Environmental factors, pasture composition, growth rate and puberty in Growing Thoroughbreds

By

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ABSTRACT

A rapid growth phase often occurs with the onset of spring in the young horse. This coincides with changes in day length, temperature, and progesterone concentrations. The change in growth, from slow to rapid in young horses has been associated with various forms of developmental orthopedic disease. The objective of this study was to distinguish associations between progesterone concentrations and other physiological and environmental measures from birth through 16 mo in young Thoroughbreds. Growth data and plasma samples were collected monthly from 3 annual crops of 20 foals. Plasma progesterone (P4) and insulin like growth factor one (IGF-I) concentrations were measured with previously validated radio immunoassay's (RIA). Progesterone concentrations were compared with day length, IGF-I and ADG using Spearman correlations. Concentrations of progesterone at birth (2.3 ± 0.4 ng/mL) decreased within the first week of life to basal values (0.11 ± 0.01 ng/mL) in colts and fillies. Progesterone in the geldings remained at baseline concentrations at all sample times. An abrupt increase in progesterone concentration was detected in fillies at a mean age of 385 ± 6.4 d, weight 381 ± 7.2 kg, and ADG 0.63 ± 0.04 kg/d. Elevations in progesterone concentrations coincided with a measured day length of 13 ± 0.1 hrs, and temperature of 15 ± 1.7 °C. Positive associations were established between progesterone concentration day length ($r = 0.59$; $P<0.0001$), IGF-I ($r = 0.25$; $P<0.01$) and ADG ($r = 0.34$; $P<0.0001$). Day length IGF-I and ADG began

to increase for both geldings and fillies at approximately 340 d of age, while progesterone started to increase at 385 ± 6.4 d for the fillies only. From this it could be hypothesized that an increase in ADG combined with optimal environmental conditions, may be associated with the subsequent elevation in progesterone concentrations in fillies. The relationship between IGF-I, and various reproductive hormones has been studied in the adult horse, yet the associations between environmental factors, ADG, and progesterone concentrations demonstrated in growing yearlings further emphasizes the extensive changes occurring during this crucial developmental stage.

Key Words: Progesterone, Growth, Yearlings, Environment

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Introduction

A horse's conformation and performance reflects genetic potential and environmental opportunity. The latter may act directly on the horse or via the pasture. About 80% of horses in Virginia receive all or part of their nutrition from pasture (USDA).

Pasture composition is highly variable. It is affected by plant species, soil fertility, climate, stage of growth and management practices (Minson, 1990). In the present study, attention focuses on 3 climate variables—day length (DL, h, mean for mo of daily sunrise to sunset), day temperature (DT, ^{o}C, mean for mo of daily means of 24 hourly measurements), and rainfall (RN, cm/mo, mean of daily measurements). Pasture composition was determined by proximate and mineral analysis, and emphasis is given to crude protein (CP, g/kg dry matter [DM]), because it is a major determinant of growth.

Growth rate is a common measure of energy intake and the nutrient balance of a ration. It also reflects genetic programming and, probably, some direct environmental variables. Changes in day length, for example, profoundly affect the onset of estrus in mares and puberty in fillies (Wesson and Ginther, 1982).

The nutritional status of an animal exerts a strong influence on growth and reproduction via the somatotropic axis, a set of hormones including insulin, growth hormone and insulin-like growth factor-1 (IGF-1). Growth rate (kg/d, average daily gain over a mo) is highly correlated with plasma IGF-1 concentration in young growing horses (Staniar, 2001). Growth rate also relates to puberty in cattle and other species. Puberty is detected by a sudden increase in plasma progesterone concentration (P4, McKinnon and Voss, 1992)

This thesis aims to seek relationships between changes in DL, DT, RN, pasture CP and possibly other nutrients, plasma [IGF-1], ADG and plasma [P4]. Associations will be tested for causality in terms of temporal sequences and plausible physiological mechanisms, with a view to integrating the measured variables into a single system leading to puberty.

Review of Literature

Photoperiod, temperature and nutrition are three well-studied environmental cues that influence reproduction in mammals. From a practical standpoint, nutrition commands the greatest attention because livestock producers can alter nutrition more easily than photoperiod and or temperature (Dunn and Moss, 1992). Puberty in the female mammal results from an interaction of a series of genetic and environmental changes. In the horse it is influenced by changes in endocrine profiles, age, weight, body composition, energy intake and season (Wesson and Ginther, 1982).

Virginia horse statistics

According to the 2001 Virginia Equine Report there are 170,000 horses housed on 29,000 farms throughout Virginia. Just under half of these horses are housed in Northern Virginia. The value of these horses was estimated to be $1.46 billion, the average value of a Thoroughbred was said to be $21,430. Breeding accounts for 19% of all equine usage in Virginia (VASS, 2002). About 80% of the horses in Virginia receive all or part of their nutrition from pasture (USDA, 1998).

Pasture

Pasture composition is highly variable. It is affected by plant species, soil fertility, climate, stage of growth and management practices (Minson, 1990).

Soil fertility

Applying nitrogen to the soil increases, yield, protein, and water content of the forage and reduces the proportion of leaf. The higher crude protein in nitrogen fertilized forage is offset by a reduction in the level of soluble carbohydrate (Alberda, 1965).

Climate

High temperatures generally increase the concentration of fiber in forages (Brown, 1939). It has been suggested that high rate of transpiration is a factor contributing to the low digestibility of forages grown at high temperatures (Minson and McLeod, 1970). This could be due to the development of a larger vascular system to convey the greater quantities of water passing through the plant. French (1961) suggested that high light intensity may be responsible for the poor quality of tropical forages.

Stage of growth

As grasses grow there is a reduction in the proportion of the leaf lamina and an increase in leaf sheath, stem, and inflorescence and a subsequent decline in digestibility. Growth of forage in the spring is often very rapid and digestibility remains constant for several weeks. The digestibility remains high until the flower heads start to emerge, at this point the dry matter (DM) yield reaches about one third of the maximum (Taji, 1967; Mowat et al. 1965). When there is a decrease in leaf lamina there is also a decrease in crude protein (CP) concentration and an increase in the concentration of cellulose, hemicellulose and lignin (Jarrige and Minson, 1964).

Management practices

Temperate grasses tend to have higher digestibility than tropical grasses, so where possible they should be sown with a preference over tropical grasses. Digestibility declines as forage matures, the most effective way of avoiding low digestibility is by maintaining forage in a young vegetative stage of growth by regular cutting or grazing, this is particularly important in spring.

There are many ways available ways to improve the quality of forage-based diets. Identification of the optimum forage strategies for use on an individual property or in a region requires knowledge of the different nutrients required for production (growth or reproduction), the ability of the forage to supply these nutrients, how to identify which aspect of the forage quality is failing, and ways this deficiency may be prevented (Minson, 1990).

Growth rate

Rapidly growing animals tend to reach puberty earlier than slow growing animals, as demonstrated in cattle (Short and Bellows, 1971) and sheep (Dyrmundsson, 1981). Brody (1945) implies that the onset of puberty in all species occurs at the point of inflexion on the growth curve. Studies in sheep have shown that even when lambs are born in the same season, there can be great difference in time of first ovulation due to growth rate. A group of spring born lambs was fed at varying levels to experimentally alter growth rate. The lambs fed well from birth reached puberty normally, other females born at the same time failed to initiate puberty at the normal age when growth was retarded via restricting level of nutrition from weaning. When such lambs were induced to grow rapidly during the breeding season, in response to unlimited quantities of food, ovulations began within a few weeks (Foster et al., 1986)

The yak, similar to the horse is seasonally polyestrous; the maturation rate however is much slower. Zhang (1989) reported yak heifers show their first signs of sexual behavior a t 22-34mo. A more recent study in yaks that more accurately measured puberty using plasma progesterone profiles showed puberty to occur between 24 and 60 mo. The majority were concentrated between 31 and 48mo, the author suggested this late onset in reaching puberty was likely due to the harsh climate (mean yr temperature 1.2˚C), the small BW at birth and slow growth rate during the prepubertal period (Yu and Li, 2001).

Growth rates in supplemented heifers were higher than in non supplemented heifers. Supplementary feeding during the dry season decreased age and increased ovarian volume at puberty (Tegegne et al., 1992).

Weight

Body weight at menarche in varied less than age at menarche in girls. These observations lead to the idea that attainment of a critical body weight triggers endocrine events that induce onset of puberty (Frisch and Revelle, 1970). A study in rats suggested that a critical body weight or composition exists for puberty and that this reflects attainment of a critical metabolic rate. This metabolic rate however has been postulated to vary genetically between animals, thus accounting for the variation in body weight at attainment of puberty (Kennedy and Mitra, 1963).

Lambs born early in the year (winter) usually attain an appropriate body size sometime during the 5 or 6 mo in the non-breeding season, but the onset of estrus is delayed until the beginning of the breeding season. Lambs born late in the year (fall) may not attain the minimum size for adulthood during the confines of the first breeding season, and, therefore, the first estrus is normally delayed until the next year when they are older and larger (Quirke, 1979).

Photoperiod

Seasonal breeding in animals has been adapted to ensure that their young are born in favorable environmental conditions. In the horse as in many other species, the cyclical nature of reproduction is cued primarily by photoperiod changes. These changes in daylight hours are translated to an endocrine signal in the pineal gland which in turn secretes melatonin during the phase of darkness (Nagy et al, 2000).

The seasonal factor responsible for modifying the time of puberty in the female lamb is day length

In the seasonally anovulatory mare, fixed, long daily photoperiods (16-24h light) are stimulatory (Kooistra & Ginther, 1979), while fixed short daily photoperiods (9h light) apparently are inhibitory (Ginther, 1979) to the onset of reproductive activity. A study by Wesson and Ginther (1982) indicated that a fixed 16h photoperiod was inhibitory to fillies and delayed the onset of puberty. The authors hypothesized from these results that a gradual increase in the length of photoperiod, such as naturally occurs is necessary for the establishment of sensitivity to photoperiod in fillies, once this occurs, a prolonged, fixed photoperiod would serve as an adequate stimulus as it does in the adult mare.

Photoperiod however is not the only factor affecting reproductive activity, removal of the pineal gland or the ganglion cervicale superior does not result in a cessation of reproductive activity, however this activity can no longer be influenced by changes in photoperiod. In well nourished, rapidly growing ewes the timing of puberty is primarily determined by photoperiod, however if the sheep is undernourished it is able to monitor day length yet unable to respond to stimulatory changes in photoperiod (Suttie et al., 1991).

Nutrition

Dietary nutrients promote the programming and expression of the metabolic pathways that enable animals to achieve their genetic potential for reproduction (Robinson, 1996). Steiner (1987) suggests that weight and fatness are consequences or correlates of metabolic changes occurring before and around the timing of puberty. According to this idea, blood born substances signal the brain about metabolic readiness for puberty, these blood born signals may be metabolites, hormones or a combination (Steiner, 1987). Different factors including body fat, insulin, non-esterified fatty acids, amino acids and availability of metabolic fuel such as glucose, may interact to regulate secretion of hormones that modulate age at puberty (Schillo, 1992).

In heifers, increased nutrient intake after an extended period of restricted nutrient intake, hastens the onset of puberty (Yelich et al., 1996). Upon examination of the influence of dietary intake on the development of dominant ovarian follicles in prepubertal heifers it was found that heifers in both groups developed ovulatory follicles of similar size, however the pubertal ovulation was a mean of 63d later in heifers on the lower energy diet compared with those fed greater amounts of dietary energy (Bergfeld et al., 1994).

A severe feed restriction will delay puberty because all somatic growth will be slowed and, as the reproductive system has a low priority for feed energy and protein puberty must be delayed (Kirkwood et al., 1987). Moderate feed restriction (77% of ad lib. Intake) from 28 to 60 kg BW delayed the onset of puberty in gilts, however restriction between 60 kg BW and puberty had no effect (Etienne et al., 1983) .

The authors suggested there may be a critical period of development in which moderate feed restriction will delay puberty, and beyond which only very severe feed restriction will delay puberty. A study in pony mares that restricted intake and reduced fat thickness from 20mm to 5mm showed the mares to be so deep in anestrus that photostimulation treatment failed to advance the first ovulation (Guillaume et al., 2002).

Nutritional status influences pulsatile release of LH in developing heifers. Heifers maintained on a low energy diet failed to exhibit an increase in LH pulse frequency at a time when heifers fed a diet adequate for growth exhibited increased LH pulse frequencies and attained puberty (Day et al., 1986). This was again observed when heifers fed restricted levels of dietary energy had suppressed frequency and amplitude of LH pulses as compared to heifers fed a diet sufficient in energy (Short et al., 1989).

Restriction of dietary energy prevented the prepubertal increase in LH pulse frequency in ovary intact, ovariectomized and estradiol treated heifers. Increased energy intake resulted in an increase in LH pulse frequency within 14 d regardless of endocrine status (Kurz et al., 1990). The author suggested that one of the ways dietary energy restriction delays the onset of puberty is by delaying the prepubertal rise in LH pulse frequency.

Lambs raised on low nutrition did not enter estrus or ovulate in the first breeding season even though an appropriate age had been achieved, this was suggested to have been because growth was retarded (Foster et al., 1991).

In well nourished, thus well growing sheep photoperiod is the primary determinant for the onset of puberty, however if undernourished, lambs are able to monitor photoperiod but are unable to respond to it. When lambs where fed ad-libitum they reached puberty earlier and at higher body weights than those severely restricted which did not enter puberty. Those moderately restricted attained puberty at a delayed rate than those unrestricted, it was suggested this was because internal cues indicated that physiological development was insufficient when photoperiod first became stimulatory (Suttie et al., 1991).

IGF-I

It has been observed in sheep that systemic IGF-I levels increase during puberty (Lackey et al. 1999). Mice carrying null mutations in the IGF-I gene are born small and fail to grow after birth (Powell-Braxton et al., 1993). Plasma IGF-I concentrations in humans usually correlate to body size, constitutional tall children have elevated plasma IGF-I levels (Gourmelen et al., 1984). Infusions of recombinant IGF-I also enhances body weight and size in a number of models (Blair et al., 1998).

IGF-I appears to play a role in linking somatic growth and development to activation of the reproductive axis. In rodents circulating concentrations of IGF-I increase during puberty (Handelsman et al., 1987). In Pigs, the pubertal increase in circulating IGF-I concentration occurs along with an age related decrease in pituitary response to GHRH and an increased LH secretion (Dubreuil et al., 1987). An increase in circulating IGF-I was found to stimulate LH secretion in sheep (Adam et al. 1997). As circulating concentrations of IGF-I increase over the course of pubertal development; it may reach a putative stimulatory threshold, which permits activation of the reproductive axis.

Increased IGF-I concentrations were found in the serum of heifers fed to achieve a high rate of gain compared with those fed to achieve a low rate of gain, indicating that IGF-I concentration in serum in positively associated with increased nutrient intake (Granger et al., 1989).

Puberty

Original theories on puberty described it as the period at which the organism becomes sexually mature, and is marked by the occurrence of changes whereby the two sexes become fully differentiated (Marshall, 1922). Developments in the those ideas describe puberty as the time when reproduction becomes possible, and sexual maturity as the time when the animal reaches it full reproductive power (Crew, 1931 and Asdell, 1946).

Puberty has been stated to occur between 8 and 20 mo of age by Cupps et al. (1969), between 15 and 18 mo of age by Nishikawa and Hafez (1968) and between 12 and 24 by Squires (1992). While the age at which onset of puberty occurs, differs between authors, all indicate that pubertal onset can be influenced by a range of factors such as nutrition, season, and inherited characteristics. The onset of puberty is a result of a series of complex developmental events that occur within the reproductive endocrine axis (Schillo, et al. 1992). In the human puberty is a dynamic period of development marked by rapid changes in body size, shape and composition. As puberty approaches, the growth velocity slows Most female mammals in temperate climates are seasonally rather than continuously fertile. The evolution of seasonal breeding influences the time when fertility is first attained in the young female. Puberty occurs only in the breeding season (Foster, et al. 1986).

Control of reproductive function in the female involves numerous interactions among the central nervous system, anterior pituitary gland, and ovary. Higher centers of the brain receive neuronal inputs that transmit various types of information concerning both external and internal environments. Inputs that influence reproduction seem to convey information about such variables as photoperiod, ambient temperature, stress, social interactions, and nutritional status (Schillo, et al. 1992).

Luteinizing Hormone

The hypothalamic regulation of tonic secretion of LH is potentially functional before puberty, but remains dormant due to either the presence of inhibitory mechanisms, the absence of stimulatory inputs, or a combination of both. The idea of an inhibitory mechanism agrees with the gonadostat theory (Hohlweg and Dohrn, 1932), which postulates that the onset of puberty depends on a decrease in the sensitivity of the hypothalamus to negative feedback of estradiol on tonic secretion of GnRH/LH.

Several studies have suggested that components of the reproductive endocrine axis are functional long before the onset of puberty. As early as 1mo of age, pulses of luteinizing hormone (LH) have been observed in the peripheral circulation of heifers (Schams et al., 1981). The fetal hypothalamo-pituitary-gonadal axis possesses the components of the regulatory mechanisms to perform most of its endocrine functions. In the pig, LH gene expression starts around day 45 post coitus (Ma et al., 1996). Measurable amounts of LH released from the pituitary are observed around day 60 of gestation in the fetal pig (Elsaesser et al., 1988).

Squires (1992) summarizes that low-frequency endogenous pulses of LH in prepubertal sheep, cattle and possibly fillies are not capable of stimulating follicular growth to the preovulatory stage. Ovarian estradiol acts on the hypothalamus to suppress the generation of pulses of GnRH that maintain the secretion of low levels of LH. As estradiol receptors decline, LH pulses increase, allowing follicles to develop to a preovulatory stage. Estradiol induces a preovulatory surge of gonadotropins, and ovulation hence puberty occurs.

Association and causation

The correlation coefficient (*r*) is a commonly used estimate of the degree of association between two variables (y,x). It often brings up the question of causation, which may be established by 9 criteria, including the following four (Rothman, 1999).:

- The degree of the association;
- The repeatability of the association;
- The temporal sequence or time-line;
- The plausibility of explanatory mechanisms.

Causation is sometimes inferred from the coefficient of determination (r^2), which is the fraction of variation in a dependent variable (y) that is explained or accounted for by variation in an independent variable (x). An example is heritability (h^2), the fraction of phenotypic variation accounted for by genetic factors. Geneticists usually assume that environmental factors are represented by 1.00 - h^2. This assumption brings up two questions. Where do genetic environmental factors fit? Is 1.00 justified, or can the sum of coefficients of determination be > 1.00? Rothman (1999) argues that the upper limit for the sum of coefficients of determination is infinity.

Calculation of r or r^2 often involves measures of y and x taken at the same time (t). One of the main criteria of a cause-effect relationship is that the cause (x) always precedes the effect (y). Cause-effect relationships may be immediate or have intermediary steps that involve a delay (induction time or latent period), which may be brief or long without diminishing the degree of causation. Simply calculating correlation without adjusting for a delay, however, will reduce the degree of a correlation. Adjustment for the delay will give a clearer indication of the strength of an association.

This process may be illustrated by a precursor-product relationship. If a marker is introduced by mouth and feces are collected for several days, the curve of fecal marker concentration plotted against time achieves a better fit if the time following marker administration (t, h) is adjusted for the delay (θ, h). So that the time factor becomes $t - \theta$ (Blaxter et al., 1956). The value of θ can be readily determined by iteration using an index of goodness of fit such as adjusted R-square (Holland et al., 1998). It represents the time between administration of the marker and first appearance of marker in the feces.

By analogy, θ may also be taken to represent an induction time or latent period between a cause and subsequent effect. The use of $t - \theta$ instead of t will increase the value of r or r^2, hence the likelihood of a causal relationship. The importance of strength of association depends on the prevalence of other causative factors, and it is subject to confounding. Consequently, other criteria are emphasized, such as the plausibility of biological mechanisms.

EXPERIMENT I

SEASONAL VARIATION IN FORAGE COMPOSITION FOR PASTURES AT THE VIRGINIA TECH MARE CENTER

by

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Hypothesis

Environmental factors such as day length, temperature and rainfall may influence pasture composition (proximate and mineral analysis).

Pastures may not supply adequate nutrients for reproduction or growth therefore horses grazing on pastures may have suboptimal nutrition for all or part of the year unless supplemented with a concentrate ration.

Objectives

The first objective of the study was to test for associations between environmental factors and pasture variables.

The second objective was to compare equine requirements for growth and reproduction, as recommended by the NRC (1989), to proximate analysis of pasture samples taken at monthly intervals for 5 y at the Middleburg Agricultural Research and Extension Center.

Materials and Methods

Pasture Management

The four pastures used in this study were established in 1989 each covering 30 acres (Fig 2). They were sown with a Kentucky bluegrass (*Poa pratensis*), white clover (*Trifolium repens*) mix and tall fescue (*Festuca arundinacea*) was planted in the high traffic areas. Each pasture contains a 5.5 × 18.3 m three sided shelter and two automatic water troughs.

The pastures were grazed by mares and foals from March to October and yearlings from November to October.

The pasture management carried out at the M.A.R.E. Center was routine. Soil in each of the pastures was tested in 1998, 2001 and 2002 for the following minerals, P, K, Mg, Zn, Mn, Cu, Fe, and B. Samples were analyzed by the Virginia Tech Soil Testing Laboratory (Table 1). In the fall a maintenance fertilizer was applied which consisted of a potassium compound (potash 62), diammonium phosphate (18-46-00 Dap), urea, ammonium sulphate, and borate 10%. Lime was also applied to the pastures annually to maintain pH at a range of 6.5 to 7.0.

Pastures were mowed regularly during late spring and summer to maintain a vegetative state, where new leaf growth predominated and also control weeds

Samples

Pasture samples were collected monthly from 1998 to 2002. Oven dried brown paper bags were used to collect samples. Forage was cut using electric clippers, a fistful of forage was grasped and cut 2 to 3 cm above the ground. If the pasture was taller than 40 cm, the sample was taken from the top 15 cm. A zig zag pattern was driven through the field approximately every 25 m, pasture was sampled (Fig 1).

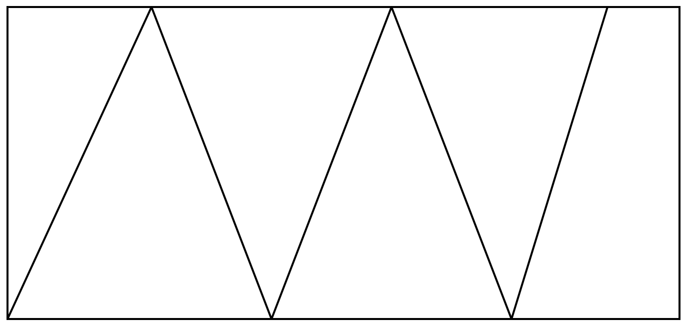

Figure 1 Sampling pattern across pastures at the MARE Center

Between 300 g and 1000 g of sample was collected. During the spring and summer mo closer to 1000 g of sample was collected as the pasture contained more moisture. In the fall and winter mo closer to 300g of sample was collected.

Day length was calculated for each day of the year using the U.S. Naval Observatory at a latitude of 38 º 58' 4.8" N, and longitude of -77º 44' 6.0" W (Fig . Temperature was measured from the National Weather Service, for the Washington Dulles International Airport which is approximately 27 km from the MARE Center. Monthly averages for temperature from 1998 to 2002 were calculated.

Preparation

The paper bags were dried and weighed prior to forage sampling. Samples were placed in hot air drying ovens with in 30 min of cutting and maintained at 70ºC until a constant dry weight was achieved (≈ 48h). Dry samples were then weighed and ground, first in a hammermill then milled into more fine particles using a Thomas/Wiley Standard Model 4 Laboratory Mill and placed into plastic vials and submitted for proximate and mineral analysis (using Dairy One, Ithaca, NY).

Analysis

Analysis of the pasture variables was conducted using standard AOAC procedures. Crude protein (CP) was analyzed using a Leco FP-528 combustion analyzer. Acid detergent fiber (ADF) and neutral detergent fiber (NDF) were analyzed using the ANKOM A200 filter bag technique (FBT). Minerals including Ca, P, Mg, K, Na, Fe, Zn, Cu, Mn, and Mo were analyzed using a Thermo Jarrell Ash IRIS Advantage Inductively Coupled Plasma (ICP) Radial Spectrometer. Sulfur was analyzed using a Leco Model SC-432. Crude fat was analyzed using ether extract, Tecator Soxtec System HT6. Ash was analyzed using the AOAC Method 942.05. Starch was analyzed using a YSI 2700 SELECT Biochemistry Analyzer. Non fiber carbohydrates (NFC) were calculated as 100% - (CP% + (NDF% - NDICP%) + Fat% +Ash%) where NDICP is neutral detergent insoluble crude protein . Non structural carbohydrates (NSC) were calculated as (starch + sugar), where sugar was analyzed using the West Virginia University procedure described by Hoover and Webster (1999).

Dry matter percentage was calculated using the following equation: [(wt of dry sample + dry bag – wt of dry bag) / (wt of wet sample + dry bag – wt of dry bag)] X 100

Statistical Analysis

Normality was tested using the Shapiro-Wilk statistic. Pearson correlations were conducted to detect any associations among the environmental variables and forage composition variables using SAS. A proc glm model was used to determine differences in forage composition between years. A quadratic curve was fitted to CP versus temperature using SAS (SAS Inst. Inc., Cary, NC).

Results

Protein

The crude protein concentrations in the pasture were sufficient in all years and mo to meet the requirements of the broodmare (Fig 11). The pastures concentrations of crude protein in December of 2001 and January and April of 2002 were not sufficient to meet the requirements of the growing horse.

The highest monthly average in 1998 was 21.2 ± 2.46 % in November, and the lowest monthly average was 13.9 ± 0.7 % in April. The highest monthly average in 1999 was 23.6 ± 0.6 % in September, and the lowest monthly average was 16.9 ± 1.67 % in July. The highest monthly average in 2000 was 21.5 ± 0.84 % in April, and the lowest monthly average was 14.6 ± 0.63 % in October. The highest monthly average in 2001 was 17.2 ± 2.18 % in Apr, and the lowest monthly average was 13.1 ± 0.17 % in December. The highest monthly average in 2002 was 17.9 ± 0.99 % in October, and the lowest monthly average was 11.8 ± 0.95 % in January.

Minerals

Calcium concentrations in the pastures meet the requirements for broodmares in all mo of 1998. In 1999 January is slightly below the required level, in 2000 all mo meet the required amounts. In 2001 and 2002 the pastures meet the requirements of the broodmare.

The growing horse's calcium requirements are not met by the pastures at the MARE center in May, September and November of 1998, January to April and September to December of 1999, September to December again in 2000 do not meet the requirements of growing horses. In 2001 February, March and April are slightly below required levels and in 2002 January, September, October and November are also slightly below required levels (Fig 10).

Phosphorus concentrations in the pastures at the MARE Center were insufficient to meet requirements of broodmares in January and March of 1998, March in 2000, July and August of 2001 and January, March and April in 2002 (Fig 7).

Concentrations of phosphorus in the pastures did not meet the requirements of growing horses in January, March, September and November of 1998, March, and September to November in 2000. September, October and December were insufficient in 2001 and January, March, April, September and November in 2002 (Fig 7).

Zinc was insufficient in all mo for both broodmares and growing horses except January and October of 2001 and August to November in 2002 (Fig 8). Copper (Fig 9) and sodium concentrations in the pastures were always insufficient.

Environment

Using visual assessment of temperature patterns over 1998 to 2002, the mo of June to September are generally the hottest (Fig 4) and day length is the longest in June (Fig 3). Fitting a quadratic curve to CP versus temperature showed a strong positive relationship ($r = 0.45$, $P < 0.0001$). The amount of variation in CP that could be accounted for by temperature was $r^2 = 0.20$, $P < 0.\ 0001$ (Fig 13). Rainfall is sporadic each year and not pattern can be detected (Fig 5). Dry matter content of the pasture reaches a low point in May and increases throughout the summer and starts to decline again around September (Fig 6). The reports from the Virginia Tech Soil Sampling Laboratory indicate that the soil profiles where adequate in all nutrient analyzed (Table 1).

Discussion

The results indicate that day length reaches a peak in June and temperature follows closely with a peak between July and August. Plant growth is most rapid in spring between the months of April and May and by mid to late summer the pasture is very dense. Crude protein shows a peak in the spring and in the fall, the pattern in crude protein has a frequency approximately double that of day length and temperature. Seasonal variation in CP concentration may be caused in part by differences in day length. The CP concentration in forage is decreased by high light intensity (Bathurst and Mitchell, 1958), this reduction is associated with the increase in forage yield and dilution of the available CP. Variation in the pasture due to season occurs as grasses grow from leafy to steamy stages and as the dry matter yields increase with higher fiber and lignin, whereas protein and non-structural carbohydrates decrease (Blaser et al., 1986).

Horses are natural forage eaters as is shown by the fact that wild horses survive and reproduce in open range situations. Feeding of concentrates becomes necessary only when there are great demands on the animal, such as pregnancy, lactation and growth. At other times when animals only require a maintenance level of nutrition for example barren mares, good quality pastures can meet the requirements of these horses. If only low quality pastures are available supplemental feeding will be more likely especially for lactating, pregnant or growing horses.

The requirements (NRC, 1998) for the pregnant mares at the MARE Center are highest between the months of January to April when the horses are in their 3rd trimester. This coincides with mid to late winter and early spring, the pasture is lacking in quantity. Throughout these months P was deficient in 1998 and 2002; in 2000 and 2001 P was only deficient from January to March. The spring growth in the pasture occurring in April was enough to satisfy the requirements of the pregnant mares. Sodium, zinc and copper were deficient in all years for the requirements of the pregnant mare from January to April.

The lactating mare has the highest requirements from May to August, this lessens from September to October but the requirements are still above maintenance. During these summer months the pasture has a high moisture content which dilutes the concentration of the minerals, however pasture availability is abundant. During May to August which coincides with early lactation P is deficient in 2001 and 2002, in 1998 deficiency only occurs in May and June. Sodium, Zn, Cu are deficient in all years. Calcium is deficient in 1998, July and August of 1999 and June and July of 2000.

The young horse has the highest requirements for growth occur between October and March, which coincides with winter at the MARE Center. During this time P is deficient in all years except 1999, Zn, Cu and Ca are all deficient through out this time. Crude protein is sufficient through out the winter, however is deficient during the middle of summer, the reasons for this are mentioned above.

Pasture composition was found to be influenced by environmental factors. Dry matter content in the pasture was responsive to day length, temperature and rain. Crude protein was influenced by day temperature, with optimum concentrations at 12ºC. Crude protein was generally deficient in July for pregnant, lactating and growing horses, which coincided with highs in temperature and increase in day length occurring in summer. When comparing the average CP concentration over all yrs to the optimum requirements for growing horses as described by Staniar et al., 2001 which has a range between 13 and 17%, the concentration of CP in the pastures is always between these ranges. It is necessary to supplement CP, P, NA, Zn, and Cu to sustain pregnancy, lactation, and growth in the horses at the MARE Center.

Implications

When pastures are a primary source of nutrition for horses, it is important to determine the availability of nutrients. Pasture composition is highly variable from year to year, reflecting the weather.

Season and weather influence pasture DM and CP, understanding the temporal patterns of day length, temperature, rainfall, DM and CP, we may devise a pro-active feeding management system for growing horses.

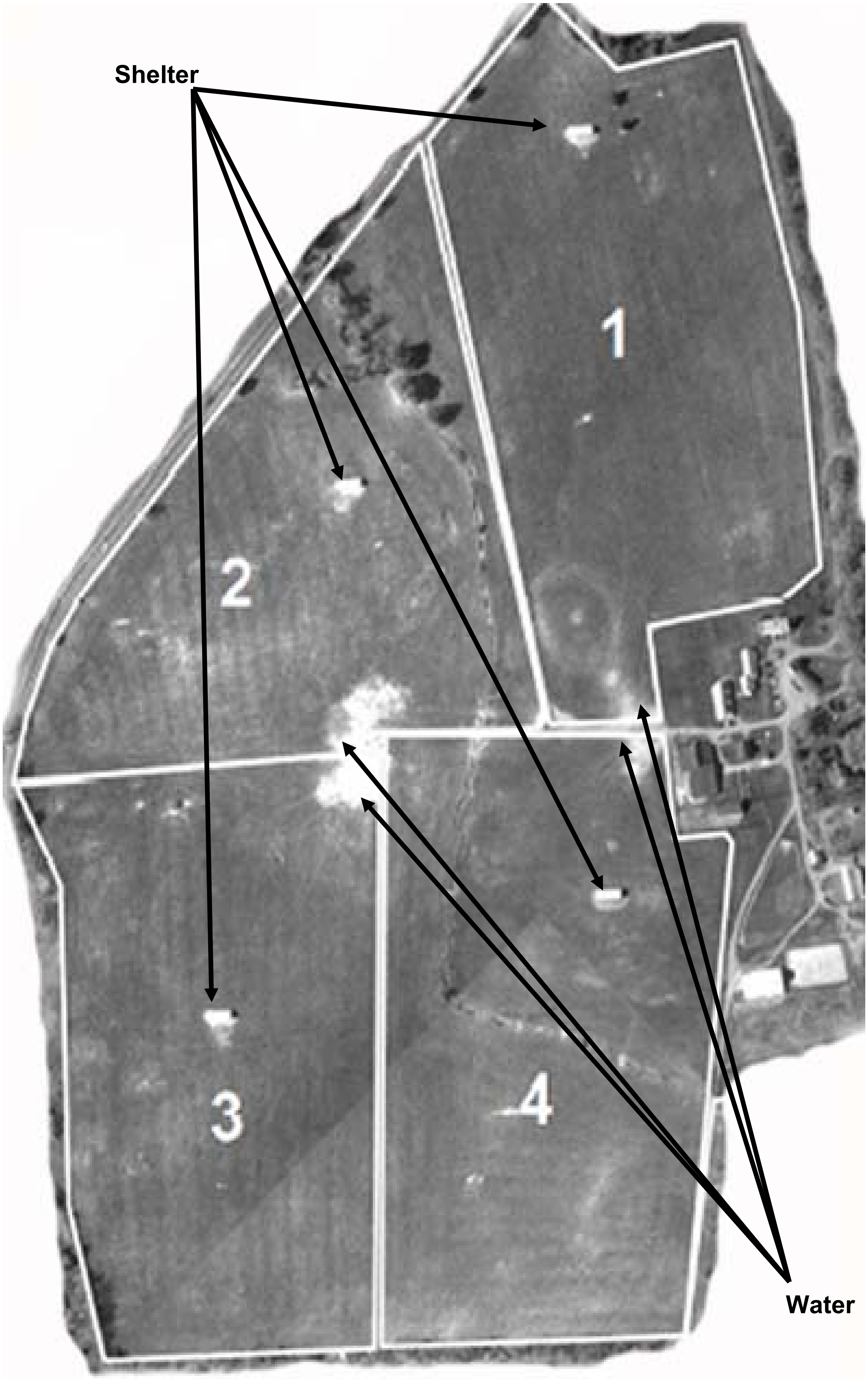

Figure 2 Pasture layouts at the MARE Center

Table1. Comparison of soil samples at the MARE Center

	Field 1			Field 2			Field 3			Field 4		
	1998	2001	2002	1998	2001	2002	1998	2001	2002	1998	2001	2002
Soil pH	6.6	6.3	6.4	6.0	6.8	6.8	6.4	6.6	6.6	6.6	6.4	6.3
P lb/A	31	32	49	24	29	41	27	35	46	28	25	74
K lb/A	138	157	315	209	114	181	254	157	430	238	157	522
Ca lb/A	2400	1200	1817	1992	1200	2604	2184	1200	2264	2160	1200	2062
Mg lb/A	137	96	142	218	99	121	166	106	146	173	94	139
Zn g/kg	1.7	3.7	1.3	2.4	3.0	1.0	5.0	2.4	0.9	3.6	1.9	1.2
Mn g/kg	12.4	16.1	11.7	12.4	16.1	13.0	15.4	11.9	12.6	12.9	14.5	12.4
Cu g/kg	0.4	0.7	0.5	0.4	0.7	0.5	0.5	0.6	0.5	0.5	0.7	0.5
Fe g/kg	30.1	16.2	23.3	11.6	27.5	20.4	27.6	9.1	12.6	28.2	17.3	21.1
B g/kg	0.5	0.7	0.4	0.4	0.8	0.4	0.5	0.7	0.4	0.5	0.7	0.4

Table 2. Forage composition for pastures at the MARE Center in 1998

Mo	Jan	SE	Mar	SE	Apr	SE	May	SE	Jun	SE	Jul	SE	Aug	SE	Sep	SE	Oct	SE	Nov	SE
DL[1]	9.26		11.0		12.4		13.7		14.8		14.2		13.1		11.60		10.3		9.40	
Temp	4.5	0.9	7.5	1.2	12.7	0.5	18.8	0.6	21.3	0.6	24.1	0.4	24.6	0.6	22.6	0.5	13.4	0.6	4.9	1.2
Rain[2]	13.8		14.2		0.5		0.7		14.9		3.4		1.1		0.0		3.8		3.1	
DM									33.0	1.64	39.1	3.63	50.2	4.41	36.1	2.50	42.5	1.92	50.3	1.75
CP	14.7	1.60	15.6	0.30	14.0	0.70	16.6	2.62	17.2	1.63	15.6	1.06	19.6	2.70	17.6	1.26	16.4	2.55	21.2	2.46
ADF	34.5	2.20	34.3	2.10	35.3	0.19	33.3	2.86	31.0	2.88	34.9	0.89	28.5	2.11	32.6	1.24	34.1	2.40	26.6	3.26
NDF	64.0	0.10	56.3	2.80	59.2	1.44	61.2	2.47	56.1	3.45	58.0	1.66	52.5	2.69	59.7	1.75	60.0	1.34	52.1	5.19
NFC	19.5				17.3				19.1	3.50	22.7	0.30	22.5		16.6	2.50	19.2	1.57		
NSC	7.70	0.50	18.4	2.50	15.4	3.20	12.0	2.10	14.1	2.64	12.3	1.15	14.7	1.81	10.2	1.53	8.83	2.46	12.2	4.80
Starch	0.80				0.70				2.55	1.15	3.15	0.25	2.30		0.55	0.35	2.23	0.57		
Sugar	6.40				6.20				8.90	0.40	7.30	0.70	14.2		8.75	2.45	9.00	1.46		
C Fat	2.65	0.45	2.35	0.15	2.33	0.18	2.55	0.56	2.95	0.49	3.00	0.56	3.38	0.30	2.80	0.38	3.05	0.23	3.80	0.17
Ash	7.13	1.33	7.28	0.36	7.83	1.16	7.80	1.12	8.53	1.14	7.70	0.80	9.31	0.40	8.94	0.42	9.34	1.94	10.8	0.42
Ca	0.51	0.04	0.57	0.01	0.59	0.03	0.50	0.02	0.50	0.02	0.52	0.04	0.51	0.02	0.50	0.02	0.55	0.02	0.54	0.05
P	0.28	0.06	0.30	0.01	0.25	0.01	0.32	0.06	0.34	0.05	0.38	0.03	0.33	0.02	0.34	0.03	0.37	0.03	0.33	0.06
Mg	0.15	0.01	0.18	0.02	0.20	0.03	0.18	0.01	0.22	0.13	0.21	0.02	0.18	0.01	0.22	0.00	0.22	0.02	0.19	0.01
K	1.07	0.20	1.37	0.55	1.19	0.21	1.46	0.56	2.38	0.13	2.34	0.14	2.50	0.02	2.40	0.17	2.52	0.08	2.36	0.11
Na	0.01	0.002	0.01	0.002	0.01	0.002	0.01	0.002	0.01	0.003	0.01	0.001	0.01	0.001	0.01	0.002	0.01	0.002	0.02	0.003
Fe	444	172	828	292	932	356	730	117	557	112	243	58	272	37	235	18.9	587	277	560	281
Zn	21.0	5.00	24.00	4.00	25.0	2.35	24.3	3.15	24.8	3.33	20.0	3.20	21.8	3.28	21.8	2.84	23.3	4.03	20.5	3.66
Cu	3.50	0.50	5.00	0.00	5.25	1.80	3.75	0.48	3.75	0.63	3.50	0.65	3.75	0.25	4.25	0.75	4.75	0.48	0.25	0.63
Mn	70.5	13.5	75.5	13.5	76.5	7.67	64.5	6.81	48.3	7.97	40.8	2.96	43.5	3.48	44.8	8.38	60.8	20.2	53.3	6.97
Mo			1.75	1.75	0.28	0.28	0.33	0.33	0.55	0.32	0.55	0.32					0.25	0.25	0.78	0.46
S	0.21				0.22	0.01	0.20	0.04	0.23		0.24	0.01	0.24	0.03	0.26	0.01	0.27		0.25	0.01

[1]DL h, Temp ºC, DM %, CP %, ADF %, NDF %, NFC %, NSC %, Starch %, Sugar %, C fat %, Ash %, Ca %, P %, Mg %, K %, Na %, Fe g/kg, Zn g/kg, Cu g/kg, Mn g/kg, Mo g/kg, S %, monthly averages.
[2]Rainfall cm, actual amount per mo

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Table 3. Forage composition for pastures at the MARE Center in 1999

Mo	Jan	SE	Feb	SE	Apr	SE	May	SE	Jun	SE	Jul	SE	Aug	SE	Sep	SE	Oct	SE	Nov	SE	Dec	SE
DL[1]	9.42		9.87		12.4		13.9	0.03	14.7		14.7		13.9		13.0		10.9		9.76		9.23	
Temp	1.7	1.0	3.1	0.8	11.7	0.5	17.1	0.5	21.6	0.5	25.7	0.6	24.1	0.7	19.4	0.6	11.7	0.5	10.1	0.7	3.4	0.8
Rain[2]	13.7		6.7		8.7		6.8		5.6		1.7		2.7		13.8		23.7		6.5		4.4	
DM					44.9	13.2	19.4	6.26	32.9	0.74	50.4	2.29	43.8	1.84	50.2	5.62	20.9	0.60	25.6	1.44	22.6	1.25
CP	17.2	4.20	17.1	1.53	20.7	1.44	17.6	1.10	19.9	2.38	16.9	1.67	19.5	1.61	23.6	0.60	20.7	2.54	17.3	0.58	20.4	0.70
ADF	32.3	1.70	32.9	2.08	30.0	2.13	32.4	1.39	30.7	2.13	32.2	1.89	29.9	2.41	27.4	2.38	30.0	2.62	31.7	0.57	30.8	2.03
NDF	56.8	2.05	55.9	2.90	54.0	2.17	57.9	1.66	57.3	3.45	55.0	1.35	56.5	3.03	51.3	1.97	55.0	3.65	59.6	1.82	54.0	3.49
NFC	20.5		20.4	1.05	17.1		17.4	3.10	14.9	1.30	20.8	0.50			16.4						19.9	4.75
NSC	14.2	3.35	14.7	1.85	12.2	0.35	10.5	1.09	7.40	3.34	15.2	1.24	10.2	1.46	11.3	0.96	10.8	1.25	11.0	2.26	11.4	2.56
Starch	0.90		1.63	0.55	2.20		1.25	1.05	0.93	0.38	0.70				1.10						1.30	0.40
Sugar	16.7		14.5	1.51	9.30		9.00	1.60	6.53	3.20	16.7	0.15			9.40						13.6	2.05
C Fat	3.05	0.45	3.38	0.37	2.88	0.57	3.73	0.67	4.47	0.12	3.70	0.24	3.55	0.17	4.03	0.57	3.75	0.65	3.90	0.10	3.40	0.22
Ash	9.35	1.22	9.36	0.43	10.4	0.35	9.37	0.13	9.36	0.53	9.49	0.72	10.3	0.33	9.89	0.17	9.85	0.55	8.19	0.40	11.2	0.89
Ca	0.39	0.06	0.49	0.05	0.47	0.02	0.54	0.02	0.54	0.04	0.49	0.05	0.47	0.01	0.58	0.03	0.47	0.03	0.44	0.03	0.49	0.03
P	0.33	0.04	0.32	0.01	0.39	0.03	0.43	0.05	0.44	0.04	0.32	0.03	0.38	0.01	0.51	0.03	0.40	0.04	0.46	0.07	0.36	0.04
Mg	0.20	0.04	0.19	0.02	0.17	0.01	0.19	0.01	0.23	0.02	0.18	0.01	0.20	0.01	0.24	0.02	0.20	0.01	0.16		0.21	0.03
K	2.62	0.05	2.53	0.14	2.73	0.26	2.82	0.30	2.83	0.28	2.73	0.05	2.61	0.19	3.22	0.06	2.54	0.27	2.70	0.14	2.69	0.33
Na	0.01	0.003	0.01	0.003	0.01	0.003	0.01	0.001	0.01	0.001	0.01	0.003	0.01	0.002	0.01	0.002	0.01	0.004	0.01	0.004	0.01	0.002
Fe	218	17.0	384	138	398	109	297	36.9	359	30.6	356	83.3	286	81.5	282	40.3	295	54.0	445	144	542	121
Zn	19.0	2.00	23.0	3.39	23.5	4.17	30.0	4.71	36.3	4.33	20.0	0.71	17.3	1.18	34.0	5.12	26.0	1.47	33.8	9.23	36.0	10.2
Cu	5.00	2.00	4.75	0.25	5.50	1.19	5.50	0.65	5.67	0.33	5.50	0.65	4.00	0.41	6.25	0.63	4.50	0.65	5.50	0.65	4.50	0.65
Mn	39.0	8.00	50.3	7.49	52.0	8.82	36.5	2.90	49.3	6.89	52.5	7.72	42.8	4.33	48.0	4.67	47.3	3.86	41.3	2.56	52.0	3.27
Mo					0.40	0.40	0.33	0.33	0.43	0.43					1.78	1.02	0.73	0.43	0.53	0.30	0.50	0.50
S	0.30		0.20		0.25	0.01	0.22	0.01			0.22	0.04	0.25	0.03	0.26	0.03	0.24	0.03	0.19	0.01	0.27	

[1]DL h, Temp ºC, DM %, CP %, ADF %, NDF %, NFC %, NSC %, Starch %, Sugar %, C fat %, Ash %, Ca %, P %, Mg %, K %, Na %, Fe g/kg, Zn g/kg, Cu g/kg, Mn g/kg, Mo g/kg, S %, monthly averages.

[2]Rainfall cm, actual amount per mo

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Table 4. Forage composition for pastures at the MARE Center in 2000

Mo	Jan	SE	Mar	SE	Apr	SE	May	SE	Jun	SE	Jul	SE	Aug	SE	Sep	SE	Oct	SE	Nov	SE	Dec	SE
DL[1]	9.33		11.4		13.0		14.0		14.8		14.2		13.3		12.0		10.5		9.70		9.24	0.01
Temp	2.8	1.2	8.8	0.7	11.8	0.7	18.1	0.6	24.4	0.5	25.6	0.4	25.6	0.5	18.3	0.7	11.1	0.8	6.7	0.8	1.1	0.5
Rain[2]	4.5		1.9		8.5		11.1		6.5		10.3		9.7		15.0		10.8		5.8		4.0	
DM	32.3	1.04	29.9	2.13	43.6	9.56	22.1	0.88	31.0	1.93	20.7	2.42	24.9	0.90	25.2	1.05	12.5	0.36	33.2	1.87	51.7	2.61
CP	20.2	0.84	17.1	1.21	21.5	0.84	19.6	2.80	20.7	1.65	18.4	1.03	15.3	1.57	17.8	1.23	14.6	0.63	19.1	1.02	17.5	1.94
ADF	27.8	0.65	32.1	1.52	28.0	1.65	31.5	2.77	28.6	1.85	31.1	2.01	35.6	1.79	32.1	1.74	30.8	1.77	31.6	1.15	31.9	1.73
NDF	51.0	2.18	57.9	2.64	51.0	3.16	53.5	4.64	52.1	2.14	55.5	1.55	59.1	1.23	57.9	2.84	56.4	3.64	57.1	1.42	55.9	0.93
NFC	23.6		17.2	1.10	19.9	2.87	19.4	2.57			16.9		20.1	0.99	16.1	3.60	24.3		19.0	2.14	18.1	
NSC	14.8	1.74	11.9	1.40	15.0	0.71	12.0	1.27	13.2	1.48	13.7	0.74	10.4	1.36	12.0	2.60	14.2	4.41	8.60	1.62	16.1	1.34
Starch	2.30		1.30	0.10	2.57	0.09	1.53	0.38			0.90		2.00	0.36	1.90	1.10	1.70		1.17	0.32	1.20	
Sugar	15.3		8.15	0.55	12.3	1.07	10.5	1.42			10.7		7.07	0.44	8.20	0.50	1.60		8.47	1.44	11.0	
C Fat	3.30	0.32	2.45	0.25	2.88	0.67	3.28	0.73	3.65	0.31	3.23	0.34	3.38	0.08	3.40	0.39	2.85	0.27	3.58	0.21	2.58	0.35
Ash	10.4	0.47	9.41	0.56	10.1	0.30	9.80	0.55	10.4	0.91	9.25	2.10	7.45	0.40	8.60	0.33	7.69	0.44	8.69	0.51	8.14	0.18
Ca	0.54	0.04	0.59	0.03	0.66	0.05	0.56	0.04	0.49	0.03	0.51		0.59	0.05	0.51	0.03	0.51	0.07	0.50	0.03	0.47	0.02
P	0.35	0.04	0.28	0.02	0.38	0.04	0.34	0.05	0.38	0.03	0.35	0.03	0.34	0.03	0.33	0.04	0.30	0.02	0.34	0.03	0.30	0.02
Mg	0.19	0.01	0.20	0.01	0.20	0.01	0.21	0.01	0.18	0.02	0.20	0.01	0.20	0.01	0.19	0.01	0.19	0.01	0.19	0.01	0.17	0.01
K	2.68	0.17	2.15	0.13	2.86	0.45	2.47	0.36	2.37	0.03	2.43	0.16	2.26	0.11	2.08	0.11	2.12	0.08	2.02	0.13	1.91	0.07
Na	0.01	0.001	0.006	0.001	0.03	0.02	0.01	0.001	0.01	0.001	0.01	0.001	0.02	0.007	0.01	0.004	0.01	0.004	0.01	0.001	0.01	0.001
Fe	407	109	536	167	321	105	591	88.3	344	87.0	618	189	231	51.9	443	165	516	245	576	218	256	65.7
Zn	25.8	1.97	28.0	3.81	35.8	4.33	28.5	2.66	22.0	1.29	33.0	8.13	27.3	11.6	32.3	8.44	38.3	17.1	28.3	2.29	18.3	1.75
Cu	4.50	0.65	3.50	0.29	7.25	1.97	5.50	0.87	4.00	0.71	4.25	0.25	4.25	0.48	4.50	0.28	3.25	0.75	5.25	0.25	3.75	0.48
Mn	55.3	9.84	57.8	7.49	50.0	4.51	68.8	8.08	52.3	4.31	50.3	2.66	49.3	5.66	51.5	3.59	48.5	7.89	63.0	7.84	56.8	0.63
Mo					0.58	0.33	0.40	0.40	0.28	0.28	0.55	0.32			0.33	0.33						
S	0.25	0.02	0.22		0.24		0.33		0.24	0.01	0.23	0.02	0.20		0.25	0.03	0.20	0.01	0.23		0.22	0.02

[1]DL h, Temp ºC, DM %, CP %, ADF %, NDF %, NFC %, NSC %, Starch %, Sugar %, C fat %, Ash %, Ca %, P %, Mg %, K %, Na %, Fe g/kg, Zn g/kg, Cu g/kg, Mn g/kg, Mo g/kg, S %, monthly averages.

[2]Rainfall cm, actual amount per mo

T.A. Cubitt

Table 5. Forage composition for pastures at the MARE Center in 2001

Mo	Jan	SE	Feb	SE	Mar	SE	Apr	SE	May	SE	Jun	SE	Jul	SE	Aug	SE	Sep	SE	Oct	SE	Dec	SE
DL[1]	9.50		10.9		11.9	0.03	13.3		14.2		14.8		14.3		13.3		11.9		10.5		9.24	
Temp	1.1	0.6	3.6	0.7	4.7	0.6	13.1	0.8	17.0	0.6	22.2	0.6	22.3	0.5	24.7	0.5	18.6	0.5	13.2	0.7	5.2	0.9
Rain[2]	5.7		4.3		10.6		5.5		14.5		12.3		10.8		11.6		8.7		3.6		2.1	
DM	71.0	7.36	69.3	1.09	56.7	3.30	23.2	1.04	28.6	1.12	26.3	1.98	31.9	0.97	31.9	0.54	34.8	1.34	29.6	0.73	18.7	0.47
CP	16.8	0.66	17.0	3.25	14.9	0.08	17.2	2.18	15.7	0.68	17.0	1.13	15.3	0.80	15.2	0.15	15.0	1.09	14.2	0.73	13.1	10.7
ADF	31.5	1.86	33.2	3.95	35.7	0.62	31.7	3.03	33.0	1.61	33.0	1.02	32.7	1.40	35.2	0.12	36.2	0.90	35.2	0.97	36.6	0.74
NDF	55.5	1.49	57.6	5.00	61.9	1.70	57.2	1.01	57.8	1.35	61.4	1.92	57.7	1.70	60.0	2.03	65.5	0.71	62.1	3.13	62.2	2.87
NFC					16.6	0.90	19.1		18.5		13.3	1.20					12.7		18.5	2.15		
NSC	15.9	1.38	15.2	1.10	11.1	1.85	13.9	2.08	11.5	1.69	10.5	1.71	16.3	0.96	13.7	2.58	8.6	0.91	12.8	2.92	14.8	2.65
Starch					1.10	0.10	1.20		0.70		1.35	0.05					1.40		1.40	0.20		
Sugar					8.23	0.90	9.90		7.00		7.85	1.15					8.70		10.8	0.35		
C Fat	2.73	0.06	2.20	0.20	2.20	0.42	2.50	0.54	2.43	0.42	3.20	0.20	2.25	0.33	2.53	0.34	2.98	0.22	2.48	0.34	2.33	0.34
Ash	9.09	0.66	8.06	0.44	8.37	0.17	8.48	0.35	11.1	1.42	8.68	0.52	8.50	1.03	8.56	0.45	8.75	0.46	7.87	0.24	7.73	0.35
Ca	0.56	0.03	0.44	0.07	0.51	0.03	0.46	0.02	0.58	0.04	0.51	0.03	0.54	0.05	0.54	0.05	0.61	0.08	0.64	0.05	0.69	0.09
P	0.33	0.02	0.32	0.03	0.30	0.02	0.38	0.06	0.30	0.02	0.34	0.02	0.27	0.01	0.28		0.32	0.03	0.25	0.02	0.27	0.02
Mg	0.22	0.01	0.19	0.04	0.23	0.01	0.20	0.01	0.22	0.02	0.18	0.02	0.21	0.02	0.22	0.02	0.21	0.03	0.20	0.01	0.22	0.02
K	1.92	0.11	1.88	0.01	1.99	0.16	2.18	0.22	1.93	0.17	1.79	0.11	1.75	0.21	1.74	0.09	1.62	0.09	1.50	0.09	1.67	0.19
Na	0.05	0.04	0.02	0.01	0.01	0.002	0.004	0.001	0.02	0.01	0.01	0.01	0.01	0.004	0.01	0.003	0.01	0.003	0.01	0.003	0.02	0.01
Fe	532	231	228	89.5	554	89.7	424	154	840	353	652	321	465	144	288	71.4	293	124	569	199	302	62.9
Zn	59.3	35.0	21.5	2.50	30.3	3.97	28.5	6.46	23.3	1.03	26.0	3.49	24.0	3.85	18.0	0.58	18.0	1.30	48.0	26.0	18.0	2.45
Cu	9.75	6.45	3.50	0.50	4.25	0.48	4.25	0.95	4.00	0.41	4.00		3.50	0.29	3.00		2.75	0.25	4.25	0.75	4.25	1.03
Mn	54.5	4.09	53.5	7.50	57.8	6.97	54.8	5.59	68.5	12.3	58.0	6.29	59.3	6.09	56.7	3.28	66.3	4.11	73.8	9.3	61.5	13.1
Mo	0.28	0.28					0.25	0.25	0.25	0.25			0.68	0.40							0.40	0.40
S	0.24	0.02	0.26	0.06	0.23		0.24	0.01	0.23	0.02	0.23	0.01	0.23	0.02	0.23	0.02	0.25		0.23	0.01	0.21	

[1]DL h, Temp ºC, DM %, CP %, ADF %, NDF %, NFC %, NSC %, Starch %, Sugar %, C fat %, Ash %, Ca %, P %, Mg %, K %, Na %, Fe g/kg, Zn g/kg, Cu g/kg, Mn g/kg, Mo g/kg, S %, monthly averages.

[2]Rainfall cm, actual amount per mo

T.A. Cubitt

Table 6. Forage composition for pastures at the MARE Center in 2002

Mo	Jan	SE	Feb	SE	Mar	SE	Apr	SE	May	SE	Jul	SE	Aug	SE	Sep	SE	Oct	SE	Nov	SE	Dec	SE
DL[1]	9.69		10.9		11.4	0.06	12.2	0.03	14.1	0.11	14.3		13.7		11.8		10.8		9.50	0.01	9.25	
Temp	3.9	0.9	5.3	1.0	7.8	0.9	13.8	0.9	16.8	0.7	25.2	0.5	25.5	0.7	20.6	0.4	12.8	0.9	6.9	0.8	0.8	0.6
Rain[2]	3.3		2.6		2.5		8.8		12.1		9.2		6.7		7.2		5.4		12.9		10.5	
DM	71.0	12.2	61.9	3.16	57.2	0.90	44.3	2.81	23.3	1.56	29.9	1.81	40.7	2.03	34.3	1.12	21.7	0.53	20.7	4.07	31.9	1.40
CP	11.8	0.95	15.2	1.05	14.4	0.71	13.1	1.13	15.1	1.46	17.0	2.06	17.7	2.45	16.2	1.26	17.9	0.99	14.8	0.44	15.3	0.92
ADF	35.7	1.70	34.6		35.5	0.81	35.1	0.78	33.8	1.62	34.7	1.90	34.0	2.26	34.9	1.98	30.4	2.22	36.3	0.65	36.4	0.40
NDF	63.7	2.83	62.3	4.05	61.4	1.58	61.3	2.09	58.8	1.68	58.6	3.68	60.0	3.38	58.7	2.85	53.1	2.47	60.4	1.14	63.0	1.46
NFC			21.5		18.5	1.25			20.9										15.3		14.2	1.07
NSC	14.1	2.00	10.1	1.55	11.5	1.57	16.7	1.61	15.5	0.75	12.9	1.24	9.83	1.82	10.3	3.51	17.2	1.28	12.7	2.12	6.98	0.79
Starch			0.90		1.07	0.33			1.40										0.70		0.50	0.17
Sugar			10.7		8.77	0.73			11.0										6.00		6.48	0.63
C Fat	2.45	0.14	2.75	0.75	2.32	0.18	2.48	0.35	2.55	0.26	2.95	0.56	3.48	0.44	3.20	0.62	4.53	0.28	3.43	0.14	3.48	0.11
Ash	8.04	0.46	7.32	0.30	8.43	0.75	6.55	0.60	7.50	0.57	8.55	1.02	9.05	1.13	11.6	0.26	7.38	0.23	7.86	0.26	9.35	0.54
Ca	0.47	0.01	0.67	0.10	0.52	0.03	0.56	0.07	0.53	0.02	0.59	0.04	0.54	0.03	0.61	0.05	0.54	0.02	0.53	0.01	0.60	0.04
P	0.27	0.02	0.29	0.04	0.24	0.01	0.26	0.20	0.30	0.03	0.32	0.05	0.61	0.03	0.29	0.03	0.35	0.30	0.32	0.02	0.35	0.03
Mg	0.21	0.01	0.19	0.03	0.16	0.01	0.19	0.01	0.18	0.01	0.18	0.01	0.78	0.01	0.19	0.01	0.18	0.004	0.18	0.004	0.21	0.02
K	1.64	0.11	1.38	0.23	1.23	0.09	1.46	0.11	1.55	0.36	1.64	0.59	1.58	0.30	1.71	0.53	2.47	0.37	2.33	0.07	2.29	0.18
Na	0.01	0.003	0.03	0.02	0.02	0.01	0.01	0.003	0.01	0.002	0.01	0.01	0.01	0.001	0.01	0.001	0.01	0.002	0.04	0.04	0.01	0.002
Fe	290	76.1	397	67.0	809	370	387	164	579	154	1938	542	2205	853	2281	1291	800	451	402	45.9	271	47.7
Zn	13.8	0.95	29.5	9.50	23.2	3.49	18.5	3.50	20.5	1.61	35.5	8.61	61.0	30.1	42.3	5.30	47.8	5.27	46.8	15.0	21.5	0.65
Cu	3.00	0.71	3.50	0.50	4.17	0.95	3.75	0.63	4.63	0.82	7.25	1.25	8.00	0.82	9.00	2.74	6.00	0.41	5.75	0.48	5.50	0.50
Mn	62.8	7.00	75.0	24.0	78.0	17.1	88.5	29.3	65.1	8.51	102	16.3	99.0	7.04	116	25.7	73.8	6.66	66.0	9.94	45.8	3.94
Mo	0.40	0.40					0.90	0.90	0.74	0.34	1.38	1.38	0.98	0.56	0.28	0.28	1.45	0.61	1.05	0.35		
S	0.20		0.22	0.02	0.23	0.01	0.16	0.02	0.20	0.02	0.21	0.03	0.25	0.02	0.23	0.01	0.23	0.01	0.21	0.01		

[1]DL h, Temp ºC, DM %, CP %, ADF %, NDF %, NFC %, NSC %, Starch %, Sugar %, C fat %, Ash %, Ca %, P %, Mg %, K %, Na %, Fe g/kg, Zn g/kg, Cu g/kg, Mn g/kg, Mo g/kg, S %, monthly averages.

[2]Rainfall cm, actual amount per mo

Table 7. Relationships between pasture and environmental variables for 1998 to 2002

	DM	CP	ADF	NDF	NFC	NSC	Starch	Sugar	Ether ex.	Ash	P	Mg	K	Na	Fe	Cu	Mn	Mo
DL		-0.16 0.02	0.24 0.0004	0.29 <0.0001	-0.23 0.06	-0.16 0.04			0.15 0.03	-0.19 0.007	0.25 0.0003		0.23 0.0008		-0.35 <0.0001		-0.30 <0.0001	-0.17 0.01
Temp	-0.19 0.01	-0.23 0.0007	0.32 <0.0001	0.33 <0.0001	-0.26 0.03	-0.20 0.004		-0.38 0.002		-0.17 0.01		0.31 <0.0001	0.16 0.02		-0.37 <0.0001	-0.16 0.02	-0.36 <0.0001	-0.23 0.0008
Rain	-0.22 0.003				0.28 0.02	-0.25 0.0002	0.56 <0.0001				0.15 0.03			-0.14 0.05	0.15 0.03			

[1]DL h, Temp ºC, Rain cm, DM %, CP %, ADF %, NDF %, NFC %, NSC %, Starch %, Sugar %, Ether extract %, Ash %, Ca %, P %, Mg %, K %, Na %, Fe g/kg, Zn g/kg, Cu g/kg, Mn g/kg, Mo g/kg, S %.

Table 8. Nutrient requirements of mares and growing horses

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug
Maintenance [1]																				
DE Mcal/kg											2.00	2.00								
CP%											8.00	8.00								
Ca%											0.24	0.24								
P%											0.17	0.17								
Mg%											0.09	0.09								
K%											0.30	0.30								
Na%											0.10	0.10								
S%											0.15	0.15								
Fe g/kg											40.0	40.0								
Mn g/kg											40.0	40.0								
Cu g/kg											10.0	10.0								
Zn g/kg											40.0	40.0								
Pregnancy [2]																				
DE Mcal/kg	2.25	2.25	2.4	2.4																
CP%	10.0	10.0	10.6	10.6																
Ca%	0.43	0.43	0.45	0.45																
P%	0.32	0.32	0.34	0.34																
Mg%	0.10	0.10	0.11	0.11																
K%	0.35	0.35	0.38	0.38																
Na%	0.1	0.1	0.1	0.1																
S%	0.15	0.15	0.15	0.15																
Fe g/kg	50.0	50.0	50.0	50.0																
Mn g/kg	40.0	40.0	40.0	40.0																
Cu g/kg	10.0	10.0	10.0	10.0																
Zn g/kg	40.0	40.0	40.0	40.0																
Lactation [3]																				
DE Mcal/kg					2.60	2.60	2.60	2.60	2.45	2.45										
CP%					13.2	13.2	13.2	13.2	11.0	11.0										
Ca%					0.52	0.52	0.52	0.52	0.36	0.36										
P%					0.34	0.34	0.34	0.34	0.22	0.22										
Mg%					0.10	0.10	0.10	0.10	0.09	0.09										
K%					0.42	0.42	0.42	0.42	0.33	0.33										
Na%					0.10	0.10	0.10	0.10	0.10	0.10										
S%					0.15	0.15	0.15	0.15	0.15	0.15										
Fe g/kg					50.0	50.0	50.0	50.0	50.0	50.0										
Mn g/kg					40.0	40.0	40.0	40.0	40.0	40.0										
Cu g/kg					10.0	10.0	10.0	10.0	10.0	10.0										
Zn g/kg					40.0	40.0	40.0	40.0	40.0	40.0										
Growing [4]																				
DE Mcal/kg									2.90	2.90	2.90	2.90	2.90	2.90	2.90	2.90	2.90	2.80	2.80	2.80
CP%									14.5	14.5	14.5	14.5	14.5	14.5	14.5	14.5	14.5	12.6	12.6	12.6
Ca%									0.68	0.68	0.68	0.56	0.56	0.56	0.56	0.56	0.56	0.43	0.43	0.43
P%									0.38	0.38	0.38	0.31	0.31	0.31	0.31	0.31	0.31	0.24	0.24	0.24
Mg%									0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
K%									0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Na%									0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
S%									0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Fe g/kg									50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
Mn g/kg									40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Cu g/kg									10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Zn g/kg									40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0

[1]Requirements taken from NRC, [2]Pregnancy requirements for 9, 10 and 11 mo [3]Lactation requirements for foaling to 3mo and 3mo to weaning, [4]Growing requirements for 4mo, 6mo and 12 mo, moderate growth

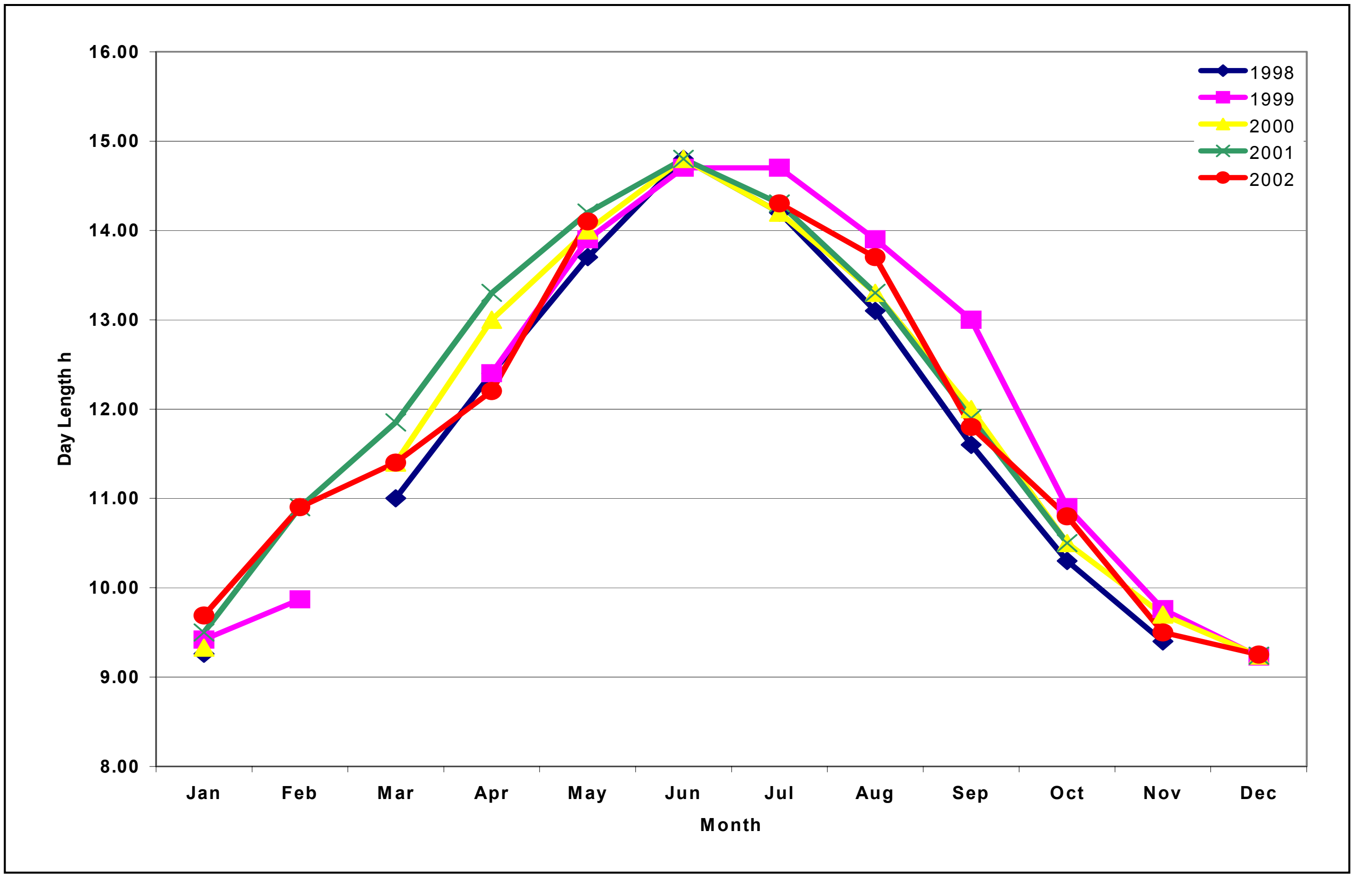

Figure 3 Comparison of day length from 1998 to 2002

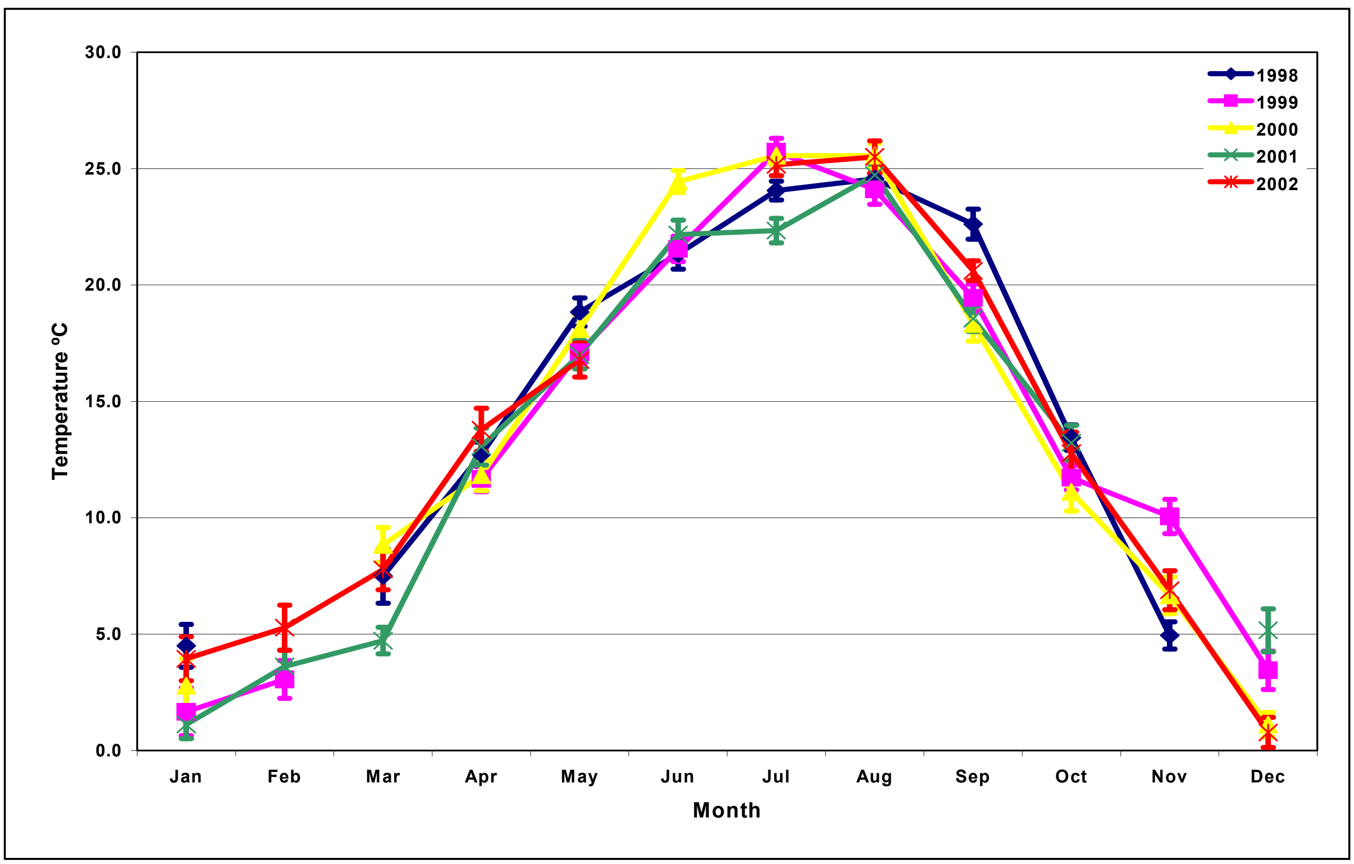

Figure 4 Comparison of temperature from 1998 to 2002

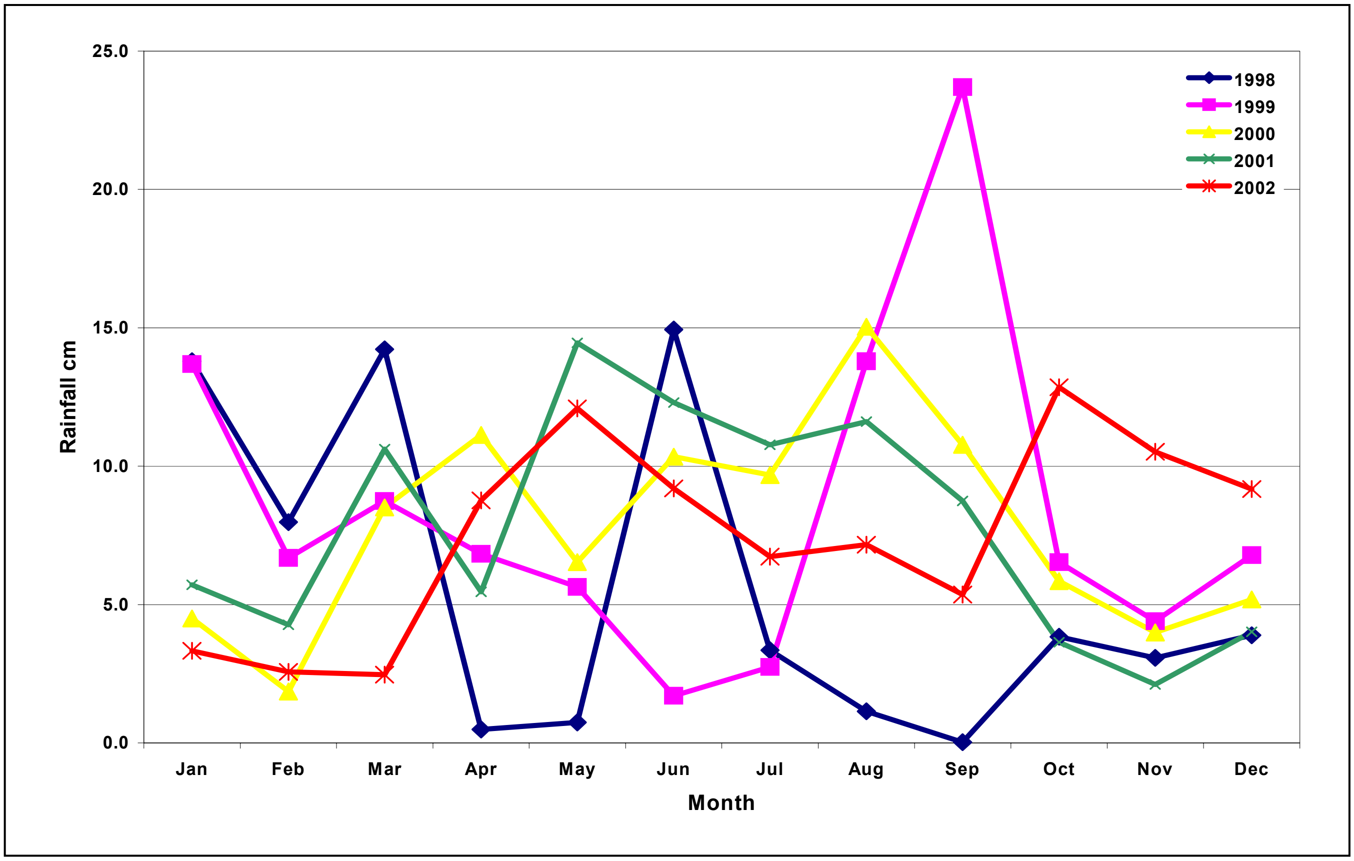

Figure 5 Comparison of rainfall patterns from 1998 to 2002

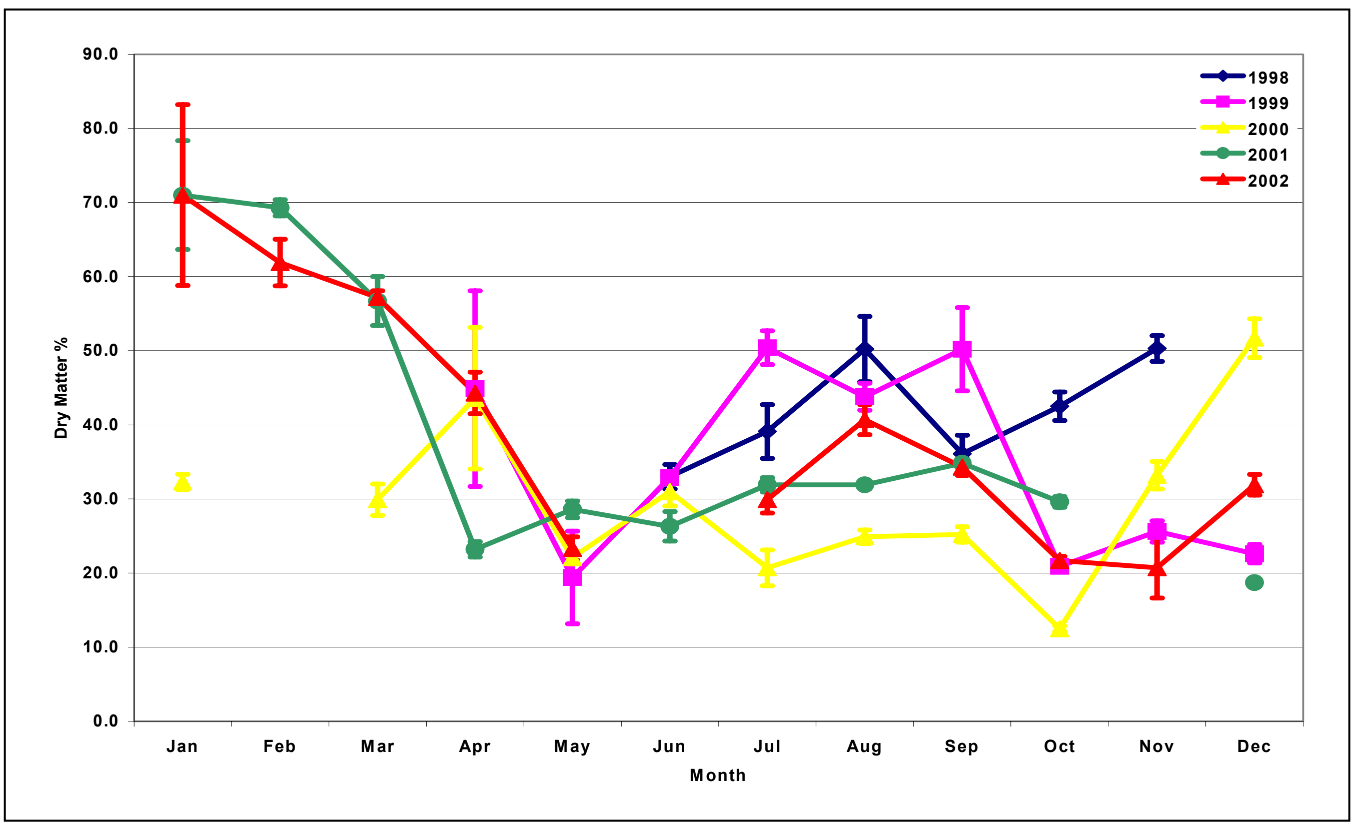

Figure 6 Comparison of dry matter in the pasture from 1998 to 2002

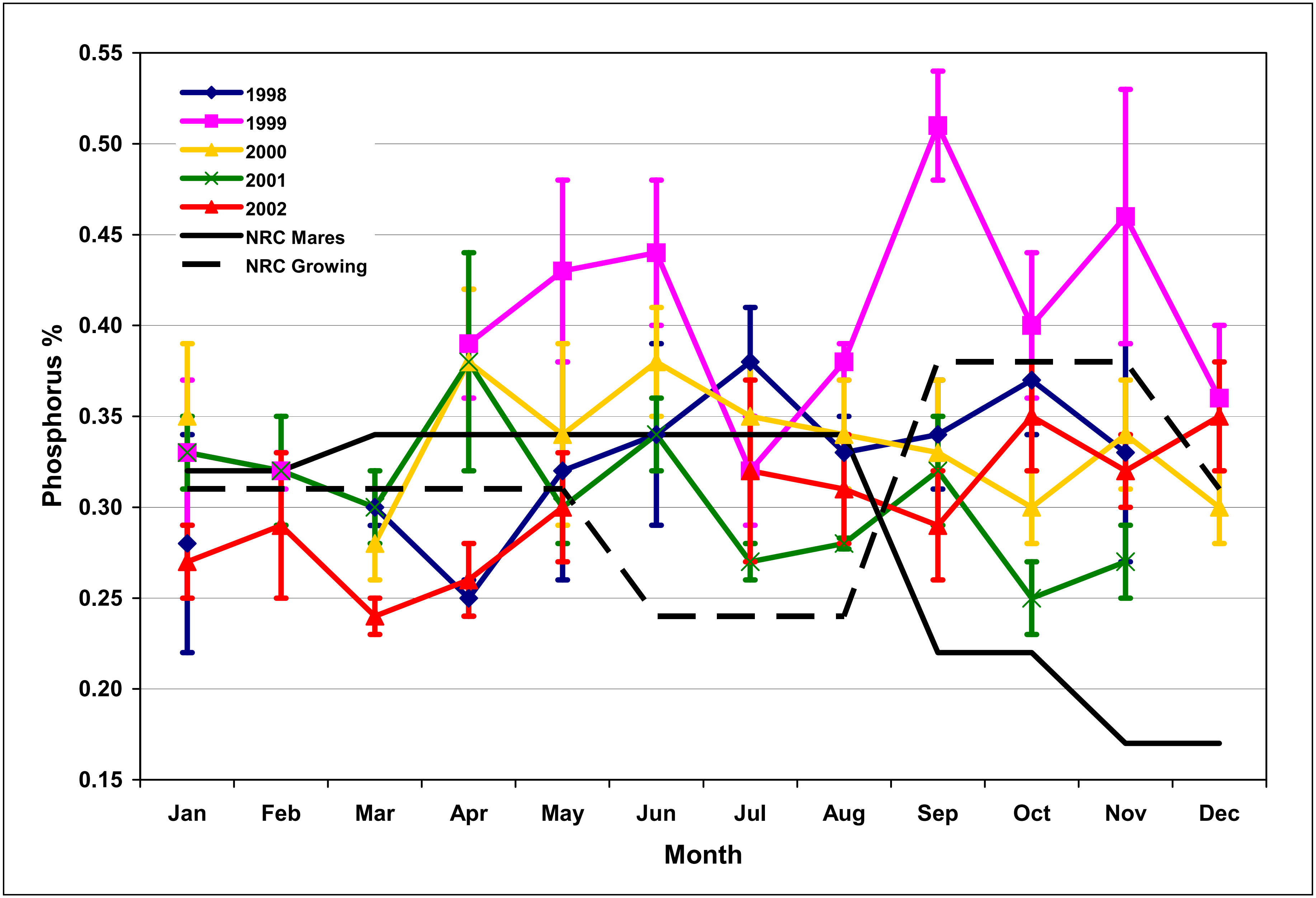

Figure 7 Comparison of requirements vs. pasture composition for phosphorus

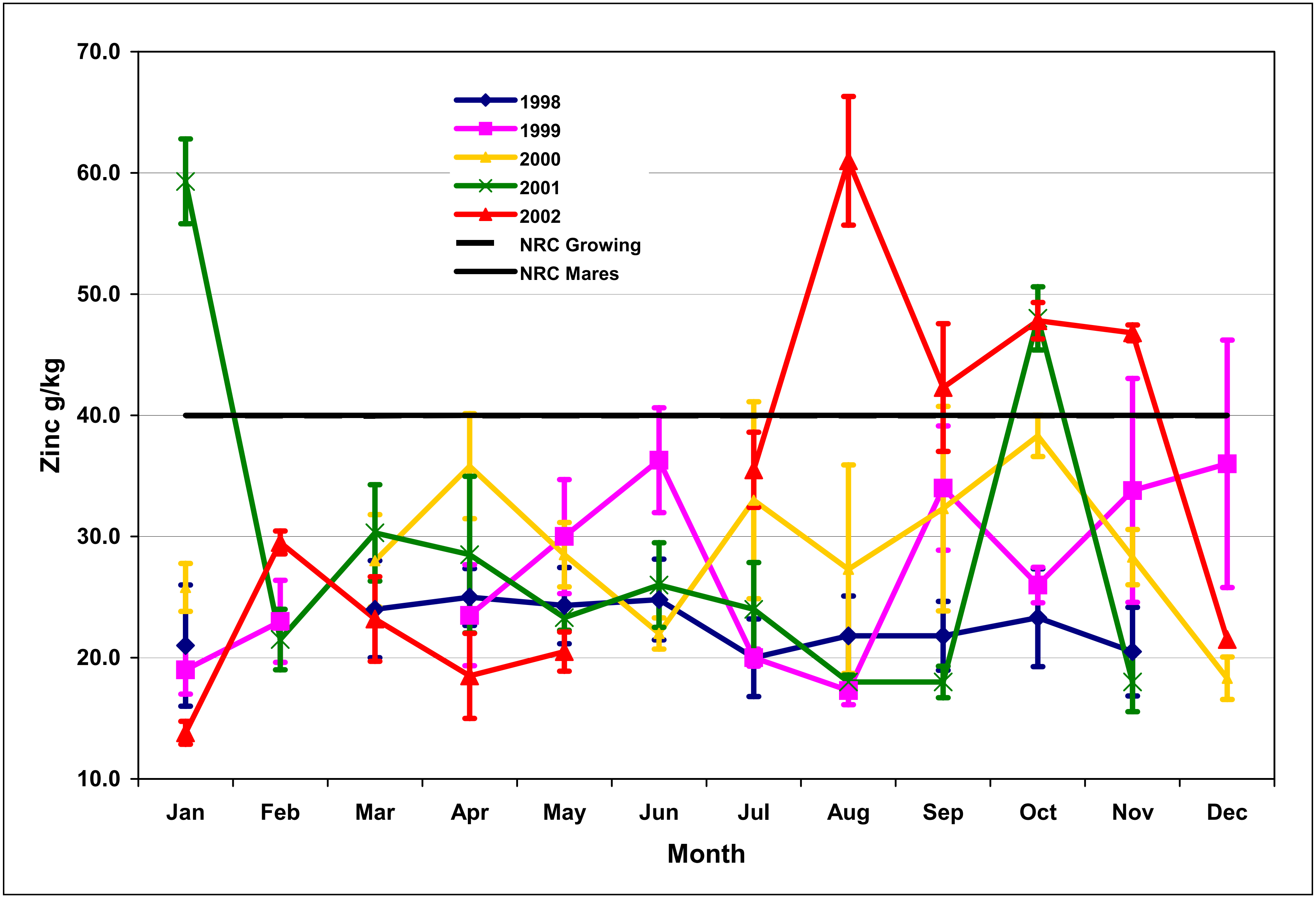

Figure 8 Comparison of requirements vs. pasture composition for zinc

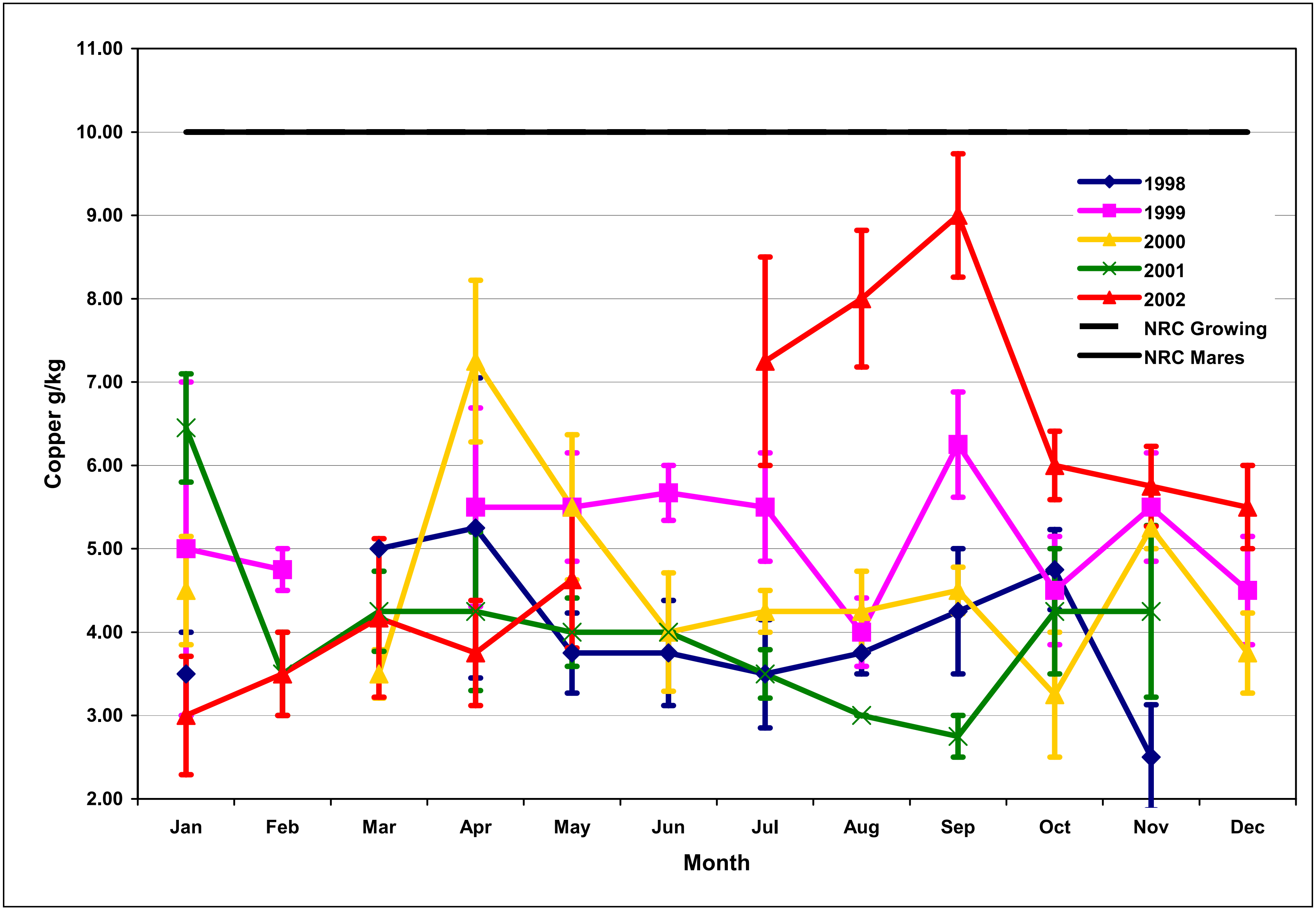

Figure 9 Comparison of requirements vs. pasture composition for copper

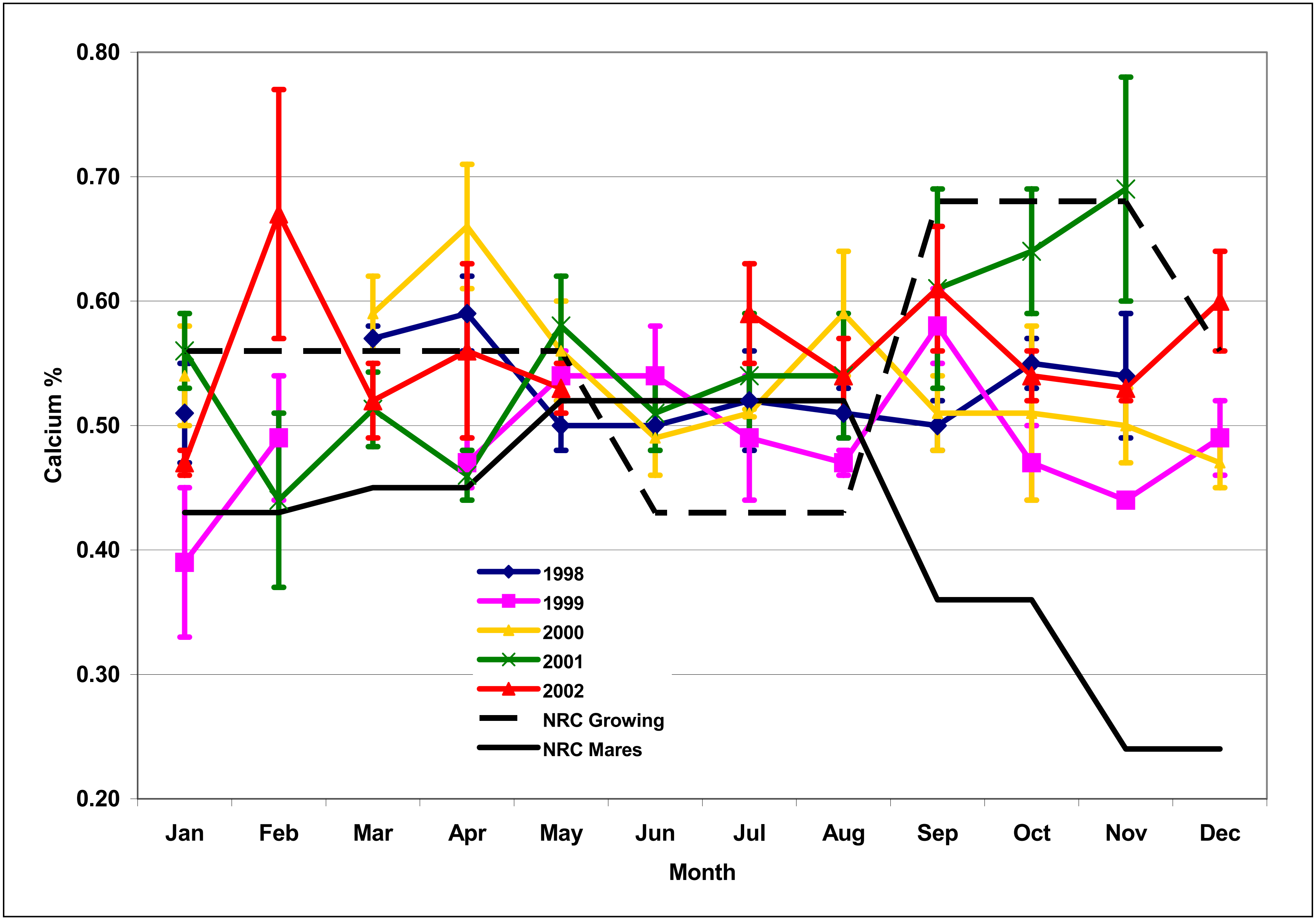

Figure 10 Comparison of requirements vs. pasture composition for calcium

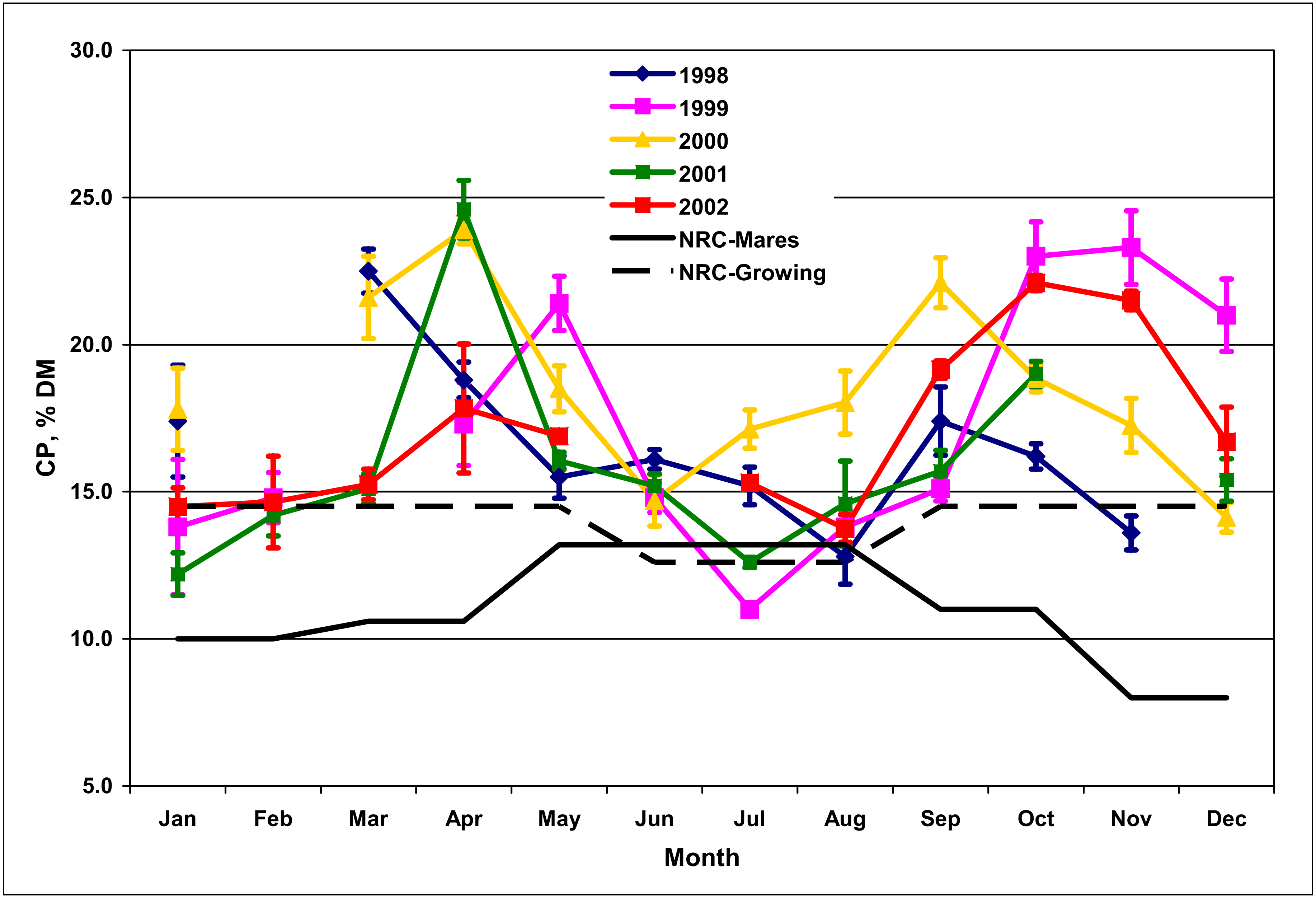

Figure 11 Comparison of requirements vs. pasture composition for crude protein

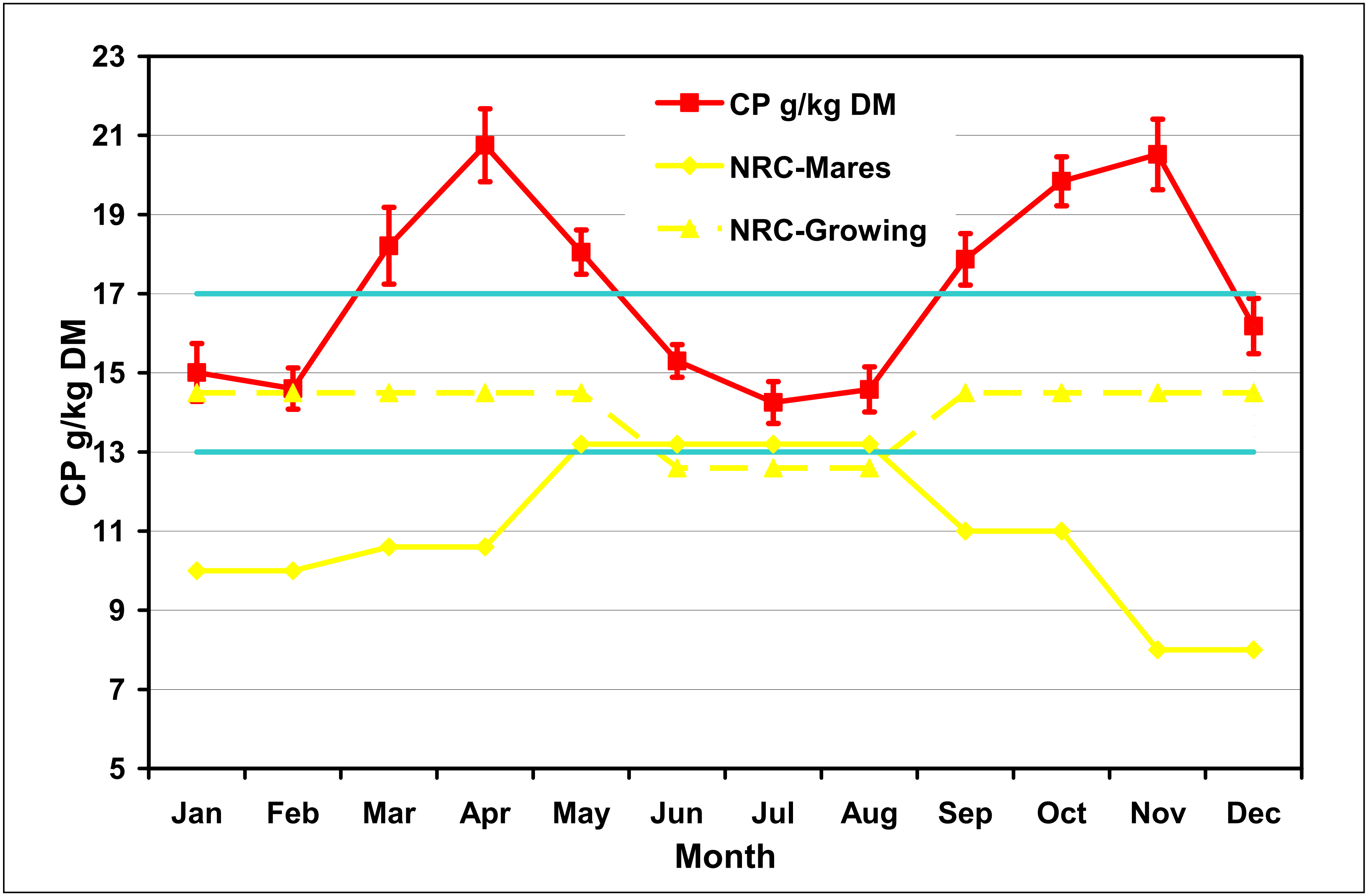

Figure 12 Nutrient requirement and optimal range of crude protein for mares and growing horses at the M.A.R.E. Center.

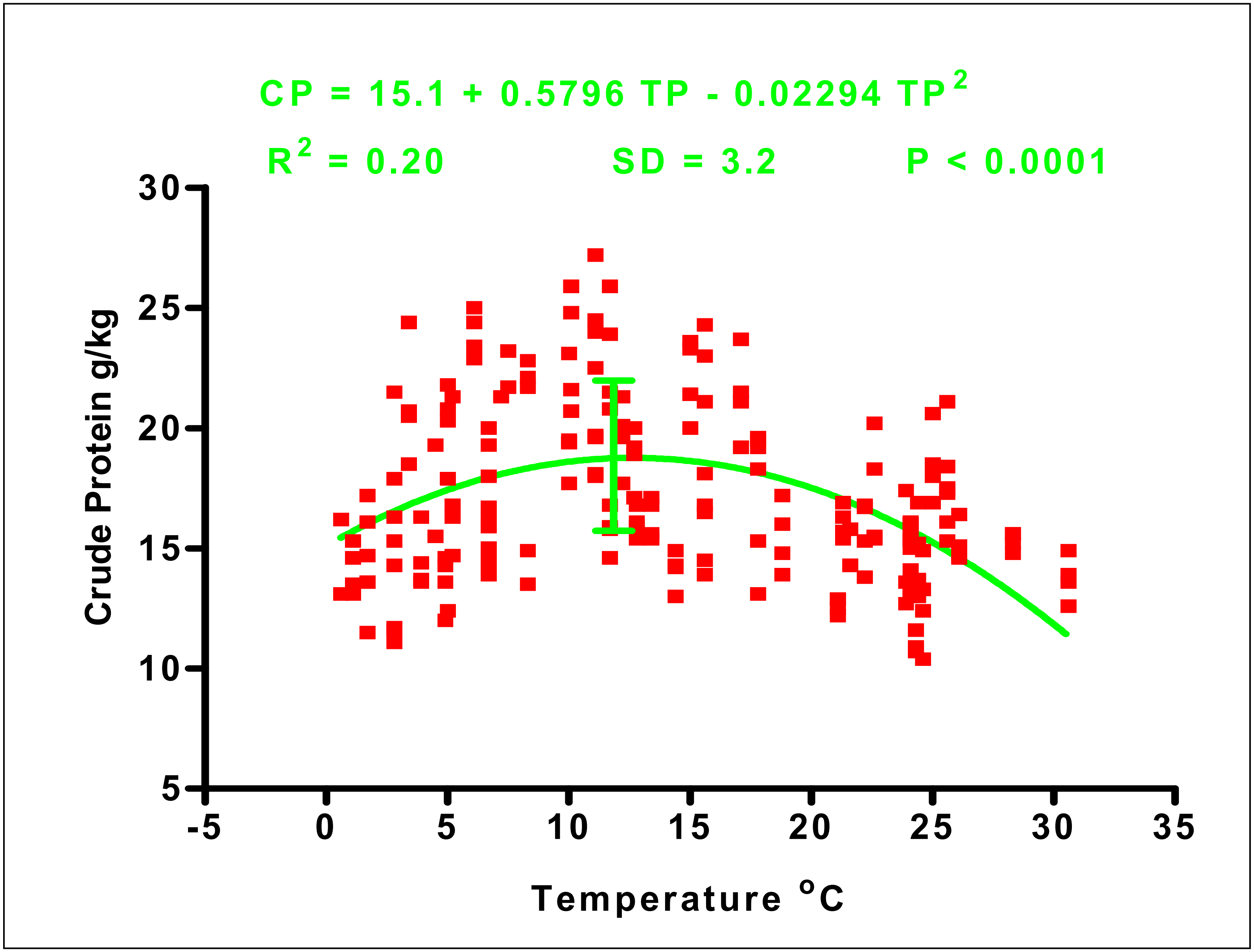

Figure 13 Quadratic curve relationship between crude protein and temperature.

Experiment II

Growth Rate and Puberty in Growing Thoroughbreds

by

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Hypothesis

Environmental factors including day length, temperature, and nutrition may affect the growth rate the growth rate, endocrine changes and onset of puberty in the young Thoroughbred.

Objectives

The objective of this study was to develop a temporal sequence of environmental, endocrine and physiological events that combine to initiate puberty.

Events or observations included in the time-line are plasma progesterone concentration, growth rate (ADG), day length, temperature, and pasture crude protein.

Materials and Methods

Animals and Management

Growth of all foals born at the Middleburg Agricultural Research and Extension Center (MAREC) is monitored for approximately 16 mo prior to the annual yearling auction in October. This protocol was approved by the Institutional Animal Care and Use Committee. Data from 3 y (1998, 2000 and 2001) for 31 fillies and 29 geldings were analyzed.

Annually, brood mares at MAREC are paired by weight, foaling date and sire, and then randomly assigned to two dietary groups; fat and fiber (FF) or sugar and starch (SS) (Table 9). These groups were kept on adjacent and similar mixed bluegrass/white clover pastures.

Mares were bred over a 9 w period from April 1st through the first week in June. The foaling season therefore extends from the end of March through the beginning of June.

Mares were placed in their respective dietary groups 3 mo prior to foaling and foaling approached the mares were placed in smaller individual paddocks for closer monitoring. Mares were maintained on the supplements until time of weaning, at which point the foals were continued on the supplements until October of the following year. Horses remained on pastures at all times, unless medical treatment was needed, in which case they were housed in stalls. Shelter was provided to each group by three sided run-in sheds (5.5 × 18.3 m) in each pasture. All horses in the pastures had ad libitum access to water. Mares and foals were on the anthelmintic, vaccination, and hoof trimming schedules at the

MAREC (Ley et al., 1992). Colts were gelded at 3 to 4 wk of age. Foals were weaned gradually, beginning at 6 mo, by the removal of two mares from each group every 4 d.

Supplements were designed to be isocaloric, with mineral and vitamin contents balanced to complement the pastures in central and north central Virginia and meet or exceed current recommendations (Griewe-Crandell et al., 1995; Hoffman and Kronfeld, 1999; NRC, 1989). The vitamin premix was formulated in collaboration with Dr. Theodore Frye and donated by Hoffman-LaRoche (Nutley, NJ).

The mares and foals were fed at 0700 and 1400 in feed tubs on the ground so that both had access to the supplement. Feed tubs were placed in a 30 m circle with (n+1) buckets available, where n was the number of mares, or weanlings in the pasture. Careful observation of feeding behavior indicated that some mares may have received more or less supplement than the desired amount, but the coefficient of variation in intake was approximately 10%, probably less than variation associated with daily pasture intake (Hoffman and Kronfeld, 1999). The amount of supplement varied in such a manner that a body condition of between 4.5 and 6 was maintained throughout the year. In order to maintain body condition, the supplement : forage ratio during mo of abundant pasture growth was 1:2, while during some of the very dry or cold mo it was increased to 1:1. For approximately a mo in February and March of 1998, yearlings were also given free access to mixed grass and alfalfa hay.

Environmental measures

Supplements were sampled every time a new batch was made, sample composites were made every 2 mo, and were submitted for proximate analysis (Table 1). Pastures were sampled monthly and were submitted for proximate analysis (Table 2 to 6,).

Day length was recorded from the US Naval Observatory for Middleburg VA, latitude 38º 58' 4.8" N, longitude -77º 44' 6.0" W (Fig 17). Temperature data was recorded from National Weather Service for the Washington Dulles International Airport (Fig 21).

Physical Measures

Foals were measured first at 24 h in 1998 and at 1 mo in 1999, 2000, 2001 and 2002. Subsequent measurements were taken at approximate 28 d intervals for the following 16 mo. Body weights were measured using a portable electronic walk on scale (Model TC-10S, Tyrel Corp.). Body condition was scored by one individual in each year of the experiment (Henneke et al., 1983). Other factors included in the analysis of growth were sex of foal, diet, year, month, season, day length, temperature, and age of foal.

Plasma Collection

Blood samples were taken each mo when the foals were brought in for measurements. The mares and foals were not fed on sampling mornings to eliminate any post prandial effect. Samples were drawn into 7 mL tubes (Lithium

Heparin Vacutainer, Becton Dickenson, Rutherford, NJ). Plasma was obtained by centrifugation and stored at -20°C for later analysis.

Hormonal Analysis

Plasma IGF-I concentrations were determined by previously described radioimmunoassay (Berry et al., 2001). Plasma IGF-I is separated from binding proteins using an acid-ethanol extraction (Breier, 1999). Plasma (100 µl) was mixed with an acid-ethanol extraction buffer (900 µl). Tubes were vortexed and centrifuged at 6,000 x g for 10 min. The supernatant (500 µl) was transferred to 12 x 75 glass tubes, and 200 µl of 0.855M Tris Base was added to each tube. These tubes were stored at -20°C for 1 h. Samples were centrifuged at 1,500 x g for 30 min, and decanted into 12 x 75 mm polypropylene tubes. Samples were stored at -20°C for later analysis by radioimmunoassay.

Recombinant human IGF-I used for standards and iodination was purchased from GrowPrep® (Adelaide, Australia). Mouse anti-human IGF-I antibody (1st antibody) was a gift of Dr. Bernard Laarveld (University of Saskatchewan). Goat anti-mouse anti-serum (2nd antibody) was purchased from Sigma Chemical Company (St. Louis, MO, USA). IGF-I was radioiodinated as described previously for α-lactalbumin (Akers et al., 1986).

For assay, standards or unknown samples were suspended in RIA buffer (30 mM sodium phosphate, 10 mM EDTA, 0.02% protamine sulfate, 0.05% Tween-20, pH 8.0) to a final volume of 500 µl. Subsequently, 100 µl of radiolabeled IGF-I (~30,000 dpm) and 100 µl of 1st antibody (1:21,000) were added to each tube. 1st antibody was diluted in mouse control serum. After 24 hr incubation at 4°C, 100 µl of 2nd antibody (1:20) was added. 2nd antibody was diluted in 0.05M EDTA-PBS at a pH of 7.5. Tubes were incubated for a further 72 hr at 4°C. Tubes had 1.5 ml of PBS added and were then centrifuged at 1,500 x g for 30 min. Tubes were then decanted and bound radioactivity was measured by gamma counting. The intraassay CV for IGF-I was 11%, and the interassay CV was 8%.

Plasma samples were analyzed for progesterone concentrations using duplicate serum samples with a previously validated, solid phase I^{125} radioimmunoassay kit (Coat-a-Count, ® DPC, Los Angeles, CA) without previous extraction. Intra- and inter-assay coefficients of variation were 4.9 % and 7.1 %, respectively.

Statistical Analysis

Normality was tested using the Shapiro-Wilk statistic. Progesterone distribution was not normal, so Spearman correlations were conducted to detect any associations between plasma progesterone concentrations and day length, crude protein in the pasture, plasma IGF-I concentrations, weight and ADG. Associations between the other variables were detected using Pearson correlations and a linear regression was fitted to ADG versus CP (SAS Inst. Inc., Cary, NC).

Predicted or interpolated values for day length , temperature and IGF-I for every day of the year (DOY), were calculated from original data taken on each measurement day, using the following sine wave equation, where B= baseline, H= amplitude, F=frequency and O=offset:

Variable = B+H*sin (F*DOY+O)

To determine a more precise timeline data sets for day length, temperature, and plasma IGF-I were shifted relative to ADG data to determine the best fit, or highest r-value.

Cause-effect relationships may be immediate or have intermediary steps that involve a delay (induction time or latent period), which may be brief or long without diminishing the degree of causation. Simply calculating correlation without adjusting for a delay, however, will reduce the degree of a correlation. An adjustment for the delay between variables was calculated to give a clearer indication of the strength of an association.

The time between variables (t, h) was adjusted for the delay (θ, h). So that the time factor becomes $t - \theta$ (Blaxter et al., 1956). The value of θ was determined by iteration using an index of goodness of fit such as adjusted R-square (Holland et al., 1998). It represents the time between a rise in a particular variable and a rise in ADG.

By analogy, θ may also be taken to represent an induction time or latent period between a cause and subsequent effect. The use of $t - \theta$ instead of t will increase the value of r or r^2, hence the likelihood of a causal relationship (Fig 16).

Results

Predicted values for day length (fig 18), IGF-I (Fig 20), and temperature (fig 22) simulated from actual data are graphically depicted. Day length and temperature show a sinusoidal pattern. Plasma IGF-I concentrations also show a similar pattern, which can be seen more clearly in the predicted data (Fig 19). The relationship between day of year (DOY) and day of age (DOA) can be seen for ADG in Figure 23 and 24. Average daily gain reaches a low point around 300 DOA which corresponds to 400 DOY.

Plasma progesterone concentrations in both the colts and fillies were median values of 1.03 ng/mL, range 0.07 ng/ml to 8.08 ng/ml 1 to 4 h after birth. These values decreased to basal concentrations of median 0.04 ng/mL, range 0.31 ng/ml within the first week of life for both sexes. The colts continued to exhibit basal plasma progesterone concentrations throughout the observational study. Visual inspection of the P4 data showed deviations from basal values starting to form in fillies between 10 and 13 mo of age, therefore correlations were conducted on data for the fillies > 230 d of age (Tables 17 to 29).

Progesterone concentrations greater than 3 standard deviations above baseline were detected in fillies at a mean age of 385 ± 6.4 d, 503 ± 5.7 DOY, day length 13 ± 0.1 h, IGF-I 194 ± 10.2 ng/mL, weight 381 ± 7.2 kg, and ADG 0.63 ± 0.04 kg/d.

Associations using raw data were tested on each year of the study as well as all 3 years combined. Positive associations were observed between day length, temperature, IGF-I and ADG (Tables 13 to 16).

Progesterone concentration positively correlated with day length (r = 0.60, $P < 0.0001$), temp (r = 0.57, $P < 0.0001$), IGF-I (r = 0.30, $P = 0.0005$) and ADG (r = 0.34, $P < 0.0001$).

A linear regression was fit to ADG versus CP. The strength of the association was average (r = 0.33) and the amount of variation in ADG accounted for by CP was small ($r^2 = 0.11$ (Fig. 14). When the data was shifted by 25d to fit the best fit of the relationship the association increased (r = 0.44) as did the amount of variation in ADG that could be accounted for by CP concentration in the pasture ($r^2 = 0.23$) (Fig. 15)

When associations were calculated to find the number of days between day length, temperature, and plasma IGF-I concentrations in relation to ADG, it was found that changes in day length occurred 51 d before ADG, temperature 16 d before ADG and IGF-I 17 d before ADG (Table 31).

The lowest point on a DOY scale in day length occurs at 349 d, temperature at 384d, plasma IGF-I concentrations at 383d and ADG at 400d, followed shortly after by an increase in progesterone concentrations in the fillies only at 503d (Fig 28 to 29).

Discussion

Elevated levels of P4 were recorded immediately after birth, this could be explained by the increase in progesterone produced by the placenta in the mare prior to birth which is rapidly metabolized to other progestogens that return to the fetus or cross the placenta and are metabolized further (Holten et al., 1991).

The results outline a set of associations; causal relationships may be inferred from a combination of factors including the time line (Fig 29) and from physiological mechanisms. Whilst the correlation coefficient (r) is only a test for association and causation can not be implied, the coefficient of determination (r^2) or the amount of variation in y that can be explained by x, can be used as evidence of causation. Using 4 of the 9 criteria set out by Rothman (1999) in his review of determining causation it is possible to begin to infer causation from the data given in this study.

The first of these criteria is the degree of association, the results indicated positive and relatively strong relationships between P4 and day length ($r = 0.60$, $P < 0.0001$), temp ($r = 0.57$, $P < 0.0001$), IGF-I ($r = 0.30$, $P = 0.0005$) and ADG ($r = 0.34$, $P < 0.0001$). The second criterion, repeatability, has been shown in the nature of the study, samples repeated monthly over a series of 3 yrs.

The third is the plausibility of explanatory mechanisms based on current understanding of physiological mechanisms. Day length exhibited an increase prior to all other variables. The female horse needs an alternating short and long photoperiod in order to be stimulatory in the onset of puberty, as demonstrated by studies using fixed levels of artificial light (Wesson and Ginther, 1982). In well nourished, rapidly growing female sheep the timing of puberty is primarily determined by day length, however if the animal is undernourished it is able to monitor day length yet unable to respond to stimulatory changes in photoperiod (Suttie et al., 1991).

Day length has been associated with increases in IGF-I in several species (Suttie et al., 1991 and Vriend et al., 1988). Extension of photoperiod from <13h/d to 18h/d of light, increases plasma concentrations of IGF-I in dairy cattle (Dahl et al., 1997). This supports the idea that day length and in particular melatonin levels act as a signal in controlling the endocrine systems involved in growth and reproduction (Dahl et al., 1997).

Insulin like growth factor-I has been proposed as one of the factors that signals nutritional status to the reproductive axis. Heifers fed low quality hay had decreased IGF-I concentrations that were positively associated with delayed puberty (Granger et al., 1989). Plasma IGF-I levels in humans usually correlate with body size, constitutional tall children have elevated plasma IGF-I concentrations (Gourmelen et al., 1984). Infusions of recombinant IGF-I enhances body weight and size in a number of models, supporting a role for IGF-I in growth (Blair et al., 1988).

Consistent with the associations found in this study it has been observed in other species, that plasma IGF-I levels increase prior to progesterone during the initiation of puberty (Lackey et al., 1999). In sheep IGF-I stimulates progesterone secretion by granulosa cells from large follicles (Monget and Moniaux, 1995). In the present study a low point in plasma IGF-I concentrations preceded a low point in ADG by approximately 17d.

Average daily gain increased between 10 and 11mo of age with delayed increase in P4 between 12 and 13 mo of age. Data reported from Sao Paulo, Brazil indicates a slightly later increase in ADG between 14 and 15 mo of age with no delay in the increase of progesterone (Nogueira et al., 1997). Yak heifers reach puberty between 24 to 60 mo of age, it has been suggested this late commencement of puberty was likely due to small BW at birth and slow growth rates during the prepubertal period (Yu and Li, 2001).

The fourth and possibly the most important criterion is temporality, which implies that the cause comes before the effect. A temporal sequence has been clearly shown using the time line outlined in figure 29. It was shown that a rise in day length preceded a rise in IGF-I concentration and temperature, which both occurred before an increase in ADG, all of these factors occurred prior to an increase in P4.

In conclusion puberty is influenced by environmental factors including nutrition, day length and temperature, also endocrine and growth factors such as IGF-I and ADG. The time line (Fig 29) developed from this study supports previous research in other species.

Implications

Thoroughbred mares are seldom bred before 4 years of age, so the timing of the onset of puberty has not the same implications that it has in production animals.

Puberty and the onset of estrus cycles in the mature mare are similar in many ways. The time line described in the present study will be useful for planning further studies of fertility and conception rate in older mares.

Table 9. Nutrient composition on a DM basis of the SS and FF supplements

Supplement	SS [1]		SS [2,3]		FF [1]		FF [2]		FF [3]	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
DM %	94.9	0.80	90.2	0.97	96.1	1.01	94.8	0.83	92.4	0.44
CP %	16.7	1.75	15.3	0.38	14.8	0.25	14.3	0.20	13.8	0.24
ADF %	10.7	1.20	11.2	0.99	21.1	0.17	22.1	0.66	24.3	0.54
NDF %	19.6	1.50	19.9	1.60	34.8	0.87	33.1	0.87	37.4	0.79
NSC %	55.3	5.00	50.4	3.43	28.6	1.82	23.0	1.27	26.0	1.27
C Fat %	1.00	0.24	3.30	0.27	9.70	0.80	11.3	0.63	15.0	0.76
Ash %	7.40	1.50	7.10	0.53	12.1	0.29	12.0	0.37	9.07	0.24
Ca %	1.30	0.30	1.30	0.12	2.36	0.19	2.39	0.12	1.67	0.04
P %	0.76	0.15	0.67	0.04	1.07	0.02	1.00	0.04	0.69	0.03
Ca:P	1.70	0.16	1.88	0.10	2.22	0.21	2.43	0.15	2.47	0.13
Mg %	0.23	0.03	0.23	0.02	0.57	0.01	0.60	0.03	0.44	0.01
K %	1.20	0.17	1.20	0.06	1.16	0.05	1.18	0.02	1.23	0.03
Na %	0.29	0.04	0.28	0.02	0.40	0.03	0.20	0.02	0.30	0.01
S %	0.23	0.02	0.20	0.01	0.20	0.01	0.20	0.01	0.20	0.01
Fe g/kg	396	91.0	298	22.7	426	6.24	328	40.6	311	20.9
Zn g/kg	146	25.1	152	11.0	166	2.60	167	10.0	166	6.50
Cu g/kg	33.7	4.91	37.6	5.29	29.0	2.52	29.0	1.45	37.6	1.50
Zn:Cu	4.30	0.15	4.51	0.23	5.79	0.49	5.91	0.35	4.50	0.22
Mn g/kg	57.7	9.40	64.3	11.6	215	26.0	223	11.7	162	8.12
Mo g/kg	1.63	0.09	1.72	0.19	0.97	0.55	0.62	0.21	3.24	2.32
DE Mcal/lb	3.0		3.0		3.0		3.0		3.0	
TDN %	79.3	1.45	78.5	1.25	66.3	0.33	65.0	0.82	62.1	0.71

[1] 1998, [2] 2000, [3] 2001; n = 6 per year

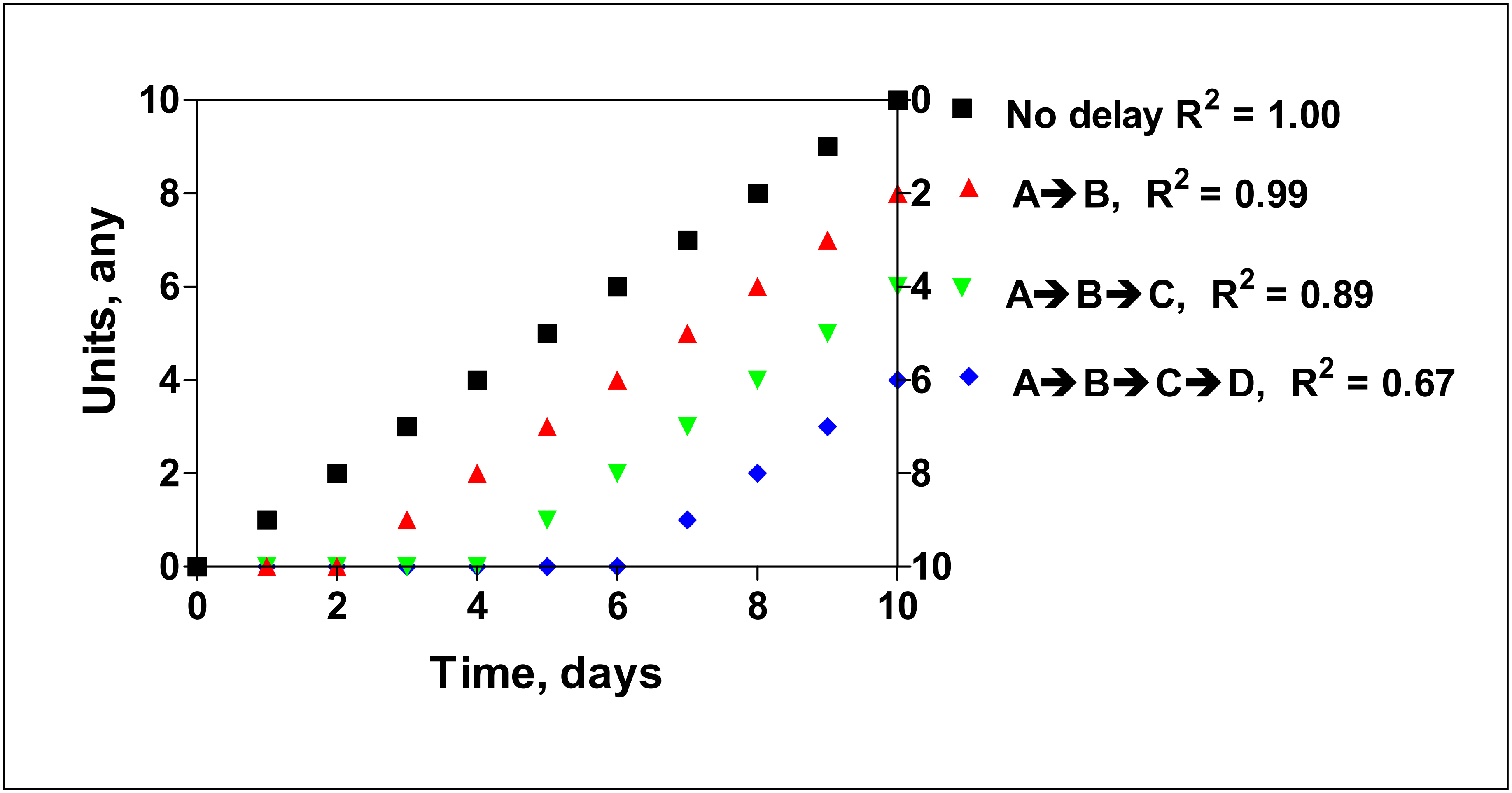

Figure 14 Differences in coefficient of determination (R^2) when a time delay is present.

Table 10. Forage composition for pastures at the MARE Center in 1998

Mo	Jan	SE	Mar	SE	Apr	SE	May	SE	Jun	SE	Jul	SE	Aug	SE	Sep	SE	Oct	SE	Nov	SE
DL[1]	9.26		11.0		12.4		13.7		14.8		14.2		13.1		11.60		10.3		9.40	
Temp	4.5	0.9	7.5	1.2	12.7	0.5	18.8	0.6	21.3	0.6	24.1	0.4	24.6	0.6	22.6	0.5	13.4	0.6	4.9	1.2
Rain[2]	13.8		14.2		0.5		0.7		14.9		3.4		1.1		0.0		3.8		3.1	
DM									33.0	1.64	39.1	3.63	50.2	4.41	36.1	2.50	42.5	1.92	50.3	1.75
CP	14.7	1.60	15.6	0.30	14.0	0.70	16.6	2.62	17.2	1.63	15.6	1.06	19.6	2.70	17.6	1.26	16.4	2.55	21.2	2.46
ADF	34.5	2.20	34.3	2.10	35.3	0.19	33.3	2.86	31.0	2.88	34.9	0.89	28.5	2.11	32.6	1.24	34.1	2.40	26.6	3.26
NDF	64.0	0.10	56.3	2.80	59.2	1.44	61.2	2.47	56.1	3.45	58.0	1.66	52.5	2.69	59.7	1.75	60.0	1.34	52.1	5.19
NFC	19.5				17.3				19.1	3.50	22.7	0.30	22.5		16.6	2.50	19.2	1.57		
NSC	7.70	0.50	18.4	2.50	15.4	3.20	12.0	2.10	14.1	2.64	12.3	1.15	14.7	1.81	10.2	1.53	8.83	2.46	12.2	4.80
Starch	0.80				0.70				2.55	1.15	3.15	0.25	2.30		0.55	0.35	2.23	0.57		
Sugar	6.40				6.20				8.90	0.40	7.30	0.70	14.2		8.75	2.45	9.00	1.46		
C Fat	2.65	0.45	2.35	0.15	2.33	0.18	2.55	0.56	2.95	0.49	3.00	0.56	3.38	0.30	2.80	0.38	3.05	0.23	3.80	0.17
Ash	7.13	1.33	7.28	0.36	7.83	1.16	7.80	1.12	8.53	1.14	7.70	0.80	9.31	0.40	8.94	0.42	9.34	1.94	10.8	0.42
Ca	0.51	0.04	0.57	0.01	0.59	0.03	0.50	0.02	0.50	0.02	0.52	0.04	0.51	0.02	0.50	0.02	0.55	0.02	0.54	0.05
P	0.28	0.06	0.30	0.01	0.25	0.01	0.32	0.06	0.34	0.05	0.38	0.03	0.33	0.02	0.34	0.03	0.37	0.03	0.33	0.06
Mg	0.15	0.01	0.18	0.02	0.20	0.03	0.18	0.01	0.22	0.13	0.21	0.02	0.18	0.01	0.22	0.00	0.22	0.02	0.19	0.01
K	1.07	0.20	1.37	0.55	1.19	0.21	1.46	0.56	2.38	0.13	2.34	0.14	2.50	0.02	2.40	0.17	2.52	0.08	2.36	0.11
Na	0.01	0.002	0.01	0.002	0.01	0.002	0.01	0.002	0.01	0.003	0.01	0.001	0.01	0.001	0.01	0.002	0.01	0.002	0.02	0.003
Fe	444	172	828	292	932	356	730	117	557	112	243	58	272	37	235	18.9	587	277	560	281
Zn	21.0	5.00	24.00	4.00	25.0	2.35	24.3	3.15	24.8	3.33	20.0	3.20	21.8	3.28	21.8	2.84	23.3	4.03	20.5	3.66
Cu	3.50	0.50	5.00	0.00	5.25	1.80	3.75	0.48	3.75	0.63	3.50	0.65	3.75	0.25	4.25	0.75	4.75	0.48	0.25	0.63
Mn	70.5	13.5	75.5	13.5	76.5	7.67	64.5	6.81	48.3	7.97	40.8	2.96	43.5	3.48	44.8	8.38	60.8	20.2	53.3	6.97
Mo			1.75	1.75	0.28	0.28	0.33	0.33	0.55	0.32	0.55	0.32					0.25	0.25	0.78	0.46
S	0.21				0.22	0.01	0.20	0.04	0.23		0.24	0.01	0.24	0.03	0.26	0.01	0.27		0.25	0.01

[1]DL h, Temp ºC, DM %, CP %, ADF %, NDF %, NFC %, NSC %, Starch %, Sugar %, C fat %, Ash %, Ca %, P %, Mg %, K %, Na %, Fe g/kg, Zn g/kg, Cu g/kg, Mn g/kg, Mo g/kg, S %, monthly averages.
[2]Rainfall cm, actual amount per mo

Table 11. Forage composition for pastures at the MARE Center in 1999

Mo	Jan	SE	Feb	SE	Apr	SE	May	SE	Jun	SE	Jul	SE	Aug	SE	Sep	SE	Oct	SE	Nov	SE	Dec	SE
DL[1]	9.42		9.87		12.4		13.9	0.03	14.7		14.7		13.9		13.0		10.9		9.76		9.23	
Temp	1.7	1.0	3.1	0.8	11.7	0.5	17.1	0.5	21.6	0.5	25.7	0.6	24.1	0.7	19.4	0.6	11.7	0.5	10.1	0.7	3.4	0.8
Rain[2]	13.7		6.7		8.7		6.8		5.6		1.7		2.7		13.8		23.7		6.5		4.4	
DM					44.9	13.2	19.4	6.26	32.9	0.74	50.4	2.29	43.8	1.84	50.2	5.62	20.9	0.60	25.6	1.44	22.6	1.25
CP	17.2	4.20	17.1	1.53	20.7	1.44	17.6	1.10	19.9	2.38	16.9	1.67	19.5	1.61	23.6	0.60	20.7	2.54	17.3	0.58	20.4	0.70
ADF	32.3	1.70	32.9	2.08	30.0	2.13	32.4	1.39	30.7	2.13	32.2	1.89	29.9	2.41	27.4	2.38	30.0	2.62	31.7	0.57	30.8	2.03
NDF	56.8	2.05	55.9	2.90	54.0	2.17	57.9	1.66	57.3	3.45	55.0	1.35	56.5	3.03	51.3	1.97	55.0	3.65	59.6	1.82	54.0	3.49
NFC	20.5		20.4	1.05	17.1		17.4	3.10	14.9	1.30	20.8	0.50			16.4						19.9	4.75
NSC	14.2	3.35	14.7	1.85	12.2	0.35	10.5	1.09	7.40	3.34	15.2	1.24	10.2	1.46	11.3	0.96	10.8	1.25	11.0	2.26	11.4	2.56
Starch	0.90		1.63	0.55	2.20		1.25	1.05	0.93	0.38	0.70				1.10						1.30	0.40
Sugar	16.7		14.5	1.51	9.30		9.00	1.60	6.53	3.20	16.7	0.15			9.40						13.6	2.05
C Fat	3.05	0.45	3.38	0.37	2.88	0.57	3.73	0.67	4.47	0.12	3.70	0.24	3.55	0.17	4.03	0.57	3.75	0.65	3.90	0.10	3.40	0.22
Ash	9.35	1.22	9.36	0.43	10.4	0.35	9.37	0.13	9.36	0.53	9.49	0.72	10.3	0.33	9.89	0.17	9.85	0.55	8.19	0.40	11.2	0.89
Ca	0.39	0.06	0.49	0.05	0.47	0.02	0.54	0.02	0.54	0.04	0.49	0.05	0.47	0.01	0.58	0.03	0.47	0.03	0.44	0.03	0.49	0.03
P	0.33	0.04	0.32	0.01	0.39	0.03	0.43	0.05	0.44	0.04	0.32	0.03	0.38	0.01	0.51	0.03	0.40	0.04	0.46	0.07	0.36	0.04
Mg	0.20	0.04	0.19	0.02	0.17	0.01	0.19	0.01	0.23	0.02	0.18	0.01	0.20	0.01	0.24	0.02	0.20	0.01	0.16		0.21	0.03
K	2.62	0.05	2.53	0.14	2.73	0.26	2.82	0.30	2.83	0.28	2.73	0.05	2.61	0.19	3.22	0.06	2.54	0.27	2.70	0.14	2.69	0.33
Na	0.01	0.003	0.01	0.003	0.01	0.003	0.01	0.001	0.01	0.001	0.01	0.003	0.01	0.002	0.01	0.002	0.01	0.004	0.01	0.004	0.01	0.002
Fe	218	17.0	384	138	398	109	297	36.9	359	30.6	356	83.3	286	81.5	282	40.3	295	54.0	445	144	542	121
Zn	19.0	2.00	23.0	3.39	23.5	4.17	30.0	4.71	36.3	4.33	20.0	0.71	17.3	1.18	34.0	5.12	26.0	1.47	33.8	9.23	36.0	10.2
Cu	5.00	2.00	4.75	0.25	5.50	1.19	5.50	0.65	5.67	0.33	5.50	0.65	4.00	0.41	6.25	0.63	4.50	0.65	5.50	0.65	4.50	0.65
Mn	39.0	8.00	50.3	7.49	52.0	8.82	36.5	2.90	49.3	6.89	52.5	7.72	42.8	4.33	48.0	4.67	47.3	3.86	41.3	2.56	52.0	3.27
Mo					0.40	0.40	0.33	0.33	0.43	0.43					1.78	1.02	0.73	0.43	0.53	0.30	0.50	0.50
S	0.30		0.20		0.25	0.01	0.22	0.01			0.22	0.04	0.25	0.03	0.26	0.03	0.24	0.03	0.19	0.01	0.27	

[1]DL h, Temp ºC, DM %, CP %, ADF %, NDF %, NFC %, NSC %, Starch %, Sugar %, C fat %, Ash %, Ca %, P %, Mg %, K %, Na %, Fe g/kg, Zn g/kg, Cu g/kg, Mn g/kg, Mo g/kg, S %, monthly averages.
[2]Rainfall cm, actual amount per mo

Table 12. Forage composition for pastures at the MARE Center in 2000

Mo	Jan	SE	Mar	SE	Apr	SE	May	SE	Jun	SE	Jul	SE	Aug	SE	Sep	SE	Oct	SE	Nov	SE	Dec	SE
DL[1]	9.33		11.4		13.0		14.0		14.8		14.2		13.3		12.0		10.5		9.70		9.24	0.01
Temp	2.8	1.2	8.8	0.7	11.8	0.7	18.1	0.6	24.4	0.5	25.6	0.4	25.6	0.5	18.3	0.7	11.1	0.8	6.7	0.8	1.1	0.5
Rain[2]	4.5		1.9		8.5		11.1		6.5		10.3		9.7		15.0		10.8		5.8		4.0	
DM	32.3	1.04	29.9	2.13	43.6	9.56	22.1	0.88	31.0	1.93	20.7	2.42	24.9	0.90	25.2	1.05	12.5	0.36	33.2	1.87	51.7	2.61
CP	20.2	0.84	17.1	1.21	21.5	0.84	19.6	2.80	20.7	1.65	18.4	1.03	15.3	1.57	17.8	1.23	14.6	0.63	19.1	1.02	17.5	1.94
ADF	27.8	0.65	32.1	1.52	28.0	1.65	31.5	2.77	28.6	1.85	31.1	2.01	35.6	1.79	32.1	1.74	30.8	1.77	31.6	1.15	31.9	1.73
NDF	51.0	2.18	57.9	2.64	51.0	3.16	53.5	4.64	52.1	2.14	55.5	1.55	59.1	1.23	57.9	2.84	56.4	3.64	57.1	1.42	55.9	0.93
NFC	23.6		17.2	1.10	19.9	2.87	19.4	2.57			16.9		20.1	0.99	16.1	3.60	24.3		19.0	2.14	18.1	
NSC	14.8	1.74	11.9	1.40	15.0	0.71	12.0	1.27	13.2	1.48	13.7	0.74	10.4	1.36	12.0	2.60	14.2	4.41	8.60	1.62	16.1	1.34
Starch	2.30		1.30	0.10	2.57	0.09	1.53	0.38			0.90		2.00	0.36	1.90	1.10	1.70		1.17	0.32	1.20	
Sugar	15.3		8.15	0.55	12.3	1.07	10.5	1.42			10.7		7.07	0.44	8.20	0.50	1.60		8.47	1.44	11.0	
C Fat	3.30	0.32	2.45	0.25	2.88	0.67	3.28	0.73	3.65	0.31	3.23	0.34	3.38	0.08	3.40	0.39	2.85	0.27	3.58	0.21	2.58	0.35
Ash	10.4	0.47	9.41	0.56	10.1	0.30	9.80	0.55	10.4	0.91	9.25	2.10	7.45	0.40	8.60	0.33	7.69	0.44	8.69	0.51	8.14	0.18
Ca	0.54	0.04	0.59	0.03	0.66	0.05	0.56	0.04	0.49	0.03	0.51		0.59	0.05	0.51	0.03	0.51	0.07	0.50	0.03	0.47	0.02
P	0.35	0.04	0.28	0.02	0.38	0.04	0.34	0.05	0.38	0.03	0.35	0.03	0.34	0.03	0.33	0.04	0.30	0.02	0.34	0.03	0.30	0.02
Mg	0.19	0.01	0.20	0.01	0.20	0.01	0.21	0.01	0.18	0.02	0.20	0.01	0.20	0.01	0.19	0.01	0.19	0.01	0.19	0.01	0.17	0.01
K	2.68	0.17	2.15	0.13	2.86	0.45	2.47	0.36	2.37	0.03	2.43	0.16	2.26	0.11	2.08	0.11	2.12	0.08	2.02	0.13	1.91	0.07
Na	0.01	0.001	0.006	0.001	0.03	0.02	0.01	0.001	0.01	0.001	0.01	0.001	0.02	0.007	0.01	0.004	0.01	0.004	0.01	0.001	0.01	0.001
Fe	407	109	536	167	321	105	591	88.3	344	87.0	618	189	231	51.9	443	165	516	245	576	218	256	65.7
Zn	25.8	1.97	28.0	3.81	35.8	4.33	28.5	2.66	22.0	1.29	33.0	8.13	27.3	11.6	32.3	8.44	38.3	17.1	28.3	2.29	18.3	1.75
Cu	4.50	0.65	3.50	0.29	7.25	1.97	5.50	0.87	4.00	0.71	4.25	0.25	4.25	0.48	4.50	0.28	3.25	0.75	5.25	0.25	3.75	0.48
Mn	55.3	9.84	57.8	7.49	50.0	4.51	68.8	8.08	52.3	4.31	50.3	2.66	49.3	5.66	51.5	3.59	48.5	7.89	63.0	7.84	56.8	0.63
Mo					0.58	0.33	0.40	0.40	0.28	0.28	0.55	0.32			0.33	0.33						
S	0.25	0.02	0.22		0.24		0.33		0.24	0.01	0.23	0.02	0.20		0.25	0.03	0.20	0.01	0.23		0.22	0.02

[1]DL h, Temp ºC, DM %, CP %, ADF %, NDF %, NFC %, NSC %, Starch %, Sugar %, C fat %, Ash %, Ca %, P %, Mg %, K %, Na %, Fe g/kg, Zn g/kg, Cu g/kg, Mn g/kg, Mo g/kg, S %, monthly averages.
[2]Rainfall cm, actual amount per mo

Table 13. Forage composition for pastures at the MARE Center in 2001

Mo	Jan	SE	Feb	SE	Mar	SE	Apr	SE	May	SE	Jun	SE	Jul	SE	Aug	SE	Sep	SE	Oct	SE	Dec	SE
DL[1]	9.50		10.9		11.9	0.03	13.3		14.2		14.8		14.3		13.3		11.9		10.5		9.24	
Temp	1.1	0.6	3.6	0.7	4.7	0.6	13.1	0.8	17.0	0.6	22.2	0.6	22.3	0.5	24.7	0.5	18.6	0.5	13.2	0.7	5.2	0.9
Rain[2]	5.7		4.3		10.6		5.5		14.5		12.3		10.8		11.6		8.7		3.6		2.1	
DM	71.0	7.36	69.3	1.09	56.7	3.30	23.2	1.04	28.6	1.12	26.3	1.98	31.9	0.97	31.9	0.54	34.8	1.34	29.6	0.73	18.7	0.47
CP	16.8	0.66	17.0	3.25	14.9	0.08	17.2	2.18	15.7	0.68	17.0	1.13	15.3	0.80	15.2	0.15	15.0	1.09	14.2	0.73	13.1	10.7
ADF	31.5	1.86	33.2	3.95	35.7	0.62	31.7	3.03	33.0	1.61	33.0	1.02	32.7	1.40	35.2	0.12	36.2	0.90	35.2	0.97	36.6	0.74
NDF	55.5	1.49	57.6	5.00	61.9	1.70	57.2	1.01	57.8	1.35	61.4	1.92	57.7	1.70	60.0	2.03	65.5	0.71	62.1	3.13	62.2	2.87
NFC					16.6	0.90	19.1		18.5		13.3	1.20					12.7		18.5	2.15		
NSC	15.9	1.38	15.2	1.10	11.1	1.85	13.9	2.08	11.5	1.69	10.5	1.71	16.3	0.96	13.7	2.58	8.6	0.91	12.8	2.92	14.8	2.65
Starch					1.10	0.10	1.20		0.70		1.35	0.05					1.40		1.40	0.20		
Sugar					8.23	0.90	9.90		7.00		7.85	1.15					8.70		10.8	0.35		
C Fat	2.73	0.06	2.20	0.20	2.20	0.42	2.50	0.54	2.43	0.42	3.20	0.20	2.25	0.33	2.53	0.34	2.98	0.22	2.48	0.34	2.33	0.34
Ash	9.09	0.66	8.06	0.44	8.37	0.17	8.48	0.35	11.1	1.42	8.68	0.52	8.50	1.03	8.56	0.45	8.75	0.46	7.87	0.24	7.73	0.35
Ca	0.56	0.03	0.44	0.07	0.51	0.03	0.46	0.02	0.58	0.04	0.51	0.03	0.54	0.05	0.54	0.05	0.61	0.08	0.64	0.05	0.69	0.09
P	0.33	0.02	0.32	0.03	0.30	0.02	0.38	0.06	0.30	0.02	0.34	0.02	0.27	0.01	0.28		0.32	0.03	0.25	0.02	0.27	0.02
Mg	0.22	0.01	0.19	0.04	0.23	0.01	0.20	0.01	0.22	0.02	0.18	0.02	0.21	0.02	0.22	0.02	0.21	0.03	0.20	0.01	0.22	0.02
K	1.92	0.11	1.88	0.01	1.99	0.16	2.18	0.22	1.93	0.17	1.79	0.11	1.75	0.21	1.74	0.09	1.62	0.09	1.50	0.09	1.67	0.19
Na	0.05	0.04	0.02	0.01	0.01	0.002	0.004	0.001	0.02	0.01	0.01	0.01	0.01	0.004	0.01	0.003	0.01	0.003	0.01	0.003	0.02	0.01
Fe	532	231	228	89.5	554	89.7	424	154	840	353	652	321	465	144	288	71.4	293	124	569	199	302	62.9
Zn	59.3	35.0	21.5	2.50	30.3	3.97	28.5	6.46	23.3	1.03	26.0	3.49	24.0	3.85	18.0	0.58	18.0	1.30	48.0	26.0	18.0	2.45
Cu	9.75	6.45	3.50	0.50	4.25	0.48	4.25	0.95	4.00	0.41	4.00		3.50	0.29	3.00		2.75	0.25	4.25	0.75	4.25	1.03
Mn	54.5	4.09	53.5	7.50	57.8	6.97	54.8	5.59	68.5	12.3	58.0	6.29	59.3	6.09	56.7	3.28	66.3	4.11	73.8	9.3	61.5	13.1
Mo	0.28	0.28					0.25	0.25	0.25	0.25			0.68	0.40							0.40	0.40
S	0.24	0.02	0.26	0.06	0.23		0.24	0.01	0.23	0.02	0.23	0.01	0.23	0.02	0.23	0.02	0.25		0.23	0.01	0.21	

[1]DL h, Temp ºC, DM %, CP %, ADF %, NDF %, NFC %, NSC %, Starch %, Sugar %, C fat %, Ash %, Ca %, P %, Mg %, K %, Na %, Fe g/kg, Zn g/kg, Cu g/kg, Mn g/kg, Mo g/kg, S %, monthly averages.
[2]Rainfall cm, actual amount per mo

Table 14. Forage composition for pastures at the MARE Center in 2002

Mo	Jan	SE	Feb	SE	Mar	SE	Apr	SE	May	SE	Jul	SE	Aug	SE	Sep	SE	Oct	SE	Nov	SE	Dec	SE
DL[1]	9.69		10.9		11.4	0.06	12.2	0.03	14.1	0.11	14.3		13.7		11.8		10.8		9.50	0.01	9.25	
Temp	3.9	0.9	5.3	1.0	7.8	0.9	13.8	0.9	16.8	0.7	25.2	0.5	25.5	0.7	20.6	0.4	12.8	0.9	6.9	0.8	0.8	0.6
Rain[2]	3.3		2.6		2.5		8.8		12.1		9.2		6.7		7.2		5.4		12.9		10.5	
DM	71.0	12.2	61.9	3.16	57.2	0.90	44.3	2.81	23.3	1.56	29.9	1.81	40.7	2.03	34.3	1.12	21.7	0.53	20.7	4.07	31.9	1.40
CP	11.8	0.95	15.2	1.05	14.4	0.71	13.1	1.13	15.1	1.46	17.0	2.06	17.7	2.45	16.2	1.26	17.9	0.99	14.8	0.44	15.3	0.92
ADF	35.7	1.70	34.6		35.5	0.81	35.1	0.78	33.8	1.62	34.7	1.90	34.0	2.26	34.9	1.98	30.4	2.22	36.3	0.65	36.4	0.40
NDF	63.7	2.83	62.3	4.05	61.4	1.58	61.3	2.09	58.8	1.68	58.6	3.68	60.0	3.38	58.7	2.85	53.1	2.47	60.4	1.14	63.0	1.46
NFC			21.5		18.5	1.25			20.9										15.3		14.2	1.07
NSC	14.1	2.00	10.1	1.55	11.5	1.57	16.7	1.61	15.5	0.75	12.9	1.24	9.83	1.82	10.3	3.51	17.2	1.28	12.7	2.12	6.98	0.79
Starch			0.90		1.07	0.33			1.40										0.70		0.50	0.17
Sugar			10.7		8.77	0.73			11.0										6.00		6.48	0.63
C Fat	2.45	0.14	2.75	0.75	2.32	0.18	2.48	0.35	2.55	0.26	2.95	0.56	3.48	0.44	3.20	0.62	4.53	0.28	3.43	0.14	3.48	0.11
Ash	8.04	0.46	7.32	0.30	8.43	0.75	6.55	0.60	7.50	0.57	8.55	1.02	9.05	1.13	11.6	0.26	7.38	0.23	7.86	0.26	9.35	0.54
Ca	0.47	0.01	0.67	0.10	0.52	0.03	0.56	0.07	0.53	0.02	0.59	0.04	0.54	0.03	0.61	0.05	0.54	0.02	0.53	0.01	0.60	0.04
P	0.27	0.02	0.29	0.04	0.24	0.01	0.26	0.20	0.30	0.03	0.32	0.05	0.61	0.03	0.29	0.03	0.35	0.30	0.32	0.02	0.35	0.03
Mg	0.21	0.01	0.19	0.03	0.16	0.01	0.19	0.01	0.18	0.01	0.18	0.01	0.78	0.01	0.19	0.01	0.18	0.004	0.18	0.004	0.21	0.02
K	1.64	0.11	1.38	0.23	1.23	0.09	1.46	0.11	1.55	0.36	1.64	0.59	1.58	0.30	1.71	0.53	2.47	0.37	2.33	0.07	2.29	0.18
Na	0.01	0.003	0.03	0.02	0.02	0.01	0.01	0.003	0.01	0.002	0.01	0.01	0.01	0.001	0.01	0.001	0.01	0.002	0.04	0.04	0.01	0.002
Fe	290	76.1	397	67.0	809	370	387	164	579	154	1938	542	2205	853	2281	1291	800	451	402	45.9	271	47.7
Zn	13.8	0.95	29.5	9.50	23.2	3.49	18.5	3.50	20.5	1.61	35.5	8.61	61.0	30.1	42.3	5.30	47.8	5.27	46.8	15.0	21.5	0.65
Cu	3.00	0.71	3.50	0.50	4.17	0.95	3.75	0.63	4.63	0.82	7.25	1.25	8.00	0.82	9.00	2.74	6.00	0.41	5.75	0.48	5.50	0.50
Mn	62.8	7.00	75.0	24.0	78.0	17.1	88.5	29.3	65.1	8.51	102	16.3	99.0	7.04	116	25.7	73.8	6.66	66.0	9.94	45.8	3.94
Mo	0.40	0.40					0.90	0.90	0.74	0.34	1.38	1.38	0.98	0.56	0.28	0.28	1.45	0.61	1.05	0.35		
S	0.20		0.22	0.02	0.23	0.01	0.16	0.02	0.20	0.02	0.21	0.03	0.25	0.02	0.23	0.01	0.23	0.01	0.21	0.01		

[1]DL h, Temp ºC, DM %, CP %, ADF %, NDF %, NFC %, NSC %, Starch %, Sugar %, C fat %, Ash %, Ca %, P %, Mg %, K %, Na %, Fe g/kg, Zn g/kg, Cu g/kg, Mn g/kg, Mo g/kg, S %, monthly averages.
[2]Rainfall cm, actual amount per mo

Table 15. Associations between environmental and physiological variables for 1998

	DL	Temp	CP%	IGF-I	ADG
DL[1]	1.00	0.85 <0.0001	-0.12 0.03	0.27 <0.0001	0.36 <0.0001
Temp		1.00	-0.20 0.002	0.37 <0.0001	0.42 <0.0001
CP%			1.00	-0.09 0.08	0.10 0.07
IGF-I				1.00	0.22 0.0002
ADG					1.00

[1]DL – day length, Temp – temperature, CP% - crude protein percent, IGF-I – insulin like growth factor 1, ADG – average daily gain

Table 16. Associations between environmental and physiological variables for 2000

	DL	Temp	CP%	ADG
DL	1.00	0.92 <0.0001	-0.02 0.65	0.42 <0.0001
Temp		1.00	0.02 0.79	0.51 <0.0001
CP%			1.00	0.15 0.01
ADG				1.00

Table 17. Associations between environmental and physiological variables for 2001

	DL	Temp	CP%	IGF-I	ADG
DL	1.00	0.86 <0.0001	-0.11 0.03	0.37 <0.0001	0.35 <0.0001
Temp		1.00	-0.22 <0.0001	0.33 <0.0001	0.39 <0.0001
CP%			1.00	-0.04 0.45	0.15 0.008
IGF-I				1.00	0.11 0.07
ADG					1.00

Table 18. Associations between environmental and physiological variables for 1998, 2000, and 2001 combined

	DL	Temp	CP	IGF-I	ADG
DL	1.00	0.87 <0.0001	-0.09 0.006	0.32 <0.0001	0.37 <0.0001
Temp		1.00	-0.12 0.0001	0.35 <0.0001	0.43 <0.0001
CP			1.00	-0.07 0.08	0.14 <0.0001
IGF-I				1.00	0.15 0.0002
ADG					1.00

Table 19. Associations between environmental endocrinologic and growth variables for fillies >230 d in 1998

	DL	Temp	CP%	IGF-I	P4	ADG
DL	1.00	0.91 <0.0001	0.17 0.02	0.43 <0.0001	0.61 <0.0001	0.40 <0.0001
Temp		1.00	-0.26 0.017	0.47 <0.0001	0.56 <0.0001	0.22 0.0247
CP%			1.00	-0.22 0.042	-0.25 <0.0065	0.41 0.0001
IGF-I				1.00	0.40 0.0006	0.30 0.004
P4					1.00	0.19 0.329
ADG						1.00

Table 20. Associations between environmental endocrinologic and growth variables for fillies >230 d in 2000

	DL	Temp	CP	P4	ADG
DL	1.00	0.96 <0.0001	0.45 <0.0001	0.31 0.0008	0.32 0.0002
Temp		1.00	0.31 0.0003	0.57 <0.0001	0.41 0.0015
CP			1.00	0.56 <0.0001	0.17 0.05
P4				1.00	0.44 0.0012
ADG					1.00

Table 21. Associations between environmental endocrinologic and growth variables for fillies >230 d in 2001

	DL	Temp	CP%	IGF-I	P4	ADG
DL	1.00	0.89 <0.0001	0.37 0.0006	0.30 0.0036	0.49 0.0001	0.32 0.002
Temp		1.00	0.21 0.05	0.15 0.17	0.53 <0.0001	0.29 0.005
CP%			1.00	0.12 0.27	-0.22 0.02	0.003 0.98
IGF-I				1.00	0.20 0.13	0.19 0.08
P4					1.00	0.33 0.0006
ADG						1.00

Table 22. Associations between environmental endocrinologic and growth variables for fillies >230 d in 1998, 2000, and 2001 combined

	DL	Temp	CP%	IGF-I	P4	ADG
DL	1.00	0.92 <0.0001	0.25 <0.0001	0.38 <0.0001	0.60 <0.0001	0.38 <0.0001
Temp		1.00	0.08 0.25	0.20 0.025	0.57 <0.0001	0.30 <0.0001
CP%			1.00	-0.02 0.73	-0.09 0.25	0.23 <0.0001
IGF-I				1.00	0.30 0.0005	0.24 0.001
P4					1.00	0.34 <0.0001
ADG						1.00

Table 23. Associations between ADG and environmental and endocrine measures using predicted data

D0	D43	**D44**	D45
0.36782 <0.0001	0.47654 <0.0001	**0.47661** **<0.0001**	0.47656 <0.0001
T0	T15	**T16**	T17
0.46126 <0.0001	0.47659 <0.0001	**0.47661** **<0.0001**	0.47649 <0.0001
I0	I16	**I17**	I18
0.36472 <0.0001	0.38599 <0.0001	**0.38613** **<0.0001**	0.38612 <0.0001

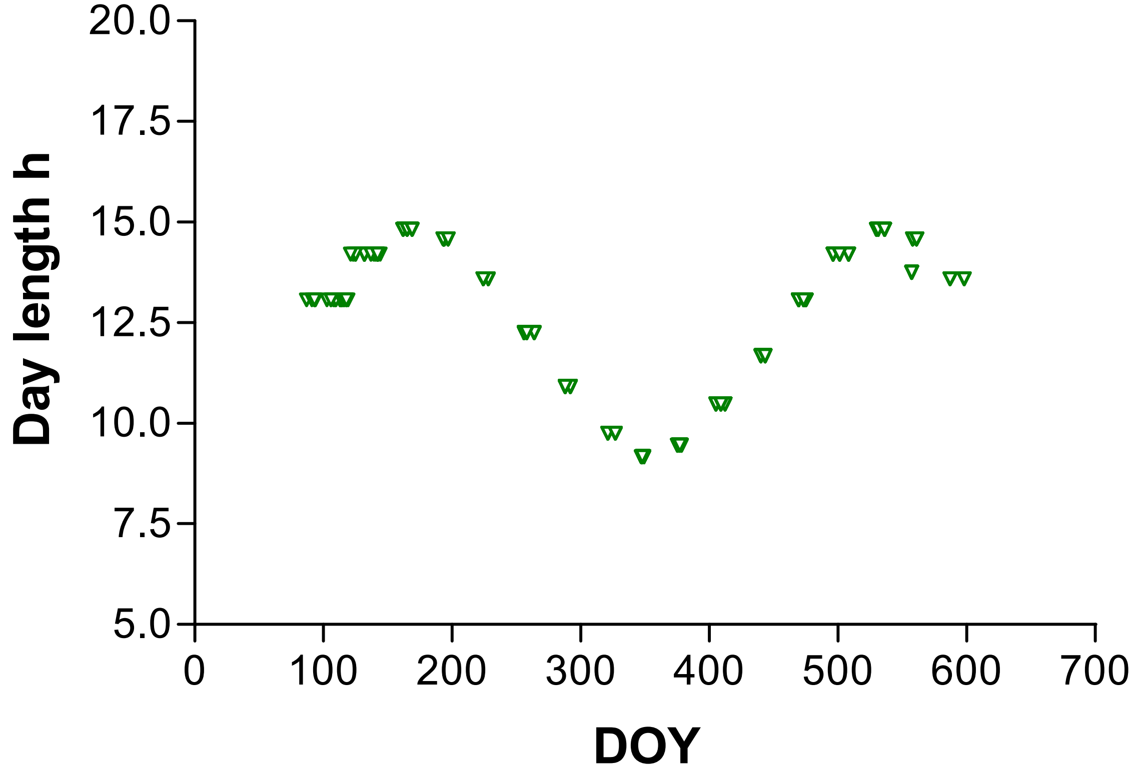

Figure 15 Actual day length for day of year

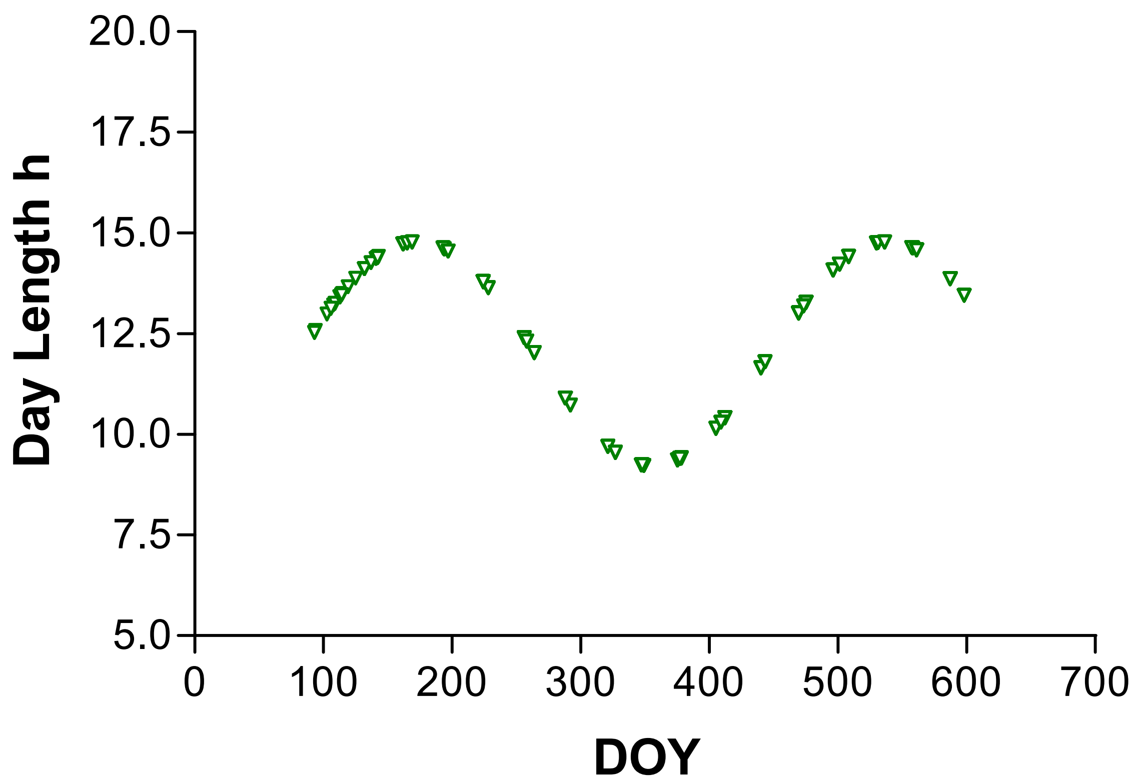

Figure 16 Predicted day length for day of year

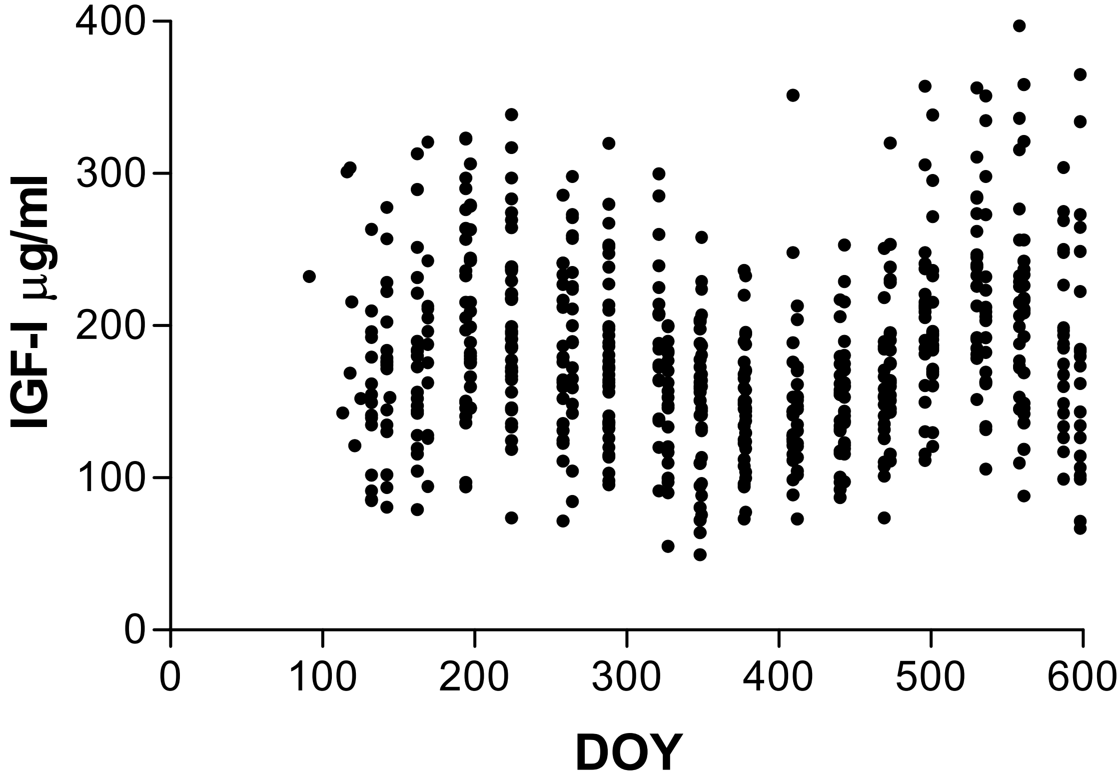

Figure 17 Actual IGF-I for day of year

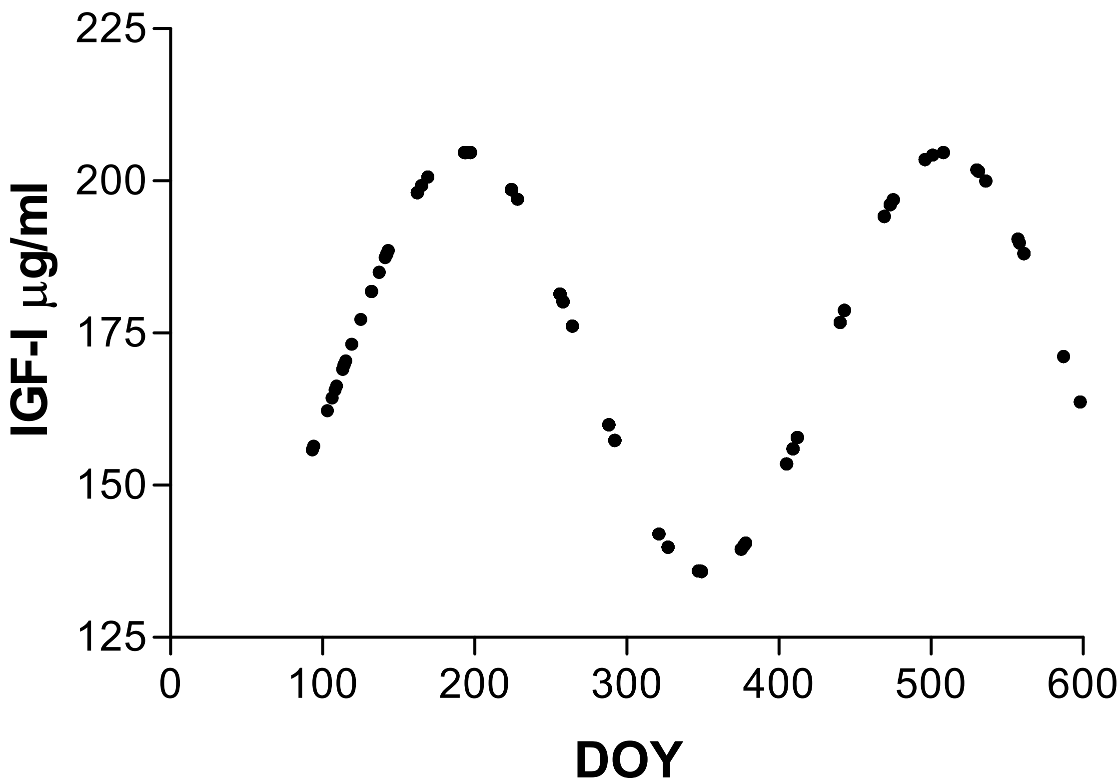

Figure 18 Predicted IGF-I for day of year

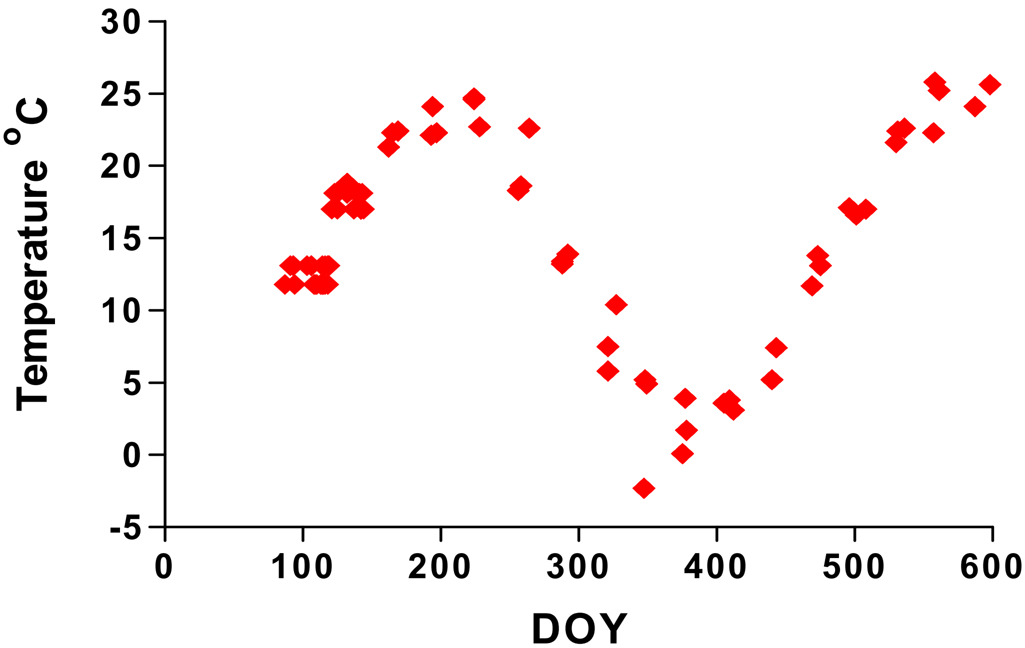

Figure 19 Actual temperature for day of year

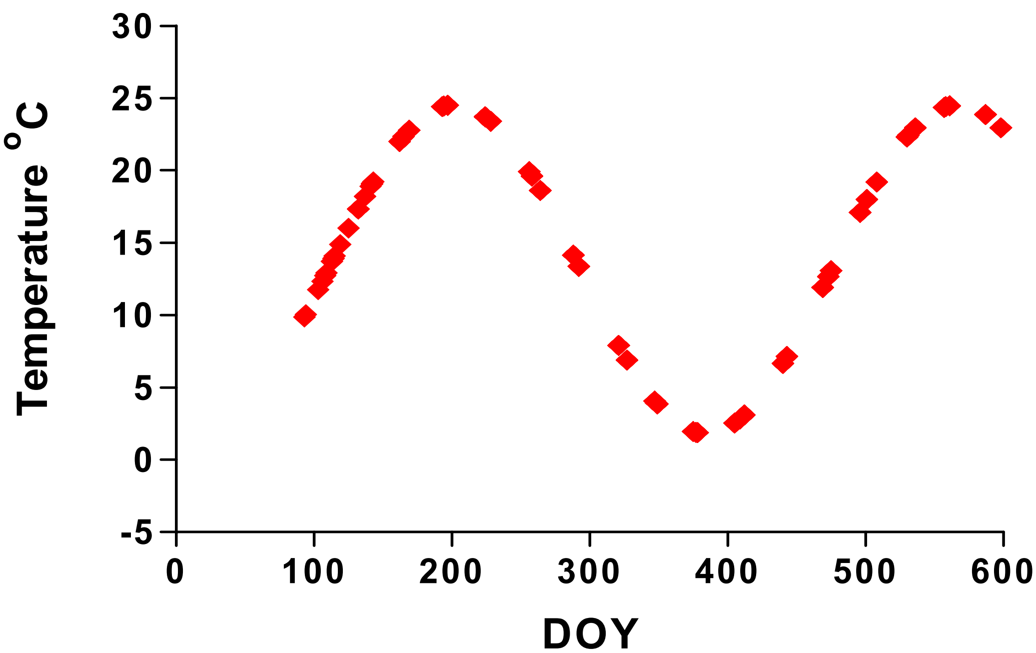

Figure 20. Predicted temperature for day of year

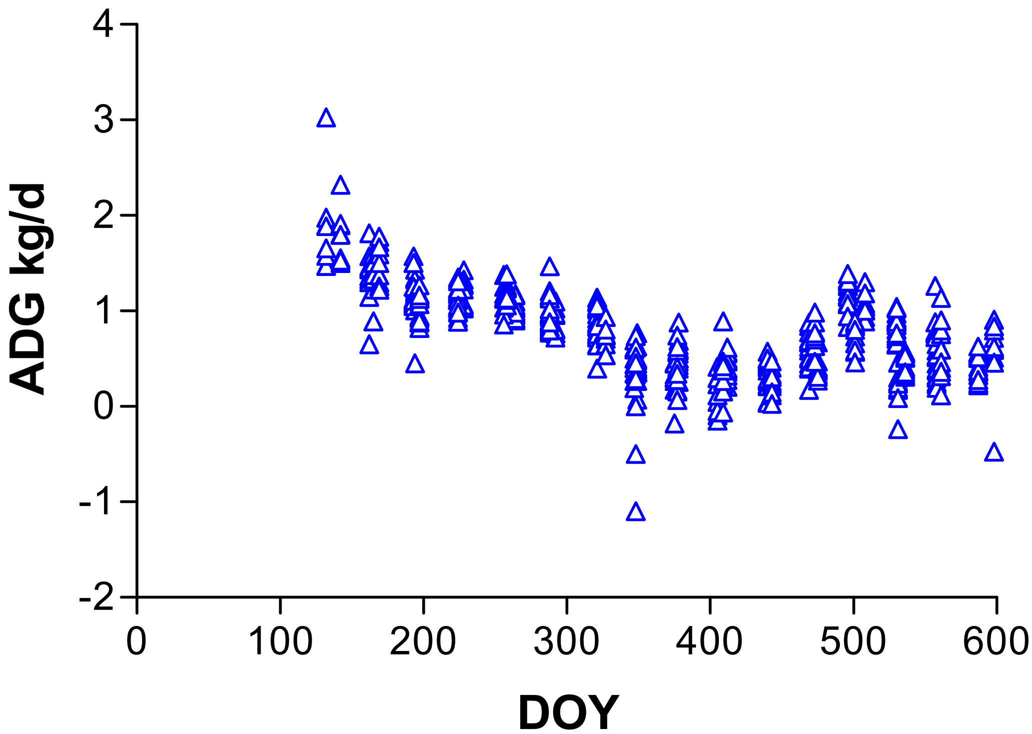

Figure 21. ADG for day of year

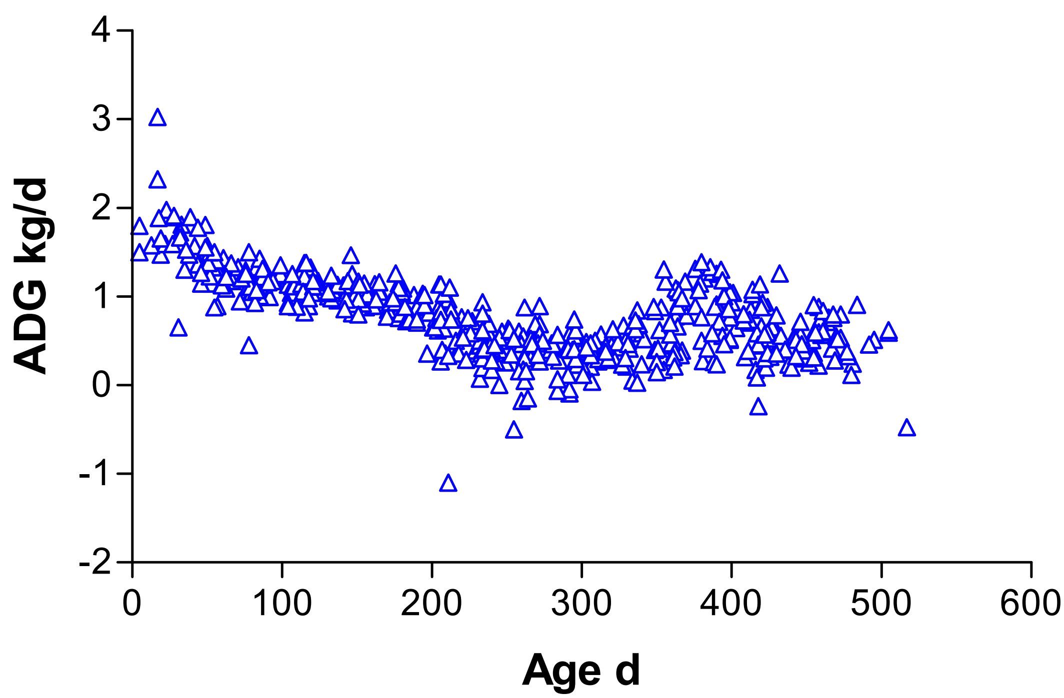

Figure 22. ADG for day of age

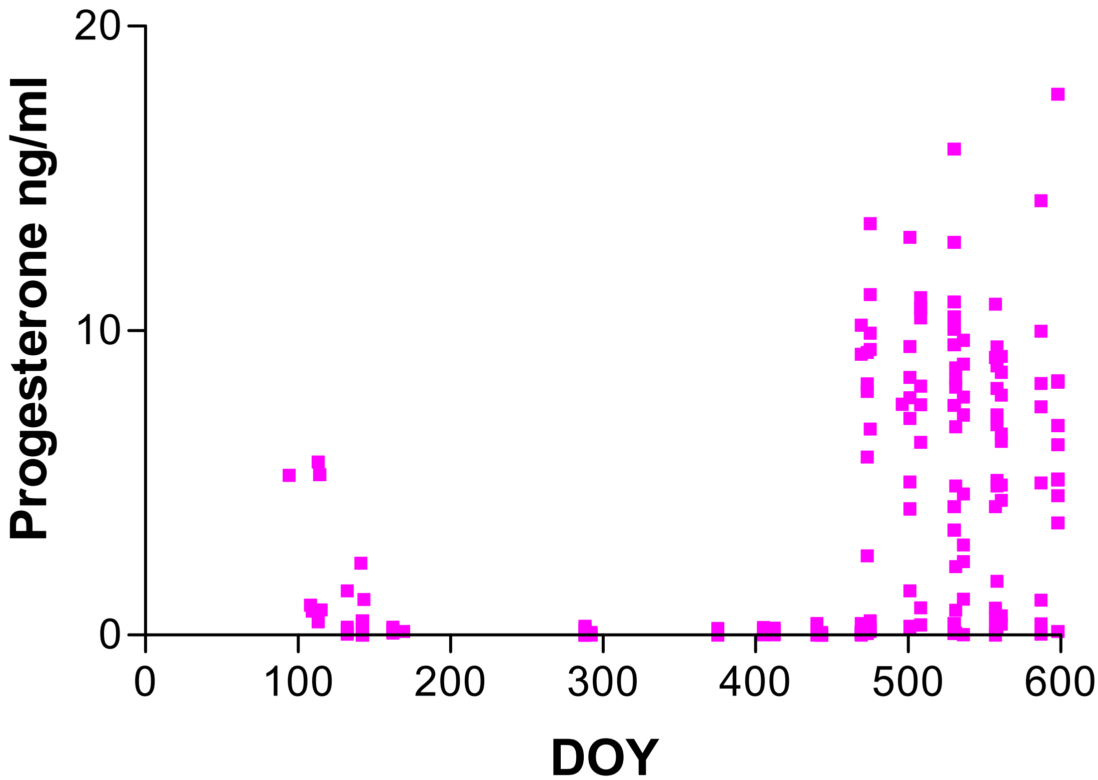

Figure 23. Plasma progesterone concentrations for day of year

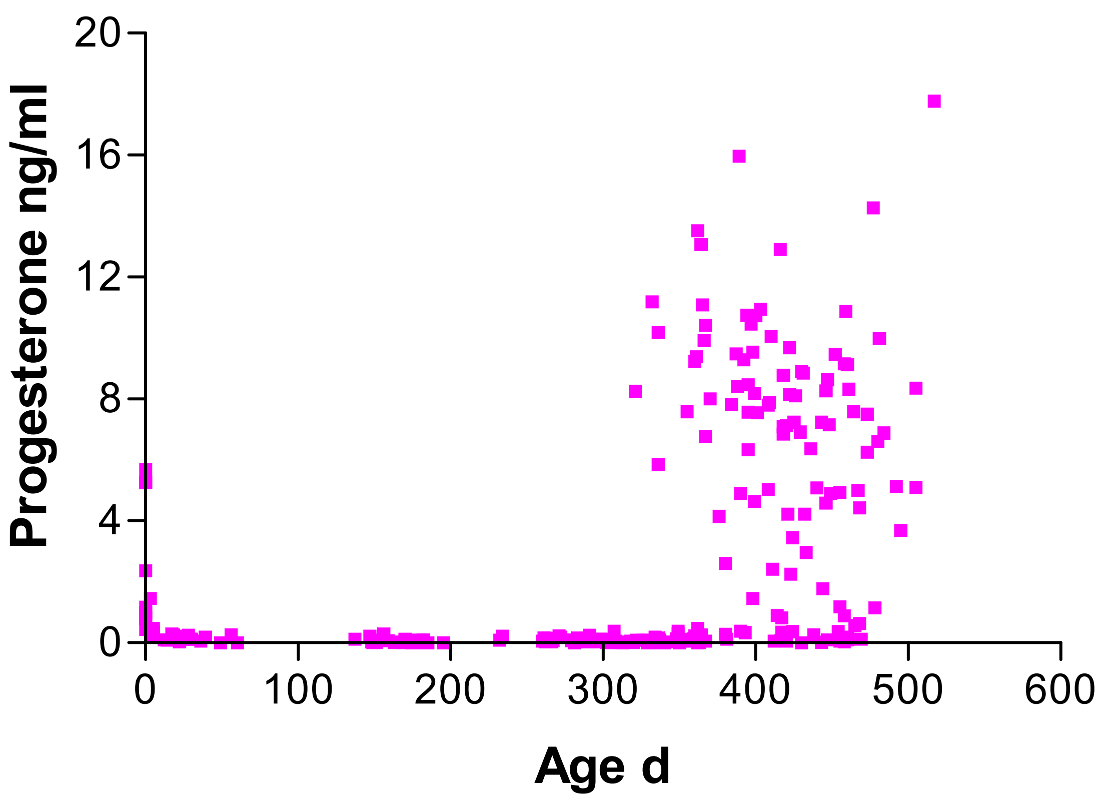

Figure 24. Plasma progesterone concentrations for days of age

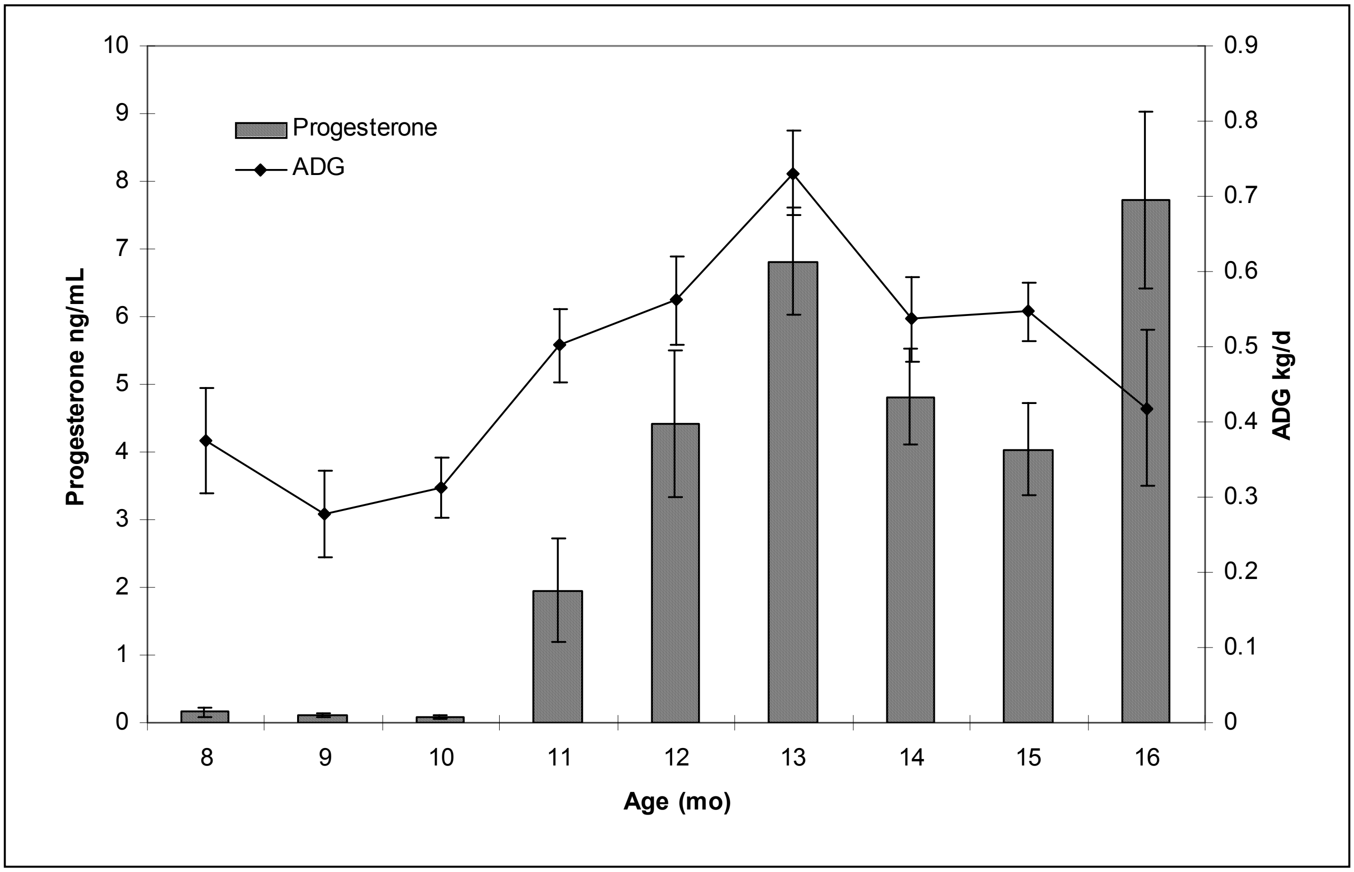

Figure 25. Plasma progesterone concentrations and ADG (mean ± SE) of fillies from 8 to 16 mo of age

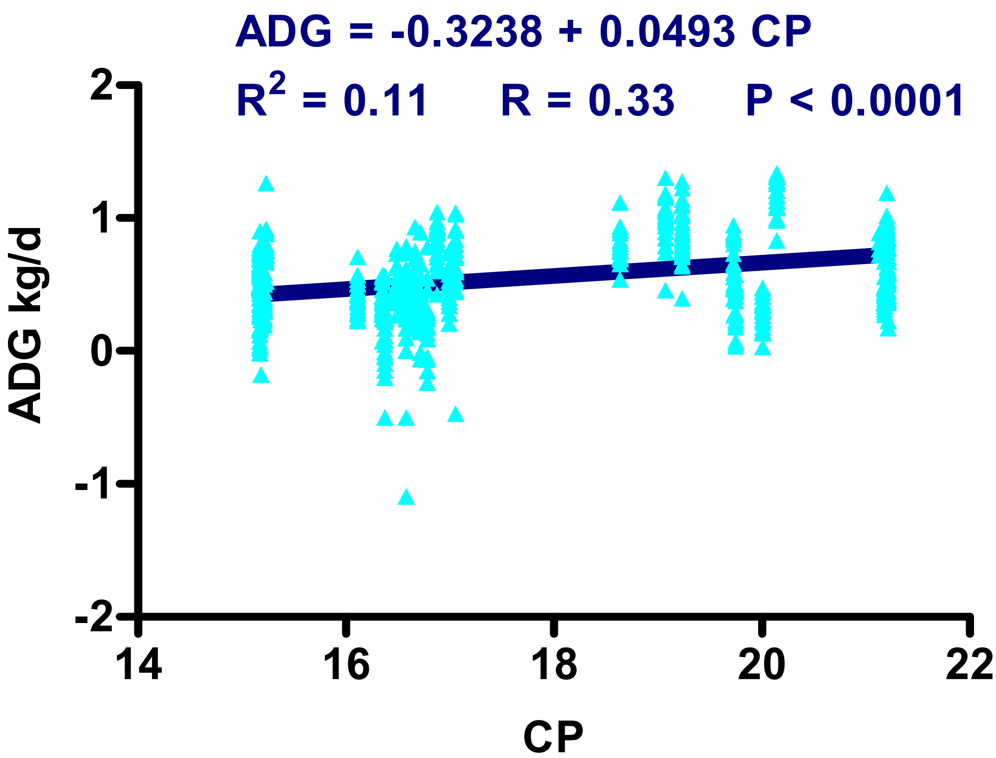

Figure 26 Average daily gain vs. crude protein

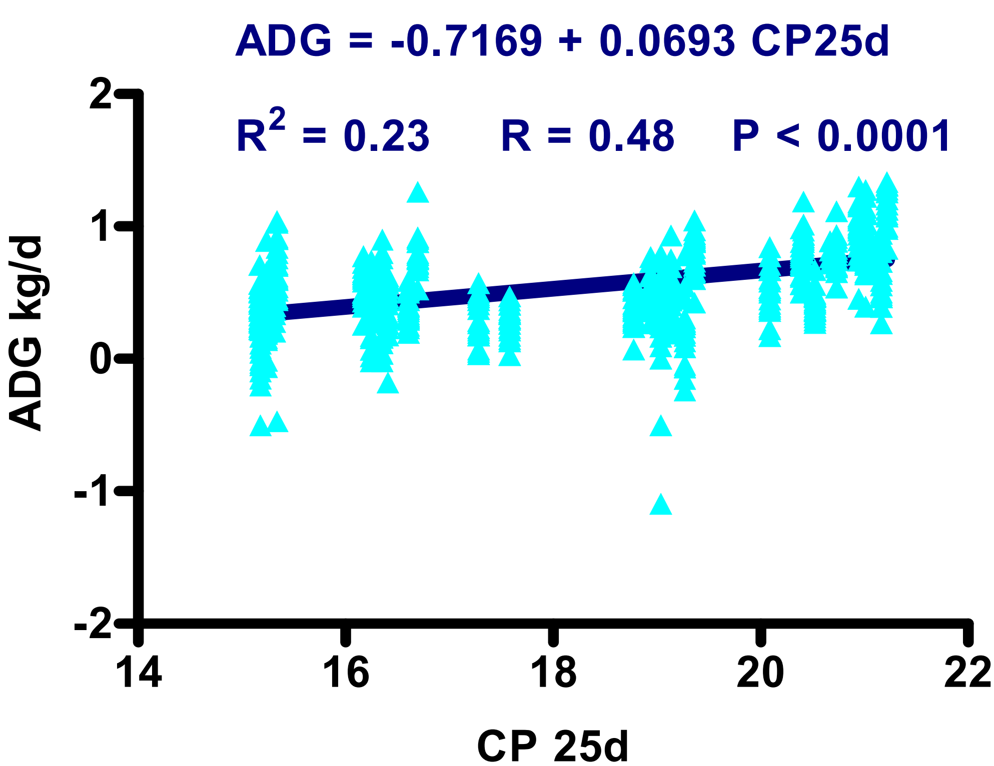

Figure 27 Average daily gain vs. shifted crude protein

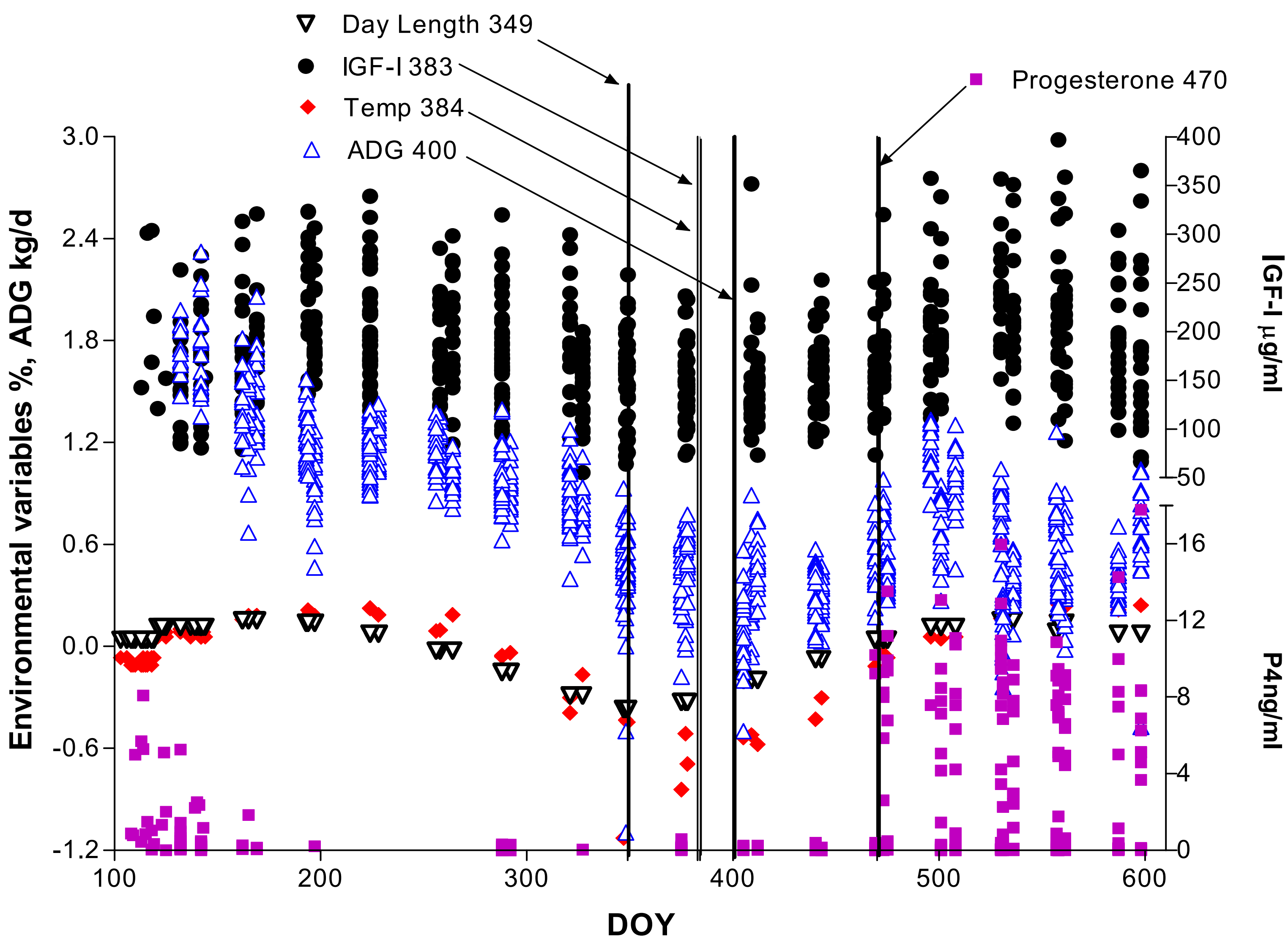

Figure 28. Actual environmental and physiological variables for DOY

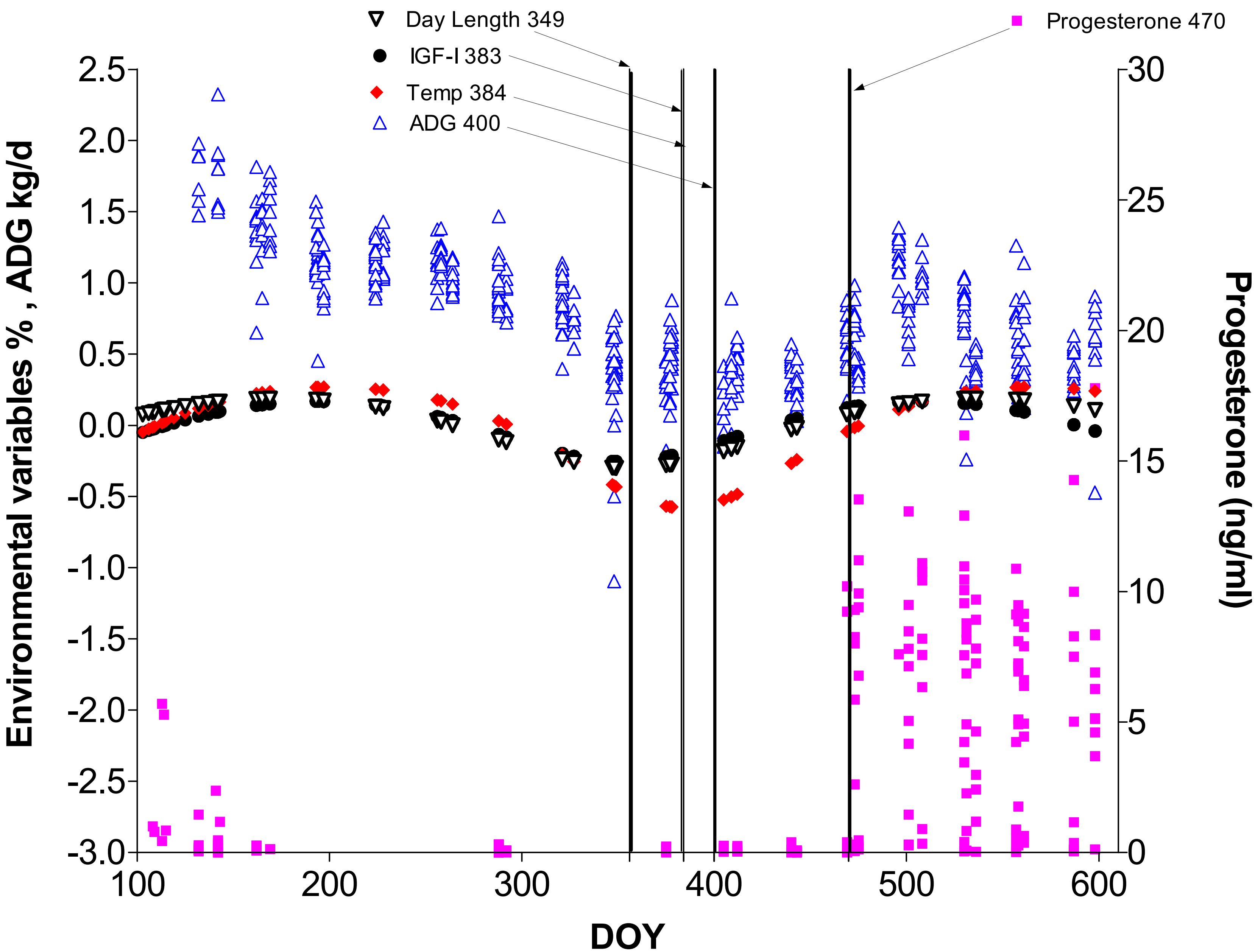

Figure 29. Predicted Day length, IGF-I, and temperature, actual ADG and P4 for day of year

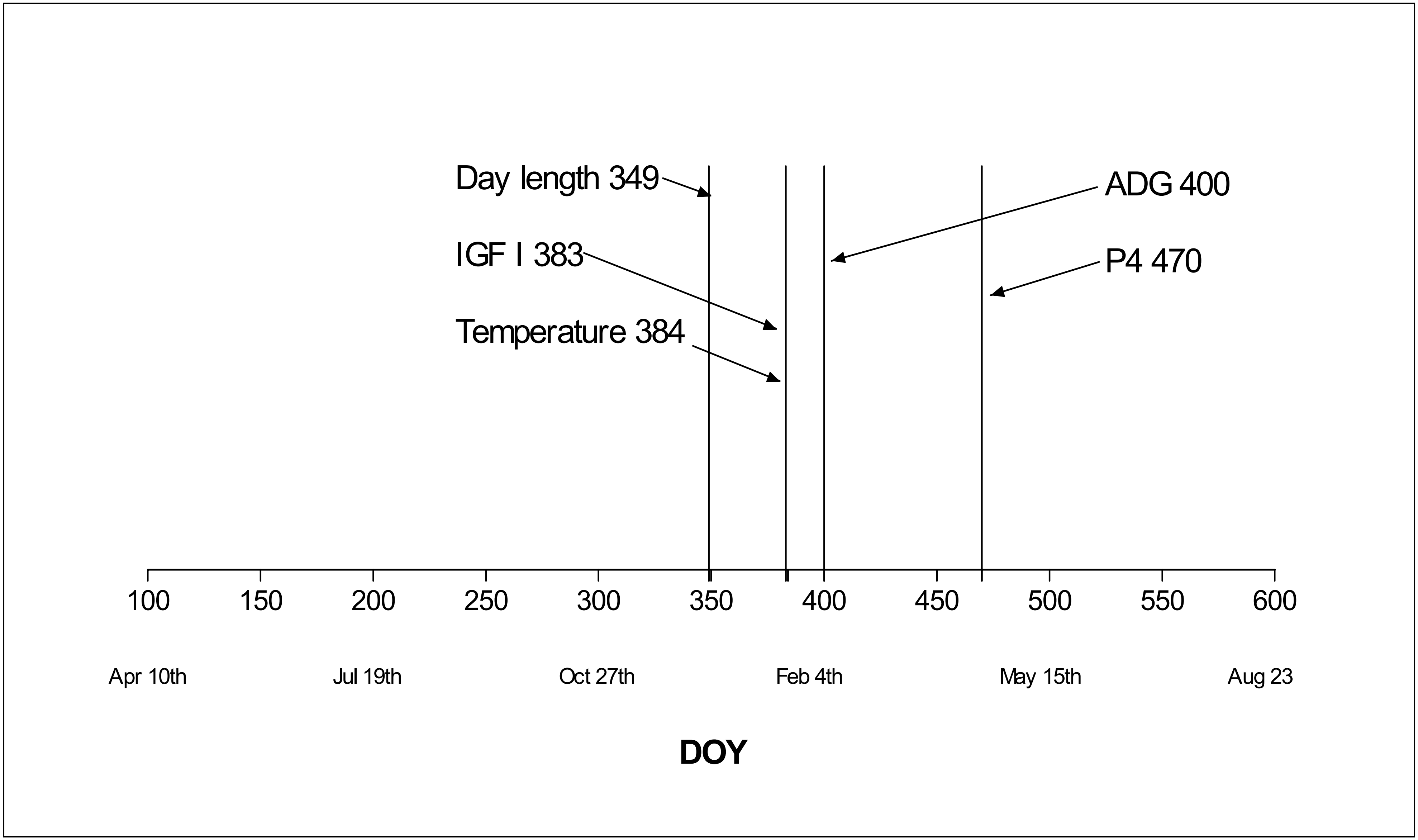

Figure 30. Timeline sequence of events, environmental and physiologic

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Vita

Tania Anne Cubitt, daughter of Annette Cubitt and Trevor Mellor, was born on August 16, 1979 in Kununnarra, Western Australia, Australia. She graduated from Rockhampton State High School in Rockhampton, Queensland in 1996. She then attended the University of Queensland, Gatton Campus, where she completed a Bachelor of Applied Science in Animal Studies, Tania graduated with Class 1 honors in 2001. She then won a Rotary International Ambassadorial Scholarship which enabled her to travel to the United States where she began working towards a Master of Science degree in Equine Nutrition in the Department of Animal and Poultry Sciences at Virginia Polytechnic Institute and State University. Tania has done the majority of her research at the Middleburg Agricultural Research and Extension Center. She will continue her studies in 2004 in pursuit of a doctoral degree in Equine Nutrition at Virginia Polytechnic Institute and State University.