

Physiology and Endocrinology: Immune Function and Health

T169 Alpha-linolenic acid exerts anti-inflammatory effect in 3T3-L1 adipocytes through mechanisms that involve activation of AMPK. K. M Ajuwon*¹, T. A Winters², B. Whisenhunt², and W. Banz², ¹Purdue University, West Lafayette, IN, ²Southern Illinois University, Carbondale.

Alpha-linolenic acid (ALA) (18:3n-3) is an omega-3 fatty acid that is found in abundance in many seeds and oils such as those from soybean, flaxseed and walnuts. It is the only plant derived omega-3 fatty acid that is consumed in large quantities in the U.S and in many parts of the world. Whereas the longer chain omega-3 fatty acids, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), have been shown to have cardiovascular and other health benefits, significant controversy still exists regarding the overall health benefits that can be obtained from ALA. Because adipose tissue inflammation is implicated in systemic inflammation that may eventually cause cardiovascular disease and insulin-resistance, we examined the possibility that ALA could exert anti-inflammatory effect in the 3T3-L1 adipocyte model. Fully differentiated adipocytes were pretreated with 150 and 250µM ALA for 6 h and then treated with lipopolysaccharide (LPS) (100 ng/mL) for 3 h. We then measured interleukin 6 (IL-6) mRNA expression with RT-PCR and protein secretion with ELISA. Treatment of adipocytes with LPS led to an induction of IL-6 mRNA and protein secretion ($P < 0.05$). This was attenuated by pretreatment of adipocytes with ALA ($P < 0.05$). To explore possible involvement of AMPK in the anti-inflammatory effect of ALA, we examined the abundance of phosphorylated and total AMPK using western blotting. We observed an induction of AMPK phosphorylation in ALA treated cells ($P < 0.05$). These results indicate that ALA exerts anti-inflammatory effect in adipocytes and also raise the possibility that AMPK is a mediator of this effect. Taken together, ALA could have health and growth benefits in both humans and animals due to its ability to attenuate inflammation.

Key Words: Adipocytes, Inflammation, AMPK

T170 Polarized interleukin-8 (IL8) secretion by swine jejunal epithelial cells (IPEC-J2) treated with soluble beta-glucan (BG). T. E. Burkey* and S. S. Shepherd, *University of Nebraska, Lincoln.*

Yeast BG may be a potential immunomodulator that could serve as a natural alternative to antimicrobial growth promoters. Beta-glucans are (1,3)-β-D-glucose polymers isolated from the yeast *Saccharomyces cerevisiae*. It has been established that BG induce enhanced phagocytic activity, increased production of reactive oxygen species and increased production of cytokines and chemokines. Beta-glucans may potentially activate receptors within the gastrointestinal tract, elicit a priming effect and initiate signaling cascades that may modulate innate as well as adaptive immune responses directed at potential pathogens. Intestinal epithelial cells are sources of chemokines, such as IL8, which lead to the recruitment of macrophages, lymphocytes, and polymorphonuclear leukocytes and may further initiate both the innate and adaptive immune responses. The objective of the current study was to evaluate the effect of soluble BG on IL8 protein secretion in IPEC-J2 cells. IPEC-J2 cells were grown to confluency on Costar Transwells, and treated apically with media alone (CTL), LPS (10 ng/ml), Zymosan A (100 µg/ml) or soluble BG (100, 10 or 1.0 µg/ml). IPECJ2 cells were exposed to the respective treatments for 6 h, and then the apical and basolateral media

were collected for subsequent quantification of IL8 by ELISA. No significant treatment by position (apical or basolateral) interaction was observed. However, this experiment resulted in a significant main effect of position where IL8 secretion was polarized in the apical direction when averaged across all treatments ($P < 0.0001$). In addition, when averaged across both positions, BG (100 µg/ml) tended to increase IL8 secretion compared to all other treatments ($P = 0.07$). The data suggest that soluble beta-glucan elicits IL8 secretion in IPEC-J2 cells and that this secretion is polarized in the apical direction.

Key Words: Swine Jejunal Epithelial Cells, Beta-Glucan, Interleukin 8

T171 The variation of IgG1, IgA and IgM concentration in blood and milk of dairy cows after implanting antigen-releasing device (ARD). C. G. Zhang^{1,2}, J. Q. Wang*¹, D. P. Bu¹, G. L. Liu¹, J. B. Cheng¹, X. L. Dong^{1,2}, K. L. Liu¹, H. Y. Wei¹, L. Y. Zhou¹, and G. Q. Zhao², ¹Chinese Academy of Agricultural Sciences, Beijing China, ²Yangzhou University, Yangzhou, China.

The objective of the study presented here was to determine if the variation in total immunoglobulin follows a similar pattern to the variation in specific anti-lipase antibody in blood and milk after implanting the ARD. Twenty healthy adult dairy cows in mid-lactation were divided into two groups ($n = 20$) according to milk yield (26.0 ± 3.5 kg), and days in milk (114 ± 34 d). Each cow of the test group was implanted with the three types of ARD (ARD1, ARD2 and ARD3) in the right iliac lymph node using an implantation gun. The trial period was carried out over 40 d. Milk samples were collected at d 0, 5, 7, 9, 15, 17, 20, 26, 30, 40 and blood samples were collected every 10 d. Total IgG1, IgA and IgM were measured by sandwich enzyme-linked immunosorbent assay (ELISA) using the Bovine IgG1/IgA/IgM ELISA Quantitation Kit. The results showed that total immunoglobulin concentration (mg/mL) in blood of test group animals (5.831 ± 0.284) was higher than control group animals (5.348 ± 0.245). IgG1 concentration (mg/mL) in milk reached a peak at d 9 (0.57 ± 0.11), 17 (0.52 ± 0.09) and 32 (0.51 ± 0.10), respectively, corresponding with the release of ARDs at d 0, 14 and 28. The IgA and IgM concentration failed to show the same regular patterns when compared with IgG1. In conclusion, the ARD implantation successfully increased total immunoglobulin, especially IgG1 concentration in blood and milk of dairy cows. (Research funded by Ministry of Science and Technology; 2006DFB32160).

Key Words: Immunoglobulin, Antigen-Releasing Device, Blood and Milk

T172 Canonical correlation of milk immunoglobulins, lactoferrin concentration and Dairy Herd Improvement data of Chinese Holstein cows. G. L. Liu¹, J. Q. Wang*¹, D. P. Bu¹, J. B. Cheng¹, C. G. Zhang^{1,2}, X. L. Dong^{1,2}, H. Y. Wei¹, L. Y. Zhou¹, and K. L. Liu¹, ¹Chinese Academy of Agricultural Sciences, Beijing, China, ²Yangzhou University, Yangzhou, China.

Immunoglobulins (Igs) together with lactoferrin (Lf) constitute an important antimicrobial system in milk and play key roles in the defense

mechanisms of mammary gland of lactating cows. The purpose of this work was to establish the relationship among the data in Dairy Herd Improvement (DHI) report and milk Igs and Lf concentration. We collected 299 individual milk samples randomly from more than 2,800 animals across six dairy farms in the greater Beijing area, and obtained the corresponding DHI data from the China DHI system. Concentrations of Igs and Lf in milk were determined by ELISA assay. The relationship among DHI data and milk Igs and Lf concentration was established using canonical correlation analysis. The results indicated that 4 canonical variables relating milk IgG1, IgA, IgM and Lf concentration as y variables with lactation number, stage of lactation, daily milk production, milk fat, protein, lactose, milk total solids and somatic cell score (SCS) as x variables were created. The canonical correlations of first and second pair of canonical variables were 0.662 and 0.469 respectively with highly significance ($P < 0.01$), and accounted for 91.6% of the data variability. Stage of lactation, daily milk production, milk protein and SCS were the significant factors affecting Lf concentration, and lactation number was the significant factors affecting IgG1. The first standardized canonical variation combination could be regarded as a predictable measure of Lf and IgM concentration, the second as a predictor of IgG1. These results may be useful for dairy producers to select cows with increased production of Igs and Lf using DHI data directly. (Research funded by Ministry of Science and Technology; 2006DFB32160)

Key Words: Dairy Herd Improvement Data, Immunoglobulin, Lactoferrin

T173 Mifepristone (RU486) modulation of dexamethasone-induced suppression of in vitro proliferation of equine lymphocytes. K.A. Gutierrez^{*1}, N. C. Burdick¹, J. G. Lyons¹, C. L. Barton¹, J. C. Laurenz², N. D. Cohen¹, N. H. Ing¹, and T. H. Welsh, Jr.¹, ¹Texas A&M University, College Station, ²Texas A&M University, Kingsville.

Adverse actions attributed to hypercortisolemia associated with Cushing's syndrome are reduced by the glucocorticoid receptor antagonist RU486 (Eur. J. Endocrinol. 157:561; 2007). Immunity may be compromised by cortisol and its synthetic analog dexamethasone (Dex; Am. J. Vet. Res. 56: 997; 1995). This study was designed to determine whether proliferation of equine lymphocytes is adversely affected by Dex, and if RU486 could modulate this negative effect. Jugular blood samples from 15 horses (4 breeds; 12 stallions; 3 geldings; 5-to-15 years of age; 450-to-800 kg BW) were collected and used to isolate lymphocytes by density gradient centrifugation. Separate cultures were established for each horse. Isolated lymphocytes (100,000 cells per well) were cultured in 96-well plates for 96 h in a humidified CO₂ incubator at 37°C in either medium alone (DMEM/F12), or medium containing concanavalin A (ConA; 0-to-5 µg/ml) with or without 1 µM Dex in the presence and absence of 1 µM RU486. Cell proliferation was determined by Promega CellTiter96 assay. Stimulation indices were determined relative to Control (medium alone) and differences in proliferation were determined by ANOVA. ConA dose-dependently increased ($P < 0.01$) lymphoproliferation (ED₅₀ 1.2 µg/ml). Dex inhibited ConA-induced proliferation

($P < 0.01$). Specifically, Dex reduced basal proliferation by 37%. At 0.625 and 1.25 µg/ml ConA, Dex reduced proliferation by 19.4 and 18.3%, respectively. Co-addition of RU486 reduced or prevented inhibitory action of Dex ($P < 0.01$). Specifically, RU486 attenuated by 1.59-fold Dex-inhibition of basal lymphoproliferation. The 4.1-fold increase in proliferation induced by 5 µg/ml ConA was reduced 24.9% by Dex; however, the presence of RU486 completely prevented Dex's action. Glucocorticoid antagonists may be used to study how immune functions are suppressed in horses that are phenotypically hypercortisolemic due to: 1) stress; 2) dexamethasone therapy; 3) Cushing's syndrome; or, 4) metabolic syndrome.

Key Words: Equine, Immune Function, RU486

T174 Bovine viral diarrhea virus, abnormal cervical mucus discharge and fertility in cows. S. Yavru^{*1}, M. Kale², M. S. Gulay², O. Yapici¹, O. Bulut¹, and A. Ata², ¹Selçuk University, Konya, Turkey, ²Mehmet Akif Ersoy University, Burdur, Turkey.

The aim of the present study was to describe whether Bovine Viral Diarrhea virus (BVDV) and appearance of cervical mucus discharge (CMD) have effects on reproductive performance of cows in estrus. For this purpose, CMD of 97 Holstein cows in estrus were evaluated visually before AI. To exclude the possible effects of reproductive problems related to nutrition deficiency, cows with body condition score lower than 2.5 were not included in the study. All cows were healthy and free of anatomical abnormalities of the reproductive tract. The CMD of cows in estrus was evaluated visually before AI. Animals having clear discharges (n = 50) with normal viscosity and without bad odor were grouped as normal cervical mucous discharge (N-CMD) group. The other cows (n = 47) with opaque mucus or mucus containing flecks of pus and purulent or mucopurulent material were grouped as abnormal cervical mucous discharge (A-CMD) group. Cows in estrus were inseminated with BVDV free frozen semen. Blood samples were tested by enzyme linked immunoassay (ELISA) for antigens (Ag) and antibodies (Ab) of BVDV. Presence of BVDV genome in mucus samples were tested by Polymerase chain reaction (PCR). No differences in hematocrit or plasma protein concentrations were observed between N-CMD (31.9 and 6.18%) or A-CMD groups (32.8 and 6.26%). First service conception rates (FSCR) were 64 and 61.7% for N-CMD and A-CMD groups, respectively ($P > 0.1$). Total of 55.7, 18.6 and 2.7% of cows were BVDV-Ag, BVDV-Ab and BVDV-PCR (+), respectively. Presence of BVDV-Ag, BVDV-Ab or BVDV-PCR (+) was not associated with A-CMD. FSCR was similar between BVDV-Ab positive (62.9%) and BVDV-Ab negative cows (62.8%). However, presence of BVDV-Ag decreased FSCR (27.8 vs. 70.9%; $P < 0.01$). Thus, the current study suggested that effect of A-CMD on FSCR is minimal. However, presence of BVDV-Ag in blood samples at the time of AI has a negative effect on fertility of Holstein cows.

Key Words: BVDV, Fertility, Cervical Mucus Discharge