

Plasma Glucose and Insulin Responses of Thoroughbred Mares Fed a Meal High in Starch and Sugar or Fat and Fiber

ABSTRACT: Plasma concentrations of glucose and insulin following a meal were compared in twelve Thoroughbred mares fed a pelleted concentrate (PC), a traditional sweet feed high in sugar and starch (SS), or a feed high in fat and fiber (FF). The feeds had similar DE and CP, but differed in fat (19, 32, and 166 g/kg DM, respectively) and NDF (199, 185, and 369 g/kg DM, respectively). Mares were paired by foaling date and weight then randomly assigned into two groups. All mares received PC in late gestation; then, after foaling, one group was fed SS, the other FF for trials in early and late lactation. For each experiment, mares were placed in stalls and deprived of feed overnight. A series of blood samples was collected via a jugular catheter from 0 to 390 min after consumption of 1.82 kg of feed. Plasma was analyzed for glucose and insulin. Baseline values, peak values and areas under curves (AUC) were compared by ANOVA. Baseline values were 74.7 ± 10.9 mg/dl for glucose and 5.86 ± 1.80 mIU/L for insulin for all diets and stages. Responses to PC did not differ between the two groups, indicating the groups were metabolically similar so there is no need for a co-variate. Peak plasma glucose ($P < 0.001$) and insulin ($P < 0.001$) concentrations were higher in SS group than in FF during both early and late lactation. Glucose ($P < 0.002$) and insulin ($P < 0.003$) AUCs were higher in SS than in FF during both early and late lactation. These results indicate that metabolic fluctuations are moderated by the replacement of sugar and starch with fat and fiber. This replacement may reduce the risk of certain digestive and metabolic disorders that have been linked to feeding meals of grain-based concentrates.

(Key Words: Horse, Glucose, Insulin, Dietary fat, Dietary fiber)

Introduction

Concentrates are commonly fed as two meals a day to supplement forage (pasture and/or hay) in the diet of horses, especially those with energy and nutrient requirements above maintenance. A meal containing a high percentage of a grain-based concentrate has been associated with digestive, circulatory, metabolic and hormonal changes (Clarke et al., 1990). Abrupt increases or high intakes of soluble carbohydrates may create large fluctuations in the growth curve (Hoffman, 1997) and increase the risk of certain digestive and metabolic disorders (Kronfeld, 1998).

Replacement of starch with fat in the feed has been used to prevent rhabdomyolysis (Kronfeld, 1973; Valentine et al., 1998; MacLeay et al., 1999), to enhance aerobic (Slade et al., 1975) and anaerobic exercise (Oldham et al., 1990), and to reduce excitability (Holland et al., 1996). Our laboratory has been developing horse feeds in which sugar and starch are replaced with fat and fiber (Kronfeld, 1996; Hoffman and Kronfeld, 1999). The present study compares the glycemic and insulinemic responses of typical concentrates and a supplement formulated with sufficient fat to achieve the same energy density of a typical concentrate and sufficient fiber to allow its use as a complete feed.

Materials and Methods

Thirty-six meal feeding experiments were conducted, 3 on each of 12 Thoroughbred mares, aged 5 to 18 yr. The protocol was approved by the institutional animal care and use committee and performed at the Virginia Tech Middleburg Agriculture Research and Extension Center.

The mares were maintained on adjacent bluegrass/white clover pastures with free access to alfalfa/orchard grass hay. They were paired by weight and foaling date, then randomly assigned to two groups as of January. For three months prior to foaling the pregnant mares were fed a pelleted concentrate (PC, PurePride-200, Purina Mills, St Louis, MO). The mares were introduced to the experimental feeds after foaling; one group received a control diet (SS) which was formulated to closely resemble a textured sweet

feed high in sugar and starch, the other group received a test feed high in fat and fiber (FF). The feeds were formulated (Table 1) to be isocaloric and isonitrogenous, and their mineral and vitamin contents were balanced with the pastures to meet or exceed recommendations (NRC, 1989). Each horse received two meals daily, a total of 3.5 to 4.0 kg, which was one-half to one-third of recommended energy requirements (NRC, 1989). Samples of the three supplements were submitted to Dairy One (DHI Forage Testing Laboratory, Ithaca, NY) for proximate and mineral analysis (Table 2). The FF feed was higher in fat ($P < 0.001$) and NDF ($P < 0.001$), but lower in NSC ($P = 0.010$) than SS and PC.

Trials were conducted in April (3-6 weeks before foaling), June (3-6 weeks after foaling), and August (10-14 weeks after foaling). Each mare was weighed on an electronic scale (Tyrel platform, TC-105, Alweights Hamilton Scale Corp, Richmond, VA) then placed in a stall overnight (12 to 18 h) with free access to water but no feed. A series of blood samples (30 ml drawn into Vacutainer tubes containing sodium heparin, Becton Dickinson and Company, Franklin Lakes, NJ) was collected via a jugular catheter at 0, 30, 60, 90, 150, 210, 270, 330, and 390 min after a meal of 1.82 kg of respective feed. The first sample was obtained immediately before the feed was given. The SS meals were consumed in 20 to 30 min, the FF in 30 to 40 min. Blood samples were immediately centrifuged at 1600 g for 10 min and plasma was removed and stored at -20°C until analysis. Plasma glucose concentrations were determined by glucose oxidase method using a chemical autoanalyser (Kit # 442640, Beckman Synchron CX5CE, Brea, CA). Plasma insulin was determined by radioimmunoassay (Coat-A-Count Insulin, kit # TKINX, Diagnostic Products Corporation, Las Angeles, CA). Areas under the concentration-time curve (AUC) were calculated as summed trapezoids.

The effects of horse, diet and stage of the reproductive cycle were evaluated by ANOVA (SAS, 1996). In the general linear models procedures, the degrees of freedom were insufficient to run a full model with all interactions. Without interactions, the main effects of horse had P values of 0.28 to 0.72, and the main effects of stage had P values of 0.20 to 0.76. Because feed, stage and their interaction were of primary interest, a mixed model was used with horse as a random effect. Means were compared by the Tukey test with $P < 0.05$.

Results

The horses weighed 669 ± 193 kg in April, 583 ± 168 kg in June, and 575 ± 173 kg in August. All the horses remained in good health throughout the observational period. No differences were found between the feeds (PC, SS or FF) and stages (late gestation, and early and late lactation) in baseline data for plasma glucose ($P = 0.36$) and insulin ($P = 0.15$); mean concentrations were 74.7 ± 10.9 mg/dL and 5.86 ± 1.80 mIU/L, respectively.

The glucose and insulin curves for the combined PC data of twelve horses are illustrated in Figure 1. The glucose and insulin AUC were the same between experimental groups of horses receiving PC ($P = 0.482$, and $P = 0.339$, respectively), which eliminated the need for using these data as co-variates in later experiments. Peaks of plasma glucose and insulin were the highest values, usually observed in the 90-min samples, although mean values were not significantly different from 60 to 150 min.

The effects of feed and stage on peak glucose and glucose AUC for mares fed the different diets are shown in Figure 2a and 2b, respectively. Glucose response for PC and SS were similar, except that peak glucose was numerically lower following the SS meal during late lactation than after the PC meal. Glucose peak and AUC were lower following FF than after SS or PC ($P < 0.001$, and $P < 0.002$, respectively) at all stages.

The effects of feed and stage on peak insulin and AUC are shown in Figure 3a and 3b, respectively. Insulin response for PC and SS were similar. Insulin peak and AUC were lower in FF than SS or PC ($P < 0.001$, and $P < 0.003$, respectively) at all stages.

Comparison of responses to feeds during early and late lactation revealed the same pattern of glucose and insulin responses through all the sets of data (Figures 2a and 2b, 3a and 3b). There were consistent decreases in glucose peak and AUC for the SS group, compared to increases that were less marked in the FF group. The corresponding P values for the feed*stage interaction were 0.20 and 0.17 for glucose peak and AUC, respectively. The slight changes in opposite directions were evident for insulin peak and AUC, but the interactions had P values of 0.26 and 0.38, respectively.

Discussion

Procedurally, these experiments resemble glucose tolerance tests, which give information about the glucose status of the animal, and glycemic indices, which give information about the glucose-equivalents in the diet (Roberts and Hill, 1973; Jenkins et al., 1981). The present glycemic and insulinemic responses are intended to give information on isocaloric meals with differing contents of glucose-equivalents, following previous studies in horses (Stull and Rodiek, 1988; Ralston, 1992; Pagan et al., 1999). In the present experiments, replacement of starch and sugar with fat and fiber moderated the postprandial glycemic and insulinemic effects of a meal fed to pregnant and lactating mares.

Changes in plasma glucose and insulin are two of the primary features of the glucose-fatty acid cycle, which is associated with meal feeding (Randle, 1998). Not measured here were other variables in the feeding-fasting cycle, such as free fatty acids, triglycerides, cortisol, thyroid hormones, growth hormone and insulin-like growth factors (Kronfeld, 1998). Daily changes in insulin and counter-regulatory hormones may contribute to the development of certain metabolic disorders (Glade and Reimers, 1985; Clarke et al., 1990; Ralston, 1996). Our results suggest that the metabolic and health impacts are likely to be similar for our experimental SS and commercial feeds, such as PC, but moderated for meals of FF.

Interpretation of these experiments is complicated in two regards. One involves the possible interaction of the feed and reproductive stage. The other concerns the relative importance of decreasing glucose-equivalents and increasing fat or fiber on the results.

A single pattern of lower glucose and insulin responses between SS and FF was seen in all trials. The slight decreases from early to late lactation in the peak glucose and insulin, and the AUCs of glucose and insulin in the SS groups were accompanied by slight increases in these variables in the FF group. Early lactation experiments in the present study encompass the first two months after foaling when the milk yield of a mare is at its peak, approximately 11.8 kg/d or 2.3 % of body weight (Gibbs et al., 1982). In contrast, tests in late lactation were performed within the fifth or sixth month period after foaling

when the milk yield has decreased to 9.8 kg/d or 1.9 % body weight. The possibility of a feed and stage trend is likely to represent an overall decrease in glucose removal from blood later in lactation, when the rate of glucose removal from blood specifically for milk lactose synthesis and volume production is decreased (Evans, 1971). This repeated and consistent tendency for an interaction between feed and stage invites further investigation, especially because changes of plasma concentrations of glucose and insulin, as well as changes in tracer glucose kinetics, have been associated previously with changes in reproductive stage (Evans, 1971; Fowden et al., 1984).

Either lowering the glucose-equivalents in the feed or increasing the fat or fiber contents could contribute to changes in the glycemic responses to a meal. Higher fat in the diet may delay or decrease the peak glycemic response by retarding gastric emptying in human subjects (Wolever, 1990). However, this delay should not affect the AUC. The effect of a high fat meal on gastric emptying has not been studied in horses to our knowledge. The glucose-equivalents would be approximated by NSC (Table 2), which amounts to 1,045, 1,035 and 339 g in the meals of PC, SS and FF, respectively. This 3-fold difference in NSC intake may be compared to the 2.5-fold difference between the SS and FF feeds in glucose AUC early lactation and the 1.6-fold difference during late lactation.

The increase in plasma insulin concentration reflects an increased rate of insulin release into the blood in response to glucose absorption and hyperglycemia (Mayes, 1996). The subsequent decline probably represents a moderation in the rate of insulin release and an increase rate of insulin removal from blood. The differences in insulin AUC between SS and FF groups were much greater (12-fold and 6-fold in early and late lactation, respectively) than corresponding differences in glucose AUC (154 % and 62 %, respectively). This finding suggests that the amount of glucose absorption, thus the change in glucose concentration in the plasma, had a greater impact than a certain level of plasma glucose concentration per se on the magnitude of the insulin response.

Several studies have compared the effects of a corn grain meal to those of corn fortified with 10 % corn oil. In horses fed a meal of alfalfa hay or corn fortified with 10 % corn oil they produced plasma glucose peaks of 103 or 116 mg/dL, respectively (Stull and

Rodiek, 1988). For meals of corn only or corn and alfalfa (50:50) the glycemic response reached a level of about 142 mg/dL for both meals. Another study used six meals fed to horses at maintenance and found smaller differences in peak glucose concentrations, which ranged from 99 mg/dL for alfalfa hay to 108 mg/dL for a sweet feed (Pagan et al., 1999). These studies have found various glucose responses ranging from slight variation between different diets, to a large range of concentration peaks.

Compared to previous studies, the magnitude of responses in our experiments was similar for glucose (Stull and Rodiek, 1988; Pagan et al., 1999), but tended to be lower for insulin, especially following a meal of FF (Figure 3). Insulin AUCs were about 1,700 min-mIU/L following an alfalfa meal, compared to 5,000 to 9,200 min-mIU/L for grain mixes (Stull and Rodiek, 1988).

The higher fiber content in the FF most likely caused a higher production of acetate, propionate and butyrate in the gut. These can control plasma glucose concentration by acting as a precursor (propionate) or by sparing glucose oxidation (mainly acetate and betahydroxybutyrate). Such effects are likely to be smaller than that of direct glucose absorption, and thereby explain the results of several studies that evaluated the effects of feeding hay and other roughages. Peak insulin concentrations were 29 and 49 mIU/L following meals of corn (7-fold increase from baseline), and corn and alfalfa (12-fold increase), respectively (Stull and Rodiek, 1988). In contrast, plasma insulin increased only 1.6 times following an alfalfa meal. In our study, peak insulin concentrations were 8.7 times baseline for PC and SS compared to 1.7 times following a FF meal. Thus, the FF feed, which has a similar energy density of a typical concentrate, has the insulinogenic effect of a roughage, alfalfa hay.

In practical human dietetics, the glycemic index (GI) is used to design meals for the feeding of athletes before and after an event (Burke et al., 1998) as well as diets for the management of non-insulin dependant diabetes mellitus (Brand-Miller and Foster-Powell, 1999). On this basis, foods are classified as low-GI or high-GI. Low-GI meals may be used to reduce the risk of diabetes, along with coronary heart disease and obesity. Consuming a high-GI meal may exaggerate the insulin and glucose responses, which could exacerbate certain metabolic problems. In regard to exercise, high-GI meals consumed

after exercise may increase glycogen storage. In contrast, low-GI meals consumed before endurance exercise may prolong glucose availability and enhance performance (Burke et al., 1998).

Similarly, in equine nutrition, the circulatory, digestive, metabolic and hormonal responses to a meal (Clarke et al., 1990; Kronfeld, 1998), including the glycemic and insulinemic responses, may be applied to feeding management during and after an athletic event (Stull and Rodiek, 1995) and reducing the risks of gastric ulcers (Murray, 1999), colic (Cohen et al., 1999), laminitis, osteochondrosis (Ralston et al., 1996) and certain types of rhabdomyolysis (Kronfeld, 1973; Valentine et al., 1998; MacLeay et al., 1999).

Implications

Horse feeds containing abundant starch from grain and sugar from molasses, such as typical pelleted or textured concentrates, have profound metabolic and hormonal effects that may be reduced or eliminated by the replacement of glucose-equivalents with fat and fiber. This exchange of nutrients may be useful in the feeding management of horses performing intense work, and in reducing the risks of certain digestive and metabolic disorders. However, the difference between the use of these energy sources in mares during different reproductive stages needs to be explored in more detail.

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

Ingredient	SS	FF
Dent yellow grain corn	60	11
Soybean meal 48 %	15.5	2
Oat straw	7	7
Alfalfa	0	13.5
Soybean hulls	4	4
Beet pulp	0	10
Cereal grain by-product	0	45
Molasses (cane)	10	5
Dicalcium phosphate	1.5	0.5
Limestone	1	1
Mineral premix ^a	0.5	0.5
Vitamin premix ^b	0.5	0.5

^aThe mineral premix provided the following per kilogram of supplement: ; NaCl, 3,774 g; Zn, 422 g; Fe, 208 g; Cu, 89.5 g; Mn, 50.3 g; Se, 1.095 g; and KI, .415 g.

^bThe vitamin premix provided the following per kilogram of a supplement: vitamin A, 1,380,080 IU; vitamin D₃, 258,000 IU; vitamin E, 26,455 IU; riboflavin, 701mg; niacin, 3009 mg; folic acid, 66 mg; thiamin, 1400 mg; biotin, 42 mg; and β -carotene, 3527 mg.

Table 2. Nutrient composition of the sugar and starch (SS), fat and fiber (FF) and the pelleted concentrate (PC) feed as analyzed in the DHI Forage Testing Laboratory (Ithaca, NY). Data are summarized on a DM basis as means \pm SE

Nutrient	SS (n = 12)	FF (n = 12)	PC (n = 3)
DM, %	90.2 \pm 0.67	92.2 \pm 0.36	92.7 \pm 0.42
DE Mcal/kg ^c	3.34 \pm 0.05	3.50 \pm 0.05	3.51 \pm 0.09
CP, %	13.7 \pm 0.59	15.4 \pm 0.22	17.8 \pm 0.98
ADF, %	10.7 \pm 1.02	22.6 \pm 0.74	11.4 \pm 0.3
NDF, %	18.5 \pm 1.11	36.9 \pm .99	19.9 \pm 2.1
Fat, %	3.2 \pm 0.28	16.6 \pm 0.76	1.9 \pm 0.2
NSC, % ^b	64.5 \pm 3.61	24.7 \pm 2.02	57.4 \pm 4.32
Ash, % ^a	6.70 \pm 0.64	9.74 \pm 0.24	6.0 \pm 0.1
Ca, %	1.15 \pm 0.14	1.37 \pm 0.19	0.76 \pm 0.05
P, %	0.60 \pm 0.05	1.35 \pm 0.06	0.73 \pm 0.02
Mg, %	0.19 \pm 0.01	0.67 \pm 0.03	0.33 \pm 0.02
K, %	1.07 \pm 0.06	1.28 \pm 0.02	0.74 \pm 0.19
Na, %	0.25 \pm 0.03	0.22 \pm 0.02	0.002 \pm .0003
S, %	0.22 \pm 0.01	0.21 \pm 0.01	0.002 \pm .0001

^aAsh = 9.425 * K + 1.442 * P - 4.231 \pm 0.867; adj r² = 0.992, P = 0.004

^bNonstructural carbohydrate; NSC = 100 - (water + CP + Fat + Ash + NDF)

^cDE = 3.6 * NSC + 1.4 * NDF + 8 * Fat + 3.6 * CP / (NSC + NDF + Fat + CP)

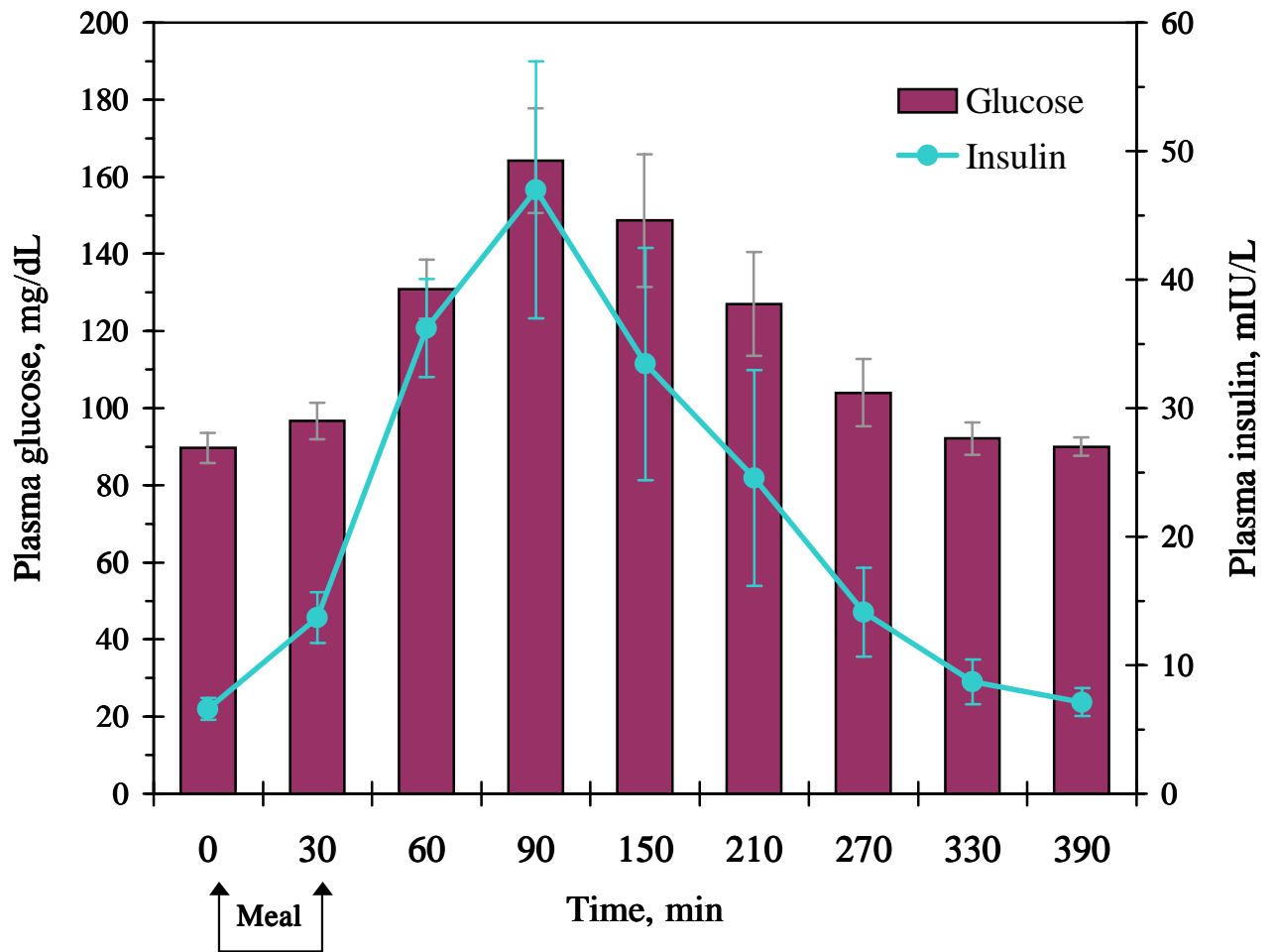


Figure 1. Mean concentration of the plasma glucose (bars) and insulin (solid line) for twelve mares fed the pelleted concentrate (PC) during late gestation. The two groups of mares were combined to illustrate of the glucose and insulin curves

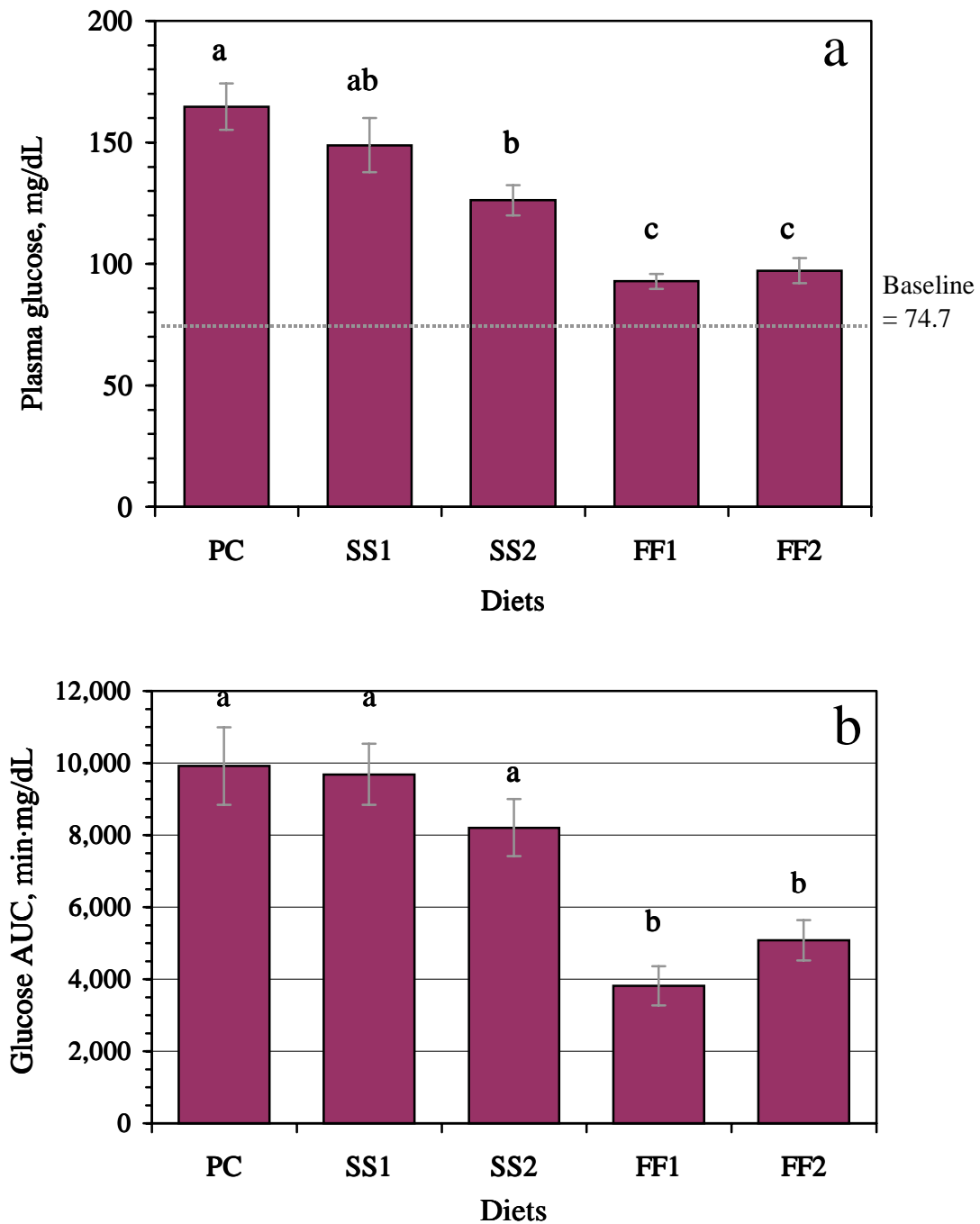


Figure 2. Peak plasma glucose (A) and glucose area under the curve (B) in mares fed the pelleted concentrate (PC), a feed high in sugar and starch (SS) and feed high in fat and fiber (FF) during early lactation (1) and late lactation (2). Groups without a common superscript differ by $P < 0.05$

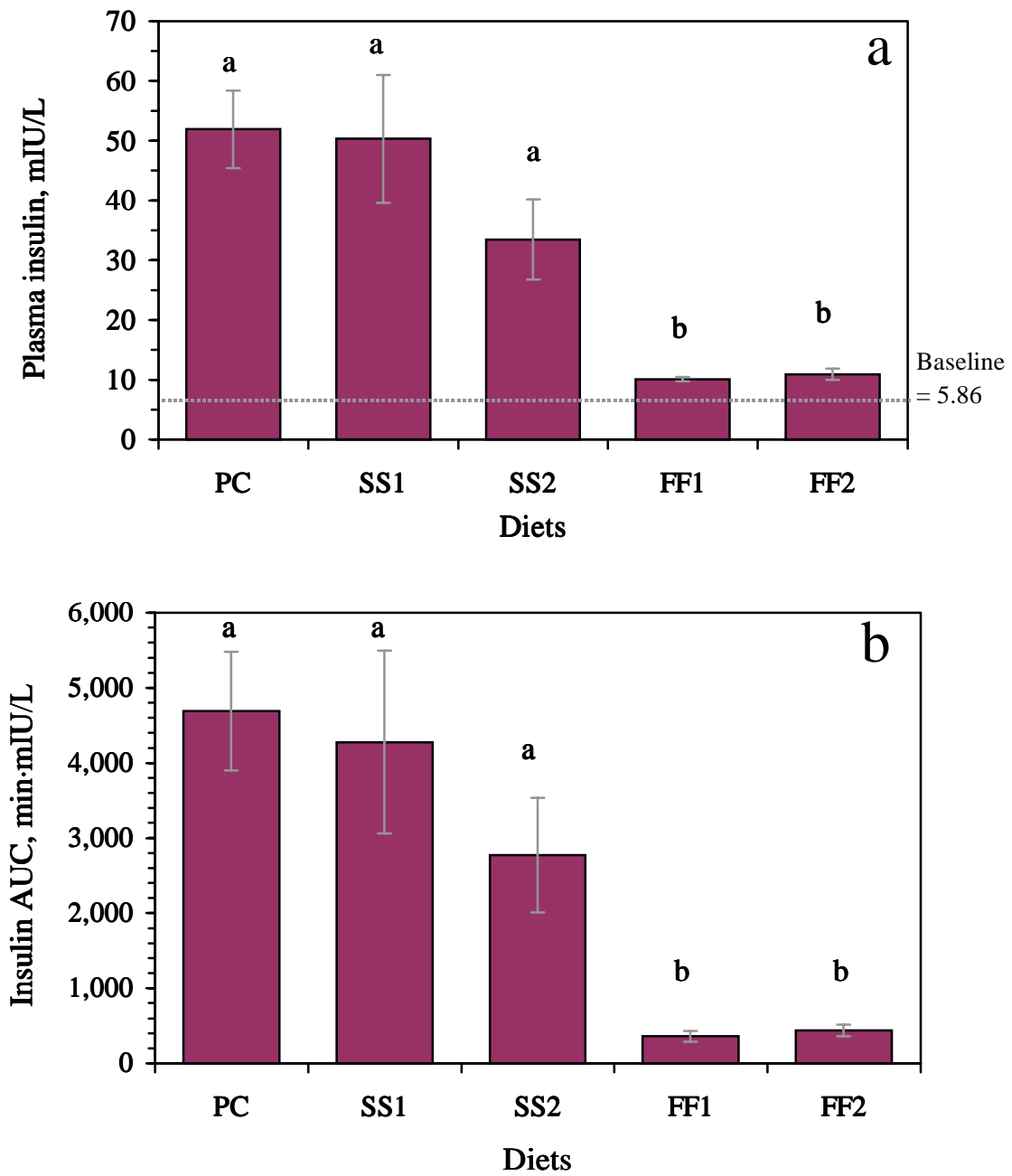


Figure 3. Peak plasma insulin (A) and area under the curve (B) for insulin in mares fed the pelleted concentrate (PC), a feed high in sugar and starch (SS) and a feed high in fat and fiber (FF) during early lactation (1) and late lactation (2). Groups without a common superscript differ by $P < 0.05$