Effect of feeding increasing quantities of starch on glycaemic and insulinaemic responses in healthy horses

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Abstract

The aim of this study was to investigate the effect of increasing the intake of starch on the glycaemic and insulinaemic responses of horses. A cross-over study design was used in which four horses were fed increasing amounts of a compound feed (0.5–3.5 kg) to provide 0.3, 0.6, 0.8, 1.1, 1.4 and 2 g starch/kg bodyweight (BW)/meal. The glycaemic response increased with starch intake ($P < 0.05$), while feeding $<1.1$ g starch/kg BW resulted in a lowered response, compared to when 1.1–2 g starch/kg BW was fed ($P < 0.01$). The results suggested that insulin responses may be more appropriate to define the effect of feeding different starch levels than glycaemic responses. A starch intake of $<1.1$ g/kg BW/meal produced only moderate glucose and insulin responses, even though highly processed cereals were used. It is therefore recommended that a starch intake of $<1.1$ g/kg BW/meal or a meal size of 0.3 kg/100 kg BW (starch content of 30–40%) is used for horses.

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Introduction

Cereal carbohydrates contribute an important part of the diet of performance horses and provide the principal energy source. The effects of isolated grains after different processing methods on the glycaemic and insulinaemic responses were recently studied in the horse (Vervuert et al., 2003, 2004, 2007). It should be noted, however, that grains are generally not eaten in isolation, but are combined with other ingredients, such as alfalfa and oats, in different feeding orders (Vervuert et al., 2008b). Alternatively, single cereals may be consumed as components of compound feeds. The feeding of compounded feeds is gaining in popularity, particularly for performance horses, as these feeds provide a more balanced diet (Richards et al., 2006). However, the safe intake of concentrates is limited to an upper level of 2 g starch/kg or 0.5 kg concentrates/100 kg bodyweight (BW) (Potter et al., 1992; Meyer and Coenen, 2002).

Compound feeds generally contain thermally processed grains, such as barley, maize, or wheat, and a high pre-caecal starch digestibility is assumed (Vervuert et al., 2008a). An increased availability of starch for enzymatic digestion should alter the metabolic response of the horse as more glucose will be absorbed in the small intestine. This is associated with higher glycaemic and insulinaemic responses (Vervuert et al., 2008a). Knowing the post-prandial effects of feeding isolated cereals or compound feeds is of special

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interest in the management of performance horses as substrate utilisation during subsequent exercise may be affected (Lawrence et al., 1993).

This study was undertaken to evaluate the glycaemic and insulinaemic responses in horses to the feeding of different quantities of starch contained within a compound feed.

Materials and methods

Animals

Four horses, one mare and three geldings, mean age (±SD) 10.8 (±0.5) years, mean BW (±SD) 560 (±36) kg and mean body condition score 5.5 (on a scale of 1–9), were individually housed in box stalls, bedded on wood shavings, and turned out onto a dry lot for 3 h/day. The horses had free access to water at all times.

The project was approved by the Ethics Committee for Animal Rights Protection of the Hannover District government, in accordance with German legislation for animal rights and welfare.

Diets

A commercial compounded feed was used as a source of starch. The ingredients of the compounded feed were oats and micronised cereals in the following descending order: oats, wheat, maize, barley, and wheat feed. The compounded feed also contained soya, soya oil, linseed, peas and ryegrass meal, minerals, fruits, and herbs. Different quantities of compounded feed were fed to supply 0.3, 0.6, 0.8, 1.1, 1.4, and 2 g starch/kg BW/meal in a randomised order according to a 6 incomplete Latin square design. As a result, dry matter (DM) intake of the compounded feed increased from a mean of 0.45 kg DM to supply 0.3 g starch/kg DM up to a mean of 3.1 kg DM to supply 2 g starch/kg BW/meal. The chemical composition of the compounded feed is shown in Table 1.

Each period consisted of 4 days acclimatisation to the treatment diet (fed once per day at 0730 h), except for the 2 g starch/kg BW/meal treatment, which involved a 10 day acclimatisation period. Horses were also fed 1 kg grass hay/100 kg BW/day, divided into three equal portions offered at 0900, 1400, and 1900 h.

Blood collection

Blood was collected at the end of each acclimatisation period before and after the test meal was fed at 0730 h following a 12 h overnight fast. At 0700 h, an indwelling catheter (1.8 × 2.35 mm/12 G, Braun) was inserted into the jugular vein. The catheter was connected to a 50 cm extension set (Vygon) and sutured in place. The extension set and catheter were flushed with physiological saline after every blood sampling.

Blood samples were collected 30 min before feeding the test meal, and thereafter at 30 min intervals for 5 h, and then at 60 min intervals for the following 3 h. Blood was collected into tubes containing no anticoagulant and allowed to clot for 30 min before centrifugation at 2012 × g for 10 min. Serum was removed and stored at −20 °C until analysed.

Analytical methods

Starch was estimated polarimetrically (Polartronic E, Schmidt and Haensch). Sugar was determined using the Luff Schoorl method (Matissek et al., 1992). Other dietary components were analysed using the Weende system (Naumann and Brassler, 1999).

Serum glucose concentrations were determined by glucose oxidase assay (Unimate 7 GLUC GDH, Roche Diagnostics), and serum insulin was determined by radioimmunoassay (Insulin RIA, Coat-A-Count [125I], DPC Biermann).

Statistical methods

The following parameters were calculated: mean serum glucose and serum insulin concentrations, peak serum glucose and serum insulin, time to peak serum glucose and serum insulin, incremental area under the serum glucose and serum insulin curve (AUC), using simple non-overlapping polygons, and the area over the baseline without consideration of the area beneath the curve.

Data were subjected to an analysis of variance (ANOVA) for repeated measures (Statistica, StatSoft), factoring the effects of diet and time post-prandially. Significant differences between means were identified by use of the Student–Newman–Keul test. Linear and nonlinear correlation tests were used to test for any relationship between serum glucose and serum insulin. Statistical significance was accepted at P < 0.05.

Results

A significant increase (P < 0.05) in serum glucose from baseline values was measured 30–60 min post-prandially for all quantities of starch fed (Fig. 1), and significantly (P < 0.05) higher mean serum glucose values were measured when the starch intake exceeded 1.1 g starch/kg BW/meal. These differences were also apparent for the peak glucose values and AUCs (Table 2). Time to reach peak serum glucose values ranged from 75 min (0.3 g

![Graph showing mean serum glucose concentrations (mmol/L) for different starch/kg BW doses.](image)
starch/kg BW/meal) to 158 min for 0.8 g starch/kg BW. Except for the highest starch intake (2.0 g starch/kg BW/meal), serum glucose concentrations returned to resting values between 240 min (0.3 and 0.6 g starch/kg BW/meal) and 420 min (1.4 g starch/kg BW/meal) after feeding (Fig. 1).

Serum insulin concentrations increased 30–60 min after feeding at all levels of starch intake, while absolute insulin values and AUCs depended on the quantity of starch consumed (Fig. 2, Table 3). The changes in insulin concentrations were biphasic for all levels of starch intake, except for the higher starch intakes (1.4 and 2.0 g starch/kg BW/meal), and were only measured for starch intakes below and above 1 g starch/kg BW/meal (Fig. 2, Table 3). Serum insulin concentrations were measured between 143 min (2 g starch/kg BW/meal) and 225 min (1.1 g starch/kg BW) post-prandially (Fig. 2, Table 3).

The relationship between mean serum glucose and serum insulin concentrations (Fig. 3) was described either by a linear equation, \( y = -167 + 36.1x \), where \( y \) is mean serum glucose and \( x \) is mean serum insulin, or by an exponential equation, \( y = -173 + \exp(4.51306 + 146385)x \), where \( y \) is mean serum insulin and \( x \) is mean serum glucose.

Discussion

This study showed that increasing the starch intake per meal increased the post-prandial glucose and insulin responses of horses, presumably as a direct result of more glucose being available within the small intestine. These results were not unexpected for two reasons: firstly, more starch and sugar increased the potential for glucose release from the small intestine. These responses of horses, presumably as a direct result of more glucose being available within the small intestine. These results were not unexpected for two reasons: firstly, more starch and sugar increased the potential for glucose release from the small intestine. These results were not unexpected for two reasons: firstly, more starch and sugar increased the potential for glucose release from the small intestine.

Changes in glucose and insulin concentrations observed in the current study were comparable to an earlier study in which single cereals were fed to supply similar quantities of starch (2 g starch/kg BW/meal) and were also cooked in the same way (Vervuert et al., 2008a). However, changes in serum glucose were not precisely related to changes in starch intake and clear differences in glycaemic response were only measured for starch intakes below and above 1 g starch/kg BW/meal. In contrast, serum insulin seemed to better reflect differences in starch intake. Generally, a close correlation between post-prandial blood glucose and insulin response is assumed, and for this reason, the nature of the glycaemic response of a horse following a meal can be used as an indicator of insulin resistance in horses (Kienzle et al., 1992).

Table 2

<table>
<thead>
<tr>
<th>Starch intake (g starch/kg BW)</th>
<th>Peak glucose (mmol/L)</th>
<th>Time peak glucose (min)</th>
<th>AUC (mmol × min/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3</td>
<td>7.3 ± 1.3b</td>
<td>75 ± 17b</td>
<td>272 ± 168b</td>
</tr>
<tr>
<td>0.6</td>
<td>7.6 ± 0.4b</td>
<td>120 ± 49bc</td>
<td>419 ± 109c</td>
</tr>
<tr>
<td>0.8</td>
<td>7.7 ± 1.1bc</td>
<td>158 ± 57c</td>
<td>559 ± 49g</td>
</tr>
<tr>
<td>1.1</td>
<td>8.5 ± 0.6c</td>
<td>143 ± 38c</td>
<td>732 ± 162d</td>
</tr>
<tr>
<td>1.4</td>
<td>8.9 ± 1.2cd</td>
<td>143 ± 29c</td>
<td>857 ± 527d</td>
</tr>
<tr>
<td>2.0</td>
<td>8.8 ± 0.9cd</td>
<td>120 ± 42bc</td>
<td>821 ± 177d</td>
</tr>
</tbody>
</table>

\(^{a}\) Values are means ± SD.

\(^{b,c,d,e}\) Means in the same column with unlike superscripts are different with \( P < 0.05 \).

Table 3

<table>
<thead>
<tr>
<th>Starch intake (g starch/kg BW)</th>
<th>Peak insulin (μU/mL)</th>
<th>Time peak insulin (min)</th>
<th>AUC (μU × min/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3</td>
<td>43 ± 26b</td>
<td>75 ± 39b</td>
<td>5463 ± 3726b</td>
</tr>
<tr>
<td>0.6</td>
<td>73 ± 27c</td>
<td>158 ± 29f</td>
<td>10339 ± 4055c</td>
</tr>
<tr>
<td>0.8</td>
<td>88 ± 69d</td>
<td>188 ± 105d</td>
<td>19301 ± 16275d</td>
</tr>
<tr>
<td>1.1</td>
<td>162 ± 32d</td>
<td>225 ± 97d</td>
<td>33612 ± 5646d</td>
</tr>
<tr>
<td>1.4</td>
<td>169 ± 131d</td>
<td>225 ± 97d</td>
<td>40386 ± 30615d</td>
</tr>
<tr>
<td>2.0</td>
<td>202 ± 72de</td>
<td>143 ± 45e</td>
<td>43771 ± 22549d</td>
</tr>
</tbody>
</table>

\(^{a}\) Values are means ± SD.

\(^{b,c,d,e}\) Means in the same column with unlike superscripts are different with \( P < 0.05 \).
Results from recent studies with horses (Jose-Cunilleras et al., 2004; Vervuert et al., 2003, 2004) showed only a weak correlation between the glycaemic and insulinaemic indexes for different cooked cereals in agreement with the current results. In consequence, the analysis of serum glucose does not properly define insulin response. Thus further work is required to clarify this relationship to predict normality. In practice, the measurement of both parameters is necessary to describe glucose metabolism.

Starch intakes >1.1 g/kg BW/meal tended to induce a biphasic insulin response. It is generally accepted that the first insulin peak results from the secretion of an immediately releasable pool of insulin granules (storage-limited model; Straub and Sharp, 2002). The second phase can last several hours if the β-cells of the pancreas are continuously exposed to glucose (signal-limited pool), and it is characterised by insulin pulses occurring at 5–15 min intervals (Nunemaker et al., 2006).

The biphasic response measured in this study was difficult to understand since a response of this magnitude normally only occurs when extracellular glucose concentrations are abruptly raised from a sub-stimulatory to a stimulatory level and then maintained at that high level (Aizawa et al., 2002). The so-called ‘glucose jump’ which would be required to induce a biphasic response in isolated mouse and rat pancreatic islets is from 3 to 17 mmol/L (Aizawa et al., 2002), which would be unlikely to occur in healthy horses under normal conditions.

In this study, fasting serum glucose concentrations were about 5 mmol/L, and increased to 7–9 mmol/L post-prandially over 30–150 min. Similar changes in serum glucose have been described in studies where oats, barley, or maize were fed to healthy horses (Vervuert et al., 2003, 2004, 2007), although it was not possible to distinguish a biphasic response in these studies. Since a compounded feed can be quite different from a simple cereal in terms of its composition, feed components other than those likely to increase glucose levels in the intestinal chyme, such as sugar, protein, fat, or fibre, may have some impact on the nature of the insulin response.

It was interesting to note that values for glucose and insulin did not return to baseline within 480 min following the consumption of 1.4 (only serum insulin) and 2 g starch/kg BW/meal. This contrasts with results from previous work, where baseline glucose and insulin values were achieved between 300 and 360 min post-prandially, respectively, when similar amounts of starch were fed using a single starch source (Vervuert et al., 2008a). This apparent inconsistency may be explained by differences in composition (see above) and/or meal size during each study. Meal size can affect the rate of gastric emptying in horses (Métais et al., 2004). Furthermore, digesta passage rate through the pre-caecal part of the gastrointestinal tract is variable and depends on a number of factors, including the quantity of food consumed, the rate of food consumption and the type of food fed (McLean et al., 2000).

All of these factors could have an impact on the speed with which starch is digested in the small intestine and thus the quantity of glucose available for absorption. De Fombelle et al. (2004) reported a shorter pre-caecal passage rate.

![Fig. 3. Relationship between serum glucose (mmol/L) and serum insulin (μU/mL) for the different starch levels. The linear relationship is described by the equation insulin = −167 + 36.1 × glucose, r = 0.73, P < 0.001. The exponential equation is described by insulin = −173 + exp (4.51306 + (0.146385) × glucose), r = 0.74, P < 0.001.](image-url)
of digesta when a starch-rich diet was fed in several small portions using nylon bags, than when it was fed as a single, large meal. However, meal size did not specifically appear to influence the glycemic response of horses fed either 1.3 kg/100 kg BW of a cereal/alfalfa mixture in one large meal or a meal divided and fed in three portions (van Weyenberg et al., 2007).

By 480 min after feeding, insulin values above resting concentrations may adversely affect racing performance in horses. Attempts to delay fatigue have been made by providing carbohydrates fed as starch (1–3 kg cereals) or glucose 2–4 h before exercise, but this practice resulted in increased insulin secretion followed by pronounced hypo-glycaemia, decreased lipolysis, and increased depletion of muscle glycogen during exercise (Lawrence et al., 1993; Stull and Rodiek, 1995). In these studies, high glycemic and insulinemic responses were monitored before the start of exercise and the fall in plasma glucose during exercise was directly proportional to blood glucose and insulin levels at the beginning of exercise. Insulin is known to inhibit lipolysis and fatty acid oxidation in skeletal muscle, resulting in an impaired availability of fatty acids in energy contribution, but increased reliance on carbohydrate stores (Jose-Cunilleras et al., 2002). It is therefore possible that persistently high serum insulin values may have a negative effect on exercise performance if the period of time between feeding and exercise is too short.

Currently, there is no general consensus about the possible negative effects that a high dietary starch intake might have on insulin metabolism in horses. In humans, it has been postulated that the consumption of foods that produce high glycemic responses and, subsequently, high insulin responses, may be associated with a higher risk for obesity, type 2 diabetes and cardiovascular disease in these people (Jenkins et al., 2002). A high intake of digestible carbohydrates has been shown to be associated with decreased insulin sensitivity in Thoroughbred foals (Treiber et al., 2005). However, large quantities of cereals are commonly fed to racing Thoroughbreds (Richards et al., 2006), while obesity and insulin resistance are rarely recorded in these breeds. This could be related to the fact that racehorses undertake intensive exercise which is known to act as a regulatory factor in insulin metabolism (Freestone et al., 1992).

Conclusions

This study showed that a starch intake \( \leq 1.1 \text{ g/kg BW/meal} \) produced only moderate glucose and insulin responses when highly digestible starch was fed in the form of a compounded feed. Earlier work suggested that a safe intake of concentrates by horses could be achieved by limiting starch intake to an upper level of 2 g starch/kg BW/meal or 0.5 kg concentrate/100 kg BW/meal (Potter et al., 1992; Meyer and Coenen, 2002). The results from the current study suggested that a meal size of 0.3 kg/100 kg BW/meal or a starch intake <1.1 g starch/kg BW/meal should be used in compounded feeds and cereals that contain 30–40% starch, while further limitations may be necessary for feeds that contain >40% starch.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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