall (36.9 ± 2.5 vs 32.0 ± 2.9 AU). Serum IGF-1 concentrations increased, whereas IGFBP-2 and IGFBP-3 decreased with greater forage availability. These components of the somatotropic axis may be useful indicators of nutritional status in free-ranging populations of Alaskan moose.

Key Words: somatotropic axis, moose (*Alces alces*), forage availability

T295 Effects of dietary probiotic supplementation and posthatching holding time on intestinal pH and microflora of male broilers. H. Unsal[1], A. G. Onol[1], M. Daskiran[2], O. Cengiz*[1], O. Tatli[1], and O. Sevim[1], [1]*Adnan Menderes University*, *Aydin, Turkey*, [2]*Johnson & Johnson Corporate Science and Technology*, *New Brunswick, NJ*.

A study was conducted to determine the effects of a dietary probiotic, a commonly used feed additive, and posthatching holding time (0, 12, 24, and 36 h post-hatching) on the intestinal pH and microflora of male broiler chicks. A 2 × 4 factorial design was implemented. Eight experimental groups were formed by two levels of dietary probiotic supplementation (Control and Protexin, 0.5 kg/ton) and four levels of posthatching holding time. Four posthatching holding times were 0 (chicks were given feed and water immediately after their arrival), 12, 24, and 36 hours. There were 4 replications for each treatment group and each replication consisted of 20 day-old birds. Chicks were received

from a commercial hatchery and transferred to the Experimental station within 2 hours after feather-sexing procedure. A corn-soybean meal based diet was used in the study. Water and feed were available for ad libitum consumption throughout the study and the experiment lasted 42 days. A significant decrease in day-10 intestinal pH ($P < 0.05$) was noted in groups with dietary probiotic supplementation (6.59 vs. 6.42). Dietary probiotic supplementation also numerically increased the number of colony forming units of lactobacilli at days 10 (7.35 vs. 7.60 cfu) and 21 (6.74 vs. 7.05 cfu) of the study. The number of colony forming units (cfu) of lactobacilli in groups with no feed and water restriction were either numerically or significantly (12 and 36 hour feed and water restriction treatments) higher (7.96 vs. 7.13, 7.28 cfu) than that of the groups with posthatching holding time prior to feeding ($P < 0.01$). Total bacteria count was similar among treatment groups during the experiment. In brief, this study indicated that early exposure to lactobacilli, which is found in either a dietary probiotic supplement or in feed naturally helps broiler chicks to develop a healthier gastrointestinal tract environment and microflora and this microflora may, in turn, inhibit pathogenic microorganisms in broiler gastrointestinal tract.

Key Words: broiler, post-hatching holding time, probiotic

T296 Maintenance energy requirements of gestating beef cows, rumen temperature, and plasma concentration of thyroxine and triiodothyronine. T. A. Pye*, B. H. Boehmer, and R. P. Wettemann, *Oklahoma Agricultural Experiment Station*, *Stillwater*.

Spring calving, Angus cows, (n = 32) were used to determine the effects of maintenance energy requirement (MR) on rumen temperature (RuT), and concentrations of thyroxine (T4) and triiodothyronine (T3) in plasma. Cows (4 to 7 yr of age) with an initial BCS of 4.4 ± 0.1 and BW of 556 ± 5.9 kg were individually fed a complete ration for 17 wk during 4–8 mo of gestation. After 2 wk on a diet calculated to supply MR (Model 1, NRC 1996) the diet was adjusted weekly until constant BW was achieved (regression analyses). BW was maintained for 31 d for 25 cows and the amount of feed consumed was actual MR. Blood samples were collected before and after consumption of feed on 2 d when cows consumed MR. Rumen temperature was recorded hourly, using rumen boluses (Smart Stock, LLC), for 4 consecutive days when cows consumed the MR diet and when cows consumed ad libitum roughage. Cows were classified based on MR as low (>0.5 SD less than mean, LMR), mod (±0.5 SD of the mean, MMR) and high (>0.5 SD greater than mean, HMR). Average MR was 84.04 (SD = 7.13) $Kcal \bullet kg^{-0.75} \bullet day^{-1}$. The difference in MR between the least efficient and the most efficient cow was 32%. Rumen temperature during maintenance and during ad libitum roughage was not influenced by MR. When cows were exposed to warmer temperatures (15°C) plasma T4 was not influenced by MR ($P = 0.92$). When exposed to cooler temperatures (−5°C), LMR cows had greater plasma T4 ($P = 0.003$) compared with HMR. Plasma T3 was not influenced by MR when cows were exposed cooler ambient temperatures ($P = 0.64$). When exposed to warmer temperatures, compared with LMR, HMR cows had greater plasma T3 ($P = 0.007$). During late gestation MR were associated with plasma concentrations of T3 and T4, but RuT was not influenced by MR. Thyroid hormone may be involved in the regulation of MR of beef cows during late gestation. Identification of cows with lower MR and greater efficiency could improve the profitability of beef production.

Key Words: beef cows, maintenance, thyroxine

T297 Effects of cobalt supplementation and vitamin B_{12} injections on energy metabolism of dairy cows. M. S. Akins*[1], S. J. Bertics[1], M. T. Socha[2], and R. D. Shaver[1], [1]*University of Wisconsin*, *Madison*, [2]*Zinpro Corporation*, *Eden Prairie, MN*.

The objective of this study was to determine metabolic responses of primi- and multiparous dairy cows fed different levels and sources (inorganic and organic) of cobalt or given weekly vitamin B_{12} injections. Forty-five primi- and multiparous cows 60 d prepartum were blocked by parity (1 or > 1) and expected calving date, and then randomly assigned to 1 of 5 treatments in a randomized complete block design. The treatments were: no supplemental Co (Control), 25 mg Co from Co carbonate (CoCarb), 25 mg (LcoGH) or 75 mg (HCoGH) Co from Co glucoheptonate, and Control with weekly 10 mg vitamin B_{12} injections. Cows were on trial until 150 DIM. Cobalt (ppm DM) in the lactating diet was 1.0, 1.9, 2.3, and 5.2 for Control and IB12, CoCarb, LCoGH, and HCoGH, respectively. Far-off, close-up, and lactating diets were 13.8, 15.1, and 18.0% CP and 48.8, 40.2, and 32.9% NDF (DM basis), respectively. Intake was not affected ($P > 0.10$) by treatment and was 19.4 ± 0.5 and 23.1 ± 0.8 kg DM/d for primi- and multiparous cows, respectively. Body weight and condition score and calculated energy balance were not affected by treatment ($P > 0.10$). Plasma glucose, non-esterified fatty acids, and β-hydroxybutyrate were not affected by treatment ($P > 0.10$). Effect of sampling day was significant ($P < 0.001$). Glucose decreased from 60 d prepartum (65 ± 1.1 mg/dL) to 30 DIM (55 ± 1.0 mg/dL), and increased at 90 DIM (60 ± 1.0 mg/dL); however, primiparous cows had a larger decrease at 30 DIM and smaller increase thereafter. Non-esterified fatty acids increased from 60 d prepartum (249 ± 39.8 mmol/L) to 1 DIM (724 ± 40.7 mmol/L), then decreased at 30 DIM (398 ± 40.1 mmol/L), with multiparous cows having a larger increase at 1 DIM. Beta-hydroxybutyrate increased from 60 d prepartum (4.2 ± 0.95 mg/dL) to 30 DIM (15.9 ± 0.95 mg/dL). Addition of Co above requirements or vitamin B_{12} supplementation did not improve energy metabolism of dairy cows.

Key Words: cobalt, vitamin B_{12}, dairy cow

T298 The relationship of tissue copper concentrations and genes involved in copper homeostasis in the cow, pig, and goat. H. So, E. Domby*, T. Engle, and H. Han, *Colorado State University*, *Fort Collins*.

Copper (Cu) serves as a cofactor for enzymes involved in a variety of biological functions. Copper transport/distribution within the cell is mediated by the expression of the copper transporter (CTR1), ATPase7A (ATP7A), ATPase7B (ATP7B) which helps Cu trafficking. Copper is also required for activity of lysyl oxidase like 1 (LOXL1) for the production of elastin and collagen in arterial tissue. Liver and pulmonary artery tissues tissue from 5 Angus crossbred steers, 6 Nubian goats, and 6 American Landrace pigs were collected. Liver and pulmonary artery samples were collected at the time of harvest and snap frozen. Liver and pulmonary artery Cu concentrations were determined via flame atomic absorption and gene expression was measured by real time PCR. Data were analyzed using PROC CORR of SAS. Liver Cu concentrations (ppm ± SE) were higher in cows (396.4 ± 109.1) and goats (181.4 ± 37.0) than in pigs (19.2 ± 3.5). All liver Cu concentrations were within normal ranges and considered adequate for each species. Liver Cu concentration was more variable in cows and goats compared with pig liver Cu concentrations. Real Time PCR revealed that goat liver *ATP7A* was positively correlated ($r^2 = 0.920$; $P < 0.003$) to liver Cu concentrations while cow and pig *ATP7A* was not correlated to liver Cu concentration. In the pig, liver *ATP7A* expression was positively correlated to *ATP7B* ($r^2 = 0.662$; $P < 0.049$). Pulmonary artery Cu concentration was highest in cows (14.9 ± 4.7), intermediate in pigs (8.9 ± 3.3), and lowest in goats (3.9 ± 1.1). Goat pulmonary artery Cu concentration was not correlated

to *CTR1* expression, however, *ATP7A* expression was positively correlated with *CTR1* ($r^2 = 0.897$; $P < 0.004$). In cow pulmonary artery, *LOXL1* expression was positively correlated to elastin expression ($r^2 = 0.912$; $P < 0.012$). Pulmonary artery Cu concentration was not correlated to gene expression of Cu homeostatic genes in the pig. This data indicates that genes involved in Cu homeostasis (*CTR1*, *ATP7A*, *ATP7B*, *LOXL1* and *elastin*) are differently regulated in different species. This may contribute to different responses to elevated pulmonary arterial pressure in different species.

Key Words: copper, liver, pulmonary artery

T299 Modification and validation of a bovine TNFα enzyme-linked immunosorbent assay with improved sensitivity. J. K. Farney*, L. K. Mamedova, and B. J. Bradford, *Kansas State University*, *Manhattan*.

Tumor necrosis factor α (TNFα) is an inflammatory cytokine that is involved in immune function and is proposed to play a role in metabolic disorders. Until recently, no bovine-specific antibodies were available for detection of TNFα. While some bovine-specific methods have been published recently, assays used for determining plasma TNFα concentration in bovine disease models often do not offer acceptable precision for measurement of basal concentrations in healthy animals. The objective of this work was to develop an effective, low-cost enzyme-linked immunosorbent assay (ELISA) procedure with improved sensitivity. A protocol developed for use with cell culture supernatant was modified for use with bovine plasma by optimizing antibody concentrations, incubation times and temperatures, and standard diluents. The coating antibody concentration was decreased from 10 μg/mL to 6.8 μg/mL, while the detection antibody concentration remained 2.5 μg/mL. Sample incubation was increased from 1 h at room temperature to an overnight incubation at 4°C, which increased the sensitivity of the assay. Multiple matrices were tested for dilution of standards and were assessed by determining recovery of bovine TNFα spiked into bovine serum and plasma. Standard curve matrices were fetal bovine serum (FBS), dialyzed FBS, lyophilized human serum (rehydrated), and phosphate-buffered saline (PBS) with 4% bovine serum albumin. Recoveries were < 50% when quantified with standards diluted in PBS, FBS, or dialyzed FBS. However, recoveries were acceptable in both bovine serum and plasma (85–120%) when quantified with standards diluted in lyophilized human serum. The modified bovine TNFα ELISA offers a detection range of 2 to 500 pg/mL. This detection limit is at least an order of magnitude lower than previously reported, and will allow for greater precision in determining basal TNFα concentrations in bovine plasma. The improved sensitivity of this ELISA will be critical to assessing current hypotheses concerning the metabolic effects of moderately elevated TNFα concentrations.

Key Words: Tumor necrosis factor alpha, bovine, ELISA

T300 Plasma cortisol, corticosteroid-binding globulin and free cortisol index in pre-and post-weaned pigs supplemented with omega-3 polyunsaturated fatty acid. H. G. Kattesh*, C. J. Kojima, M. P. Roberts, and G. M. Pighetti, *University of Tennessee*, *Knoxville*.

A dietary supplement of omega-3 polyunsaturated fatty acid (PUFA) has been shown to decrease corticosteroid-binding globulin (CBG) response to an LPS challenge in the post-weaned pig, suggesting a reduction in immune system activation and less of a need for the biologically active free form of cortisol. The aim of this study was to examine the effects of PUFA supplementation to lactating sows and their offspring on growth and indicators of stress in the pre- and post-weaned pig. Upon farrowing (d 0), sows received either a standard lactation diet (SC; n = 4) or SC supplemented (1.0% by weight) with a commercial PUFA source (SP) throughout lactation. At 11–14 d of age, pigs within sow-diet treatment group were creep-fed a diet similarly supplemented with or without PUFA. Pigs (n = 6/litter) were weaned at 21–24 d of age into 8 pens in a replicated 2 × 2 factorial design and fed a nursery diet with (PP; 12 SC and 12 SP) or without (PC) PUFA. Pigs were weighed (BW) and bled on d 14, 21 (before weaning), 22 and 28, for determination of plasma cortisol (CORT) and CBG concentrations, free cortisol index (FCI), and neutrophil: lymphocyte ratio (N:L). Pre- and post-weaning BW was not different ($P > 0.10$) among pigs regardless of treatment. On d 21, SP pigs exhibited a lower ($P < 0.01$) CORT (54.8 ± 7.2 vs. 90.0 ± 9.0 nmol/L) and higher ($P < 0.05$) CBG (9.1 ± 0.7 vs. 7.0 ± 0.5 mg/L) compared with SC pigs. The resultant FCI (nmol cortisol/mg CBG) was lower ($P < 0.001$) for the SP pigs. On d 22, CORT, CBG and N:L increased ($P < 0.001$) but returned to pre-weaning levels by d 28. No significant differences were found among the post-weaned treatment groups for any of the stress indicators measured. The results suggest that PUFA supplementation can reduce the amount of circulating free cortisol in the pig before weaning; however, this benefit is negated due to the stress of weaning.

Key Words: pig, PUFA, stress