463 The activation of insulin and nutrient signaling components leading to translation initiation in skeletal muscle of neonatal pigs is developmentally regulated. A. Suryawan*, R. A. Orellana, A. S. Jeyapalan, H. V. Nguyen, J. R. Fleming, and T. A. Davis, *USDA/ARS Children's Nutr. Res. Ctr., Department Pediatrics, Baylor Coll. of Med.*, *Houston, TX*.

Insulin (INS) and amino acids (AA) act independently to stimulate protein synthesis in skeletal muscle of neonatal pigs and the responses decrease with development. The purpose of this study was to compare the effect of INS and AA on the activation of signaling components leading to translation initiation and how these responses change with development. To examine the independent role of INS, hyperinsulinemic-euglycemic-euaminoacidemic clamps were performed in fasted 6-d-old (n=4) and 26-d-old (n=6) pigs to raise plasma insulin from 5 (fasting level) to 30 (fed level) μU/ml while AA and glucose were maintained at fasting levels. To elucidate the independent role of AA, a balanced AA mixture was infused into fasted 6-d-old (n=4) and 26-d-old (n=6) pigs to raise branched-chain amino acids from 500 (fasting level) to 1000 μmol/L (fed level) while INS and glucose were maintained at fasting levels. INS, but not AA, increased the phosphorylation of protein kinase B. Both INS and AA increased the phosphorylation of mammalian target of rapamycin (mTOR), ribosomal protein S6 kinase-1, and eukaryotic initiation factor (eIF) 4E-binding protein 1 (4E-BP1) and these responses were higher in 6-d-old compared to 26-d-old pigs ($P<0.05$). In 6-d-old pigs, both INS and AA reduced the binding of raptor to mTOR ($P<0.05$). Both INS and AA decreased the binding of 4E-BP1 to eIF4E ($P<0.05$) and increased eIF4E binding to eIF4G ($P<0.05$); these effects were greater in 6-d-old than in 26-d-old pigs ($P < 0.05$). Furthermore, neither INS, AA, nor age had any effect on the phosphorylation of eukaryotic elongation factor 2. Our results suggest that the activation of many of the insulin and nutrient signaling components leading to translation initiation is developmentally regulated and parallels the developmental decline in protein synthesis in skeletal muscle of neonatal pigs.

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Key Words: Protein Synthesis, Skeletal Muscle, Pig

Immunology - Livestock and Poultry II

464 Effects of maternal nutrition and selenium supplementation on absorption of IgG and survival of lambs. C. J. Hammer*[1], K. A. Vonnahme[1], J. B. Taylor[2], D. A. Redmer[1], J. S. Luther[1], T. L. Neville[1], J. J. Reed[1], J. S. Caton[1], and L. P. Reynolds[1], [1]*North Dakota State University*, *Fargo*, [2]*USDA-ARS, U.S. Sheep Experiement Station*, *Dubois, ID*.

To examine the effects of maternal nutrition with or without additional selenium (Se) on absorption of IgG and survival of lambs, 82 Rambouillet ewe lambs were allotted randomly to one of six treatments in a 3 x 2 factorial design. Groups included dietary levels of Se [Adequate Se (ASe, 7.4 μg/kg BW) vs. High Se (HSe, 85 μg/kg BW)], and plane of nutrition [60% (RES), 100% (CON), and 140% (HIGH) of NRC requirements for gestating lambs]. Basal diets were fed once daily in a complete pelleted form and HSe ewes received a Se pellet to meet the required Se level. Upon parturition, lambs were immediately separated from their dams, weighed, and serum samples obtained. Lambs were fed artificial colostrum for the first 20 h after which lambs were fed milk replacer. The artificial colostrum contained 64.3 g IgG/L and lambs received 10.6 g IgG/kg BW divided into seven feedings. At 24 h post-parturition serum samples were obtained to assess IgG status. Lambs were reared similarly in a temperature controlled (12°C), and ventilated facility for the duration of the study. Signs of illness prompted treatment following protocol. Morbidity was assessed as number of days treated for respiratory or gastrointestinal symptoms. Mortality was calculated as days in flock. Plane of maternal nutrition affected ($P < 0.05$) ability of lambs to absorb IgG after birth, with 24 h IgG concentrations of 2276, 1586, and 1214 mg/dl for lambs from RES, CON, and HIGH respectively. Selenium supplementation of ewes decreased ($P < 0.01$) 24 h IgG concentrations in lambs from 1912 mg/dl for ASe to 1472 mg/dl for HSe. Morbidity was not different for lambs from ewes in any of the treatment groups. Mortality was greater ($P < 0.01$) for offspring of ewes in the HIGH group; this group also had the lowest 24 h IgG concentration. Lambs born from ewes on a high or low plane of nutrition or with high dietary Se appear to have an altered ability to absorb IgG after birth. Further research is needed to determine the mechanisms by which this occurs.

Key Words: IgG, Mortality, Lamb

465 Effect of supplementation with a *Bacillus*-based direct-fed microbial on immune development of dairy calves. K. Novak*[1], E. Davis[1], C. Wehnes[1], T. Rehberger[1], D. Shields[2], and J. Coalson[2], [1]*Agtech Products, Inc.*, *Waukesha, WI*, [2]*Merrick's, Inc.*, *Union Center, WI*.

Immune development was evaluated in 65 Holstein bull calves in response to the addition of a *Bacillus*-based direct-fed microbial (DFM) to electrolyte scour treatment. Calves were assigned to three treatments based on the presence of scours: non-scouring, electrolyte, and electrolyte+DFM. Scouring calves received electrolyte for a mandatory two days. Blood was sampled from eight calves from each treatment on d 3, 7, 14, 21, 28, and 42 post-placement for an analysis of leukocyte populations by flow cytometry. Immune cell populations were determined using a panel of monoclonal antibodies specific for various cell surface markers, including, CD4 (T helper cells), CD8 (cytotoxic T and natural killer cells), CD25 (IL-2 receptor), CD62L (L-selectin), TCR1 (γδ T cell receptor), AM-2 (activated γδ T cells), CD45RO (memory T cells), CD172a (monocytes), and CD14 (LPS receptor). Activated immune cells ($CD8^-CD25^+$) were greater ($P = 0.05$) in electrolyte+DFM calves compared to electrolyte and negative control calves. L-selectin expression on leukocytes ($CD8^-CD62L^+$) was greater ($P = 0.05$) in calves on either electrolyte treatment compared to negative control calves. Electrolyte+DFM calves had a greater ($P = 0.05$) γδ T cell population ($CD8^-TCR^+$) compared to electrolyte and negative control calves. Calves provided the electrolyte treatment had a greater ($P = 0.05$) cytotoxic memory T cell ($CD8^+CD45RO^+$) population than the negative control calves; whereas, the electrolyte+DFM calves

showed an intermediate response between the two. The proportion of activated γδ T cells ($TCR1^+AM\text{-}2^+$) was greater ($P = 0.05$) in electrolyte+DFM calves compared to electrolyte and negative control calves. Monocytes expressing LPS ($CD172a^+CD14^+$) did not differ on d 3, 7, 21, and 42 post-placement, but on d 28 this population was lower ($P = 0.07$) in electrolyte+DFM calves compared to negative control calves (treatment x day interaction, $P = 0.10$). The results of this study indicate that supplementation with a *Bacillus*-based DFM at the incidence of scours has the potential to enhance innate and adaptive immune development in calves.

Key Words: Probiotic, Immunity, Bovine

466 Effects of an immunostimulatory feed additive on neutrophil function and development of titer in ruminant livestock. N. E. Forsberg*[1,3], Y. Wang[3], S. Puntenney[3], and J. Burton[2], [1]*Oregon State University*, *Corvallis*, [2]*Michigan State University*, *East Lansing*, [3]*OmniGen Research*, *Corvallis, OR*.

OmniGen-AF increases expression of molecular markers of innate immunity. These markers include neutrophil L-selectin, interleukin-1B and interleukin-8R. Ability of this product to augment expression of markers has been noted particularly in animals which have been immunosuppressed. Goals were to determine whether changes induced by the product at the molecular level translated into changes in neutrophil function and in development of titer against a vaccination program. In the first study, immunosuppressed sheep (0.1 mg Azium/hd/d) were fed either a control ration or a ration containing OmniGen-AF (5 g/d) for 31 d. At the end of the study, blood samples were recovered and neutrophils were purified using a Percoll gradient. Phagocytosis and respiratory burst assays were completed using commercial ELISA kits. In the second study, Angus cattle (275 kg) were assigned to one of three diets (0, 15 and 30 g/hd/d of OmniGen-AF). Calves were vaccinated with the Pfizer J5 E. coli vaccine on d 7, 21 and 35 and J5 titer was assessed within IgM, IgG1 and IgG2 fractions at d 56. Beginning on d 56 all animals were placed on the control diet and titer was again assessed on d 82. Neutrophils recovered from immunosuppressed sheep displayed enhanced function. Phagocytosis was increased ($P<0.01$) by 40% and respiratory burst activity was increased 2-fold ($P<0.01$). In growing cattle no differences in J5 titer were detected within the IgM fraction. At d 56, the additive did not affect ($P>0.05$) J5 titer in the IgG1 fraction; however, J5 titer in the IgG2 fraction was elevated 2-fold ($P<0.05$) in the animals fed 30 g/head OmniGen/d. At d 82, J5 titer in control-fed animals had declined to levels which were similar to those on d 0; however, animals which had been fed OmniGen-AF at the 15g or 30g levels had elevated J5 titer ($P<0.05$) in the IgG1 fraction. No differences ($P>0.05$) in J5 titer within the three experimental groups were noted in the IgG2 fraction on d 82. Anecdotal reports indicate benefit of the OmniGen-AF product for herd health. Enhanced neutrophil function and enhanced responses to vaccination programs may underlie these reports.

Key Words: Immunity, OmniGen-AF, Titer

467 Induction Of proinflammatory cytokines and constitutive expression Of Nramp1 in bovine blood neutrophils after exposure to *E. coli* endotoxin (LPS). A. Morris*, Z. Liu, and M. Worku, *North Carolina Agricultural and Technical State University*, *Greensboro*.

The pattern of constitutive and inducible expression of genes associated with the inflammatory response is important in defining innate (natural) resistance/susceptibility to diseases. Natural Resistance Associated Macrophage Protein1 (Nramp1) is expressed in macrophages and polymorphonuclear leukocytes. Studies have shown that Nramp1 is critical for resistance to several diseases. Mutations in Nramp1 are associated with increased disease susceptibility. Human polymorphonuclear leukocytes (PMN) are the major site of Nramp1 expression, followed to a lesser degree by monocytes (MN). Expression of Nramp1 by bovine PMN and the effect of mediators of inflammation such as *E. coli* endotoxin (LPS) have not been defined. The objectives of this study were to evaluate the expression of Nramp1 mRNA and determine how Nramp1 expression is regulated in bovine blood PMN upon LPS stimulation. Bovine blood PMN were isolated from three clinically healthy Holstein Friesian cows. The PMN were then incubated (37°C, 5% CO2, 15 min) in the presence or absence of LPS (100ng). Control cells were maintained in phosphate buffered saline (PBS). RNA was then isolated using Tri reagent (SIGMA) and the quality and quantity of RNA was determined using a Bioanalyzer (Agilent). Isolated RNA (600ng) was used to prepare cDNA (Ambion-Retroscript). Specific primers for Nramp1, IL-1β, IL-8 and GAPDH as loading control were used for RT-PCR. Nramp1 was constitutively expressed in both LPS and PBS treated bovine PMN. There was an induction of IL-1β and IL-8 genes upon LPS stimulation. These results indicate that Nramp1 is constitutively expressed in bovine blood PMN. No further up regulation was observed by exposure to LPS (100 ng) for 15 min. However this dose and time of exposure was sufficient to induce the transcription of the proinflammatory cytokines like IL-1β and IL-8. Variation was observed in the levels of gene expression between cows. Further studies will fine tune the response to activation by LPS.

Key Words: Polymorphonuclear Neutrophils, Nramp1, LPS

468 Growth performance and immunocompetence of heat stressed broilers fed different sources of dietary fatty acids. M. O. Smith*[1] and J. R. Bartlett[2], [1]*University of Tennessee*, *Knoxville*, [2]*Tuskegee University*, *Tuskegee, AL*.

One hundred and forty four male broilers were used to evaluate the effects of two sources of fatty acids on growth and immune competence of heat stressed broilers. Four replicate groups of twelve chicks each were assigned to three dietary treatments consisting of a basal diet supplemented with corn oil, fish oil (menhaden), or fish oil plus zinc and raised in battery brooders. On day 22, chicks were transferred to either a thermoneutral, TN (23.9 C) or a heat stress, HS (23.9 - 35 C, diurnal cycling) environmental chamber. Humoral immunity was assessed by injecting birds intravenously with 1 mL of 7% sheep red blood cell suspension followed by evaluation of sera for total, mercaptoethanol-resistant (ME-R), and mercaptoethanol-sensitive (ME-S) antibody titers. Cell-mediated immunity was assessed by using a sephadex stimulation method to recruit and harvest abdominal exudate cells (AEC), and phagocytic ability of macrophages determined. On day 50, birds were weighed, slaughtered, and thymus, spleen, liver, and bursa of Fabricius collected and weighed. Sources of dietary fatty acids did not impact growth, however, HS birds consumed 11% less feed and gained 8% less weight than TN birds. Bursa and thymus weights were reduced by 23% and 24% respectively in HS birds but diets had no effect. Number of AEC and incidence of macrophages were not affected by fatty acid source but the percent phagocytic

macrophages decreased under HS. Total ME-S (IgM) and ME-R (IgG) antibody titers were highest (P< 0.03) for fish oil + Zn while total and IgM titers were reduced (P<0.01) by HS. Results indicate that feeding fish oil can assist birds to mount an effective immune response during HS.

Key Words: Heat stress, Broiler, Immunocompetence

469 Immunopathology and cytokine responses in broiler chickens coinfected with eimeria maxima and clostridium perfringens using an animal model of necrotic enteritis. H. S. Lillehoj*[1], S. S. Park[1], P. C. Allen[1], S. FitzCoy[2], and D. A. Bautista[3], [1]*U.S. Department of Agriculture-ARS*, *Beltsville, MD*, [2]*Schering-Plough Animal Health*, *Millsboro, DE*, [3]*University of Delaware*, *Georgetown*.

The incidence of necrotic enteritis (NE) due to Clostridium perfringens (CP) infection in commercial poultry has been increasing at an alarming rate. While pre-exposure of chickens to coccidia infections is believed to be one of the major risk factors leading to NE, the underlying mechanisms of CP virulence remain undefined. The objectives of this study were to utilize an experimental model of NE produced by Eimeria maxima (EM) and CP coinfection to investigate the pathological and immunological parameters of the disease. Broilers coinfected with EM plus CP exhibited more severe gut pathology compared with animals given EM or CP alone. Additionally, EM/CP coinfection increased the numbers of intestinal CP bacteria compared with chickens exposed to an identical challenge of CP alone. Coinfection with EM and CP repressed nitric oxide synthase gene expression that was induced by EM alone, leading to lower plasma NO levels. Intestinal expression of a panel of cytokine and chemokine genes following EM/CP coinfection showed a mixed response depending on the transcript analyzed and the time following infection. In general, IFN-α, IFN-γ, IL-1β, IL-2, IL-12, IL-13, IL-17, and TGF-β4 were repressed, while IL-8, IL-10, IL-15, and LITAF were increased during coinfection compared with challenge by EM or CP alone. These results are discussed in the context of EM and CP to act synergistically to create a more severe disease phenotype leading to an altered cytokine/chemokine response than that produced by infection with the individual pathogens.

Key Words: Necrotic Enteritis, Clostridium Perfringens, Eimeria Maxima

470 Intestinal cytokine responses to Salmonella enterica serovar typhimurium infection in young chicks. Y. O. Fasina*[1], P. S. Holt[2], E. T. Moran[1], R. W. Moore[2], D. E. Conner[1], and S. R. Mckee[1], [1]*Auburn University*, *Auburn, AL*, [2]*USDA-ARS Egg Safety & Quality Research Unit*, *Athens, GA*.

Vaccination has been proposed as one of the best ways to control Salmonella infection in poultry. Cytokines are essential effector molecules for innate immunity and have been proposed as adjuvants that can improve efficacy of vaccines. Thus, we designed an experiment to determine the effect of Salmonella typhimurium (ST) infection on the expression of selected pro-inflammatory cytokines (IL-1, IL-6, IFNy) and IL-10 (an anti-inflammatory cytokine) in the intestine of chicks. A 14-day experiment was conducted using 112 day-old chicks. Chicks were randomly allocated to 2 treatments; treatment 1 (CN) consisting of chicks that were not challenged with ST, and treatment 2 (STC) consisting of chicks that were challenged with ST at 4 days of age. Chicks were fed a corn-soybean meal diet. On days 4 and 9 post-challenge (PC), intestinal ST levels were enumerated on XLT4 agar. On days 5 and 10 post-challenge, intestinal tissue samples were collected (from jejunum, ileum and cecum) and analyzed by quantitative Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) procedure to determine the level of expression of cytokine genes. CN chicks remained ST-free throughout the experiment. Intestinal level of ST in STC chicks was 4.05 ± 3.30 log10 CFU at 4 days PC, indicating that ST challenge was successful. Expression levels (mRNA levels) of the pro-inflammatory cytokines were higher in STC chicks compared to CN chicks in all tissues. Specifically, at 10 days PC, jejunal fold change in mRNA was highest ($p < 0.05$) for IL-6 (2.62). Also, cecal fold change in mRNA was highest ($p < 0.05$) for IL-1 (1.71). In all tissues, IL-10 had the lowest mRNA levels. It was concluded that ST infection induced an inflammatory immune response characterized by increased expression of proinflammatory cytokines (IL-1, IL-6, and IFNy).

Key Words: Salmonella Typhimurium, Pro-inflammatory Cytokines, Broiler Chicks

471 Comparative expression of activin receptor type IIB in bovine peripheral blood mononuclear cells. S. Tanaka*, S. Hayashi, Y. Taketa, M. Miyake, K. Watanabe, S. Ohwada, H. Aso, and T. Yamaguchi, *Laboratory of Functional Morphology, Graduate School of Agricultural Science, Tohoku University*, *Sendai, Japan*.

Myostatin, a member of the transforming growth factor-β (TGF-β) superfamily, acts mainly as a negative regulator of skeletal muscle mass through the signal transduction of activin receptor type IIB (ActRIIB). Double-muscled (DM) cattle with mutations of myostatin gene result in a marked muscle hypertrophy. TGF-β1 is a critical factor in regulation of T cell-mediated immune responses and in the induction of immune tolerance. In addition, Activin A, which belongs to the same TGF-β superfamily member as myostatin, is involved in inflammatory responses. These findings indicate that myostatin may be related to immune responses. The present study was conducted to investigate the comparative expression of ActRIIB in bovine peripheral blood mononuclear cells (PBMC). The peripheral blood was collected from Holstein cattle (n=3). PBMC were prepared by density gradient centrifugation using Lympholite-H, and T cells, B cells and monocytes were isolated by magnetic cell sorting (MACS) method. Total RNA was extracted from MACS-isolated T cells, B cells and monocytes and the mRNA expression of ActRIIB was analyzed by PCR method with bovine specific primers. Furthermore, ActRIIB positive cells in PBMC, T cells, B cells and monocytes were analyzed by flow cytometry using anti-bovine CD3, anti-bovine BB2, anti-bovine CD14 and anti-human ActRIIB antibodies. The ActRIIB mRNA was expressed in T cells, B cells and monocytes but less in B cells. The ActRIIB positive cells in PBMC were 31.0±3.9%. The percentages of ActRIIB positive cells were 48.0±3.4%, 24.1±2.0% and 53.47±4.7% in T cells, B cells and monocytes, respectively. Thus, ActRIIB was preferentially expressed in T cells and monocytes. These results strongly suggest that myostatin primarily acts on T cells and monocytes, and regulates the function of their cell types to mediate immune responses in cattle.

Key Words: Activin receptor type IIB, Lymphocytes, Bovine