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FEED INTAKE PATTERNS AND ASSOCIATED BLOOD GLUCOSE, FREE FATTY ACID AND INSULIN CHANGES IN PONIES¹

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Summary

The feeding patterns of five pony geldings fed pelleted diets *ad libitum* were quantified for five 24-hr periods. Eighty percent of a given pony's total daily intake ($6.3 \pm .81$ kg or $2.9 \pm .41\%$ BW) was eaten in $10 \pm .9$ separate meals. Each meal averaged $.49 \pm .13$ kg of pellets and lasted 44 ± 10 minutes. The mean intermeal interval was 84 ± 10 min, with a maximum of 3 hour. The animals spent $38 \pm 7.2\%$ of a 24-hr period engaged in eating activities, $84 \pm 3.7\%$ of which was devoted to meals, the other 16% spent in nibbling activities. Forty-nine percent of the total daily intake was consumed between 0800 and 1700 hours. Fewer ($P < .01$), smaller ($P < .05$) and less frequent ($P < .01$) meals were observed between 1700 and 0800 hour. Wood chewing was observed only in the course of aggressive interactions between ponies.

The ponies were placed on a feeding schedule that mimicked their normal *ad libitum* feeding patterns. Venous blood samples were drawn 1) after 1 hr of feeding; 2) after a subsequent 3-hr fast; 3) at the end of a second meal after the fast. Plasma glucose dropped from a satiety level of 111.4 ± 7.1 mg/100 ml to 87.5 ± 4.9 mg/100 ml after the 3-hr fast ($P < .05$) and had risen to the previous satiety level (104.6 ± 13.4 , $P < .005$) at the end of the subsequent meal. Immunoreactive Insulin (IRI) dropped from 85.2 ± 43 μ U/ml to 52.4 ± 43 ($P < .005$) during the fast and rose to 91.4 ± 48 ($P < .05$) after the

second meal. Plasma free fatty acids (FFA) did not change significantly from the first satiated state ($.187 \pm .04$ meq/ml) to the normal hunger state ($.196 \pm .03$ meq/ml) or at the second satiated state ($.184 \pm .05$ meq/ml). There were interpony variations ($P < .01$) in the plasma IRI at all three sampling times. The plasma glucose level at normal hunger was negatively correlated ($P < .01$) with the subsequent meal size and rate of eating. It is postulated that plasma glucose levels may play a role in the control of feed intake in ponies.

(Key Words: Ponies, Feeding Patterns, Feed Intake Controls, Plasma Glucose, Plasma Free Fatty Acids, Immunoreactive Insulin.)

Introduction

Studies of spontaneous feed intake patterns and associated changes in blood metabolites and hormones have provided important background data for the investigation of the factors controlling feed intake in ruminants (Baile, 1975; Baile *et al.*, 1974; Chase *et al.*, 1976; Gary *et al.*, 1970) and nonruminants (Beck and Kisselef, 1974; Davis and Campbell, 1973; Panksepp, 1976). The relative importance of gastric fill, blood levels of glucose, volatile fatty acids, free fatty acids and cholecystokinin differs between the two groups of animals in the determination of satiety and hunger (Baile, 1975; Della Fera and Baile, 1979).

Despite the large amount of data available on the digestion and metabolism of ponies (Argenzio *et al.*, 1974; Glinsky, 1976; Hintz, 1975; Hintz *et al.*, 1971; Koller *et al.*, 1978), few studies have addressed the potential controls of feed intake in equine animals. Palatability of diets (Haenlein *et al.*, 1966) and aberrant behaviors such as wood chewing (Willard *et al.*, 1977) are the only aspects of equine feeding behavior on which there is current data avail-

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able. Ponies are capable of both ruminant-like and nonruminant modes of digestion and metabolism, apparently alternating between the two dependent upon the fiber and soluble carbohydrate content of their diets or length of fast (Argenzio and Hintz, 1970, 1972; Hintz, 1975; Glinsky, 1976; Koller *et al.*, 1978; Van der Noot and Gilbreath, 1970). This flexibility makes the pony an interesting intermediate model for the study of controls of feed intake relative to the other species on which there are data available.

This study was designed to provide background data for the study of controls of equine feeding. The two experiments presented in this paper were designed to: 1) describe and analyze the spontaneous daily feeding activities of mature ponies given *ad libitum* pelleted feeds and 2) measure the relationship of blood glucose, free fatty acids (FFA) and immunoreactive insulin (IRI) in "normally" hungry and satiated ponies.

Experimental Procedure

Five mature pony geldings (average weight 213 ± 19 kg; range 197 to 245 kg) were maintained indoors in individual free stalls. The floor was concrete and bedded with wood shavings. Partitions between the stalls were metal bars that allowed the animals to interact with each other. The room was temperature controlled (24 ± 2 C) and under continuous light. Diurnal variations in activity levels occurred during the "working day" (0800 to 1700 hr) due to other experiments being conducted in the area. All handling (grooming, exercise and feeding) was done during this period.

Individual feeders in each stall were equipped with load cells connected to a Data Acquisition System (DAS; Baile, 1975; Baldwin *et al.*, 1977) in the adjacent room. Weight of feed that remained in each feeder could be read from a digital display on the DAS.

The ponies were fed a complete pelleted diet (table 1) free choice during the experiments unless otherwise noted. By the end of a 4-week adaptation period the animals' *ad libitum* daily intakes and body weights had stabilized. All ponies were transferred to indoor exercise pens for 1 hr each day, at which time the stalls were cleaned. Feed was replenished in the morning (0800 hr), during exercise periods and in the evening (1700 hr) to insure *ad libitum* condi-

TABLE 1. COMPOSITION OF PELLETTED DIET FED TO PONIES AS % DRY MATTER

Moisture	13%
Dry matter	87%
Crude protein	15.2
Dig. protein (est)	12.2
Crude fiber	10.7
Fat	3.45

tions. This daily routine was maintained throughout the experimental period unless otherwise noted.

Experiment 1. Behavioral observations were taken over a 17-day period. Data collection was divided into "working day" sessions from 0800 to 1700 hr and "night" sessions from 1700 to 0800 hr. During these sessions the activity of each pony was recorded at 5-min intervals on a time sheet, except when the animals were in the exercise pens. Activities were classified as: consummatory (eating and drinking) and nonconsummatory (resting, lying down, defecation, urination and social activity). Weights of feed remaining in each feeder were recorded every 15 minutes. Only data from complete sessions, in which all animals were healthy and observations were recorded for the full session, were utilized in the data analyses presented in this paper. Data presented herein represent the averages for five ponies for five "working day" and "night" sessions, representative of five 24-hr periods.

Each recorded behavioral observation was considered to represent an average period of 5 minutes. "Eating bouts" were defined as any series of eating observations (one or more 5-min periods) separated from the next eating observation by at least 20 min (four behavioral observations). "Meals" were defined as any eating bout in which more than 150 g of feed was consumed, based on preliminary observations which showed the animals would "nibble" (Baile, 1975) small amounts (<150 g) of feed when disturbed.

Individual pony averages were used to calculate the overall means for the feeding parameters presented in tables 2 and 4. Correlation coefficients were calculated as suggested by Panksepp (1976) and Kisselef (1970) for the premeal interval *vs* meal size and the postmeal interval *vs* meal size; both for individual ponies and across all animals and days. The Student's *t*

test was used to compare means between day and night feeding patterns.

Experiment II. The experimental period lasted 9 days. The same ponies and feed were used. Based on the results of experiment I, a feeding schedule was designed to mimic the spontaneous meal patterns. The animals were fasted from 0800 to 0900 hr, then presented with 1.5 kg of feed for 1 hr. At 1000 hr the remaining feed weights were recorded and all feed was removed for 3 hours. At 1300 hr the animals were presented with 3 kg of feed. At the end of the ensuing meal, the amount of pellets that remained was again recorded but the animals were allowed *ad libitum* access to feed until the following morning. Jugular blood samples were drawn by venipuncture. Samples were taken: 1) 1 hr after the morning feed was presented (first satiated state), 2) at the end of the 3 hr fast (normal hunger) and 3) 10 min after the second meal had apparently ended (second satiated state). If a pony resumed eating for more than 5 min immediately after the third blood sample was drawn, a fourth sample was taken 10 min after the animal had again stopped eating. The fourth sample was then considered to represent the "second satiated state" in the final analysis. Plasma and serum samples were frozen for subsequent analyses.

One of the ponies from experiment I was excluded from experiment II because it became unmanageable when approached for venipuncture. Only complete series of three consecutive samples drawn from ponies with a minimum of excitement were used for data analysis. The data presented in this paper represent a minimum of 5 replicate days for three animals and 2 replicate days on a fourth, for a total of seven replicates of three sampling periods on four animals.

Samples were analyzed for: glucose (glucose oxidase method on deproteinized plasma; Huggett and Nixon, 1957), free fatty acids (Dole, 1956; Novak, 1965) and immunoreactive insulin (porcine standard³, RIA; Morgan and Lazarow, 1963).

Results

Experiment I. Mean meal parameters for a 24-hr period are presented in table 2. Eighty percent of the total daily intake was accounted

³Eli Lilly Co., Lot No. 615-D63-10; 25.5 U/mg.

TABLE 2. PONY FEEDING PATTERNS BASED ON MEANS FROM FIVE PONIES OBSERVED FOR FIVE 24-HR PERIODS^a

Total daily intake (kg, TDI)	TDI as % BW	Total meal intake (kg, TMI)	TMI as % BW	TMI as % TDI	Total eating time (TET, min)	TET as % of 24 hr	Total time in meals (TTM, min)	TTM as % TET	Number of meals	Meal size (kg)	Meal size as % BW	Meal duration (min)
6.3 ± .81	2.9 ± .41	5.0 ± 1.0	2.4 ± .35	80 ± 6.0	537 ± 97	38 ± 7.2	451 ± 74	84 ± 3.7	10 ± .9	.49 ± .13	.23 ± .06	44 ± 10
Eating rate (g/min)	14 ± 3	Pre-meal interval (min)	84 ± 10	Postmeal interval (min)	88 ± 5							

^aValues are means ± standard deviations.

for with the criterion of a meal being at least 150 g consumed within at least 15 minutes. The remaining 20% was eaten between meals "nibbling" (Baile, 1975). Nibbling bouts usually lasted less than 5 min and occurred when the ponies were disturbed or in the course of social interactions. Nibbling accounted for only 16% of the total observed eating time.

The differences between pre- and post-meal intervals are due to the sampling techniques wherein the last post-meal intervals were known but the corresponding pre-meal interval was not included due to the delineation of the day-night sessions. For example: pony No. 2 finished a meal at 0600 during a "night" session and laid down until 0800 when he got up and ate another criterion meal. The post-meal interval was 2 hr, but the corresponding 2-hr pre-meal interval was not used in the data analysis, because the duration and size of the second meal were not recorded and (or) used for the subsequent working day.

Table 3 presents the percentage feed consumed as meals and the frequency distribution within the meal size intervals. Meal size data were divided into intervals of .20 kg and summed for all animals for the totals in each meal category. Almost half (49%) of the daily meals were consumed in quantities between .15 and .34 kg and represented 22% of the total daily intake. The frequency distribution of meals greater than .35 kg varied considerably between ponies.

Feeding parameters separated into "working day" (0800 to 1700 hr) and "night" (1700 to 0800 hr) periods are presented in table 4. Total intakes, eating times and numbers of meals are presented on a per hour basis to eliminate the bias of different total observation times between the day and night sessions. The ponies ate 42% of their total daily intake during the 8 hr working day. Significant differences were

found in the patterns observed during the day vs night periods. The longer post-meal intervals at night ($P < .01$) reflect the ponies' tendency to lie down and sleep for 1 hr or more several times after midnight. The animals would lie down and (or) doze during the working day too, but rarely for more than 30 minutes. There was not a significant diurnal difference in the amount of nibbling.

There were no significant correlations between either the pre-meal interval and subsequent meal size or post-meal interval and previous meal size on either body weight adjusted or unadjusted data.

Experiment II. The results of the assays for plasma glucose, free fatty acids (FFA) and immunoreactive insulin (IRI) are presented in table 5. Analysis of variance of plasma glucose and free fatty acid levels showed no difference between ponies. For IRI there was a difference ($P < .01$) between animals, explaining the large standard deviations.

The plasma glucose level after fasting (normal hunger) was inversely correlated to the subsequent meal size ($P < .01$, $r = -.95$, $n = 22$) and the rate at which it was eaten ($P < .01$, $r = -.92$, $n = 22$). No such correlation for either IRI or FFA levels were shown, either for individual ponies or the pooled samples.

The average changes in plasma glucose, FFA and IRI are presented in table 5b. There were no significant differences in the drop in plasma glucose and IRI during the 3-hr fast and the subsequent rise after the second meal (average duration 45 min). The differences between the two satiated states were not significant in any of the blood data. The blood glucose dropped an average of 5% per hour of fasting and rose an average of 3.7% per .10 kg of feed consumed. The insulin levels dropped 19 $\mu\text{U/ml}$ per hour of fast and rose 4.0 $\mu\text{U/ml}$ per .10 kg of feed

TABLE 3. PERCENTAGE OF FEED CONSUMED AS MEALS AND NUMBER OF MEALS ON EACH .20 kg MEAL SIZE CATEGORY—CUMMULATORY DATA FROM FIVE PONIES OVER FIVE 24-HR PERIODS

Category of meal size (kg)	.15 - .34	.35 - .54	.55 - .74	.75 - .94	.95 - 1.14	1.15 - 2.25
% total kg consumed	23.6	16.8	16.1	15.4	12.9	15.2
Total number of meals within category	121	46	31	22	15	13

TABLE 4. COMPARISON OF DAY VS NIGHT (SEE TEXT) FEEDING PARAMETERS IN PONIES. AVERAGES REPRESENT THE MEANS OF FIVE PERIODS FOR FIVE PONIES^a

Feeding parameters	Meal size as % BW	Meal duration (min)	Eating rate (g/min)	Premeal interval (min)	Postmeal interval (min)	Intake per hour (kg/hr)	Number of meals per hour	Total meal intake as of % total intake	Total time eating as of % total time
"Working Day" 0800 to 1700 hr	.7 ^b ± .07	50 ± 15	13 ± 3	72 ± 12	64 ^b ± 10	.33 ^d ± .05	.51 ^d ± .04	84 ± 5.7	46 ^d ± 8.7
Night 1700 to 0800 hr	.21 ^c ± .06	41 ± 11	14 ± 3	90 ± 17	101 ^c ± 6.6	.22 ^e ± .03	.40 ^e ± .05	76 ± 10	31 ^e ± 5.6

^aValues are means ± standard deviations.

^{b,c}Values with different superscripts within a column differ ($P < .05$, Student's t test) between working day and night.

^{d,e}Values with different superscripts within a column differ ($P < .01$, Student's t test) between working day and night.

consumed. There were no significant FFA changes.

Discussion

Experiment 1. Several investigators have observed confined ponies and horses fed pelleted diets. Haenlein *et al.* (1966) compared the intake of sheep and ponies given *ad libitum* access to either pelleted or loose hay. The ponies voluntarily consumed more pellets (2.8% of their body weight) than loose hay. Hintz and Loy (1966) observed that horses fed restricted diets of hay in various physical forms ate the pelleted diets more rapidly than the other forms. An accelerated eating rate may therefore account for the increased intake observed by Haenlein *et al.* (1966). The voluntary intake of pellets by ponies in Haenlein's study is within the range reported in this paper. Willard *et al.* (1973, 1977) fed horses and ponies restricted diets of pelleted concentrates or loose hay. They presented the only quantified behavioral patterns (Willard *et al.*, 1977) for confined equine animals available in the literature. The horses consumed their allotted diets in 48 minutes. The similarity of this "meal" duration is well within the average meal duration of the ponies given *ad libitum* access to pellets in this study. The animals on restricted pelleted diets, however, devoted 26.5% of the rest of a 24-hr period to aberrant consummatory behavior (wood chewing and coprophagy) or "searching activities" (presumably for food). The animals fed loose hay spent greater percentages of their time consuming their diets (39.5%) and less time on the aberrant behaviors (10.4%). Haenlein *et al.* (1966) also reported an unquantified increase in wood chewing by ponies given *ad libitum* access to pelleted feeds. The ponies in our study were not observed to chew the wooden boards on their feeders except to bite them in the course of aggressive interactions with each other. They also bit the metal bars of the stalls and nibbled at their feed or bedding during such encounters. All of these activities appeared to be forms of "displacement" behaviors (Lorenz, 1970; Tinbergen, 1952). Such activities are considered to be the expression of frustration or conflicting drives (Lorenz, 1970; and Tinbergen, 1952). Isolation and "boredom" have been the ascribed causes of aberrant consummatory activities in other confined species (Hediger, 1955). Since the ponies in the other studies were apparently

TABLE 5. LEVELS OF PLASMA GLUCOSE, FREE FATTY ACIDS (FFA) AND IMMUNOREACTIVE INSULIN (IRI) IN HUNGRY AND SATIATED PONIES.

A) ABSOLUTE LEVELS^a

Plasma constituent	Glucose ^b mg %	FFA ^b mEq/ml	IRI ^c μU/ml
Sample 1 (first satiated state)	111.4 ± 7.1 ^d	.187 ± .041	85.2 ± 43 ^f
Sample 2 (Normal hunger)	87.5 ± 4.9 ^e	.196 ± .034	52.4 ± 43 ^g
Sample 3 or 4 (second satiated state)	104.6 ± 13.4 ^d	.184 ± .052	91.4 ± 48 ^f

B) AVERAGE CHANGES IN BLOOD LEVEL^a

Plasma constituent	Glucose mg %	FFA mEq/ml	IRI μU/ml
Δ1 to 2 ^h (fasting)	-24.6 ± 9.5	-.006 ± .006	-32.7 ± 10
Δ2 to 3 (satiation)	+18.9 ± 9.4	-.018 ± .027	+39 ± 5.7
Δ1 to 3	-3.7 ± 11.6	+.003 ± .029	8.8 ± 4.4

^aValues are means ± standard deviation.

^bMeans represent six replicates on three ponies plus two replicates on one pony.

^cMeans represent five replicates on three ponies.

^{d,e}Values with different superscript within column differ ($P < .05$) based on two-way analysis of variance and Duncan's Multiple Range Test.

^{f,g}Values with different superscript within column differ ($P < .005$) based on two-way analysis of variance and Duncan's Multiple Range Test.

^hSee text for explanation and discussion.

isolated from each other, the wood chewing and coprophagy observed may have been due to deficiencies imposed by the restricted diets (Schurg *et al.*, 1977), frustration (Tinbergen, 1952) and (or) boredom (Hediger, 1955). It therefore appears that wood chewing and coprophagy are not due to the pelleted diets *per se*, as suggested by the previous investigators.

The study of spontaneous feeding patterns in various species has been quite active in recent years. Knowledge of how the animals normally eat (meal size, frequency, intermeal intervals, rates of eating, etc.) has been used to determine how different diets affect the pattern of feed intake (Baile, 1975; Sclafani and Berner, 1976; Wangsness *et al.*, 1978). Such studies also allow speculation as to the controls of a feeding "operator" under "normal" and experimental conditions (Davis and Campbell, 1973; Kisselef, 1970; Panksepp, 1976; Sanderson and Van der Wede, 1975). It is of interest to note that the feed intake pattern described in this paper for

confined ponies closely resembles those reported for other domestic herbivores such as sheep (Baile, 1975; Peterson *et al.*, 1974); goats (Baile *et al.*, 1969) and dairy cattle (Chase *et al.*, 1976). The dairy cattle were within the same median body weight ranges as the ponies. They consumed similar amounts of feed (6.03 kg per day) in equivalent meal sizes (.41 kg). The average meal duration of dairy cattle (20 min) was, however, one half that of the ponies, but the authors did not include the time spent in rumination, which might logically be included as "eating" time. The smaller sheep and goats (Baile *et al.*, 1969; Peterson *et al.*, 1974) had lower daily intakes (approx. 1.5 kg) and smaller meal sizes (.11 kg). All four species, however, consumed nine to 12 meals in 24 hour. They devoted between 413 min (sheep, Peterson *et al.*, 1974) to 537 min (ponies, this paper) to daily eating activities. All ate a proportion of their total intakes as isolated nibbling bouts (14% sheep; 16% ponies). There was no signifi-

cant correlation between meal size and pre- and post-meal intervals [such as described for the rat (Le Magnen and Tallon, 1966; Thomas and Mayer, 1968)] in any of the domestic herbivores studied. Doubt has recently been expressed as to the validity of this measure (Panksepp, 1976) in the estimation of the controls operant in the animals' feeding patterns.

Both sheep and cattle (Baile, 1975; Chase *et al.*, 1976; Peterson *et al.*, 1974) showed distinct diurnal variations in their feeding activities. Both ruminants and ponies significantly reduced their feeding activities between 0100 and 0600 hr (Gary *et al.*, 1970; Baile, 1975; Chase *et al.*, 1976; Peterson *et al.*, 1974). One might assume the time between meals at night would reflect the upper physiological levels of "hunger" the animals will tolerate before seeking food again. The intermeal intervals during the day period for the ponies appeared to be more closely governed by exogenous cues such as the presentation of fresh feed or feeding by neighboring animals.

Experiment II. Blood glucose, free fatty acids and insulin have all been implicated as potential factors in the control of feed intake of nonruminants (Oomura, 1976). The results of this experiment show significant and relatively rapid changes in plasma glucose and IRI that were correlated with the "normal" states of hunger and satiety. The negative correlation ($P < .01$) of the "normal hunger" plasma glucose and subsequent meal size and rate of eating after a 3-hr fast suggests a possible relationship in the determination of hunger in the ponies. The ponies were, however, maintaining their glucose and IRI levels within a finite range, as evidenced by the lack of significant differences between the two satiated levels of glucose and IRI. The lack of correlation of blood glucose levels with the previous meal size supports this theory. There were no significant correlations between the corresponding levels of glucose, IRI or FFA. Whether the animals ate to replete an absolute deficit or ceased eating when a set level of glucose and (or) IRI was attained, is still an open question. The extreme individual variation in IRI complicates the analysis of its potential role.

Further experimentation is needed to confirm the role (or absence thereof) of blood glucose, insulin and free fatty acids in the determination of the onset and cessation of feeding in ponies. Ruminants' postulated

controllers of feed intake (volatile fatty acids; Baile, 1975) also remain to be investigated as potential determinants of pony feeding behavior.

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