

pelleting, increases foregut starch digestibility, possibly mitigating effects of starch source on bacterial communities. The aims were to (i) determine the effect of starch source in pelleted concentrates on *Lactobacillus* spp., total starch utilizing bacteria (TSU), and cellulolytic bacteria in mares, and (ii) evaluate pre- and postpartum changes in fecal bacterial communities from 324 d of gestation to 28 d postpartum. Nineteen Thoroughbred mares were paired by last breeding date then randomly assigned to either an oat-based (OB) or a corn and wheat middlings-based (CWB) pelleted concentrate in addition to forage. Beginning at 310 d of gestation, mares were fed 3.2 kg/d (DM) of assigned concentrate (OB or CWB). After parturition, concentrate intake gradually increased to 4.8 kg/d (DM). The concentrates contained 38.0%, 36.2% starch, 6.6%, 8.8% WSC, and 5.4%, 7.5% ESC for OB and CWB, respectively. Fecal samples were collected at 324 d of gestation, before parturition, 24 h, 14 d, and 28 d postpartum. Fecal samples were collected immediately after defecation by catch or from the center of the pile into single use plastic bags and transported to the lab in an insulated cooler (37°C) under CO<sub>2</sub>. Samples were serially diluted 10-fold with phosphate buffered saline and the dilutions were used to inoculate selective media. Selective media were used for enumeration of *Lactobacillus* spp., TSU, and cellulolytic bacteria. Data were log transformed then analyzed with PROC MIXED (SAS 9.3) to test the main effects of treatment (OB or CWB), time of sample, and treatment by time interaction. Results were considered significant when  $P < 0.05$ . There was no effect of starch source on enumerated bacterial communities ( $P > 0.05$ ), in contrast to previous work. These results suggest that pelleting concentrates may alter some of the effects of starch sources. There was no effect of time on TSU ( $P > 0.05$ ), however *Lactobacillus* spp. and cellulolytic bacteria decreased 24 h postpartum ( $P < 0.05$ ). Therefore, major physiological events, such as parturition, appear to alter the hindgut microbiota.

**Key Words:** bacteria, concentrate, horse

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## HORSE SPECIES SYMPOSIUM: NUTRITION AND IMMUNOLOGY

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### 0815 Nutritional immunology for the geriatric horse.

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Over the past century, improvements in health care and advancements in science and medicine have extended the average lifespan of humans and companion animals, including horses. We are now facing new challenges with the paradox of an older horse population with increased longevity and the potential of increased age-associated diseases. One of the most recognized consequences of aging is a decline in function of the immune system. Two main terms that characterize

a declining immune system of the old horse are immunosenescence and inflamm-aging. Immunosenescence in the aged individual is characterized by changes in various aspects of cellular and humoral immunity, in particular a decline in lymphoid cell numbers and function. It has been well documented that the aged, including horses, have increased susceptibility to and prolonged recovery from infectious disease, poor vaccine responses, and increased incidence of cancers. Somewhat paradoxically, advanced age is also associated with increased production of pro-inflammatory cytokines and other inflammatory mediators, a phenomenon termed inflamm-aging. Inflamm-aging predicts both increased morbidity and mortality for a variety of chronic diseases. Together, immunosenescence and inflamm-aging may increase susceptibility to infection and contribute to aged-related health conditions such as arthritis, equine Cushing's disease, and laminitis. Nutritional immunology is a new field of study, in which nutrition is used as a modifiable factor in impacting immune function in particular to delay/reverse immunosenescence and to improve the aged resistance to infection. Further, nutritional interventions are practical, cost-effective approaches to mitigating this age-related breakdown in immune function. Natural dietary compounds found in a variety of plants, roots, fruits, vegetables, nuts, and seeds are promising candidates in helping to combat the effects of an aging immune system. Several natural dietary compounds (carotenoids, flavonoids, isothiocyanates, terpenoids, proanthocyanidins, omega fatty acids, and polyphenolic compounds) have been shown to possess broad biological activities of anti-oxidation, anti-inflammation, detoxification, regulation of signaling pathways, modulation of enzyme activities, and improvement of immune responses to vaccination. Unfortunately, few studies have been conducted to better understand what effect nutrition may have on modulating or improving immune responses of the aged horse. Previous and current nutritional studies to improve immune function in old horses by supplementation with vitamin E, n-3 polyunsaturated fatty acids (DHA), prebiotics, and polyphenols will be reviewed here. More research is needed to identify effective and optimal conditions for various nutritional intervention regimens to improve the function of the aged immune system of the horse.

**Key Words:** horse, aging, immune

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### 0816 Nutrition and immunity: General principles.

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The development, maintenance, and response of the immune system are influenced by nutrition. For most nutrients the most important nutritional strategy for optimizing immunity is meeting the established requirements for maximizing growth, reproduction, and feed efficiency and avoidance of traditional signs of deficiency. Severe deficiencies of required nutrients typically impair host immunity and resistance to disease, but such deficiencies should be rare in modern animal production.

More relevant are marginally deficient and surfeit levels of nutrients. In the case of several essential nutrients, leukocytes, especially T lymphocytes, are very sensitive to marginal deficiencies, while for many other nutrients the immune system is largely unaffected by marginal deficiencies. This difference in sensitivity is due the types and amounts of nutrient transporters expressed on each cell type. Nutrients also support the anabolic processes of pathogens and increase their pathogenicity, though this troublesome effect is likely limited to a small subset of nutrients. Iron in particular can increase the pathogenicity of some pathogens when provided in excess. Several essential and nonessential nutrients have regulatory effects on leukocytes. Required nutrients with indisputable immunoregulatory actions in rodents and livestock include the long-chain polyunsaturated fatty acids (PUFA) and vitamins A, D, and E. Many nutrients that are not normally considered as being dietary essential also modulate immunity, including carotenoids, vitamin C, and phytonutrients (e.g., capsicum, genistein, curcumin, essential oils, conjugated linoleic acids). In general those nutrients that are not structural components or cofactors for enzymes are most likely to be immunomodulatory. Unlike increases in nutrients from deficient to sufficient levels, where many indices of immunocompetence go from impairment to normal function, supplementation of immunomodulatory nutrients causes some components of immunity to be elevated and others to be diminished; in other words, the type and intensities of responses have been changed (i.e., immunomodulated). In situations where a single infectious disease dominates the production losses and where it is clearly known what type of immune response optimally protects against that disease, supplementation of a nutrient that modulates the immune system toward that optimal response is indicated. Thus, the value of an immunomodulatory nutrient to “improved” immunity is context dependent and depends on the types of disease challenges in a herd. In summary, nutritional impacts on immunity are complex and their understanding and applications requires a “first principles” approach.

**Key Words:** disease, immunity, deficiency

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**0817 Optimizing nutrition to improve immune function in horses.** L. K. Warren\*, *University of Florida, Gainesville.*

Nutrition plays a supportive role in immunity; thus, a balanced diet is critical to mount an appropriate immune response to infection or trauma. Many nutrients have widely recognized roles in host defense; however, nutrient requirements for horses who are stressed or immunocompromised are not fully known. Owners and farm managers are increasingly interested in holistic approaches to maintaining the health of horses in their care, which has encouraged research on the impact of various nutrients and dietary supplements on immune function. Investigations have typically targeted three populations: performance horses, foals, and horses with

compromised or inappropriate immune responses (e.g., senior horses, horses with recurrent airway obstruction). Sport horses face many immunosuppressive stressors, including strenuous exercise training, frequent competition, and transport over long distances, including international shipping. This group also has increased exposure to pathogens via contact with outside horses at competition venues. Foals present a different set of challenges, which center on delayed onset of adaptive immune responses. Protection against pathogens are provided to the foal through the ingestion of immunoglobulins in colostrum soon after birth; thus, investigations often focus on the diet of the mare as a means to improve colostrum quality. Immuno-nutrition research in horses has typically been inspired by positive outcomes observed in other species. Although a comparatively small body of research has been conducted in horses, several nutrients have been explored, including vitamins (E, C), trace minerals (Zn, Se), amino acids (arginine), and fatty acids (omega-3, omega-6, conjugated linoleic acid). Additionally, prebiotic fibers (mannan-oligosaccharides,  $\beta$ -glucans), probiotics (lactic acid bacteria, live yeast cultures), and nonnutritive dietary supplements (resveratrol, superoxide dismutase) have received some attention in equine research. Collectively, the impact of these nutrients and supplements on the status and functional capacity of the immune system have been variable. Differences in study outcomes may be due to high variability in responses among horses, health status, dosage, length and timing of supplementation, basal diet composition, type of immune system challenge evaluated, and immunological variables measured. The latter is often more limited in scope in equine compared to other livestock research, where tissues can be harvested postmortem for more detailed evaluation of response to diet. Ultimately, the study of nutrition’s impact on immunity in horses is in its infancy, with many other nutrients to explore and much to learn. Pairing equine nutritionists with immunologists could hasten research progress and improve study effectiveness.

**Key Words:** equine, immuno-nutrition, diet

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**0818 Effect of selenium and vitamin E supplementation on blood glutathione peroxidase activity and selenium in moderately exercised horses.**

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The objective was to evaluate the effect of selenium (Se, Se-yeast) and vitamin E (E,  $\alpha$ -tocopheryl acetate) supplementation on red cell glutathione peroxidase activity (GPx) and Se blood concentrations in moderately exercised horses. The Institutional Animal Care and Use Committee of the School of Veterinary Medicine, National University of Mexico, approved the experimental procedures. Twenty-four clinically healthy horses (5–15 yr, 450 kg BW) from the Mexico City Police, without physical activity the month before this trial,

Table 0818.

Blood glutathione peroxidase activity (GPx, U/L) and selenium (Se, ng/mL) in supplemented moderately exercised horses

Day	Adaptation period					Exercise				Re-adaptation period		
	0	7	14	21	28	35	42	49	56	Unsupplemented		
	63	70	77									
GPx <sup>1</sup> (effect of interaction day×Se×E)												
LSeLE <sup>2</sup>	1179	1071	1009	1069	1343	1513	1734	1516	1318	1114	1031	851
LSeHE	1082	971	899	935	1117	1559*	1564*	1484*	1631	1050	1002	876
HSeLE	1235	1112	1126	1015	1397	1638	1757	1598	1660	1201	1054	951
HSeHE	1422	1331	1261	1132	1502	1869*	1955*	1862*	1705	1285	1163	994
Se <sup>3</sup> (effect of day)												
	41.9 <sup>d</sup>	61.6 <sup>cd</sup>	74.0 <sup>cd</sup>	117.0 <sup>b,c</sup>	210.8 <sup>a</sup>	118.4 <sup>b</sup>	76.9 <sup>cd</sup>	80.7 <sup>b,c,d</sup>	105.1 <sup>b,c</sup>	60.4 <sup>cd</sup>	76.1 <sup>cd</sup>	54.6 <sup>cd</sup>

<sup>1</sup>SEM: GPx, 98.9; Se, 7.0.

d0, baseline concentrations

<sup>2</sup>LSe, 0.1; HSe, 0.3 mg Se/kg DM; LE, 1.6; HE, 2.0 IU/kg BW<sup>\*</sup>Groups LSeHE and HSeHE are different ( $P < 0.05$ ) within the same column<sup>3</sup>Mean values within a line with different letters are different ( $P < 0.05$ )

were used. They were individually stabled and randomly allocated in a factorial experiment (2 Se × 2 E levels) with repeated measures. The groups, with 6 experimental units in each one, were: LSeLE, HSeLE, LSeHE, and HSeHE (LSe, 0.1; HSe, 0.3 mg Se/kg DM and LE, 1.6; HE, 2 IU vitamin E/kg BW; NRC, 2007). Se and E were given to supplement the deficient daily ration (Se, < 2µg; E, 14.4IU by kg DM, respectively). The study lasted 77 d distributed in 3 periods: adaptation (d 0 to d 32); moderate exercise (d 33 to d 56) and readaptation (d 57 to d 77). Exercise period consisted of 30 min (5:20:5 min. warm up: gallop: cool down) in 3 consecutive days and 4 d without exercise. At d 64, supplementation was stopped. Once a week, jugular blood samples were taken; during the exercise period it was taken 10 min after activity of the third day. Day zero corresponded to baseline measurement of studied variables. GPx was quantified by spectrophotometry (Randox Daytona) while Se was quantified by hydride generation atomic absorption spectrometry. Data were analyzed by a mixed model (PROC MIXED, SAS 9.1.3) with the design described above. Day, Se, E, and their interactions were the fixed effects, while horse nested in treatment was the random effect. Statistical significance was set at  $P < 0.05$ . Tukey-Kramer's test was used to compare LSM. The interaction: day × Se × E affected ( $P < 0.05$ ) blood GPx; differences were observed at d 35–49 between HSeHE and LSeHE groups ( $1915.0 \pm 65.9$  U/L;  $1510.1 \pm 54.1$  ng/mL, respectively). Day and Se affected blood Se ( $P < 0.05$ ). Values from d 0 to d 14 were not different ( $P > 0.05$ ) from d 63 to 77 ( $61.4 \pm 2.1$  ng/mL). Blood Se increased at d 28 ( $210.8 \pm 7.0$  ng/L) and decreased again at d 35 ( $118.38 \pm 7.0$  ng/L). In general, Se was higher in HSe ( $97.5 \pm 3.8$  ng/mL) than in LSe horses ( $82.01 \pm 3.8$  ng/mL). Conclusion: supplementation levels of both Se and E affect blood GPx and Se and are closely related to physical activity.

**Key Words:** selenium,  $\alpha$ -tocopheryl, glutathione peroxidase

### 0819 Age-related changes in select fecal bacteria

**in foals.** M. B. Pyles<sup>1</sup>, A. L. Fowler<sup>1</sup>, V. Bill<sup>1</sup>, B. E. Harlow<sup>1,2</sup>, A. Crum<sup>1</sup>, S. H. Hayes<sup>1</sup>, M. D. Flythe<sup>1,2</sup>, and L. M. Lawrence<sup>1</sup>, <sup>1</sup>University of Kentucky, Lexington, <sup>2</sup>United States Department of Agriculture, Agricultural Research Service, Forage-Animal Production Research Unit, Lexington, KY.

Adult horses depend on the microbial community in the hindgut to digest fiber and produce VFAs that are utilized for energy. Microbial colonization in the gastrointestinal tract of foals is essential to develop a healthy symbiotic relationship and prevent proliferation of pathogenic bacteria. However, colonization is not well understood. The objectives were to evaluate the age-related changes and effects of maternal diet on select fecal bacterial groups in foals from 1 d to 28 d of age. Thoroughbred foals ( $n = 19$ ) were from dams fed one of two concentrates: an oat-based (OB) or corn and wheat middlings-based (CWB) pelleted concentrate. The mares began the experimental diet at 310 d of gestation and remained on the assigned diets until 28 d postpartum. The foals had access to assigned concentrates, and a mixed grass and alfalfa hay and cool-season grass pasture were available ad libitum. Fecal samples were collected from foals at 1 d (14–36 h), 4 d, 14 d, and 28 d. Foals were continuously monitored on sample days to collect fecal samples immediately after defecation by catch into sterile specimen cups or from the center of the pile using sterile gloves. Fecal samples were transported to the laboratory in an insulated cooler (37°C) under CO<sub>2</sub>. Samples were serially diluted 10-fold before inoculation of selective media. Enumerations were performed for *Lactobacillus* spp., total starch utilizing bacteria (TSU), and cellulolytic bacteria (CB). Enumeration data were log transformed then analyzed with PROC MIXED (SAS 9.3) to test the main effects of maternal diet (OB or CWB), time of sample, and interaction between maternal diet and time. Results were considered significant when  $P < 0.05$ . There was no effect of maternal diet on bacterial enumerations ( $P > 0.05$ ). There was an interaction between maternal

diet and time in *Lactobacillus* spp. with CWB foals having more lactobacilli than OB at 1 d and 4 d ( $P < 0.05$ ); however, there were no differences observed at 14 d ( $P > 0.05$ ). These results indicate that maternal diet may influence some bacteria in foals. Fecal lactobacilli, TSU, and CB increased with age in foals ( $P < 0.05$ ) with CB first appearing between 4 d and 14 d. It is evident that colonization of the hindgut is a sequential process beginning early in the foal's life.

**Key Words:** bacteria, maternal diet, foal

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**0820 Changes in equine hindgut fermentation and carbohydrate digestion in response to varying sources of nitrogen.** M. O. Lass\*, J. S. Drouillard, J. M. Kouba, C. I. Vahl, Y. Wei, and T. L. Douthit, *Kansas State University, Manhattan.*

Casein or urea were administered as dietary N sources in a replicated  $3 \times 3$  Latin square experiment to evaluate impact on hindgut fermentation and carbohydrate digestion in the horse. A basal diet consisting of native prairie hay (5.9% CP; 72.7% NDF) was fed to 9 cecally cannulated horses ( $469 \pm 109$  kg BW) at 0.6% of BW DM basis every 8 h for 6 wk. Periods consisted of a 7-d acclimation to the basal diet and a 7-d dosing phase. Horses were dosed via the cecal cannula  $3 \times /d$  at the time of feeding for 7 d with 200 mL of water (W), or with 200 mL of water containing casein (C) or urea (U) to provide 1.12% N in the diet. During the final 4 d of each dosing phase, total fecal output was collected, and cecal digesta was collected 4 h after each feeding. Cecal digesta and feces were analyzed for pH and concentrations of ADF, NDF, ADIA, total VFA, and ammonia. Dosing with U resulted in greater concentrations of cecal ammonia (3.00 mM,  $P < 0.05$ ) compared to horses dosed with W (1.69 mM), or C (2.01 mM). Cecal pH increased ( $P < 0.01$ ) in horses dosed with C (7.72) or U (7.85) compared to W (7.45), but fecal pH was not different between treatments ( $P > 0.05$ ). Total VFA concentrations in cecal digesta were greater ( $P < 0.01$ ) for horses dosed with U (50.27 mM) compared to those dosed with W (32.17 mM) or C (32.17 mM). Fecal NDF and ADF were decreased ( $P < 0.05$ ) in horses dosed with U (63.07% NDF, 52.78% ADF) or C (59.68% NDF, 49.98% ADF) compared to those treated with W (66.50% NDF, 54.49% ADF), but ADIA content was not different ( $P > 0.05$ ). Taken together, differences in cecal ammonia, pH, and total VFA, along with changes in fecal NDF and ADF as a result of treatment, indicate that protein and non-protein N sources introduced directly into the cecum lead to changes in microbial fermentation in the hindgut that increase fiber digestion in the horse.

**Key Words:** equine, fiber digestion, nitrogen

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**0821 Effects of meal size and frequency on the equine cecal microbiota.** E. B. Venable\*, S. S. Bland<sup>1</sup>, H. Holscher<sup>2</sup>, T. W. Liu<sup>2</sup>, and K. S. Swanson<sup>2</sup>,  
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The effects of meal size and frequency on the equine cecal microbiota are not well documented. We hypothesized that meal size will alter the profile of the microbiota present in the equine cecum. Southern Illinois University Institutional Animal Care and Use approval (#13-070) was obtained before the initiation of this research. Cecally cannulated horses ( $n = 6$ ) with a BCS of 5 ( $\pm 0.5$ ) were utilized in this replicated Latin Square ( $3 \times 3$ ) design. All horses received group pasture turnout daily for approximately 6 h and were stalled overnight in identical box stalls ( $3 \times 4$  m). Treatment diets of Strategy® pelleted grain-based concentrate were as follows: A = one meal, 2.72 kg, 0600 h; B = two meals, 1.36 kg, 0600 and 1600 h; C = three meals, 0.91 kg, 0600, 1200, and 1600 h. Each treatment period consisted of 8 d of acclimation followed by 3 d of collection. All horses received ad libitum access to water, a white salt block, and 3 kg of mixed alfalfa/grass hay offered overnight. Body weight was recorded weekly and data were analyzed using a Latin square design with the proc MIXED procedure of SAS and was not affected by treatment ( $P > 0.05$ ). Cecal samples (216 total) were collected four times daily over a three-day collection within each period. Cecal bacterial DNA was extracted, and 16S rRNA amplicon sequencing using Illumina technology was followed by analysis using QIIME 1.8.0 and proc MIXED. Significance was set at ( $P \leq 0.05$ ). Weighted principal coordinates analysis (PCoA) of UniFrac distances between samples based on their 97% OTU composition indicated that Treatment A was different than Treatment C ( $P = 0.028$ ). In addition, PCoA also revealed a significant difference associated with breed ( $P < 0.05$ ) for both weighted (abundance) and unweighted (community composition) measures. Alpha diversity measures indicated that bacterial diversity is higher in geldings as compared to mares ( $P < 0.01$ ). Predominant bacterial phyla included Firmicutes (58.8–63.2%); Bacteroidetes (28.78–34.2%); Proteobacteria (2.2–2.8%); and Spirochaetes (2.5–2.7%) for all treatments. Furthermore, when treatment effects were examined at the genus level, six different genera were significantly affected by treatment (*Prevotella*, *YRC22*, *Lactobacillus*, *Streptococcus*, *Coprococcus*, and *Phascolarctobacterium*). These data demonstrate that size and frequency of pelleted concentrate meals affect both the abundance and the composition of the bacteria present in the equine cecum.

**Key Words:** horse, microbiota, meal size