SERUM LEPTIN CONCENTRATION VARIES WITH MEAL SIZE AND FEEDING FREQUENCY

A Thesis

by

SAMANTHA MICHELLE BRUCE

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2004

Major Subject: Animal Science

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Approved as to style and content by:

Gary D. Potter (Chair of Committee)	Clifford Honnas (Member)
Pete G. Gibbs (Member)	John McNeill (Head of Department)

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ABSTRACT

Serum Leptin Concentration Varies with Meal Size

and Feeding Frequency. (August 2004)

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Horses with high energy requirements are generally fed two large concentrate meals per day, either in the form of grain or pellets. The postprandial elevation of blood glucose resulting from this type of feeding has the potential to alter production of hormones such as leptin. Leptin is an adipose-derived protein that promotes satiety in normal animals. The objective of this study was to determine if feeding large amounts of concentrate twice each day would alter serum leptin concentration. Nine horses were placed into three groups (A, B, and C) and each group was rotated through three feeding schedules (2x, 3x, and 4x) in a 3 x 3 Latin square design. Horses were fed twice per day on the 2x schedule, three times per day on the 3x schedule, and four times per day on the 4x schedule. Horses were fed the same total amount of concentrate per day throughout the study, although meal size varied with the number of times the horse was fed per day. Horses were weighed and scored for body condition on the first day of each period. Each treatment period lasted for 11 days. Blood was drawn on days one, four, and seven of each period and leptin concentration was determined by radioimmunoassay. On the afternoon of the tenth day of each period, horses were fitted with jugular catheters and blood was drawn every two hours for 24-hours to determine the circadian rhythm of

leptin secretion. Additionally, blood was taken 30 minutes prior to and every 30 minutes after the morning meal to determine postprandial plasma glucose concentrations. Mean and peak glucose values were higher on the 2x schedule than the 3x or 4x schedules ($P < 0.05$). Leptin concentration was highest in horses on the 3x schedule, although when these data were normalized to baseline (day one) values, leptin was highest on the 2x schedule ($P < 0.05$). Serum leptin concentration was highly correlated with body condition score ($P < 0.01$), but not gender ($P = 0.82$), and leptin increased throughout the study ($P < 0.05$). Data from the 24-hour collection showed that serum leptin concentration varied with time in horses on the 2x but not the 4x schedule ($P < 0.05$). Linear regression of data from the 2x schedule indicates that the pattern of change may be modeled by a quadratic equation ($P < 0.05$). This study demonstrates that feeding horses large carbohydrate meals twice per day disrupts the normal pattern of leptin in the horse, possibly affecting appetite and other physiological processes.

DEDICATION

This thesis is dedicated to my parents, Drs. Eugene and Margaret Bruce. Their advice, although not always heeded, was generally correct. It is also dedicated to Drew Steelman for the endless supply of both love and caffeine.

ACKNOWLEDGEMENTS

The author would like to extend thanks to the following:

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And finally, to my parents, for the money, the computer(s), the truck, the constant advice on how to survive graduate school, and most importantly, for the chance to prove myself.

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INTRODUCTION

Many of today's horses are being pushed harder than their ancestors ever were. Whether in the performance arena or the breeding shed, horses are expected to mature early, to perform well during training and showing, and then to produce high-quality offspring year after year. All of these tasks require a great amount of energy, which generally cannot be supplied by roughage alone. Therefore horses are fed concentrate feed in the form of grains or pellets, usually once or twice daily. However, this can not only lead to an increased incidence of colic, it can also disrupt the horse's normal homeostasis by increasing blood concentrations of glucose, as well as hormones such as insulin and growth hormone. These changes in hormone concentrations have the potential to alter appetite along with many other physiological processes. Therefore the effect of meal feeding on the release of hormones, especially those involved in appetite, needs to be determined.

This thesis follows the style and format of the Journal of Animal Science.

OBJECTIVES OF THE STUDY

The objectives of this study were as follows:

1. To determine if the amount of grain fed in a single meal alters serum leptin concentration.

2. To determine any circadian rhythm of leptin in the horse and if it is affected by timing of meals.

REVIEW OF LITERATURE

An Introduction to Leptin

Leptin was first discovered in 1994 (Zhang et al., 1994) and was dubbed the "anti-obesity" hormone. For the first time in history, the simple administration of a protein to a genetically obese knockout mouse reduced body fat mass and increased energy levels (Campfield et al., 1995; Halaas et al., 1995; Pelleymounter et al., 1995). The implication for human applications was enormous. In the following years, however, it was found that congenital leptin deficiency was probably not a cause of obesity in humans as few people are actually leptin deficient. Therefore, research focus has shifted to investigating acquired leptin resistance as a contributing factor in today's obesity epidemic. Additionally, as the mechanisms of action of leptin have been elucidated, it has shown itself to be more ubiquitous than thought originally. Today, research has implicated leptin in the control not only of energy metabolism, as well as processes of growth, reproduction, immune function and osteogenesis.

Leptin is a 16 kDa protein produced by the *Ob* gene. It is similar in structure and signaling to a cytokine, with 146 amino acid residues arranged in four alpha helices (Margetic et al., 2002). Although leptin mRNA has been isolated from varied locations such as skeletal muscle, stomach, placenta and ovaries, and bone (Margetic et al., 2002), its production occurs in the greatest amounts in brown and white adipose tissue. Plasma leptin concentrations therefore reflect the amount of adipose tissue in the body and tend to increase and decrease with weight gain and loss, respectively. In normal animals,

leptin acts an afferent signal to the central nervous system (CNS) indicating that the body is either in positive or negative energy balance. This causes an appropriate change in appetite in an attempt to correct the imbalance and maintain body weight. Leptin deficient (*ob/ob*) mice, however, are hyperphagic and massively obese, due to their inability to regulate appetite. These pathologies can be corrected by administration of exogenous leptin (Campfield et al., 1995; Halaas et al., 1995; Pelleymounter et al., 1995).

Interestingly, plasma leptin concentrations depend upon much more than simply body fat mass. Females tend to have higher plasma leptin than males, and older animals higher concentrations than younger animals, independent of adiposity (Montague et al., 1997; Buff et al., 2002). In humans, subcutaneous fat produces much more leptin than omental fat (Hube et al., 1996; Montague et al., 1997), although the opposite has been found in rodents (Hube et al., 1996). High carbohydrate diets increase plasma leptin to a greater degree than high fat diets (Dirlewanger et al., 2000) and timing of meals may influence its circadian rhythm in humans (Elimam and Marcus, 2002), although this has not been tested in animals.

As leptin mRNA began to be identified in locations other than adipose tissue, early investigators searched for other possible roles of leptin in metabolism. Evidence accumulated that demonstrated the involvement of leptin in insulin secretion, hematopoeisis, thyroxine production, cardiovascular function, fluid balance, and fertility, among others (Margetic et al., 2002). Seemingly, there was no physiological system that was not under the control of leptin in some way. This aspect of leptin research has led to

the hypothesis that leptin is much more than a mere satiety signal; it is, in fact, a "master hormone," communicating information about energy availability between the CNS and the periphery and acting as a permissive factor for energy-intensive processes such as growth, reproduction, and initiation of an immune response.

Metabolic Effects of Leptin

Leptin influences metabolism in two ways: first, by regulating appetite via the CNS, and second, by peripheral changes in glucose transport and metabolism. Leptin begins its work in the CNS by binding to its receptor, *Ob-R* (Harvey and Ashford, 2003). This receptor has six splice variants, designated *a* through *e*. The long form of the receptor, also called *Ob-Rb*, is the only one that has signaling capabilities. It is a member of the class I family of cytokine receptors and acts through the JAK-STAT pathway to stimulate gene transcription. Most evidence has shown that JAK1 is activated preferentially, although activation of JAK2 has also been reported (Harvey and Ashford, 2003). Additionally, *Ob-R* has been shown to activate both PI3-kinase and the Ras-MAPK pathway in several peripheral cell types (Harvey and Ashford, 2003).

High plasma leptin appears to downregulate the production of both neuropeptide Y (NPY) and α-melanocyte stimulating hormone (α-MSH) (Stephens et al., 1995; Satoh et al., 1998), thus suppressing the appetite. These orexigenic peptides stimulate food intake via the CNS. New evidence suggests that leptin may exercise its control over NPY and α-MSH through a novel gastric hormone called ghrelin. In vitro, ghrelin has been shown to increase expression of both NPY and α-MSH (Nakazato et al., 2001).

Likewise, feed-restricted mice receiving exogenous leptin maintained normal ghrelin concentrations, while pair-fed control mice experienced a 40% increase in ghrelin (Barazzoni et al., 2003). These investigators drew the conclusion that leptin negatively regulates ghrelin, and that ghrelin rose in the control mice due to a lack of inhibition from leptin. However, the loose correlation found between plasma concentrations of leptin and ghrelin have made this hypothesis a controversial one (Cummings and Foster, 2003).

As a complement to its central regulation of energy intake, leptin also exerts control over how energy is used in the periphery. This control takes two forms: regulating the balance between oxidation and storage of fatty acids, and influencing transport and metabolism of glucose. In the first case, leptin reduces fat storage through stimulation of oxidative pathways as well as through enhancement of triacylglyceride hydrolysis (Muoio and Dohm, 2002). For example, leptin deficient mice tend to partition fatty acids toward storage and away from oxidation, while administration of exogenous leptin reverses this tendency (Pelleymounter et al., 1995). Furthermore, Minokoshi et al. (2001) have shown leptin to activate a skeletal muscle protein kinase A, which in turn inactivates acetyl CoA carboxylase and limits the transport of long-chain fatty acids into the mitochondria. Leptin also increases expression levels of uncoupling proteins 1, 2 and 3 (UCP1, UCP2, and UCP3) in adipose tissue as well as in skeletal muscle, pancreas and liver (Muoio and Dohm, 2002). Uncoupling proteins dissociate oxidative phosphorylation from electron transport, thus generating heat and wasting energy. Muoio and Dohm (2002) present an intriguing hypothesis as to the ultimate

physiological purpose of leptin's involvement in fatty acid partitioning. Their "lipotoxicity hypothesis" is based on the fact that obesity-related diseases such as dyslipidemia, coronary heart disease, and type II diabetes are all associated with the accumulation of excess amounts of lipids in non-adipose tissues. This leads to cell dysfunction and disease. In short, the hypothesis states that adipocytes produce leptin to promote fatty acid usage and prevent ectopic lipid accumulation. Although further investigation is required, this hypothesis provides a useful model to help explain previous findings and point investigators in new directions of research (Muoio and Dohm, 2002).

The effects of leptin on glucose metabolism are somewhat more complicated than those on fat. Several in vitro studies indicated that leptin had no effect on glucose transport in various muscle cell cultures (Muoio et al., 2002; Ranganathan et al., 1998). However, in vivo studies showed that leptin infusion increased whole-body glucose turnover, glucose uptake into brown adipose tissue (BAT), brain, heart, and skeletal muscle (Burcelini et al., 1999; Kamohara et al., 1997). Interestingly, Kamohara et al. (1997) demonstrated that denervation of soleus muscle attenuated the change in glucose transport, indicating that this effect of leptin may after all be centrally mediated.

Leptin and Insulin

Although leptin mediates changes in fatty acid storage and glucose transport, its major influence on energy metabolism is effected through its interplay with insulin. The *Ob* receptor is found on insulin-secreting β cells of the pancreas as well as on γ cells,

which produce glucagon (Chen et al., 1997). Most in vitro studies show that leptin downregulates β cell production of insulin, possibly through increased potassium channel permeability (Muoio and Dohm, 2002). Discrepancies in these results have been noted and attributed to the multiple signaling pathways that control intracellular calcium concentration, which is sometimes used as an indicator of receptor activation. Differences in the preparations of leptin used in various studies may also be responsible (Harris, 2000). In *ob/ob* mice, however, β cells are hyperpolarized and hypersensitive to acetylcholine, supporting the hypothesis that leptin affects potassium channel permeability (Chen et al., 1997). Furthermore, *ob/ob* mice are hyperinsulinemic, a condition which is reversed by leptin replacement (Pelleymounter et al., 1995). Complicating the matter, Harris et al. (1998) inhibited glucose-stimulated release of insulin in leptin-treated lean mice, although others have shown that a depletion of intracellular triglycerides prevents normal pancreatic islet function even in hyperleptinemic mice (Koyama et al., 1997; Kristensen et al., 1999). Thus Harris et al. (2002) concluded that, at least in vivo, insulin inhibition may be due primarily to the metabolic state of the β cells and not leptin per se.

Conversely, dependency of leptin on insulin appears much more simple. An absence of insulin inhibits leptin synthesis, as evidenced by low leptin concentrations in streptozotocin-induced diabetic rats, while insulin replacement results in a restoration of leptin (MacDougald et al., 1995; Patel et al., 1999) In vitro, pharmacological amounts of insulin stimulate leptin mRNA transcription (Barr et al., 1997), while physiological amounts administered in vivo only increase leptin after several days (Boden et al., 1997;

Kolaczynski et al., 1996). This in vivo elevation of leptin may actually be due to an increase in adipose tissue secondary to the hyperinsulinemia (Harris, 2000). However, in a review article exploring leptin receptor signaling, Zabeau et al. (2003) suggest that leptin and insulin may interact in different ways depending upon cell line and tissue type.

Because of the fascinating regulation of leptin and insulin, several studies have attempted to manipulate one by regulating the other. Most notably, Mueller et al. (1998) investigated the mechanism of insulin-induced leptin secretion in vitro by using various agents to block either glucose transport or glycolysis. They determined by multiple regression analysis that leptin secretion was correlated significantly with a decrease in glucose concentration in the cell culture, but it was not correlated with insulin concentration. Additionally, blocking glucose uptake attenuated leptin release, even in the presence of insulin. Building on this work, Dirlewanger et al. (2002) investigated the effects of three days of overfeeding either a fat (FA) or carbohydrate (CHO) diet on serum leptin in healthy women. Any form of positive energy balance should increase leptin, but they hypothesized that the CHO diet would result in a greater increase due to the large postprandial peaks in blood glucose. This hypothesis was confirmed since the CHO diet resulting in a 27% increase in leptin, while the FA diet had no effect.

Leptin Research in Equines

Despite the large amount of leptin research that has been carried out in vitro as well as in rodent models and in humans, surprisingly few articles have been published

regarding leptin in the horse. Numbering fewer than fifteen as of January 2004, most of these articles have focused on leptin as a mediator of fertility and cyclicity in the mare. McManus and Fitzgerald (2000) at the University of Kentucky were the first to publish data on leptin in horses, and they validated a radioimmunoassay (RIA) kit (Linco Research, St. Charles MO) for detection of leptin in equine serum. Their first study investigated the effects of 24-hours of feed restriction on serum leptin, LH, FSH, and prolactin concentrations in cycling mares. Though none of these variables were significantly altered, the authors later concluded that perhaps it was due to maintenance of leptin above some threshold concentration. In a much more informative study, Buff et al. (2002) at the University of Missouri attempted to both identify and characterize equine leptin. They cloned a partial sequence of the *Ob* and *Ob-R* genes in order to develop reverse transcription polymerase chain reaction (RT-PCR) primers for the detection of leptin RNA from various tissues. Subsequently, they developed an RIA to measure peripheral leptin and then used this to assay serum from 71 horses of various ages and body condition scores (BCS). These findings were similar to those in humans: older horses and those with higher body condition scores tended to have higher serum leptin concentrations. Interestingly, stallions and geldings had higher serum leptin than mares, which is contrary to other species. This study emphasizes the fact that leptin concentrations are highly variable even among individuals with the same BCS, and because of this, BCS is not very tightly correlated with serum leptin concentration. Nevertheless, Buff et al. (2002) made a very valuable contribution to equine leptin research with this experiment.

Of the remaining nine leptin studies performed in horses, four have attempted to correlate leptin with seasonal anestrus in the mare, two have looked at the effect of dexamethasone treatment on serum leptin, two have identified leptin in mares' milk, and one has isolated leptin from stallions' seminal plasma. Conflicting results from the seasonal anestrus studies have been reported. Three studies reported that a change in BCS or body fat percentage had no effect on timing of anestrus (McManus and Fitzgerald, 2003). However, one group of researchers noted that leptin, IGF-1, and prolactin were greatly reduced in mares with a BCS of 3.0 to 3.5 (Gentry et al., 2002a). These somewhat contradictory results may be explained by the theory that leptin does not, in fact, play a role in seasonal anestrus until a threshold BCS is achieved (Fitzgerald et al., 2002). It is also important to note that, although two studies have found an in vivo increase in leptin after administration of dexamethasone, the cause of this response has yet to be determined (Gentry et al., 2002b; Cartmill et al., 2003). It may very well be due to the secondary increase in blood glucose and insulin, rather than a direct effect of dexamethasone itself.

With the exception of the initial characterization of equine leptin and its correlation with BCS (Buff et al., 2002), no studies have been carried out relating leptin to nutrition in equines. In its most basic function as a regulator of appetite, the manipulation of leptin in the horse may provide a means to increase feed intake in hard-working performance horses and lactating mares. Additionally, many horses eat large carbohydrate meals, and this may result in large variations in leptin with unknown physiological consequences. Because leptin is involved in growth, reproduction, bone

density, and immune function, it is an area of research that equine scientists should consider in the future.

EXPERIMENTAL PROCEDURES

Nine yearling Quarter horses were used in this experiment. Three geldings and six fillies were used. All horses were between 13 and 16 months of age and weighed an average of 324 kg at the start of the study. Horses were divided into three groups, designated A, B, and C, and each horse was identified by its group letter plus horse number 1, 2, or 3. Each group was randomly assigned one gelding and two fillies. Eight of the horses were housed according to group in 15 m x 20 m dry lots. Due to a leg injury, one gelding (Horse 1B) was kept in a stall for the duration of the project. All horses had access to shade and water throughout the study.

The horses underwent a 10 day adaptation period to accustom them to the dry lots and to increasing amounts of feed. They were fed a 14% crude protein, pelleted concentrate[a] and coastal Bermudagrass hay in approximately a 60:40 ratio. The amount of pelleted feed was increased from 2.7 kg per day to approximately 4.5 kg per day until horses were consuming a total of 2.5% of their average body weight per day in hay plus concentrate. During the adaptation period, horses in each pen ate both hay and concentrate out of a communal trough. After the first day, no fighting was observed at feeding time and all horses had equal access to feed.

During the experimental period, each group of horses was rotated through three different feeding schedules during three time periods in a replicated 3x3 Latin Square

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[a] 14% Horse Pellet, Producers Cooperative Association, Bryan, TX 77806

design. The periods were 11 days long and the feeding schedules are described in Table 1.

Table 1. Description of meal times on the 2x, 3x and 4x feeding schedules

Feeding Schedule	Description
2x	horses fed concentrate and hay twice per day at 7:00am and 7:00pm
3x	horses fed concentrate three times per day at 7:00am, 3:00pm, and 11:00pm horses fed hay twice per day at 7:00am and 7:00pm
4x	horses fed concentrate four times per day at 7:00am, 1:00pm, 7:00pm, and 1:00am horses fed hay twice per day at 7:00am and 7:00pm

Regardless of feeding schedule, the total amount of concentrate fed per day was held constant at 1.5% BW. During the experimental period, the horses were separated at feeding time and concentrate was fed in individual metabolism stalls. Hay was group-fed at 1.0% of the average body weight of the horses in the pen.

Data Collection

On the first day of each period, horses were weighed and scored for body condition. Blood samples were collected between 6:00 am and 7:00 am, prior to the morning meal. Samples were also collected prior to the morning meal on the fourth and seventh days of each period. All blood was drawn from the jugular vein into Vacutainers and then temporarily stored on ice until processing.

On the afternoon of the tenth day, all horses were catheterized intravenously. Catheterization was alternated between the left and right jugular veins throughout the study to minimize scarring. An area superficial to the vein was clipped and scrubbed with an iodine scrub solution and then rinsed with 70% isopropyl alcohol. This was repeated three times. A 14 cm catheter was then introduced into the vein and secured to the skin with Superglue. An extension set filled with heparinized saline was attached to the catheter and held in place with an Elastikon bandage wrapped loosely around the neck. 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 (100 mg/ml) or acepromazine (10 mg/ml) or a combination of both. Appendix 3 lists which horses were sedated and which drugs they received. All catheters were flushed with a solution of heparinized saline after catheterization and at least every four hours following that until their removal approximately 26 hours later.

The 24-hour blood collection began at 6:00 pm on the tenth day. Blood was drawn from horses on the 2x and 4x schedules every two hours for 24-h, and the last draw occurred at 6:00 pm on the 11th day. Every time blood was taken, approximately 12 ml of heparinized saline was emptied from the catheter and then discarded, and a clean syringe used to take sample blood. Blood was immediately transferred from the syringe to an empty Vacutainer and the catheter flushed with approximately 12 ml heparinized saline to prevent clotting. Blood was always taken from the horses in the same order, beginning with horse 1A and ending with 3C. During these collections, horses were kept in the dry lots but were tied to prevent them from rubbing their

catheters. Individual water buckets were hung for each horse and all were still fed according to the previously described schedules. Additionally, horse 1B was relocated to the dry lots and tied with the other horses for ease and speed of collection. All samples were taken within about 10 minutes.

Blood samples were also taken before and after the 7:00 am meal to determine the postprandial blood glucose response. These samples were transferred into tubes with NaFl added and the tubes then inverted multiple times to allow mixing. The first draw was at 6:30 am, horses were fed at 7:00 am, and blood was taken again at 7:30 and then every thirty minutes thereafter until 1:00 pm.

After the last blood draw at 6:00 pm on day 11, catheters were removed. A gauze pad, held in place by the Elastikon bandage, was left covering the wound for several hours to promote clotting and prevent contamination. Horses were untied and allowed to move freely about their pens. Horse 1B was returned to his stall immediately.

Laboratory Analyses

All blood samples were processed within twenty-four hours of collection. Until processing, they were held at 4ºC. Blood tubes were centrifuged at 1200 x g for 25 minutes at 4°C, or until adequate separation had occurred. When necessary, a clean wooden applicator stick was used to break the plasma proteins away from the side of the tubes and the centrifugation was repeated. The resulting serum or plasma was transferred to 1.5 ml microcentrifuge tubes, labeled appropriately, and stored at -20ºC until analyses.

Plasma glucose content was determined by a YSI 2300 Glucose/Lactate Analyzer. All samples were thawed at 4ºC and then vortexed briefly before being analyzed. Leptin was analyzed by radioimmunoassay (RIA) using established methods (Fitzgerald and McManus, 2000). A multi-species leptin RIA kit was used[b]. This particular RIA was a double-antibody competitive binding assay in which sample leptin competes with radiolabeled leptin for a limiting amount of primary antibody. The steps of the assay procedure are as follows: on the first day of the assay, 100 µl of serum was added to 100 µl of assay buffer (0.025M EDTA, 0.08% sodium azide, 1% BSA, and 0.05% Triton X-100 in 0.05M phosphosaline at pH 7.4). Then an anti-leptin antibody was added. Samples were vortexed and incubated at 4ºC for 20-24 hours. Next, 100 µl (approximately 12,000 cpm) of I^{125} radiolabeled leptin was added to each tube. Samples were vortexed again and incubated for another 20-24 hours. Finally, a polyethylene glycol-based second antibody precipitating reagent was added. The samples were vortexed, incubated for 20 minutes, and centrifuged at 3000 x g for 25 minutes. Sample tubes were inverted to remove supernatant and allowed to dry upside down for approximately 5 minutes. Tubes were then counted with a gamma counter[c]. Samples were run according to kit protocol, except that dilutions were performed on the human standards. 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

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[b]Linco Research, St. Charles, MO

[c]Packard Cobra II Gamma Counter, Packard Biosciences, Boston, MA

leptin. This was to compensate for the extremely low concentrations of leptin generally found in the horse. Results were reported in ng/ml human equivalents (HE). All standards and controls were run in triplicate and samples were run in duplicate.

Statistical Analyses

Statistical analyses were performed using a statistical software package[d]. Analyses of variance were performed on all data to determine influence of various parameters on serum leptin concentration. When significant differences were found, means were separated using a Fisher-Hayter pairwise comparison or Student's t-test as appropriate. Additionally, some data were normalized to either a day one or a time zero value, which was representative of a baseline. This was done to better visualize changes in the concentration of leptin.

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[d]Stata 7.0, Stata Corporation, College Station, TX

RESULTS AND DISCUSSION

Feed Intake and Refusals

Horses on the 2x, 3x, and 4x 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 (Table 2). Amounts of feed refused per meal were negligible. No difference in satiety was noted between the three feeding schedules.

Table 2. Mean amounts of concentrate offered and refused per horse per meal when yearling horses were fed two (2x), three (3x), or four (4x) times per day

Feeding	Concentrate	
Schedule	Offered, kg	Refused, kg
2x	2.58	0.02
3x	1.74	0.02
4x	1.30	0.00

Weight and Body Condition Score

Although the horses used in this study were young, growing yearlings, body weight did not increase significantly ($P = 0.99$) during the experimental period as determined by analysis of variance. Furthermore, regression analysis of weight showed no correlation ($P = 0.459$) with period (Figure 1). 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. On the first day of the experimental period, the mean

body weight was 342 ± 8.7 kg, and on the last day that weights were measured the mean was 354 ± 11.0 kg (Appendix 5A). Average daily gain was 0.39 ± 0.14 kg. Body weight was not correlated with body condition score (BCS) (P = 0.19) nor with gender (P = 0.69) (Figure 2). This was most likely due to the lack of variation in weight and body condition score in this population of horses.

Although mean BCS increased from 4.83 ± 0.14 during period one to 5.11 ± 0.14 during period three, this increase was not significant (P = 0.28) (Appendices 6A and 6B). Body condition score was influenced heavily (P < 0.01) by gender, with females having a higher mean BCS (5.14 ± 0.09 versus 4.61 ± 0.14) than males (Figure 3). No effect of feeding schedule was observed (P = 0.90).

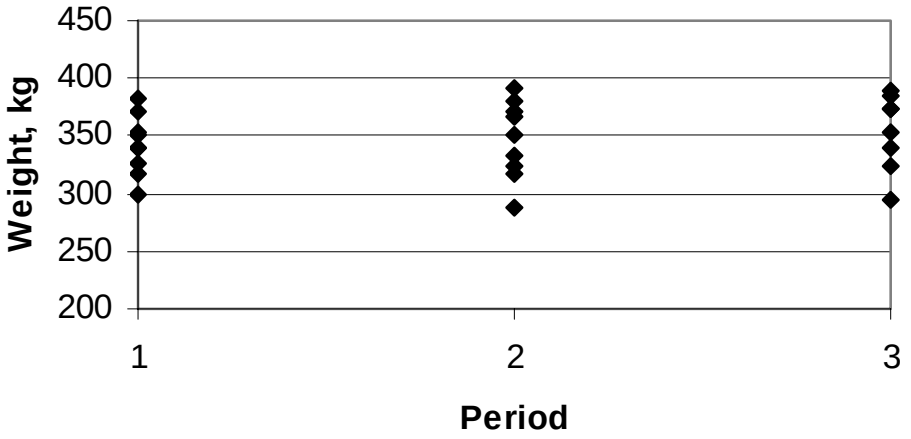

Figure 1. Correlation between body weight and experimental period in nine yearling horses (r^2 = 0.459, P = 0.71)

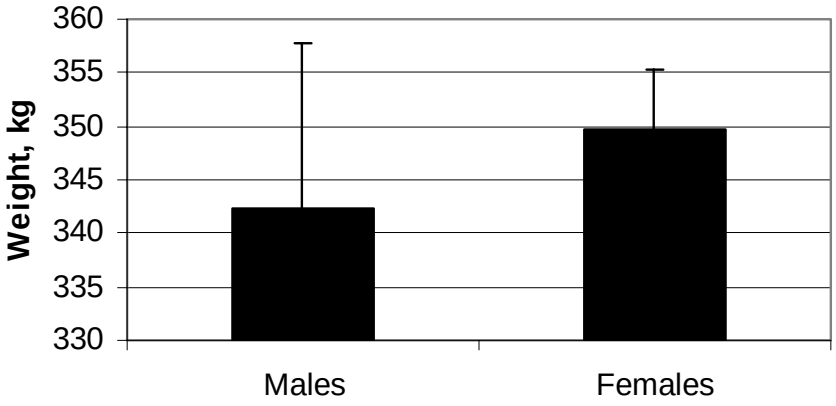

Figure 2. Mean body weight of male and female yearling horses

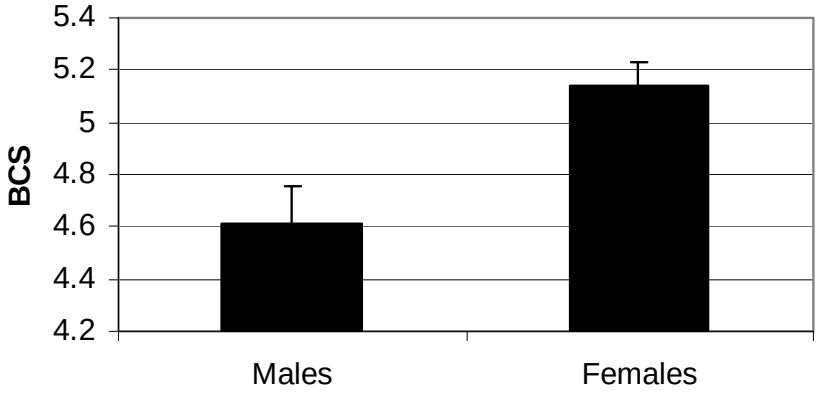

Figure 3. Mean body condition score of male and female yearling horses

Postprandial Plasma Glucose

Baseline glucose concentrations did not differ between feeding schedules ($P = 0.83$). Postprandial concentrations of plasma glucose varied over the six hours that blood was drawn ($P < 0.05$), as shown in Figure 4. Plasma glucose concentration differed significantly between feeding schedules at only one time point: at 30 min post-feeding, glucose concentration was higher ($P < 0.05$) in horses on the 2x schedule (Appendix 4A). Mean glucose concentrations on the 2x schedule also tended to be higher than those on the 3x and 4x schedules at two ($P = 0.08$) and four ($P = 0.07$) hours after feeding. Mean peak glucose was higher ($P < 0.05$) on the 2x (151 mg/dl) compared to the 4x schedule (110 mg/dl), but did not differ between 2x and 3x or 3x and 4x (Figure 5). Mean glucose varied with feeding schedule ($P = 0.001$): mean concentrations of glucose were higher in horses on the 2x schedule (98.81 ± 1.59 mg/dl) than those on the 3x (92.52 ± 1.30 mg/dl) or 4x (93.26 ± 0.97 mg/dl) schedules ($P < 0.05$), although there was no difference between the 3x and 4x schedules (Figure 6). Figure 7 illustrates that changes in glucose concentration from baseline to peak values tended to be higher on the 2x schedule when compared with the 4x schedule ($P = 0.07$). Areas under the curves (AUC) for the 2x, 3x, and 4x schedules were 1253.67 ± 37.8, 1168.73 ± 26.7, and 1176.00 ± 20.4, respectively (data not shown). The area under the curve on the 2x schedule was significantly higher ($P < 0.05$) than the AUC on the 3x schedule, but neither the 2x nor 3x schedules differed from the 4x. These results indicate that the larger amounts of concentrate fed per meal on the 2x schedule did in fact result in a larger plasma glucose response than the smaller amounts fed on the 3x and 4x schedules.

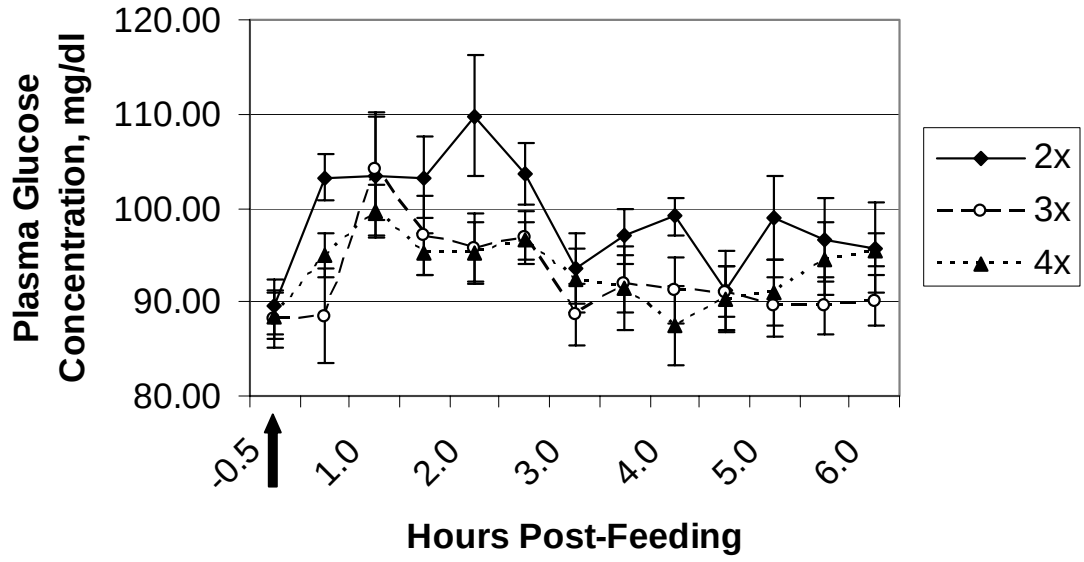

Figure 4. Postprandial plasma glucose concentrations in yearling horses fed two (2x), three (3x), or four (4x) times per day (arrow indicates time of feeding)

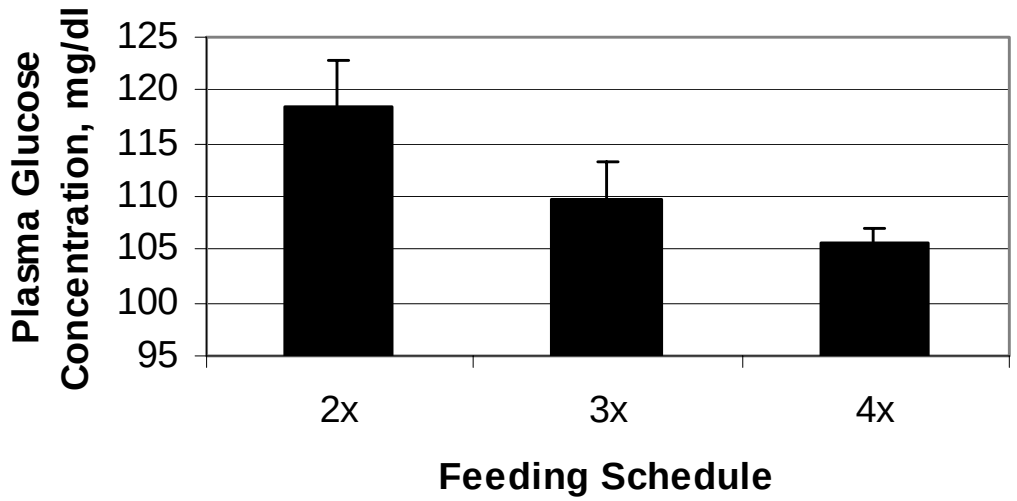

Figure 5. Peak postprandial plasma glucose concentrations in yearling horses fed two (2x), three (3x), or four (4x) times per day

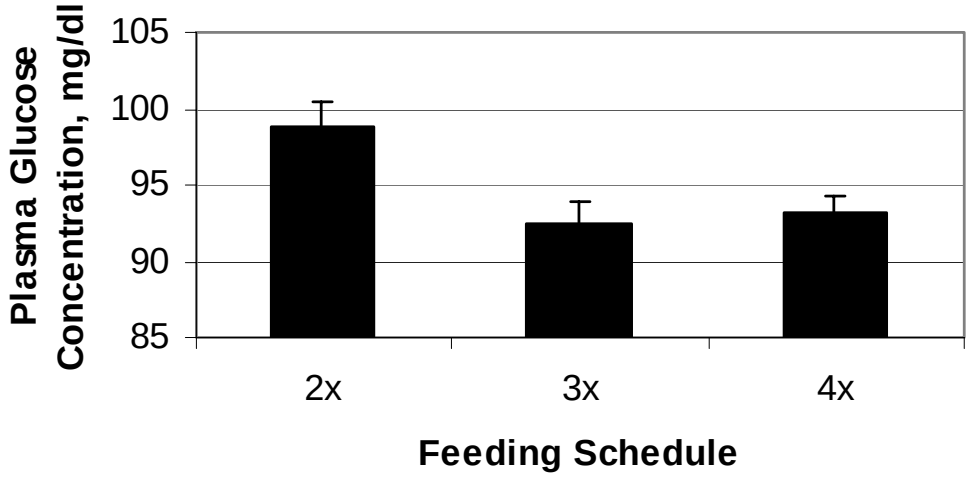

Figure 6. Mean postprandial plasma glucose concentrations in yearling horses fed two (2x), three (3x), or four (4x) times per day

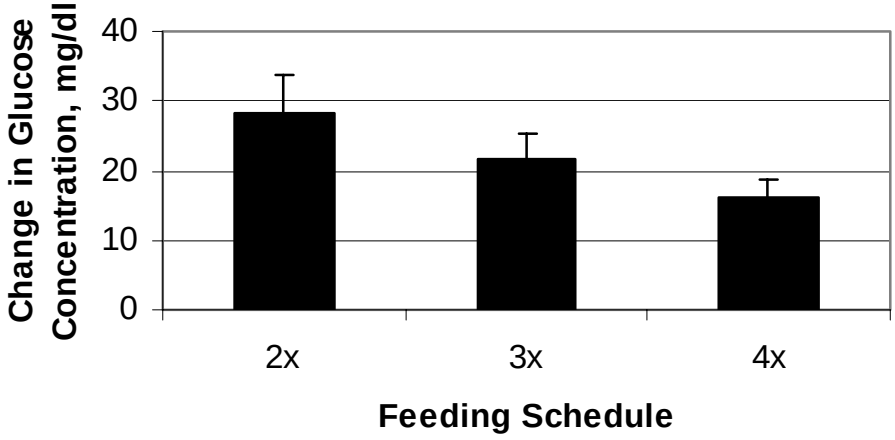

Figure 7. Change in glucose concentration from baseline to peak in yearling horses fed two (2x), three (3x), or four (4x) times per day

Glucose concentration also varied with period, as shown in Table 3. Horses had a lower mean glucose during period one ($P < 0.05$) than during the other two periods. Peak glucose concentration was higher in period three (119.12 ± 5.27) than in period one (106.5 ± 2.66) ($P < 0.05$), but was not significantly different than in period two (109.67 ± 2.47). Mean change from baseline to peak was higher during period three (35.27 ± 4.43) than during either period one (17.53 ± 2.57) or period two (16.84 ± 3.26) ($P < 0.01$). Interestingly, baseline concentrations of glucose were lower during period three ($P < 0.05$) than during period two. This variation in plasma glucose concentration may be due to the development of insulin resistance. Hoffman et al. (2003) demonstrated an

acquired insulin resistance and decreased glucose sensitivity in horses after eight weeks of concentrate feeding. This is an intriguing possibility and is a possible area of future research.

Table 3. Baseline, peak, change from baseline to peak, and mean glucose concentrations, and area under the glucose curve during experimental periods one, two, and three in yearling horses

	Glucose Concentrations, mg/dl				
Period	Baseline	Peak	Change from Peak to Baseline	Mean	AUC
1	88.97[a]	106.50[a]	17.53[a]	90.72[a]	1144.67[a]
2	92.82[b]	109.67[a]	16.84[a]	97.81[b]	1234.82[b]
3	83.85[a]	119.12[b]	35.27[b]	96.40[b]	1224.27[a,b]

[a,b] Column means not sharing superscripts differ ($P < 0.05$)

Leptin: Influence of Feeding Schedule

Analysis of variance on data from days 1, 4, and 7 of each period indicated that feeding schedule influenced mean serum leptin concentration ($P < 0.05$) (Appendix 7B). Additionally, the mean concentration of leptin in horses on the 3x schedule tended to be higher ($P = 0.1$) than those on the 2x or 4x schedules. Means from the 2x, 3x, and 4x schedules were 2.24 ± 0.11, 2.35 ± 0.10, and 2.13 ± 0.11 ng/ml HE, respectively (Figure 8).

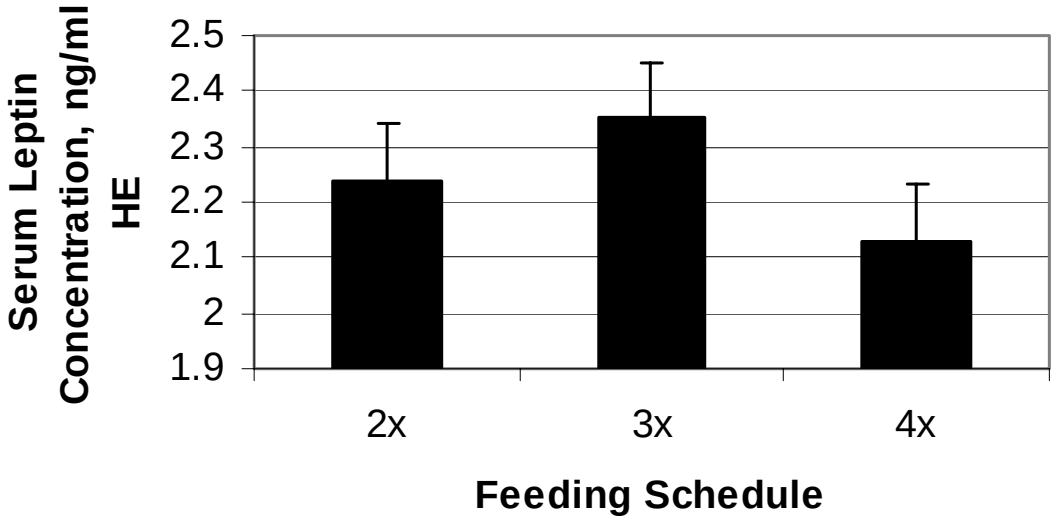

Figure 8. Mean serum leptin concentration in yearling horses fed two (2x), three (3x), or four (4x) times per day

Mean serum leptin concentrations on the first day of each period, which served as baseline values, were 2.08 ± 0.17 ng/ml HE on the 2x schedule, 2.42 ± 0.20 ng/ml HE on the 3x schedule, and 2.08 ± 0.17 ng/ml HE on the 4x schedule (Appendix 7A). The differences between these means were significant ($P < 0.05$), so all data were normalized to leptin concentration on day one of each period to minimize variation. It is also important to note that, although the 3x schedule resulted in higher absolute concentrations of leptin than the other two schedules, it also began with the highest

values on day one. Additionally, normalization is appropriate in this case because leptin concentrations generally require 24 – 48 hrs change after a stimulus (Hickey, 2000), so concentrations on day one are essentially a product of the previous period. Thus, normalization permits better visualization of changes occurring in leptin production as a result of each feeding schedule.

By day four of each period, analysis of variance on normalized data indicated an effect of feeding schedule, with the 2x schedule having a greater change in leptin than either the 3x or 4x schedules ($P < 0.05$) (Appendix 7B). By day seven, this difference was significant at $P < 0.01$. This is illustrated in Table 4.

Table 4. Normalized serum leptin concentration in yearling horses fed two (2x), three (3x), or four (4x) times per day on days one, four, and seven of the experimental periods

	Normalized Serum Leptin Concentration, ng/ml HE				
Feeding	Day				
Schedule	1	4	7	Mean	SEM
2x	0	0.11[a]	0.36[a]	0.16[a]	0.07
3x	0	0.08[b]	-0.01[b]	0.02[b]	0.07
4x	0	0.08[b]	0.09[b]	0.05[b]	0.09

[a,b] Column means not sharing superscripts differ ($P < 0.05$)

Mean changes in leptin concentration by day seven on the 2x, 3x, and 4x schedules were 0.36 ± 0.13, -0.01 ± 0.21, and 0.09 ± 0.09 ng/ml HE, respectively. Additionally, a Student's t-test revealed that the changes in leptin concentration from

day one to day seven were significantly different than zero only on the 2x feeding schedule ($P < 0.05$) (Figure 9). The change in leptin on the 3x and 4x schedules was not different from zero ($P > 0.05$).

Changes in leptin concentration occurred primarily from day four to day seven (Table 4), which indicates that horses may adjust their production of leptin more slowly than people, who generally respond in 24 – 48 hours. Its is possible that further increases in leptin would have been apparent if the periods had been longer. Future leptin research should allow for this extended adaptation period.

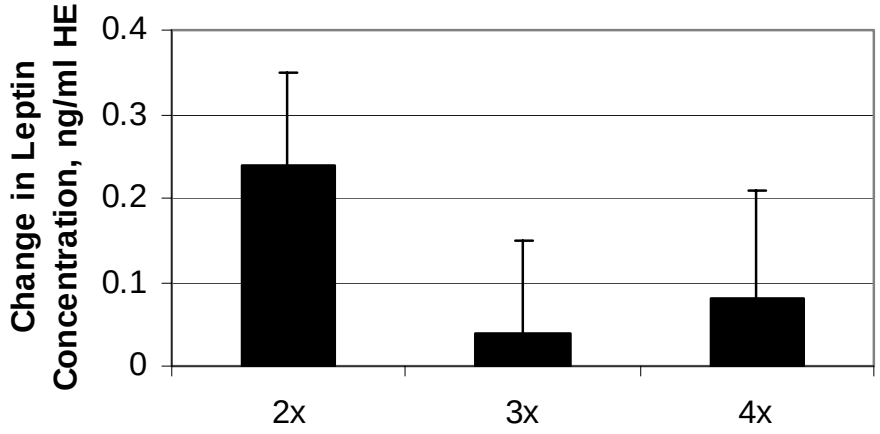

Figure 9. Mean changes in serum leptin concentration from day one to day seven of the experimental periods in yearling horses fed two (2x), three (3x), or four (4x) times per day

These data lend support to the hypothesis that large carbohydrate meals can elevate serum leptin concentrations in the equine over a period of days. However, because the physiological range of serum leptin concentrations in the horse is so small, the differences in leptin between feeding schedules are also small, especially when the standard errors of the means are taken into consideration. A larger number of experimental animals may be necessary in future research. Because leptin can potentially affect so many aspects of growth, performance, and reproduction, further investigation of the influence of meal size on leptin is warranted.

Leptin: Influence of Body Condition Score and Gender

Serum leptin concentrations were found to be influenced by BCS ($P < 0.05$) (Appendix 7B), confirming the results of Buff et al. (2002). Figure 10 demonstrates the positive correlation between BCS and leptin concentration. Contrary to previous studies (Buff et al., 2002; Gentry et al., 2002), gender did not influence leptin concentration ($P = 0.81$). Mean leptin was 2.33 ± 0.12 ng/ml HE in males and 2.17 ± 0.09 ng/ml in females (Figure 11), despite the fact that females had a much higher BCS than males (Figure 3), which could have caused an attenuation of any gender effects. In other studies, the differences in leptin concentration between the two sexes may have been due to inequalities in BCS between the two groups or to the influence of androgens and estrogens. Because all the horses in this study were of approximately the same BCS and of a fairly young age, these variables may not have come into play, thus rendering the gender effect insignificant.

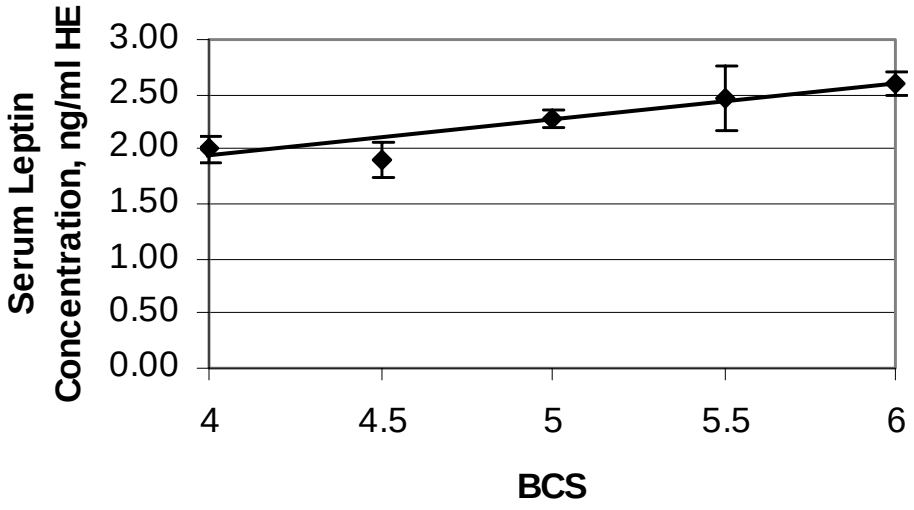

Figure 10. Correlation between mean leptin and mean body condition score in yearling horses ($r^2 = 0.87$, $P = 0.02$)

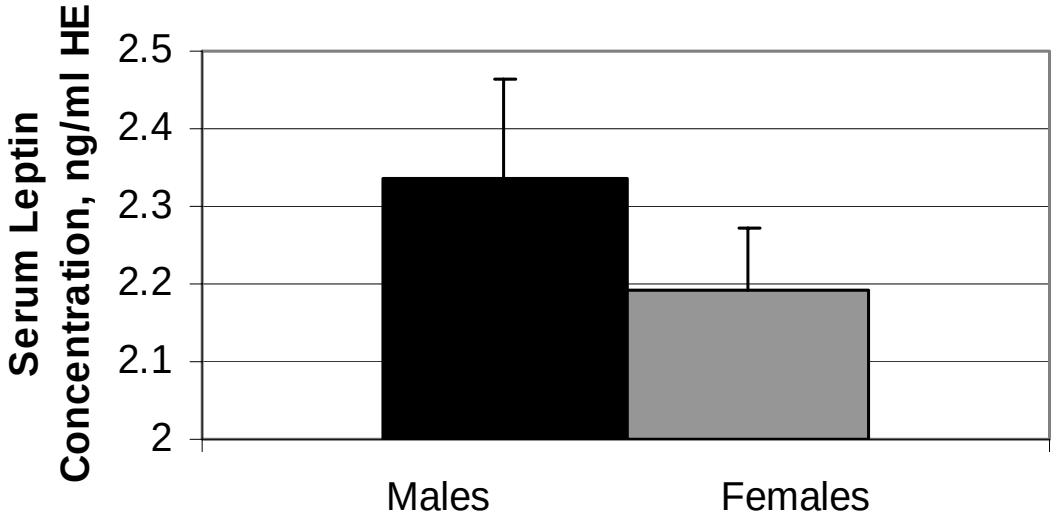

Figure 11. Mean serum leptin concentration in male and female yearling horses

Leptin: Influence of Period

Mean leptin concentration for all horses increased over all three periods ($P < 0.01$), as shown in Figure 12. This 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, nevertheless it may have been enough to influence leptin concentration. Mean serum leptin concentration and mean body condition score were correlated ($r^2 = 0.83$, $P = 0.001$) throughout the study (Figure 12). Furthermore, the higher plasma glucose concentrations experienced by the horses during period three may have also contributed to the elevation in leptin from period two to period three.

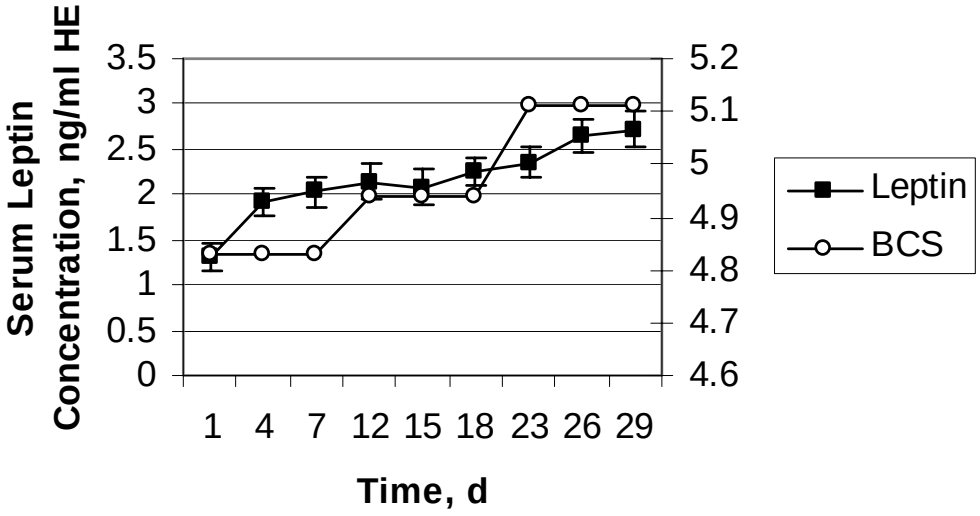

Figure 12. Changes in mean body condition score and mean serum leptin concentration over time are correlated in yearling horses ($r^2 = 0.83$, $P = 0.001$)

Period also influenced the effects of gender, body condition score, and feeding schedule on leptin ($P < 0.05$) (Appendix 7B). However, this is not so much a result of differences in the periods per se as it is of the rotation of horses through the blocks of the Latin square. Table 5 illustrates the fact that one group of horses had significantly ($P = 0.01$) higher leptin concentrations throughout the study, and as this group went through the various feeding schedules, the data appears to be influenced by period, when in fact the variation is due almost solely to the group effect.

Table 5. Effects of experimental grouping and experimental period on mean serum leptin concentrations in yearling horses

Group	Feeding Schedule	Period	Mean Leptin ng/ml HE	SEM
A	2x	1	2.74	0.18
	3x	2	1.99	0.17
	4x	3	1.88	0.12
		Total	1.99 [a]	0.16
B	2x	1	2.23	0.09
	3x	2	1.83	0.13
	4x	3	1.84	0.09
		Total	1.84 [a]	0.10
C	2x	1	2.15	0.19
	3x	2	2.76	0.12
	4x	3	2.67	0.2
		Total	2.15 [b]	0.17

[a,b] Column means not sharing common superscripts differ ($P < 0.05$)

Similarly, the influence of period on the effect of body condition score results from the fact that not all body condition scores were observed during all periods. For example, no horses had a BCS of 4.5 or 6 during the first period, none had a BCS of 5.5 or 6 during the second period, and so on (Appendix 6A). Additionally, the larger number of samples from horses with a BCS of 5 gives a more accurate representation of leptin concentration during any given period than a few samples from one horse with a BCS of 4.5. These two phenomena add up to produce an interaction of variables that, although statistically significant, does not impact the results of this study.

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., 2002b). 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 ($P > 0.5$) (Table 6). 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 leptin research.

Table 6. Serum leptin concentrations before and after administration of acepromazine, xylazine, or both to yearling horses

				Leptin Concentration, ng/ml HE	
Horse	Date	Medication		Before Medication	After Medication
1B	7/24/03	1.0 cc acepromazine, 1.0 cc xylazine		2.18	2.37
2B	7/13/03	1.5 cc acepromazine, 0.3 cc xylazine		2.10	1.7
3B	7/13/03	3 cc acepromazine		1.68	1.81
1C	7/13/03	3 cc acepromazine		3.15	3.41
	7/24/03	1.5 cc acepromazine, 1.0 cc xylazine		2.98	3.11
2C	7/13/03	3 cc acepromazine		2.19	2.36
			Mean	2.38	2.46
			SEM	0.17	0.21

Several horses also received Banamine during the course of the study for treatment of colic. No research has investigated the effect of analgesics on leptin production, but comparison of leptin concentrations before and after administration of Banamine does not suggest that there was any effect of the drug on leptin ($P > 0.05$). It is interesting to note that four incidences of colic occurred in three horses during the study, and that all three horses were on the 3x feeding schedule when they colicked. One horse from each group colicked, and horse 3C colicked twice within four days. Though the colics may not be a cause of the increased serum leptin concentrations, and vice versa, the relationship between the two is interesting to note.

Circadian Rhythm of Leptin

Analysis of variance on data taken over a 24-h period shows many of the same results as the data taken on days one, four, and seven. The 24-h leptin concentrations were heavily influenced by feeding schedule ($P < 0.01$), body condition score ($P < 0.01$), and period ($P < 0.01$) (Appendix 8B). The effect of period and its resulting interactions with other variables is most likely due to the reasons discussed above (see Table 5). Unlike leptin concentration data from samples obtained once per day, the 24-h data indicated a strong effect of gender ($P < 0.01$). As shown in Figure 13, average leptin concentration in males was 2.48 ± 0.09 and only 2.17 ± 0.06 in females. This is surprising given the difference in BCS between the genders (Figure 3), but corroborates previous results (Buff et al., 2002). Mean body condition score was not correlated with mean serum leptin concentration in this set of data (Figure 14), perhaps indicating that horses with different body condition scores have a different pattern of leptin secretion over a 24-h period. Data from Figure 15 show that mean leptin concentration was lowest during period two ($P < 0.05$), perhaps due to some environmental effect.

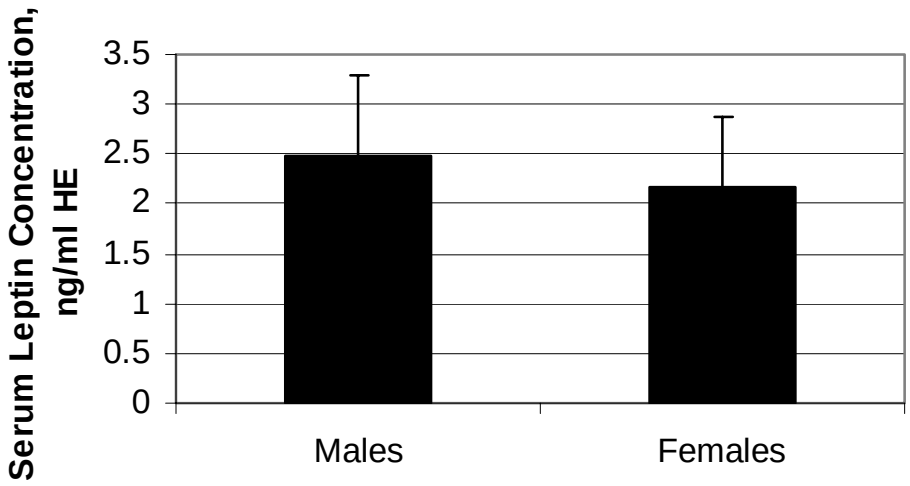

Figure 13. Mean serum leptin concentrations in male and female yearling horses during a 24-h period

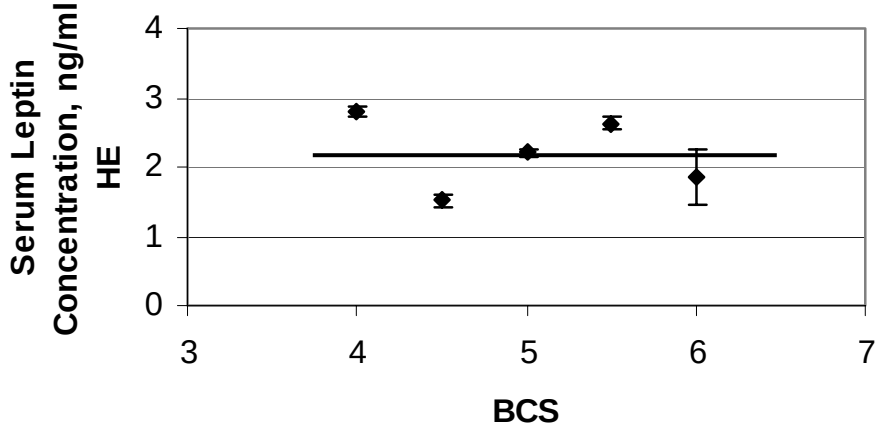

Figure 14. Correlation between mean serum leptin concentration and mean body condition score during a 24-h period in yearling horses ($r^2 = 0.05$, $P = 0.71$)

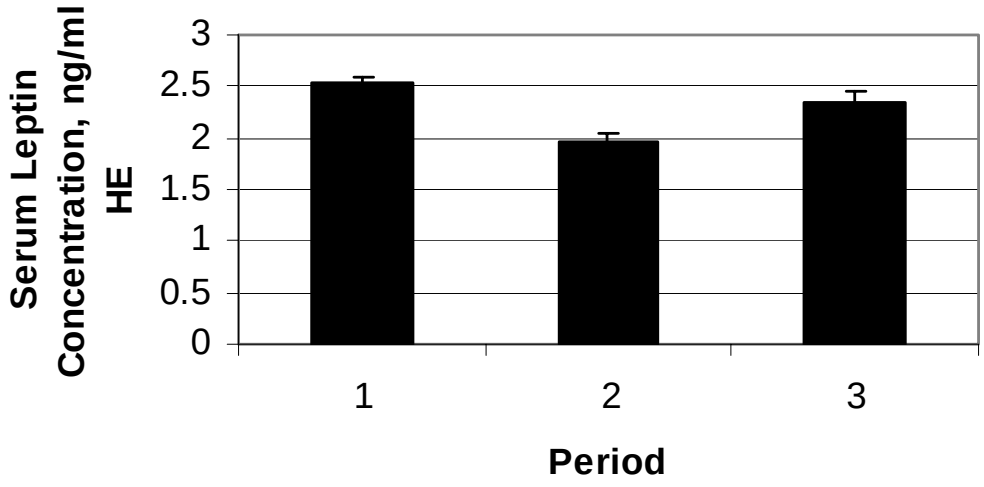

Figure 15. Mean serum leptin concentration in yearling horses during experimental periods one, two, and three

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 2x and 4x schedules, while some did not differ at all. Likewise, some horses showed variations in serum leptin concentration with time, whereas some did not (Figures 16 and 17).

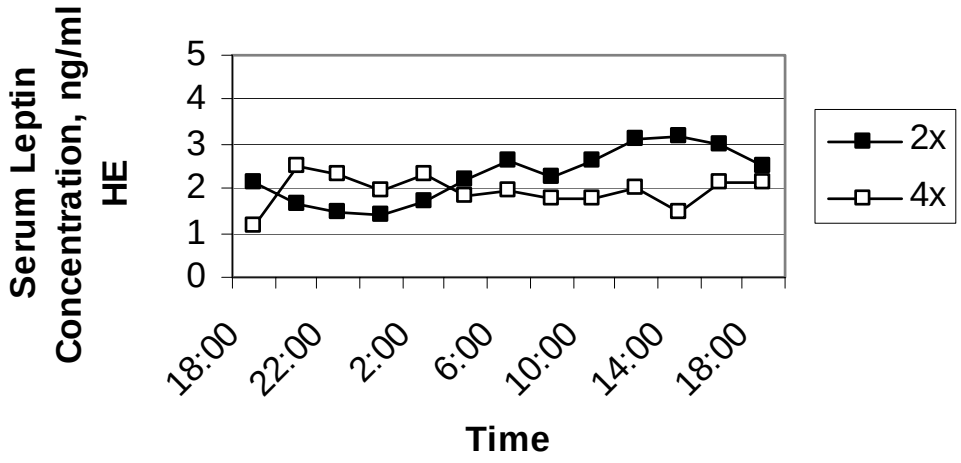

Figure 16. Serum leptin concentration over a 24-h period in a yearling horse (Horse #3A) fed two (2x) or four (4x) times per day

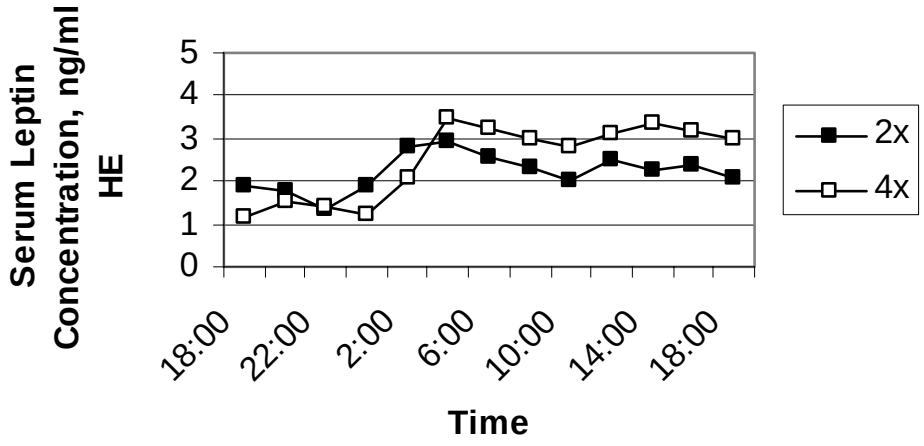

Figure 17. Serum leptin concentration over a 24-h period in a yearling horse (Horse #2C) fed two (2x) or four (4x) times per day

Mean leptin concentration was 2.24 ± 0.08 ng/ml HE on the 2x schedule and 2.32 ± 0.07 ng/ml HE on the 4x schedule (Appendix 8C). Because of the differences in baseline concentrations of leptin between horses, data was normalized to the first data point, which was taken at 1800 h. Three hours after feeding, the normalized leptin concentration was lower on the 2x schedule than on the 4x schedule ($P < 0.05$) (Figure 18). At this point, leptin concentration on the 2x 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 remain elevated postprandially. The postprandial decrease in leptin (time 19:00 to 22:00) has been noted previously in cattle (Delavaud et al., 2002), although small postprandial peaks have been found in sheep two to eight hours after a meal (Marie et al., 2001). There are several possible explanations as to why leptin decreased after the evening but not the morning meal. First, leptin may have a natural circadian rhythm in horses that peaks during the morning and then dips during the evening. This is not likely, however, since horses on the 4x schedule did not experience the same fluctuations. The second explanation is that leptin actually falls postprandially, but failed to do so after the morning meal. This is possible in light of the fact that many of the horses were stressed during the catheterization procedure, and the resulting release of cortisol could have affected leptin's response to the morning meal.

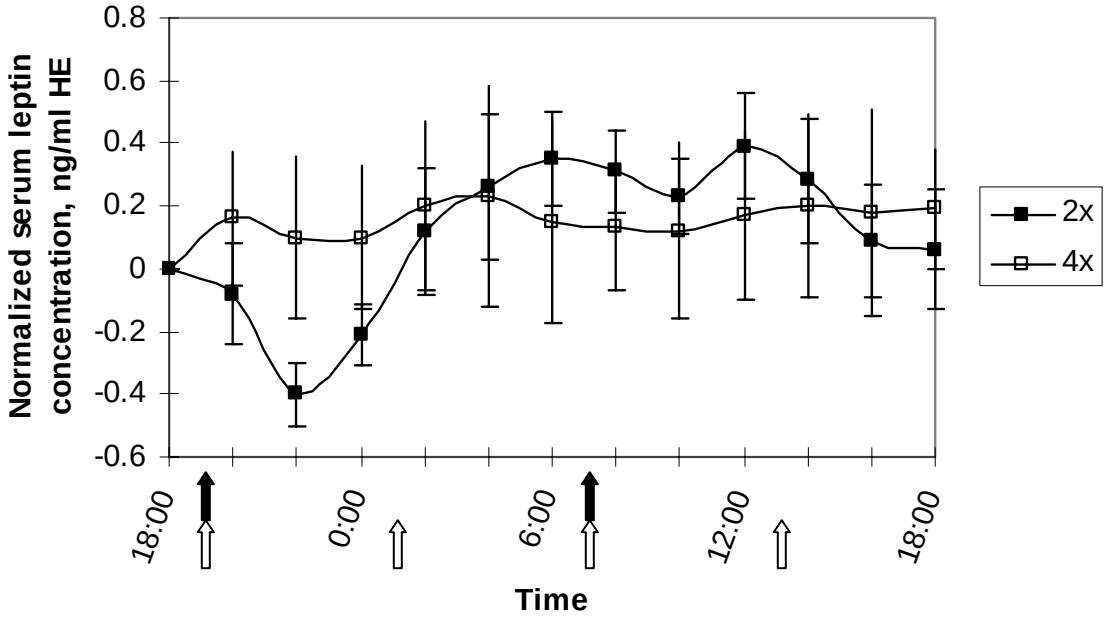

Figure 18. Mean normalized serum leptin concentration over a 24-h period in yearling horses fed two (2x) or four (4x) times per day (solid arrows indicated feeding times on 2x schedule, open arrows indicate feeding times on 4x schedule)

Analysis of variance on data from the 2x schedule shows a definite effect of time ($P < 0.05$), while leptin concentration did not vary with time on the 4x schedule ($P > 0.9$) (Appendix 8B). Regression analysis of the normalized data from the 2x feeding schedule indicates that leptin concentrations vary significantly with time in a quadratic model ($P < 0.05$), although no significant correlation was found when fitting these data to a linear model ($P = 0.18$). Regression analysis of data from the 4x schedule showed no variance with time in either a linear ($P > 0.4$) or quadratic ($P > 0.3$) model. This indicates that, when horses are fed two large concentrate meals per day, there are definite changes in leptin secretion over a 24-h period. On the other hand, when horses

are fed multiple small meals per day, serum leptin does not change with time. This is in accordance with a study done by Marie et al. (2001), which found no innate circadian rhythm of leptin in sheep. This indicates that our imposition of meal feeding upon horses affects more than simply gut function. It also has the power to disrupt the release of hormones and change the homeostasis of the animal. For optimum health, perhaps we should attempt to maintain a steady-state of serum leptin in our domesticated horses.

SUMMARY AND CONCLUSIONS

Energy is an essential nutrient to 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 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 horses were rotated through three feeding schedules in a replicated 3x3 Latin square design. Horses were fed two, three, or four times per day, although the total daily amount of feed was held constant. Horses were weighed and scored for body condition on the first day of each period. Blood samples were drawn before the morning meal on days one, four, and seven of each period. Additionally, blood was drawn every two hours for 24-hours beginning on day 10 of each period. Blood was also drawn every 30 minutes after the morning meal during the 24-hour collection for determination of postprandial plasma glucose concentration.

Neither weight nor body condition score changed significantly during the study ($P = 0.99$, $P = 0.28$). Both mean and peak plasma glucose was highest in horses fed twice per day ($P < 0.05$), as was mean area under the curve. Although mean leptin concentration tended to be highest when horses were fed three times per day ($P = 0.1$), this was most likely due to differences in baseline values. Normalization of data to day

one values showed leptin to increase in horses fed twice per day ($P < 0.05$), while no change was noted in horses fed three or four times per day ($P > 0.05$). Leptin was elevated in horses with higher body condition scores ($P < 0.01$) and increased steadily throughout the study ($P < 0.01$).

Data from the 24-hour collection indicated that horses fed twice per day had definite fluctuations in leptin production throughout the day ($P < 0.01$), while horses fed four times per day did not ($P > 0.05$)

Overall, this study indicates that feeding horses two large concentrate meals per day may increase mean serum leptin concentrations and can cause fluctuations in leptin production over a 24-h period. This does not appear to happen in horses fed multiple small meals. Therefore, the feeding of two large meals per day may cause variations in leptin production that are not innate to the horse. This departure from baseline leptin concentration has the potential to affect appetite, along with innumerable physiological processes. Thus, further exploration of the effects of common equine management practices on leptin is warranted. Specifically, because so many horses experience stress during training and showing, the effect of cortisol on leptin needs to be determined in vivo. Additionally, the correlation between serum leptin concentration and appetite needs to be explored in both normal and obese horses, as well as in “hard keepers”.

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APPENDICES

APPENDIX 1. CONCENTRATE OFFERED PER MEAL ON EACH FEEDING SCHEDULE, KG

	Concentrate, kg		
	Feeding Schedule		
Horse	2x	3x	4x
1A	2.38	1.65	1.24
2A	2.64	1.74	1.32
3A	2.45	1.63	1.22
1B	2.55	1.7	1.27
2B	2.79	1.86	1.39
3B	2.65	1.77	1.32
1C	2.87	1.92	1.44
2C	2.38	1.59	1.23
3C	2.47	1.8	1.24

APPENDIX 2. CONCENTRATE REFUSED ON EACH FEEDING SCHEDULE, KG

	Concentrate, kg		
	Feeding Schedule		
Horse	2x	3x	4x
1A	0.00	0.00	0.00
2A	0.00	0.00	0.00
3A	0.00	0.19	0.00
1B	0.00	0.00	0.00
2B	0.15	0.00	0.00
3B	0.00	0.00	0.00
1C	0.00	0.00	0.00
2C	0.00	0.00	0.00
3C	3.44	4.53	0.00
Total	3.59	4.73	0.00

APPENDIX 3. HORSES RECEIVING MEDICATIONS WHILE ON THE STUDY

Horse	Date	Medication
1A	7/13/03	7.0 cc Banamine
1B	7/24/03	1.0 cc acepromazine, 1.0 cc xylazine
	8/4/03	1.0 cc acepromazine, 1.0 cc xylazine
2B	7/13/03	1.5 cc acepromazine, 0.3 cc xylazine
	7/24/03	3.0 cc acepromazine, 1.0 cc xylazine
	8/2/03	7.0 cc Banamine
3B	7/13/03	3.0 cc acepromazine
	8/4/03	1.0 cc acepromazine, 1.0 cc xylazine
1C	7/13/03	3.0 cc acepromazine
	7/24/03	1.5 cc acepromazine, 1.0 cc xylazine
	8/4/03	3.0 cc acepromazine
2C	7/13/03	3.0 cc acepromazine
	8/4/03	1.0 cc acepromazine, 1.0 cc xylazine
3C	7/18/03	8.0 cc Banamine
	8/4/03	1.0 cc acepromazine, 1.0 cc xylazine

APPENDIX 4A. MEAN GLUCOSE BY FEEDING SCHEDULE AND TIME, MG/DL

	Glucose, mg/dl		
	Feeding Schedule		
Time	2x	3x	4x
-0.5	89.54	88.14	88.50
0.5	103.21[a]	88.45[b]	95.01[b]
1.0	103.46	104.11	99.70
1.5	103.19	97.16	95.12
2.0	109.78	95.78	95.20
2.5	103.70	96.78	96.54
3.0	93.62	88.71	92.39
3.5	97.06	91.94	91.50
4.0	99.11	91.19	87.51
4.5	91.11	91.05	90.37
5.0	98.92	89.49	90.01
5.5	96.63	89.60	94.60
6.0	95.72	90.16	95.51
Mean	98.81	92.52	93.26
SEM	1.59	1.30	0.97

[a,b] Row means not sharing a common superscript differ ($P < 0.05$)

APPENDIX 4B. ANOVA TABLE FOR MEAN, BASELINE, PEAK, AND CHANGE IN GLUCOSE CONCENTRATION AND AREA UNDER THE GLUCOSE CURVE

Source	df	SS	MS	F-value	P-value
Glucose, mg/dl					
Mean Concentration					
Total	336	46795.37	139.2719		
Model	68	14862.79	218.5705	1.83	0.0004
Error	268	31932.58	119.1514		
schedule	2	2612.605	1306.302	10.96	0.0000
time	12	4976.814	414.7345	3.48	0.0001
period	2	3253.096	1626.548	13.65	0.0000
schedule*time	24	1916.156	79.83985	0.67	0.8788
schedule*period	4	50.89084	12.72271	0.11	0.9801
time*period	24	1815.146	75.63107	0.63	0.9080
Baseline Concentration					
Total	25	1693.691	67.74764		
Model	8	926.7376	115.8422	2.57	0.0486
Error	17	766.9535	45.11491		
schedule	2	17.03402	8.517012	0.19	0.8297
period	2	361.2673	180.6337	4.00	0.0376
schedule*period	4	570.9455	142.7364	3.16	0.0409
Peak Concentration					
Total	25	3226.12	129.0448		
Model	8	1471.6	183.95	1.78	0.1506
Error	17	1754.52	103.2071		
schedule	2	669.0649	334.5325	3.24	0.0642
period	2	582.1292	291.0646	2.82	0.0876
schedule*period	4	113.7505	28.43762	0.28	0.8897
Change from Baseline to Peak					
Total	25	4153.285	166.1314		
Model	8	2745.731	343.2164	4.15	0.0066
Error	17	1407.553	82.79725		
schedule	2	472.6429	236.3215	2.85	0.0854
period	2	1628.698	814.3491	9.84	0.0015
schedule*period	4	468.5725	117.1431	1.41	0.2715

APPENDIX 4B. CONTINUED

Source	df	SS	MS	F-value	P-value
Glucose, mg/dl					
Area Under the Curve					
Total	25	216952.562	8678.10246		
Model	8	82241.2682	10280.1585	1.30	0.3086
Error	17	134711.293	7924.19373		
schedule	2	38221.712	19110.856	2.41	0.1197
period	2	42011.2623	21005.6311	2.65	0.0995
schedule*period	4	766.282576	191.570644	0.02	0.9987

APPENDIX 5A. BODY WEIGHT BY PERIOD, KG

	Weight, kg		
	Period		
Horse	1	2	3
1A	300	288	294
2A	352	371	373
3A	326	333	339
1B	340	352	*
2B	371	381	385
3B	353	366	373
1C	383	392	389
2C	317	323	323
3C	340	317	353
Mean	342	347	354
SEM	8.7	11.3	11

APPENDIX 5B. ANOVA TABLE FOR WEIGHT BY GENDER, BCS, AND PERIOD

Source	df	SS	MS	F-value	P-value
Weight, kg					
Total	25	111952.654	4478.10615		
Model	14	57911.4538	4136.53242	0.84	0.6254
Error	11	54041.2	4912.83636		
gender	1	807.572643	807.572643	0.16	0.6929
BCS	4	35472.0869	8868.02173	1.81	0.1981
period	2	77.2947888	38.6473944	0.01	0.9922
gender*BCS	1	5607.69231	5607.69231	1.14	0.3082
gender*period	2	9034.1633	4517.08165	0.92	0.4273
BCS*period	4	4429.46529	1107.36632	0.23	0.9185

APPENDIX 6A. BODY CONDITION SCORES BY PERIOD

	Horse	Gender	Feeding Schedule	BCS
Period 1	1A	M	3x	4.5
	2A	F	3x	5
	3A	F	3x	4.5
	1B	M	4x	4
	2B	F	4x	5
	3B	F	4x	5
	1C	M	2x	5
	2C	F	2x	5
	3C	F	2x	5.5
Period 2	1A	M	4x	4.5
	2A	F	4x	5
	3A	F	4x	5
	1B	M	2x	4
	2B	F	2x	5
	3B	F	2x	5
	1C	M	3x	5
	2C	F	3x	5
	3C	F	3x	6
Period 3	1A	M	2x	5
	2A	F	2x	5.5
	3A	F	2x	5
	1B	M	3x	4.5
	2B	F	3x	5
	3B	F	3x	5
	1C	M	4x	5
	2C	F	4x	5
	3C	F	4x	6

APPENDIX 6B. ANOVA TABLE FOR BODY CONDITION SCORE BY PERIOD, GENDER, AND FEEDING SCHEDULE

Source	df	SS	MS	F-value	P-value
BCS					
Total	26	5.46296296	0.21011396		
Model	13	3.76388889	0.289529915	2.22	0.0824
Error	13	1.69907407	0.130698006		
period	2	0.36111111	0.180555556	1.38	0.2858
gender	1	1.6712963	1.6712963	12.79	0.0034
schedule	2	0.02777778	0.013888889	0.11	0.9000
period*gender	2	0.06481482	0.032407407	0.25	0.7840
period*schedule	4	1.59259259	0.398148148	3.05	0.0563
gender*schedule	2	0.06481482	0.032407407	0.25	0.7840

APPENDIX 7A. SERUM LEPTIN CONCENTRATIONS ON DAYS 1, 4, AND 7 OF EACH PERIOD, NG/ML HE

	Horse	Day	Feeding Schedule	Sex	Leptin (ng/ml HE)	Normalized Leptin (ng/ml ME)
Period 1	1A	1	3x	M	*	0
	2A	1	3x	F	2.72	0
	3A	1	3x	F	2.02	0
	1B	1	4x	M	1.94	0
	2B	1	4x	F	2.15	0
	3B	1	4x	F	1.68	0
	1C	1	2x	M	2.62	0
	2C	1	2x	F	1.36	0
	3C	1	2x	F	1.87	0
	1A	4	3x	M	1.22	*
	2A	4	3x	F	2.45	-0.27
	3A	4	3x	F	2.00	-0.02
	1B	4	4x	M	2.26	0.32
	2B	4	4x	F	1.67	-0.48
	3B	4	4x	F	1.53	-0.15
	1C	4	2x	M	2.67	0.05
	2C	4	2x	F	1.61	0.25
	3C	4	2x	F	1.86	-0.01
	1A	7	3x	M	1.46	*
	2A	7	3x	F	1.95	-0.07
	3A	7	3x	F	2.14	0.2
	1B	7	4x	M	1.60	-0.34
	2B	7	4x	F	2.10	-0.05
	3B	7	4x	F	1.68	0
	1C	7	2x	M	3.15	0.53
	2C	7	2x	F	2.19	0.83
	3C	7	2x	F	2.03	0.16
Period 2	1A	1	4x	M	1.36	0
	2A	1	4x	F	2.34	0
	3A	1	4x	F	2.13	0
	1B	1	2x	M	1.76	0
	2B	1	2x	F	1.71	0
	3B	1	2x	F	1.81	0
	1C	1	3x	M	3.41	0
	2C	1	3x	F	2.36	0
	3C	1	3x	F	2.36	0
	1A	4	4x	M	1.28	-0.08
	2A	4	4x	F	1.99	-0.35
	3A	4	4x	F	1.84	-0.29
	1B	4	2x	M	2.28	0.52
	2B	4	2x	F	1.52	-0.19

APPENDIX 7A. CONTINUED

	Horse	Day	Feeding Schedule	Sex	Leptin, ng/ml HE	Normalized Leptin, ng/ml HE
	3B	4	2x	F	1.51	-0.30
	1C	4	3x	M	3.14	-0.27
	2C	4	3x	F	2.55	0.09
	3C	4	3x	F	2.62	0.24
	1A	7	4x	M	1.79	0.43
	2A	7	4x	F	2.17	-0.17
	3A	7	4x	F	2.01	-0.12
	1B	7	2x	M	2.18	0.42
	2B	7	2x	F	1.82	0.11
	3B	7	2x	F	1.89	0.08
	1C	7	3x	M	2.98	-0.43
	2C	7	3x	F	2.61	0.25
	3C	7	3x	F	2.89	0.53
Period 3	1A	1	2x	M	2.40	0
	2A	1	2x	F	3.03	0
	3A	1	2x	F	2.18	0
	1B	1	3x	M	2.37	0
	2B	1	3x	F	*	0
	3B	1	3x	F	1.72	0
	1C	1	4x	M	3.11	0
	2C	1	4x	F	1.73	0
	3C	1	4x	F	2.26	0
	1A	4	2x	M	2.70	0.30
	2A	4	2x	F	2.37	-0.66
	3A	4	2x	F	3.23	1.05
	1B	4	3x	M	2.63	0.27
	2B	4	3x	F	1.89	*
	3B	4	3x	F	2.21	0.49
	1C	4	4x	M	2.42	-0.69
	2C	4	4x	F	3.65	1.92
	3C	4	4x	F	2.75	0.49
	1A	7	2x	M	2.06	-0.34
	2A	7	2x	F	3.70	0.67
	3A	7	2x	F	2.98	0.80
	1B	7	3x	M	2.58	0.19
	2B	7	3x	F	*	*
	3B	7	3x	F	2.23	0.51
	1C	7	4x	M	3.32	0.21
	2C	7	4x	F	2.12	0.39
	3C	7	4x	F	2.70	0.44

APPENDIX 7B. ANOVA TABLE FOR LEPTIN (DAYS 1, 4, & 7) BY PERIOD, DAY, FEEDING SCHEDULE, GENDER, AND BCS

Source	df	SS	MS	F-value	P-value
Leptin, ng/ml HE					
Total	77	24.51375	0.31836		
Model	34	18.90926	0.556155	4.27	0.0000
Error	43	5.604488	0.130337		
period	2	2.988253	1.494127	11.46	0.0001
day	2	0.248518	0.124259	0.95	0.3934
schedule	2	0.995925	0.497962	3.82	0.0297
gender	1	0.007466	0.007466	0.06	0.8120
BCS	4	1.827748	0.456937	3.51	0.0146
period*day	4	0.53119	0.132798	1.02	0.4084
period*schedule	4	3.035328	0.758832	5.82	0.0008
period*gender	2	2.832936	1.416468	10.87	0.0002
period*BCS	5	4.733093	0.946619	7.26	0.0001
day*schedule	4	0.262629	0.065657	0.50	0.7331
day*gender	2	0.066446	0.033223	0.25	0.7762
schedule*gender	2	2.530992	1.265496	9.71	0.0003
schedule*BCS	0	0			
gender*BCS	0	0			

APPENDIX 8A. MEAN LEPTIN CONCENTRATIONS DURING 24-H BLOOD COLLECTIONS, NG/ML HE

		Feeding Schedule			
	Time	2x	4x	Total	SEM
Period 1	18:00	2.54	2.57	2.56	0.18
	20:00	2.52	2.68	2.60	0.32
	22:00	2.30	2.22	2.26	0.29
	0:00	2.46	2.36	2.41	0.24
	2:00	2.86	2.27	2.57	0.29
	4:00	2.91	2.23	2.57	0.26
	6:00	3.12	2.03	2.58	0.34
	8:00	2.98	2.23	2.61	0.29
	10:00	2.67	2.19	2.43	0.27
	12:00	2.92	2.41	2.66	0.15
	14:00	2.71	2.44	2.55	0.24
	16:00	2.66	2.18	2.42	0.25
	18:00	2.78	2.40	2.58	0.25
	Time	2x	4x	Total	SEM
Period 2	18:00	2.12	1.65	1.88	0.28
	20:00	2.09	2.28	2.19	0.16
	22:00	1.72	2.27	2.00	0.29
	0:00	1.98	2.17	2.08	0.19
	2:00	1.86	2.24	2.05	0.36
	4:00	1.84	1.83	1.84	0.26
	6:00	1.73	1.94	1.84	0.17
	8:00	2.16	1.92	2.04	0.36
	10:00	2.48	1.67	1.99	0.44
	12:00	2.09	1.93	2.01	0.41
	14:00	1.96	1.83	1.89	0.44
	16:00	1.73	1.99	1.86	0.33
	18:00	1.97	2.28	2.10	0.38
	Time	2x	4x	Total	SEM
Period 3	18:00	1.73	1.70	1.71	0.38
	20:00	1.72	1.94	1.83	0.21
	22:00	1.19	2.31	1.75	0.32
	0:00	1.32	1.69	1.51	0.18
	2:00	2.02	2.94	2.39	0.28
	4:00	2.42	3.18	2.72	0.36
	6:00	2.27	3.18	2.63	0.27
	8:00	2.16	3.67	2.76	0.48
	10:00	2.15	3.04	2.50	0.38
	12:00	2.57	3.28	2.85	0.25
	14:00	2.58	3.38	2.90	0.32
	16:00	2.41	3.27	2.76	0.31
	18:00	2.52	3.02	2.73	0.22

APPENDIX 8B. ANOVA TABLE FOR LEPTIN (24-HOUR COLLECTION) BY PERIOD, TIME, FEEDING SCHEDULE, GENDER, AND BCS

Source	df	SS	MS	F-value	P-value
Leptin, ng/ml HE					
Total	224	128.284	0.572697		
Model	114	98.69791	0.865771	3.22	0.0000
Error	110	29.58612	0.268965		
period	2	13.77541	6.887707	25.61	0.0000
time	12	3.725548	0.310462	1.15	0.3251
schedule	1	4.199579	4.199579	15.61	0.0001
gender	1	7.402777	7.402777	27.52	0.0000
BCS	4	23.23927	5.809819	21.6	0.0000
period*schedule	2	4.399801	2.199901	8.18	0.0005
time*schedule	12	1.542699	0.128558	0.48	0.9239
gender*schedule	1	6.638777	6.638777	24.68	0.0000
BCS*schedule	1	12.82673	12.82673	47.69	0.0000
period*time	24	10.64739	0.443641	1.65	0.0433
period*gender	1	8.922531	8.922531	33.17	0.0000
period*BCS	1	1.131325	1.131325	4.21	0.0427
gender*time	12	2.377062	0.198089	0.74	0.7131
time*BCS	40	7.211893	0.198089	0.67	0.9245
gender*BCS	0	0			

APPENDIX 8C. SERUM LEPTIN CONCENTRATIONS DURING 24-H BLOOD COLLECTIONS, NG/ML HE

Horse	Period	Time	Feeding Schedule	Time Fed	Leptin (ng/ml HE)	Normalized Leptin (ng/ml ME)
1A	2	18:00	4x	x	1.60	0.00
1A	2	20:00	4x		1.74	0.14
1A	2	22:00	4x		*	*
1A	2	0:00	4x	x	1.94	0.34
1A	2	2:00	4x		1.81	0.21
1A	2	4:00	4x		1.45	-0.15
1A	2	6:00	4x	x	1.43	-0.17
1A	2	8:00	4x		1.42	-0.18
1A	2	10:00	4x		0.85	-0.75
1A	2	12:00	4x	x	1.34	-0.26
1A	2	14:00	4x		1.45	-0.15
1A	2	16:00	4x		1.03	-0.57
1A	2	18:00	4x		2.03	0.43
1A	3	18:00	2x	x	1.08	0.00
1A	3	20:00	2x		1.87	0.79
1A	3	22:00	2x		1.07	-0.10
1A	3	0:00	2x		0.95	-0.13
1A	3	2:00	2x		1.82	0.74
1A	3	4:00	2x		1.62	0.54
1A	3	6:00	2x	x	1.75	0.67
1A	3	8:00	2x		1.40	0.32
1A	3	10:00	2x		1.06	-0.02
1A	3	12:00	2x		2.00	0.92
1A	3	14:00	2x		1.68	0.60
1A	3	16:00	2x		1.65	0.57
1A	3	18:00	2x		1.64	0.56
1B	1	18:00	4x	x	2.51	0.00
1B	1	20:00	4x		2.62	0.11
1B	1	22:00	4x		2.54	0.03
1B	1	0:00	4x	x	3.03	0.52
1B	1	2:00	4x		3.08	0.57
1B	1	4:00	4x		2.76	0.25
1B	1	6:00	4x	x	2.35	-0.16
1B	1	8:00	4x		2.47	-0.04
1B	1	10:00	4x		2.38	-0.13
1B	1	12:00	4x	x	2.39	-0.12
1B	1	14:00	4x		2.15	-0.36
1B	1	16:00	4x		2.58	0.07
1B	1	18:00	4x		2.55	0.04
1B	2	18:00	2x	x	3.12	0.00
1B	2	20:00	2x		2.53	-0.59

APPENDIX 8C. CONTINUED

Horse	Period	Time	Feeding Schedule	Time Fed	Leptin (ng/ml HE)	Normalized Leptin (ng/ml ME)
1B	2	22:00	2x		2.69	-0.43
1B	2	0:00	2x		2.67	-0.45
1B	2	2:00	2x		3.26	0.14
1B	2	4:00	2x		2.59	-0.53
1B	2	6:00	2x	x	3.01	-0.11
1B	2	8:00	2x		3.61	0.49
1B	2	10:00	2x		3.46	0.34
1B	2	12:00	2x		3.81	0.69
1B	2	14:00	2x		3.74	0.62
1B	2	16:00	2x		2.76	-0.36
1B	2	18:00	2x		3.02	-0.10
1C	1	18:00	2x	x	2.92	0.00
1C	1	20:00	2x		3.24	0.22
1C	1	22:00	2x		2.78	-0.14
1C	1	0:00	2x		2.48	-0.44
1C	1	2:00	2x		2.59	-0.33
1C	1	4:00	2x		2.89	-0.03
1C	1	6:00	2x	x	4.01	1.09
1C	1	8:00	2x		3.79	0.87
1C	1	10:00	2x		3.33	0.41
1C	1	12:00	2x		3.15	0.23
1C	1	14:00	2x		3.05	0.13
1C	1	16:00	2x		2.88	-0.04
1C	1	18:00	2x		2.93	0.01
1C	3	18:00	4x	x	3.25	0.00
1C	3	20:00	4x		2.86	-0.39
1C	3	22:00	4x		2.69	-0.56
1C	3	0:00	4x	x	2.26	-0.99
1C	3	2:00	4x		3.17	-0.08
1C	3	4:00	4x		2.87	-0.38
1C	3	6:00	4x	x	3.16	-0.09
1C	3	8:00	4x		4.35	1.10
1C	3	10:00	4x		3.27	0.02
1C	3	12:00	4x	x	3.49	0.24
1C	3	14:00	4x		3.41	0.16
1C	3	16:00	4x		3.40	0.15
1C	3	18:00	4x		3.30	0.05
2A	2	18:00	4x	x	2.21	0.00
2A	2	20:00	4x		2.58	0.37
2A	2	22:00	4x		2.80	0.59
2A	2	0:00	4x	x	2.61	0.40
2A	2	2:00	4x		2.58	0.37
2A	2	4:00	4x		2.25	0.04

APPENDIX 8C. CONTINUED

Horse	Period	Time	Feeding Schedule	Time Fed	Leptin (ng/ml HE)	Normalized Leptin (ng/ml ME)
2A	2	6:00	4x	x	2.45	0.24
2A	2	8:00	4x		2.60	0.39
2A	2	10:00	4x		2.41	0.20
2A	2	12:00	4x	x	2.44	0.23
2A	2	14:00	4x		2.60	0.39
2A	2	16:00	4x		2.81	0.60
2A	2	18:00	4x		2.66	0.45
2A	3	18:00	2x	x	2.00	0.00
2A	3	20:00	2x		1.63	-0.37
2A	3	22:00	2x		1.05	-0.95
2A	3	0:00	2x		1.60	-0.40
2A	3	2:00	2x		2.56	0.56
2A	3	4:00	2x		3.42	1.42
2A	3	6:00	2x	x	2.44	0.44
2A	3	8:00	2x		2.81	0.81
2A	3	10:00	2x		2.73	0.73
2A	3	12:00	2x		2.61	0.61
2A	3	14:00	2x		2.87	0.87
2A	3	16:00	2x		2.58	0.58
2A	3	18:00	2x		2.63	0.63
2B	1	18:00	4x	x	3.01	0.00
2B	1	20:00	4x		3.67	0.66
2B	1	22:00	4x		2.78	-0.23
2B	1	0:00	4x	x	2.49	-0.52
2B	1	2:00	4x		2.57	-0.44
2B	1	4:00	4x		2.63	-0.38
2B	1	6:00	4x	x	2.32	-0.69
2B	1	8:00	4x		2.62	-0.39
2B	1	10:00	4x		2.79	-0.22
2B	1	12:00	4x	x	2.41	-0.60
2B	1	14:00	4x		3.26	0.25
2B	1	16:00	4x		2.76	-0.25
2B	1	18:00	4x		2.96	-0.05
2B	2	18:00	2x	x	1.59	0.00
2B	2	20:00	2x		*	*
2B	2	22:00	2x		1.31	-0.28
2B	2	0:00	2x		1.50	-0.09
2B	2	2:00	2x		0.65	-0.94
2B	2	4:00	2x		0.83	-0.76
2B	2	6:00	2x	x	1.35	-0.24
2B	2	8:00	2x		1.34	-0.25
2B	2	10:00	2x		*	*
2B	2	12:00	2x		1.27	-0.32

APPENDIX 8C. CONTINUED

Horse	Period	Time	Feeding Schedule	Time Fed	Leptin (ng/ml HE)	Normalized Leptin (ng/ml ME)
2B	2	14:00	2x		1.25	-0.37
2B	2	16:00	2x		1.36	-0.23
2B	2	18:00	2x		0.74	-0.85
2C	1	18:00	2x	x	1.90	0.00
2C	1	20:00	2x		1.74	-0.16
2C	1	22:00	2x		1.33	-0.57
2C	1	0:00	2x		1.90	0.00
2C	1	2:00	2x		2.83	0.93
2C	1	4:00	2x		2.91	1.01
2C	1	6:00	2x	x	2.58	0.68
2C	1	8:00	2x		2.30	0.40
2C	1	10:00	2x		2.04	0.14
2C	1	12:00	2x		2.51	0.61
2C	1	14:00	2x		2.24	0.34
2C	1	16:00	2x		*	*
2C	1	18:00	2x		2.08	0.19
2C	3	18:00	4x	x	1.18	0.00
2C	3	20:00	4x		1.51	0.33
2C	3	22:00	4x		1.43	0.25
2C	3	0:00	4x	x	1.22	0.04
2C	3	2:00	4x		*	*
2C	3	4:00	4x		3.50	2.32
2C	3	6:00	4x	x	3.21	2.03
2C	3	8:00	4x		2.99	1.01
2C	3	10:00	4x		2.81	1.63
2C	3	12:00	4x	x	3.08	1.90
2C	3	14:00	4x		3.36	2.18
2C	3	16:00	4x		3.15	1.97
2C	3	18:00	4x		2.99	1.01
3A	2	18:00	4x	x	1.14	0.00
3A	2	20:00	4x		2.52	1.38
3A	2	22:00	4x		2.29	1.15
3A	2	0:00	4x	x	1.97	0.83
3A	2	2:00	4x		2.33	1.19
3A	2	4:00	4x		1.80	0.66
3A	2	6:00	4x	x	1.94	0.80
3A	2	8:00	4x		1.74	0.60
3A	2	10:00	4x		1.74	0.60
3A	2	12:00	4x	x	2.02	0.88
3A	2	14:00	4x		1.44	0.30
3A	2	16:00	4x		2.13	0.99
3A	2	18:00	4x		2.15	1.01
3A	3	18:00	2x	x	2.11	0.00

APPENDIX 8C. CONTINUED

Horse	Period	Time	Feeding Schedule	Time Fed	Leptin (ng/ml HE)	Normalized Leptin (ng/ml ME)
3A	3	20:00	2x		1.67	-0.44
3A	3	22:00	2x		1.45	-0.66
3A	3	0:00	2x		1.41	-0.70
3A	3	2:00	2x		1.69	-0.42
3A	3	4:00	2x		2.21	0.10
3A	3	6:00	2x	x	2.62	0.51
3A	3	8:00	2x		2.26	0.15
3A	3	10:00	2x		2.65	0.55
3A	3	12:00	2x		3.09	0.98
3A	3	14:00	2x		3.19	1.08
3A	3	16:00	2x		3.01	0.89
3A	3	18:00	2x		2.48	0.37
3B	1	18:00	4x	x	2.20	0.00
3B	1	20:00	4x		1.76	-0.44
3B	1	22:00	4x		1.33	-0.87
3B	1	0:00	4x	x	1.55	-0.65
3B	1	2:00	4x		1.18	-1.02
3B	1	4:00	4x		1.30	-0.90
3B	1	6:00	4x	x	1.43	-0.77
3B	1	8:00	4x		1.60	0.80
3B	1	10:00	4x		1.41	-0.79
3B	1	12:00	4x	x	2.43	0.23
3B	1	14:00	4x		1.79	-0.41
3B	1	16:00	4x		1.21	-0.99
3B	1	18:00	4x		1.69	-0.51
3B	2	18:00	2x	x	1.64	0.00
3B	2	20:00	2x		1.76	0.12
3B	2	22:00	2x		1.15	-0.49
3B	2	0:00	2x		1.77	0.13
3B	2	2:00	2x		1.67	0.03
3B	2	4:00	2x		2.09	0.45
3B	2	6:00	2x	x	1.73	0.09
3B	2	8:00	2x		1.54	-0.10
3B	2	10:00	2x		1.51	-0.13
3B	2	12:00	2x		1.18	-0.46
3B	2	14:00	2x		0.88	-0.76
3B	2	16:00	2x		1.07	-0.57
3B	2	18:00	2x		0.81	-0.83
3C	1	18:00	2x	x	2.80	0.00
3C	1	20:00	2x		2.59	-0.21
3C	1	22:00	2x		2.78	-0.02
3C	1	0:00	2x		3.00	0.20
3C	1	2:00	2x		3.15	0.35

APPENDIX 8C. CONTINUED

Horse	Period	Time	Feeding Schedule	Time Fed	Leptin, ng/ml HE	Normalized Leptin, ng/ml HE
3C	1	4:00	2x		2.94	0.14
3C	1	6:00	2x	x	2.78	-0.02
3C	1	8:00	2x		2.86	0.06
3C	1	10:00	2x		2.65	-0.15
3C	1	12:00	2x		3.09	0.29
3C	1	14:00	2x		2.84	0.04
3C	1	16:00	2x		2.71	-0.09
3C	1	18:00	2x		3.32	0.52

VITA

Samantha Michelle Bruce was born on June 29, 1979 in Cleveland, Ohio to Eugene and Margaret Bruce. Her family moved to Lexington, Kentucky when she was 12, and in 1997 she graduated from Tates Creek High School. Samantha earned her B.S. in equine science from Colorado State University in 2001. She began her graduate career at Texas A&M University in August of 2002 under Dr. Gary Potter. During this time, Samantha served as both a research and teaching assistant, as well as a coach's assistant for the Texas A&M Equestrian Team. She is a member of the Equine Nutrition and Physiology Society.

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