Feeding grape seed extract to horses: effects on health, intake and digestion

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A feeding trial involving four Thoroughbred race horses was undertaken to establish whether inclusion of grape seed extract (GSE) in the diet of horses undergoing mild exercise had any effects on their general health, intake and digestion. Supplementation with GSE had no effect on either feed or water intake of the horses and the supplement was readily palatable to the horses at all levels of inclusion. Feeding GSE caused no adverse effects in terms of animal health (temperature, pulse and respirations rates), and there were some positive effects related to a presumed alteration in fermentation in the hindgut. Feeding GSE increased faecal pH, changing from acid faeces (pH 6.6) when no GSE was fed to neutral faeces (pH 7.0) when 150 mg GSE/kg body weight (BW) was fed. In addition, blood glucose concentrations were significantly (P < 0.05) decreased when GSE was fed at 100 and 150 mg/kg BW (5.50 ± 0.26 and 5.32 ± 0.72 mmol/l, respectively) compared with the control diet (5.77 ± 0.31 mmol/l). The actual mechanisms causing these alterations are yet to be elucidated, but could have important implications for the prevention of acidosis.

Keywords: grape seed extract, horses, tannins

Introduction

Grape seed extract (GSE) is sold as a supplement for horses in the USA, Australia and Europe. Despite a thorough review of the literature, no information relating to the effects of feeding GSE specifically or proanthocyanidins in general to horses could be found. GSE is derived from the residual inner epidermis and the outer parenchymous cell layers of the seed. It contains a heterogeneous mixture of gallic acid (Palma and Taylor, 1999) and proanthocyanidins (Fuleki and Ricardo da Silva, 1997).

The paper reports on the findings of a preliminary investigation undertaken to elucidate the effects of GSE on horses, with a focus on identifying any adverse effects of GSE inclusion in the diet on animal health, intake and digestion.

Material and methods

Animals and management

Four Thoroughbreds, aged between 5 and 16 years, were used (three geldings and one mare). They were on loan (with informed consent) from their owners for the duration of the trial. Mean body weight (BW) was 534 ± 22.6 kg.

The horses were stabled during the night in individual stalls (3 m × 4.2 m, concrete bases with good drainage). The horses were bedded on barley straw. The water buckets in each stable were filled with fresh water to the required level. Oaten hay was placed into a net and tied to the wall in each stable.

The horses were fed a concentrate feed twice a day at 0730 and 1700 h. Following their morning concentrate feed, the horses were turned out (daily) into individual paddocks, which were bare of pasture. The horses were provided with 40% of their daily allocation of hay via hay nets placed in their day paddock; the remaining hay was provided in their stables at night. GSE daily inclusion was maintained consistently as a percentage of total BW. Daily feed intake was determined by subtracting the weight of feed refusal from the amount of feed offered (both on dry matter (DM) basis).

Experimental design and diets

The trial was based on a Latin square design, involving four dietary treatments and four experimental periods. The horses were brought to the appropriate level of fitness prior to the commencement of the actual feeding trial. This took
Grape seed extract for horses

approximately 4 weeks of light lunge work. Following this was the trial proper with each experimental period within the Latin square design taking 3 weeks. The total experimental period was thus 16 weeks.

Horses were randomly allocated a dietary treatment for each period. Each of the four experimental periods was of 21-day duration, comprising of a 14-day adaptation by a 7-day sampling period.

The four experimental diets were as follows: (1) basal diet – consisting of 3 kg horse muesli (concentrate feed), 1.5 kg oaten chaff, 1 kg lucerne chaff and 10 kg oaten hay; (2) basal diet + 50 mg GSE/kg BW; (3) basal diet + 100 mg GSE/kg BW and (4) basal diet + 150 mg GSE/kg BW. The basal ration contained (on DM basis) 7.7% crude protein and 8.9 MJ digestible energy per kilogram DM. At the level of feeding used, this equated to 1.5 times the NRC (2007) protein and energy requirements for a 500 kg horse undertaking light exercise work.

The horse muesli was formulated and manufactured by Thompson and Redwood Produce Supplies (Perth, Western Australia). The GSE was a proprietary product (Vinlife®; Tarac Technologies Pty Ltd, South Australia). The GSE was a light brown, non-fibrous powder with a characteristic herbal/tannic odour. It is freely soluble in water. Phenolic compounds are the major constituent of GSE, with a total phenolic content as gallic acid equivalent (GAE) of 46% and containing 40% oligomeric proanthocyanidins (as GAE).

The actual amount of GSE fed varied and depended on the BW of the horse. The horses were weighed each week and the total amount of GSE fed was adjusted where needed.

Live weight, temperature, heart and respiration rates

Once weekly during the trials, the horses were weighed after their morning feed using a horse weighbridge (Sun Beam True Test AG500 Series Version 3.1). Temperature (rectal), heart and respiration rates were also measured weekly after the horses had completed their evening feed. Respiration rate was measured while the horse was standing and still eating the evening feed.

Exercise

Prior to the start of the trial, a period of 4 weeks was allowed for the horses to undergo basic training in lunging and to gain the necessary level of fitness (Marlin and Nankervis, 2002). The trial required the horses to work at low-intensity speeds of approximately 2 to 4 m/s, which meant the horses only needed to be worked at the gaits of walk and trot (Evans, 2000). The horses were worked five times a week on a 20 m circle in an outdoor-fenced sand arena. The horse worked on each rein (right- or left-hand side) for the same duration each session at either gait. The duration and intensity of the lunge session increased as the horses’ fitness improved.

The horses’ fitness was maintained during the 12-week trial by undertaking three lunge sessions per week on a 20 m circle in the outdoor sand arena. Each session involved 20 min at walk (average 1.76 m/s) and trot (average 3.8 m/s) (Marlin and Nankervis, 2002).

A regular lunge session, when the horses were in a fit condition, consisted of 2 min walk (left rein), change the rein, 2 min walk (right rein), transition to trot, 7 min trot (right rein), change rein, transition to trot, 7 min trot (left rein), transition to walk and 2 min walk to cool the horse down.

A lunge test, adapted from Marlin and Nankervis (2002), was undertaken during weeks 3, 6, 9 and 12 of the trial. Heart rate was recorded using a Polar Equine® (Pursuit Performance Pty Ltd, South Australia) heart-rate monitor.

Analysis and calculations

Fresh faecal samples (100 to 500 g) were collected weekly (a.m.) and the pH was recorded. The samples were taken from the centre of the faecal mass within 30 s of its deposition (Nicol et al., 2002). Three pH readings were taken using an EcoScan pH 5/6 m (Eutech Instruments Pty Ltd, Singapore). The pH probe was placed directly into the faecal balls to obtain the readings.

Once a week, prior to their morning feed, 50 ml urine samples were collected from each horse using a plastic bucket with a long wooden handle. A sub-sample was taken and then stored frozen at −18°C. The urine samples were subsequently analysed for specific gravity as well as minerals and creatinine levels to enable determination of the animals’ fractional excretion ratio (FER) for minerals.

In total, 10 ml venous blood samples were drawn on a weekly basis (immediately following urine collection) directly from the jugular vein using 18 gauge needles, into labelled vacutainers containing lithium heparin as an anticoagulant (BD Vacutainers™ LH PST™II; AllMed). The samples were centrifuged at 3000 g for 15 min. Plasma was harvested within an hour of collection and frozen at −18°C for subsequent analyses of minerals, creatinine, glucose and lactate concentrations.

The mineral (Na, K, Cl and Mg) and creatinine analyses of blood and urine and analysis of blood glucose and lactate concentrations were conducted by Vetpath Laboratory Services, Perth, Western Australia (NATA Accreditation no. 14776). All analyses were carried out using the Olympus AU400 analyser using (appropriate) Olympus reagents. For the determination of Na, K and Cl concentrations in both urine and plasma, analysis involved using the ion-specific electrode on the AU400. For plasma lactate analysis, BioMerieux reagents were used. For all analyses, the samples were diluted as necessary to fall within the dynamic/linear range of the AU400.

The FER for minerals was used to determine electrolyte balance in the horses (King, 1994). The determination of electrolyte clearance as a percentage of creatinine (Cr) clearance was calculated using the following equation:

\[
\frac{([M]_{\text{urine}} - [M]_{\text{plasma}})}{[C]_{\text{plasma}} - [C]_{\text{urine}}} 
\]

where M is the mineral under investigation, urine, the urinary concentration of a substance and plasma, the plasma concentration of a substance.
Results and discussion

Animal health
All animals maintained good health throughout the trial. Supplementation with GSE had no effect on either temperature or respiration rate (measured at rest); however, feeding 50 mg GSE/kg BW resulted in a significantly lower (P < 0.05) heart rate (Table 1) and there is no explicable reason for this effect. However, for all the horses for all treatments, the heart rates were within the normal range for an adult horse (Pillner and Davies, 2000).

The results from this study are similar to findings in other animal studies where GSE had no adverse effects on animal health (Reed, 1995; Mittalet al., 2003; Iwasaki et al., 2005). However, Wren et al. (2002) found feeding GSE to rats at levels of up to 2.0% for a period of 90 days resulted in all groups of animals gaining weight during the trial and that food consumption in the 2.0% group was significantly higher than that of the control group. However, Moreno et al. (2003) and Vogel et al. (2004) reported decreased BWs in mice and rats while other researchers (Yamakoshi et al., 2002; Gontheiret al., 2003) found GSE treatments did not produce any significant changes in BW in rats.

Table 1: Effect of supplementation with GSE on temperature, heart and respiration rates (measured at rest) (mean ± s.d.) of exercised horses

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Temperature (°C)</th>
<th>Respiration rate (breaths/min)</th>
<th>Heart rate (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>37.5 ± 0.10</td>
<td>10 ± 1.3</td>
<td>36 ± 3.46</td>
</tr>
<tr>
<td>50 mg GSE/kg BW</td>
<td>37.5 ± 0.05</td>
<td>10 ± 1.3</td>
<td>33 ± 3.44</td>
</tr>
<tr>
<td>100 mg GSE/kg BW</td>
<td>37.5 ± 0.08</td>
<td>10 ± 1.6</td>
<td>35 ± 3.46</td>
</tr>
<tr>
<td>150 mg GSE/kg BW</td>
<td>37.5 ± 0.08</td>
<td>11 ± 1.3</td>
<td>36 ± 4.36</td>
</tr>
</tbody>
</table>

GSE = grape seed extract; BW = body weight.
Values within a column with unlike superscript letters were significantly different (P < 0.05).

Table 2: Effect of supplementation with GSE on feed and water intakes (mean ± s.d.) of exercised horses

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Water intake (l/day)</th>
<th>Hay intake (kg DM/day)</th>
<th>Total feed intake (kg DM/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>26.5 ± 3.79</td>
<td>6.6 ± 1.05</td>
<td>11.5 ± 1.07</td>
</tr>
<tr>
<td>50 mg GSW/kg BW</td>
<td>28.5 ± 6.63</td>
<td>7.9 ± 2.63</td>
<td>12.9 ± 2.68</td>
</tr>
<tr>
<td>100 mg GSE/kg BW</td>
<td>26.5 ± 4.20</td>
<td>7.1 ± 1.41</td>
<td>12.1 ± 1.43</td>
</tr>
<tr>
<td>150 mg GSE/kg BW</td>
<td>28.5 ± 5.16</td>
<td>7.3 ± 2.59</td>
<td>12.3 ± 2.63</td>
</tr>
</tbody>
</table>

GSE = grape seed extract; DM = dry matter; BW = body weight.
however, including GSE in the diet resulted in increased faecal pH up to neutral (pH 7.0) when 150 mg GSE/kg BW was fed. It appears that feeding GSE has either decreased the amount of starch entering (the caecum) or changed the nature of fermentation in the caecum.

Fermentation of starch in the hindgut is associated with an increase in populations of Gram-positive bacteria (Bailey et al., 2003). Jayaprakasha et al. (2003) found Gram-positive bacteria were completely inhibited by GSE treatment (850 to 1000 p.p.m.). Baydar et al. (2006) also reported antibacterial properties of GSE. Therefore, it is postulated that including GSE in the diet of exercising horses modified the bacterial population of the hindgut, resulting in altered VFA production and more alkaline faeces.

This is a potentially significant finding and warrants further research. Overgrowth of Gram-positive Streptococcal species in the hindgut has been associated with the development of carbohydrate-induced laminitis in horses and whether this is specific to feeding GSE has been determined. Compared with the control horses, the GSE-supplemented horses gained weight and had more alkaline faeces. The actual mechanisms causing these alterations are yet to be elucidated, but could have important implications for the prevention of acidosis.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Faecal pH</th>
<th>Faecal N (%)</th>
<th>Faecal water (mL/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.6 ± 0.11a</td>
<td>2.52 ± 0.12</td>
<td>0.79 ± 0.02</td>
</tr>
<tr>
<td>50 mg/kg BW</td>
<td>6.8 ± 0.27b</td>
<td>2.68 ± 0.21</td>
<td>0.80 ± 0.01</td>
</tr>
<tr>
<td>100 mg/kg BW</td>
<td>6.9 ± 0.52b</td>
<td>2.57 ± 0.55</td>
<td>0.80 ± 0.01</td>
</tr>
<tr>
<td>150 mg/kg BW</td>
<td>7.0 ± 0.12b</td>
<td>2.68 ± 0.29</td>
<td>0.79 ± 0.02</td>
</tr>
</tbody>
</table>

GSE = grape seed extract; BW = body weight. Values within a column with unlike superscript letters were significantly different (P < 0.05).

Table 4 Effect of supplementation with GSE on the fractional excretion ratio (mean ± s.d.) of major electrolytes in exercised horses

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fractional excretion ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Na</td>
</tr>
<tr>
<td>Control</td>
<td>0.04 ± 0.02</td>
</tr>
<tr>
<td>50 mg GSE/kg BW</td>
<td>0.03 ± 0.01</td>
</tr>
<tr>
<td>100 mg GSE/kg BW</td>
<td>0.04 ± 0.02</td>
</tr>
<tr>
<td>150 mg GSE/kg BW</td>
<td>0.03 ± 0.01</td>
</tr>
</tbody>
</table>

GSE = grape seed extract; BW = body weight. Values within a column with unlike superscript letters were significantly different (P < 0.05).

References


Baydar NG, Sagdic O, Ozkan G and Cetin S 2006. Determination of antibacterial effects and total phenolic contents of grape (Vitis vinifera L.)


