# The relationship between body condition, leptin, and reproductive and hormonal characteristics of mares during the seasonal anovulatory period[1]

**L. R. Gentry, D. L. Thompson, Jr.[2], G. T. Gentry, Jr., K. A. Davis, R. A. Godke, and J. A. Cartmill**

Department of Animal Science, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Baton Rouge 70803-4210



**ABSTRACT:** An experiment was conducted to determine the effects of high vs low body condition scores (BCS) produced by restricted feeding on reproductive characteristics, hormonal secretion, and leptin concentrations in mares during the autumnal transition and winter anovulatory period. Mares with BCS of 6.5 to 8.0 were maintained on pasture and/or grass hay, and starting in September, were full fed or restricted to produce BCS of 7.5 to 8.5 (high) or 3.0 to 3.5 (low) by December. All but one mare with high BCS continued to ovulate or have follicular activity during the winter, whereas mares with low BCS went reproductively quiescent. Plasma leptin concentrations varied widely before the onset of restriction, even though all mares were in good body condition. During the experiment, leptin concentrations gradually decreased ($P < 0.0001$) over time in both groups, but were higher ($P < 0.009$) in mares with high vs low BCS after 6 wk of restriction, regardless of initial concentration. No differences ($P > 0.1$) between groups were detected for plasma concentrations of LH, FSH, TSH, GH, glucose, or insulin in samples collected weekly; in contrast, plasma prolactin concentrations were higher ($P < 0.02$) in mares with high BCS, but also decreased over time ($P < 0.008$). Plasma IGF-I concentrations tended ($P = 0.1$) to be greater in mares with high vs low BCS. The prolactin response to sulpiride injection on January 7 did not differ ($P > 0.1$) between groups. During 12 h of frequent blood sampling on January 12, LH concentrations were higher ($P < 0.0001$), whereas GH concentrations ($P < 0.0001$) and response to secretagogue (EP51389; $P < 0.03$) were lower in mares with high BCS. On January 19, the LH response to GnRH was higher ($P < 0.02$) in mares with high BCS; the prolactin response to TRH also was higher ($P < 0.01$) in mares with high BCS. In conclusion, nutrient restriction resulting in low BCS in mares resulted in a profound seasonal anovulatory period that was accompanied by lower leptin, IGF-I, and prolactin concentrations. All but one mare with high BCS continued to cycle throughout the winter or had significant follicular activity on the ovaries. Although leptin concentrations on average are very low in mares with low BCS and higher in well-fed mares, there is a wide variation in concentrations among well-fed mares, indicating that some other factor(s) may determine leptin concentrations under conditions of high BCS.

Key Words: Body Condition, Hormones, Leptin, Mares, Reproduction

 J. Anim. Sci. 2002. 80:2695–2703

## Introduction

Inadequate nutrition impairs reproductive function in farm animals (Rutter and Randel, 1984; Schillo, 1992), leading to delayed onset of puberty (Short and Bellows, 1971; Day et al., 1986), induction of anestrus (Richards et al., 1989; Rhodes et al., 1996), and prolonged postpartum anestrus (Wiltbank et al., 1964; Selk et al., 1988). A similar relationship between nutrition and reproductive efficiency has been noted for horses (Henneke et al., 1983, 1984; Hines et al., 1987). In cattle and sheep, dietary energy restriction suppresses episodic release of LH (Schillo, 1992). The relationship between nutrition and reproductive hormones is less understood in the mare (Hines et al., 1987).

Leptin is a hormone suggested to be a signal between body fat and the hypothalamus (Houseknecht et al., 1998). In other species, leptin concentrations vary directly with percentage of body fat (Prolo et al., 1998; Chilliard et al., 2000). Administration of leptin to female mice induces early puberty (Chehab et al., 1997),

[1]Approved for publication by the Director of the Louisiana Agric. Exp. Sta. as manuscript no. 02-11-0098. We thank A. F. Parlow and the NIDDKD, National Hormone and Pituitary Program, Harbor-UCLA Medical Center, Torrance, CA, for reagents.

[2]Correspondence: phone: 225-578-3445; fax: 225-578-3279; E-mail: dthompson@agctr.lsu.edu.

Received February 18, 2002.

Accepted June 25, 2002.

and in leptin-deficient ob/ob mice, which are infertile, leptin restores LH secretion and gonadal function (Barash et al., 1996). McManus and Fitzgerald (2000) reported that mares deprived of feed for 24 h exhibited a decrease in plasma leptin concentrations, but had no change in gonadotropin or prolactin secretion. Fitzgerald and McManus (2000) described a profound seasonal variation in leptin concentrations in mature mares that was barely discernable in young mares.

The present experiment was designed to test the hypothesis that nutritional restriction leading to low body condition would intensify the depth and/or length of the seasonal anovulatory period in mares. Specifically, the effects of body condition on follicular patterns, pituitary hormone secretion, ovarian progesterone secretion, and leptin concentrations were studied during the autumnal transition and winter anovulatory period in mares either maintained in good condition or fed restricted diets starting in September.

## Material and Methods

*Animals and Treatments.* Twenty-four light horse mares, 3 to 19 yr of age, with a mean BW of 496 kg and a mean body condition score (**BCS**) of 7 (6.5 to 8.0; Henneke et al., 1983) were separated into two groups of 12, such that breed type, age, and BW were evenly distributed across groups. One group was then randomly selected to be the high-BCS group and the other the low-BCS group. Beginning in September, mares in the high-BCS group were given (continued on) free choice access to pasture (dormant Alicia bermudagrass and winter ryegrass) and were supplemented with a good quality bermudagrass hay as needed. Mares in the low-BCS group were slowly limit-grazed for decreasing periods of time on equivalent pastures until they reached the desired BCS of 3.0 to 3.5, at which time they were grazing approximately 2 h/d. Good quality bermudagrass hay was supplemented as necessary. All mares had free access to water and a trace-mineralized salt block (Champions Choice, Cargill, Inc., Minneapolis, MN). Mares were housed at the Louisiana State University Agricultural Center, Idlewild Research Station in Clinton, LA, and were maintained on routine herd health and deworming regimens (Evans, 2001). During the experiment, mares in both groups were dewormed at least once with moxidectin dewormer (Quest Paste, Fort Dodge Animal Health, Overland Park, KS). Fecal egg counts were used to assess deworming requirements, and over the course of the experiment, mares in the low-BCS group had to be dewormed more frequently than those in the high-BCS group, likely due to copraphagy.

*Determination of BCS and Stage of Cyclicity.* Body weights and BCS were recorded weekly throughout the experiment. Body condition score was determined each time by the same technician by visual appraisal and palpation of the six areas suggested by Henneke et al. (1983). Once the mares approached their target BCS (December), ovarian examinations by transrectal ultrasonography (Aloka 550V with 5-MHz linear-array transducer, Aloka Science and Humanity, Wallingford, CT) and estrous detection with a stallion was initiated. Mares were checked for estrus and their ovaries assessed for ovarian size, follicle number and size, and number of corpora lutea every 72 h. Follicles were assigned to categories based on their size (small, 10 mm or less; medium, 11 to 19 mm; large, 20 mm or larger). When a mare achieved a follicle of at least 30 mm, her ovaries were examined daily until the follicle ovulated or regressed to below 20 mm.

*Blood Sampling and Challenges.* Beginning in September, weekly blood samples were collected at 0730 by jugular venipuncture into two 7-mL evacuated tubes (one containing potassium oxalate and sodium fluoride and one containing sodium heparin; Vacutainer, Becton and Dickinson, Franklin Lakes, NJ). These samples were centrifuged at $1{,}500 \times g$ at 4°C for 15 min and the plasma harvested and stored at −15°C. Concentrations of LH (Thompson et al., 1983a), FSH (Thompson et al., 1983b), GH (Thompson et al., 1992), prolactin (Colborn et al., 1991), IGF-I (Sticker et al., 1995b), insulin (DePew et al., 1994), TSH (Sticker et al., 2001), and leptin (McManus and Fitzgerald, 2000) were determined by RIA previously validated for horse tissues. Intra- and interassay coefficients of variation and assay sensitivities were 6%, 9%, and 0.2 ng/mL for LH; 7%, 11%, and 1.4 ng/mL for FSH; 8%, 11%, and 0.5 ng/mL for GH; 7%, 12%, and 0.2 ng/mL for prolactin; 5%, 12%, and 8 ng/mL for IGF-I; 5%, 8%, and 0.1 ng/mL for insulin; 5% and 0.02 ng/mL for TSH; and 4%, 8%, and 0.8 ng/mL for leptin. Glucose concentrations were determined colorimetrically using commercial kits (Sigma Tech. Bull. No. 315, Sigma Chemical, St. Louis, MO).

During January, several hormonal challenges and a period of frequent sampling were conducted to determine the effects of high vs low BCS on hormonal profiles and secretagogue responses. On these days, each mare was fitted with an indwelling jugular catheter and allowed a brief (about 1 h) period of rest. Blood sampling on a set schedule was then started, and test substances, when appropriate, were administered via the jugular catheter. During the sampling periods, mares did not eat but were provided water at regular intervals.

The first challenge was on January 7, when all mares were administered sulpiride (Sigma), a dopamine receptor antagonist and prolactin secretagogue. Blood samples were collected at −15, 0, 15, 30, 45, 60, 75, 90, 120, 150, 180, 210, and 240 min, relative to injection of sulpiride (0.1 mg/kg BW) in saline. Plasma was harvested for determination of prolactin concentrations.

On January 12, a period of frequent sampling was performed to assess basal patterns in plasma LH, FSH, prolactin, and GH concentrations. Blood samples were collected every 15 min for 12 h starting in the morning. Immediately following the last (12-h) blood sample, a GH secretagogue was administered to assess GH responsiveness. The secretagogue used was EP51389 (Deghenghi, 1997) at 10 μg/kg BW in saline, which previously has been used to induce GH secretion in horses (Bynum et al., 1998; Kennedy et al., 2002). Additional blood samples were drawn at 10, 20, 30, 40, 50, 60, 75, and 90 min after secretagogue injection for GH determination.

On January 19, mares were administered a combination of GnRH (1 μg/kg BW; Sigma) and TRH (4 μg/kg BW; Sigma) in saline to assess LH, FSH, TSH, and prolactin responsiveness to these secretagogues. Blood samples were collected at –10, 0, 10, 20, 30, 45, 60, 90, 120, 150, 180, 210, and 240 min relative to injection.

*Statistical Analyses.* Data were analyzed by the SAS GLM procedure (SAS Inst. Inc., Cary, NC). Follicle data in January were analyzed in a completely randomized ANOVA (Steele and Torrie, 1980). Hormonal and glucose data over time (weekly data and challenges) were analyzed in split-plot ANOVA (Gill and Hafs, 1971) for a completely randomized design. In these analyses, the main effect of treatment was tested with the mare within treatment as the error term; the time factor and its interaction with treatment were tested with residual error. Differences between groups in each time period in the treatment-time interactions were assessed by the LSD test (Steel and Torrie, 1980).

Analysis of leptin concentrations indicated a prerestriction difference ($P < 0.05$) between groups in September. Further examination of the individual mares on that date indicated there was a cluster of mares that had low (4 ng/mL or less; n = 15) leptin concentrations and others that had high (7 ng/mL or higher; n = 8) concentrations (one mare was missing the prerestriction blood sample). Due to chance, there was an uneven distribution of mares with low vs high leptin concentrations in the two groups. To account for this, separate analyses were performed with only the low-leptin and only the high-leptin mares, and means for all three analyses are presented. Two alternate approaches also were compared (use of the pre-experimental leptin concentration as a covariate and a natural log adjustment), and all approaches produced similar outcomes.

## Results

From September to the end of December, BW of the mares in the high-BCS group increased slightly (Figure 1a), whereas the BW of mares in the low-BCS group decreased ($P < 0.005$) by about 90 kg. By the end of December, all mares in the low group had BCS of 3 or 3.5, whereas mares in the high group ranged from 7.5 to 8.5 ($P < 0.001$; Figure 1b).

None of the mares with low BCS ovulated or formed functional luteal tissue (as indicated by ultrasound examination and progesterone concentrations) after 12 wk of restriction (Figure 2a). In spite of this, some of these mares did show strong signs of behavioral estrus, such as squatting, urinating, and eversion of the clitoris when exposed to the stallion. Seven of the mares with high BCS continued to ovulate and cycle normally

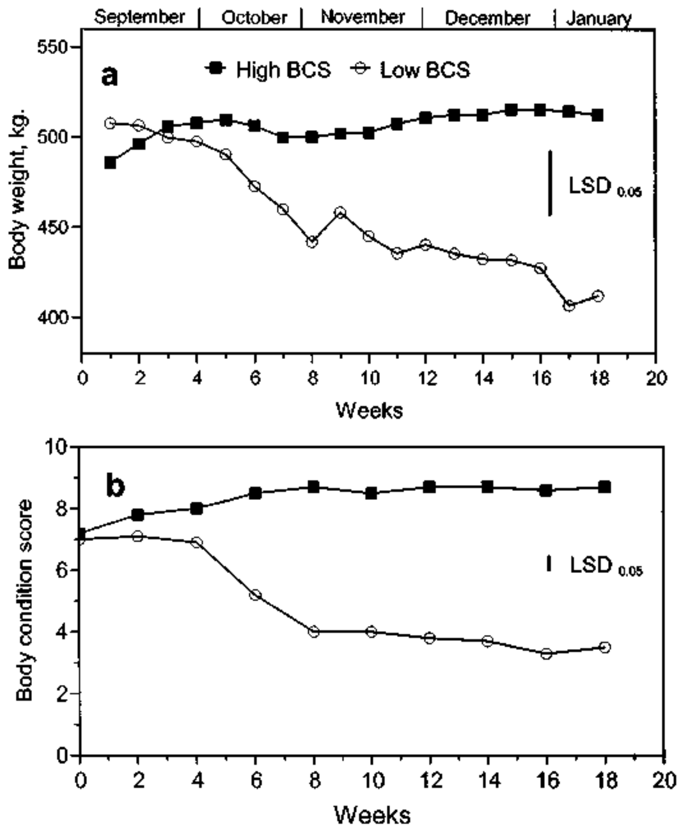


**Figure 1.** BCS = body condition score. Mean body weights (a) and body condition scores (b) in mares either full fed (High BCS) or feed-restricted to achieve low body condition (Low BCS) by December. Pooled SEM were 13.2 kg for body weights and 0.1 for body condition scores. The vertical lines in each graph indicate the LSD value ($P < 0.05$) for differences between groups for each time period.

throughout January. As a group, the mares in the high-BCS group had higher ($P = 0.034$) progesterone concentrations, a greater ($P = 0.006$) number of corpora lutea (7 vs 0; SEM = 0.04) and more large ($P = 0.008$) and medium ($P = 0.07$) follicles during January (Table 1) than did mares with low BCS. Individually, one mare with high BCS went quiescent (mid November until late spring), and four mares experienced a brief period with low progesterone concentrations (late December to mid February); however, they continued to have several large follicles on their ovaries at ultrasound examination.

Plasma concentrations of LH, FSH, TSH, GH, glucose, and insulin in weekly samples drawn from September through January were not affected ($P > 0.10$) by BCS (data not shown). Plasma prolactin concentrations (Figure 2b) were higher ($P < 0.02$) in mares of high BCS compared with mares in low BCS, however average concentrations in both groups decreased over time ($P < 0.008$). Plasma IGF-I concentrations also tended ($P = 0.10$) to be higher in mares with high BCS (Figure 2c).

There was an interaction ($P < 0.0001$) between treatment and week as revealed by the overall ANOVA for

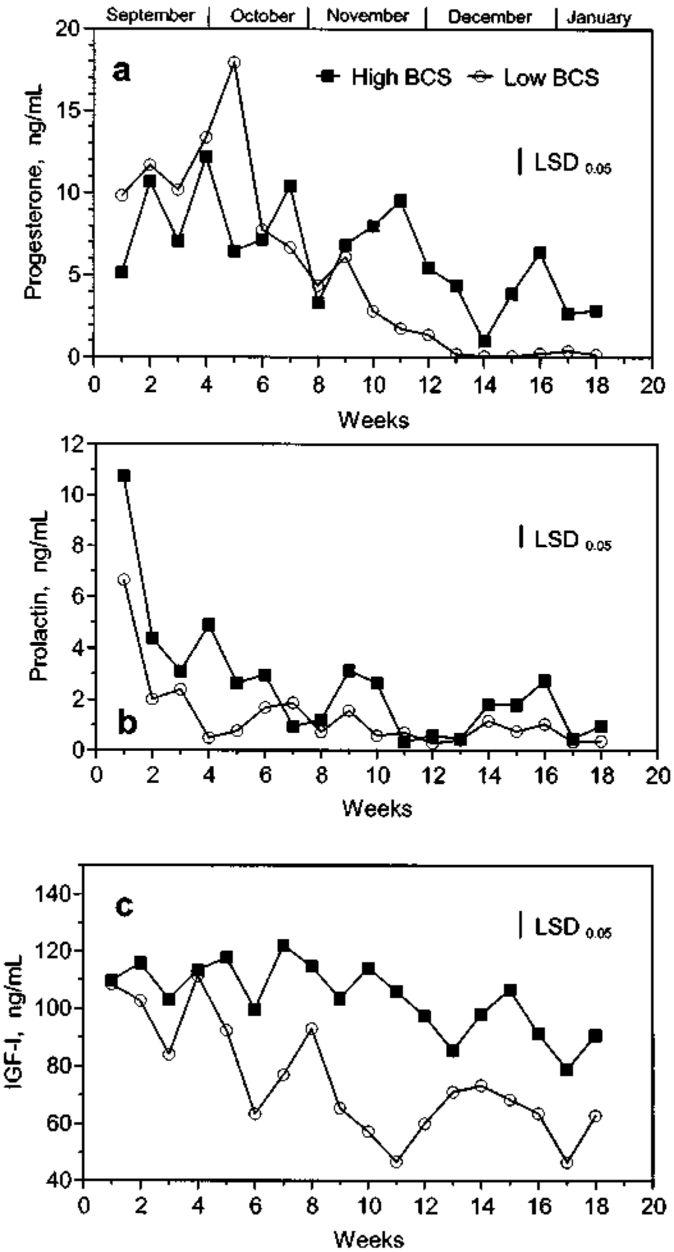


**Figure 2.** BCS = body condition score. Mean plasma progesterone (a), prolactin (b), and IGF-I (c) concentrations in mares either full fed (High BCS) or feed-restricted to achieve low body condition (Low BCS) by December. Pooled SEM were 1.7, 0.7, and 6.9 ng/mL for progesterone, prolactin, and IGF-I concentrations, respectively. The vertical lines in each graph indicate the LSD value ($P <$ 0.05) for differences between groups for each time period.

plasma leptin concentrations in all mares (Figure 3a) and in mares that had low (Figure 3b) or high (Figure 3c) leptin concentrations before onset of restriction in September. Leptin concentrations for both groups showed a downward trend from September to January. Regardless of pretreatment leptin status, the leptin concentrations in mares restricted to obtain low BCS dropped lower ($P$ < 0.01) than those fed to maintain high BCS.

**Table 1.** Mean daily number of large, medium, and small follicles in mares of high and low BCS during January[a]

| Follicles: | High BCS | Low BCS | SEM | $P$-value |
|---|---|---|---|---|
| Large, 20 mm and larger | 0.86 | 0.03 | 0.20 | 0.008 |
| Medium, 11 to 19 mm | 4.75 | 2.90 | 0.59 | 0.07 |
| Small, 10 mm or less | 9.19 | 7.86 | 0.69 | 0.23 |

[a]Averaged over three assessments made January 5, 11, and 17. BCS = body condition score.

The prolactin responses to sulpiride injection on January 7 are shown in Figure 4a. Prolactin concentration increased ($P$ < 0.0001) within 15 min in both groups, but there was no difference ($P$ > 0.1) in response between the mares with high vs low BCS. In contrast, the prolactin response to TRH administered on January 19 (Figure 4b) was greater ($P$ < 0.01) in mares with high BCS relative to those with low BCS.

During the 12-h period of frequent blood sampling on January 12, plasma LH (Figure 5a) concentrations were greater ($P$ < 0.0001) and GH concentrations (Figure 5b) were lower ($P$ < 0.0001) in mares with high BCS relative to those with low BCS. Plasma FSH and prolactin concentrations were not affected ($P$ > 0.10; data not shown). The GH response to EP51389 that immediately followed the 12-h period of frequent sampling (Figure 6) was also lower ($P$ < 0.03) in mares with high vs low BCS.

On January 19, the LH response to GnRH injection (Figure 7) was greater ($P$ < 0.02) in mares with high BCS relative to those with low BCS. The FSH response to GnRH and the TSH response to TRH on the same date were not affected ($P$ > 0.10) by BCS (data not shown).

## Discussion

The impact of low BCS on the occurrence of the seasonal anovulatory period in these mares was profound. All mares with low BCS had low progesterone concentrations, lacked significant follicular activity, and were anovulatory for 6 to 7 mo, based on ultrasound scans and progesterone analyses. In contrast, the majority of mares with high BCS remained reproductively active throughout the winter, and those with brief periods of anovulation, except for one, retained significant follicular activity on their ovaries. According to Nequin et al. (2000), a progressive decrease in mean luteal phase progesterone concentration is the first endocrine change observed when mares pass through transition into anestrus and that there are actually four phases that mares pass through during this time. It is interesting to note that none of the mares in this study exhibited distinct four phase periods. The low-BCS mares did not have aberrant ovulatory cycles during late autumn or a period of anovulatory follicular growth preceding total reproductive quiescence. Also, only one of the mares in

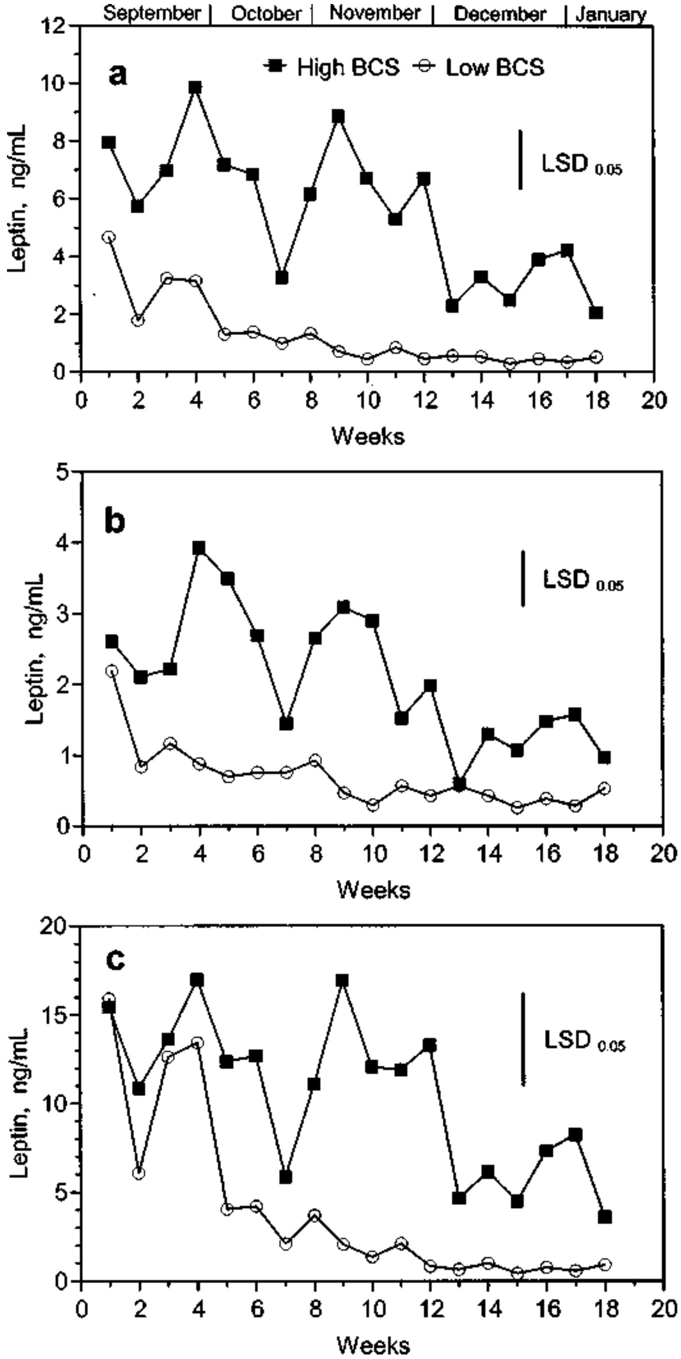


**Figure 3.** BCS = body condition score. Mean plasma leptin concentrations in all mares (a) and in mares that had low (b) or high (c) leptin concentrations before onset of restriction in September. Mares were either full fed (High BCS) or feed-restricted to achieve low body condition (Low BCS) by December. Number of mares in the high vs low BCS groups were 12 and 12 (a), 7 and 9 (b), and 5 and 2 (c), respectively; one mare was missing the prerestriction blood sample and could not be grouped in b or c. Pooled SEM were 0.71, 0.34, and 2.8 ng/mL for a, b, and c, respectively. The vertical lines in each graph indicate the LSD value ($P < 0.05$) for differences between groups for each time period.

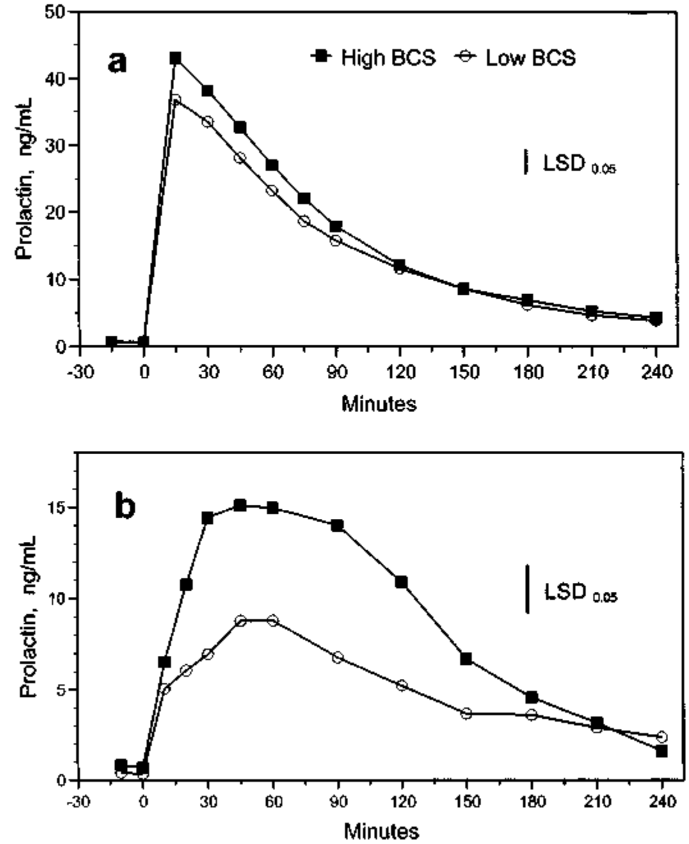


**Figure 4.** BCS = body condition score. Mean plasma prolactin concentrations in response to sulpiride injection (a) on January 5 and TRH injection (b) on January 19 in mares either full fed (High BCS) or feed-restricted to achieve low body condition (Low BCS) by December. Pooled SEM were 1.2 and 1.0 ng/mL for a and b, respectively. The vertical lines in each graph indicate the LSD value ($P < 0.05$) for differences between groups for each time period.

the high-BCS group went into a totally reproductive quiescent state. Given that the results presented herein were generated from mares on a rapidly declining plane of nutrition during the fall transitional period, whether they are directly applicable to mares sustained in low body condition throughout the breeding season and into the fall transition needs to be studied further.

Henneke et al. (1984) showed that breeding efficiency was enhanced in mares entering the breeding season or foaling at a BCS of 5.0 or greater. Those researchers also reported that initial excess stores of body fat enhance fertility. Also, nonfoaling mares with BCS >5 had average intervals from February 1 to first estrus and to first ovulation of 26 and 37 d, respectively; corresponding intervals for mares with BCS <5 were much longer (39 and 63 d, respectively). Ovarian activity in mares in the present experiment was actually followed with ultrasound, teasing, and blood sample collection for progesterone analysis until all mares went through vernal transition and ovulated or until the end of May.

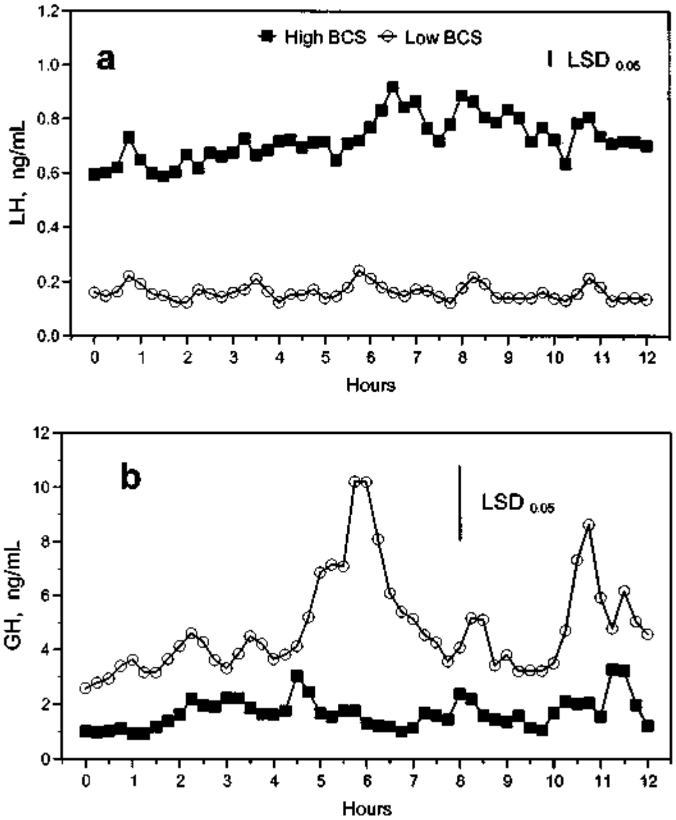


**Figure 5.** BCS = body condition score. Mean plasma LH (a) and GH (b) concentrations during a period of frequent sampling on January 12 in mares either full fed (High BCS) or feed-restricted to achieve low body condition (Low BCS) by December. Pooled SEM were 0.05 and 1.0 ng/mL for LH and GH concentrations, respectively. The vertical lines in each graph indicate the LSD value ($P < 0.05$) for differences between groups for each time period.

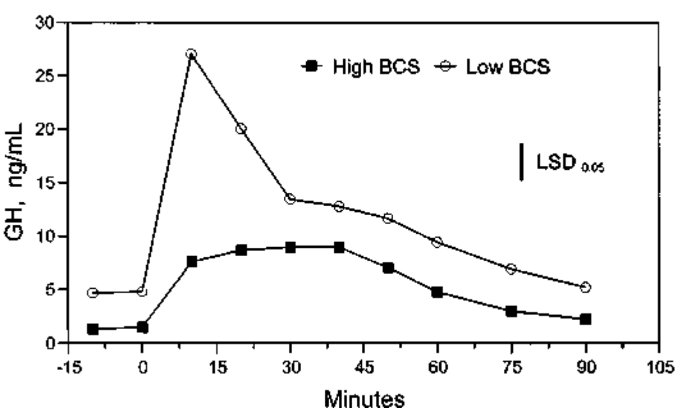


**Figure 6.** BCS = body condition score. Mean plasma GH concentrations in response to EP51389, a GH secretagogue, on January 19 in mares either full fed (High BCS) or feed-restricted to achieve low body condition (Low BCS) by December. Pooled SEM was 1.3 ng/mL. The vertical line indicates the LSD value ($P < 0.05$) for differences between groups for each time period.

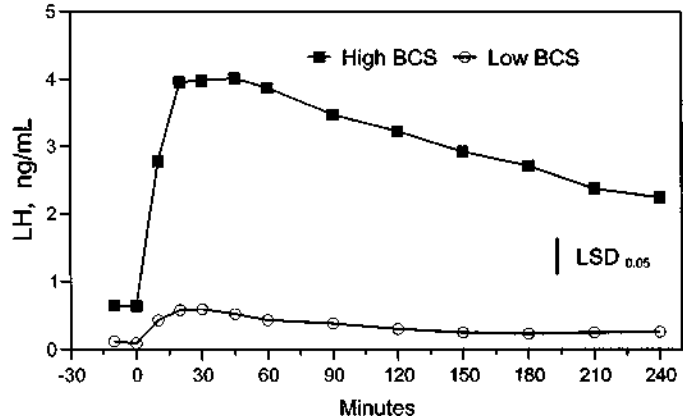


**Figure 7.** BCS = body condition score. Mean plasma LH in response to GnRH on January 19 in mares either full fed (High BCS) or feed-restricted to achieve low body condition (Low BCS) by December. Pooled SEM was 0.4 ng/mL. The vertical line indicates the LSD value ($P < 0.05$) for differences between groups for each time period.

The anovulatory period in the mares with low BCS extended from around mid-October to April or May, a period of 6 to 7 mo. In Henneke's study, whether mares were a BCS >5 or <5, ovulation still occurred by April 1. The time frame differences between the two experiments may be due to the fact that mares in the present experiment were at the two extremes of BCS from approximately late October. Although some mares in the experiment of Henneke et al. (1984) started the experimental period at a BCS of 3.0 to 3.5, they were fed to gain weight. Similar to these data and those of Henneke et al. (1984), Fitzgerald and McManus (2000) reported that young (2 to 5 yr of age) mares, with inherently less body fat than their mature (>10 yr of age) counterparts, entered the seasonal anestrous period earlier than the mature mares, and the mature mares were more likely to continue estrous cycles throughout the winter.

Leptin is an adipocyte-derived protein that has been shown to vary directly with body mass index and percentage body fat in other species (Prolo et al., 1998; Chilliard et al., 2000). Fitzgerald and McManus (2000) reported that their young mares with inherently less body fat also had lower leptin concentrations in summer and fall than did their older, fatter mares. Our results in the present experiment indicate that lower nutrient intake and hence lower BCS does indeed result in reduced leptin concentrations, regardless of the mare's age. Moreover, this was the case for mares with relatively high leptin concentrations in September (7 ng/mL or greater) as well as for those with low concentrations in September (4 ng/mL or less). Because leptin concentrations were not assessed until the experiment was over, it was not possible to detect the unequal distribution of mares with low vs high leptin in September, before nutrient restriction was started. As it turned out, only two of the 12 mares destined to be restricted had high leptin concentrations in September, whereas five of the mares not destined to be restricted had high leptin concentrations in September, even though all mares had BCS between 6.5 and 8.0 at that time. This may indicate that there is a genetic component to leptin production and secretion that determines relative levels (low vs high) under conditions of good body condition. Under conditions of poor body condition, leptin concentrations were consistently low, regardless of their initial level in September.

The decrease in leptin concentrations in mares of high BCS in the winter also was consistent, even though BCS and BW were relatively constant throughout the experiment. January concentrations averaged about 50% of those in September. Fitzgerald and McManus (2000) also reported a profound seasonal variation in leptin concentrations in mature mares, with average concentrations increasing from July through September and then decreasing precipitously from October to December. In contrast, leptin concentrations in young mares did not increase during the summer, and dropped only slightly in the winter. Examination of the individuals in the present experiment and their leptin data indicated that age did not account for the differences in pretreatment leptin concentrations among mares in September.

Although the mares with low BCS were reproductively inactive from the ovarian standpoint, three of them did show a behavioral (paradoxical) estrus when exposed to the stallion. These mares showed all the outward signs of estrus (squatting, urinating, clitoral eversion); however, upon examination of the ovaries and analysis of blood for progesterone, it was determined that they were reproductively inactive. Ginther (1992) described similar estrous displays in seasonally inactive mares, and suggested that the term "seasonally anovulatory" be used rather than seasonally anestrous for that reason. Likewise, Nequin et al. (2000) observed estrous behavior during periods of low progesterone and very low estrogen concentrations. Therefore, although estrous detection with a stallion is helpful for determining estrus in cyclic mares, behavioral displays of estrus are possible in the absence of significant follicular activity.

In the present study, no differences were detected in weekly plasma samples for LH, FSH, GH, TSH, glucose, or insulin concentrations. Plasma prolactin, however, was greater in mares with high BCS and decreased over time from September to January. This is in agreement with studies that have shown prolactin to be lower during the winter months (Thompson et al., 1986a,b; Johnson, 1987). DePew et al. (1994) reported that plasma prolactin increased relative to feeding in both mares and stallions, which may explain why concentrations were greater in mares with high BCS. Similarly, Bryant et al. (1970) reported that plasma concentrations of prolactin were higher after feeding and were lower during feed restriction in goats; McAtee and Trenkle (1971) reported similar results in cattle. In contrast, McManus and Fitzgerald (2000) reported that circulating concentrations of prolactin were no different in feed-restricted mares than in controls; however, the restricted period was short term (24 h).

Although no differences were detected in GH concentrations, plasma IGF-I concentrations tended to be higher in mares of high vs low BCS. Leptin may be a hormonal signal indicating body fat stores; however, IGF-I seems to be more responsive to short-term changes in nutrition, and decreases within 48 h of feed restriction (Sticker et al., 1995a). As in cattle (Granger et al., 1989), the decrease in plasma IGF-I concentrations with long-term nutrient restriction is accompanied by an increase in GH characteristics in horses (Sticker et al., 1995a,b), although the GH changes in horses tend to be minor relative to other species. In a report by Breier (1999), decreased nutrition led to elevated GH secretion but reduced hepatic GH receptor number and plasma concentrations of IGF-I. The increased GH concentrations in the 12-h period of frequent sampling and the increased GH response to secretagogue in the mares with low BCS in the present experiment are consistent with those previous reports, and may be indicative of nutritional stress induced by low BCS.

In the present experiment, prolactin was secreted in both groups in response to sulpiride on January 7 and TRH on January 19; however, effects of BCS (lower response in mares with low BCS) were only evident after TRH injection. In general, the magnitude of the secretagogue-induced secretion is proportional to pituitary stores, which usually reflect pituitary synthetic activity. We have reported seasonal differences in pituitary prolactin content (Thompson et al., 1986a) and responsiveness to TRH (Thompson et al., 1986b) in vivo, as others have in vitro (Evans et al., 1991), and like plasma concentrations, both are lowest in the winter. The greater prolactin response to TRH in mares with high BCS is consistent with their higher average prolactin concentrations in weekly plasma samples, and together they may indicate a greater prolactin production and/or storage rate in these mares. It is not known whether the elevated prolactin concentrations contributed to the more active reproductive status in mares with high BCS, but previous research in our laboratory (Thompson et al., 1997) indicated that daily injection of 4 mg of recombinant porcine prolactin into seasonally anovulatory pony mares resulted in ovulation and/or luteal formation within 28 d. In contrast, Fitzgerald and Davison (1997) reported that prolactin concentrations did not differ between mares that underwent seasonal anestrus and mares that continued to cycle during the winter.

Relative to mares of low BCS, mares with high BCS secreted more LH in response to GnRH on January 19. The greater LH response was indicative of cyclic vs seasonally anovulatory mares (Hines et al., 1991; Ginther, 1992), which was consistent with the mares with high BCS continuing to grow follicles and ovulate. Several investigators have shown that both LH and

FSH (Evans and Irvine, 1976; Alexander and Irvine, 1986; Johnson, 1987) are released from the pituitary when seasonally anestrous mares are given exogenous GnRH; however, those studies used mares of average body condition and the GnRH was given over several week's time. The mares with high BCS in the present experiment also had higher LH concentrations during the 12-h sampling period on January 12 compared with mares with low BCS, which was likely due to those in the follicular phase when LH concentrations are elevated (Ginther, 1992).

In conclusion, nutrient restriction resulting in low BCS in mares resulted in a profound seasonal anovulatory period that was accompanied by lower leptin, IGF-I, and prolactin concentrations. All but one mare with high BCS continued to ovulate throughout the winter or had significant follicular activity on the ovaries. Although leptin concentrations on average are very low in mares with low BCS and higher in well-fed mares, there is a wide variation in concentrations among well-fed mares, indicating that some other factor(s) may determine leptin concentrations under conditions of high BCS.

## Implications

The present experiment further demonstrates the relationship between the level of nutrition and reproductive status of the mare, particularly for mares in low body condition. Mares of low body condition consistently experienced a longer and deeper anestrus than those of good body condition. It is possible that leptin may be an important link in this relationship between nutrition and reproductive status in the horse as has been suggested for other species. However, based on the variation among mares, other factors may be involved in determining whether a mare in good body condition has high or low plasma leptin concentrations.

## Literature Cited


Alexander, S. L., and C. H. G. Irvine. 1986. Effect of graded doses of gonadotropin-releasing hormone on serum LH concentrations in mares in various reproductive states: Comparison with endogenously generated LH pulses. J. Endocrinol. 110:19–26.

Barash, I. A., C. C. Cheung, D. S. Weigle, H. Ren, E. B. Kabigting, J. L. Kuijper, D. K. Clifton, and R. A. Steiner. 1996. Leptin is a metabolic signal to the reproductive system. Endocrinology 137:3144–3147.

Breier, B. H. 1999. Regulation of protein and energy metabolism by the somatotropic axis. Domest. Anim. Endocrinol. 12:209–218.

Bryant, G. D., J. L. Linzell, and F. C. Greenwood. 1970. Plasma prolactin in goats measured by radioimmunoassay: The effects of teat stimulation, mating behavior, stress, fasting and of oxytocin, insulin and glucose injections. Hormones (Basel) 1:26–35.

Bynum, R. J., D. L. Thompson, Jr., L. R. Gentry, S. R. Morris, P. J. Burns, and R. Deghenghi. 1998. Physiologic responses to a novel growth hormone-releasing tripeptide (EP51389) in yearling horses. J. Anim. Sci. 76(Suppl. 1):119. (Abstr.)

Chehab, F. F., K. Mounzih, R. Lu, and M. E. Lim. 1997. Early onset of reproductive function in normal female mice treated with leptin. Science (Wash., DC) 275:88–90.

Chilliard, Y., A. Ferlay, Y. Faulconnier, M. Bonnet, J. Rouel, and F. Bocquier. 2000. Adipose tissue metabolism and its role in adaptations to undernutrition in ruminants. Proc. Nutr. Soc. 59:127–134.

Colborn, D. R., D. L. Thompson, Jr., T.L. Roth, J. S. Capehart, and K. L. White. 1991. Responses of cortisol and prolactin to sexual excitement and stress in stallions and geldings. J. Anim. Sci. 69:2556–2562.

Day, M. L., K. Imakawa, D. D. Zalesky, R. J. Kittok, and J. E. Kinder. 1986. Effects of restriction of dietary energy intake during the prepubertal period on secretion of luteinizing hormone and responsiveness of the pituitary to luteinizing hormone-releasing hormone in heifers. J. Anim. Sci. 62:1641–1646.

Deghenghi, R. 1997. The development of 'impervious' peptides as growth hormone secretagogues. Acta Pediatr. (Suppl.) 423:85–87.

DePew, C. L., D. L. Thompson, Jr., J. M. Fernandez, L. S. Sticker, and D. W. Burleigh. 1994. Changes in concentrations of hormones, metabolites, and amino acids in plasma of adult horses relative to overnight feed deprivation followed by a pelleted-hay meal at noon. J. Anim. Sci. 72:1530–1539.

Evans, J. W. 2001. Horses. W. H. Freeman and Co., New York.

Evans, M. J., S. L. Alexander, C. H. G. Irvine, J. H. Livesey, and R. A. Donald. 1991. In vitro and in vivo studies of equine prolactin secretion throughout the year. J. Reprod. Fertil. (Suppl.) 44:27–35.

Evans, M. J., and C. H. G. Irvine. 1976. Measurement of equine follicle-stimulating hormone and luteinizing hormone: Response of anestrous mares to gonadotropin releasing hormone. Biol. Reprod. 15:477–484.

Fitzgerald, B. P., and L. A. Davison. 1997. Comparison of the effects of *N*-methyl-DL-aspartic acid on gonadotropin and prolactin secretion in anestrous mares and mares exhibiting estrous cycles during anestrus. Biol. Reprod. 57:36–42

Fitzgerald, B. P., and C. J. McManus. 2000. Photoperiodic versus metabolic signals as determinants of seasonal anestrus in the mare. Biol. Reprod. 63:335–340.

Gill, J. L., and H. D. Hafs. 1971. Analysis of repeated measurements of animals. J. Anim. Sci. 33:331–336.

Ginther, O. J. 1992. Reproductive Biology of the Mare: Basic and Applied Aspects. Equiservices, Cross Plains, WI.

Granger, A. L., W. E. Wyatt, W. M. Craig, D. L. Thompson, Jr., and F. G. Hembry. 1989. Effects of breed and wintering diet on puberty and plasma concentrations of growth hormone and insulin-like growth factor 1 in heifers. Domest. Anim. Endocrinol. 6:253–262.

Henneke, D. R., G. D. Potter, and J. L. Kreider. 1984. Body condition during pregnancy and lactation and reproductive efficiency of mares. Theriogenology 21:897–909.

Henneke, D. R., G. D. Potter, J. L. Krieder, and B. F. Yeates. 1983. Relationship between body condition score, physical measurements and body fat percentage in mares. Equine Vet. J. 15:371–372.

Hines, K. K., K. J. Affleck, S. P. Barrows, W. L. Murdoch, B. P. Fitzgerald, and R. G. Loy. 1991. Follicle-stimulating hormone pulse amplitude decreases with the onset of the breeding season in the mare. Biol. Reprod. 44:516–521.

Hines, K. K., S. L. Hodge, J. L. Kreider, G. D. Potter, and P. G. Harms. 1987. Relationship between body condition and levels of serum luteinizing hormone in postpartum mares. Theriogenology 28:815–825.

Houseknecht, K. L., C. A. Baile, R. L. Matteri, and M. E. Spurlock. 1998. The biology of leptin: A review. J. Anim. Sci. 76:1405–1420.

Johnson, A. L. 1987. Seasonal and photoperiod-induced changes in serum prolactin and pituitary responsiveness to thyrotropin-releasing hormone in the mare. Proc. Soc. Exp. Biol. Med. 184:118–122.

Kennedy, S. R., D. L. Thompson, Jr., H. E. Pruett, P. J. Burns, and R. Deghenghi. 2002. Growth hormone response to a novel GH releasing tripeptide in horses: Interaction with GnRH, TRH, and sulpiride. J. Anim. Sci. 80:744–750.

McAtee, J. W., and A. Trenkle. 1971. Effects of feeding, fasting, glucose or arginine on plasma prolactin levels in the bovine. Endocrinology 89:730–734.

McManus, C. J., and B. P. Fitzgerald. 2000. Effects of a single day of feed restriction on changes in serum leptin, gonadotropins, prolactin and metabolites in aged and young mares. Domest. Anim. Endocrinol. 19:1–13.

Nequin, L. G., S. S. King, J. F. Roser, B. L. Soderstrom, E. M. Carnevale, and K. R. Neumann. 2000. Uncoupling of the equine reproductive axes during transition into anoestrus. J. Reprod. Fert. (Suppl.) 56:153–161.

Prolo, P., M. L. Wong, and J. Licinio. 1998. Leptin. Int. J. Biochem. Cell Biol. 30:1285–1290.

Rhodes, F. M., K. W. Entwistle, and J. E. Kinder. 1996. Changes in ovarian function and gonadotropin secretion preceding the onset of nutritionally induced anestrus in *Bos indicus* heifers. Biol. Reprod. 55:1437–1443.

Richards, M. W., R. P. Wetteman, and H. M. Schoenemann. 1989. Nutritional anestrus in beef cows: Body weight change, body condition, luteinizing hormone in serum and ovarian activity. J. Anim. Sci. 67:1520–1526.

Rutter, L. M., and R. D. Randel. 1984. Postpartum nutrient intake and body condition: effect on pituitary function and onset of estrus in beef cattle. J. Anim. Sci. 58:265–274.

Schillo, K. K. 1992. Effects of dietary energy on control of luteinizing hormone secretion in cattle and sheep. J. Anim. Sci. 70:1271–1282.

Selk, G. E., R. P. Wetteman, K. S. Lusby, J. W. Oltjen, S. L. Mobley, R. J. Rasby, and J. C. Garmendia. 1988. Relationships among weight change, body condition and reproductive performance of range beef cows. J. Anim. Sci. 66:3153–3159.

Short, R. E., and R. A. Bellows. 1971. Relationships among weight gains, age at puberty and reproductive performance in heifers. J. Anim. Sci. 32:127–131.

Steele, R. G. D., and J. H. Torrie. 1980. Principles and procedures of statistics: A biometrical approach. 2nd Ed. McGraw-Hill Publishing Co., New York.

Sticker, L. S., D. L. Thompson, Jr., L. D. Bunting, J. M. Fernandez, C. L. DePew, and M. R. Nadal. 1995a. Feed deprivation of mares: Plasma metabolite and hormonal concentrations and responses to exercise. J. Anim. Sci. 73:3696–3704.

Sticker, L. S., D. L. Thompson, Jr., J. M. Fernandez, L. D. Bunting, and C. L. DePew. 1995b. Dietary protein and(or) energy restriction in mares: Plasma growth hormone, IGF-I, prolactin, cortisol, and thyroid hormone responses to feeding, glucose, and epinephrine. J. Anim. Sci. 73:1424–1432.

Sticker, L. S., D. L. Thompson, Jr., and L. R. Gentry. 2001. Pituitary hormone and insulin responses to infusion of amino acids and N-methyl-D, L-aspartate in horses. J. Anim. Sci. 79:735–744.

Thompson, D. L., Jr., R. Hoffman, and C. L. DePew. 1997. Prolactin administration to seasonally anestrous mares: Reproductive, metabolic, and hair-shedding responses. J. Anim. Sci. 75:1092–1099.

Thompson, D. L., Jr., R. A. Godke, and T. M. Nett. 1983a. Effects of melatonin and thyrotropin releasing hormone on mares during the nonbreeding season. J. Anim. Sci. 56:668–677.

Thompson, D. L., Jr., L. Johnson, R. L. St. George, and F. Garza, Jr. 1986a. Concentrations of prolactin, luteinizing hormone and follicle stimulating hormone in pituitary and serum of horses: Effect of sex, season and reproductive state. J. Anim. Sci. 63:854–860.

Thompson, D. L., Jr., M. S. Rahmanian, C. L. DePew, D. W. Burleigh, C. J. DeSouza, and D. R. Colborn. 1992. Growth hormone in mares and stallions: Pulsatile secretion, response to growth hormone-releasing hormone, and effects of exercise, sexual stimulation, and pharmacological agents. J. Anim. Sci. 70:1201–1207.

Thompson, D. L., Jr., S. I. Reville, M. P. Walker, D. J. Derrick, and H. Papkoff. 1983b. Testosterone administration to mares during estrus: Duration of estrus and diestrus and concentrations of LH and FSH in plasma. J. Anim. Sci. 56:911–918.

Thompson, D. L., Jr., J. J. Wiest, and T. M. Nett. 1986b. Measurement of equine prolactin with an equine-canine radioimmunoassay: Seasonal effects on the prolactin response to thyrotropin releasing hormone. Domest. Anim. Endocrinol. 3:247–252.

Wiltbank, J. N., W. W. Rowden, J. E. Ingalls, and D. R. Zimmerman. 1964. Influence of post-partum energy level on reproductive performance of Hereford cows restricted in energy intake prior to calving. J. Anim. Sci. 23:1049–1053.