Effect of Soluble Carbohydrate Content of Pelleted Diets on Postprandial Glucose and Insulin Profiles in Horses

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Key words: Horse, soluble carbohydrate, glucose, insulinx

Introduction

Hyperglycemia and hyperinsulinemia are associated with an increased risk of laminitis, especially in ponies (*Freestone* et *al.*, 1992; *Jeffcott* et *al.*, 1986; *Yelle*, 1986). In ponies pelleted grain-based rations cause significant postprandial increases in blood glucose and insulin (*Argenzio* and *Hintz*, 1972; *Ralston* et *al.*, 1979) relative to diets with lower soluble carbohydrate (SCHO) content such as grass hay. High grain diets also are considered to increase behavioral reactivity in horses (*McClure* et *al.*, 1992), which, based on data from other species, may be caused by metabolic effects of elevated blood glucose and insulin (*Fernstrom* and *Wurtman*, 1971).

Despite the common use of pelleted or textured concentrates in horse rations, relatively little is known regarding the metabolic effects of such rations in this species (*Glade*, 1987). Dietary concentration of SCHO may be correlated with the glycemic potential of horse feeds, however field trials in horses fed high SCHO pelleted rations failed to show expected glycemic responses (*Ralston*, 1990, unpublished data). Processing techniques and composition of grain-based foods can alter glycemic responses in humans irrespective of SCHO content (*Wolever* et *al.*, 1991). Previous diet, breed and clinical syndromes such as obesity and pituitary adenomas also significantly affect glucose and

Summary

Hyperglycemia and hyperinsulinemia have been associated with increased risk of laminitis in ponies and, in other species, changes in behavioral reactivity. Postprandial glucose and insulin are significantly increased in ponies fed pelleted or textured concentrates relative to rations containing lower levels of soluble carbohydrate (SCHO) such as hay. Metabolic responses may differ, however, between ponies and horses and glycemic indices of horse feeds have not been well defined. This study was designed to test the hypothesis that increased SCHO in pelleted feed would increase postprandial glucose and insulin in mature horses. Seven mares were assigned to one of two treatment groups in a replicated crossover design. 50 % of the calories in the total daily rations were provided by grass hay and 50 % by one of two pelleted rations: Pellet 1: 30 % of the calories from SCHO; Pellet 2: 80 % of the calories from SCHO. Protein and mineral concentrations of the diets were comparable (± 10 %). At the end of each four week trial, blood for insulin and glucose analysis was drawn immediately before feeding 1/2 the total daily pellets and at 30 min intervals for four hours after feeding. Two mares were hyperglycemic and hyperinsulinemic regardless of treatment relative to the other mares ($P < .01$) and were excluded from the final analysis of the data. Glucose concentrations did not differ ($P > .1$) between treatment groups at any time nor did the area under the glucose curve.

Insulin concentrations were higher in mares fed pellet 2 at 180 minutes post feeding (pellet 1: 88 ± 12 pmole/l vs pellet 2: 130 ± 16 pmole/l, $P = .050$) and the area under the insulin curve was higher ($P = .054$) for mares fed the high soluble carbohydrate diet. Glucose/insulin ratios were lower ($P < .05$) in mares adapted to the high SCHO diet at $t = 180$ and 210 min post feeding than when fed pellet 1. The differences observed, however, were not as pronounced as expected. Availability of SCHO from pelleted feeds and/or differences in glucose/insulin metabolism between horses may account for the lack of expected differences. Glycemic indices of horse feeds and invidiual variation in response to SCHO content of feeds warrant further investigation.

Einfluß des Gehaltes an löslichen Kohlenhydraten in pelletierten Rationen auf den postprandialen Glucose- und Insulinspiegel von Pferden

Hyperglycämie und Hyperinsulinämie sind mit erhöhtem Risiko für Reheerkrankungen bei Ponies in Zusammenhang gebracht worden, bei anderen Species dagegen mit Veränderungen der Regulation der Nahrungsaufnahme. Ponies, die pelletiertes Kraftfutter erhielten, wiesen postprandial höhere Glucose- und Insulinspiegel auf als Tiere mit Heufütterung. Zwischen Ponies und Pferden könnten aber Unterschiede im Kohlenhydratstoffwechsel bestehen. Der Effekt von Futtermitteln auf den Blutzuckerspiegel bei Pferden ist bisher kaum überprüft. In der vorliegenden Untersuchung sollte daher überprüft werden, ob bei erwachsenen Pferden erhöhte Gehalte an löslichen Kohlenhydraten in Kraftfuttermitteln postprandial zum Anstieg des Blutglucose- und Insulinspiegels führen. 7 Stuten wurden in 2 Fütterungsgruppen (Cross-over-Verfahren) eingeteilt. Die Hälfte der Energie wurde aus Wiesenheu bereitgestellt, die andere Hälfte jeweils aus pelletiertem Kraftfutter, wobei bei einer Gruppe 80 Prozent, bei der anderen 30 Prozent der Energie im Kraftfutter aus löslichen Kohlenhydraten stammten. Der Eiweiß- und Mineralstoffgehalt war vergleichbar (± 10 Prozent). Nach einer jeweils 4wöchigen Fütterungsperiode wurden unmittelbar vor sowie 4 Stunden nach der Fütterung der halben Tagesration halbstündlich Blutproben gezogen. 2 Stuten zeigten unabhängig von der Fütterung Hyperglycämie und Hyperinsulinämie verglichen mit den anderen Tieren ($p < 0{,}01$). Diese beiden Stuten wurden daher nicht in die Auswertung einbezogen. Der Blutglucosegehalt zeigte zu keinem Zeitpunkt fütterungsbedingte Unterschiede, ebensowenig wie die Fläche unter der postprandialen Verlaufskurve. Der Insulingehalt war bei Stuten, die das Kraftfutter mit nur 30 Prozent löslichen Kohlenhydraten erhielten, 180 Minuten nach der Fütterung höher (88 ± 12 pmol/l im Vergleich zu 130 ± 16 pmol/l, $p = 0{,}050$), die Fläche unter der Insulinkurve war dagegen in der Gruppe mit dem Kraftfutter mit hohem Gehalt an löslichen Kohlenhydraten größer ($p = 0{,}054$). Das Glucose-Insulin-Verhältnis war bei der Ration mit hohem Gehalt an löslichen Kohlenhydraten 180 und 210 Minuten postprandial kleiner ($p < 0{,}05$) als in der anderen Gruppe. Die Unterschiede waren jedoch weit weniger ausgeprägt als erwartet. Dieser Befund könnte mit einer geringen Verfügbarkeit der Kohlenhydrate aus dem pelletierten Kraftfutter und/ oder mit individueller Variation im Glucose-Insulin-Stoffwechsel zwischen den Pferden zusammenhängen. Zur Abklärung dieser Fragen sind weitere Untersuchungen erforderlich.

insulin metabolism in equine animals (*Argenzio* and *Hintz*, 1972; *Freestone* et *al.*, 1992; *Garcia* and *Beech*, 1986; *Jacobs* and *Bolton*, 1982; *Jeffcott* et *al.*, 1986; *Ralston* et *al.*, 1988). Previous reports of glycemic responses of ponies or horses to diet or glucose challenge have compared grain-based concentrates to predominantly hay rations (*Argenzio* and *Hintz*, 1972; *Garcia* and *Beech*, 1986; *Jacobs* and *Bolton*, 1982; *Mehring* and *Tyznik*, 1970). No studies of similar diets which differ primarily in SCHO fed to horses were found. This study was designed as a preliminary investigation in an extensive program to determine the metabolic and behavioral effects of high SCHO diets in horses. The purpose was to document alterations in postprandial plasma glucose and insulin profiles in mature horses fed pelleted feeds with differing SCHO content.

Materials and Methods

Six mature Standardbred mares and 1 Anglo Arabian mare in similar body condition (body condition score = 7.0 to 7.5 on a scale of 1–9, *Henneke* et *al.*, 1983) were used. The mares were maintained in stalls, with 6 hours of turnout on drylot daily throughout the study (January – May, 1991). Routine anthelmintic, vaccination and hoof trimming schedules were observed. All mares had their teeth checked and had rough points filed off if necessary before initiation of the trials. Mares were paired according to body weight and assigned to one of two treatment groups. 50 % of the total calories estimated to be required by each mare (see below) were provided by one of two pelleted rations containing different levels of SCHO: Pellet 1[2]: 30 % of the total calories (2.2 kcal/kg feed as fed) from SCHO; Pellet 2[3]: 80 % of the total calorie (2.86 kcal/kg feed as fed) from SCHO. Caloric content of the pellets had been determined previously by feeding trials (*Breuer*, 1990, Personal communication). The SCHO caloric contribution was estimated based on nitrogen free extract calculations from proximate analyses of the pellets. Measured amounts of a single source of timothy grass hay provided the rest of the caloric requirement for each mare. Caloric content was estimated from proximate analysis of the hay (Triple S Laboratories, Loveland, Colorado and NRC, 1989). Protein and mineral concentrations of the diets were comparable (± 10 %). Caloric requirements for each individual were calculated based on National Research Council (*NRC*, 1989) recommendations for maintenance plus a 10 % increase to compensate for cold weather and possible overestimation of the caloric content of the hay (*NRC*, 1989). The rations were fed individually in two equal feedings at 08.00 h and 16.00 h and orts were recorded daily. Water and trace mineralized salt were available free choice and mares were weighed weekly throughout the study.

Dietary treatments were tested in a 2 × 2 replicated cross-over design with a four week adaptation period preceding each test. To reduce excitement during repeated blood sampling, a jugular catheter was inserted in each mare and infused with 0.9 % heparin saline 12 to 16 hrs before testing (*Roberts* and *Norman*, 1979). At 08.00 h of each test day, blood for insulin and glucose analysis was drawn immediately before feeding 1/2 the total daily ration of pellets (2.73 – 3.64 kg, depending on body weight and treatment group) and at 30 min intervals for four hours after feeding. Hay was not fed during this period. Blood was placed immediately in tubes treated with sodium fluoride[4]. Tubes were centrifuged and the plasma drawn off within 30 minutes of collection. Samples were stored frozen pending analysis. Plasma glucose was determined by glucose oxidase assay[5] and plasma insulin was analyzed by a radioimmunoassay kit[6] validated for horses (*Freestone* et *al.*, 1991).

Data were analyzed using GLM-ANOVA to factor the effects of treatment, individual, trial and time on plasma glucose and insulin at each time point and the areas under the curves. Student's T-test was used to compare treatment means at each sampling time if trial and individual were determined not to contribute significantly (p > .10) to variability.

Table 1: Glucose and insulin profiles after feeding isocaloric amounts of high vs low SCHO pellets to 2 mares with abnormal responses. Values are means ± (SE) for two trials for each mare on each diet.

Time post feeding (min)	Glucose (mmole/l)		Insulin (pmole/l)	
	Low SCHO Pellet 1	High SCHO Pellet 2	Low SCHO Pellet 1	High SCHO Pellet 2
0	3.60 (.07)	3.40 (.19)	38 (3)	36 (4)
30	4.71 (.26)	4.08 (.26)	150 (17)	145 (26)
60	5.88 (.58)	4.37 (.36)	292 (32)	252 (43)
90	6.50 (.34)	5.27* (.38)	425 (51)	323 (54)
120	6.32 (.73)	5.55 (.35)	474 (82)	384 (70)
150	5.72 (.94)	5.65 (.53)	510 (110)	446 (77)
180	6.14 (.93)	5.19 (.63)	523 (123)	488 (114)
210	6.10 (.40)	4.89* (.42)	575 (166)	492* (120)
240	6.18 (.92)	5.08 (.36)	600 (265)	516 (120)

* Values within time period differ (P>.05, Student's T-test) between treatments

[2] Purina Horse Chow 100, Purina Mills Inc., St. Louis, Mo.
[3] Purina Pure Pride 100, Purina Mills Inc., St. Louis, Mo.
[4] Vacutainer, Becton Dickinson, Inc, Rutherford, NJ.
[5] Kit 510-A, Sigma Chemical Co., St. Louis, Mo.
[6] Coat-A-Count, Diagnostic Products, Los Angeles, Ca.

Results

The mares maintained body weight (± 10 kg) throughout the experimental period, justifying the addition of 10 % above the *NRC* (1989) caloric recommendations for maintenance. Two mares were hyperglycemic and hyperinsulinemic regardless of diet relative to the other mares (tables 1 and 2) and were excluded from T-test analyses of the data. Glucose/insulin ratios were lower ($p < .05$) in mares adapted to the high SCHO diet at t = 180 and 210 min after feeding than when fed pellet 1 (table 2). Postprandial plasma glucose and insulin responses were lower and had fewer differences between treatments than had been expected based on the literature on ponies fed commercial pelleted feed (table 3) (*Argenzio* and *Hintz*, 1972; *Freestone* et *al.*, 1992; *Ralston* et *al.*, 1979). Glucose concentrations did not differ ($P > .1$) between treatment groups at any time point nor did the area under the glucose curve. Insulin concentrations were higher ($P = .05$) in mares fed pellet 2 at 180 minutes post feeding (table 3) and the area under the insulin curve was higher ($P = .054$) for mares fed the high soluble carbohydrate diet (pellet 1: 432 ± 76 pmole/l vs pellet 2: 696 ± 103 pmole/l). Insulin variances were greater ($P < .05$) in mares fed pellet 2 than when fed pellet 1 at T = 60 to 150 min postfeeding.

Table 2: Glucose/insulin ratios after feeding isocaloric amounts of high vs low soluble carbohydrate pellets to mature mares with normal (n = 5) and abnormal (n = 2) glycemic responses. Values are means ± (SE) for 5 horses.

Time post feeding (min)	Normal mares		Abnormal mares	
	Low SCHO Pellet 1	High SCHO Pellet 2	Low SCHO Pellet 1	High SCHO Pellet 2
0	1.38 (.14)	1.43 (.20)	0.96 (.08)	0.97 (.07)
30	0.89 (.12)	0.85 (.13)	0.32 (.03)	0.30 (.04)
60	0.55 (.06)	0.47 (.06)	0.20** (.01)	0.18** (.03)
90	0.57 (.08)	0.44 (.07)	0.16** (.01)	0.18** (.02)
120	0.60 (.06)	0.46 (.07)	0.14** (.01)	0.16** (.03)
150	0.64 (.09)	0.44 (.06)	.12** (.02)	0.13** (.02)
180	0.63 (.07)	0.40* (.05)	0.13** (.01)	0.13** (.03)
210	0.62 (.08)	0.41* (.06)	0.12** (.02)	0.13** (.05)
240	0.58 (.08)	0.41 (.06)	0.29 (.18)	0.13** (.04)

* Values within time period differ (P>.05, Student's T-test) between treatments within group

** Values within time period differ between normal and abnormal mares on the same treatment (P<.01)

Table 3: Glucose and insulin profiles after feeding isocaloric amounts of high vs low soluble carbohydrate pellets to mature mares. Values are means ± (SE) for 5 horses fed each diet twice.

Time post feeding (min)	Glucose (mmole/l)		Insulin (pmole/l)	
	Low SCHO Pellet 1	High SCHO Pellet 2	Low SCHO Pellet 1	High SCHO Pellet 2
0	3.54 (.16)	3.49 (.01)	26.8 (1.7)	27.8 (2.9)
30	3.95 (.13)	3.86 (.12)	51.0 (5.8)	54.9 (8.1)
60	4.27 (.10)	4.57 (.23)	83.4 (7.2)	114.6 (17.0)
90	4.50 (.15)	4.68 (.23)	93.7 (13.2)	144.8 (32.4)
120	4.90 (.28)	4.91 (.23)	91.4 (12.5)	147.4 (34.5)
150	4.75 (.23)	4.94 (.40)	90.1 (14.6)	138.4 (26.5)
180	4.92 (.34)	4.60 (.25)	87.8 (12.2)	130.0* (16.5)
210	4.62 (.24)	4.48 (.32)	91.1 (16.6)	127.0 (19.3)
240	4.19 (.18)	4.14 (.15)	89.9 (1.6)	95.7 (9.6)

* Values within time period differ (P>.05, Student's T-test) between treatments

Discussion

The original hypothesis had been that the higher SCHO content of pellet 2 would result in greater glucose absorption and insulin release than pellet 1. Pellet 2 did cause slightly higher ($P = .054$) insulin secretion after feeding than did pellet 1. However the lack of difference ($P > .1$ at all times) in plasma glucose was not expected. Carryover metabolic adaptation to the diets should not have been a factor since differences in glucose metabolism were recorded as little as one week after introduction of a new ration in ponies (*Jacobs* and *Bolton*, 1982). Each mare was tested on each feed twice and individual variation does not account for the lack of expected differences between the diets.
Either the mares fed pellet 2 had increased sensitivity to insulin with higher rates of glucose absorption or SCHO availability in pellet 2 was not as great as anticipated.
In ponies fed pelleted diets, there was an increase of at least 2.2 mmole/l in plasma glucose within 60 to 90 minutes of feeding (*Ralston* et *al.*, 1979). The relatively low increase in blood glucose with both diets in the normal mares (1.38 and 1.45 mmole/l maximal increases at 150 and 180 min after feeding) is unexplained at this time. The amounts fed were comparable to amounts used in previous studies (*Argenzio* and *Hintz*, 1972; *Ralston* et *al.*, 1979). Obesity may reduce glucose tolerance and weight reduction results in lower blood glucose and insulin following standardized

oral glucose challenge (*Freestone* et *al.*, 1992). Ponies used in at least one of the previous studies (*Ralston* et *al.*, 1979, unpublished data) were obese (body condition scores of 8 – 9, *Henneke* et *al.*, 1983). Mares used in the current study were fat but not obese, which may have contributed to the lower than expected postprandial glycemia. Effects of pelleting on SCHO availability or alteration in rate of passage, digestion or absorption may have contributed to the unexpected results of this study.

All of the mares used were over 12 years old with a mean age of 16 yrs, but only two were grossly abnormal in their postprandial glucose and insulin profiles. The two mares (aged 14 and 16 years) with abnormal glucose and insulin profiles were not obese at the time of testing. Pituitary tumor are a common cause of hyperglycemia and hyperinsulinemia in aged horses (*Garcia* and *Beech*, 1986; *Ralston* et *al.*, 1988). Neither mare had gross clinical signs (hirsutism, polyuria, polydypsia, laminitis), but may have been experiencing preclinical metabolic alterations associated with pituitary adenomas (*Ralston* et *al.*, 1988). Why these individuals showed greater glycemic and insulinemic response to the lower SCHO pellet 1 is unknown. One of the mares was lost to follow up, but the other (14 yr old) exhibited progressively worse glucose tolerance and hyperinsulinemia over the course of the next 9 months.

The greater individual variation in response to the high SCHO diet warrants further investigation. Horse owners frequently report individual variation in behavioral responses to sweet feed or concentrated feed (*Ralston*, personal observation) which may be reflected in the variable glycemic/insulinemic responses to the feed. More data are needed on the glycemic indices of horse feed and the metabolic and behavioral responses of horses to these feeds.

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