Growth and the Somatotropic Axis in Young Thoroughbreds

by

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Key Words: Growth, Somatotropic axis, Glycemic response, Thoroughbred foals

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(ABSTRACT)

This group of experiments focused on relationships between diet, somatotropic axis, and growth. Growth hormone (GH) and insulin-like growth factor I (IGF-I) are factors in the somatotropic axis, and important to development of growth cartilage in the young animal. The entire study was divided into four main experiments. Characteristics of growth in 113 Thoroughbred foals born over a five year period were described with a series of empirical and physiological equations. Glycemic and insulinemic responses to different feed compositions were evaluated with glycemic response tests. The 24 hr pattern of plasma glucose, insulin, GH, and IGF-I was described in yearlings fed two meals a day. Finally, an association between ADG and IGF-I was described in Thoroughbreds from birth to 16 mo of age. Feeding diets to the foal that influence the somatotropic axis during growth may affect development of growth cartilage in unexpected or detrimental ways.

The pattern of weight in Thoroughbred foals from birth to 16 mo of age was most closely described by multiple regression with a combination of age, girth, body length, and physeal circumference (R^2 = 0.99), while ADG was described with age, day length, temperature and girth (R^2 = 0.59). Correlations of ADG with temperature and day length further strengthen this association (r = 0.41 and 0.33, $P < 0.0001$; respectively).

Glycemic and insulinemic responses were significantly higher in yearlings fed a sugar and starch supplement when compared to those fed a fat and fiber supplement ($P = 0.043$ and 0.031; respectively). The estimated hydrolyzable carbohydrate made up 72 % of the total DE in the sugar and starch and only 16 % in the fat and fiber. The decrease in hydrolyzable carbohydrates moderated the glycemic and insulinemic responses in those yearlings fed the fat and fiber supplement.

Glucose and insulin secretion was significantly affected by the feeding of two meals in a 24 hr period ($P < 0.0001$). Plasma glucose concentrations peaked 1.3 hr and plasma insulin peaked 2 hr following the meals. There were peaks in GH approximately 90 and 40 min following the glucose and insulin peaks. The consistency of these peaks for all horses in the experiment suggests a possible relationship with the meals. Plasma IGF-I concentrations were not affected by the meals ($P = 0.99$).

Plasma IGF-I was positively correlated with ADG from birth to 16 mo of age in foals fed either a fat and fiber or sugar and starch supplement (r = 0.34, $P < 0.0001$). Plasma IGF-I was higher in foals fed the sugar and starch in May, June, July and October of 1998, and January, February, March, April, and May of 1999 ($P < 0.10$). The greatest differences ($P < 0.05$) corresponded with some of the highest ADG in June of 1998 and April and May of 1999.

The results from the series of experiments described here suggest a possible role of dietary mangement in reducing the risk of skeletal disorders that involve the influence of IGF-I on chondrocyte maturation.

(Key Words: Growth, Somatotropic axis, Glycemic response, Thoroughbred foals)

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Chapter 1:
Introduction to the problem

The athletic ability of the horse is the attribute that has most defined its relationship with humans. Horse producers have selected and bred horses for speed and power for over 2000 years. Osteochondrosis (OC), an abnormal differentiation of the growth cartilage, is a major economic problem in the equine industry, partially due to selection forces (Jeffcott, 1996; McIlwraith, 2001). The etiopathogenesis of OC certainly contains genetic and environmental components, both of which have been investigated (Jeffcott and Henson, 1998). The somatotropic axis is a likely involved in both the genetic and environmental components. The somatotropic axis consists of multiple levels of hormones, receptors, binding proteins and proteases interacting with one another to control growth and metabolism. Growth hormone (GH) and insulin-like growth factor I (IGF-I) are principal to the control exerted by the axis. Other hormones that are components of the axis include insulin, thyroid hormones, glucocorticoids, leptin, sex steroids and locally produced growth factors (Breier, 1999). Complex interactions within the axis establish a dynamic control of metabolism. Ultimately, the axis adapts to changing environmental and physiological conditions.

In the young horse, growth cartilage is responsible for the lengthening of bones and development of joint cartilage. Growth cartilage is composed of resting, proliferative, hypertrophic and apoptotic chondrocytes. Endocrine, paracrine and autocrine signaling mechanisms are responsible for the movement of chondrocytes through these stages (Leach and Twal, 1994). This process of development can be seen as an extension of the spatial organization that occurs in the embryo. In this context, it is understandable how genetic or environmental cues that result in abnormal chondrocyte maturation may produce characteristics that are a detriment to a horse's soundness.

Research investigating the role of nutrition, as a portion of the environmental component of OC, has focused on several factors. Included in these are the source and level of dietary energy in the diet (Glade and Reimers, 1985; Savage et al., 1993). Quarterhorse foals fed on an *ad libitum* basis had a higher occurrence of conformational and musculoskeletal abnormalities related to developmental orthopedic disease (DOD) at 25 mo when compared to a group fed on a limited basis (Cymbaluk et al., 1990). A higher incidence of dyschondroplasia was observed in foals fed 129 % of NRC recommended dietary energy levels in the form of a pelleted concentrate with 5% added fat (Savage et al., 1993). The relationship between the increased energy and incidence of DOD may be related to an increased insulin response to the carbohydrates absorbed from the diet (Ralston, 1996). The above relationships are the basis for a sequence of events from the environment, to the somatotropic axis, to changes in growth that may result in the occurrence of OC. This relationship is further strengthened by *in vitro* work with equine chondrocytes. Henson and coworkers (1997) reported that equine chondrocytes incubated in serum containing increasing concentrations of IGF-I exhibitted increasing rates of proliferation. Articular cartilage collected *post mortem* was examined for the presence of dyschondroplastic lesions (Shingleton et al., 1997). Histological analysis of these lesions showed a vascular supply with proliferative chondrocytes surrounding the vessels. This suggests that a systemic factor may be preventing the differentiation of those chondrocytes surrounding the vessels.

The somatotropic axis acts as a connection between changes in environment and growth. In rapidly growing foals, circulating IGF-I concentrations are higher than those in adult horses, likely related to rapid growth rates (Davicco et al., 1994). During compensatory growth, following feed restriction, GH secretion is elevated (Hornick et al., 2000). Synovial fluid from exercised ponies had higher concentrations of IGF-I than synovial fluid from unexercised ponies (van de Lest et al., 2000). The synovial fluid with an increased concentration of IGF-I stimulated

chondrocytes to enhance their production of proteoglycans. In the first case IGF-I is likely related to rapid growth rates, and in the last two cases, IGF-I may be acting as a regulator of tissue growth in response to changes in the environment. It is then interesting to hypothesize a possible role of IGF-I in studies where its concentrations were not measured. A significant loss of bone mineral content was observed after 12 wk of deconditioning in Arabian horses (Porr et al., 1998). A reverse of the process seen in the exercised ponies above may have resulted in a decrease in stimulation of the somatotropic axis and a resultant decrease in bone mineral content. Average daily gain was higher in Thoroughbred foals that were later diagnosed with cervical vertebral malformation, a form of DOD affecting the spinal column, in comparison to normal foals (Ruff et al., 1993). It is possible that increased concentrations of IGF-I resulted in foals exhibiting increased growth rates.

The direct effects of nutrition on the somatotropic axis in the horse remain unclear. The relationship begins as food enters the body and is absorbed. Supplements fed to the horse customarily contain energy in the form of soluble and insoluble carbohydrates. Soluble carbohydrates, or starches, are absorbed as glucose. The increase in plasma glucose following a meal results in a cascade of other metabolic changes (Clarke et al., 1990). This series of events is typically repeated twice a day at feeding times and results in a "feeding/fasting" cycle of hormones and metabolites (Kronfeld, 1998). This is in contrast to the manner in which the horse evolved as a grazing and browsing herbivore, eating continuously or multiple small meals through the day. This start of this cycle has been demonstrated in Thoroughbred mares fed supplements with non-structural carbohydrate (NSC) concentrations of 64.5 $\pm$ 3.61 and 24.7 $\pm$ 2.02 % (Williams et al., 2001). Peak plasma glucose and insulin concentrations were significantly higher in the group of horses fed the high NSC supplement. Differences in diet composition are also likely to affect GH and IGF-I (Elsasser et al., 1989).

Characterizing patterns of growth is a first step to understanding how various environmental factors can affect growth. The objectives of this investigation are to describe growth and be able to predict growth patterns in situations other than those found in the experiment. Types of equations used to analyze growth data include empirical, allometric, and logistic equations. The empirical equations are based on data collected directly from the experiment. The coefficients in these equations are chosen simply due to their ability to reduce the residual sums of squares. Empirical equations are likely to be the best fit to the data, yet lack the flexibility to be applied to data collected under circumstances other than the current experiment. Allometric equations are useful in examining the relationship between variables. They relate a particular body dimension to some power of the weight. Theoretically, a linear measure should vary with weight to the 1/3 power. However, this is not usually the physiological best fit. Girth, body length, and height varied in their relationship to the weight of elands from the 0.41 to 0.26 power (Jeffery and Hanks, 1981). Logistic growth equations were developed as a modification of exponential growth with the understanding that there was a theoretical upper limit to growth of a population over time (Tsoularis, 2001). There are a number of different variations of the basic logistic equation:

$$y = \frac{A}{\left(1 \pm be^{-kt}\right)} \qquad [1]$$

The two used in this paper are the monomolecular:

$$y = A\left(1 \pm be^{-kt}\right) \qquad [2]$$

and the Richards form (Richards, 1959):

$$y = A\left(1 \pm be^{-kt}\right)^{M} \qquad [3]$$

In each of these equations weight is being predicted as a function of time. The form of these equations is such, that there is some biological interpretability of the parameters (Fitzhugh, 1976). The parameter A is an asymptotic value for the

variable being evaluated, b is a scaling parameter that adjusts for a postnatal start of the curve, k is a maturing index, and M is the parameter that determines the point of inflection of the curve. When M = 1, the point of inflection is at time zero, and when 0 < M < 1, there is no postnatal inflection point (Notter et al., 1990). A final equation type uses the relationship between mass and volume as a basis for prediction.

Weight to volume relationships have been used to successfully estimate weight in various species (Carroll and Huntington, 1988; Jeffery and Hanks, 1981). These equations use a cylinder, with a circumference equal to the animals girth and a length equal to the body length of the animal, to estimate the portion of the animals volume most closely related to the animals weight. The volume thus determined is then multiplied by a coefficient that is determined by the best fit line through the data with an intercept of zero. In absolute terms weight is a function of volume and density. If accurate volume calculations could be made, the predicted coefficient would approximate density. For management purposes, accurate measurement of a horse's volume is cumbersome. There are three issues that must be addressed in developing this type of equation. The first is that any error that is made in the calculation of volume becomes part of the error in the estimation of the density coefficient. The second is that density is not a constant, but instead a value that varies with the age and condition of the animal. The third is the value of the equation to the farm manager, and this value has components of ease of use and accuracy.

Two lines of research are started separately as presented in this paper and brought together in chapter five. The first of these is an investigation of characteristics of growth for Thoroughbred foals through the first year and a half of life. The second is the effect of diet on fluctuations of circulating hormones and metabolites related to the somatotropic axis over periods from 6 to 24 hr. Finally, concentrations of IGF-I are examined over a period of 16 months in foals fed either

a sugar and starch or fat and fiber supplement. For the purpose of this dissertation, supplement will be used to represent the feed offered to horses, as we consider this feed a supplement to the nutrients and energy found in the pasture. The effects of diet and environment on concentrations of circulating plasma IGF-I are examined, and concentrations of IGF-I over time are compared to growth.

Chapter 2:
Characteristics of growth in Thoroughbred foals

Introduction

Equine growth has been studied extensively due to the importance of development to future athletic ability (Carroll and Huntington, 1988). The objective of optimal skeletal and muscular development is a driving force in many branches of the equine industry. Yearlings at annual sales are often evaluated by their size and degree of development. This has led to breeding practices that select for horses with increased growth rates and greater mature size. Rapid growth has been associated with increased occurrence of various forms of DOD, including osteochondrosis, epiphysitis, and flexural abnormalities (Cymbaluk et al., 1990; Ruff et al., 1993). Various studies have attempted to define environmental factors that perturb the genetically programmed growth rate. These factors include diet, age, climate, month of birth, onset of puberty, Quantifying longitudinal bone growth may also be important to addressing various forms of DOD that can occur in the young growing foal (Fretz et al., 1984). Characterizing normal growth rates is also important in determining nutrient requirements for the growing foal. Development of accurate equations for assessing normal growth will help those in the equine industry better assess foal development, and aid in the prevention of abnormalities associated with above or below normal growth rates.

The first objective of this study was to quantify characteristics of growth in five groups of Thoroughbred foals raised in five consecutive years on an agricultural research and extension center in north-central Virginia. A second objective was to develop empirical and predictive equations that represent the first year of growth in Thoroughbreds. The results from this study should help to further characterize the growth of the young Thoroughbred and draw attention to periods of retarded and compensatory growth.

Materials and Methods

Growth of all foals born at the Middleburg Agricultural Research and Extension Center (MAREC) is monitored for approximately 16 months prior to the annual yearling auction in October. This protocol has been approved by the institutional animal care and use committee. Records for five years (1996-2000) were analyzed with the objectives of characterizing patterns of growth, examining environmental perturbations to growth, and producing empirical and physiological equations for growth.

Annually, brood mares at MAREC are paired by weight, foaling date and sire, and then randomly assigned to two dietary groups. These groups are pastured on adjacent and similar mixed grass/legume fields, and are exchanged between pastures on a monthly basis to decrease possible pasture effects. Mares are bred over a 9 week period from April 1st through the first week in June. The foaling season therefore runs from the end of March through the beginning of June.

Mares and foals were placed in their respective groups five to seven days following foaling. Mares were maintained on the supplements from May until time of weaning, at which point the foals were continued on the supplements until July or August of the following year. Horses remained on mixed grass and ladino clover pasture 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 $\times$ 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 routine at the MAREC (Ley et al., 1992). Colts were gelded at three to four weeks of age. Foals were weaned gradually, beginning at six months, by the removal of two mares from each group every four days.

Foals were first measured at 24 hr or one month depending on the year. Subsequent measurements were taken at approximate 28 day intervals for the following 16 months. Measurements of BW, ADG, body condition score (BC), wither

and hip heights, length of body, forearm, and cannon bones, girth, and circumference of the carpus and fetlocks (Table 3) were taken at each period. 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 effects of sire, dam, dam age at birth, sex of foal, diet, year, month, season, day length, temperature, and age of foal. Ten different supplements were fed to the mares and foals over the five years examined. Research during those years focused on developing fat and fiber supplement suitable for brood mares and young horses. Nutrient and ingredient compositions are described in Table 1 and 2. Supplements were designed to be isoenergetic, 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, and after weaning, the number of weanlings in the pasture. This arrangement allowed each horse to eat its portion of the total amount of supplement offered to the group. 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 months when pasture growth was good was 1:2, while during some of the very dry or cold months

it was increased to 1:1. For approximately a month in February and March of 1998, yearlings were also given free access to mixed grass and alfalfa hay (18.7% CP).

Analysis of variance with repeated measures was used to determine the effect of year and month on weight. Growth was evaluated using both empirical and physiological equations to estimate weight for 113 foals for periods of growth ranging from 14 to 16 mo. A total of 1,676 measurement points were included. At each of these points 14 measurements were performed, including weight. A total of 23,464 data points were available for analysis. Stepwise selection in linear regression was utilized to determine those variables to be included in the empirical equations (SAS Inst. Inc., Cary, NC). Variables included in the empirical equations each improved the R^2 greater than 0.001. The empirical equation was then fit to each year of data individually, as well as all five years combined.

Physiological equations were based on the relationship between weight and volume, or variations of the Richards growth equation (Richards, 1959). Coefficients and parameters for physiological equations were determined with non-linear regression performed using GraphPad Prism version 3.02 for Windows (GraphPad Software, San Diego California USA). Two different measures of volume, derived from linear body dimensions, were used to predict weight (Table 10). Equations used to predict ADG with age used constants previously determined in the prediction of weight with age. Residuals from the prediction of ADG in relation to age were compared with sine wave patterns of temperature and day length. A sine wave component was then added to the Richards equation in an attempt to take into account some of the variation in weight and ADG caused by changes in day length and temperature.

Results

The effect of year was not significant for the five years included in the models ($P = 0.076$), while month was highly significant ($P < 0.0001$). Age, girth, body length, and physeal circumference were variables chosen for the empirical equation

that best fit the weight data (Table 5). The equation fit each year individually and the complete data set with R^2 values of greater than 0.98. Age, temperature, day length, and girth were variables chosen for the empirical equation that best fit the ADG data (Table 6). The R^2 values for the individual years ranged from 0.50 to 0.73. The R^2 value for the complete data set was 0.59. Individual p-values are listed for each coefficient, expressing the probability that the value of the coefficient is not different from zero. For both empirical equations there are several coefficients during individual years that have a high probability of being not different from zero. During those years the variables represented by those coefficients did not contribute significantly to the predictive power of the equations.

Analysis of the change in growth of linear body dimensions in relation to weight was evaluated using allometric equations (Table 7). Exponents in these equations ranged from 0.36 to 0.06, with corresponding R^2 values of 0.99 to 0.26. Best fit lines are depicted in figures 1 – 3.

The change in linear body dimensions was examined in relation to age with Richards and monomolecular growth equations (Table 8). The values of R^2 for the different equations ranged from 0.97 to 0.27. The residual sum of squares was reduced for each variable using the Richards equation. The relationship between physeal circumference, front and hind cannon length, and age could not be determined with the Richards equation due to lack of convergence. The value of the inflection parameter (M) in the Richards equations remained in the range of $0 < M \geq 1$, hence no point of inflection was defined. Best fit lines are depicted in figures 4 – 6.

The relationship between weight and age was fit using the Richards equation (Table 9). The asymptotic coefficient was 852 ± 136, and the inflection parameter was 0.59, with an R^2 of 0.96. The value of the inflection parameter (M) in the Richards equations remained in the range of $0 < M \geq 1$, hence no point of inflection

was defined. Average daily gain's relationship with age was fit using the first derivative of the Richards equation.(R^2 = 0.46) (Figure 8).

Day length and temperature were positively correlated with ADG (r = 0.30 and 0.43, $P < 0.0001$; respectively). Both day length and temperature were characterized by sine wave equations (R^2 = 0.93 and 0.89; respectively) (Figure 9). Addition of the sine wave component improved the R^2 and decreased the residual sum of squares for the weight and ADG prediction equations (Figure 11). The frequency of one complete oscillation for the sine wave estimating temperature and day length was different than that for weight and ADG (365 $\pm$ 2 and 251 $\pm$ 2 d; respectively). Best fit lines for the modified Richards equation are shown in figure 10.

Two different estimations of volume were used to form weight prediction equations of the form, weight = volume × m + b. The first was based on the equation used by Carroll and Huntington (1988), which uses girth and body length ($Volume_1$) , and the second uses these as well as physeal circumference, and forearm and front cannon length ($Volume_2$) (Table 10). The equations were derived with and without a y-intercept. The best fit slope for the $Volume_1$ equation without a y-intercept was 1145 $\pm$ 1.5 (R^2 = 0.98), while the best fit slope for the $Volume_2$ equation without a y-intercept was 1062 $\pm$ 1.2 (R^2 = 0.98). The best fit to the data was the equation using $Volume_2$ with a y-intercept (R^2 = 0.984). The fit of the equations without y-intercepts using the predetermined slopes was compared on a raw data set of 151 data points. The $Volume_2$ equation without a y-intercept was the most appropraite in describing a raw data set of 151 weights in Thoroughbred foals from 0 to 6 mo of age (R^2 = 0.99) (Figure 13).

Discussion

Monitoring the development of foals is important for various reasons. The first is understanding the nutritional needs of the foal through its first year of life. The second is that developmental problems are often initiated early in life with

clinical significance only occurring when the horse enters training. During this period of growth, foundations are being laid for the muscular and skeletal systems that will facilitate the athletic ability later in life. These systems are changing rapidly, and changes in nutrition will have substantial effects. Accurately estimating growth characteristics is important in the proper nutritional management of foals.

No differences were found between colts and fillies for any of the variables measured. This is in contrast to other studies that have examined growth in young Thoroughbreds (Hintz et al., 1979; Thompson and Smith, 1994). One possible explanation is the early gelding of colts at the MARE Center. Typically, Thoroughbred colts are not castrated at all, or castrated later than six weeks. Average body weights (Table 4) were greater than those of foals raised on Windfield Farms from 1958 – 1975 (Hintz et al., 1979), and more closely resembled those of foals raised in Kentucky from 1989 – 1995 (Pagan et al., 1996; Thompson and Smith, 1994). Means of several body dimensions decrease in the last sampling month. This decrease is due to a smaller number of horses being sampled at this last sampling point.

Empirical equations developed for predicting weight using variables recorded over five years were the best fit to the data in this experiment. This is to be expected as these variables and their coefficients were chosen simply due to their ability to reduce deviations of predicted from actual values. This is not to say that there is not useful data to be taken away from these equations. The variables included in the equations explain a large portion of the variability in the dependent variable. These variables will likely be useful if used in creating equations that have more biological significance. Simple linear regression of weight with girth demonstrates that girth explained 96% of the variation in the weight data in this experiment. This explains why girth is the most commonly used measure for estimating the weight of horses. After girth, the next three most important determinants of predicted body weight were age, physeal circumference and body

length, with corresponding partial R^2 improvements of 0.012, 0.006 and 0.002. The physeal circumference's inclusion in the equation is likely due to the fact that its pattern in relation to age (Figure 6) shows similar characteristics as weight to age. The decrease and subsequent increase in growth between 250 and 450 days of age seen in the weight data is also visible in the physeal circumference data.

Average daily gain was best predicted by a combination of girth, temperature, age and day length. Temperature and age were also the only variables whose coefficients were always statistically different from zero in equations for individual years as well as the conglomerate of all years. Environmental effects on growth and factors related to growth have been investigated in various species of livestock (Cymbaluk and Christison, 1989; Lincoln et al., 2001; Sarko et al., 1994). The relationship between environmental variables and growth supports the hypothesis that changes in the environment are important influences of growth.

Allometric relationships are useful in examining the association between weight and linear body dimensions. Comparing the proportionality coefficient and exponent of each best fit equation, suggests these are variables that change in a similar manner to one another in comparison to weight. The equations for girth and body length are similar and have the highest R^2 values (0.99 and 0.95; respectively). Groupings after this include hip and wither height, fetlock and physeal circumference, and hind and front cannon length. Forearm length is left alone with a relationship that is somewhere between that of fetlock circumference and body length. It is clear that girth and body length change to the greatest extent in relation to weight, and that this change is also close to being proportional to weight to the 1/3 power. These facts further demonstrate why these measures are most useful in predicting weight.

Logistic growth models both fit the data in this experiment and provided useful characteristics of the growth curves. Logistic curves generally model population dynamics, and the analogy is extended to the growth of organisms as

self contained populations of cells (Majkowski and Uchmanski, 1980; Tsoularis, 2001). Two different forms of the basic logistic model were used to fit the data. The first was the monomolecular, with a point of inflection set at the origin, and the second was the Richards, with a variable point of inflection. The Richards curve fit seven out of the ten variables examined, and in each of these cases the residual sums of squares were reduced with the Richards equations, compared with other logistic models. Estimations of linear body dimensions and weight from these equations are comparable with data from Thoroughbred foals, yearlings and two year olds in England as well as Kentucky (Green, 1976, 1969; Thompson, 1995). Kentucky bred Thoroughbred colts weighed 83.5 and 364.5 kg at 14 and 364 days of age, and had corresponding wither heights of 108.2 and 146.3 cm. Wither heights of English bred Thoroughbreds were 153 cm at 24 months of age. Weights predicted from Richards equation in this study at 14, 365, and 730 d were 83.5, 373, and 517 kg. Predicted wither heights at these time points were 111, 149, and 154 cm. 95 % prediction intervals may be useful to breeding farm managers monitoring growth of their foals (Figure 7).

The residuals from the prediction of ADG using the first derivative of the Richards equation showed systematic deviations from zero at at least two points over time (Figure 11). The first of these is between 225 and 325 d, and the second follows between 325 and 400 d. The first reveals that the actual weights are lower than predicted weights during this time period, and the second, that actual weight is greater than predicted. Comparison of this pattern in residuals to patterns of day length and temperature suggest a relationship, which is further substantiated by the use of these variables in the empirical equation used to predict ADG. Day length and temperature's relationships with time are both characterized by sine waves that finish one complete oscillation every 365 days (Figure 9). In an attempt to better fit the weight and ADG data, a sine wave component was added to the Richards equation. The modified Richards increased the R^2 and decreased the residual sums

of squares for the predictions of weight and ADG. The modified Richards equation was characterized by a frequency that completed one complete oscillation every 253 d. It is noted that this frequency synchronizes the fluctuations in ADG with the fluctuations that occur in day length and temperature from birth to 500 d of age. In figure 12 the curve formed by the best fit equations were simulated past 500 d. It is clear from the figure that this modified Richards is only valid through the end of sampling in this study. After this point, the sine waves no longer align.

Weight to volume relationships have been used to successfully estimate weight in various species (Carroll and Huntington, 1988; Jeffery and Hanks, 1981). These equations use a cylinder, with a circumference equal to the horses girth and a length equal to the body length of the horse, to estimate the portion of the horses volume most closely related to the horses weight. The volume thus determined is then multiplied by a coefficient that is determined by the best fit line through the data with an intercept of zero. In absolute terms weight is a function of volume and density. If accurate volume calculations could be made, the predicted coefficient would approximate density. For management purposes, accurate measurement of a horse's volume is cumbersome. There are three issues that must be addressed in developing this type of equation. The first is that any error that is made in the calculation of volume becomes part of the error in the estimation of the density coefficient. The second is that density is not a constant, but instead a value that varies with the age, condition of the horse, and gut fill. The third is the value of the equation to the farm manager, and this value has components of ease of use and accuracy.

The equation developed by Carroll and Huntington (1988) for the prediction of weight was from data collected on 372 horses of different breeds, sizes and condition scores. The value of the density coefficient in their equation was 0.001058 kg/cm^3. For the purpose of this paper the units were changed to kg/m^3, which yields a value of 1058. Using the same volume calculation, a density

coefficient of 1145 ± 1.5 kg/m^3 was determined for the 1614 measurements taken in this study (Table 10). In an attempt to improve the calculation of volume, the volume of the legs was approximated using the physeal circumference and the forearm and front cannon length. The legs accounted for 18.7 to 5.5 % of the total measured volume, which is comparable to a value of approximately 3.8% measured in adult Warmbloods (Buchner et al., 1997). The new volume calculation resulted in a density coefficient of 1062 ± 1.2 kg/m^3. The above equations were then tested by fitting each to a raw data set of 151 measurements of Thoroughbred foals between 0 and 300 d old (Table 11 and Figure 13). The calculation that used body and leg volume had the highest R^2 (0.99) and the lowest residual sums of squares. This is due to the fact that; the densities of the foals in this study were likely greater than those of the horses used in the Carroll and Huntington study, and the measure of volume in the present study took into account the change in leg volume in relation to the total volume. Taking the leg volume into account removed some of the error from the density coefficient estimation. Finally, while this equation is somewhat more cumbersome than the previous, it improves the accuracy of weight estimation in Thoroughbred foals.

Four different techniques were used to examine characteristics of growth in Thoroughbred foals. Empirical equations provide the best fit to the data from this experiment. Variables included in those equations either changed in a similar way or affected the change of weight and ADG. Allometric equations allow investigation of how linear body dimensions change with weight. Girth and body length appear to change in a similar manner to weight. Logistic growth models provide useful information about changes in growth with time, and emphasize effects of the environment over time. The 95 % prediction intervals for growth variables over time will be useful in monitoring foal development. Examination of systematic deviations in the data from the best fit line for ADG emphasizes the importance of the environment in determining growth rates. Finally, approximations of volume

provide a useful and accurate method of estimating weight. However, equations developed from the data in this experiment are most appropriate for estimating weight in horses between birth and 500 days of age.

Table 1 Ingredient composition of supplements[a] fed over the five years growth data was collected.

Ingredient	Ca-1	Ca-2	CP-3	CP-4	SS-1	FF-1	SS-2	FF-2	SS-3	FF-3
Oat straw	23	22.7	23	25	7	7	7	7	7	7
Alfalfa	0	0	0	0	0	13.5	0	13.5	0	13.5
Dent yellow grain corn	4	3.9	4	20	60	11	60	11	60	1.5
Beet pulp	16.5	16.3	16	16	0	10	0	10	0	10
Soybean hulls	15	14.8	15	15	4	4	4	4	4	4
Corn oil	11	10.8	11	11	0	0	0	0	0	0
Molasses (cane)	5	4.9	5	5	10	5	10	5	10	5
Soybean meal	22	21.7	22	3	15.5	2	15.5	2	15.5	2
Limestone	0.8	2.2	2.2	1.8	1	1	1	1	1	0
Calcium phosphate, dibasic	1.7	1.7	1.7	1.8	1.5	0.5	1.5	0.5	1.5	0
Lysine	0	0	0	0.6	0	0	0	0	0	0
Vitamin premix	0.5[b]	0.5[b]	0.5[b]	0.5[b]	0.5[b]	0.5[b]	0.5[c]	0.5[c]	0.5[b]	0.5[b]
Mineral premix[d]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Threonine	0	0	0	0.4	0	0	0	0	0	0
Processed cereal by-product[e]	0	0	0	0	0	45	0	45	0	56

[a] 10 different supplements; Ca-1 and Ca-2 are low and high calcium, CP-1 and CP-2 have different amino acid compositions, SS and FF supplements are variations of sugar and starch, or fat and fiber compositions.

[b] Provided the following amounts per kg of supplement: vitamin A, 6,900 IU; β-carotene, 17.6; vitamin D_3, 1,290 IU; vitamin E, 132 mg; vitamin C, 333 mg; Niacin, 15 mg; Thiamin, 7 mg; Riboflavin, 3.5 mg; Folic acid, .33 mg; Biotin, .21 mg.

[c] Provided the following amounts per kg of supplement: vitamin A, 6,900 IU; β-carotene, 17.6; vitamin D_3, 1,290 IU; vitamin C, 333 mg; Niacin, 15 mg; Thiamin, 7 mg; Riboflavin, 3.5 mg; Biotin, .21 mg.

[d] Provided the following amounts per kg of supplement: Fe, 46.1mg; Zn, 105.8mg; Cu, 25.11mg; Mn, 18.02 mg; Se, .55 mg; I, .35 mg; NaCl used as carrier, 4160 mg.

[e] Processed cereal by-product contains 92.5% DM, 21-26% EE, 15% CP, 14-24% NSC, and 31% NDF.

Table 2. Nutrient composition on a DM basis of supplements[ab] fed over the five years growth data was collected.

	Ca-1 (n = 14)		Ca-2 (n = 14)		CP-3 (n = 6)		CP-4 (n = 6)		SS-1 (n = 3)		FF-1 (n = 3)		SS-2 (n = 12)		FF-2 (n = 12)		SS-3 (n = 6)		FF-3 (n = 6)	
Item	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
CP, %	15.4	0.5	15.3	0.7	13.7	0.2	8.5	0.2	16.7	1.8	14.8	0.3	13.7	0.6	15.4	0.2	15.3	0.7	14.5	0.3
Fat, %	14.2	1.0	13.1	1.0	14.5	0.6	14.6	0.7	1.0	0.2	9.7	0.8	3.2	0.3	16.6	0.8	3.1	0.5	11.9	1.1
ADF, %	28.7	1.2	30.0	1.0	31.2	0.6	32.1	0.7	10.7	1.2	21.1	0.2	10.7	1.0	22.6	0.7	9.4	1.7	20.7	1.1
NDF, %	43.2	1.6	45.3	1.6	43.0	0.6	44.4	1.0	19.6	1.5	34.8	0.9	18.5	1.1	36.9	1.0	18.0	2.7	31.4	1.0
NSC, %	20.2	1.5	17.7	1.0	19.0	1.5	17.5	2.8	55.3	4.9	28.6	1.8	64.5	3.6	24.7	2.0	57.2	3.9	29.7	2.3
Ca, %	1.2	0.13	1.6	0.20	1.8	0.07	1.8	0.07	1.3	0.3	2.4	0.2	1.2	0.1	1.4	0.2	1.0	0.2	2.7	0.2
P, %	0.5	0.04	0.5	0.05	0.5	0.02	0.6	0.02	0.8	0.15	1.1	0.03	0.6	0.05	1.4	0.06	0.6	0.07	1.1	0.05
Mg, %	0.2	0.01	0.2	0.01	0.2	0.01	0.2	0.01	0.2	0.03	0.6	0.01	0.2	0.01	0.7	0.03	0.3	0.04	0.7	0.04
K, %	1.1	0.02	1.2	0.02	1.2	0.04	0.9	0.02	1.2	0.17	1.2	0.05	1.1	0.06	1.3	0.02	1.0	0.05	1.2	0.03
Na, %	0.3	0.02	0.2	0.03	0.3	0.02	0.3	0.03	0.3	0.04	0.4	0.03	0.3	0.03	0.2	0.02	0.2	0.02	0.3	0.02
Fe, mg/kg	426	21.8	484	80.4	543	19.7	562	32.8	396	90.9	426	6.2	257	32.1	356	15.7	258	32.1	384	92
Zn, mg/kg	142	10.5	143	17.0	176	21.3	174	9.5	146	25.1	166	2.6	124	11.1	169	11.6	124	11.0	176	5.6
Cu, mg/kg	30	3.3	35	5.2	39	2.3	41	1.9	34	4.9	29	2.5	25	2.5	31	1.9	24.6	2.5	27.3	2.3
Mn, mg/kg	60	1.9	61	3.2	71	2.0	70	1.7	58	9.4	215	26.0	64	5.7	207	20.9	44	5.7	245	16
S, %	0.2	0.00	0.2	0.01	0.2	0.00	0.2	0.01	0.2	0.02	0.2	0.01	0.2	0.01	0.2	0.01	0.2	0.01	0.2	0.01
Cl, ion %	0.4	0.03	0.4	0.05	0.6	0.01	0.8	0.05	N/A	N/A	N/A	N/A	0.5	0.04	0.4	0.03	0.5	0.04	0.5	0.06

[a] 10 different supplements; Ca-1 and Ca-2 are low and high calcium, CP-1 and CP-2 have different amino acid compositions, SS and FF supplements are variations of sugar and starch, or fat and fiber compositions.

[b] Analysis performed by Dairy One, Ithaca, NY

Table 3. Description of body measurements adapted from Hoffman et al., 1996 used to monitor growth of foals

Variable	Measurement description
Wither height	Distance from the ground to the highest point of the withers
Hip height	Distance from the ground to the highest point of the croup
Body length	Distance from the point of the shoulder to the point of the buttock
Girth	Circumference of the girth behind the elbow and an 2.54 cm behind the highest point of the withers
Forearm	Distance from the point of the elbow to the accessory carpal bone
Front cannon	Distance from the accessory carpal bone to the proximal sesamoids
Carpus	Circumference of the knee at the metaphysis of the distal radius, just above the accessory carpal bone
Fetlock	Circumference of the fetlock at the metaphysis of the distal third metacarpal bone, just above the proximal sesamoids
Hind cannon	Distance from the point of the hock (calcaneus) to the proximal sesamoids

Table 4. Summary of growth variables[a] examined in Thoroughbred foals[b]

Variable Measured		Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug
n		16	93	110	113	113	113	113	111	110	110	110	90	110	110	108	67	24
Age d			15.3	42.2	70.5	102.2	133.9	164.2	193.8	222.4	251.4	283.7	311.4	345.5	377.2	406.8	429.4	463.5
	SE		1.11	1.44	1.49	1.55	1.62	1.67	1.74	1.72	1.80	1.71	1.97	1.72	1.67	1.64	2.19	3.46
Weight kg		58.6	85.5	123.4	155.7	191.5	224.5	255.4	282.2	298.8	312.4	322.1	329.4	350.0	378.9	395.6	410.9	436.6
	SE	1.53	2.06	2.19	2.13	2.20	2.14	2.22	2.18	2.10	2.10	2.28	2.62	2.47	2.47	2.68	3.25	4.44
ADG[c] kg/d			1.81	1.36	1.15	1.13	1.05	1.02	0.90	0.59	0.47	0.31	0.26	0.59	0.91	0.55	0.37	0.44
	SE		0.11	0.05	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.03	0.03	0.05	0.03
BC[de]				5.4	5.1	4.9	5.0	5.0	5.0	5.0	5.0	4.9	5.0	4.9	5.1	5.1	5.1	5.1
	SE			0.09	0.04	0.04	0.03	0.03	0.04	0.04	0.03	0.04	0.04	0.04	0.04	0.04	0.05	0.06
Wither height cm		107.0	111.4	118.3	124.4	130.1	134.1	137.8	140.5	143.2	144.2	145.7	146.6	148.4	149.9	150.2	150.8	150.0
	SE	1.14	0.50	0.51	0.52	0.41	0.40	0.35	0.32	0.36	0.36	0.31	0.38	0.35	0.33	0.34	0.37	0.72
Hip height cm		108.0	112.9	121.3	127.8	132.8	137.6	141.8	144.6	146.9	147.9	149.4	150.4	152.2	153.2	153.9	155.1	155.4
	SE	1.28	0.57	0.54	0.53	0.42	0.39	0.41	0.41	0.38	0.38	0.32	0.38	0.37	0.34	0.35	0.39	0.71
Girth cm		86.3	96.2	109.6	118.7	127.5	134.7	141.6	147.8	151.2	153.8	155.5	157.3	160.3	165.0	167.4	169.2	171.5
	SE	0.81	0.84	0.72	0.62	0.55	0.48	0.49	0.46	0.41	0.37	0.36	0.46	0.38	0.42	0.38	0.47	0.78
Body length cm		78.0	90.0	102.5	113.1	121.2	128.1	133.7	138.2	142.2	144.3	147.5	148.8	151.7	154.7	158.1	158.5	164.7
	SE	0.93	0.86	0.76	0.69	0.58	0.52	0.56	0.49	0.51	0.50	0.53	0.61	0.58	0.61	0.54	0.81	0.78
Forearm cm		29.7	31.0	33.7	35.0	36.0	37.3	38.8	39.9	39.7	41.0	41.4	42.1	42.5	43.2	44.2	43.5	42.2
	SE	0.30	0.20	0.18	0.20	0.19	0.16	0.15	0.16	0.18	0.18	0.17	0.17	0.15	0.15	0.14	0.23	0.27
Front Cannon cm		27.8	28.9	29.9	30.7	31.0	31.5	31.8	31.7	31.6	32.0	32.1	31.8	31.9	32.1	32.2	32.1	30.2
	SE	0.29	0.17	0.14	0.13	0.12	0.13	0.14	0.13	0.14	0.14	0.15	0.18	0.15	0.17	0.17	0.26	0.15
Hind Cannon cm		39.5	39.8	41.2	42.2	42.7	43.4	44.0	44.2	44.4	44.7	44.8	45.1	45.0	45.0	45.5	45.5	42.8
	SE	0.46	0.23	0.17	0.14	0.12	0.15	0.14	0.15	0.17	0.16	0.17	0.19	0.17	0.18	0.18	0.27	0.23
Physeal circumference cm		24.0	24.7	26.7	27.8	28.5	29.1	29.8	30.0	29.9	30.2	30.6	30.7	31.4	31.8	31.8	31.8	31.3
	SE	0.24	0.15	0.13	0.15	0.11	0.13	0.13	0.11	0.11	0.13	0.10	0.11	0.10	0.10	0.12	0.13	0.15
Fetlock circumference cm		20.2	20.7	22.1	22.8	23.6	24.2	24.8	25.2	25.3	25.5	25.6	25.8	26.2	26.4	26.6	26.6	26.3
	SE	0.25	0.11	0.10	0.10	0.10	0.09	0.10	0.10	0.11	0.09	0.08	0.09	0.09	0.09	0.09	0.11	0.11

[a]A description of growth variables is given in Table 3.
[b] number of horses measured in a particular month.
[c]ADG = ((Weight(month 1) – Weight(month 2))/number of days between sampling periods.
[e]Body condition measured on a scale of 1 to 9 (Henneke et al., 1983).
[f]Body condition scores were first measured in June

Table 5. Coefficients in the best fit empirical equation[a] estimating body weight from variables measured in Thoroughbred foals (0 – 17 mo). P values represent the probability that the coefficient is not different from zero

Year	Intercept	*P*	C	*P*	A	*P*	L	*P*	G	*P*	C2	*P*	R^2
1996 (n = 320)	309.1 ± 62	< 0.0001	-28.1 ± 4.4	< 0.0001	$8.1 \times 10^{-5} \pm 2.5 \times 10^{-5}$	0.0013	$0.002 \pm 6.0 \times 10^{-4}$	< 0.0001	$0.012 \pm 5.0 \times 10^{-4}$	< 0.0001	0.56 ± 0.07	< 0.0001	0.989
1997 (n = 308)	484.3 ± 93	< 0.0001	-40.4 ± 6.6	< 0.0001	$-5.3 \times 10^{-6} \pm 2.6 \times 10^{-5}$	0.8378	$0.007 \pm 5.0 \times 10^{-4}$	< 0.0001	$0.011 \pm 4.0 \times 10^{-4}$	< 0.0001	0.73 ± 0.11	< 0.0001	0.989
1998 (n = 377)	142.5 ± 61	0.0192	-16.2 ± 4.4	0.0002	$3.0 \times 10^{-4} \pm 1.8 \times 10^{-5}$	< 0.0001	$0.005 \pm 4.0 \times 10^{-4}$	< 0.0001	$0.008 \pm 4.0 \times 10^{-4}$	< 0.0001	0.38 ± 0.08	< 0.0001	0.993
1999 (n = 338)	224 ± 104	0.0328	-23.3 ± 7.2	0.0013	$2.0 \times 10^{-4} \pm 2.2 \times 10^{-5}$	< 0.0001	$0.006 \pm 5.0 \times 10^{-4}$	< 0.0001	$0.009 \pm 4.0 \times 10^{-4}$	< 0.0001	0.49 ± 0.12	< 0.0001	0.990
2000 (n = 273)	147.8 ± 49	0.0029	-15.2 ± 3.4	< 0.0001	$9.0 \times 10^{-5} \pm 2.0 \times 10^{-5}$	< 0.0001	$0.005 \pm 4.0 \times 10^{-4}$	< 0.0001	$0.011 \pm 4.0 \times 10^{-4}$	< 0.0001	0.29 ± 0.06	< 0.0001	0.993
All years (n = 1615)	173 ± 34	< 0.0001	-18.3 ± 2.4	< 0.0001	$2.0 \times 10^{-4} \pm 1.1 \times 10^{-5}$	< 0.0001	$0.004 \pm 2.0 \times 10^{-4}$	< 0.0001	$0.011 \pm 2.0 \times 10^{-4}$	< 0.0001	0.38 ± 0.04	< 0.0001	0.988

[a] Weight = intercept + C * physeal circumference + A * age^2+ L * body length^2 + G * girth^2 + C2 * physeal circumference^2

Table 6. Coefficients in the best fit empirical equation[a] estimating ADG from variables measured in Thoroughbred foals. P values represent the probability that the coefficient is not different from zero

Year	Intercept	*P*	A	*P*	T	*P*	D	*P*	G	*P*	T2	*P*	D2	*P*	G2	*P*	R^2
1996 (n = 299)	19.5 ± 1.9	< 0.0001	$-0.005 \pm 7.0 \times 10^{-4}$	< 0.0001	0.13 ± 0.02	< 0.0001	-3.0 ± 0.4	< 0.0001	-0.07 ± 0.02	< 0.0001	$-0.001 \pm 1.0 \times 10^{-4}$	< 0.0001	0.12 ± 0.01	< 0.0001	$3.0 \times 10^{-4} \pm 6.1 \times 10^{-5}$	< 0.0001	0.726
1997 (n = 285)	-2.2 ± 2.1	0.2991	$-0.005 \pm 5.0 \times 10^{-4}$	< 0.0001	0.13 ± 0.02	< 0.0001	-0.9 ± 0.3	0.0067	0.06 ± 0.03	0.0333	$-0.001 \pm 1.0 \times 10^{-4}$	< 0.0001	0.04 ± 0.01	0.0050	$-1.0 \times 10^{-4} \pm 9.1 \times 10^{-5}$	0.1321	0.636
1998 (n = 352)	3.3 ± 1.4	0.0185	$-0.003 \pm 4.0 \times 10^{-4}$	< 0.0001	0.09 ± 0.01	< 0.0001	-1.0 ± 0.2	< 0.0001	0.01 ± 0.01	0.5919	$-0.001 \pm 1.0 \times 10^{-4}$	< 0.0001	0.05 ± 0.01	< 0.0001	$5.9 \times 10^{-6} \pm 5.3 \times 10^{-5}$	0.9111	0.663
1999 (n = 312)	6.1 ± 2.8	0.0336	$-0.006 \pm 9.0 \times 10^{-4}$	< 0.0001	0.09 ± 0.02	< 0.0001	-1.2 ± 0.5	0.0117	-0.03 ± 0.03	0.4090	$-0.001 \pm 8.1 \times 10^{-5}$	< 0.0001	0.06 ± 0.02	0.0034	$2.0 \times 10^{-4} \pm 1.0 \times 10^{-4}$	0.1703	0.501
2000 (n = 252)	2.9 ± 3.1	0.3549	$-0.007 \pm 7.0 \times 10^{-4}$	< 0.0001	0.08 ± 0.02	< 0.0001	-0.4 ± 0.6	0.4458	-0.04 ± 0.02	0.0247	$-0.001 \pm 2.0 \times 10^{-4}$	< 0.0001	0.03 ± 0.02	0.2461	$2.0 \times 10^{-4} \pm 6.9 \times 10^{-5}$	0.0010	0.731
All years (n = 1500)	7.0 ± 0.9	< 0.0001	$-0.005 \pm 3.0 \times 10^{-4}$	< 0.0001	0.08 ± 0.01	< 0.0001	-0.9 ± 0.2	< 0.0001	-0.05 ± 0.01	< 0.0001	$-0.001 \pm 5.1 \times 10^{-5}$	< 0.0001	0.04 ± 0.01	< 0.0001	$2.0 \times 10^{-4} \pm 3.0 \times 10^{-5}$	< 0.0001	0.591

[a] ADG = intercept + A * age + T * temperature + D * day length + G * girth + T2 * temperature^2 + D2 * day length^2 + G2 * girth^2

Table 7. Allometric relationships ($y = a*x^b$) between linear body dimensions and live body mass of Thoroughbred foals[a], where y = body dimension and x = live body mass.

Variable	Proportionality coefficient (a)[b]	Exponent (b)[b]	R^2
Girth, cm	19.34 ± 0.13	0.361 ± 0.001	0.99
Body length, cm	18.30 ± 0.24	0.360 ± 0.002	0.95
Forearm length, cm	11.36 ± 0.16	0.223 ± 0.003	0.84
Hip height, cm	45.52 ± 0.33	0.205 ± 0.001	0.95
Wither height, cm	45.83 ± 0.34	0.199 ± 0.001	0.94
Fetlock circumference, cm	10.02 ± 0.10	0.163 ± 0.002	0.84
Physeal circumference, cm	12.51 ± 0.13	0.156 ± 0.002	0.83
Hind cannon length, cm	27.61 ± 0.33	0.083 ± 0.002	0.50
Front cannon length, cm	22.09 ± 0.33	0.064 ± 0.003	0.26

[a] n = 1615
[b] Asymptotic standard deviations are listed

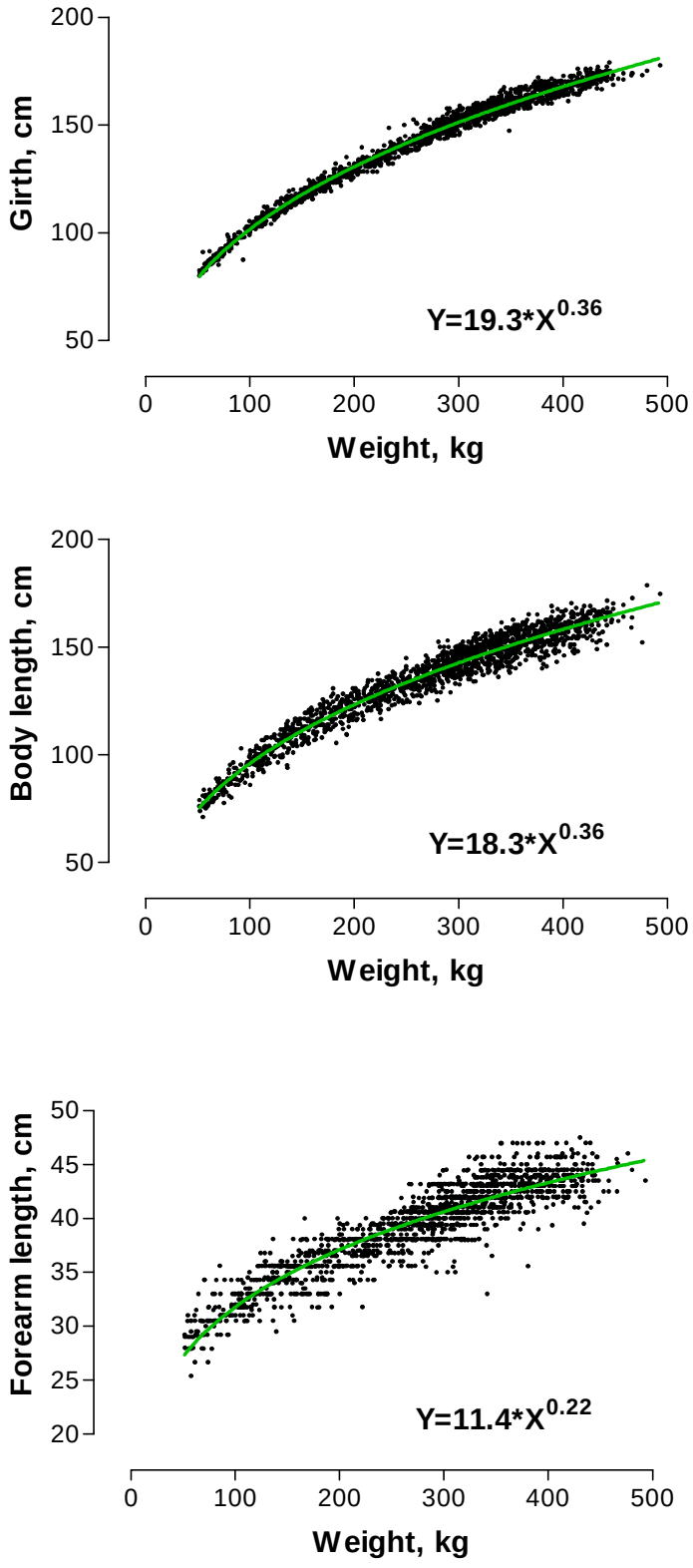


Figure 1. Allometric relationships (y = a*xb) between linear body dimensions and live body mass of Thoroughbred foals (n = 1615), where y = body dimension and x = live body mass.

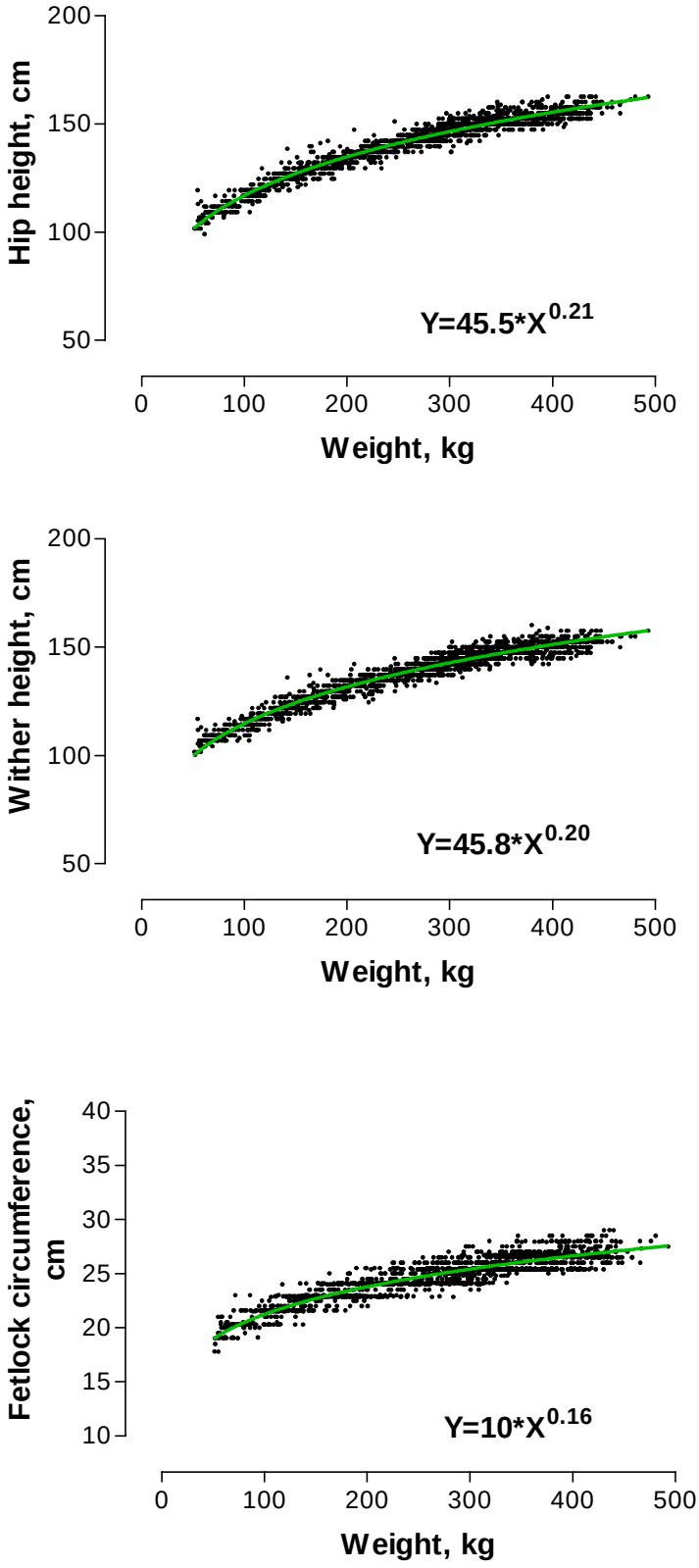
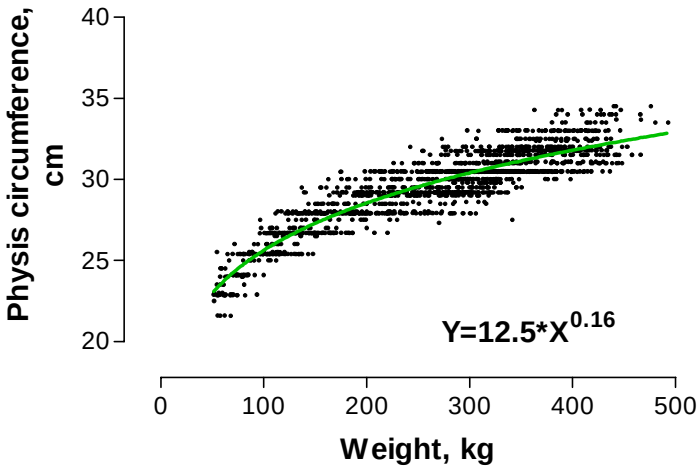
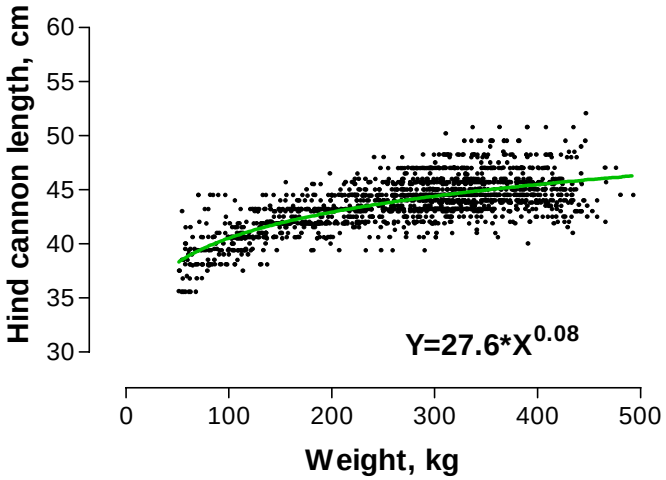


Figure 2. Allometric relationships ($y = a*x^b$) between linear body dimensions and live body mass of Thoroughbred foals (n = 1615), where y = body dimension and x = live body mass.





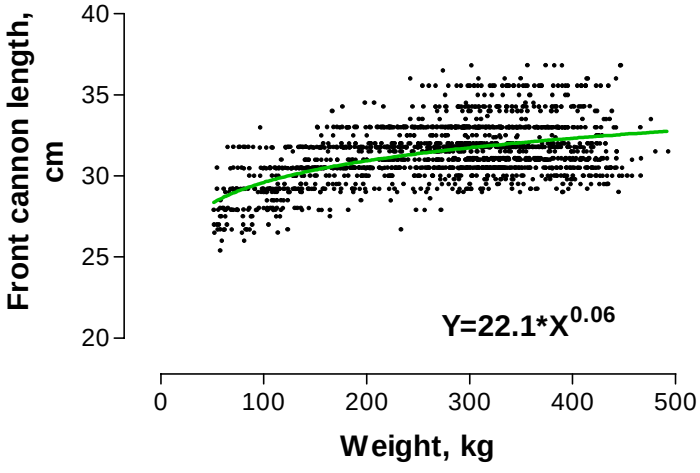


Figure 3. Allometric relationships ($y = a*x^b$) between linear body dimensions and live body mass of Thoroughbred foals (n = 1615), where y = body dimension and x = live body mass.

Table 8. Estimated parameter values[a] of asymptote (A), scaling parameter (b), maturing index (k), inflection parameter (M), percent variation accounted for (R^2), and residual sum of squares (RSS) for the growth of linear body dimensions with age (t) in Thoroughbred foals[b].

Variable	A	b	k	M	R^2	RSS
Wither height, cm						
Monomolecular[c]	153 ± 0.3	-0.30 ± 0.002	$0.007 \pm 1.4 \times 10^{-4}$	1.00	0.93	19247
Richards[d]	154 ± 0.8	0.56 ± 0.11	$0.006 \pm 5.6 \times 10^{-4}$	0.45 ± 0.12	0.93	19200
Hip height, cm						
Monomolecular[c]	157 ± 0.3	-0.31 ± 0.002	$0.007 \pm 1.5 \times 10^{-4}$	1.00	0.92	21798
Richards[d]	158 ± 0.9	0.72 ± 0.07	$0.005 \pm 5.2 \times 10^{-4}$	0.31 ± 0.05	0.92	21614
Girth, cm						
Monomolecular[c]	174 ± 0.5	-0.48 ± 0.002	$0.006 \pm 8.8 \times 10^{-4}$	1.00	0.97	24624
Richards[d]	194 ± 3.6	0.95 ± 0.01	$0.002 \pm 2.7 \times 10^{-4}$	0.28 ± 0.01	0.97	22282
Body length, cm						
Monomolecular[c]	163 ± 0.6	-0.48 ± 0.003	$0.006 \pm 1.3 \times 10^{-4}$	1.00	0.94	47352
Richards[d]	186 ± 5.8	0.97 ± 0.01	$0.002 \pm 3.7 \times 10^{-4}$	0.25 ± 0.02	0.94	44006
Forearm length, cm						
Monomolecular[c]	45 ± 0.3	-0.33 ± 0.003	$0.005 \pm 2.1 \times 10^{-4}$	1.00	0.84	4636
Richards[d]	51 ± 3.5	0.94 ± 0.05	$0.001 \pm 8.3 \times 10^{-4}$	0.19 ± 0.03	0.84	4593
Fetlock circumference, cm						
Monomolecular[c]	27 ± 0.1	-0.25 ± 0.003	$0.007 \pm 2.7 \times 10^{-4}$	1.00	0.79	1373
Richards[d]	28 ± 0.4	0.91 ± 0.05	$0.003 \pm 8.5 \times 10^{-4}$	0.14 ± 0.02	0.79	1353
Physeal circumference, cm[e]						
Monomolecular[c]	32 ± 0.1	-0.24 ± 0.003	$0.008 \pm 3.1 \times 10^{-4}$	1.00	0.75	2074
Front cannon length, cm[e]						
Monomolecular[c]	32 ± 0.1	-0.12 ± 0.006	0.016 ± 0.001	1.00	0.27	3811
Hind cannon length, cm[e]						
Monomolecular[c]	45 ± 0.1	-0.14 ± 0.004	0.011 ± 0.004	1.00	0.47	4784

[a] Asymptotic standard deviations are listed
[b] n = 1615
[c] $y = A(1-be-kt)$
[d] $y = A(1-be-kt)^M$
[e] The Richards equation would not converge for these variables.

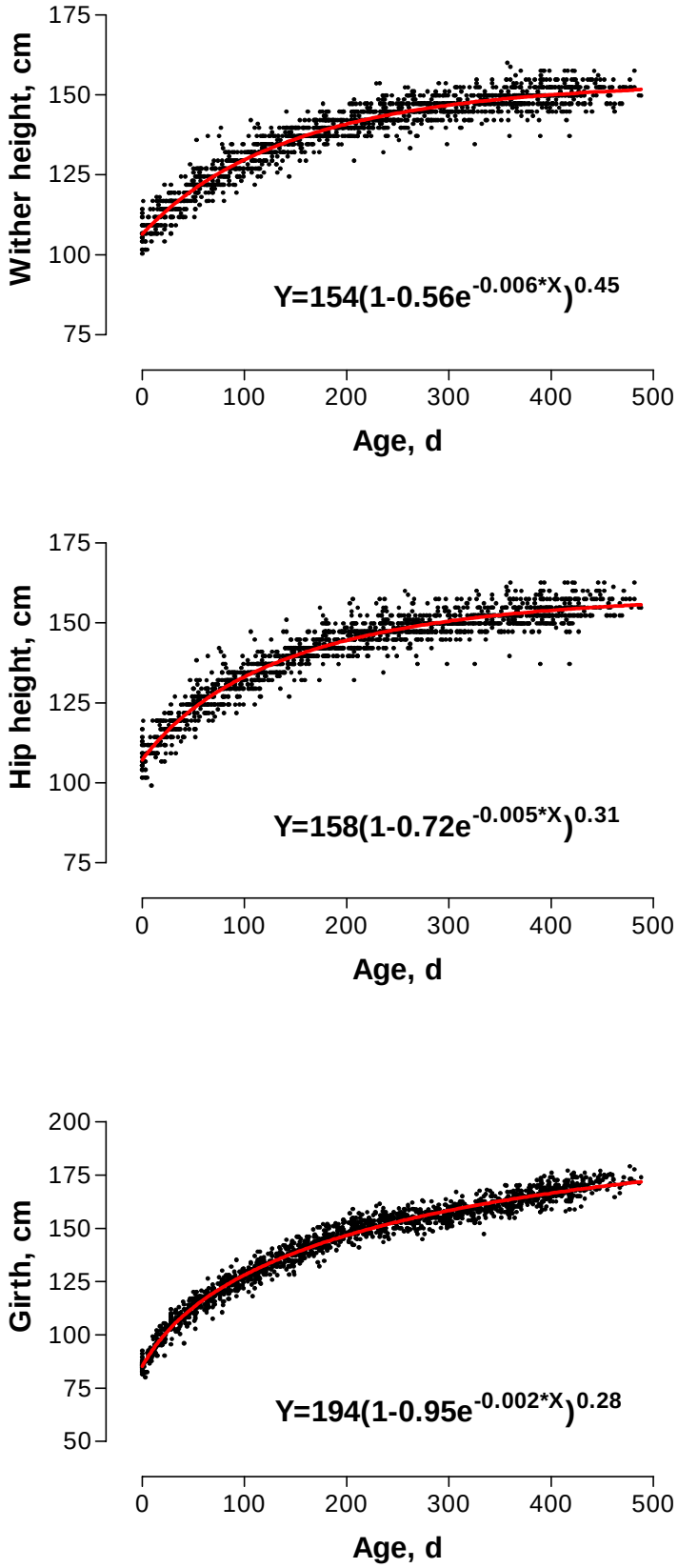


Figure 4. Curves obtained by fitting Richards equation to linear body dimensions in Thoroughbred foals(n = 1615), where y = body dimension and x = age.

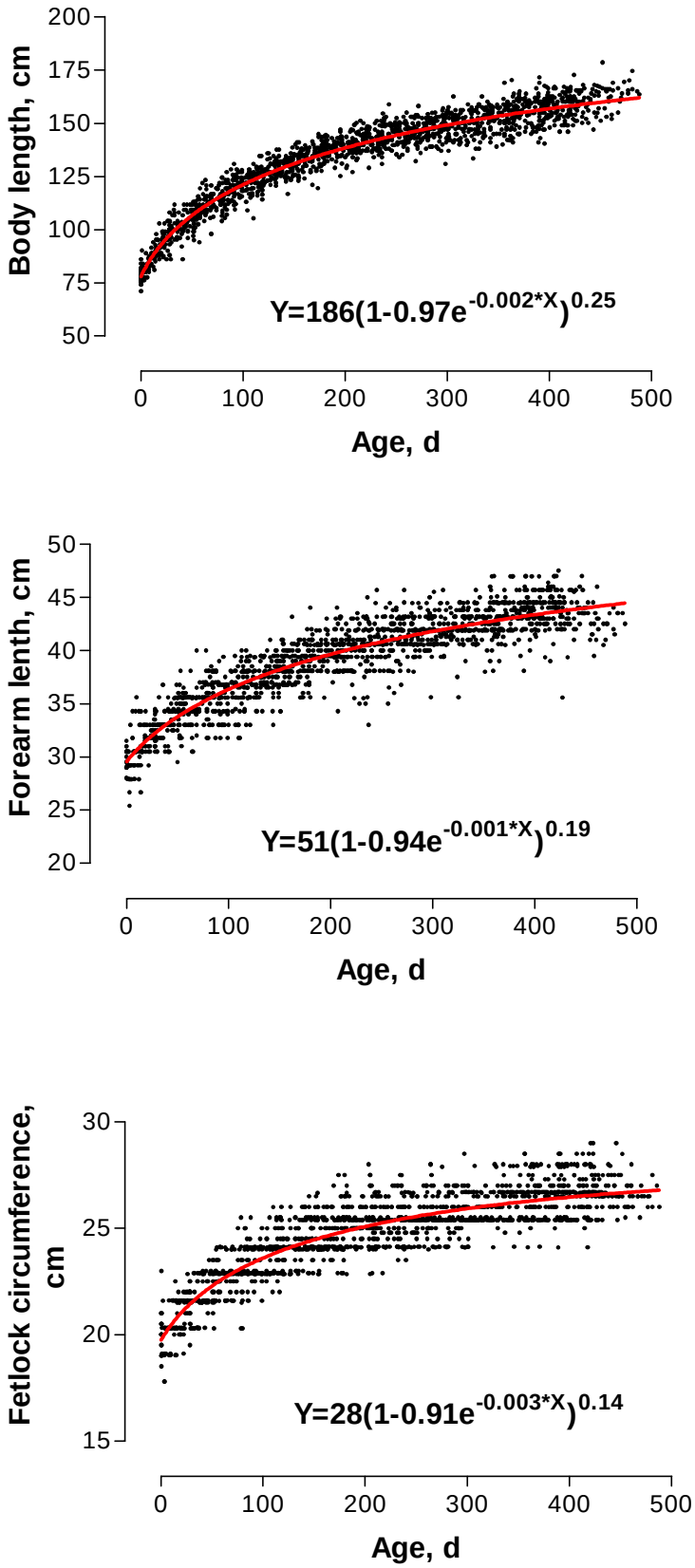
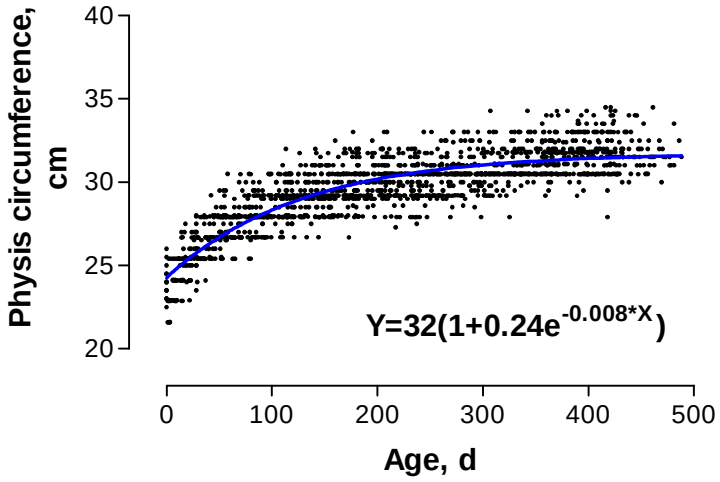
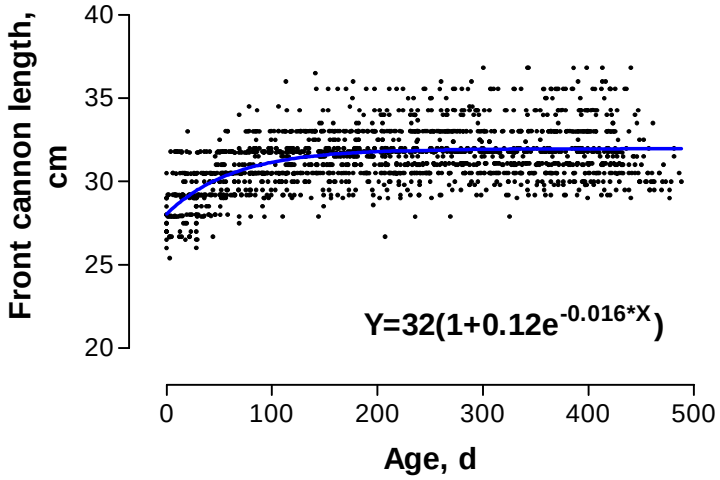


Figure 5. Curves obtained by fitting Richards equation to linear body dimensions in Thoroughbred foals(n = 1615), where y = body dimension and x = age.





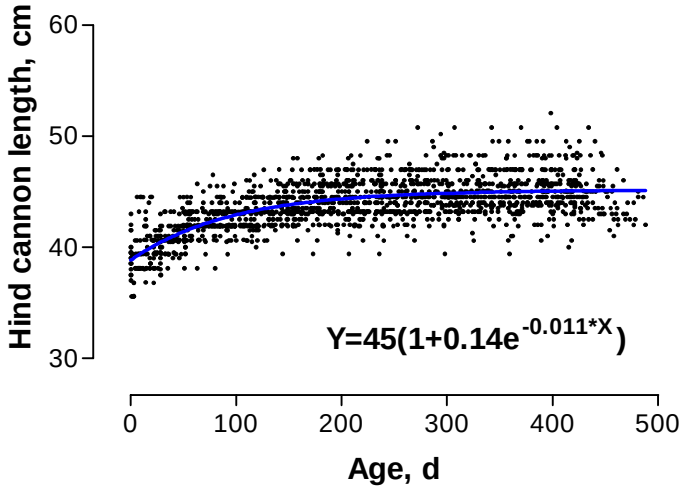


Figure 6. Curves obtained by fitting a monomolecular equation to linear body dimensions in Thoroughbred foals(n = 1615), where y = body dimension and x = age.

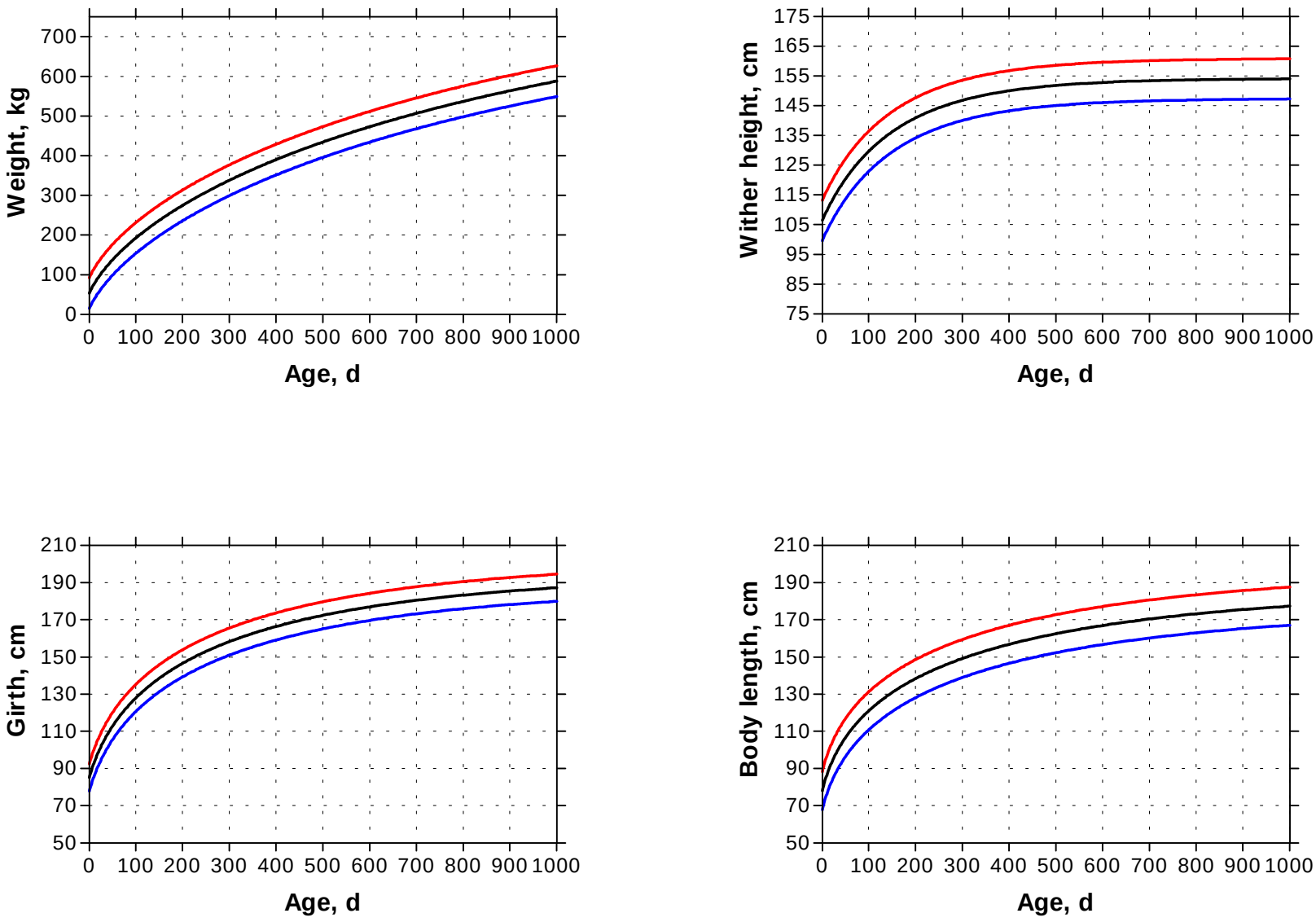


Figure 7. 95% prediction intervals for weight, wither height, girth and body length in Thoroughbred foals at ages from 0 to 1000 d.

Table 9. Estimated parameter values[a], percent of variation accounted for (R^2), and residual sum of squares (RSS) for Richards and modified Richards growth equations used to characterize weight and ADG as a function of age (t) in Thoroughbred foals[b].

Parameter	Richards[c]	Richards and sine wave[d]
Asymptote (A)	852 ± 136	852
Scaling parameter (b)	0.99 ± 0.005	0.99
Maturing index (k)	$7.6 \times 10^{-4} \pm 2.9 \times 10^{-4}$	7.6×10^{-4}
Inflection parameter (M)	0.59 ± 0.03	0.59
Amplitude (H)		
Weight, kg	N/A	-3.9 ± 0.7
ADG, kg/d	N/A	0.2 ± 0.01
Frequency (F)		
Weight, radians/d[e]	N/A	0.0249 ± 0.001
ADG, radians/d[e]	N/A	$0.0247 \pm 3.8 \times 10^{-4}$
Offset (O)		
Weight, radians[f]	N/A	-0.19 ± 0.32
ADG, radians[f]	N/A	-1.8 ± 0.10
Weight R^2	0.961	0.964
Weight RSS	625757	579340
ADG R^2	0.459	0.582
ADG RSS	149.3	115.3

[a] Asymptotic standard deviations are listed
[b] n = 1615
[c] Weight prediction ($y = A(1-be-kt)^M$), ADG prediction ($y = MAkbe-kt(1-be-kt)^M(1-be-kt)^{-1}$)
[d] Weight prediction ($y = (A(1-be-kt)^M)+H(\sin(Ft+O))$), ADG prediction ($y = (MAkbe-kt(1-be-kt)^M(1-be-kt)^{-1})+H(\sin(Ft+O))$)
[e] Time of one complete cycle = $2\pi/F$
[f] Offset in d = O/F

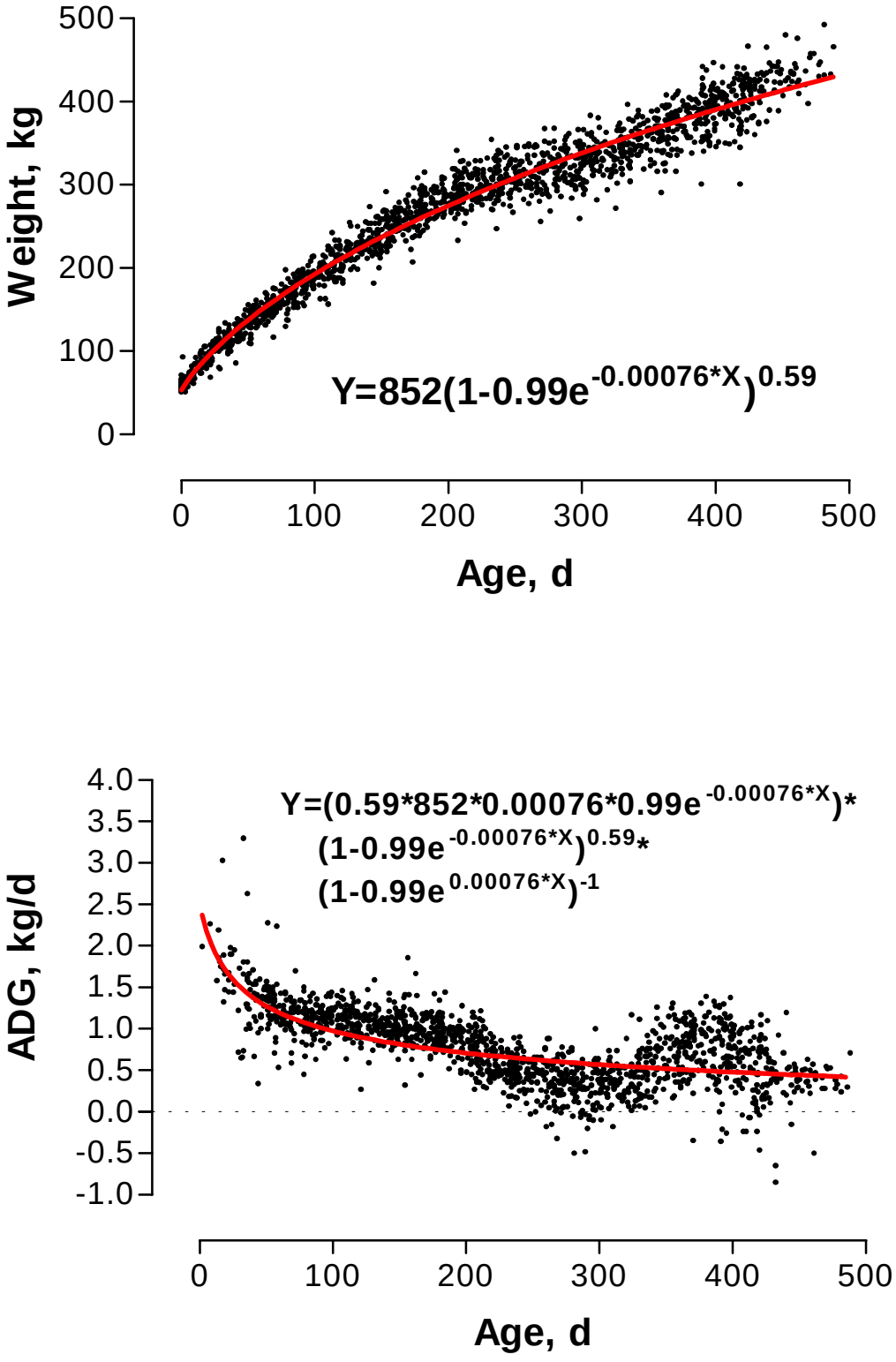


Figure 8. Curves obtained by fitting Richards equation to live body mass and ADG in Thoroughbred foals(n = 1615), where y = weight and ADG, and x = age.

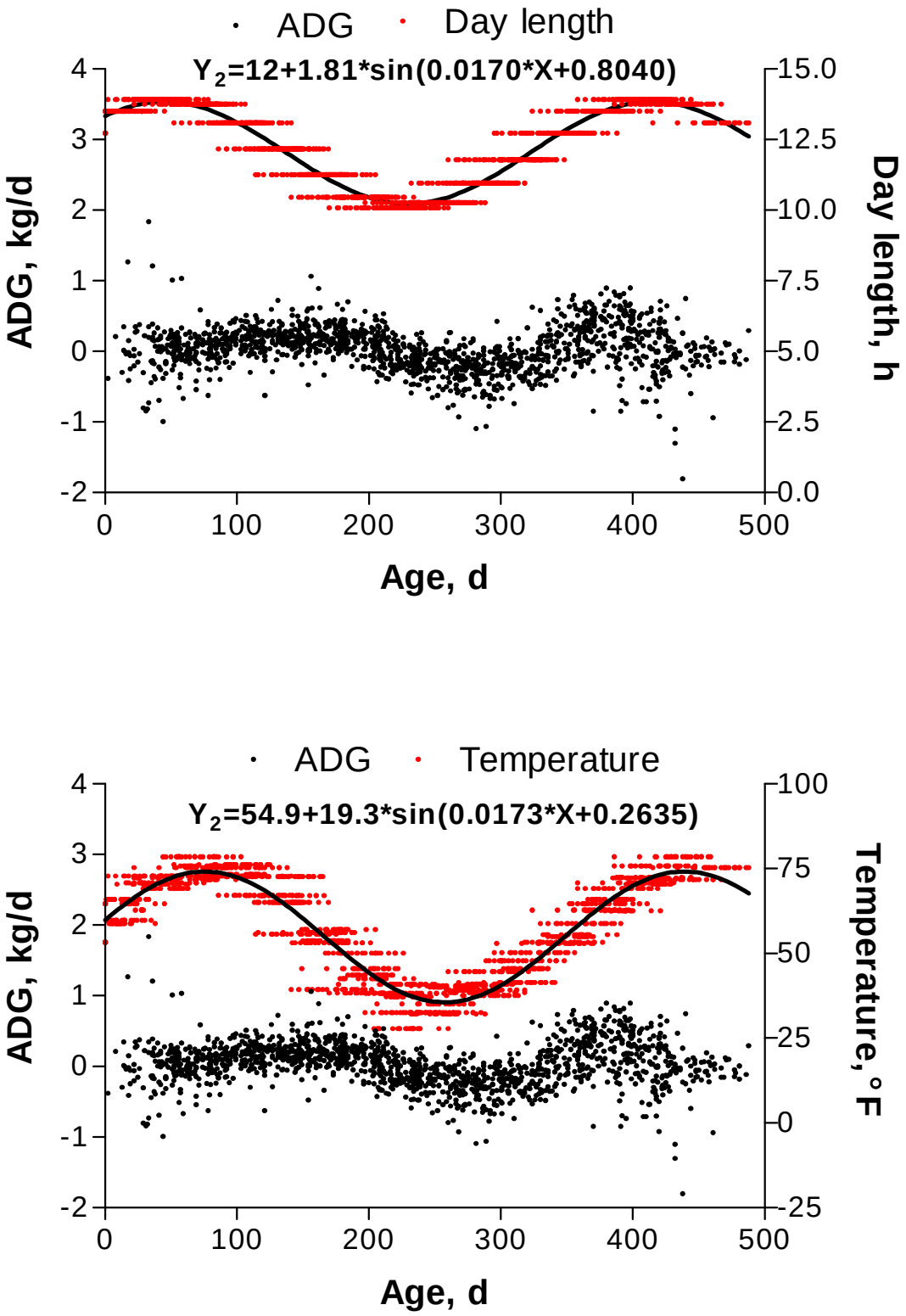


Figure 9. The relationship between the residuals of Richards equation for ADG and day length and temperature at different ages in Thoroughbred foals. The equations are best fit sine wave equations to the day length and temperature data.

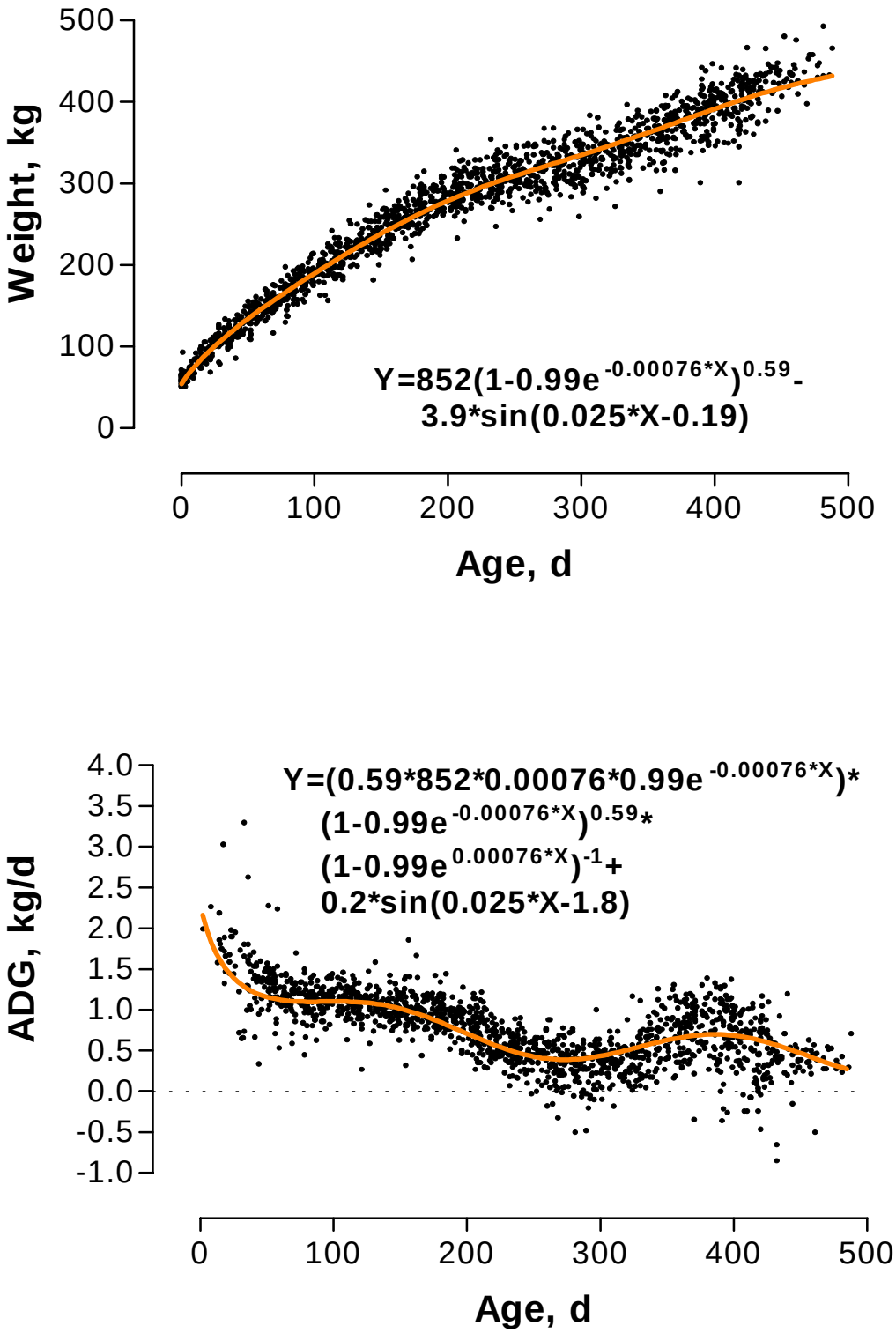
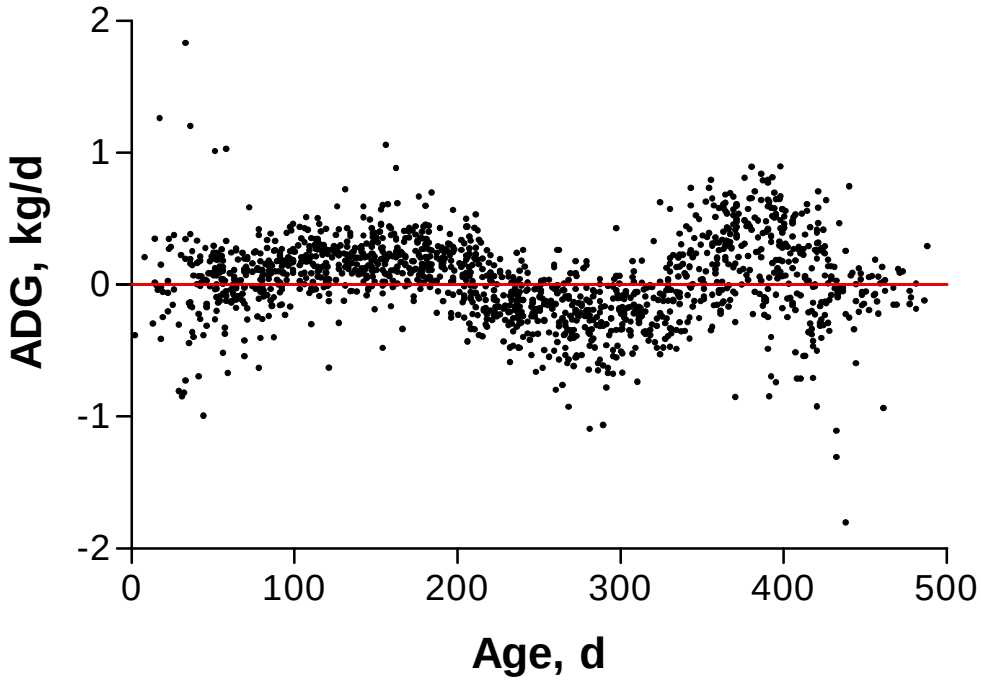


Figure 10. Curves obtained by fitting a modified Richards equation to live body mass and ADG in Thoroughbred foals(n = 1615), where y = weight and ADG, and x = age.



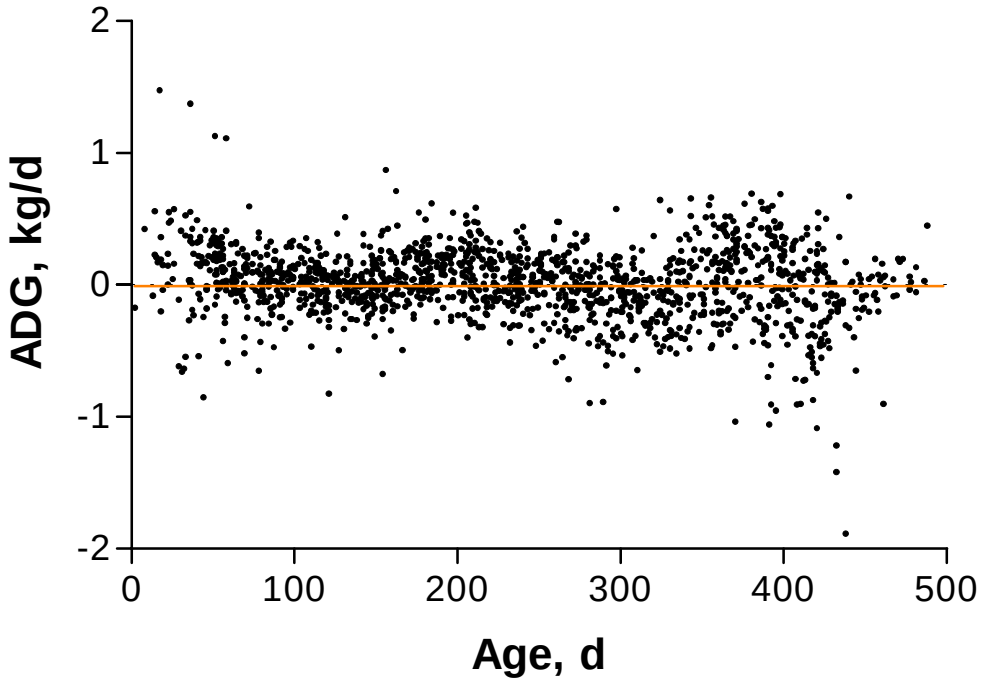
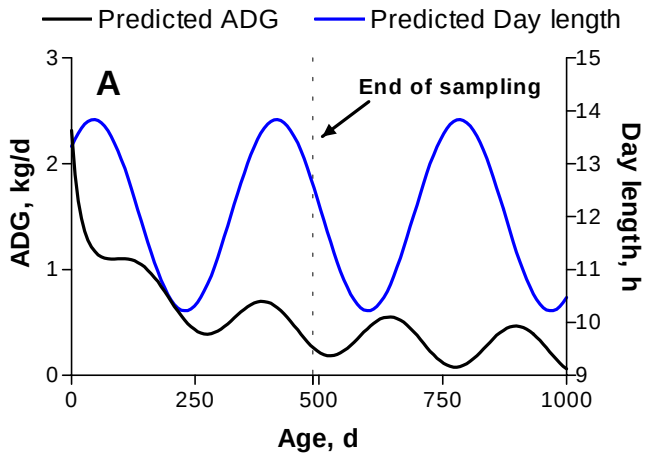
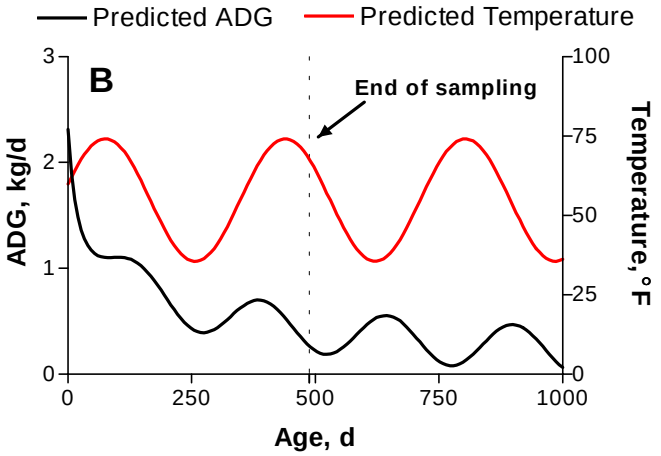


Figure 11. Residuals of the Richards and modified Richards equation's predictions of ADG.





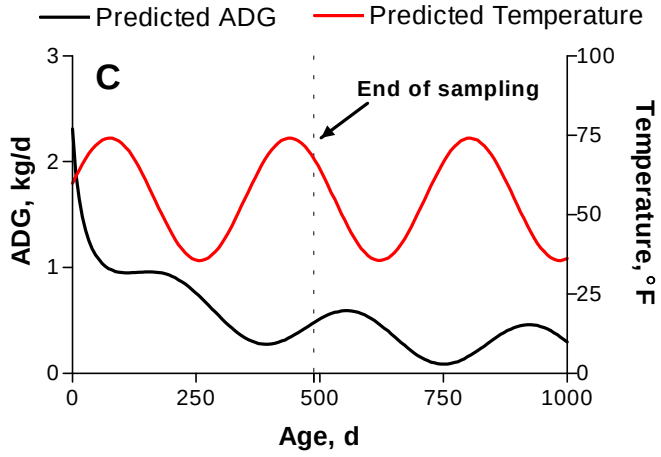


Figure 12. Predicted ADG, day length and temperature over a 1000 day period. In charts A and B, ADG is predicted using a sine wave with a frequency of 0.0247 radians/d. In chart C, ADG is predicted using a sine wave with a frequency of 0.0172 radians/d. In charts A, B and C day length and temperature have a frequency of 0.017 radians/d.

Table 10. Parameters[a] of weight estimation equations for Thoroughbred foals[b] using two measures of volume that incorporate girth (G), body length (L_1), physeal circumference (C), and forearm + front cannon length (L_2).

Equation	Slope (m)	y-intercept (b)	R^2	RSS
$Volume_1 = \frac{G^2 \times L_1}{4\pi}$				
Weight = m($Volume_1$) + b	1069 ± 3.6	20.7 ± 0.92	0.982	294152
Weight = m($Volume_1$)	1145 ± 1.5	N/A	0.976	386152
$Volume_2 = \frac{\left(G^2 \times L_1\right) + 4\left(C^2 \times L_2\right)}{4\pi}$				
Weight = m($Volume_2$) + b	1028 ± 3.3	9.6 ± 0.90	0.984	263612
Weight = m($Volume_2$)	1062 ± 1.2	N/A	0.982	282347

[a] Asymptotic standard deviations are listed
[b] n = 1615

Table 11. Fit of models predicting weight from volumes that incorporate girth (G), body length (L_1), physeal circumference (C), and forearm + front cannon length (L_2).in Thoroughbred foals[ab] using a line with a slope (m) and no y-intercept. Fit was evaluated by examining percent variation accounted for (R^2), and residual sum of squares (RSS).

Prediction model	Equation	Slope (m)	R^2	RSS
Carroll and Huntington	$Weight = \frac{G^2 \times L_1}{4\pi} \times m$	1058	0.895	78368
Carroll and Huntington (fitted)	$Weight = \frac{G^2 \times L_1}{4\pi} \times m$	1145	0.977	16984
Author's results	$Weight = \frac{(G^2 \times L_1) + 4(C^2 \times L_2)}{4\pi} \times m$	1062	0.991	6861

[a] n = 151 (raw data, not used in determining equation coefficients)
[b] Actual mean weight was 172 ± 5.7 kg with a range of 50 to 314 kg

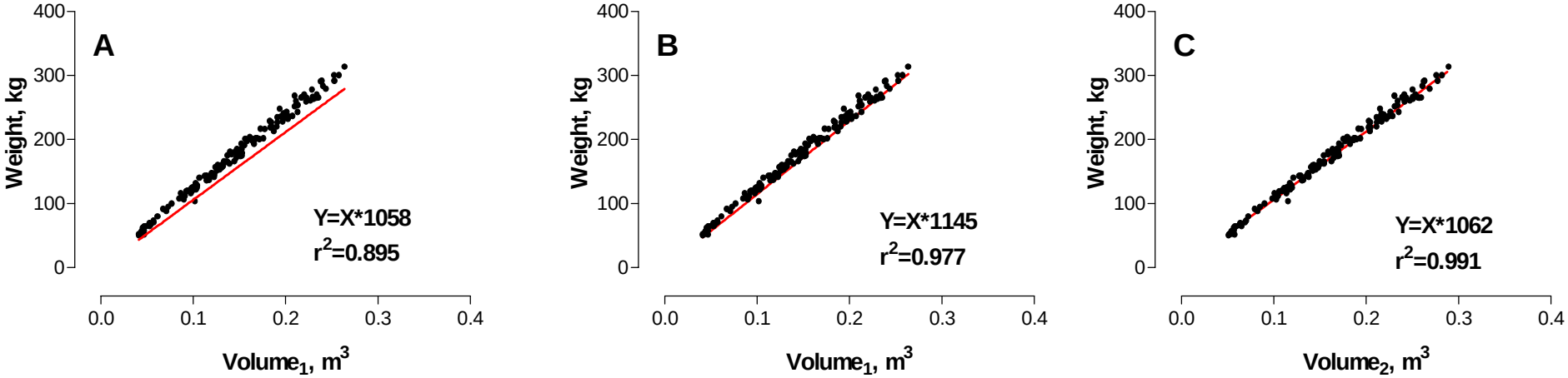


Figure 13. Volume and weight relationships for three different forms of the equation; weight = volume × m, for Thoroughbred foals. In chart A and B, volume is calculated from girth and body length. In chart C, volume is calculated from girth, body length, physeal circumference, and forearm and front cannon length. Chart A uses the coefficient determined by Carrol and Huntington (1988). Chart B and C use coefficients determined from the previous 5 yr of growth data.

Chapter 3:
Glycemic response tests in Thoroughbred yearlings

Introduction

Management practices typically allow for the twice daily feeding of supplements to horses. The goal of feeding supplements is to match daily nutrient and energy intake with requirements for growth, reproduction, and work (Hoffman and Kronfeld, 1999; NRC, 1989). Typical concentrates contain hydrolyzable carbohydrates as the principal energy source. Intake of such supplements results in physiologic perturbations to digestive, circulatory, metabolic and hormonal systems (Clarke et al., 1990). Disturbances to these systems in turn affect the normal functioning of the horse. These perturbations may be manifested as changes in growth and body condition, and increased risk of digestive and metabolic disorders (Kronfeld, 1998). Significant fluctuations in the growth pattern of horses as well as other species have been connected with the DOD (Jeffcott and Henson, 1998).

Exchanging fat and fiber for a portion of the hydrolyzable carbohydrates may help to reduce disturbances caused by the sudden availability of a large portion of hydrolyzable carbohydrates. The benefits of added fat included its high energy density, higher digestive and metabolic efficiency, and a lower risk of certain digestive and metabolic disorders (Kronfeld et al., 2001). The increased fiber content would sustain an active microbial population in the hind-gut, and increased energy from the production of acetate, propionate, and butyrate. The present experiment focuses on the glycemic response in Thoroughbred yearlings fed supplements with either a high concentration of hydrolyzable carbohydrates and low concentration of fat, or a low concentration of hydrolyzable carbohydrates and a high concentration of fat.

Materials and Methods

This experiment was conducted in conjunction with a long-term study investigating growth and development. Pregnant mares were initially paired by weight and foaling date and then randomly assigned to two groups. These two groups were maintained for 19 months. The groups were continuously grazed on mixed grass and legume pasture. Pregnant mares were started on dietary supplements three months prior to foaling, and foals were weaned at six months of age.

Twelve Thoroughbred yearlings that were 449.5 ± 4.5 d of age were used. They were raised on one of the two experimental supplements used in the experiment, with equal numbers on each supplement. The yearlings were accustomed to being weighed and handled. This protocol had been approved by the institutional animal care and use committee.

Two supplements contained different carbohydrate profiles (Table 12 and 13). The first (SS-2) contained a high level of rapidly digestible carbohydrates (non-structural carbohydrates (NSC): 64.5 ± 3.6 %) and lower levels of slowly fermentable carbohydrates (ADF 10.7 ± 1.0 %; NDF 18.5 ± 1 %). The second (FF-2) contained a low level of NSC (24.7 ± 2.0 %) and high levels of ADF (22.6 ± 0.7 %) and NDF (36.9 ± 1.0 %). The fat content was 16.6 ± 0.8 % in the FF-2 and 3.2 ± 0.3 % in the SS-2 supplement. The supplements were also approximately isonitrogenous (CP: SS-2 13.7 ± 0.6 %; FF-2 15.4 ± 0.2 %). Supplements were designed 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).

Glycemic response tests were conducted in July of 1999. Yearlings were weighed (Tyrel platform, TC-105, Alweights Hamilton Scale Corp., Richmond, VA),

then stalled overnight (12 to 18 h) with *ad libitum* access to water but no supplement. The following morning at 0700 catheters were placed in the left jugular vein to facilitate multiple sampling through the day.

Baseline blood samples were taken 45 min after catheter placement. Yearlings were fed (1.82 kg of SS-2 or FF-2) immediately after baseline sampling. The SS-2 and FF-2 meals were consumed in approximately 35 min. Subsequent blood samples were taken at 40, 70, 100, 160, 220, 280, 340, and 400 min after the baseline sample. Blood was collected into 10–ml tubes (Lithium Heparin Vacutainer, Becton Dickinson, Rutherford, NJ). Samples were centrifuged immediately after collection for 10 min at 1600g at 20°C. Plasma was separated and frozen at -4°C. Plasma glucose concentrations were determined using a glucose oxidase colorimetric assay in a chemical autoanalyzer (Kit # 442640, Beckman Synchron CX5CE, Brea, CA). Plasma insulin was determined by radioimmunoassay (Coat-A-Count Insulin, Kit # THINX, Diagnostic Products Corp., Los Angeles, CA). Both the glucose and insulin assays had been previously validated in the horse (Ralston, 1992).

Data are presented as means ± SE's. The responses of plasma concentrations of glucose and insulin to a meal were calculated as increments over a baseline value. Two sample t-tests revealed no difference between dietary groups in initial, pre-meal values, so the data for all 12 horses were used to calculate the baseline means ± SE's for glucose and insulin.

Incremental glucose and insulin were evaluated for differences between the supplements over the entire sampling period by analysis of variance with repeated measures using the mixed procedures of SAS (SAS Inst. Inc., Cary, NC). The model statement included diet, time, and diet × time interaction, and horse within diet was defined as the subject effect. The covariance structure was set to autoregressive order one. Tukey's multiple-comparison procedure was used to test differences between time periods, with P set to 0.05 for significance and to 0.10 for a trend.

Two sample t-tests were used to determine if differences were present in variables between diets at a given sample time.

In this experiment plasma glucose and insulin concentrations were expressed as increments of the fasting or baseline concentrations to reduce the effects of the baseline concentrations on plasma glucose and insulin responses (Wolever, 1990). Incremental areas under the curve were calculated using the equation:

$$\text{Area} = (t_1 - t_0)*((I_{t0}+I_{t1})/2)+\ldots(t_{n+1}-t_n)*((I_{tn}+I_{tn+1})/2) \quad [1]$$

Where t is the time at which a particular sample is taken and I is the incremental change in the variable being measured at a specific time. Incremental area under the curve (AUC) of the concentration versus time data was calculated by summed trapezoids using GraphPad Prism version 3.02 for Windows (GraphPad Software, San Diego California USA). Two approaches were used in examining AUC. The first was to create points on the curves that represented the mean of all six yearlings (Figure 14), and then determine the AUC. This technique does not allow for the calculation of a standard error of the AUC value. The second technique was to evaluate each yearling's AUC individually, and present the mean ± SE of the AUC's for each dietary group (Table 14).

Results

The yearlings weighed 436 ± 11 kg in the SS-2 group and 421 ± 6.0 kg in the FF-2 group ($P = 0.29$). Baseline plasma glucose concentration was 112 ± 5.0 mg/dl. Plasma glucose concentrations changed with time ($P < 0.0001$). They were higher ($P = 0.021$) in the SS-2 than in the FF-2 group.

Plasma glucose concentration changed with time ($P < 0.0001$). The first and last samples were not significantly different from one another ($P = 0.60$). Diet significantly affected plasma glucose concentrations ($P = 0.02$). Peak glucose concentrations were 63% higher in the SS-2 group than the FF-2 group ($P = 0.01$). Peaks were reached at 108 ± 11 min, and both groups had returned to baseline at 400 min. Plasma glucose means were higher in the SS-2 group than in the FF-2

group at 70 and 100 min ($P < 0.05$). There was a pattern of higher plasma glucose in the SS-2 group at 160, 220, 280 and 340 min ($P < 0.18$). The mean areas under the individual glucose response curves for the two supplements were higher in the SS-2 group ($P = 0.04$) (Figure 15).

Plasma insulin concentration changed with time ($P < 0.0001$). The first and last samples were not significantly different from one another ($P = 0.62$). Diet significantly effected plasma insulin concentrations ($P = 0.031$). Peak insulin concentrations were 100% higher in the SS-2 group than the FF-2 group ($P = 0.02$). Peaks were reached at 113 ± 26 min, and both groups had returned to baseline at 400 min. Plasma insulin means were higher in the SS-2 group than in the FF-2 group at 70, 100 and 160 min ($P < 0.05$). There was a pattern of higher plasma insulin in the SS-2 group at 220, 280 and 340 min ($P < 0.19$). The mean areas under the individual insulin response curves for the two supplements were higher in the SS-2 group ($P = 0.03$) (Figure 15).

Discussion

The results demonstrated that the glycemic and insulinemic responses to a meal can be greatly reduced by substituting fat and fiber for starch and sugar. These response tests incorporate aspects seen in both glucose tolerance tests, which provide information about the glucose status of the animal, and glycemic indices, which provide information about glucose-equivalents in the diet (Wolever, 1990). The glycemic response test has been previously validated in brood mares (Williams et al., 2001), using two meals that are isocaloric for horses and yet differ in glucose equivalents . The changes in method met our objective of testing the effects of the whole meal, rather than only its carbohydrate, potentially glucose yeilding component.

Isocaloric glycemic response tests have been used to investigate the glucose and insulin response to numerous feeds in the horse. These feeds have included corn-based concentrates, concentrates with added corn oil, and various kinds of hay

(Stull and Rodiek, 1988). Offering feeds with relatively higher soluble carbohydrate concentrations results in an increased glucose and insulin response (Ralston, 1992; Smyth et al., 1989). The magnitudes of the responses in this trial are comparable with previous results.

Plasma glucose and insulin concentrations increased following ingestion of both the SS-2 and FF-2 supplements. Both plasma glucose and insulin returned to baseline values within 400 min. These were the only similarities between the responses of the two diets. Concentrations differed between diets in samples taken from 70 to 340 min. Repeated measures analysis resolved the differences due to diet and sample over the sampling period, and points to a difference in both glucose and insulin response over time due to diet (i.e. a significant interaction between diet and sample, $P < 0.05$). Incremental glucose and insulin response data was evaluated by two similar measures illustrated in Table 14. The first uses means at each sampling point for individual diets to form a curve. This technique does not permit the statistical determination of differences. The second technique uses means from individual curves from the data from each horse. The differences in the data from the two techniques were minor. Thus, horses fed the SS-2 supplement had a greater plasma glucose and insulin response than the FF-2 group. Incremental area under the curve, peak concentration, and concentrations at several sampling points were also higher in the SS-2 group. The time taken to reach peak values and time to return to baseline tended to be longer in the SS-2 group.

Various techniques in evaluating data have been used in previous studies that have investigated glucose and insulin responses to meals or glucose infusions (Murphy et al., 1997; Roberts and Hill, 1973; Stull and Rodiek, 1988). In these trials an initial baseline blood sample is taken after a period of fasting and just prior to administration of glucose or presentation of the meal. Blood samples are taken every 30 to 60 min for the following 4 to 9 hr. Following the meal or glucose dose, plasma glucose and insulin concentrations rise to a peak and then return to baseline

concentrations, although this return to baseline is not always observed within the sampling period. Baseline plasma glucose concentrations in these previous studies ranged from 83 to 100 mg/dl, with subsequent peaks following a meal of between 103 to 142 mg/dl. Baseline plasma insulin concentrations ranged from 2 to 5 mIU/L, with subsequent peaks of between 9 and 50 mIU/L. The lower peaks were noted in horses fed a 100% alfalfa diet, while the highest peaks were in horses fed a 50% corn and 50% alfalfa meal.

Factors that may affect glycemic response include diet composition, rate of consumption, gastric emptying, hydrolysis of carbohydrates, absorption of glucose from the gut, entry, and removal of glucose from the blood. The two supplements used in this experiment were isocaloric, yet differed in fractions of non-structural carbohydrates (NSC), NDF, ADF, and crude fat. Rate of consumption was 25 to 30 min for both groups. The other factors were not measured in this experiment. The diets' effect on glycemic response was likely due to the reduced NSC content in the FF-2, or its increased concentration of fat and fiber. Non-structural carbohydrate concentrations were 160% higher, and glucose and insulin AUC's were 109% and 157% in the SS-2 supplement.

Although some basal secretion of insulin may occur, it is generally believed that changes in glucose are required for acute insulin release (Karam, 1997). Stimulation for release of insulin is mainly accomplished by plasma glucose concentration. Glucose is passively transported across the β cell membrane by the GLUT-2 transporter. Less potent stimulators of insulin release include vagal stimulation and the amino acid leucine. Several other molecules have been shown to potentiate the release of insulin. Most of these are connected to the progression of digestion in the gastrointestinal tract. Examples include cholecystokinin, secretin, and gastrin. Finally, certain molecules inhibit the release of insulin. These include somatostatin and the α-adrenergic effects of catecholamines. It is likely that the

increased NSC concentrations in the SS-2 supplement led to increased availability of glucose for absorption from the small intestine.

The fluctuations in the glucose and insulin levels, that result from feeding two meals with a high concentration of hydrolyzable carbohydrates each day, will affect the production of other metabolites and hormones. Examples of these include free fatty acids, triglycerides, thyroid hormones, growth hormone, insulin-like growth factors, and insulin-like growth factor binding proteins (Glade and Reimers, 1985; Kronfeld, 1998). It is likely that this feeding/fasting cycle is foreign to the metabolism of the horse, which evolved as a grazing species. Results from this experiment demonstrate that the feeding/fasting cycle is moderated in yearlings fed supplements that have a portion of the hydrolyzable carbohydrates replaced with fat.

Moderation of the feeding/fasting cycle may reduce the risk of several digestive and metabolic disorders (Clarke et al., 1990; Kronfeld, 1998). Exertional rhabodomylosis has been mitigated or prevented by replacing starch and sugar sources with vegetable oils or rice bran (Valentine et al., 2001). Developmental orthopedic disease has been associated with exaggerated plasma glucose and insulin responses to a concentrate meal (Pagan et al., 2001; Ralston, 1995). Some of the data suggests that there may be a connection between the elevated response and osteochondrosis (Ralston, 1996). The Rutgers studies led to a patent of a diagnostic test for individuals. A subsequent small survey of 6 herds associated the incidence of osteochondrosis with a single estimate of plasma glucose or insulin 60 min after a meal. The regressions included an obvious outlier that invalidates the claimed associations, which would require confirmation by further studies.

Plasma glucose and insulin responses are initial events in the feeding/fasting cycle. Further on in the cycle are changes in hormones more likely to influence chondrocyte maturation to bone, such as the thyroid hormones (Glade and Reimers, 1985) and the somatotropic axis, involving GH and IGF-I (Staniar et al., 2001).

Therefore, studies of the somatotropic axis became the major intent of this dissertation.

Table 12. Ingredient composition (%) of the sugar and starch supplement (SS-2) and the fat and fiber supplement (FF-2)

Ingredient	SS-2	FF-2
Oat straw	7	7
Alfalfa	0	13.5
Dent yellow grain corn	60	11
Beet pulp	0	10
Soybean hulls	4	4
Molasses (cane)	10	5
Soybean meal	15.5	2
Limestone	1	1
Calcium phosphate, dibasic	1.5	0.5
Vitamin premix[a]	0.5	0.5
Mineral premix[b]	0.5	0.5
Processed cereal by-product[c]	0	45

[a] Provided the following amounts per kg of supplement: vitamin A, 6,900 IU; β-carotene, 17.6; vitamin D3, 1,290 IU; vitamin C, 333 mg; Niacin, 15 mg; Thiamin, 7 mg; Riboflavin, 3.5 mg; Biotin, .21 mg.
[b] Provided the following amounts per kg of supplement: Fe, 46.1mg; Zn, 105.8mg; Cu, 25.11mg; Mn, 18.02 mg; Se, .55 mg; I, .35 mg; NaCl used as carrier, 4160 mg.
[c] Processed cereal by-product contains 92.5% DM, 26% EE, 15.6% CP, 14.1% NSC, and 33.2% NDF.

Table 13. Nutrient composition[a] on a DM basis of the sugar and starch supplement (SS-2), and the fat and fiber supplement (FF-2).

	SS-2 (n = 3)		FF-2 (n = 3)	
Item	Mean	SE	Mean	SE
Crude protein, %	13.7	0.59	15.4	0.22
Crude fat, %	3.2	0.28	16.6	0.76
Acid detergent fiber, %	10.7	1.02	22.6	0.74
Neutral detergent fiber, %	18.5	1.11	36.9	0.99
Non-structural carbohydrates, %	64.5	3.61	24.7	2.02
Ca, %	1.2	0.14	1.4	0.19
P, %	0.6	0.05	1.4	0.06
Mg, %	0.2	0.01	0.7	0.03
K, %	1.1	0.06	1.3	0.02
Na, %	0.3	0.03	0.2	0.02
Fe, mg/kg	257	32.1	356	15.7
Zn, mg/kg	124	11.1	169	11.6
Cu, mg/kg	24.6	2.54	30.5	1.89
Mn, mg/kg	63.5	5.71	207	20.9
S, %	0.2	0.01	0.2	0.01

[a] Analysis performed by Dairy One, Ithaca, NY

Table 14. Incremental glucose and insulin response data of yearlings fed the sugar and starch (SS-2) or fat and fiber (FF-2) supplements.

	AUC of mean curves[a]		Mean AUC of individual curves[b]			
	SS-2	FF-2	SS-2 (n = 6)		FF-2 (n = 6)	
			Mean	SE	Mean	SE
Glucose						
Peak value, mg/dl	55.4	33.5	57.0[c]	6.6	35.0[d]	3.9
Time to peak, min	100	100	110	10	105	12
Time to return to baseline, min	396	275	329	31	293	22
Area under the curve, min*mg*dl^{-1}	10841	5103	11074[c]	2633	5302[d]	638
Insulin						
Peak value, mIU/L	8.4	4.4	10.0[c]	1.8	5.0[d]	0.5
Time to peak, min	100	100	145	34	80	18
Time to return to baseline, min	400	288	349	32	269	37
Area under the curve, min*mIU*L^{-1}	1932	733	2017[c]	506	786[d]	127

[a] Curves formed by mean values at each sampling point
[b] Data from individual curves for each horse
[c,d] Values with different superscripts are different ($P < 0.05$)

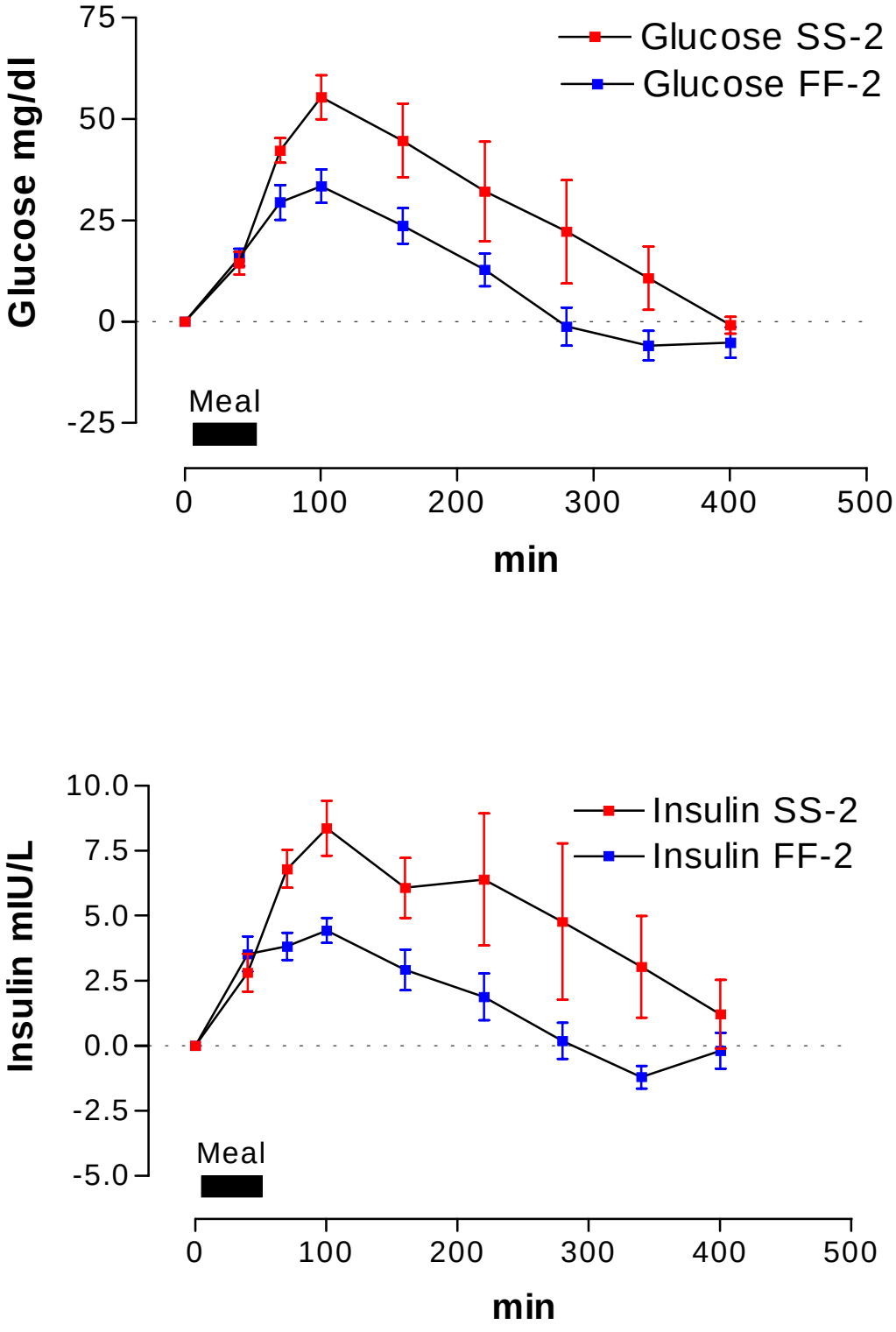
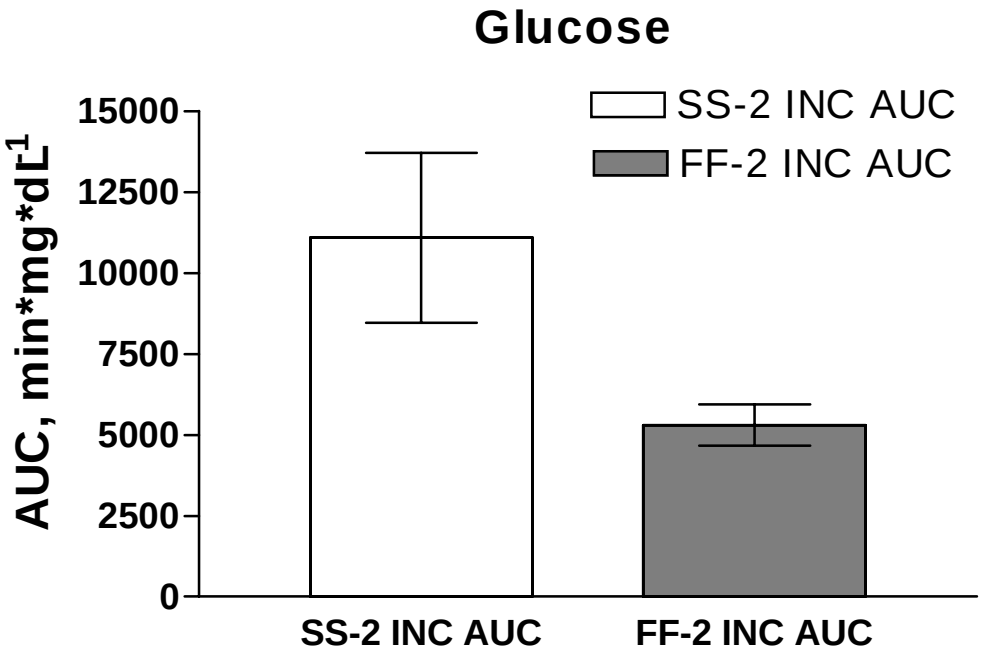


Figure 14. Incremental plasma glucose and insulin responses to meals fed immediately after the 0 min blood sample. Individual points represent means ± standard errors for yearlings in that group (n = 6).



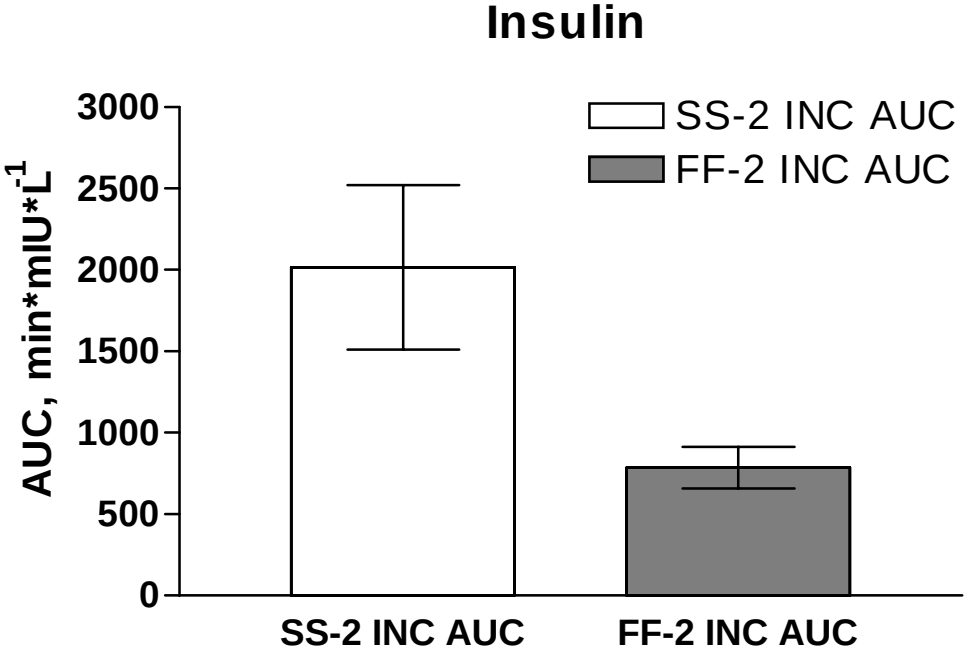


Figure 15. Glucose and insulin areas under the curve for yearlings fed the sugar and starch (SS-2) and fat and fiber (FF-2) supplements. Bars represent means ± standard error (n = 6).

CHAPTER 4:
Circadian patterns of glucose, insulin, GH, and IGF-I in Thoroughbred yearlings

Introduction

A feeding/fasting cycle of factors in the somatotropic axis in meal fed horses has been hypothsized in previous research (Williams et al., 2001). An experiment that examines the somatotropic axis's circadian pattern will characterize the effect of the meals as well as periods between meals spent grazing or eating hay. Previous work at the Middleburg Agricultural Research and Extension Center (MAREC) demonstrated different plasma glucose and insulin responses in mares fed fat and fiber versus sugar and starch meals (Williams et al., 2001). Similar differences were observed in yearlings fed these diets. These glycemic response tests were conducted for six hr periods and only examined glucose and insulin fluctuations. This experiment was intended to investigate the circadian patterns in glucose, insulin, growth hormone (GH), and insulin-like growth factor I (IGF-I) in yearlings fed a sugar and starch supplement in comparison to those fed a fat and fiber supplement.

Energy intake affects the secretion of GH and IGF-I. Growth hormone and IGF-I affect cartilage metabolism (Butler and LeRoith, 2001; Isaksson et al., 1985). It is for this reason that it is important to understand how the energy source in the diet affects these factors in the somatotropic axis. The importance of IGF-I to cartilage metabolism was realized in conjunction with its discovery in 1957 (Salmon and Daughaday, 1957). This sulphation factor increased the metabolic activity of cartilage explants, and acted as the direct messenger mediating the effects of growth hormone. Curiously, experiments with cartilage were some of the early work examining the growth promoting effects of GH (Freud et al., 1939). Growth hormone is secreted from the anterior pituitary in a pulsatile manner in many species, and this secretion is under the control of the hypothalamus (Jaffe et al., 1998; Lefcourt et al., 1995; Thompson et al., 1992). Circulating GH stimulates various tissues to produce IGF-I, with the liver being the main source of circulating IGF-I.

Our interest in examining GH and IGF-I was drawn from *in vitro* work examining the effects of IGF-I on chondrocytes in culture, as well as histological studies (Henson et al., 1997; Shingleton et al., 1997). Articular cartilage, collected *post mortem* from horses, was examined for the presence of dyschondroplastic lesions (Shingleton et al., 1997). Histological analysis of these lesions showed a vascular supply with proliferative chondrocytes surrounding the vessels. This leads to the possibility that a systemic factor prevents the differentiation of those chondrocytes surrounding the vessels. The importance of GH and IGF-I is due to their relationship to one another within the somatotropic axis. Various studies have addressed the relationship between energy level in the equine diet and the occurrence of DOD (Glade and Reimers, 1985; Kronfeld et al., 1990; Ralston, 1996). These studies present evidence for a possible positive relationship, but do not communicate solid evidence as to the chain of events between the diet and DOD. The effect of feeding various energy sources on circadian patterns of secretion of somatotropic axis hormones has not been investigated in the young, growing Thoroughbred.

Materials and Methods

This experiment was conducted in conjunction with a long-term study investigating growth and development. The horses in this experiment were those in the fifth year of the survey of growth. Dams were initially paired by weight and foaling date and then randomly assigned to two groups. These two groups were maintained for 19 months. The groups were continuously grazed on mixed grass and legume pasture. Dams were started on dietary supplements three months prior to foaling, and foals were weaned at six months of age.

Twelve thoroughbred yearlings, aged 321.4 $\pm$ 3.7 d and weighing 331.3 ± 8.2 kg, were used in this experiment. Yearlings were raised on one of the two experimental supplements used on the day of the experiment, with equal numbers on each supplement. Yearlings had been accustomed to being brought into the stalls prior to the start of this experiment.

Two supplements containing different carbohydrate profiles were formulated for this experiment (Table 15 and 16). The first (SS-3) contained a high level of rapidly digestible carbohydrates (non-structural carbohydrates (NSC) 57.2 ± 3.9 %) and lower levels of slowly fermentable carbohydrates (ADF 9.4 ± 1.7 %; NDF 18.0 ± 2.7 %). The second (FF-3) contained a low level of rapidly digestible carbohydrates (NSC 29.7 ± 2.3 %) and high levels of slowly fermentable carbohydrates (ADF 20.7 ± 1.1 %; NDF 31.4 ± 1.0 %). The FF-3 supplement had a higher concentration of fat in comparison to the SS-3 supplement (11.9 ± 1.1 and 3.1 ± 0.5 %; respectively). The two supplements were approximately isonitrogenous (CP SS-3 15.3 ± 0.6 %; FF-3 14.5 ± 0.3 %). Supplements were designed 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; Kronfeld et al., 1996; NRC, 1989). The vitamin premix was formulated in collaboration with Theodore Frye and donated by Hoffman-LaRoche (Nutley, NJ).

Twelve yearlings were brought into stalls with *ad libitum* access to hay and water. Yearlings were paired and randomly assigned to SS-3 or FF-3. One jugular catheter was placed in each weanling at 0530 to 0600. Two meals of 1.6 kg (3.0 Mcal/kg DE) were fed, separated by 6.5 hr . Blood samples (20 ml) were drawn at 30 min intervals into sodium heparinized tubes and immediately placed on ice. Within 30 min. of sampling, blood was centrifuged at 3000 g for 10 min and plasma removed and frozen at –20^{o}C.

Plasma glucose concentration was measured using a colorimetric assay (Kit # 442640, Beckman Synchron CX5CE, Brea, CA). Plasma insulin was determined by a radioimmunoassay (Coat-A-Count Insulin, Kit # THINX, DPC, Los Angeles, CA). Duplicate assays of plasma glucose had an intraassay CV of <1%, and insulin had a CV of 5%. The interassay CV for glucose was 2% and for insulin was 5%.

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 then stored at -20°C for one hr. Samples were then centrifuged at 1,500 x g for 30 min, and decanted into 12 x 75 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 was 8%.

Growth hormone concentration was measured with a double antibody radioimmunoassay (Standards and antibodies for this assay have been generously provided by Dr. A. F. Parlow and the National Hormone and Peptide Program.). Highly purified equine GH (AFP7112B) was radioiodinated as a standard for this assay. Monkey anti-porcine GH was used as the 1st antibody, and goat anti-monkey antiserum was used as the 2nd antibody (Antibodies Incorporated). 1st antibody was diluted in PBS containing 0.05M EDTA and 1:250 normal (non-immune) monkey serum. 2nd antibody was diluted in 1:12.5 in 0.05M EDTA-PBS at a pH of 7.5. A

standard curve was set-up with concentrations of GH from 0.1 to 10 ng/tube . On the first day of the assay 300 μl of serum were added to 200 μl of assay buffer and 100 μl of 1st antibody. Tubes were incubated at room temperature. On the second day 100 μl of radiolabeled equine GH was added and placed in a cold room at 4°C, and on the third day 100 μl of 2nd antibody was added. Tubes were incubated for 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 interassay CV for the GH assay was 7%.

Dependent variables were evaluated for differences between the supplements over the entire sampling period by analysis of variance with repeated measures using the mixed procedures of SAS (SAS Inst. Inc., Cary, NC). The model statement included terms for diet, horse within diet, time, and diet × time interaction. The covariance structure was set to autoregressive order one. Tukey's multiple-comparison procedure was used to test differences between time periods, with P set to 0.05 for significance and to 0.10 for a trend. Two sample t-tests were used to determine if differences were present in variables between diets during a given sample time. Differences were considered significant at a $P < 0.05$, and a trend at $P \leq 0.10$.

Incremental areas under the curve were calculated using the equation:

$$\text{Area} = (t_1 - t_0)*((I_{t0}+I_{t1})/2)+\ldots(t_{n+1}-t_n)*((I_{tn}+I_{tn+1})/2)$$

Where t is the time at which a particular sample is taken and I is the incremental change in the variable being measured at a specific time. Incremental area under the curve (AUC) of the concentration versus time data was calculated by summed trapezoids using GraphPad Prism version 3.02 for Windows (GraphPad Software, San Diego California USA). Data from the last 20 plasma samples were pooled to determine a baseline value for glucose and insulin. Two different approaches were used in examining AUC, both using the same baseline measurements. The first was to create points on the curves that represented the mean of all six yearlings (Figure 16), and then determine the AUC. This technique

did not allow for the calculation of a standard error of the AUC value. The second technique was to evaluate each yearling's AUC individually, and present the mean ± SE of the AUC's for each dietary group (Table 17). Concentrations of GH were subjected to peak analysis using the PC - Pulsar computer program (PC – Pulsar Version 3.01, Urbana, IL) (Gitzen and Ramirez, 1976). The algorithms used are described in Merriam and Wachter (1982).

Results

The yearlings were 321.4 ± 3.7 days of age on the day of the trial was conducted. There was no difference in the weights of the yearlings in the SS-3 group (333.5 ± 13.9 kg) versus those in the FF-3 group (329.1 ± 10.2 kg) ($P = 0.40$).

The estimated baseline plasma glucose concentrations in the SS-3 group were not different than the estimated baseline in the FF-3 group (112.1 ± 2.5 and 107.2 ± 1.5 mg/dl, $P = 0.14$; respectively). Diet significantly affected plasma glucose concentrations ($P = 0.03$), with plasma glucose being lower in the SS-3 group. Analysis of incremental glucose and insulin data is presented in Table 17. Following the morning meal, peak glucose concentrations were reached in approximately 1.5 hr. The first glucose peak was higher in the FF-3 group than the SS-3 group ($P = 0.04$), while there was no difference in between the two groups for the second peak ($P = 0.18$). The first peak occurred later in the FF-3 group ($P = 0.03$) and tended to take longer to return to baseline than the curve for the SS-3 group ($P = 0.05$). All of the above resulted in a greater AUC for the first curve in the FF-3 group ($P = 0.02$). The differences in the second peak were not statistically significant.

The estimated baseline plasma insulin concentrations in the SS-3 group were not different than the estimated baseline in the FF-3 group (6.04 ± 0.63 and 5.14 ± 0.37 mIU/L, $P = 0.12$; respectively). There was a tendency for diet to affect plasma insulin concentrations ($P = 0.06$), with plasma insulin being lower in the SS-3 group. The only significant differences in the plasma insulin peaks were the time to reach the peak for the first peak and AUC for the second peak ($P = 0.02$ and 0.04; respectively).

The circadian pattern of GH is presented in figures 17 - 18. The number of peaks in the 24 hr period was 6.6 ± 0.5, with a range between individuals from 4 to 9 peaks. There was no difference in peak analysis data between geldings and fillies , or between dietary groups ($P > 0.05$) (Table 18). Peak amplitude was 14.5 ± 1.4 ng/ml, with peaks occurring at a rate of 0.28 ± 0.02/hr.

The circadian pattern of IGF-I is presented in figure 19. Repeated measures analysis demonstrated no effect of sample, diet, or sample × diet interactions ($P > 0.05$). The mean plasma IGF-I concentration over the 24 hr period was 239 ± 3.8 ng/ml. The variance in the SS-3 group was significantly different from that of the SS-3 group ($F = 4.17$, df_n and $df_d = 143$, $P < 0.0001$). The data from two horses in the SS-3 group were removed due to being greater than three standard deviations away from the mean throughout the trial. The mean plasma IGF-I concentrations for these two horses were 378 ± 8.8 and 124 ± 6.9 ng/ml. Upon removal of these horses the difference in the variance between the two groups was removed ($F = 0.74$, $df_n = 95$, $df_d = 143$, $P = 0.12$).

Discussion

This experiment focused on the glucose, insulin, GH, and IGF-I concentrations in the blood over a 24 hr. period in Thoroughbred yearlings. This data further characterizes the aspects of the feeding/fasting cycle that occur with the twice daily feeding of supplements that elicit a high glycemic response. The results from this experiment are particularly interesting in that they are contrary to prior research that showed a low glycemic response to fat and fiber supplements. This is also the first documentation of the circadian pattern of plasma GH and IGF-I concentrations in Thoroughbred yearlings.

The glycemic response to the diets in this experiment was unexpected. The only statistical differences in the glucose and insulin responses revealed a higher glycemic response for the FF-3 supplement. Previous results showed the glycemic response of the fat and fiber supplements to be significantly lower than the sugar and starch supplements. One possible explanation is that changes to the

supplements ingredient composition affected glycemic response. Changes to the FF-3 from previous years formulations included a 24 % increase in the percentage of cereal by-product and a change to the starch content of the cereal by-product that resulted in a 68% increase in NSC for the by-product. Regretfully, these differences were unknown prior to this experiment. Moreover, these differences were not indicated by the nutrient composition of the supplements. This means that caution should be taken before making assumptions about the predicted glycemic response of a supplement simply from its nutrient composition. It is likely that several characteristics of the fat and fiber supplement resulted in its eliciting a similar glycemic response to the sugar and starch supplement. The cereal by-product ingredient had a very small particle size, making the surface area available for digestion much greater than that of the corn in the sugar and starch supplement. Ingredients with different physical characteristics vary in their digestibilities and physiologic responses they produce (Cuddeford, 2000). It is hypothesized that these factors resulted in diets with similar concentrations of available hydrolyzable carbohydrates in the small intestine of yearlings fed these diets.

The glycemic responses to both supplements in this experiment are very similar to that seen in previous studies for diets with a high level of hydrolyzable carbohydrates (Stull and Rodiek, 1988; Williams et al., 2001). There was a distinct response pattern of the feeding/fasting cycle when the yearlings are fed the supplement. All animals had *ad libitum* access to hay, and were observed eating for a majority of the experiment, yet the only significant glycemic responses were those associated with feeding of supplements. While hay is not similar to pasture it is likely that constant grazing results in a similar pattern of glucose and insulin secretion to that seen during the night time hours of this experiment. It is clear that feeding supplements increases circulating concentrations of insulin and glucose in the Thoroughbred yearling.

Because of the pulsatile nature of GH's secretion, detailed analysis of its circulating concentrations requires measurement of individual peaks and the pattern

of those peaks over the experiment period. Growth hormone's episodic release is clearly evident in the profiles from horses in this experiment (Appendix 1). The secretion profile was characterized by more frequent peaks with similar amplitudes compared to studies in adult horses. The frequency of peaks in grade, lighthorse stallions was 4.1 $\pm$ 0.6 peaks/d, with a mean amplitude 19.9 $\pm$ 6.3 ng/ml, while frequency in geldings was 3.2 $\pm$ 0.3 peaks/d, with a mean amplitude of 4.2 $\pm$ 0.4 ng/ml (Christensen et al., 1997b; Thompson et al., 1992). The pattern of peaks did not appear random between horses in this experiment. Specifically, GH peaks appeared more consistently surrounding the feed periods, with peaks occurring at 3.2 $\pm$ 0.17, 5.7 $\pm$ 0.13, and 8.5 $\pm$ 0.36 hr. These peaks were characterized by an initial larger peak followed fairly quickly by a smaller one just prior to the afternoon feeding. The third peak was typically not as large as the first and occurred approximately 2 hr following the afternoon feeding. Secretion of GH is controlled by growth hormone releasing hormone and somatostatin (Aron et al., 1997). Growth hormone is released in states of relative hypoglycemia, yet in this case these pulses occur just as plasma glucose and insulin concentrations are beginning to decrease from peak values. Perhaps the rate of decrease in the glucose and insulin levels stimulates these pulses in GH secretion. The second peak does appear to be cut short at the time of the afternoon feeding. Further investigation into the differences between GH secretory patterns in young and adult horses may better give account for these patterns.

Insulin-like growth factor I concentrations remained relatively constant during the 24 hr experiment. It is likely that changes in circulating IGF-I reflect more long-term changes in nutrient partitioning. The yearlings fed the SS-3 supplement had a significantly greater variation in concentrations of IGF-I in comparison to those fed the FF-3. This variation appears to be due to circulating concentrations in two yearlings. One of these had concentrations that were consistently higher, while the other was lower than 3 standard deviations from the mean concentrations. These horses were not excluded because of the consistent nature of the IGF-I

concentrations and the likelihood that they were part of the normal population. Plasma IGF-I concentrations in Standardbred geldings, 7-21 yr of age, ranged from 168 to 209 ng/ml (Christensen et al., 1997a). The lower values were in geldings that had been fasted for a period of 48 hr, while the higher values were 24 hr after refeeding. The effect of aging on plasma IGF-I was examined in Standard bred fillies and mares (Malinowski et al., 1996). Plasma IGF-I ranged from a high at 2 mo of 604 $\pm$ 50 ng/ml to a low at birth of 285 $\pm$ 50 ng/ml. The higher concentration of IGF-I in this experiment when compared to that of the Standardbred gelding is likely due to the fact that the yearlings in this experiment were in a period of rapid growth. The differences between this experiment and that of Malinowski et al. (1996) may also reflect differences in experimental procedures.

The most important point from this experiment is the understanding that nutrient composition does not translate directly into predicted physiological responses to a meal. There is interesting work examining the effect of different carbohydrate fractions on the glycemic responses that emphasizes the need for a more physiological relevant measure of carbohydrate content in equine rations (Hoffman et al., 2001). The consistent pattern of GH secretion between horses in relation to meals warrants further study to examine the effect of supplements that do elicit different glycemic responses. Due to the lack of differences found in the other variable measured, it is not surprising that IGF-I concentrations were not different between the two dietary groups. Changes in the somatotropic axis result in changes in growth, hence it is important to be familiar with the effects of the environment on the somatotropic axis.

Table 15. Ingredient composition (%) of the sugar and starch supplement (SS-3) and the fat and fiber supplement (FF-3)

Ingredient	SS-3	FF-3
Oat straw	7	7
Alfalfa	0	13.5
Dent yellow grain corn	60	1.5
Beet pulp	0	10
Soybean hulls	4	4
Molasses (cane)	10	5
Soybean meal	15.5	2
Limestone	1	0
Calcium phosphate, dibasic	1.5	0
Vitamin premix[a]	0.5	0.5
Mineral premix[b]	0.5	0.5
Processed cereal by-product[c]	0	56

[a] Provided the following amounts per kg of supplement: vitamin A, 6,900 IU; β-carotene, 17.6; vitamin D3, 1,290 IU; vitamin E, 132 mg; vitamin C, 333 mg; Niacin, 15 mg; Thiamin, 7 mg; Riboflavin, 3.5 mg; Folic acid, .33 mg; Biotin, .21 mg.
[b] Provided the following amounts per kg of supplement: Fe, 46.1mg; Zn, 105.8mg; Cu, 25.11mg; Mn, 18.02 mg; Se, .55 mg; I, .35 mg; NaCl used as carrier, 4160 mg.
[c] Processed cereal by-product contains 92.5% DM, 21% EE, 15% CP, 24% NSC, and 30% NDF.

Table 16. Nutrient composition[a] on a DM basis of the sugar and starch supplement (SS-3), and the fat and fiber supplement (FF-3).

	SS-3 (n = 6)		FF-3 (n = 6)	
Item	Mean	SE	Mean	SE
Crude protein, %	15.3	0.66	14.5	0.25
Crude fat, %	3.1	0.49	11.9	1.13
Acid detergent fiber, %	9.4	1.73	20.7	1.05
Neutral detergent fiber, %	18.0	2.67	31.4	1.03
Non-structural carbohydrates, %	57.2	3.85	29.7	2.25
Ca, %	1.0	0.15	2.7	0.19
P, %	0.6	0.07	1.1	0.05
Mg, %	0.3	0.04	0.7	0.04
K, %	1.0	0.05	1.2	0.03
Na, %	0.2	0.02	0.3	0.02
Fe, mg/kg	258	32.1	384	92.1
Zn, mg/kg	124	11.0	176	5.6
Cu, mg/kg	24.6	2.54	27.3	2.29
Mn, mg/kg	43.8	5.71	245	15.8
S, %	0.2	0.01	0.2	0.01
Cl, ion %	0.5	0.04	0.5	0.06

[a] Analysis performed by Dairy One, Ithaca, NY

Table 17. Incremental glucose and insulin response data of yearlings fed the sugar and starch (SS-3) or fat and fiber (FF-3) supplements.

	AUC of mean curves[a]		Mean AUC of individual curves[b]			
	SS-3	FF-3	SS-3 (n = 6)		FF-3 (n = 6)	
			Mean	SE	Mean	SE
Glucose						
Peak # 1 value, mg/dl	26.8	38.1	28.7[c]	4.8	41.7[d]	4.4
Peak # 2 value, mg/dl	36.7	41.3	37.8	5.0	43.7	3.3
Time to peak # 1, hr	1.3	1.3	1.2[c]	0.1	1.5[d]	0.1
Time to peak # 2, hr	1.3	1.3	1.3	0.2	1.4	0.1
Time to return to baseline # 1, hr	3.4	4.7	3.2	0.3	4.1	0.4
Time to return to baseline # 2, hr	3.4	4.1	3.3	0.2	3.9	0.7
Area under the curve # 1, $hr*mg*dl^{-1}$	49.6	90.5	50.9[c]	10.3	96.3[d]	17.4
Area under the curve # 2, $hr*mg*dl^{-1}$	70.1	87.2	71.7	13.6	90.8	8.9
Insulin						
Peak value # 1, mIU/L	8.2	10.0	8.7	1.4	13.1	2.0
Peak value # 2, mIU/L	13.0	13.7	13.8	3.4	15.3	1.2
Time to peak # 1, hr	1.9	1.9	1.6	0.3	2.3	0.4
Time to peak # 2, hr	1.5	2.5	1.7[c]	0.2	2.4[d]	0.2
Time to return to baseline # 1, hr	4.4	6.2	4.4	0.6	4.9	0.4
Time to return to baseline # 2, hr	5.3	9.0	5.9	0.6	6.5	1.1
Area under the curve # 1, $hr*mIU*L^{-1}$	19.6	36.8	17.8[c]	3.2	36.2[d]	8.8
Area under the curve # 2, $hr*mIU*L^{-1}$	36.8	45.6	34.6	9.0	42.7	4.6

[a] Curves formed by mean values at each sampling point
[b] Data from individual curves for each horse
[c,d] Values with different superscripts are different ($P < 0.05$)

Table 18. Analysis of plasma growth hormone concentrations in Thoroughbred yearlings fed a sugar and starch supplement (SS-3) or fat and fiber supplement (FF-3).

	SS-3 (n = 6)		FF-3 (n = 6)	
Item	Mean	SE	Mean	SE
Number of peaks	6.0	0.52	7.2	0.70
Amplitude, ng/ml	16.7	1.29	12.2	2.31
Peak duration, hr	1.9	0.22	1.5	0.28
Peak frequency, hr	0.3	0.02	0.3	0.03
Interpeak interval, hr	4.0	0.33	3.3	0.61

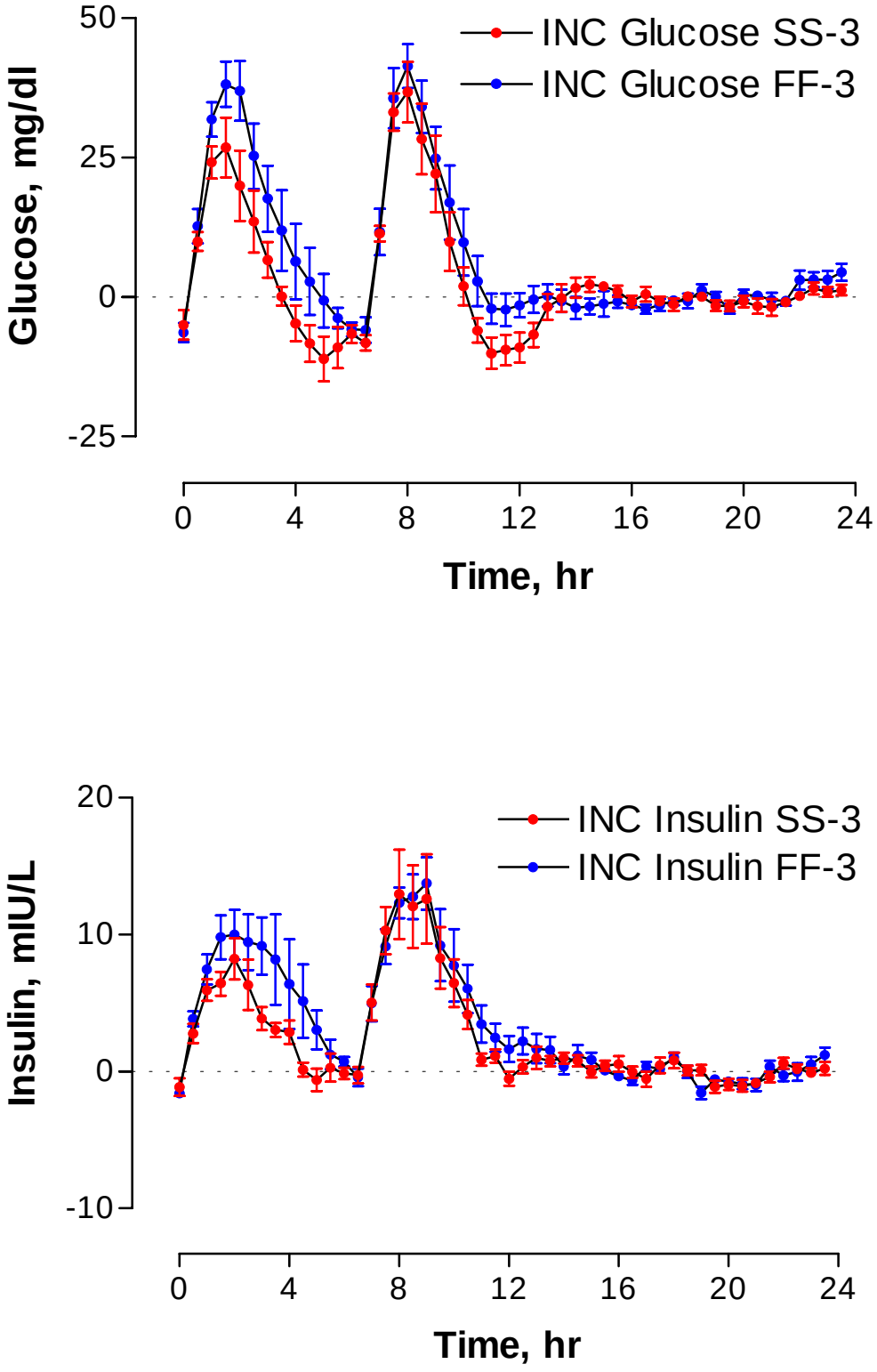


Figure 16. Circadian patterns of incremental plasma glucose and insulin concentrations in Thoroughbred yearlings fed a sugar and starch (SS-3) or fat and fiber (FF-3) supplement at 0 and 6.5 hr.

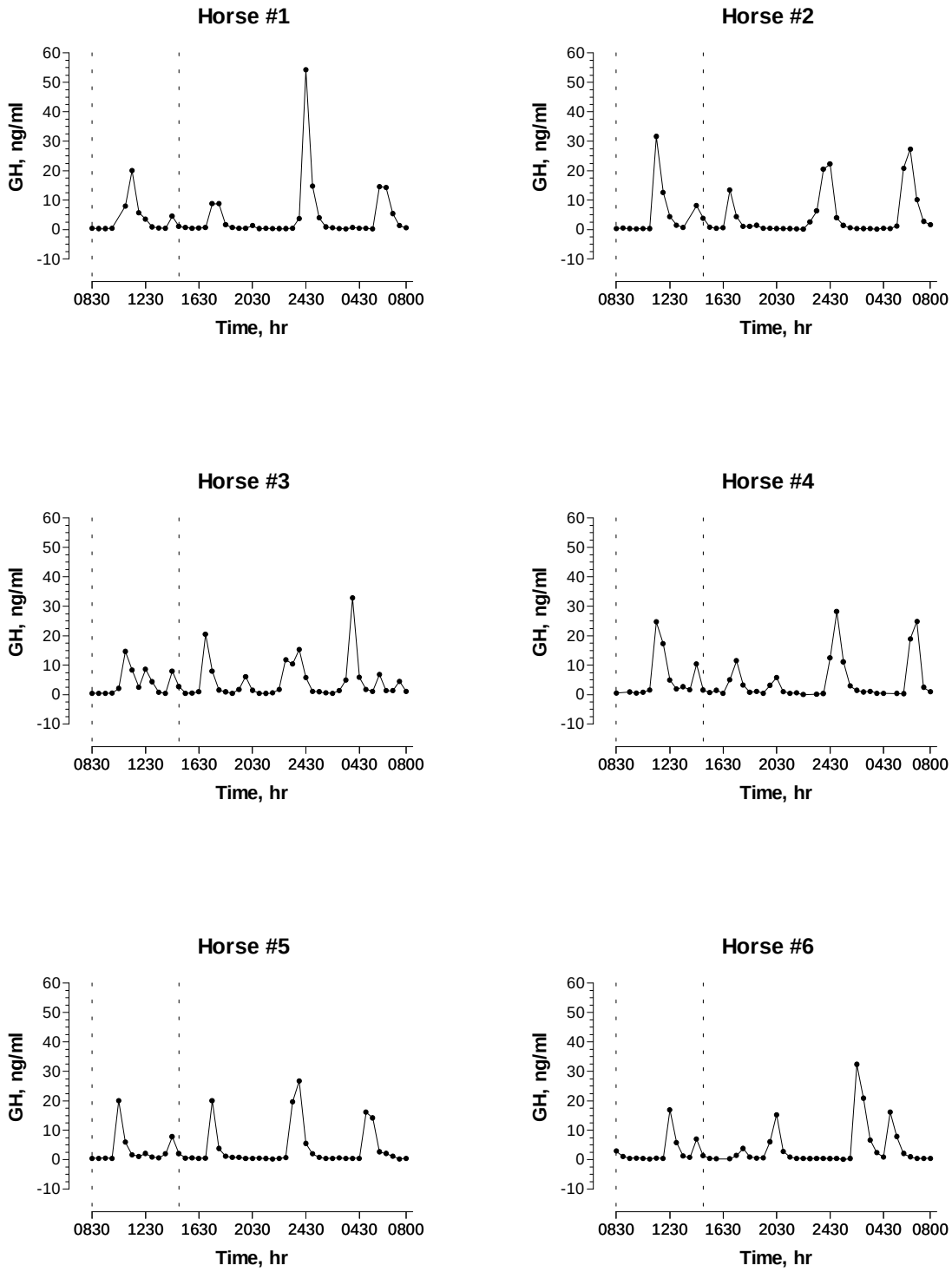


Figure 17. Circadian pattern of plasma growth hormone (GH) concentrations in Thoroughbred yearlings fed a sugar and starch (SS-3) supplement. Vertical lines represent time of feeding.

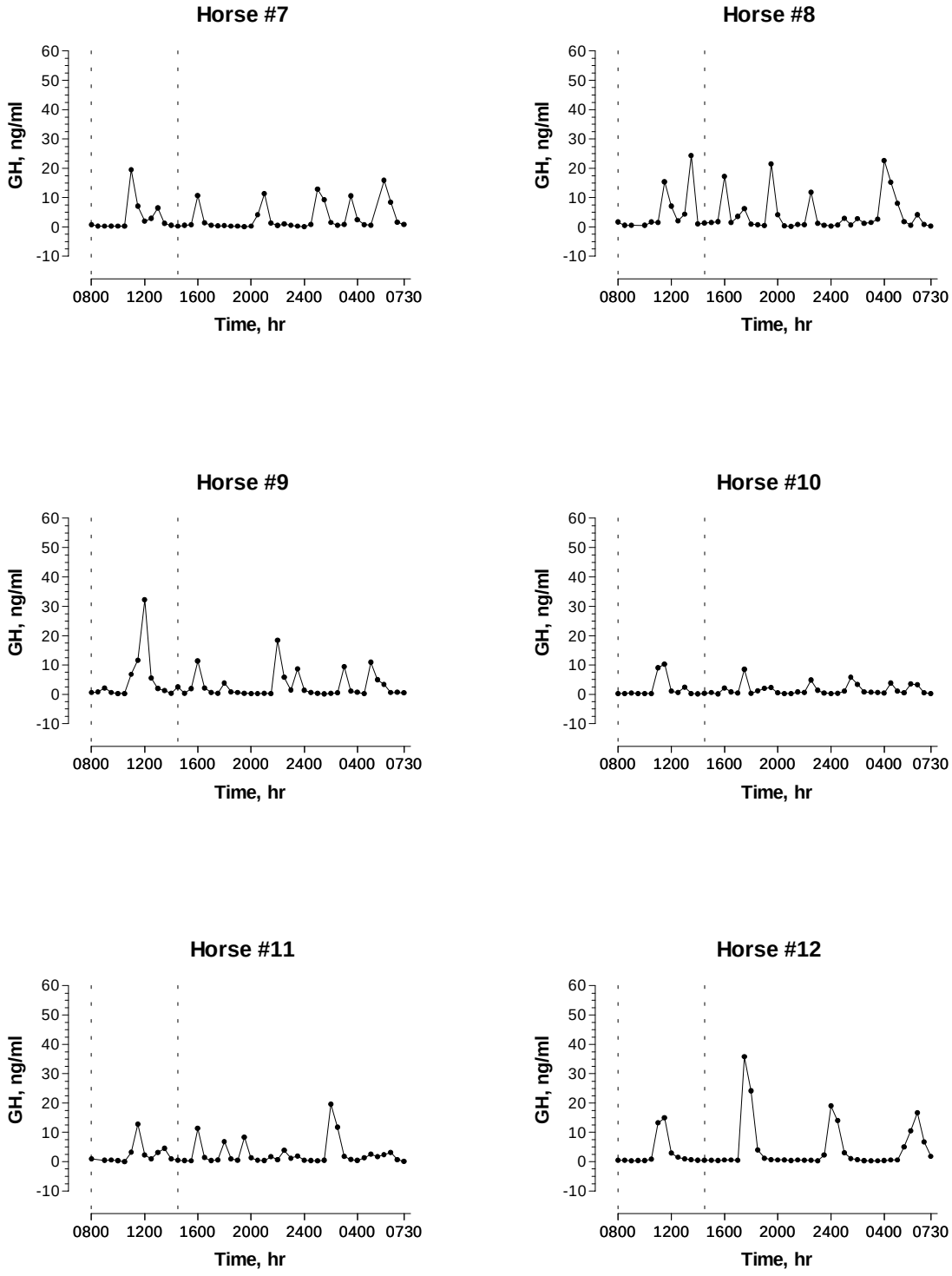


Figure 18. Circadian pattern of plasma growth hormone (GH) concentrations in Thoroughbred yearlings fed a fat and fiber (FF-3) supplement. Vertical lines represent time of feeding.

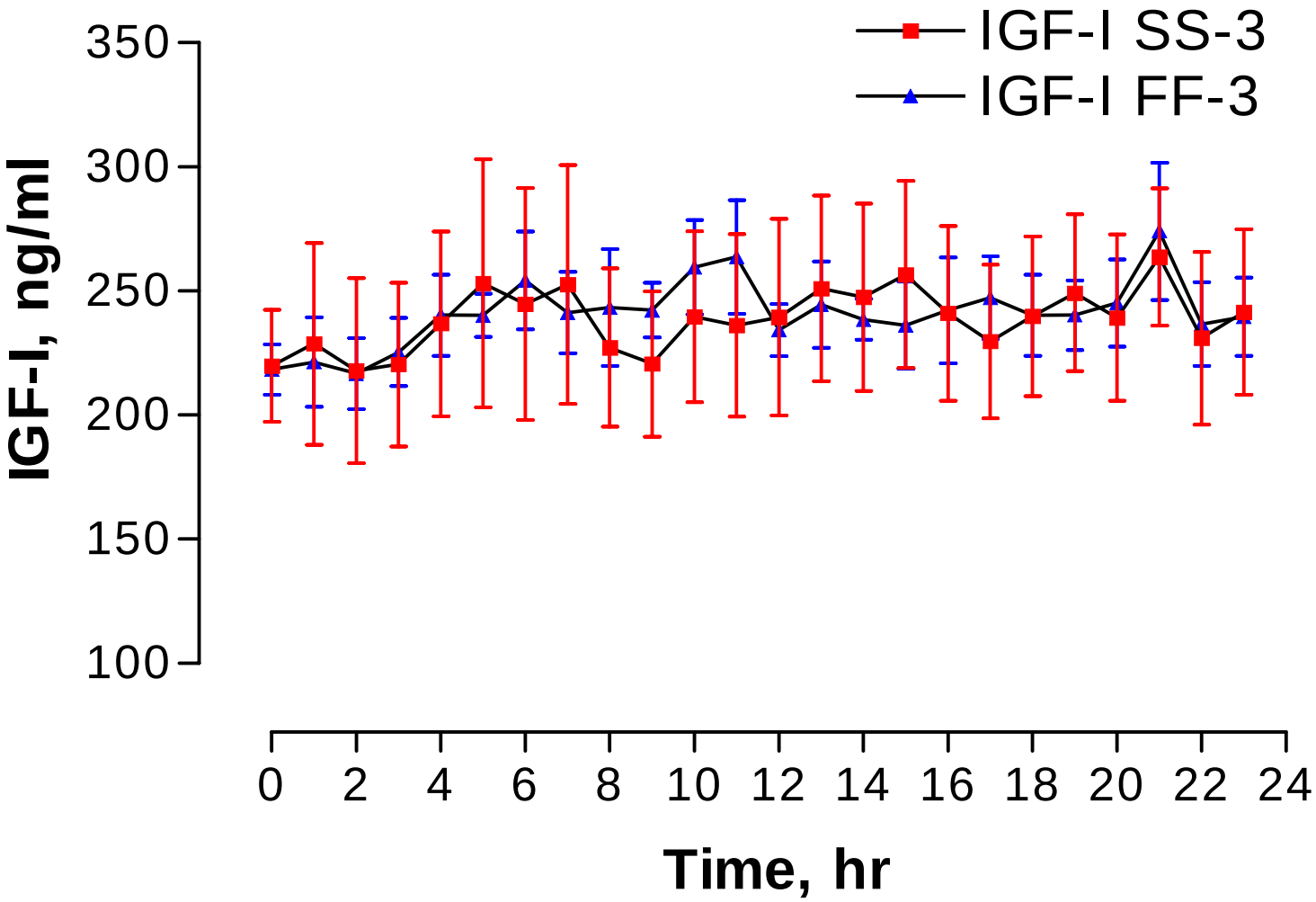


Figure 19. Circadian pattern of plasma insulin-like growth factor I (IGF-I) concentrations in Thoroughbred yearlings fed a sugar and starch (SS-3) or fat and fiber (FF-3) supplement at 0 and 6.5 hr.

CHAPTER 5:
Association between growth and IGF-I in young Thoroughbreds

Introduction

Developmental orthopedic disease is a major source of economic loss to the equine industry (Jeffcott and Henson, 1998). Osteochondrosis, one form of DOD, is characterized by the abnormal differentiation of chondrocytes from regions of proliferation and hypertrophy, to regions of ossification and invasion of osteoblasts and osteocytes. Insulin and insulin-like growth factor-I's (IGF-I) role in the normal maturation of growth and articular cartilage has been well documented (Henson et al., 1997). In the horse, the normal maturation of chondrocytes present in the epiphyseal growth plate is of interest due to the occurrence of DOD. Feeding meals high in hydrolyzable carbohydrates may produce a cyclical pattern in circulating concentrations of factors in the somatotropic axis . Disturbances to normal circulating levels of insulin, IGF-I as well as other factors in the somatotropic axis may result in abnormal maturation of chondrocytes, dyschondroplasia and osteochondrosis. The objective of this experiment was to examine how the chemical form of energy in the diet affects circulating concentrations of factors in the somatotropic axis over the first 16 mo of growth in Thoroughbred foals.

Materials and Methods

Growth was studied in 24 Thoroughbred foals from birth to 16 mo of age at the Middleburg Agricultural Research and Extension Center (MAREC). Dams were grouped by age, breeding date and sire of the foal, and then randomly assigned to one of two groups. Mares and foals were placed in their respective groups five to seven days following foaling. Mares and foals were maintained on the supplements from June of 1998 until time of weaning, at which point the foals were continued on the diets until September of 1999. Horses remained on mixed grass and ladino clover pasture 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 $\times$ 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 routine at the MAREC (Ley et al., 1992). Colts were gelded at three to four weeks of age. Foals were weaned gradually, beginning at six months, by the removal of two mares from each group every four days.

Two supplements containing different carbohydrate profiles were formulated for this experiment (Table 19). The sugar and starch (SS-1) contained a high level of rapidly digestible carbohydrates (non-structural carbohydrates (NSC) 55.3 $\pm$ 4.9 %) and lower levels of slowly fermentable carbohydrates (ADF 10.7 $\pm$ 1.2 %; NDF 19.6 $\pm$ 1.5 %). The fat and fiber (FF-1) contained a low level of rapidly digestible carbohydrates (NSC 28.6 $\pm$ 1.9 %) and high levels of slowly fermentable carbohydrates (ADF 21.1 $\pm$ 0.2 %; NDF 34.8 $\pm$ 0.9 %). The FF-1 supplement had a higher concentration of fat in comparison to the SS-1 supplement (9.7 $\pm$ 0.8 and 1.0 $\pm$ 0.2 %; respectively). The two supplements were also approximately isonitrogenous (CP SS-1 16.7 $\pm$ 1.8 %; FF-1 14.8 $\pm$ 0.3 %). Mares were fed a pelleted concentrate prior to June (PurePride-200, Purina Mills, St. Louis, MO).

Foals were weighed once every 28 days. Measurements of BW, ADG, body condition score (BC), wither and hip heights, length of body, forearm, and cannon bones, girth, and circumference of the carpus and fetlocks were taken on the same day the horses were weighed (Table 21). Body weights were measured using a portable electronic walk–on scale (Model TC-10S, Tyrel Corp.). Body condition was scored by one evaluator through the entire experiment, using the method of (Henneke et al., 1983).

Blood samples were taken each month 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.

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 then stored at -20°C for one hr. Samples were then centrifuged at 1,500 x g for 30 min, and decanted into 12 x 75 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 (1^{st} antibody) was a gift of Dr. Bernard Laarveld (University of Saskatchewan). Goat anti-mouse anti-serum (2^{nd} 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 1^{st} antibody (1:21,000) were added to each tube. 1^{st} antibody was diluted in mouse control serum. After 24 hr incubation at 4°C, 100 μl of 2^{nd} antibody (1:20) was added. 2^{nd} 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 was 8%.

Dependent variables were evaluated for differences between the supplements over the entire sampling period by analysis of variance with repeated measures

using the mixed procedures of SAS (SAS Inst. Inc., Cary, NC). The model statement included diet, horse within diet, time, and diet × time interaction. Tukey's multiple-comparison procedure was used to test differences between time periods, with *P* set to 0.05 for significance and to 0.10 for a trend. Two sample t-tests were used to determine if differences were present in variables between diets during a given sample time. Pearson correlations were used to examine relationships between dependent variables of interest. Differences were considered significant at a $P < 0.05$, and a trend at $P \leq 0.10$.

Results

Growth variables for the 24 foals in this experiment were similar to those in past and subsequent years (Table 22). Average daily gain in the SS-1 group was not different from that in the FF-1 group (0.79 ± 0.03 kg/d; 0.80 ± 0.03 kg/d; respectively) ($P = 0.86$) over the period of the experiment. There was a month and diet × month effect on ADG ($P < 0.0001$ and $P = 0.003$; respectively) and plasma IGF-I concentrations ($P < 0.0001$ for both effects). The effect of diet was not significant for ADG or plasma IGF-I ($P = 0.78$ and 0.98; respectively).

There were no differences between the diets for ADG during specific months ($P > 0.05$). The pattern of ADG consisted of rapid gains during the first two months followed by a slow decrease from July through October. A significant drop in ADG occurred in November, followed by relatively low gains through the month of March. A significant increase in gain occurred in April and May, followed by a steady decline through August. The ADG in June (1.3 ± 0.04 kg/d) was significantly greater than all other gains ($P < 0.01$) with the exceptions of July and the following April (1.2 ± 0.04 kg/d, $P = 0.8$ and 1.2 ± 0.04 kg/d, $P = 0.7$; respectively). Average daily gain was it lowest in March (0.3 ± 0.04 kg/d). The ADG was positively correlated with day length and temperature ($r = 0.34$ and $r = 0.41$, $P < 0.0001$).

Plasma IGF-I over the whole experiment period was higher in the SS-1 group (223 ± 1.1 ng/ml) than in the FF-1 group (217 ± 1.1 ng/ml, $P = 0.0001$) (Figure 20).

Plasma IGF-I was higher in the SS-1 group than in the FF-1 group in May and June of 1998 ($P = 0.04$, and 0.03; respectively), and February, April and May of 1999 ($P = 0.05$, 0.003, and 0.03; respectively). Plasma IGF-I tended to be higher in the SS-1 group than in the FF-1 group in July and October of 1998 ($P = 0.06$, and 0.09; respectively), and January and March of 1999 ($P = 0.10$, and 0.11; respectively) Plasma IGF-I levels were partially correlated with the average daily gain ($r = 0.34$, $P < 0.001$) (Figure 21). Plasma IGF-I concentrations were also positively correlated with day length and temperature ($r = 0.38$ and $r = 0.33$, $P < 0.0001$).

Discussion

Weight gains observed during the summer months began to decrease in October and November. Reasons for this decline may be due; first, to cooler temperatures and increased energy demands for thermoregulation; second, foals were weaned in November; and third, pasture quality begins to decrease at this time of year, supplying the weanlings with less of the energy they need to grow. In April, a considerable increase in ADG was observed in both treatment groups. This change was attributed to milder temperatures, improving pasture conditions, and presumably some compensatory growth after depressed gains during the winter months.

There has been limited investigation of circulating IGF-I in the growing foal. A study of 95 Standardbred mares and fillies examined plasma IGF-I concentrations at 0, 1, 7, and 14 d; 1, 2, 4, 6, and 9 mo; 5 to 8 yr; 16 to 22 yr (Malinowski et al., 1996). The concentrations of IGF-I increased from 285 $\pm$ 50 ng/ml at birth to 572 $\pm$ 50 ng/ml at 14 d of age. Concentrations decreased from 604 $\pm$ 50 ng/ml at month 2 to 530 $\pm$ 50 ng/ml at 9 mo. Mares in the range from 5 to 22 years had plasma IGF-I concentrations from 295 to 369 $\pm$ 50 ng/ml. The results from the current experiment contrast with these results. The difference in the results may be due to several factors. The samples in the Malinowski study were all taken from different horses on three different farms, with each time period representing a different

group of horses. Samples in this experiment were taken from 24 Thoroughbred foals raised on the same farm and sampled repeatedly for a period of 16 mo. The difference in total IGF-I may also reflect differences in assay methods, because no values found in this experiment were comparable to the high values in the Malinowski study. If there is an environmental effect on IGF-I, it would not have been visible because samples were only taken in July, August and September.

The positive correlation between ADG and IGF-I was not unexpected in this trial. Insulin-like growth factor I's role in growth is well documented (Butler and LeRoith, 2001). Longer daily photoperiod has been associated with an increased circulating IGF-I in dairy cows, and this increase resulted in increased milk yields (Dahl et al., 1997). This example makes a similar connection of changes in the environment resulting in a change in the somatotropic axis that causes a physiologic change in the organism. This is further confirmed by positive correlations of temperature and day length with ADG and circulating IGF-I.

Insulin-like growth factor-I was consistently higher in the SS-1 group in comparison to the FF-1 group. Differences in circulating IGF-I in steers have been attributed to changes in diet composition (Elsasser et al., 1989). The results, in the current experiment, are evidence that plasma IGF-I concentration is decreased by replacement of soluble carbohydrates with fiber and fat. This decrease is probably associated with subdued plasma glucose and insulin responses to FF-1, which represent the circadian feeding/fasting cycle of metabolites and hormones. The differences in IGF-I were particularly prominent during periods of rapid growth, which are also periods of concern for the development of DOD. An earlier experiment at the MAREC demonstrated higher bone densities in foals fed a diet similar to the SS-1 in comparison to foals fed a fat and fiber diet (Hoffman et al., 1999). The difference in bone density was hypothesized to be due to changes in calcium absorption, but it is possible that a difference in circulating IGF-I similar to that seen here would result in an increased bone density.

The results from this experiment in combination with research showing an increased DOD in horses fed high levels of energy (Savage et al., 1993), and *in vitro* work demonstrating the effect of increased insulin and IGF-I on cultured equine chondrocytes (Henson et al., 1997), indicate that the increase in circulating IGF-I observed in foals fed diets that produce an exaggerated glycemic response may increase the risk of OC. The fact that the greatest differences between the two supplements occur during periods of rapid growth is an added concern because of the connection between rapid growth and OC (Ruff et al., 1993).

Table 19. Ingredient composition (%) of the sugar and starch supplement (SS-1) and the fat and fiber supplement (FF-1)

Ingredient	SS-1	FF-1
Oat straw	7	7
Alfalfa	0	13.5
Dent yellow grain corn	60	11
Beet pulp	0	10
Soybean hulls	4	4
Molasses (cane)	10	5
Soybean meal	15.5	2
Limestone	1	1
Calcium phosphate, dibasic	1.5	0.5
Vitamin premix[a]	0.5	0.5
Mineral premix[b]	0.5	0.5
Processed cereal by-product[c]	0	45

[a] Provided the following amounts per kg of supplement: vitamin A, 6,900 IU; β-carotene, 17.6; vitamin D3, 1,290 IU; vitamin E, 132 mg; vitamin C, 333 mg; Niacin, 15 mg; Thiamin, 7 mg; Riboflavin, 3.5 mg; Folic acid, .33 mg; Biotin, .21 mg.
[b] Provided the following amounts per kg of supplement: Fe, 46.1mg; Zn, 105.8mg; Cu, 25.11mg; Mn, 18.02 mg; Se, .55 mg; I, .35 mg; NaCl used as carrier, 4160 mg.
[c] Processed cereal by-product contains 92.5% DM, 26% EE, 18% CP, and 29% NDF.

Table 20. Nutrient composition[a] on a DM basis of the sugar and starch supplement (SS-1), and the fat and fiber supplement (FF-1).

	SS-1 (n = 3)		FF-1 (n = 3)	
Item	Mean	SE	Mean	SE
Crude protein, %	16.7	1.75	14.8	0.25
Crude fat, %	1.0	0.24	9.7	0.80
Acid detergent fiber, %	10.7	1.18	21.1	0.17
Neutral detergent fiber, %	19.6	1.47	34.8	0.87
Non-structural carbohydrates, %	55.3	4.86	28.6	1.82
Ca, %	1.3	0.29	2.4	0.20
P, %	0.8	0.15	1.1	0.03
Mg, %	0.2	0.03	0.6	0.01
K, %	1.2	0.17	1.2	0.05
Na, %	0.3	0.04	0.4	0.03
Fe, mg/kg	396	90.9	426	6.2
Zn, mg/kg	146	25.1	165.7	2.6
Cu, mg/kg	33.7	4.91	29.0	2.52
Mn, mg/kg	57.7	9.39	214.7	26.0
S, %	0.2	0.02	0.2	0.01

[a] Analysis performed by Dairy One, Ithaca, NY

Table 21. Description of body measurements adapted from Hoffman et al., 1996 used to monitor growth of foals

Variable	Measurement description
Wither height	Distance from the ground to the highest point of the withers
Hip height	Distance from the ground to the highest point of the croup
Body length	Distance from the point of the shoulder to the point of the buttock
Girth	Circumference of the girth behind the elbow and an 2.54 cm behind the highest point of the withers
Forearm	Distance from the point of the elbow to the accessory carpal bone
Front cannon	Distance from the accessory carpal bone to the proximal sesamoids
Carpus	Circumference of the knee at the metaphysis of the distal radius, just above the accessory carpal bone
Fetlock	Circumference of the fetlock at the metaphysis of the distal third metacarpal bone, just above the proximal sesamoids
Hind cannon	Distance from the point of the hock (calcaneus) to the proximal sesamoids

Table 22. Summary of growth variables[a] examined in the comparison between supplements[b] in year three of the growth survey.

Variable Measured	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	SE[c]
Age d																	
SS-1	17	40	72	102	142	166	199	227	256	290	318	347	374	408	436	465	4.36
FF-1	15	41	69	99	139	163	196	224	253	287	315	344	371	405	433	462	5.54
Weight kg																	
SS-1	81.9	124.4	136.4	195.8	236.0	259.7	287.6	300.5	316.3	334.8	345.6	359.3	390.3	418.5	428.6	441.3	7.15
FF-1	73.5	110.7	146.7	182.7	222.2	244.4	275.8	289.5	307.6	321.8	328.0	348.1	379.8	405.8	418.8	431.8	7.15
ADG[d] kg/d																	
SS-1		1.43	1.22	1.08	1.00	0.99	0.85	0.46	0.55	0.54	0.39	0.47	1.15	0.83	0.36	0.44	0.05
FF-1		1.25	1.13	1.20	0.99	0.93	0.95	0.49	0.63	0.42	0.22	0.69	1.18	0.76	0.47	0.45	0.05
BC[ef]																	
SS-1			5.0	4.9	5.0	5.0	4.9	5.0	4.9	5.0	5.0	4.9	5.3	5.1	5.2	5.2	0.09
FF-1			4.7	4.8	4.8	5.0	4.9	4.9	4.9	5.1	5.0	4.9	5.3	5.0	4.9	5.1	0.09
Wither cm																	
SS-1	109.6	118.3	122.3	129.1	135.0	136.5	139.5	142.9	142.5	144.6	145.2	146.3	147.7	148.2	149.9	150.1	1.24
FF-1	109.0	116.7	121.1	128.3	133.8	136.5	139.5	141.6	143.1	145.0	144.4	147.5	148.8	148.8	151.6	149.9	1.24
Hip cm																	
SS-1	110.7	120.0	124.7	131.0	137.0	139.9	141.8	146.1	145.2	147.7	149.0	150.7	153.3	153.9	155.6	155.2	1.29
FF-1	110.8	118.5	123.2	130.2	135.7	138.6	141.2	145.0	145.2	147.7	148.0	152.2	152.9	154.1	156.0	155.6	1.29
Girth cm																	
SS-1	94.6	107.8	119.2	126.7	136.3	140.8	148.2	151.2	153.0	157.0	159.2	161.6	166.2	169.3	170.5	171.2	1.87
FF-1	91.4	104.9	114.7	125.0	134.7	139.5	146.8	150.5	154.2	156.3	160.3	160.5	166.4	168.5	171.1	171.7	1.87
Length cm																	
SS-1	92.6	107.6	118.9	127.0	133.5	138.8	142.7	147.8	150.5	153.1	155.8	157.9	162.2	165.0	166.1	166.0	1.77
FF-1	90.0	103.4	113.8	123.2	131.4	134.8	139.5	146.5	147.9	153.0	153.0	155.8	160.2	160.9	163.9	163.3	1.77
Forearm cm																	
SS-1	30.1	33.2	36.2	36.5	37.9	38.5	40.5	40.4	41.8	41.9	42.8	42.7	42.8	43.7	43.0	42.4	0.46
FF-1	30.0	33.1	35.1	36.3	38.1	38.9	40.0	40.2	41.3	41.9	41.9	43.2	42.5	43.3	43.0	41.9	0.46
Front Cannon cm																	
SS-1	28.4	29.3	30.4	30.9	31.7	31.3	31.2	30.5	31.1	30.9	30.6	30.7	30.3	30.5	30.3	30.0	0.28
FF-1	28.0	29.2	30.2	31.2	31.5	31.8	31.0	30.3	31.2	31.0	31.4	31.1	30.8	30.7	31.1	30.3	0.28
Hind Cannon cm																	
SS-1	38.5	40.6	42.3	43.0	43.9	43.6	43.5	42.7	43.8	44.1	43.8	44.0	44.1	44.0	43.6	42.8	0.37
FF-1	39.1	40.2	42.0	42.5	44.6	44.5	45.0	42.5	44.1	43.3	44.4	44.1	43.8	43.7	44.0	42.8	0.37
Carpus cm																	
SS-1	24.3	26.0	27.6	28.5	29.4	29.5	30.3	29.4	30.3	30.4	30.9	31.0	31.8	31.9	31.7	31.4	0.30
FF-1	23.6	25.7	26.6	27.6	28.3	29.1	29.6	29.6	29.6	29.9	30.2	30.9	31.4	31.4	31.3	31.3	0.30
Fetlock cm																	
SS-1	20.7	21.5	22.9	23.5	24.2	24.5	25.2	24.8	25.6	25.5	26.0	26.0	26.4	26.6	26.7	26.3	0.23
FF-1	19.9	21.2	22.0	23.1	23.8	24.3	24.9	24.7	25.1	25.3	25.8	25.9	26.4	26.2	26.4	26.2	0.23

[a]A description of growth variables is given in Table 21.
[b]SS-1 = Sugar and Starch supplement; FF-1 = Fat and Fiber supplement.
[c]Least squares standard error of the means.
[d]ADG = ((Weight(month 1) – Weight(month 2))/number of days between sampling periods.
[e]Body condition measured on a scale of 1 to 9 (Henneke et al., 1983).
[f]Body condition scores were first measured in July, 1998

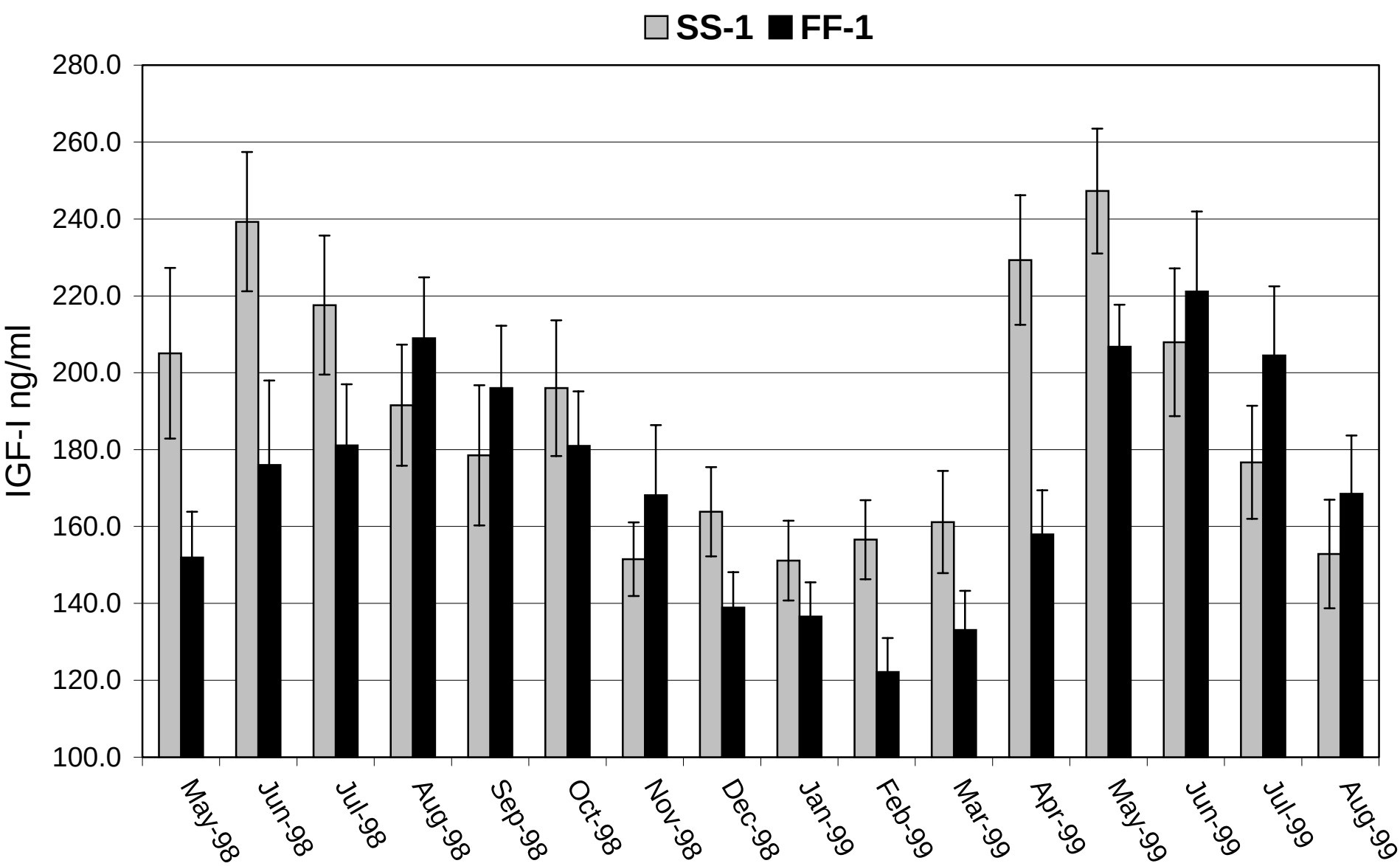


Figure 20. Circulating insulin-like growth factor I (IGF-I) concentrations in Thoroughbred foals fed a sugar and starch (SS-1) or fat and fiber (FF-1) supplement over a period of 16 mo.

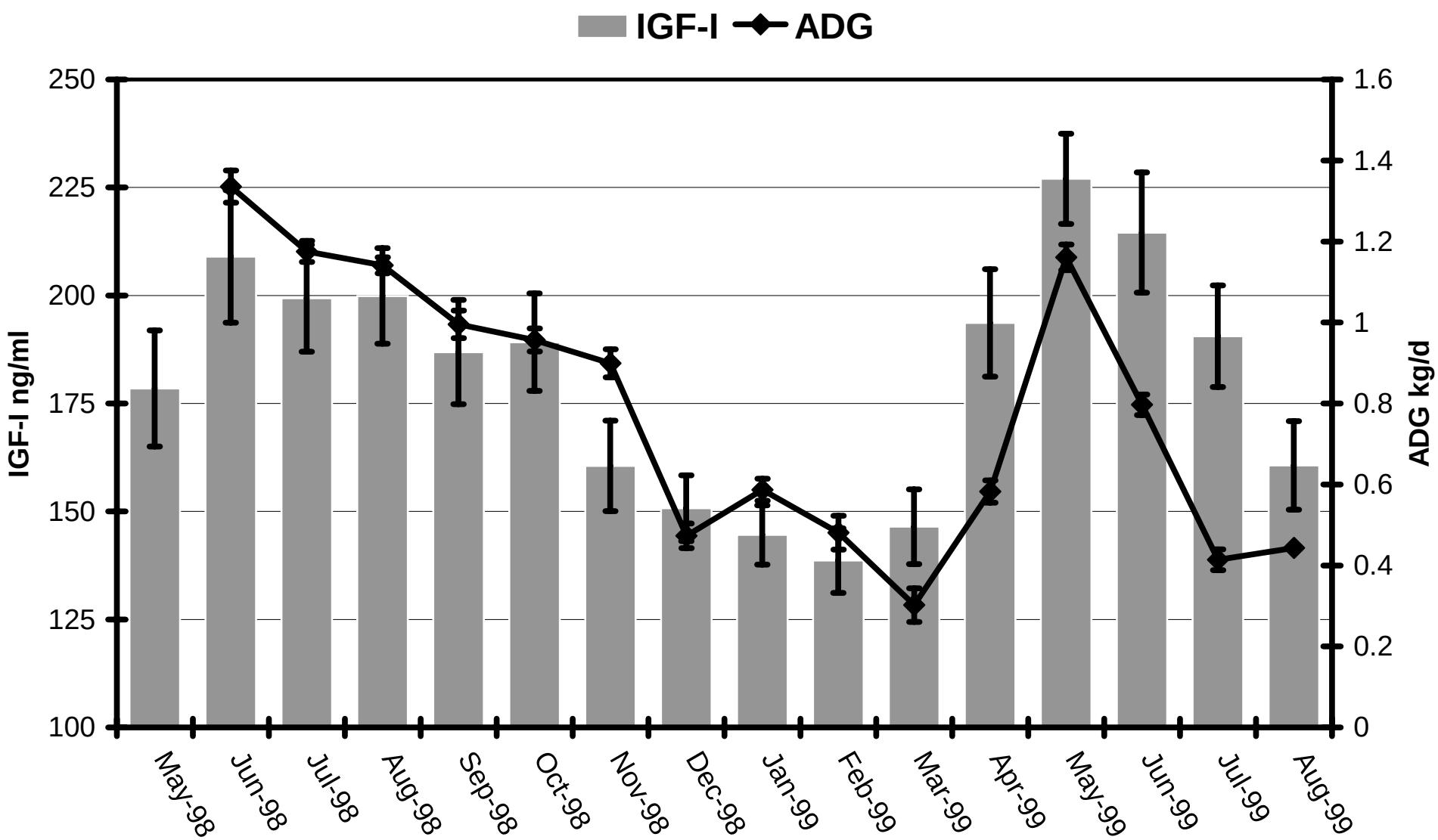


Figure 21. Circulating insulin-like growth factor I (IGF-I) and average daily gain (ADG) in Thoroughbred foals during the first 16 mo of life.

IMPLICATIONS

Nutrition's effect on the somatotropic axis is likely to be a major component in its role in the etiopathogenesis of osteochondrosis. The somatotropic axis is one of the fundamental controllers of bone and cartilage development, yet this is part of a larger role that the somatotropic axis plays in mediating the organism's growth in a changing environment. This environment includes components that can be controlled, such as diet and biomechanical influences, and components that cannot, such as temperature and day length. It is likely that part of the somatotropic axis's role is to allow an organism to survive the changes to the environment that cannot be controlled. The somatotropic axis in the horse has had 60 million years to adjust to changes in temperature, day length, and even pasture conditions, yet only about 3,000 years to adjust to domestication. In order to realize the full potential of the horse we must better understand the effect of domestication.

Some of the most easily measured effects of the somatotropic axis are changes in growth. The somatotropic axis may act as an accelerator pedal, changing the rate of growth to suit the environmental conditions. By examining how the growth of the horse changes over a time, a general idea of how the somatotropic axis reacts to certain stimuli can be gauged. In this experiment changes in growth were seen at the time of weaning, the following spring, and the following summer. There are a number of changes to the environment at each of these points that may have affected the somatotropic axis. Two of these changes that are easily measured and whose effects can be quantified are day length and temperature. As was seen above, these factors accounted for a significant portion of the variability in the changes of growth. These factors were also directly correlated with average daily gain and circulating insulin-like growth factor I. These are also factors that are difficult to control for most of the equine community. While changes in growth are an easily measured indicator of the status of the somatotropic axis, they are likely not a very precise measure. Dyschondroplasia, the early stages of osteochondrosis, is characterized by subtle changes in the

development of the growth cartilage. Dyschondroplasia in horses is usually undetectable until the lesion has progressed to a clinically significant stage. It is possible then, that changes in the somatotropic axis, that are not detectable through measures of growth, may have an effect at the tissue level.

Two factors that have been connected with the occurrence of osteochondrosis are rapid growth rates and a high dietary energy intake. We have already discussed growth rates relationship with the somatotropic axis. Traditionally, high energy horse feeds have contained high concentrations of hydrolyzable carbohydrates. Digestion of the starch and absorption of the glucose from this meal stimulates an insulin response. Further, the increase in glucose following the meal results in lower growth hormone secretion. These changes then reverse as plasma glucose levels begin to fall. These changes directly tie carbohydrate intake to changes in the somatotropic axis. The changes in hormones and metabolites result in a feeding/fasting cycle of glucose and insulin in horses that are fed two meals daily. This cyclical pattern is unlikely to have been seen in the grazing herbivore prior to domestication. The above results also connect an increase in hydrolyzable carbohydrate intake to an increase in plasma insulin-like growth factor I. This is important because collection and analysis of growth hormone data is much more cumbersome than insulin-like growth factor I.

It appears then that the commonality between osteochondrosis, rapid growth rates, and high hydrolyzable carbohydrate intakes is the somatotropic axis. To best clarify this idea, take the following example of a year in the life of a feral horse and a domesticated horse.

The feral horse is born April 1st, into an environment of brush and plains. Gradually, through his first few months, plants become a larger part of his diet and he spend most of the day and a portion of the night grazing with the herd to meet the needs of his growing body. His level of energy intake, the temperature and day length result in the somatotropic axis maintaining a fairly constant level of growth. As the day length shortens, temperature falls, and energy become more difficult to

find, the somatotropic axis backs off so that the energy that is available can be put towards thermoregulation. When spring finally arrives, and the days become longer, the grass on the plains begins to grow, and the somatotropic axis acts to accelerate growth so that our foal may reach his optimal size for survival in this environment. The amount which the somatotropic axis changes growth over the this period of the foals life has been perfected over millions of years, so that the horses that survive are best suited to their environment.

The domesticated horse is born April 1st, in a stall on 1000 acre farm. Gradually, through his first few months, plants and a supplement become a larger part of his diet. He spends most of the day grazing and comes into the stall at night where hay is supplied. Eating the supplement results in a cyclical pattern of insulin that tells the somatotropic axis there is more energy available, so the growth rate of this foal is greater than that of the feral horse. Like the feral horse the growth of this foal decreases in the winter for many of the same reasons, and in the spring, in a similar manner the somatotropic axis accelerates growth, but it pushes growth a bit harder because the signals that the somatotropic axis is getting from the environment are that there is more energy available.

The problem is that the system that was finely tuned over millions of years to interpret changes in the natural environment and respond accordingly, was not finely tuned for the environment of the domestic horse. Rapid growth that results in the development of osteochondrosis in the feral horse would result in that horse's inability to survive, so the somatotropic axis adjusted to compensate for changes However, the changes the natural environment presented were very different from those presented by domestication. To best care for the horse, and enable them to reach their full athletic ability, we must take into account their evolutionary heritage. In a way, we must understand their relationship with nature more than their relationship with us.

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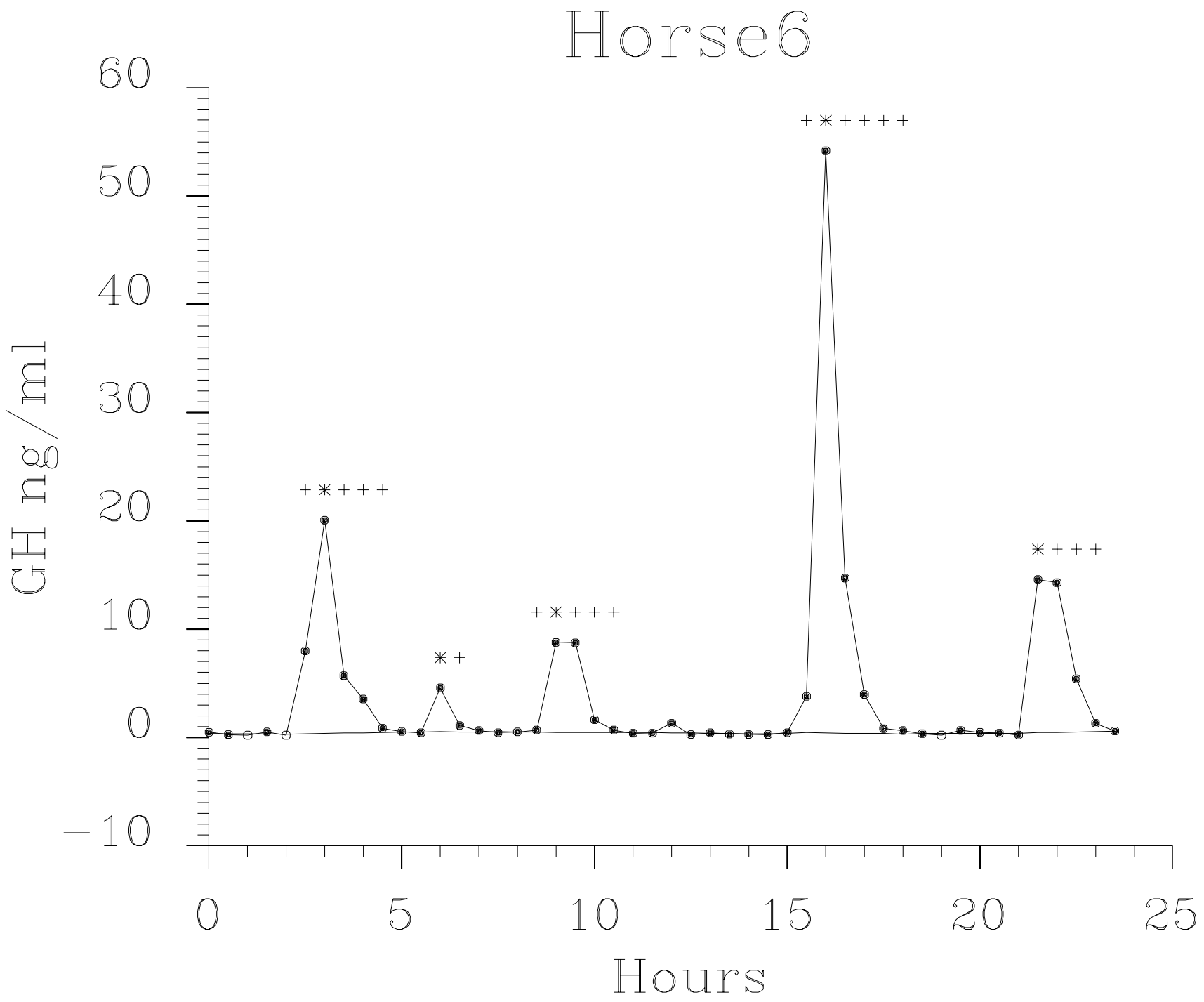
APPENDIX 1

This appendix contains 12 figures of growth hormone concentrations measured in Thoroughbred foals. This data is the same as that presented in Chapter 4. These figure were generated in DOS using the PC-Pulsar program. They were included because the peaks in growth hormone are more clearly defined and all the points that were include in each peak are identified by an asterick above the point. Horse numbers 6, 63, 78, 114, 125, and 134 were in the SS-3 group, and horse numbers 38, 81, 124, 126, 129, and 135 were in the FF-3 group.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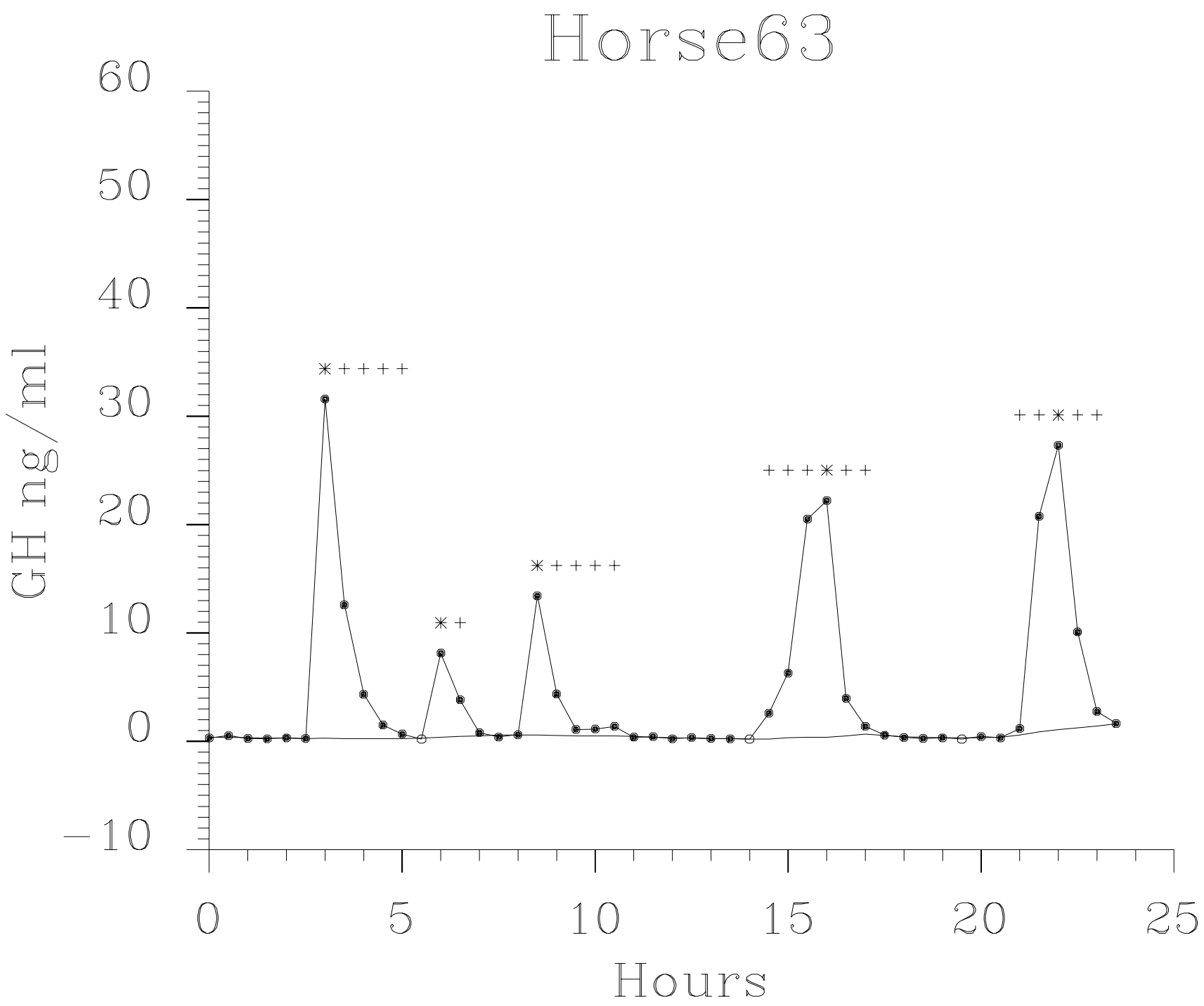
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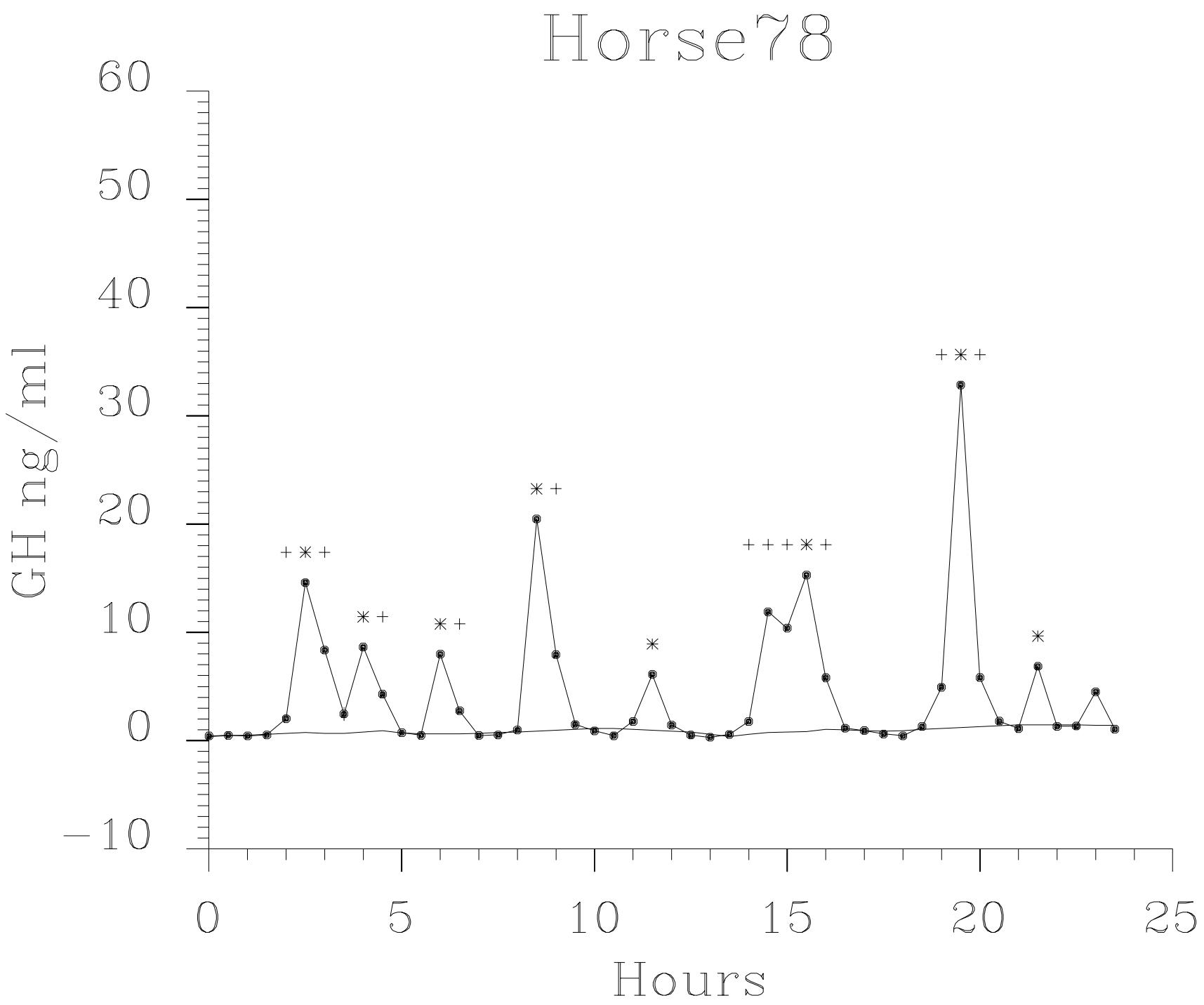
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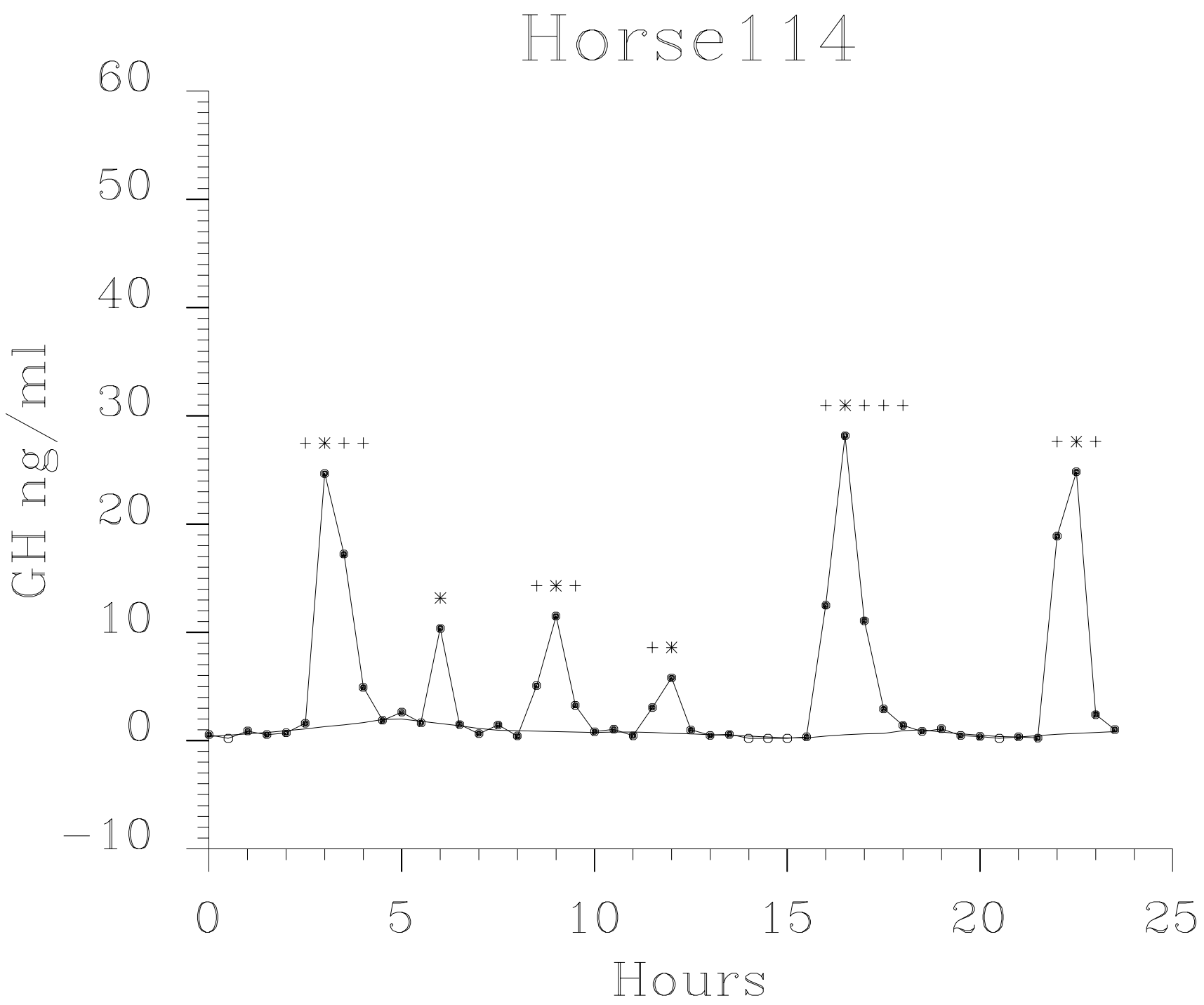
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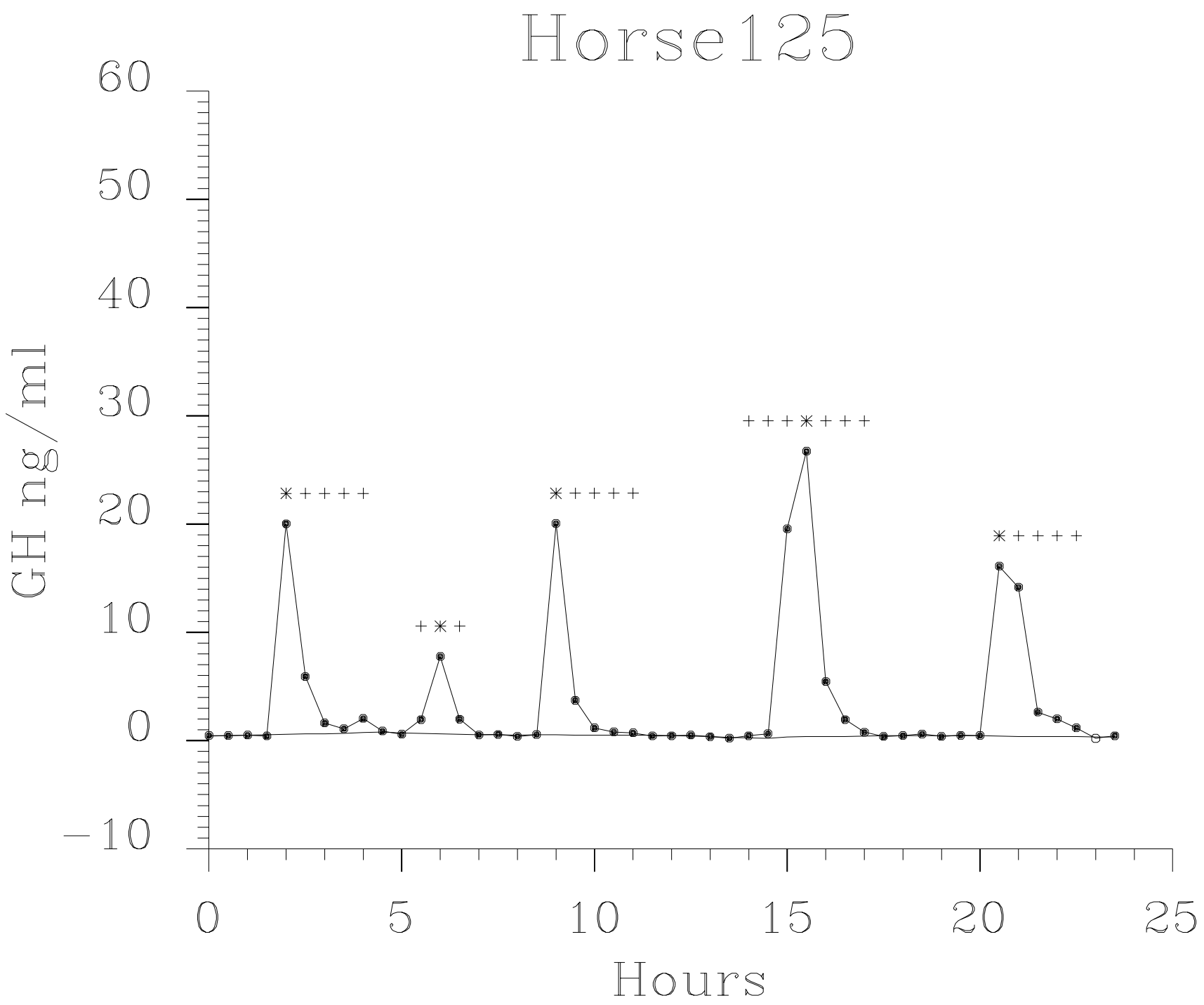
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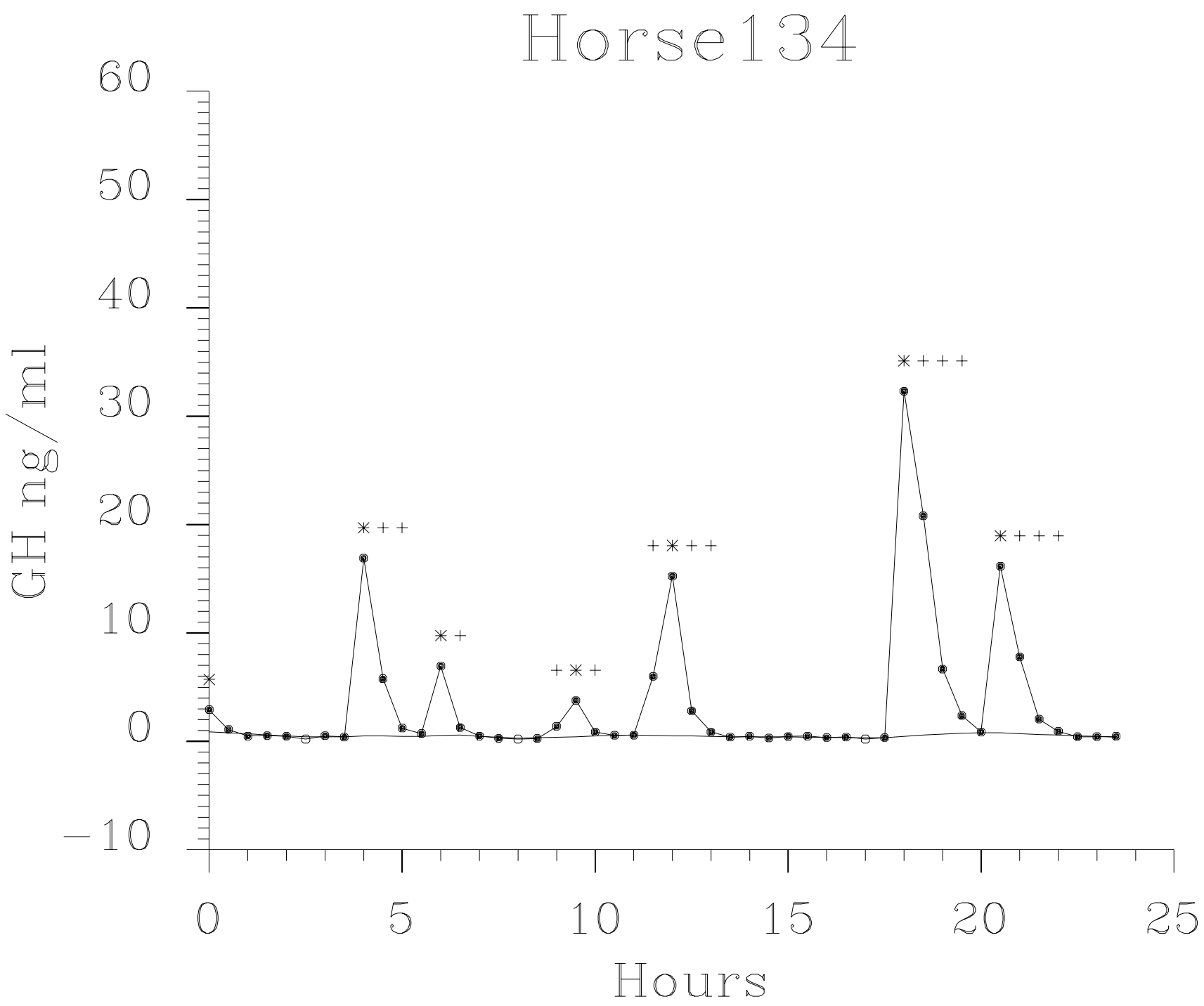
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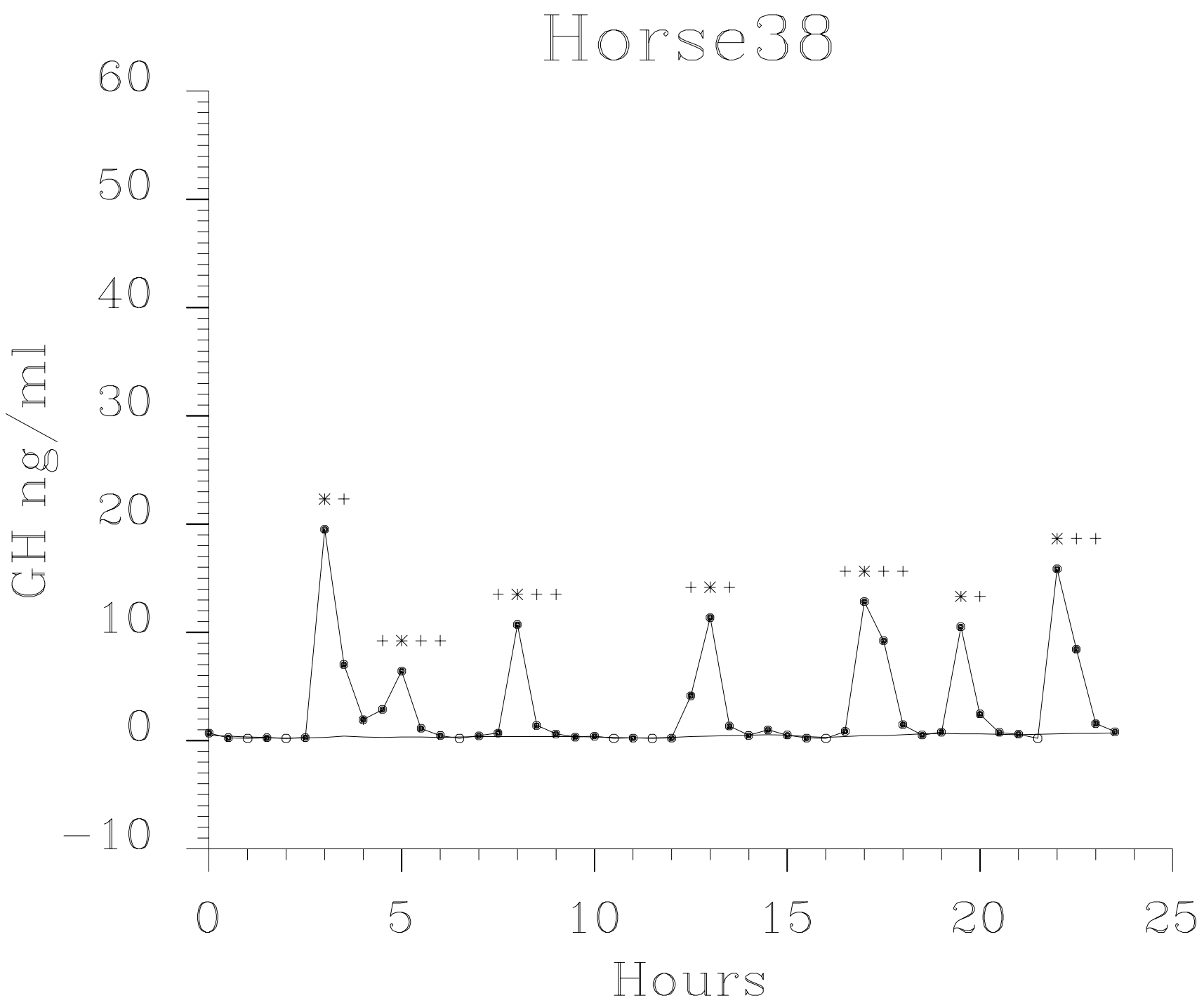
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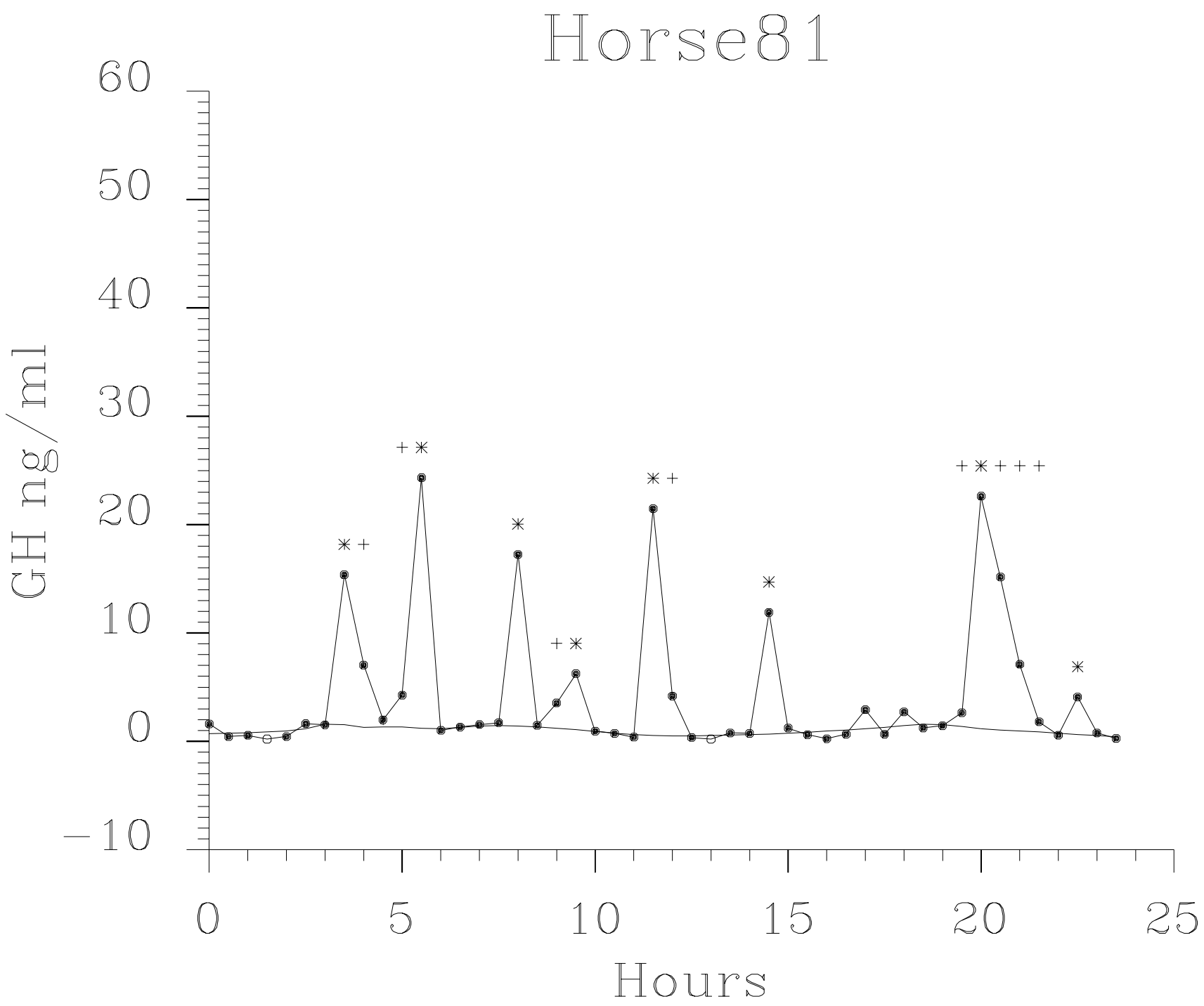
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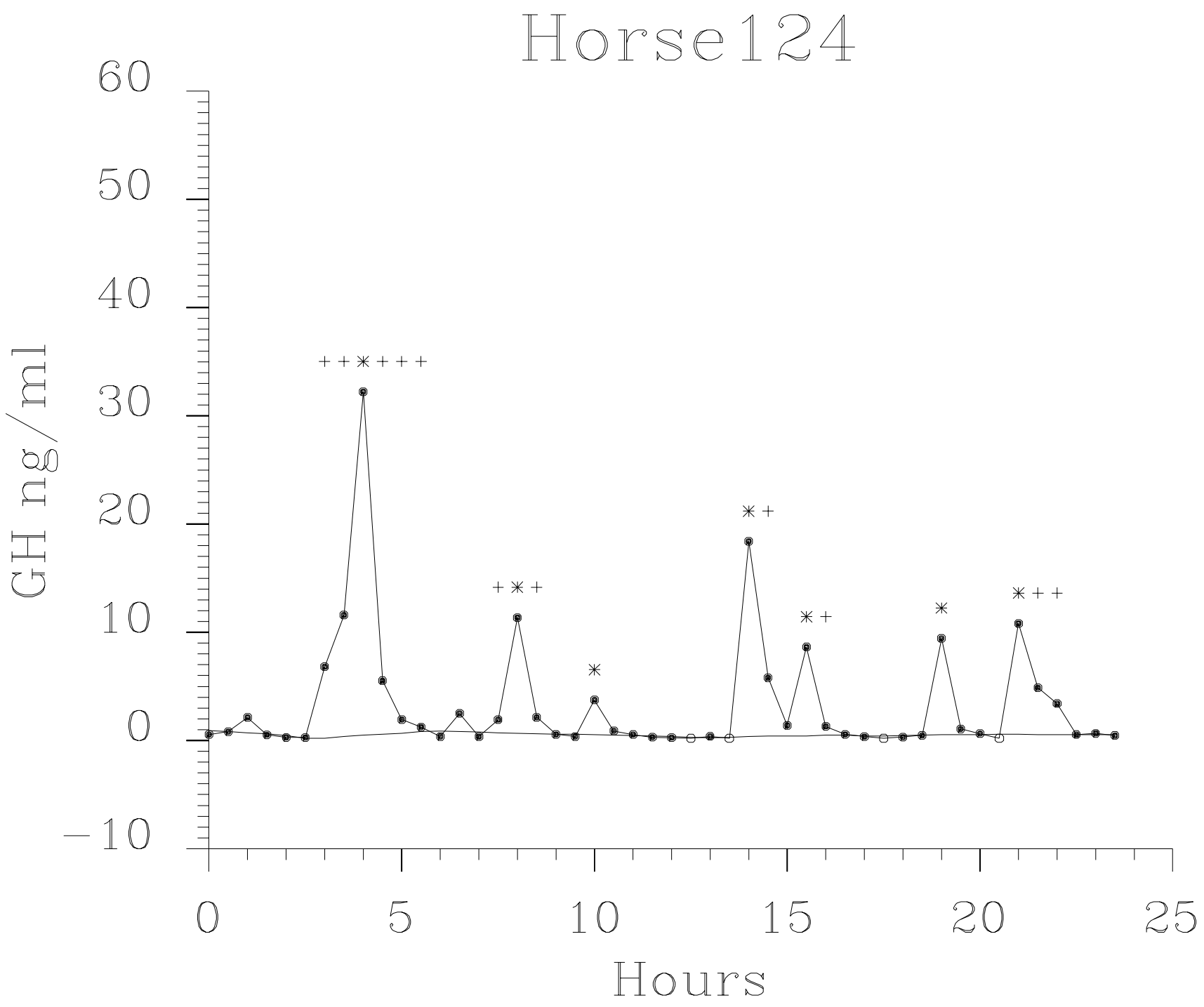
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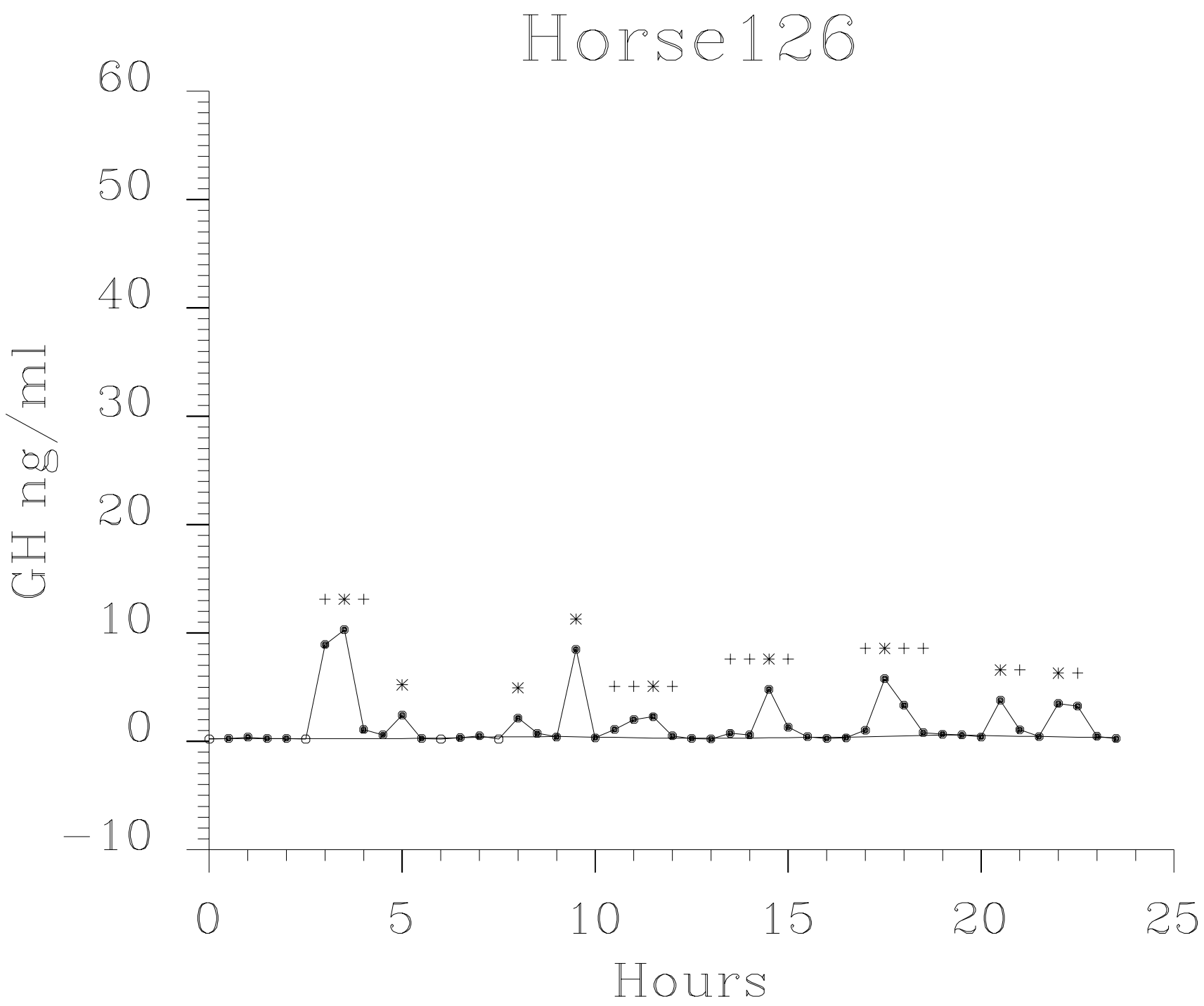
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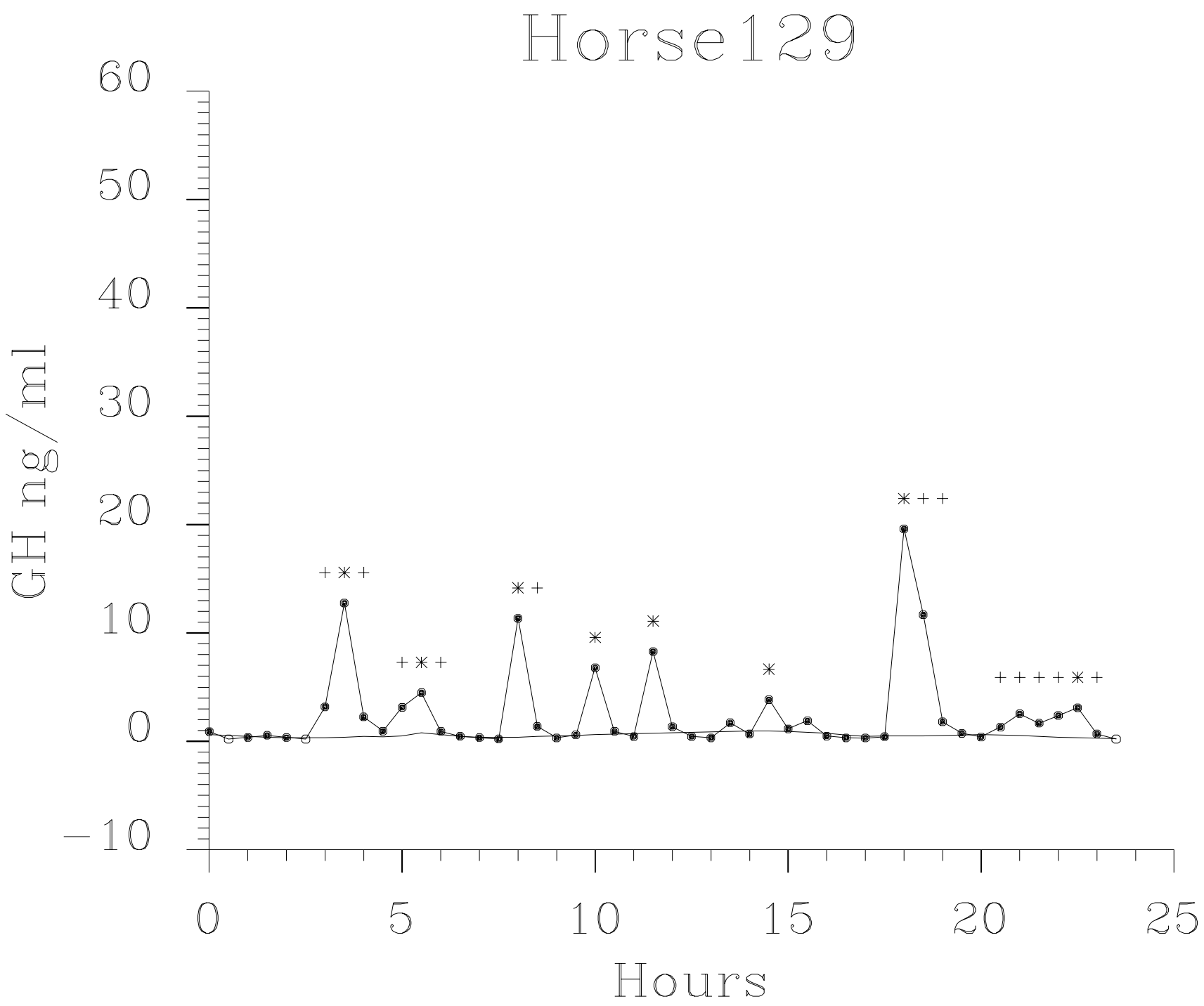
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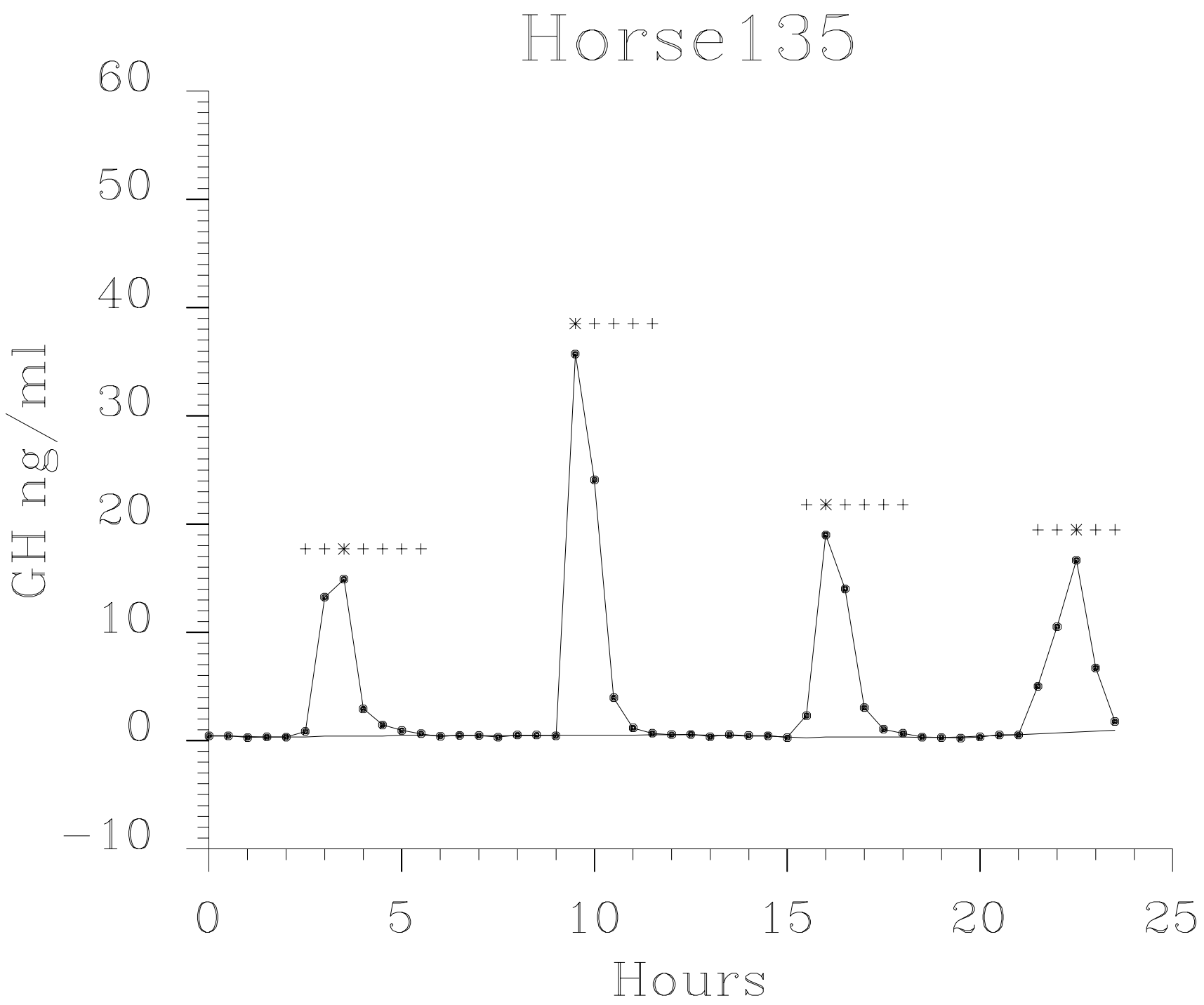
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APPENDIX 2

To validate the acid ethanol extraction and radioimmunoassy used to measure plasma concantrations of IGF-I, a pilot study was run in which Thoroughbred mares were injectected with equine somatotropin (eST). One of the recognized effects of GH is stimulation of IGF-I release from the liver. The objective of this study was to quatify the release of IGF-I in Thoroughbreds in response to 5 days of daily intramuscular eST (EquiGen, BresaGen Limited, Australia) injection.

Materials and Methods

Four nonpregnant mares were used in this trial. Two received intramuscular injection of 25 μg/kg BW eST, and two, 0.9% sodium chloride vehicle equal in volume to that containing eST in the first two mares over a period of five days. Prior to the initial injection, baseline weight and body condition scores were recorded. Simultaneously, jugular vein blood samples were drawn to determine baseline levels of IGF-I. Following collection of this data, eST and the placebo dose were administered and the mares placed back on pasture. At 0700 on days 2–4 of the study sample collection and eST administration followed the above schedule. A final sample was collected on day 5 (Table 1A).

Blood samples were collected via jugular venipuncture into 10 ml tubes (Lithium Heparin Vacutainer, Becton Dickenson, Rutherford, NJ). Samples were centrifuged within an hour of collection for 10 minutes at 3000 g at 20°C. Plasma was then pipetted off and frozen at -20°C.

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 then stored at -20°C for one hr. Samples were then centrifuged at

1,500 x g for 30 min, and the supernatent decanted into 12 x 75 polypropylene tubes. Samples were stored at -20°C for later analysis by radioimmunoassay.

Dilutions of samples were made to determine the percentage IGF-I recovery the extraction and radioimmunoassay would yield. Dilutions of 1:1, 2:1, and 4:1 were made using acid ethanol extracted samples and water or standard (GrowPrep, Adelaide, AU), which had a concentration of 20 ng/ml. These dilutions were taken through the entire extraction procedure and concentrations of IGF-I measured using the gamma counter.

Insulin-like growth factor I plasma concentrations were quantified using a double antibody radioimmunoassay. Recombinant IGF-I and plasma IGF-I in extracts were measured using a mouse anti-human IGF-I monoclonal primary antibody. Primary antibody and [^{125}I]IGF-I were added to the extracted sample and buffer for a 24 hr incubation. Secondary antibody was then added and samples are left to incubate for three days. Samples were then centrifuged and the supernatant removed. Radioactivity of remaining pellets was quantified with a gamma counter. Radioactivity is inversely related to levels of IGF-I in the sample.

Results

The acid ethanol extraction enabled detection of IGF-I concentrations from 5 to 80 μl. The dilutions revealed recoveries from 97% to 114% (Table 2A). The extraction and radioimmunoassay were further validated by the results from the trial conducted with the four Thoroughbred mares. The two mares that received intramuscular injections of eST had significantly increased plasma concentrations of IGF-I by the second day of the trial and these continued to increase over the period of the trial (Figure 1A). This was in contrast to those mares that were only receiving the vehicle, whose plasma IGF-I had no change over the 5 d.

Discussion

This is the first use of the acid ethanol extraction for equine plasma. While the method of acidification of plasma before column chromatography is considered a

more reliable method, it is time consuming and impractical for studies with a large number of samples. The above results confirm the efficiency of the acid ethanol extraction for removing binding proteins and enabling the measurement of circulating IGF-I.

Table 1A. Schedule for eST injection of Thoroughbred mares for IGF-I validation experiment.

	Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**
Group 1 (Control Group) **2 nonpregnant Thoroughbred mares**	Weigh, Body Condition Score, Baseline Blood sample Dose #1 = 0.9% sodium chloride	Sample #2 ↓ Dose #2 = 0.9% sodium chloride	Sample #3 ↓ Dose #3 = 0.9% sodium chloride	Sample #4 ↓ Dose #4 = 0.9% sodium chloride	Sample #5 END
Group 2 (Experimental Group) **2 nonpregnant Thoroughbred mares**	Weigh, Body Condition Score, Baseline Blood sample Dose #1 = 25µg/kg eST	Sample #2 ↓ Dose #2 = 25µg/kg eST	Sample #3 ↓ Dose #3 = 25µg/kg eST	Sample #4 ↓ Dose #4 = 25µg/kg eST	Sample #5 END

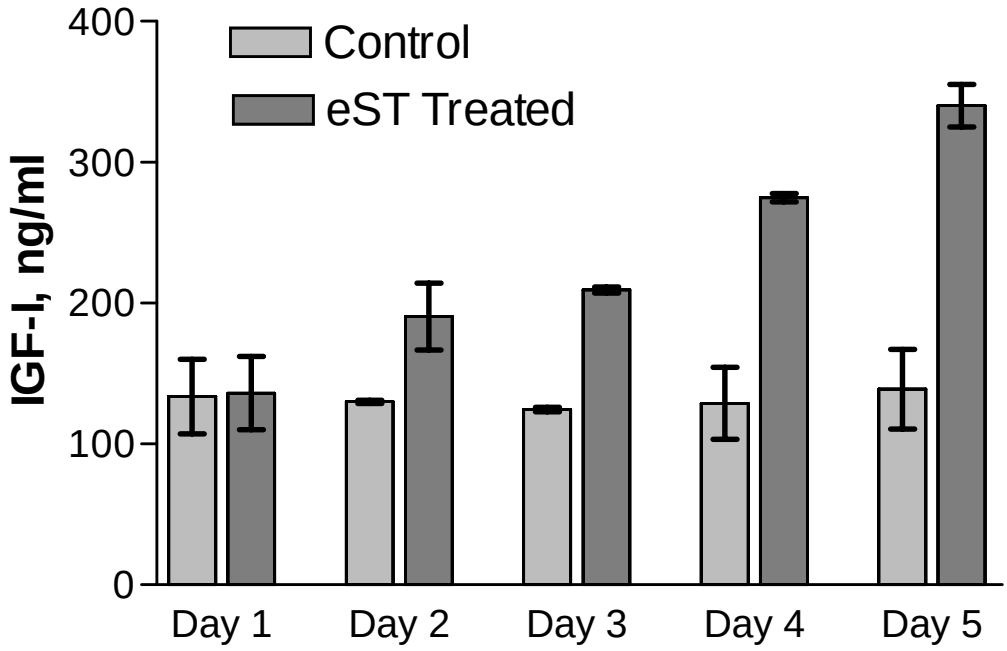

Figure 1A. Plasma IGF-I concentrations in Thoroughbred mares given intramuscular injections of 25 μg/kg BW eST or 0.9% sodium chloride vehicle over a 5 d period.

Method	Dilutions	IGF-I, ng/ml	% Recovery
Sample to H_2O	1:1	135 ± 8.7	104
	2:1	179 ± 5.2	104
	4:1	187 ± 2.6	97
Sample to Standard, 20 ng/ml	1:1	159 ± 10.5	114
	2:1	194 ± 7.9	109
	4:1	237 ± 16.7	113

Table 2A. Recoveries of IGF-I from dilutions of sample with either water or IGF-I standard

Vita

William Burton Staniar, son of Mrs. Dale Staniar and Mr. William Staniar, was born on August 26, 1974 in Princeton, New Jersey. He graduated from Pomfret School in Pomfret, Connecticut in 1992. He then attended the University of Richmond, where he majored in Biology. William received his Bachelor of Arts degree in May 1996. He received his Master of Science in Equine Nutrition in the Department of Animal and Poultry Sciences at Virginia Polytechnic Institute and State University. His thesis focused on the protein content of supplements fed to young, growing yearlings. William stayed at Virginia Tech to pursue his Ph.D. investigating the patterns of growth in young Thoroughbreds, and how diet affects endocrine systems important to optimal athletic development. William has done the majority of his research at the Middleburg Agricultural Research and Extension Center. He received a John L. Pratt Fellowship to pursue a doctoral degree in Equine Nutrition at Virginia Polytechnic Institute and State University. Following the completion of his Ph.D. William will be continuing a similar line of research in a post-doctoral position at Virginia Polytechnic Institute and State University.