

via contrasts that averaged data by hour over days corresponding to CON, ME and PER respectively. An increase was detected at h 2 in total VFA, acetate, and propionate in PER compared with CON ($P < 0.05$). Isobutyrate, butyrate and valerate were also increased at 2 h in PER compared with both CON and ME ($P < 0.05$). At h 4 there was an increase in total VFA, acetate, propionate, and butyrate in PER compared with both CON and ME ($P < 0.05$). Accumulation of isobutyrate and isovalerate was also increased in PER compared with CON at h 4 ($P < 0.05$). Valerate concentration in PER was increased compared with ME at h 4 ($P < 0.015$). It appears that *M. elsdenii* survives transit through the foregut and affects microbial fermentation. Because differences were only detected in PER, it appears that it takes several days following dosing to establish a viable population of *M. elsdenii* in the equine hindgut.

Key Words: *Megasphaera elsdenii*, hindgut, probiotic

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Colonic bacterial composition and blood cells in horses undergoing a change of diet

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Colic has been associated with high-starch diet and hindgut microbial disturbances. Moreover, horses with large colon colic showed hematological profile changes. This study aimed to evaluate the hematological parameters in horses undergoing a change of diet, and to correlate those parameters with the colonic bacterial composition. Six adult geldings (466 ± 40 kg BW) fistulated in the right ventral colon were used in a longitudinal study composed of two 3-week consecutive periods (P). In P1, they were fed with 100% hay (H). After a gradual transition of 5 d, they received in P2 a (B) diet composed of 57% hay and 43% rolled barley (>2 g of starch/kg BW in the morning meal). Colonic bacterial composition and complete blood count were assayed in samples collected 4 h after the morning meal twice in each period at 10-d intervals. Orthogonal contrasts testing variations for the H diet (H10 vs. H20), B diet (B10 vs. B20) and between the 2 diets (H vs. B) were performed (SAS). Correlations between blood and colonic parameters were evaluated. Results, presented in Table 1, highlighted unexpected differences of Neutrophil (N) and Lymphocyte (L) percentages and their ratio during the H diet. This could be explained by preservation problems for the H20 blood sample. Our data confirmed that B diet induced bacterial

disturbances in the colon. Simultaneously, leukocyte counts, N percentage and N/L ratio significantly increased whereas L percentage significantly decreased. These hematological variations may indicate an alimentary stress. Several significant correlations were found between blood and colonic parameters. The major ones were leukocytes count, correlated with the cellulolytic bacteria ($r = -0.47$; $P = 0.02$) and with the total anaerobic bacteria counts ($r = +0.49$; $P = 0.02$), and the N percentage correlated with the lactate-utilizing bacteria count ($r = +0.47$; $P = 0.02$). This study suggests that hematological parameters could be useful indicators to evaluate colonic microbial state.

Key Words: colonic bacteria, blood cells, correlations

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Magnesium aspartate supplementation and reaction speed response in horses

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Magnesium (Mg) containing supplements and feeds typically claim, among many things, to have a calmative effect on horse behavior without any published evidence for such an effect. The aim of this study was to investigate if oral Mg supplementation above NRC recommendations increases the Mg status in the horse and has an observable effect on horse behavior. Six mature Standardbred geldings were used. Prior to Mg supplementation, all horses had their reaction speed recorded with and without mild sedation (0.04 mg/kg BW Acepromazine) using a random cross over design. Horses were then allocated a treatment using a Latin square design with each horse acting as its own control. All horses were fed a base (control) ration comprising low energy pellets (Cool Conditioner, CopRice, Leeton, Australia) plus clover/ryegrass hay fed at 2.0% BW (total mean Mg of ration: 11.2 ± 2.0 g). Three treatments were used in the trial: control ration, control plus 2.5 g added Mg (as magnesium aspartate) and control plus 10 g added Mg. Horses were fed each ration in 2 meals per day for a period of 7 d. All horses were kept in individual dry lots that allowed free exercise. On Day 6, a series of blood and urine samples were collected over 24 h to determine Mg status and excretion. On Day 7, each horse underwent a reaction speed test. This measured the time taken for a horse to cover 2 m in a custom-built chute after being startled. Repeated

Table 1

Evolution of colonic bacterial counts and blood cells of horses undergoing a change from hay (H) to hay/barley (B) diet

	Samplings				MSE	Contrast, P-value		
	H10	H20	B10	B20		H10 vs. H20	B10 vs. B20	H vs. B
Bacterial counts¹								
Total anaerobics	6.74	6.79	7.35	7.58	0.43	NS	NS	**
Cellulolytics	4.96	4.66	3.96	4.28	0.52	NS	NS	**
Lactate-utilizers	5.47	5.47	6.78	7.16	0.43	NS	NS	***
Amylolytics	5.27	4.01	4.94	4.99	0.38	***	NS	*
Blood cells								
Erythrocytes ($\times 10^6/\text{mm}^3$)	7.91	7.52	7.61	7.55	0.36	NS	NS	NS
Hematocrit (%)	38.6	38.0	37.6	37.4	1.9	NS	NS	NS
Leukocytes ($\times 10^3/\text{mm}^3$)	7.90	7.72	8.91	8.68	0.77	NS	NS	**
N (%)	61.5	50.4	63.8	63.5	3.2	***	NS	***
L (%)	29.4	38.8	27.3	29.3	3.6	***	NS	**
N/L ratio	2.10	1.37	2.42	2.24	0.28	***	NS	***

¹log₁₀ cfu/mL.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS = not significant.

measures ANOVA of blood and urine concentrations at allocated sampling times showed an increase in total blood Mg concentrations and urinary Mg excretion between the control group and the Mg supplemented groups. There was a trend for Mg supplementation to decrease reaction speed ($P = 0.07$) when the control was compared with both the 2.5 g and 10 g added Mg groups. There was a significant difference shown between the control and 10 g added Mg treatment ($P = 0.025$) using a paired Students *t*-test. Mean reaction speed (\pm SD) was Control, 5.3 ± 2.2 m/s; 2.5 g added Mg, 3.7 ± 1.8 m/s and 10 g added Mg, 3.1 ± 1.5 m/s, with no effect of day. This was comparable to the pre-study testing where unsedated horses had a mean reaction speed of 5.3 ± 1.8 m/s compared with 3.5 ± 1.5 m/sec when sedated. This is the first time an objective measurement of behavioral change due to oral magnesium supplementation has been reported in the horse.

Key Words: behavior, magnesium, equine

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Comparison of inflammation, nutritional status, muscle mass, pituitary function, and age in geriatric horses

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Pituitary pars intermedia dysfunction (PPID) is an endocrinopathy commonly associated with aging in the equine population. Inflamm-aging (systemic low-grade chronic inflammation) also occurs with aging. Little is currently known about whether the inflammation or nutritional status of geriatric horses may be associated with the occurrence of PPID. Sarcopenia frequently accompanies PPID, thus muscle mass is also of interest. To determine whether inflamm-aging, nutritional status, muscle mass, and PPID status may be correlated, various measures of these parameters were compared in geriatric horses. Forty-three old horses (mean 24.4 ± 3.0 yrs) were used to measure immune, endocrine, muscle, fatty acid, vitamin, and mineral parameters. Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized blood, purified, antibody-stained intracellularly, and analyzed via flow cytometry to determine lymphocyte production of interferon- γ (IFN γ) and tumor necrosis factor- α (TNF α). RNA was also isolated from PBMCs and underwent polymerase chain reactions to determine expression of various inflammatory cytokines. Serum interleukin-6, C-reactive protein, and TNF α levels were determined via enzyme-linked immunosorbent assays (ELISAs). Serum vitamin, fatty acid, and mineral levels were also measured. Muscle and fat mass were determined via ultrasound; muscle scores were also assigned (0–3). Thyrotropin releasing hormone (TRH) stimulation was performed to determine PPID status, in which adrenocorticotropin hormone (ACTH) levels were measured in plasma pre and 10 min post (T-10) intravenous administration of TRH (1mg/mL saline/horse). Pearson correlation testing was performed to determine correlations between various parameters. Baseline ACTH and T-10 ($R = 0.631$; $P < 0.001$) yielded a strongly significant relationship. Age and T-10 ACTH ($R = 0.256$; $P = 0.0973$) exhibited a trend, while age and basal ACTH ($R = 0.389$; $P = 0.0109$) were significantly correlated. ACTH was not significantly correlated with muscle, fatty acid, vitamin, or mineral measures. Inflammatory markers also did not appear correlated with ACTH, thus PPID and inflamm-aging may not be associated. Age, however, exhibited a

positive correlation with fatty acids C18:2n6c, C20:4n6c, and C24:1n9c, as well as with lymphocyte production of IFN γ and TNF α , and serum selenium and vitamin E ($P < 0.05$). Age showed a negative correlation with fatty acids C16:0 and C20:1n9c, fat free mass, fat weight, and muscle score ($P < 0.05$). The correlation of age with various measured parameters indicates that age plays a role in regulating inflammatory and metabolic function of the horse.

Key Words: inflammation, pituitary pars intermedia dysfunction (PPID), age

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Influence of oat β -glucan on neutrophil function and lymphocyte subsets in response to stress

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Physiological stress has a pronounced effect on immune cells and their ability to mount an effective defense against pathogens. Oat β -glucan (BG) has been shown to enhance innate immunity, particularly in response to stress induction. We hypothesized that elevated intake of oat BG would improve innate immunity and/or mitigate stress-induced immunosuppression. Twelve horses (mean \pm SEM, 552 ± 10 kg; 11.5 ± 1.4 yr) were used in a 4×4 Latin square design study. Horses were fed 4 diets differing in amount and source of BG for 22 d: corn (<0.6% BG, as-fed), regular feed oats (2.85% BG), high BG oat variety (4.0% BG), and corn top-dressed with a concentrated oat BG powder (79.4% BG). High BG oats and oat BG powder were fed in quantities to deliver 170 mg BG/kg BW/d. All diets were isocaloric. Horses also had free access to pasture forage (<0.6% BG) and were fed a vitamin/mineral supplement. On d 18 horses were tethered for 12 h with their heads elevated at a height of 1.5 m to induce physiological stress. Nasopharyngeal flush (NPF) and whole blood samples were obtained before initiation of dietary treatment (d 0), before head elevation (d 18), and immediately after (0 h), and 12, 24, and 72 h post-head elevation. At the completion of each 22-d period, horses underwent a 13-d dietary washout followed by treatment reallocation. Neutrophil function in whole blood and lymphocyte subset populations in both NPF and whole blood were assessed by flow cytometry. Data were compared using mixed model ANOVA with repeated measures. Oxidative burst and phagocytosis of propidium iodide-labeled *Streptococcus equi* by whole blood neutrophils were not affected by diet, but were affected by stress ($P < 0.05$). Neutrophil capacity for phagocytosis ($P = 0.07$) and oxidative burst ($P = 0.01$) as measured by mean fluorescence intensity were suppressed 72 h after head elevation. Percent of neutrophils able to phagocytose and subsequently undergo oxidative burst tended to be lower ($P = 0.07$) 72 h after head elevation. Percent of neutrophils capable of bacterial stimulation was increased at 12 h post ($P = 0.002$). Prolonged head elevation caused an increase ($P < 0.0001$) in the percent of circulating CD8+ T cells and consequently a decrease ($P = 0.01$) in the ratio of CD4+ to CD8+ T cells. Circulating B cells increased ($P = 0.003$) 12 h after the cessation of head elevation which caused the ratio of T cells to B cells to decrease ($P = 0.02$); both values returned to baseline by 24 h post. Although head elevation for 12 h did induce short-term upper respiratory and systemic immune distress, the level and form of oat BG in the diet did not appear to mitigate responses.

Key Words: horse, soluble fiber, immunosuppression