Meal size and feeding frequency influence serum leptin concentration in yearling horses

S. M. Steelman,*1 E. M. Michael-Eller,† P. G. Gibbs,† and G. D. Potter†

*Department of Medical Physiology, Texas A&M University Health Science Center, College Station, 77843; and †Department of Animal Science, Texas A&M University, College Station, 77843

ABSTRACT: Energy is an essential nutrient for all horses, and it is especially important in performance horses, pregnant and lactating mares, and young growing horses. A negative energy balance in horses such as these may result in unsatisfactory performance, decreased fertility, or slow growth. Therefore, ensuring adequate energy intake is an important aspect of the nutritional management of the equine. This study was undertaken to investigate the effects of feeding large, carbohydrate-rich, concentrate meals on the satiety-inducing hormone, leptin. Three groups of yearling horses were rotated through 3 feeding schedules in a replicated 3 × 3 Latin square design. Horses were fed 2, 3, or 4 times per day (2 ×, 3 ×, and 4 × feeding schedules, respectively), each for a period of 11 d, with the total amount of daily feed held constant. Horses were weighed and BCS was determined on the first day of each period. Blood samples were collected before the morning meal on d 1, 4, and 7 of each period. Additionally, blood was sampled for the last 24 h of the 2 × and 4 × dietary periods. Neither weight nor BCS changed during the study (P = 0.99 and P = 0.28, respectively). Both mean and peak plasma glucose were greatest in 2 × horses (P < 0.05), as were mean areas under the curve. Serum leptin concentration increased in 2 × horses (P < 0.05), but not in horses fed 3 or 4 times daily. Leptin was elevated in horses with greater BCS (P < 0.05) and increased steadily throughout the study (P < 0.05). Data from the 24-h collection indicated that 2 × horses had fluctuations in leptin production throughout the day (P < 0.05), whereas horses fed 4 times daily did not. Overall, this study indicates that feeding horses 2 large concentrate meals daily can increase mean serum leptin concentrations and may cause fluctuations in leptin production over a 24-h period. This departure from baseline leptin concentration has the potential to affect appetite, along with numerous other physiological processes.

Key words: glucose, horse, leptin

INTRODUCTION

In today’s equine industry, many horses are fed concentrate feed, usually once or twice daily. This not only leads to an increased incidence of colic (Tinker et al., 1997), but can also disrupt the horse’s homeostasis by increasing blood concentrations of glucose and hormones such as insulin and GH (Ropp et al., 2003). These changes in hormone concentrations have the potential to alter appetite in rodents (Menendez and Atrens, 1991; Wynne et al., 2005) along with other physiological processes, especially in the horse (DeKock et al., 2001; Sessions et al., 2004). Therefore, the effect of meal feeding on the release of hormones, especially those involved in appetite, needs to be determined.

One key hormone that regulates appetite is leptin (Campfield et al., 1995; Pelleymounter et al., 1995). Leptin is produced in the adipose tissue in response to glucose uptake and is an indicator of energy availability (reviewed in Muoio and Dohm, 2002). In many species, when serum leptin concentrations are high, appetite is decreased; however, when leptin concentrations fall, physiological processes such as reproduction and growth are put on hold to conserve energy. This relationship may hold true in the equine as well. Serum leptin concentrations increase with BCS (Buff et al., 2002) and both short-term and chronic feed restriction in mature mares result in a decline in serum leptin concentrations (Gentry et al., 2002a; McManus and Fitzgerald, 2000). The manipulation of leptin in the horse may provide a way to increase feed intake in growing horses, performance horses, and lactating mares.

Thus, the main objective of this study was to determine if feeding yearling horses multiple small carbohy-
drate meals daily would result in a lower serum leptin concentration than feeding 2 large meals daily. The effect of meal size and timing on the pattern of leptin secretion over a 24-h period as well as the effects of sex and BCS were also investigated.

**MATERIALS AND METHODS**

All experimental procedures were approved by the Texas A&M University Animal Care and Use Committee. This experiment utilized 9 Quarter Horse yearlings between the ages of 13 and 16 mo. Eight of the horses were housed in 15- × 20-m drylot pens, whereas 1 horse was confined to a stall for the duration of the study due to a leg injury. Horses were divided into 3 groups, with each group randomly assigned 2 fillies and 1 gelding. All horses underwent a 10-d adaptation period to acclimate them to the pens and to eating increased amounts of feed. Horses were fed a 14% CP, pelleted concentrate (Producer’s CoOp, Bryan, TX) and coastal Bermuda grass hay in a 60:40 (wt/wt) ratio at a total intake of 2.5% of BW/d. During the adaptation period, horses were fed twice daily. Before the adaptation period, horses had been maintained on pasture and fed approximately 0.83 kg of 14% CP concentrate twice daily.

After the adaptation period, each group of horses was rotated through 3 feeding schedules in a replicated 3 × 3 Latin square design. The feeding schedules are summarized in Table 1. The horses were fed 2, 3, or 4 times per day (2×, 3×, and 4× feeding schedules, respectively); amounts of hay and concentrate offered each day were held constant throughout the study at a total intake of 2.5% of BW/d. Concentrate was fed in individual stalls, whereas hay was group fed by pen. No fighting was observed during group feeding, and all horses had equal access to hay.

Each feeding schedule was fed for a period of 11 d. The following period was begun on the day after the end of the previous period, so that the duration of the entire experiment was 33 d. The study was conducted during the months of July and August. On the first day of each period, horses were weighed, BCS was determined, and blood samples were collected between 0600 and 0700, before the morning meal. Samples were also collected before the morning meal on d 4 and 7 of each period. Blood samples were collected by jugular venipuncture into Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) and then stored at 4°C until serum was harvested.

All horses on the 2× and 4× schedules were intravenously catheterized between 1400 and 1700 on d 10. During this procedure, horses were held in stocks and restrained with an upper-lip twitch. It was necessary to sedate several of the horses with either xylazine or acepromazine or a combination of both. The 24-h blood collection began at 1800 on d 10. Blood was collected from horses every 2 h for 24 h, and the last collection occurred at 1800 on d 11. The 2× and 4× schedules were chosen for 24-h sampling because it was hypothesized that the differences in serum leptin concentrations would be most pronounced between horses fed 2 and 4 times daily. We also took into consideration the technical difficulties and animal discomfort associated with sampling that involved repeatedly placing intravenous catheters. Omitting the 3× schedule from the 24-h protocol provided the horses with a break between samplings. Blood was always taken from the horses in the same order, and all samples were taken within approximately 10 min. During these collections, the horses remained tethered to a rail, but were still fed according to the previously described schedules. Horses were tied in such a way that the concentrate could still be fed individually and hay could be group fed.

Blood samples were also collected before and after the 0700 meal on d 11 to determine the postprandial blood glucose response. These samples were taken via jugular venipuncture, and then the blood was transferred into tubes containing NaF. The first collection was at 0630, horses were fed at 0700, and blood was taken again at 0730 and then every 30 min thereafter until 1300. Catheters were removed after the last blood collection at 1800 on d 11, and the horses were untied and allowed to move freely about their pens.

Blood tubes were centrifuged at 1,200 × g for 25 min at 4°C, or until adequate separation had occurred. The resulting serum or plasma was stored at ~20°C until analysis. Plasma glucose content was determined with a YSI 2300 Glucose/Lactate Analyzer (YSI, Inc., Yellow Springs, OH). Leptin was analyzed by RIA with a multispecies leptin RIA kit (Linco Research, St. Louis, MO) using established methods (McManus and Fitzgerald, 2000). According to the method of McManus and Fitzgerald (2000), our standard curve was created using solutions of 0.5, 1.0, 2.0, 5.0, 10.0, and 20.0 ng/mL of human leptin. This assay has previously been validated for use in horses (Buff et al., 2002). The intra- and interassay coefficients of variation for control serum samples were 6.7 and 15.7%, respectively. The limit

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**Table 1. Description of the feeding schedules**

<table>
<thead>
<tr>
<th>Feeding schedule</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>2×</td>
<td>Horses fed concentrate and hay twice daily at 0700 and 1900</td>
</tr>
<tr>
<td>3×</td>
<td>Horses fed concentrate 3 times daily at 0700, 1500, and 2300; horses fed hay twice daily at 0700 and 1900</td>
</tr>
<tr>
<td>4×</td>
<td>Horses fed concentrate 4 times daily at 0700, 1300, 1900, and 0100; horses fed hay twice daily at 0700 and 1900</td>
</tr>
</tbody>
</table>

1The total amounts of hay and concentrate offered each day were held constant throughout the study at a total intake of 2.5% of BW/d.
of detection of the assay was 0.5 ng/mL. Results are reported in nanograms of human equivalents (HE) per milliliter.

Statistical analyses were performed using a statistical software package (Stata 7.0, StataCorp, College Station, TX). Areas under the plasma glucose concentration–time curves were calculated by using the trapezoidal rule. Plasma glucose concentrations were subjected to ANOVA. Variables within the model included time, feeding schedule, and the time by feeding schedule interaction. Analyses of variance were also performed on serum leptin concentrations to determine the influence of BCS, sex, feeding schedule, square, and period. When differences were found at $P < 0.05$, means were separated using a Fisher–Hayter pairwise comparison or Student’s $t$-test as appropriate. Additionally, some data were normalized to either a d 1 or a time zero value, which was representative of a baseline. This was done to better visualize changes in the concentration of leptin. Simple regression analysis was performed to determine the correlation between mean serum leptin concentrations and mean BCS. Regression analysis was also used to fit normalized serum leptin concentrations from the 24-h blood collections to either a linear or quadratic model.

RESULTS AND DISCUSSION

Feed Intake and Refusals

Horses on the 2×, 3×, and 4× feeding schedules were offered an average of 2.58 ± 0.06 kg, 1.74 ± 0.04 kg, and 1.30 ± 0.08 kg concentrate per meal, respectively. Amounts of feed refused per meal were negligible. No difference in satiety was noted among horses on the 3 feeding schedules.

Weight and BCS

Although the horses used in this study were young, growing yearlings, BW did not increase appreciably ($P = 0.99$) during the experimental period (342 ± 10.1 kg during period 1 to 354 ± 10.1 kg during period 3). Furthermore, regression analysis of BW showed no correlation with period ($r^2 = 0.459, P = 0.71$; data not shown). This may have been due to the short duration of the study or to variations in the horses’ hydration status or amount of digesta in the gut at the time of weighing. Average daily gain was 0.39 ± 0.14 kg. Body weight was not correlated with BCS ($P = 0.19$) or with sex. This was most likely due to the lack of variation in BW and BCS in this population of horses. Body condition score was influenced heavily by sex, with females having a greater mean BCS than males ($P < 0.01; 5.14$ vs. $4.61$; data not shown). No effect of feeding schedule was observed ($P = 0.90$) on either BW or BCS (Table 2).

Postprandial Plasma Glucose

Baseline glucose concentrations did not differ among horses on feeding schedules ($P = 0.83$; Table 3). Postprandial concentrations of plasma glucose varied over the 6 h that blood was drawn ($P < 0.05$; Figure 1). Plasma glucose concentration differed significantly among feeding schedules at only 2 time points: at 30 and 120 min postfeeding, glucose concentration was greatest ($P < 0.05$) in horses on the 2× schedule. Peak glucose was greater ($P < 0.05$) on the 2× compared with the 4× schedule, but did not differ among horses on the 2× and 3× or 3× and 4× feeding schedules (Table 3). Mean glucose varied with feeding schedule ($P < 0.05$); mean concentrations of glucose were greater in horses on the 2× schedule than those on the 3× or 4× schedules ($P < 0.05$), although there was no difference between the 3× and 4× schedules. The area under the curve on the 2× schedule was greater ($P < 0.05$) than that on the 3× and 4× schedules, but the 3× schedule did not differ from the 4×. These results in concert indicate that the larger amounts of concentrate fed per meal on the 2× schedule did in fact result in a larger plasma glucose response than the smaller amounts fed on the 3× and 4× schedules.

Glucose concentration also varied with period, as shown in Table 4. Horses had a lower mean glucose concentration during period 1 ($P < 0.05$) than during the other 2 periods. Peak glucose concentration was greater in period 3 than in periods 1 or 2 ($P < 0.05$). Mean change from baseline to peak was greater during

### Table 2. Body condition score and BW in yearling horses fed 2 (2×), 3 (3×), or 4 (4×) times daily

| Feeding schedule | BCS | BW (

<table>
<thead>
<tr>
<th>Feeding schedule</th>
<th>Baseline</th>
<th>Peak</th>
<th>Mean</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>2×</td>
<td>89.5</td>
<td>118.3a</td>
<td>98.8a</td>
<td>1,253.7a</td>
</tr>
<tr>
<td>3×</td>
<td>88.1</td>
<td>109.8ab</td>
<td>92.5b</td>
<td>1,168.7b</td>
</tr>
<tr>
<td>4×</td>
<td>88.5</td>
<td>106.1b</td>
<td>93.3b</td>
<td>1,176.0b</td>
</tr>
<tr>
<td>Pooled SEM</td>
<td>2.24</td>
<td>3.48</td>
<td>1.00</td>
<td>29.3</td>
</tr>
</tbody>
</table>

a,bWithin a column, means without a common superscript differ, $P < 0.05$.
aMeasurements taken between 0630 and 1130 on d 11 of each period. Values represent the means of 9 yearling horses rotated through 3 periods of a replicated 3 × 3 Latin square design.

Area under the curve.
Figure 1. Postprandial plasma glucose concentrations in yearling horses fed 2 (2×), 3 (3×), or 4 (4×) times daily. Measurements taken on d 11 of each period. Each point represents the mean ± SEM of 9 horses; arrow indicates time of feeding. Asterisks indicate difference (P < 0.05) between glucose concentrations of horses on the 2× feeding schedule and horses on the 3× and 4× feeding schedules at 0.5 and 2 h postfeeding.

period 3 (35.27 mg/dL) than during either period 1 (17.53 mg/dL) or period 2 (16.84 mg/dL; P < 0.01). Interestingly, baseline concentrations of glucose were lower during period 3 (P < 0.05) than during period 2.

**Leptin: Influence of Feeding Schedule**

Serum leptin concentrations on the first day of each period served as baseline values (Table 5). These values differed among feeding schedules (P < 0.05) and would have been reflective of the previous feeding schedule rather than the current feeding schedule, so all data were normalized to leptin concentration on d 1 of each period. Additionally, the nonnormalized mean concentration of leptin did not differ among horses on different feeding schedules. Analysis of normalized data indicated an effect of feeding schedule, with the 2× schedule having a greater change in leptin than the 3× schedule, which changed more than the 4× schedule (P < 0.05). Additionally, the changes in leptin concentration from d 1 to d 7 were different than zero only on the 2× and 3× feeding schedules (Table 5; P < 0.05). The change in leptin concentration on the 4× schedule was not different from zero. It is possible that further increases in leptin would have been apparent if the experimental periods had been longer.

These data lend support to the hypothesis that large carbohydrate meals can elevate serum leptin concentrations in the equine over a period of days, independent of total daily amount of feed consumed. Several short reports have indicated that serum leptin concentration rises postprandially in the equine (Cartmill et al., 2005; Crowley et al., 2005; Storer et al., 2005), perhaps stimulated by a rise in serum insulin concentration. However, this is the first evidence in any species that meal size alone influences the production of leptin. In a study of 5 obese women, Fogteloo et al. (2004) showed that increasing feeding frequency from 3 to 8 times daily did not affect mean leptin concentration over a 24-h period. However, the differences in species, age, sex, and nutritional status between subjects used in this study and by Fogteloo et al. (2004) may very well have contributed to the disparate results.

**Leptin: Influence of BCS and Sex**

Serum leptin concentrations were found to vary with BCS (P < 0.05), confirming the results of Buff et al. (2002). Figure 2 demonstrates the positive correlation between BCS and leptin concentration. Contrary to previous studies (Buff et al., 2002; Gentry et al., 2002b), sex did not significantly influence leptin concentration (P = 0.81). Mean leptin was 2.33 ± 0.12 ng of HE/mL in males and 2.17 ± 0.09 ng/mL in females, despite the fact that females had a much greater BCS than males, which could have caused a skewing of any sex effects. In other studies, the differences in leptin concentration between the 2 sexes may have been due to the presence of androgens and estrogens. Because all the horses in this study were fairly young, these variables may not have come into play, thus rendering the sex effect not significant. However, several studies have reported

**Table 4. Plasma glucose responses during the 3 experimental periods**

<table>
<thead>
<tr>
<th>Period</th>
<th>Baseline</th>
<th>Peak</th>
<th>Mean</th>
<th>AUC 3</th>
<th>Mean serum leptin concentration 3</th>
<th>Mean BCS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>89.0 a</td>
<td>106.5 a</td>
<td>90.7 a</td>
<td>1,144.7 a</td>
<td>2.00 a</td>
<td>4.83</td>
</tr>
<tr>
<td>2</td>
<td>92.8 a</td>
<td>109.7 a</td>
<td>97.3 b</td>
<td>1,234.8 b</td>
<td>2.16 b</td>
<td>4.94</td>
</tr>
<tr>
<td>3</td>
<td>83.8 b</td>
<td>119.1 b</td>
<td>96.4 b</td>
<td>1,224.3 b</td>
<td>2.57 b</td>
<td>5.11</td>
</tr>
<tr>
<td>Pooled SEM</td>
<td>2.24</td>
<td>3.48</td>
<td>1.00</td>
<td>29.3</td>
<td>0.07</td>
<td>0.12</td>
</tr>
</tbody>
</table>

a-c Within a column, means without a common superscript differ, P < 0.05.

1 Measurements taken between 0630 and 1130 on d 11 of each period. Values represent the means of 9 yearling horses rotated through 3 periods of a replicated 3×3 Latin square design.

2 Serum leptin concentration given as ng of human equivalents (HE)/mL. Values represent the mean of the serum leptin concentration on d 1 of each period.

3 Area under the curve.
that sex hormones may not, in fact, be responsible for the sex differences seen in leptin concentration. Ovariectomy did not change serum leptin concentration in mares (Buff et al., 2005), and no differences have been seen between stallions and geldings fed a similar diet (Buff et al., 2002). It is also necessary to remember that many factors besides sex hormone concentrations differ between the young animals used in this study and the mature horses used in previously published work. For example, GH is greater in younger horses than in older ones (Stewart et al., 1993); it is also greater in stallions and geldings than in mares (Thompson et al., 1994). Because GH has been shown to increase serum leptin concentrations in other species (Lissett et al., 2001), this must be kept in mind when comparing this study to others.

**Leptin: Influence of Period**

Mean leptin concentration for all horses increased over all 3 periods (P < 0.05), as shown in Table 4. The increase in leptin is most likely attributable to the rise in BCS; a larger fat mass has a greater capacity to produce leptin. Although BCS did not increase significantly during the course of the study, the pattern of change is uniform and it may have been enough to change leptin concentration. Furthermore, the greater plasma glucose concentrations experienced by the horses during period 3 may have also contributed to the elevation in leptin from period 2 to period 3.

**Other Factors**

Several variables differed among the groups, most notably the administration of medications. Horses not receiving sedation may have been more stressed during catheterization, resulting in a release of cortisol. This could have increased production of leptin because glucocorticoids have been shown to stimulate leptin production in the horse (Gentry et al., 2002a). Alternatively, many of the horses were sedated for the catheterization procedure, and the effect of sedatives on leptin production is unknown. However, data from those individuals receiving sedation does not indicate that this had any effect on leptin concentration (data not shown). Because many equine studies involve stressful situations such as catheterization, as well as the administration of sedatives, the effects of both cortisol and sedatives on leptin production needs to be taken into consideration in future research.

Previous studies have also shown variations in serum leptin concentrations with season. For example, leptin concentrations are greater in the winter than in the summer in both young and mature mares (McManus and Fitzgerald, 2003), although the opposite was found in undernourished mares (Buff et al., 2005). However, due to the short duration of this study, it is unlikely that our results reflect seasonal changes in leptin concentration.

**Circadian Rhythm of Leptin**

Analysis of data taken over a 24-h period showed many of the same results as the data taken on d 1,
Table 6. Effect of period on mean serum leptin concentration over 24 h in yearling horses fed either twice (2×) or 4 times (4×) daily

<table>
<thead>
<tr>
<th>Period</th>
<th>2×</th>
<th>4×</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.73 xa</td>
<td>2.32 ab</td>
<td>2.52^a</td>
</tr>
<tr>
<td>2</td>
<td>1.93 y</td>
<td>2.00 y</td>
<td>1.97 y</td>
</tr>
<tr>
<td>3</td>
<td>2.06 ya</td>
<td>2.71 zb</td>
<td>2.35^x</td>
</tr>
<tr>
<td>Mean</td>
<td>2.24</td>
<td>2.32</td>
<td>2.28</td>
</tr>
<tr>
<td>Pooled SEM</td>
<td>0.11</td>
<td>0.11</td>
<td>0.06</td>
</tr>
</tbody>
</table>

^a,bWithin a row, means without a common superscript differ, \( P < 0.05 \). Values represent mean of 9 yearling horses rotated through 3 periods of a replicated 3 × 3 Latin square design.

x,y,zWithin a column, means without a common superscript differ, \( P < 0.05 \).

4, and 7. The 24-h leptin concentrations were heavily influenced by period \( (P < 0.05; \) Table 6). Although we found a strong correlation between BCS and leptin in samples taken on d 1, 4, and 7, we were unable to demonstrate a relationship between mean 24-h serum leptin concentration and BCS (Figure 3). This suggests that BCS may only be correlated with prefeeding serum leptin concentrations. Unlike leptin concentration data from samples obtained once per day, the 24-h data indicated a strong effect of sex \( (P < 0.01) \). Average leptin concentration in males was 2.48 ng of HE/mL and only 2.17 ng of HE/mL in females. This is surprising given the difference in BCS between the sexes, but corroborates previous results (Buff et al., 2002).

One of the most prominent features of this portion of the study was the highly individualized pattern of leptin secretion over the 24-h period. Some horses responded very differently to the 2× and 4× schedules, whereas some did not differ at all. Likewise, some horses showed variations in serum leptin concentration with time, whereas some did not. This is illustrated in Figures 4A and 4B. The 2 horses represented in Figure 4 were both fillies of approximately the same BCS, yet they show 2 very different patterns of leptin release over the 24-h period.

Mean leptin concentration over the 24-h period was 2.24 ± 0.08 ng of HE/mL on the 2× schedule and 2.32 ± 0.07 ng of HE/mL on the 4× schedule (Table 6). Because of the differences in baseline concentrations of leptin between horses, data were normalized to the first data point, which was taken at 1800 on d 10. Three hours after feeding, the normalized leptin concentration was lower on the 2× schedule than on the 4× schedule \( (P < 0.05; \) Figure 5). At this point, leptin concentration on the 2× schedule was also lower \( (P < 0.05) \) than at 0600, 0800, 1000, 1200, and 1400. Because horses were fed at 1900 and 0700, these data seem to indicate that leptin concentration fell after the evening meal, only to rise again before the morning feeding, and then remain elevated postprandially.

Figure 3. Correlation between mean leptin concentration [ng of human equivalents (HE)/mL] in samples taken during a 24-h period and mean BCS \( (r^2 = 0.05; P = 0.61) \).

Figure 4. Serum leptin concentration [ng of human equivalents (HE)/mL] over a 24-h period in 2 yearling fillies fed 2 (2×) or 4 (4×) times daily. A) shows data from horse 3A, and B) shows data from horse 2C.
tethered for the entire 24-h period, it is possible that the stress of restraint caused a prolonged release of cortisol, and that this affected the pattern of leptin secretion. Synthetic glucocorticoids have been shown to elevate serum leptin concentration in equines (Gentry et al., 2002a; Cartmill et al., 2003); thus, elevated cortisol levels may account for the sustained rise in leptin seen from 0200 to 1600. A postprandial decrease in leptin (from 1900 to 2200) has been noted previously in cattle (Delavaud et al., 2002), although small postprandial peaks have been found in sheep 2 to 8 h after a meal (Marie et al., 2001). Two studies have found that, in mature horses, leptin concentrations are greater in the evening than in the morning (Buff et al., 2005; Cartmill et al., 2005). Furthermore, horses that are not grain-fed do not display a diurnal variation in leptin (Cartmill et al., 2005; Buff et al., 2005). The fact that horses on the 4× schedule did not experience the same fluctuations in leptin as those on the 2× schedule suggests that feeding 4 small meals daily results in a pattern of leptin secretion that more closely mimics that of grazing horses. Analysis of leptin concentration in 2× horses shows a significant effect of time (P < 0.05), whereas leptin concentration did not vary with time in horses fed 4 times daily (Figure 5). Regression analysis of the normalized data from the 2× feeding schedule indicates that leptin concentrations vary significantly with time in a quadratic model (P < 0.05). Regression analysis of data from the 4× schedule showed no variance with time in either a linear or quadratic model. This indicates that, when horses are fed 2 large concentrate meals daily, there are definite changes in leptin secretion over a 24-h period. On the other hand, when horses are fed multiple small meals daily, serum leptin does not change with time. However, a study done by Piccione et al. (2004) found an innate circadian rhythm of leptin in horses, wherein leptin peaked during dark hours and fell during the day. This pattern was not abolished by feed deprivation. The reason for the discordance in results is unknown, but may be due to experimental procedure or the age of the horses used in this study, as mentioned previously. As stated earlier, horses may require several days for their serum leptin concentrations to reach a new steady-state level after an acute stimulus: if Piccione et al. (2004) only measured leptin concentration after 24 h of fasting, the effects may not have been visible at that time point. Furthermore, our results are in accordance with a study done by Marie et al. (2001), which found no innate circadian rhythm of leptin in sheep. This suggests that the imposition of meal feeding upon horses affects more than simply gut function; it may also disrupt the release of hormones and change the homeostasis of the animal.

**LITERATURE CITED**


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