

## Animal Health: Immunity and health

**55 Effect of dietary organically bound selenium and D- $\alpha$ -tocopherol acetate bolus on serum antioxidants status of transit stressed wether lambs.** N. K. Chirase<sup>\*1,2</sup>, J. B. Taylor<sup>3</sup>, T. Thelen<sup>3</sup>, and L. W. Greene<sup>1,2</sup>, <sup>1</sup>Texas Agricultural Experiment Station, Amarillo, <sup>2</sup>West Texas A&M University, Canyon, <sup>3</sup>Agriculture Research Service, Dubois, ID.

Animals often encounter many stressors and pathogens associated with current production systems which could compromise the antioxidant and immune defense systems. An experiment was conducted to determine the effects of pretransit dietary Se (provided by wheat grain; Se = 6.1 mg/kg) and daily D- $\alpha$ -tocopherol acetate bolus (TOCO; 3.8 IU/kg BW) on pre- and posttransit serum free retinol (VitA),  $\alpha$ -tocopherol (aVitE),  $\gamma$ -tocopherol (gVitE), Se concentrations (ug/ml), and partial antioxidant capacity (PACA) of wether lambs. Twenty-nine lambs (BW = 27 0.36 kg) of similar type and origin were weaned, stratified by BW; assigned randomly to and fed one of the following treatments: adequate Se (< 0.3 mg/kg), no TOCO (CON; n = 9); high Se, no TOCO (HSE; n = 9); adequate Se, TOCO (HVE; n = 5); high Se, TOCO (SEVE; n = 6). Diets for all treatments were of similar nutrient composition, isonitrogenous and isocaloric. Lambs were fed the diets for 20 d pretransit and fed a common diet for an additional 21 d posttransit. Blood samples were taken on d 0, 7, 14 and 20 d and the serum harvested was used for Se, VitA, aVitE, gVitE and PACA assays. On d 21, lambs were transported (864 km) by truck and a trailer for 12 h after 24 h of fasting. Sampling and analysis procedures were repeated every 7 d for 21 d posttransit. The data were analyzed using Mixed Models procedures of SAS. Weaning stress (7 d post weaning) depressed (P < 0.05) serum aVitE concentrations and PACA of all treatment groups, except those fed SEVE. Lambs fed HVE and SEVE had 1.6 and 2.0 times greater (P < 0.01) serum aVitE concentrations than those fed CON or HSE pretransit, respectively but decreased (P < 0.05) posttransit. Serum Se increased linearly pretransit, and subsequently decreased posttransit in HSE and SEVE fed groups. Weaning and transit stress altered serum antioxidant concentrations of lambs.

**Key Words:** Lambs, Organically bound selenium,  $\alpha$ -tocopherol

**56 Intracellular glutathione concentration in bovine natural killer cells after infection with bovine respiratory syncytial virus or bovine viral diarrhoea virus.** L. A. Matulka<sup>\*1</sup>, L. Wilkie<sup>2</sup>, C. Kuszynski<sup>2</sup>, S. Justice<sup>1</sup>, D. Wylie<sup>1</sup>, K. M. Eskridge<sup>1</sup>, D. R. Brink<sup>1</sup>, and C. L. Kelling<sup>1</sup>, <sup>1</sup>University of Nebraska, Lincoln, NE, <sup>2</sup>University of Nebraska Medical Center, Omaha, NE.

Glutathione (GSH), a cysteine-containing tripeptide, is found in millimolar concentration in all mammalian cells. Cellular glutathione concentrations may be altered nutritionally, since cysteine availability is markedly influenced by diet. Glutathione deficiency contributes to immunological dysfunction in human immunodeficiency virus-infected patients. A similar mechanism may underlie pathogenesis of bovine respiratory disease complex (BRDC). In BRDC, *Mannheimia haemolytica* colonization of lungs resulting in pneumonia may be triggered by impaired host immunological responses following infection with bovine respiratory syncytial virus (BRSV) and/or bovine viral diarrhoea virus (BVDV). Bovine peripheral blood mononuclear cells (PBMC) were infected with BRSV and BVDV to determine effect on intracellular glutathione concentration. Peripheral blood was obtained from a donor animal and mononuclear cells by Ficoll-Paque density centrifugation. Cells were infected with either BRSV, BVDV, or left untreated and incubated for 48 h at 37° C and 5% CO<sub>2</sub>. Samples were enriched for natural killer (NK) cells (1000U/ml of interleukin-2 during 48 h incubation). After incubation, cells were stained with antibodies to identify NK cells (CD2+, CD3-). NK cells were stained with monochlorobimane and intracellular GSH levels were determined as the fluorescence produced from the GSH-S-transferase conjugation of monochlorobimane with GSH. Intracellular GSH levels were decreased in the BRSV and BVDV infected NK cells compared to the control (P<.01). Reduced GSH levels in NK cells may contribute to development of BRDC.

	Control	BRSV	BRSV+IL-2	BVDV	BVDV+IL-2	SE
MFU <sup>c</sup>	48.7 <sup>a</sup>	15.9 <sup>b</sup>	17.0 <sup>b</sup>	14.7 <sup>b</sup>	14.6 <sup>b</sup>	4.6
						n=4

<sup>a, b</sup>Means with different superscripts differ (P<.01) <sup>c</sup>Mean Fluorescence Units

**Key Words:** Glutathione, Natural killer cells, Bovine

**57 Effects of intravenous infusion of triglyceride emulsions varying in lipid source on lymphocyte functions in the bovine.** D. Scalia<sup>1</sup>, U. Bernabucci<sup>\*1</sup>, D. G. Mashek<sup>2</sup>, B. Ronchi<sup>1</sup>, R. R. Grummer<sup>2</sup>, and N. Lacetera<sup>1</sup>, <sup>1</sup>Universit della Tuscia, Viterbo, Italy, <sup>2</sup>University of Wisconsin, Madison.

Previous in vitro studies from our laboratory have shown that fatty acids represented in plasma NEFA affect immune functions both in sheep and bovine. However, little is known in ruminants about the effects of fatty acids in vivo. Therefore, our objective was to assess the effects of intravenous infusion of triglyceride (TG) emulsions derived from different lipid sources on peripheral blood mononuclear cell (PBMC) functions during a period of fatty liver induction. Six multiparous, non-pregnant, non-lactating Holstein cows were used in a replicated 3x3 Latin Square design. For 4 d, cows were fasted and infused intravenously with a 20% TG emulsion derived from linseed oil (LO), fish oil (FO), or tallow (Ta). The emulsions were administered for 20 to 30 min every 4 h throughout the 4 d fast at a rate of 0.54 g TG/kg BW/d. Blood samples were taken before the first infusion, and then every 24 h during the fast. Cows were fed ad libitum for 24 d between the fasts. After isolation, the PBMC were stimulated by phytohemagglutinin (PHA), concanavalin A (ConA), or pokeweed mitogen (PWM). For all the three mitogens, DNA synthesis was lower (P < 0.05) for Ta than for LO and FO. A significant time\*treatment interaction was pointed out. Seventy two and 96 h after first infusion of FO, the DNA synthesis stimulated by PHA increased (P < 0.01). Regardless the mitogens, the infusion of Ta was responsible for a transient and dramatic reduction (P < 0.01) of DNA synthesis, which was evident on 48 and 72 h after first infusion. Infusion of LO did not affect (P > 0.10) the DNA synthesis of PBMC. Results reported here confirm those of in vitro studies and indicate that fatty acids can modify the immune cell functions of cows in a way, which depends primarily on the type of fatty acids.

**Key Words:** Lymphocyte, Fatty acids, Bovine

**58 Lymphocyte functions in obese cows during transition period.** U. Bernabucci<sup>\*1</sup>, D. Scalia<sup>1</sup>, B. Ronchi<sup>1</sup>, D. Pirazzi<sup>1</sup>, A. Nardone<sup>1</sup>, and N. Lacetera<sup>1</sup>, <sup>1</sup>Universit della Tuscia, Italy.

A previous study carried out in our laboratory has shown negative relationships between the intensity of lipomobilization and the immune response in sheep. However, little is known about the relationships between body score and immune functions in cows. Therefore, the objective of this study was to evaluate the effects of body score on lymphocyte functions in transition dairy cows. The study was carried out in 21 Holstein cows. Thirty days before the expected calving, the 21 cows were categorized as thin (n = 6), medium (n = 8), and obese (n = 7) on a condition score basis. Fourteen and 7 d before and 14 and 35 d after calving, blood samples were taken, and peripheral blood mononuclear cells (PBMCs) were isolated. After isolation, the PBMCs were stimulated, and DNA synthesis, immunoglobulin M (IgM), and interferon gamma (IFN-gamma) secretion were evaluated. The DNA synthesis was measured after stimulation with phytohemagglutinin (PHA), concanavalin A (ConA), or pokeweed mitogen (PWM); the IgM secretion was measured after stimulation with PWM; the IFN-gamma secretion was measured after stimulation with ConA. Data referred to the 21 cows, indicated that the DNA synthesis was lower (P < 0.01) on day 7 before calving, that the IgM secretion on day 7 before calving was lower (P < 0.05) than that recorded after calving, and that the IFN-gamma secretion did not change (P > 0.05) during the experimental period. Either on day 14 or 35 after calving, the IgM secretion in obese cows was lower (P < 0.01) compared to that recorded in thin cows. Seven days before calving, the IFN-gamma secretion was lower (P < 0.001) in obese cows compared to thin and medium cows. In conclusion, the immunodepression taking place in cows around calving would be more evident in overconditioned cows.

**Key Words:** Body score, Lymphocyte, Cow

**59 In vitro modulation by beta-glucan and ascorbic acid of blood leukocyte toll-like receptor and acute phase cytokine expression.** S. D. Eicher<sup>\*1</sup>, T. R. Johnson<sup>2</sup>, and K. A. McMunn<sup>1</sup>, <sup>1</sup>USDA-ARS, West Lafayette, IN, <sup>2</sup>Purdue University, West Lafayette, IN.

In a previous study, neutrophil functions were decreased for calves fed a non-water soluble beta-glucan product derived from *Saccharomyces cerevisiae* in conjunction with ascorbic acid at day 7 and 28 post-transport. The objective of this study was to determine if in vitro stimulation of whole blood leukocytes with that beta-glucan plus ascorbic acid was dependent on the age of the calf. Blood samples were taken from 12 non-transported Holstein calves at 1, 3, 7, 10, 14, 18, 21, 24, and 28 days-of-age. Leukocytes were stimulated with ascorbic acid (0.3 ug/ml) and beta-glucan (0.4 ug/ml) for 1 hour, red blood cells were lysed, and RNA extracted. The RNA was subjected to real-time RT-PCR for quantification of the expression of interleukin-1 (IL-1) and its receptor antagonist (IL-1Ra), tumor necrosis factor-alpha (TNF), and toll-like receptors 2 and 4 (TLR2 and TLR4). TLR2 and TLR4 had treatment effects ( $P < .05$ ), but not day or treatment by day interactions. TLR2 was greater ( $P < .05$ ) for treated cells on day 7, 14, and 24 and tended ( $P < .10$ ) to be greater on day 10. In contrast, TLR4 was only greater for treated cells on day 7 ( $P < .05$ ) and tended to be ( $P < .10$ ) on day 24. IL-1 had a treatment main effect and a treatment by day interaction ( $P < .05$ ), but IL-1Ra had main effects for treatment and day ( $P < .05$ ) and only a trend ( $P < .10$ ) for a treatment by day interaction. IL-1 was greater for treated cells ( $P < .05$ ) on all but day 4. IL-1Ra was greater ( $P < .05$ ) for treated cells only on days 1, 7, 10, and 24. TNF was only different for a main effect of day ( $P < .05$ ), but not for treatment or treatment by day interaction. Only IL-1 and its receptor antagonist expression were stimulated on day 1. On days 7 and 24 all tested receptors and cytokines had increased RNA expression. So, it appears that there are periods during which the blood leukocytes may be refractory for increased RNA expression of cytokines and toll-like receptors in response to beta-glucan and ascorbic acid stimulus.

**Key Words:** Innate immunity, Ascorbic acid, Beta-glucan

**60 An evaluation of rumen-protected choline and a monensin controlled release capsule on the health and metabolic function of periparturient dairy cows.** L. C. Zahra<sup>\*1</sup>, T. F. Duffield<sup>1</sup>, S. J. LeBlanc<sup>1</sup>, K. E. Leslie<sup>1</sup>, T. Overton<sup>2</sup>, and D. Putnam<sup>3</sup>, <sup>1</sup>Department of Population Medicine, Guelph Ontario, Canada, <sup>2</sup>Department of Animal Science, Ithaca NY, <sup>3</sup>Balchem Corporation, Slate Hill NY.

During early lactation, high-producing dairy cows undergo a phase of negative energy balance. This can lead to metabolic disorders and subsequently cause losses in production. To prevent this, ionophores are often administered to ruminants. Administration of monensin controlled release capsules (CRC) in early lactation cows improves energy balance, while choline aids in fat metabolism and transport. Choline, however, can be a limiting nutrient in lactating dairy cows. The objective of this study was to determine whether there is an interaction between these two supplements on metabolic parameters. In this study, 53 primiparous and multiparous Holsteins were randomly assigned to receive a monensin CRC 3 weeks before their expected calving date, or a topdress of 56g rumen-protected choline (RPC; Reashure<sup>®</sup> choline, Balchem Encapsulates, New Hampton, NY) once daily from 3 weeks before calving until 28 DIM, or both (RPC & CRC), or neither (CON).

**62 Transhumance and dry-season supplementation for cattle in the Sahel.** S. Fernandez-Rivera<sup>\*</sup>, A. Salla, P. Hiernaux, and T. Williams, *International Livestock Research Institute, Addis Ababa, Ethiopia.*

We assessed the effect of dry-season supplementation and seasonal transhumance on ADG and weaning rates of cattle in the Sahel. 108 cows (60 in Katanga and 48 in Guro-Yena, 50 km East of Niamey, Niger) were allotted to 6 treatments, i. e. factorial combinations of 3 supplement levels (0, 360 and 720 g DM/d of millet bran, 16% CP) and two management systems (year-long sedentary management and transhumance to the pastoral zone during the rainy season and to intensely cultivated areas after grain harvest). The study lasted 4 years (1999-

Blood samples were collected from the tail vein at enrollment, one week before calving, and in the first and second weeks post-calving. Body condition (BCS) was scored at enrollment and in the second week after calving. Liver biopsies were obtained from multiparous cows randomly selected from each treatment group within 48 hours of calving and 3 weeks post-calving. Daily milk records up to 60 DIM and health records were obtained. Adjusting for parity and BCS at enrollment, beta-hydroxybutyrate (BHB) levels in the first week post-calving were lower in the RPC and CRC groups than controls (934, 916 and 1466  $\mu\text{mol/L}$  respectively,  $P = 0.05$ ). Non-esterified fatty acids (NEFA) in the first week post-calving were lower in each group than in controls ( $\text{CON} = 0.76$ ,  $\text{RPC} = 0.38$ ,  $\text{CRC} = 0.47$ ,  $\text{RPC} \& \text{CRC} = 0.63$  mEq/L,  $P \leq 0.03$ ). Plasma aspartate transaminase (AST) in the RPC group was 73 U/L compared to 103 U/L in the RPC & CRC group at the first week post-calving ( $P = 0.01$ ). Overall, urea levels were lower in the CON group (3.95 mmol/L) than either the RPC (4.53 mmol/L) or the RPC & CRC groups (4.65 mmol/L) ( $P \leq 0.05$ ). There were no significant differences in blood glucose or cholesterol levels between the treatment groups.

**Key Words:** Dairy, Choline, Metabolism

**61 Metabolism and gastric transport of ergot alkaloids in ruminants grazing endophyte-infected tall fescue.** N. S. Hill<sup>\*1</sup>, A. W. Ayers<sup>1</sup>, J. A. Stuedemann<sup>2</sup>, F. N. Thompson<sup>1</sup>, P. T. Purinton<sup>1</sup>, and G. Rottinghaus<sup>3</sup>, <sup>1</sup>University of Georgia, <sup>2</sup>USDA-ARS, J. Phil Campbell Natural Resources Laboratory, <sup>3</sup>University of Missouri.

Livestock grazing endophyte-infected (E+) tall fescue suffer from chronic ergot alkaloid toxicity. Ergovaline is repeatedly implicated as the toxin causing the anomaly, but little or no credible evidence exists as such. Towards that end our objective was to examine gastric metabolism and transport of ergot alkaloid in E+ tall fescue. First, in vitro ruminal digests of E+ and E- tall fescue were conducted for 0, 6, 12, 24, and 48 h and alkaloids in the aqueous fraction analyzed by ELISA and HPLC. Extracted alkaloids from the ruminal digests were tested for in vitro transport across ruminal and omasal tissues using parabiotic chambers. Secondly, three sheep each grazing E+ and E- tall fescue were anaesthetized and their right ruminal, right gastric, and mesenteric veins surgically catheterized. Whole blood was collected, plasma alkaloids extracted, and analyzed by ELISA. ELISA analysis from the ruminal digests found no alkaloids in ruminal fluids from E- tall fescue, but alkaloids in ruminal fluids from E+ tall fescue increased with time ( $P < .01$ ). HPLC speciation of alkaloids in E+ ruminal fluids found only 9 ppb ergovaline at 0 h, which decreased to 1 ppb at 6 h. Conversely, lysergic acid concentration increased from 20 ppb at 0 h to 240 ppb at 48 h ( $P < .01$ ). Lysergic acid was the only ergot alkaloid that transported across ruminal or omasal tissue. More lysergic acid transported across ruminal tissue than omasal tissue ( $P < 0.1$ ) in the in vitro system. In the in vivo study, there were no differences ( $P > .05$ ) in plasma ergot alkaloids from mesenteric or gastric veins regardless of whether sheep were grazing E+ or E- tall fescue. However, plasma sampled from the ruminal vein of sheep grazing E+ tall fescue had more (13.9 ppb) than that of sheep grazing E- tall fescue (0.56 ppb) ( $P < .01$ ). These data indicate lysergic acid, not ergovaline, is the toxin causing fescue toxicosis and its site of absorption is the rumen.

**Key Words:** Fescue toxicosis, Tall fescue, Alkaloid metabolism

## International Animal Agriculture

2002) in Katanga and 3 years (2000-2002) in Guro-Yena. Cows were individually supplemented for 103-134 d each year and weighed monthly, at the start and end of supplementation and at departure to and return from transhumance to the pastoral zone (98-124 d) and to the intensely cultivated areas (33-55 d). Weight loss (g/d) of non-lactating, non-late-gestating cows in the dry season was highest (-380 $\pm$ 13) in 2002 and lowest (-258 $\pm$ 16) in 1999 ( $P < 0.01$ ). Over 4 years, cows receiving 0, 360 and 720 g supplement/d had ADG during the dry season of -384 $\pm$ 10, -321 $\pm$ 12 and -282 $\pm$ 12 g/d, respectively ( $P < 0.01$ ). Providing 1 kg millet bran/d decreased ( $P < 0.01$ ) weight loss by 145 $\pm$ 21 g/d. ADG (g/d) during the rainy season was lowest (428 $\pm$ 20) in 2002 and highest (704 $\pm$ 19) in 2001 ( $P < 0.01$ ). Cows receiving 0, 360 and 720 g/d supplement during the previous dry season had ADG during the rainy season of 605 $\pm$ 16,