Growth and Plasma Leptin in Yearling Mares Fed a High-Fat Supplemented Diet

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Abstract

Our objective was to determine if feeding a high-fat supplement versus a control supplement to growing yearling mares on pasture would affect growth, body composition or endocrine parameters as assessed via body weight (BW), body condition scores (BCS) or concentrations of plasma hormones. Eight yearling mares were paired by initial BW (348 ± 19 kg) and maintained on pasture and supplemented with either a high-fat supplement (16% fat) or a control supplement (3% fat) at 0.8% of their BW in two daily meals for 8 weeks. Both BW and BCS increased for all mares throughout the study (each P, 0.0001); however, no difference in BW or BCS could be attributed to treatment effects. Nonetheless, plasma concentrations of leptin were greater in mares fed the high-fat supplement (P = 0.0001) compared with the control supplement. Plasma concentrations of growth hormone tended to be greater in high-fat-fed mares (P = 0.06). Plasma concentrations of insulin did not differ between treatment groups (P = 0.96). Although no gross difference in BW or BCS was discernable among mares fed the control versus high-fat supplement, these data provide evidence that increasing fat content in the diet may alter leptin levels independent of changes in body composition.

Keywords: leptin; equine; high-fat diet; body condition

Introduction

The practice of adding fat to horse diets has been accepted in the performance horse industry as a way of increasing energy input into the animal to meet the increased demands of performance without compromising health1,2. In contrast, feeding high-fat rations to growing horses, as a means to increase growth rates, has not been widely accepted due to lingering concerns of inducing growth abnormalities associated with rapid growth3. Consequently, many producers develop yearling replacement mares by maintaining them on pasture and supplementing their diets with concentrate starch rations to meet their nutritional requirements.

Recently, it was reported that body fat mass was positively associated with endocrine levels of leptin4. Leptin is a 16kDa protein hormone, primarily produced and secreted by white adipose tissue, which contributes to the regulation of energy homeostasis4. Circulating levels of leptin have been detected in horses and those levels increase as adiposity increases5,6. The current premise is that leptin functions as a dynamic indicator of adipose mass/nutritional status for the animal and communicates nutritional well-being of the animal to hypothalamic appetite centres. Consequently, leptin is believed to play a key role in feed intake, growth, metabolism and reproduction7,8.

In mature horses, concentrations of leptin decreased when obese pony mares were subjected to a period of feed deprivation9 and increased after a meal or infusion of insulin into euglycaemic stallions10. Our objective was to determine if feeding a high-fat supplement versus a control supplement to growing yearling mares on pasture would affect growth, body composition or endocrine parameters as assessed via body weight (BW), body condition scores (BCS) or
plasma concentrations of leptin, growth hormone (GH) and insulin.

Materials and methods

Animals

Eight yearling Quarter Horse-type maiden mares were utilized and maintained at the University of Missouri–Columbia horse farm. All procedures utilizing live animals were approved by the University of Missouri Animal Care and Use Committee.

Procedures

Horses were statistically blocked by initial BW (348 ± 19 kg) to ensure equal distribution of comparable animals. The two heaviest mares were assigned to block 1, with the allocation continuing until the two lightest mares were assigned to block 4. One horse from each block was fed a high-fat supplement and the other fed a control supplement at 0.8% of their BW per day (adjusted weekly), fed in two daily meals for 8 weeks. Horses were maintained in a single pasture, consisting mainly of tall fescue, for the duration of the experiment and allowed free access to water. Supplements were formulated to contain similar amounts of protein and fibre, and different amounts of fat (Table 1), and then pelleted. The control supplement, GROSTRONG® 13% (ADM Alliance Nutrition, Quincy, IL, USA), contained 3.1% fat (2.6 Mcal DE kg⁻¹). The high-fat supplement was formulated using the same ingredients as the control supplement and contained 16% fat (3.6 Mcal DE kg⁻¹). The fat source for both supplements was stabilized rice bran and soybean oil refinery lipid. At every feeding, each horse’s feed was top-dressed with 60 ml of sorghum molasses (to increase palatability), and an additional 28 g of GROSTRONG® Minerals for horses (ADM Alliance Nutrition).

Horses were fed individually in feeding stanchions to insure that they consumed only the feed allotted for each individual. Mares were allowed 30 min to eat at 0600 and 1800h daily. After 30 min from introduction of the meal, unconsumed feed was weighed to determine feed intake.

Blood was collected from mares following feed removal after the 06.00 feeding each Monday. Three consecutive blood samples were collected from each horse at 0, 15 and 30 min and assayed for hormones. This triplicate sampling paradigm was performed each week in an effort to minimize the within-week and pulse variation in levels of hormones. Samples were collected via jugular venipuncture into Vacutainer® (Becton Dickinson, Franklin Lakes, NJ, USA) tubes with EDTA (tri-potassium ethylenediaminetetraacetic acid). Blood samples were immediately centrifuged at 3000 × g for 25 min at 4°C and plasma was collected and stored at ~20°C until analysed for leptin, GH and insulin. Concentrations of leptin were determined using RIA (radioimmune assay) procedures previously validated for horse plasma². All samples were analysed in triplicate in a single assay, and the intra-assay coefficient of variation was <10%. GH concentrations were determined by RIA procedures previously validated for horse plasma¹. All samples were analysed in triplicate in a single assay, and the intra-assay coefficient of variation was <10%. Insulin concentrations were determined using a commercial insulin assay kit (Diagnostic Products Incorporated, Los Angeles, CA, USA), which was previously validated for use with horse plasma¹². All samples were analysed in duplicate in a single assay and the intra-assay coefficient of variation was <10%.

Each week, BW (determined using a digital scale) and BCS (using techniques reported by Henneke et al.¹³) were determined for each horse. Two trained technicians independently determined BCS of each horse and the average of the two scores was used for statistical analysis.

Statistics

Data were analysed with a repeated measures design using the Mixed Model procedures of SAS V8 (SAS Inst., Inc., Cary, NC, USA) to determine the effects of treatment (control versus high-fat) on BCS, feed intake, BW and plasma concentrations of leptin, GH and insulin. Covariance structure was determined by Akaike information criterion and Schwarz–Bayesian criterion¹⁴. The covariance structures used were compound symmetry for BCS; autoregressive for plasma concentrations of GH and insulin; autoregressive heterogeneous for BW and feed intake and Toeplitz for plasma concentrations of leptin. The Toeplitz structure makes the following assumptions in relation to time: spacing and variance are equal; covariance and correlation are not equal. The model used to determine the effects on the dependent variables included block (by BW), treatment, week and treatment by week as independent variables, where week was the repeated measure and animal within treatment by

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Control</th>
<th>High-fat</th>
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<tbody>
<tr>
<td>DM (%)</td>
<td>88.9</td>
<td>90.8</td>
</tr>
<tr>
<td>DE estimated (Mcal kg⁻¹)</td>
<td>2.6</td>
<td>3.6</td>
</tr>
<tr>
<td>CP (%)</td>
<td>13.9</td>
<td>14.7</td>
</tr>
<tr>
<td>Fibre (%)</td>
<td>11.5</td>
<td>11.6</td>
</tr>
<tr>
<td>ADF (%)</td>
<td>15.3</td>
<td>17.1</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>3.1</td>
<td>16.0</td>
</tr>
</tbody>
</table>

Table 1 Nutrient composition (represented on a dry matter basis) of the high-fat and control supplements fed to yearling Quarter Horse-type mares

Feeds tested by ADM Alliance Nutrition laboratories.
block was used as the subject. Least square means and differences were generated for the variables of treatment, week and treatment by week.

Results

We observed no difference in the effect of dietary treatment on BW ($P = 0.85$; Fig. 1); however, BW did increase from week to week ($P = 0.0001$), indicating that the horses continued to grow throughout the study. In addition, although BCS increased in all horses over the duration of the study ($P = 0.0001$), no differences could be attributed to treatment effects ($P = 0.22$; Fig. 2). Similarly, amount of supplement consumed by mares did not differ with respect to treatment ($P = 0.2$; high-fat = 2.82 ± 0.14 kg day$^{-1}$ vs. control = 3.02 ± 0.14 kg day$^{-1}$). Surprisingly, overall mean plasma concentrations of leptin within group (i.e. main treatment effect) were greater for high-fat-treated mares (1.54 ± 0.12 ng ml$^{-1}$) when compared with control mares (0.88 ± 0.12 ng ml$^{-1}$; $P < 0.05$). Over the duration of the study (treatment by time effect; $P = 0.0001$; Fig. 3), plasma concentrations of leptin were greater in the high-fat-treated mares when compared with control mares. A time-series analysis revealed that plasma concentrations of leptin did not differ prior to the initiation of feeding regimens.

After 1 week of mares consuming the high-fat diet, peripheral concentrations of leptin tended (P < 0.1) to be greater among high-fat-fed mares. At 2 weeks and thereafter, high-fat-fed mares had significantly greater peripheral concentrations of leptin ($P = 0.0001$) than control mares. Mean concentrations of GH tended to be greater in mares consuming the high-fat supplement (3.30 ± 0.37 ng ml$^{-1}$) compared with control mares (1.98 ± 0.37 ng ml$^{-1}$; $P = 0.06$). No significant treatment by time interaction was observed with GH concentrations. Mean concentrations of insulin did not differ between high-fat-supplemented mares (48.55 ± 8.00 µIU ml$^{-1}$) compared with control-fed mares (47.98 ± 8.00 µIU ml$^{-1}$; $P = 0.96$).

Discussion

Concentrations of leptin tended to differ the first week following the initiation of dietary supplementation, with significant differences occurring by week 2 and continuing throughout the duration of the experiment. Body composition parameters, as assessed by BW and BCS, did not differ between treatment groups, but did increase throughout the study. These data provide evidence that all mares were growing at the same rate. The absence of differences in body composition between treatment groups supports the argument that differences in concentrations of leptin were directly due to dietary supplementation of added fat. These observations are in agreement with reports that rodents fed a high-fat diet exhibited an increase...
in circulating concentrations of leptin\textsuperscript{15,16}. In the rodent studies, however, mice with the high-fat diet increased in BW, whereas in the present study no increase in BCS (due to treatment) was observed, suggesting that either no change in BCS occurred, or that the fat which accumulated could not be detected by BCS techniques.

Horse producers have traditionally refrained from feeding energy-dense feeds to growing horses, based on a belief that horses growing at a rapid rate are predisposed to developing osteochondrosis. However, Scott et al.\textsuperscript{17} determined that feeding growing yearling horses a concentrate diet consisting of up to 10% fat was not detrimental to the health of the horse. These investigators\textsuperscript{17} noted that feed intake was not significantly different, but horses fed the highest amount of fat (10%) tended to require less total feed per kilogram weight gained. Ott and Asquith\textsuperscript{18} reported that feeding yearling horses 60% above the recommended energy requirements resulted in no bone growth abnormalities. In the present study, no horses were observed to be in ill health, or suffered any growth defects, thus all were returned to the University of Missouri research and teaching herd.

The focus of the present study was to determine if feeding a ration high in fat to growing yearling mares that were managed on pasture would affect growth and body composition when compared with a control supplement. We did not observe any difference in the amount of concentrate consumed between the two groups. In agreement with this observation, Scott et al.\textsuperscript{17} also noted that feeding a ration consisting of 10% fat did not influence feed intake. Admittedly, the horses in the present study had free access to fescue pasture in addition to concentrate, and thus we cannot rule out the possibility that high-fat-supplemented mares may have consumed less forage to balance their nutrient intake. Further, due to facility constraints and the untrained management of the horses, we were unable to determine forage intake. Identifying faeces from individual horses within the pasture or performing faecal grabs were not possible.

Previously, we reported a linear increase in leptin concentrations in horses as BCS increased\textsuperscript{5}. We have also reported an increase in BW and BCS in thin pony mares without subsequent increases in leptin concentrations\textsuperscript{5}. In the present study, we did not observe corresponding treatment differences in either BW or BCS relating to concentrations of leptin. The observed changes in concentrations of leptin, without an increase in BW or BCS, may be explained by Kennedy’s lipostatic theory\textsuperscript{19}. Briefly, Kennedy suggested that animals regulate appetite and energy expenditure according to the amount of energy stored (i.e. body fat) and food available. This theory has now been elucidated with the discovery of leptin, which has been reported to be a key regulator in this mechanism, acting to signal adiposity and regulate energy balance\textsuperscript{7,8}. We conclude that the differences in concentrations of leptin (with respect to treatment) were attributable to regulation of the ‘lipostat’, as mares consumed the high-fat supplement for the duration of the experiment. An alternate explanation for the elevated concentrations of leptin in the high-fat group without subsequent body composition changes could be that the high-fat group had developed a greater amount of internal adipose mass as opposed to subcutaneous adipose. Cartmill et al.\textsuperscript{20} have reported different concentrations of leptin in horses with the same BCS. In beef cattle, during the fattening phase, kidney and pelvic fat is deposited at a faster rate than inter-muscular fat, and this ratio is even greater in dairy breeds\textsuperscript{21}. Unfortunately, the estimation of body composition by BCS only accounts for subcutaneous adipose accumulation, and no means were available to quantitate inter-abdominal adipose mass in the horses. We conclude that some horses may develop intra-abdominal adipose more rapidly, being more metabolically efficient.

Mean GH concentrations tended to be greater in the high-fat-supplemented mares when compared with the control group. The increased energy concentration in the high-fat diet most likely elicited a metabolic response, as indicated by the increase in GH. One of the effects of GH is the metabolism of fat, and thus we conclude from our finding that GH secretion was increased in the high-fat-supplemented mares as a response to the increased concentration of fat in the diet. In a similar study with fat-tailed Tuf lambs, GH concentrations did not increase when leptin increased during short-term high-fat feeding\textsuperscript{22}. We attribute this to the difference in physiological digestive processes of fat between these species. Added dietary fat has been shown to be highly digestible by horses\textsuperscript{23}, and sheep have been shown to have a lower digestibility of fat\textsuperscript{24}. A study investigating added fat to growing horse diets reported variable differences in GH between a carbohydrate-based diet and a 10% added fat diet\textsuperscript{25}. However, in that report it was noted that GH concentrations were greater in the fat-added diet immediately following feeding.

In the present study, we found no differences in insulin concentrations between high-fat and control diets. Previous work, investigating the effects of diet on insulin in horses, has shown no differences between a diet of corn and corn with 10% corn oil when measuring post-prandial concentrations\textsuperscript{26}. Based on the previous and current finding, we conclude that the addition of fat may not alter insulin secretion in horses.

In conclusion, while feeding a 16% fat diet did not alter BW or BCS during the 8-week study, the high-fat diet increased plasma concentrations of leptin for reasons yet unknown. Nonetheless, these data provide evidence
High-fat diet and leptin in yearling mares

that feeding high-fat rations can alter plasma leptin levels independent of changes in body composition, or alternatively that plasma leptin levels are influenced by subtle alterations in body composition that occur prior to any clinically detectible increase in adiposity.

References


