could be reduced. Furthermore, because growing horses use P differently than mature horses, there might be a difference in P digestibility due to age. This study was designed to examine the availability of dietary P in growing horses and mature horses. Four long yearling geldings (19 ± 1 mo; 478 ± 58.9 kg) and 4 mature geldings (10.5 ± 7.5 yr; 541 ± 45.9 kg) were fed a diet of timothy cubes, alfalfa cubes, and concentrate without added P. The diet contained 0.26% P (dry matter basis) and 29% of that P occurred as phytate-P. The diet was formulated to be close to each horse’s P requirement. The concentrate contributed 50% of the total P and the forage cubes contributed 50%. There was a 14 d diet adaptation period during which feed intake was adjusted to minimize orts and horses were accustomed to wearing fecal collection harnesses. Total fecal collections were then conducted for a 4 d period. Fecal and feed samples were measured using a gravimetric assay. Dry matter digestibility was 53.93% for both age groups was 53.93% measured using a gravimetric assay. Dry matter digestibility was 53.93% for both age groups was 53.93% conducted for a 4 d period. Fecal and feed samples were used to determine P and dry matter digestibility. P was measured using a gravimetric assay. Dry matter digestibility for both age groups was 53.93 ± 3.13% and was not different between ages (P > 0.05). Parental P digestibility was not different (P > 0.05) between the two groups. True digestibility was calculated using published values for endogenous losses (NRC, 2007). Estimated true P digestibility (27.63 ± 12.70%) was not different between the two groups (P > 0.05). One mature horse had a very low P digestibility, and the data were re-analyzed without the values from this horse. However, even without including this horse in the data set, age did not affect apparent or true P digestibility (P > 0.05). Long yearlings and mature horses have the same ability to digest P, thus a similar P digestibility can be used for both age groups when formulating diets.

**Effects of polyphenolic bioactive compounds (pterostilbene, resveratrol, curcuminoids, quercetin, and hydroxypterostilbene) on pro-inflammatory cytokine production in vitro**

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Advanced age in horses (>20 years) is associated with increased production of pro-inflammatory cytokines, termed “inflamm-aging”. Nutritional intervention to counteract this response in old horses remains to be studied. Non-steroidal anti-inflammatory drugs (NSAIDs) such as flunixin meglumine (banamine) and phenylbutazone (bute) are commonly used to treat inflammation in horses. However, long term use of NSAIDs can pose health problems in the horse. Thus, the use of polyphenolic bioactive compounds with anti-inflammatory properties including pterostilbene, resveratrol, curcuminoids, quercetin, and hydroxypterostilbene are of interest. The goal of this research was to determine the effects of these compounds on equine cytokine production in vitro, as a preliminary step towards in vivo research. Heparinized blood was collected aseptically from the jugular vein of aged horses (n=6; mean age 26±2 yrs). Peripheral blood mononuclear cells (PBMC) were isolated by ficoll-paque density centrifugation, resuspended in c-RPMI-2.5% media and incubated at 37°C, 5% CO₂ overnight for 18-19 hrs with each of the compounds at multiple concentrations (320, 160, 80, 40, 20, 10 μM). Stock dilutions were prepared by dissolving each of the compounds in dimethyl sulfoxide (DMSO). Aliquots (2 μL) of each stock dilution were administered to the wells to achieve the aforementioned concentrations. Following the incubation with compounds, PBMCs were stimulated with PMA-ionomycin and brefeldin A for 4 hrs. PBMC viability was measured using a Vi-Cell XR to determine whether cells incubated with DMSO alone and stimulated with PMA-ionomycin (positive control) were statistically different from those incubated with the compounds at various dilutions. Pro-inflammatory cytokine (IFN-γ and TNF-α) production by lymphocytes were quantified by intracellular staining and flow cytometry. Optimal concentrations for each compound were determined on the criteria that: 1) No statistical difference (P>0.05) in percent viability between positive control and PBMCs at the concentration existed, and 2) Of those with comparable viability, IFN-γ production was significantly (P<0.05) reduced compared to the positive control. Differences of means were analyzed using one-way ANOVAs. Results showed that the optimal concentration for each of the compounds were the following: curcuminoids - 20 μM, hydroxypterostilbene - 40 μM, pterostilbene - 80 μM, resveratrol – 160 μM, and quercitin - 160 μM. At the optimal concentrations, IFN-γ production by lymphocytes was significantly reduced (P<0.001) compared to the positive control. Likewise, TNF-α production was significantly reduced (P<0.001) for all compounds but curcuminoids. In another study, flunixin meglumine and phenylbutazone were compared to curcuminoids, the most potent of the compounds, at a concentration of 20 μM. While curcuminoids significantly reduced inflammatory cytokine production compared to the positive control, flunixin meglumine and phenylbutazone did not. This research demonstrates not only that curcuminoids, hydroxypterostilbene, pterostilbene, quercetin and resveratrol significantly reduce inflammation, but also that these compounds have the potential to outperform NSAIDs.

**Acknowledgements**

Dr. Patrick Lawless, EquiThrive™ provided the compounds for this research.

**Effect of citrulline-malate supplementation on plasma amino acids and glycemic and insulinemic responses in horses**

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Arginine supplementation has been shown to improve insulin sensitivity in several species. Because citrulline escapes splanchnic extraction, it is more effective at
increasing arginine availability in humans and pigs than oral arginine supplementation. Therefore, citrulline could provide a novel dietary intervention for the management of insulin resistant horses. The hypothesis of this study was that citrulline-malate (CIT) supplementation would increase arginine availability and consequently alter insulin response to a starch-rich meal. Twelve clinically normal horses (mean ± SEM, 10.8 ± 2.5 yr; 552 ± 13 kg; body condition score 5-6 out of 9) were randomly assigned to receive CIT (86 mg/kg BW) or urea (isonitrogenous control; 25 mg/kg BW) for 14 d. Supplements were hand-mixed into a grain concentrate (fed at 0.5% BW) and offered once daily. On d 14 after an overnight fast, glycemic and insulinemic responses to a grain meal (fed at 0.25% BW, which provided 0.9 ± 0.02 g starch + ethanol soluble carbohydrates per kg BW) containing the daily allotment of urea or CIT were evaluated. Venous blood samples were obtained before and every 30 min after the meal was consumed for 5 h and glucose, insulin and amino acid concentrations were determined. Data were analyzed as a mixed model ANOVA with repeated measures, using time, treatment, and time*treatment as fixed effects and horse within treatment as a random variable. Time to consume the meal averaged 14.9 ± 2.7 min and did not differ between horses fed CIT or urea. Plasma citrulline, arginine, ornithine and glutamate concentrations increased (P < 0.01) in response to the meal and were higher (P < 0.05) in horses supplemented with CIT compared to urea. Plasma lysine, methionine and threonine concentrations also increased (P < 0.0001) in response to the meal, but were unaffected by CIT consumption. Plasma urea and NH3 were unaltered by the meal or dietary treatment. Glycemic response to the meal was similar between treatments; however, serum insulin was lower (P < 0.05) when horses consumed a meal containing CIT versus urea. Likewise, plasma glucose area under the curve (AUC) was similar between treatments, but serum insulin AUC was lower (P = 0.05) in CIT than urea-fed horses. Insulin sensitivity (estimated by the reciprocal of the insulin square root index and homeostasis model assessment) and pancreatic β-cell responsiveness (estimated by modified glucose to insulin ratio and homeostasis model assessment) were not different (P < 0.05) between horses fed CIT or urea. Results demonstrate that CIT can increase whole-body arginine supply without negatively affecting amino acids that may be limiting in equine diets. In addition, supplementation with CIT may be useful for maintaining glycemic control while reducing hyperinsulinemia in insulin resistant horses, but deserves further investigation.

Threonine supplementation does not increase protein synthesis in weanlings receiving a grass forage and commercial concentrate

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Threonine is a potentially limiting amino acid for growth in horses (Graham et al., 1994). The threonine requirement of six month old weanlings can be estimated at 81 mg/(kg-d) using the muscle protein amino acid profile in conjunction with the lysine requirement (NRC, 2007). This may underestimate threonine requirements as large amounts are extracted by the gut for mucin synthesis in other species (Nichols and Bertolo, 2008). The study objective was to determine if threonine supplementation (+Thr) would increase whole-body protein synthesis (WBPS) in weanlings receiving grass forage and a commercial concentrate. Using a crossover design, six Thoroughbred weanling colts (269±24 kg, 176±30 d) were supplemented with either threonine (83 mg/(kg-d); total diet threonine: 223 mg/(kg-d)) or an isonitrogenous amount of glutamate (103 mg/(kg-d); total diet threonine: 140 mg/(kg-d)). Following five days of adaptation, venipuncture samples were taken immediately before and 90 minutes after the morning concentrate meal. The next day, whole-body phenylalanine kinetics were determined using a 2 h primed, constant infusion of [13C]sodium bicarbonate, to measure whole-body CO2 production, followed by a 4 h primed, constant infusion of [1-13C] Phenylalanine, to measure phenylalanine oxidation to CO2 and phenylalanine flux. Most plasma amino acid concentrations were elevated post-feeding (P < 0.01). The horses receiving +Thr had greater baseline and post-feeding plasma threonine, glycine, and methionine (P < 0.01) compared to the glutamate supplemented (+Glu) horses. Phenylalanine flux, intake, oxidation and non-oxidative disposal were similar between treatments (Table 1; P > 0.05). These results indicate that threonine intake was not limiting to WBPS when horses received this commercial concentrate with quality grass forage.

Table 1

<table>
<thead>
<tr>
<th></th>
<th>+Glu</th>
<th>+Thr</th>
<th>Pooled SE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phenylalanine flux [µmol/(kg h)]</strong></td>
<td>61</td>
<td>62</td>
<td>3</td>
<td>0.56</td>
</tr>
<tr>
<td><strong>Phenylalanine entering the free pool [µmol/(kg h)]</strong></td>
<td>24.5</td>
<td>23.0</td>
<td>1.0</td>
<td>0.18</td>
</tr>
<tr>
<td>Dietary intake</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein breakdown</td>
<td>36.1</td>
<td>39.5</td>
<td>3.0</td>
<td>0.30</td>
</tr>
<tr>
<td><strong>Phenylalanine leaving the free pool [µmol/(kg h)]</strong></td>
<td>12.2</td>
<td>11.5</td>
<td>2.2</td>
<td>0.74</td>
</tr>
<tr>
<td>Oxidation</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Non-oxidative disposal (indicator of WBPS)</td>
<td>48.4</td>
<td>51.0</td>
<td>3.1</td>
<td>0.42</td>
</tr>
</tbody>
</table>

1 Values are least squares means ± SE.
2 Flux = rate of phenylalanine entry = rate of phenylalanine leaving; rate of phenylalanine entry = phenylalanine intake + phenylalanine release from protein breakdown; rate of phenylalanine leaving = phenylalanine oxidation + non-oxidative phenylalanine disposal.

Acknowledgements

This study was funded by a WALTHAM-BUCKEYE Foundation Grant.

References