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Postprandial insulin responses to various feedstuffs differ in insulin dysregulated horses compared to non-insulin dysregulated controls

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Summary

Background: Controlling postprandial hyperinsulinaemia is important in insulin dysregulated (ID) horses to reduce the risk of laminitis.

Objectives: To evaluate postprandial insulin responses of ID vs. non-insulin dysregulated (NID) horses to feedstuffs varying in non-structural carbohydrate (NSC) and crude protein (CP).

Study design: Randomised crossover.

Methods: 18 adult mixed-breed horses (13.3 ± 2.2 years; 621 ± 78.8 kg) were individually fed (~1g/kg BW) specific feedstuffs within two crossover studies. 8ID & 8NID were used in Study A and 11ID & 5 NID in Study B. Study A, all horses were randomly fed once: cracked corn (CC: ~74% NSC & ~9% CP), ration balancer with low protein (RB-LP: ~15%NSC & ~17% CP), ration balancer with high protein (RB-HP: ~14% NSC and ~37%CP), and 50:50 mixture of RB-LP:RB-HP (MIX-P). Study B, horses were randomly fed once: CC, RB-HP, steam-flaked corn (SF: ~73% NSC & ~10%CP), oat groats (OG :~64%NSC & ~14% CP), and a low NSC pellet (L-NSC: ~6%NSC & ~12%CP). Blood was collected for insulin determination (RIA) before and 30, 60, 75, 90, 105, 120, 150, 180, 210, and 240 minutes post-feeding in study A and at 60-minutes in study B. Data were analysed via ANOVA for repeat measures post any required transformations.

Results: ID horses had significantly greater insulin responses (AUCi) than NID for all diets in both studies ($p<0.001$; ID 22,362 ± 10,298 μIU/mL·min & NID 6,145 ± 1,922 μIU/mL·min). No effect of diet on AUCi for NID ($p=0.2$) but in ID the CC (32,000 ± 13,960 μIU/mL·min) AUCi was higher than RB-LP ($p=0.01$; 18,977 ± 6,731 μIU/mL·min). ID insulin (T60) was lower for the L-NSC (57.8 ± 18.5 μIU/mL) vs. all other diets ($p<0.02$; 160.1 ± 91.5 μIU/mL).

Main limitations: Small numbers of horses; no ponies.

Conclusions: NSC appears to be the main driver of the postprandial insulin response. ID horses respond disproportionately to feeding even small amounts of low/moderate NSC feedstuffs. Data on possible dietary thresholds for postprandial insulin responses cannot be extrapolated from NID horses.

Introduction

Equine metabolic syndrome (EMS) describes a collection of risk factors that increase the risk of laminitis[1]. Insulin dysregulation (ID) is the main, consistent component[2] and is a collective term for tissue insulin resistance, basal and postprandial hyperinsulinaemia. Experimental insulin infusion can induce laminitis in healthy ponies and horses[3,4] and ingesting feedstuffs high in non-structural carbohydrates (NSC: starch + water-soluble carbohydrates) can trigger endocrinopathic laminitis[5,6]. Recent work in the ID pony, has suggested that the insulin response to oral sugars may be one of the most important predictors of laminitis risk[5]. Therefore, controlling postprandial hyperinsulinaemia is of crucial importance due to its potential risk of inducing endocrinopathic laminitis.

Current management strategies to reduce the risk of laminitic episodes, especially in horses with ID; therefore, include limiting NSC intake, increasing structured exercise, and when necessary, using pharmaceuticals[2]. Dietary recommendations[7] include feeding hay with less than 10-12% NSC (on a dry matter [DM] basis) often combined with an appropriate ration balancer to provide adequate overall amino acids, vitamin and mineral intakes. The NSC recommendations; however, appear to have been extrapolated from work with polysaccharide storage myopathy horses[8] with limited published work directly involving ID horses. To limit postprandial insulinaemic responses in healthy horses, it has been recommended that complementary feeds should either provide ≤ 1.1 g starch/kg BW or the meal limited to 0.3 kg/100 kg BW [9]. Such recommendations have not been established for the ID animal. Recently, ID horses fed 1.2g protein and 0.5g NSC/kg BW had 9-fold greater insulin responses than healthy, NID horses[10]. The authors suggested that protein (especially high in insulinogenic amino acids) intake therefore should also be considered[10]. Balancer complementary feeds[11], fed in small amounts (e.g. 100g/100 kg BW) are typically rich in protein and essential amino acids.

More information is therefore required with respect to the role of protein and NSC in driving the insulin response in ID horses so that more targeted advice can be provided to help reduce postprandial insulin responses. This may be especially important in the more severely affected individuals, which are often overweight and being fed restricted forage-based diets with a balancer. Therefore, the following study evaluated the effect of feeding small amounts of different feedstuffs varying in NSC and crude protein (CP), to determine if ID horses produce greater postprandial hyperinsulinaemic responses compared to NID horses. Our hypothesis was that ID horses when fed small amounts of moderate to low NSC providing feeds would have similar insulin responses to NID horses but would have higher responses to feeding even small amounts of high NSC cereals.

2. Materials and Methods

Two separate crossover studies were undertaken: Study A during Summer 2017 and Study B during the summer of 2018.

2.1 Horses

Overall, 18 adult horses (mean ± SD: 13.3 ± 2.2 years and 621 ± 78.8 kg) of mixed breeds, including Thoroughbred (n=2), Appaloosa (n=1), Paint (n=2), Thoroughbred cross (n=2), Tennessee Walking Horse (n=1), Warmblood (n=2), Standardbred cross (n=2), and unknown mix breed (n=6) were used. The horse demographics for each study are described in Table 1. One-week prior to the study, body condition (1-9; BCS) and cresty neck score (0-5; CNS) were determined for each horse by 3 personnel experienced in assessing BCS and CNS and the average recorded[12,13]. In addition, bodyweight (BW) was measured with a calibrated portable agriculture scale (model 700, Tru Test Inc, Mineral Wells, Texas, USA). All horses were considered to be non-PPID i.e. no signs of hypertrichosis and non-fall basal ACTHs (chemiluminescence immunoassay [Immulite® 1000][14]) below the recommended cutoff of < 30 pg/mL [1].

Study A used 8 adult horses with insulin dysregulation (ID) and 8 healthy, non-insulin dysregulated horses (NID, control group); Study B: 11 ID and 5 NID horses. Three of the NID horses in Study A had become ID in the 12 months between the studies and 2 (1 ID and 1 NID)

111 others had to be replaced for Study B due to unrelated medical issues (i.e. lameness, ophthalmic
112 issues). In order to categorise horses into their metabolic groups, they underwent an oral sugar
113 test (OST) 2 weeks prior to the beginning of each study [2]. They were placed into 3.7 x 3.7 m
114 individual pens and a basal blood sample was taken via jugular venipuncture. Immediately after,
115 0.15 mL/kg BW of Karo Light Corn Syrup (AHC Food Companies INC.) was administered
116 orally. Sixty-minutes later another venous blood sample was collected. Blood samples were
117 processed within 3 hours of collection. Blood was centrifugated at 800 g for 10 minutes,
118 aliquoted and stored in -20°C for determination of insulin concentration by Cornell University's
119 Animal Healthy and Diagnostic Center (AHDC) endocrinology laboratory (Ithaca, New York,
120 USA) via commercially available human insulin radioimmunoassay (RIA) (EMD Millipore
121 Corp, Billerica, Massachusetts, USA). Horses with basal insulin >50µIU/mL, and/or 60-minute
122 post OST insulin concentrations > 45 µIU/mL were considered ID [1].

123 *2.2 Study Design*

124 Horses were group housed in 4-acre semi-dry lots (minimal grass and weeds) and had ad
125 libitum access to grass hay when they were not being sampled. During the 2 weeks prior to the
126 start of each study horses were acclimatised to pens (individual dry lot [3.7 x 3.7 m]) and
127 feedstuffs for approximately 1 – hour (0700-0800) each day. During this hour they were fed 33%
128 of the total ration of a particular treatment diet. In order to ensure all horses used in the final
129 study would eat all the diets, the small meals of each diet were provided in a random order to
130 each horse in such a way that every horse sampled all of the study diets at least twice and all
131 diets were presented during the acclimation period.

132 For both studies blood was collected into 10 mL serum blood tubes and processed within
133 1 – 2 hours after collection by centrifugation at 800 g x 10 minutes with the serum being
134 removed aliquoted, and frozen (-20 C).

135 a) Study A

136 Horses received 2 treatment diets each week over a 2-week period with 24 hours between
137 each treatment diet. For the rest of the week the horses were kept in their paddocks with ad
138 libitum access to grass hay. Horses were split into two groups (each group containing equal
139 number of ID and NID horses) and sampled 2 times per week. The order of diet provision was

randomised. On sampling days, horses were brought up into individual pens (0700-0800) and a basal blood (T-1) sample taken via jugular venipuncture. Immediately following T-1, an intravenous catheter (16 g x 5-inch; Covetrus) was placed aseptically into the jugular vein. Thirty-minutes after catheter placement, a second basal blood (T0) sample was taken via the catheter port. The individual treatment diets were then given and consumed by all horses within ~10 minutes. After each diet had been consumed, timers were set, and blood was collected at 30, 60, 75, 90, 105, 120, 150, 180, 210, and 240-minutes postprandially.

b) Study B

Horses received weekly (same day each week) a different diet, in a randomised order on sampling days. All horses were sampled at the same time (minutes apart). All diets were fed on this day after horses had been brought up into their individual pens (0700-0800). A basal blood (T0) sample was initially taken by jugular venipuncture followed by the immediate offering of the treatment diet. After the consumption of the treatment diet (~10 minutes), a timer was set for blood to be collected 60-minutes postprandially. Both samples were collected via jugular venipuncture. For the rest of the week the horses were kept in their paddocks with ad libitum access to grass hay.

2.3 Diets

Four treatment diets (1.02 ± 0.1 g/kg BW) were fed in study A and 5 (1.25 ± 0.1 g/kg BW) in study B (Table 2). Diets were provided in a random order to the horses and all diets were represented each day. All treatment diets, grass hay, and pasture were sampled weekly and sent to Equi-Analytical Laboratory (Dairy One Forage Laboratory, Ithaca, New York, USA) for analysis via wet chemistry.

a) Study A: The ration balancer with high protein (RB-HP) was a commercially available high protein balancer (Buckeye Nutrition, Dalton, Ohio, USA) and was fed based on manufacturer's daily intake recommendation. All other diets were fed at the same rate. The ration balancer with lower protein (RB-LP; Buckeye Nutrition) had been formulated to have a similar NSC to the RB-HP but a lower protein content. The RB-HP and the RP-LP were then mixed to provide a mixed ration balancer with moderate protein (MIX-P). The cracked corn (CC; Woodford Feed Company Inc,

Versailles, Kentucky, USA) was mixed with 60 mL of molasses to increase palatability and was used as a positive control.

b) Study B: The same RB-HP was used along with the CC and a specifically formulated low NSC pelleted feed (L-NSC). In addition, in order to evaluate the insulin response to more pre-caecally digestible starch (than in CC), steam-flaked corn with 60 mL of molasses (SFC), and oat groats (OG) were also fed.

2.4 Assays

Insulin was analysed by Cornell University's AHDC endocrinology laboratory via human insulin radioimmunoassay (RIA) (EMD Millipore Corp, Billerica, Massachusetts, USA) and run in duplicates [2]. The sensitivity of the assay, as reported by the manufacturer is 2.72 μIU/mL. The mean intra- and inter-assay coefficients of variation were 7.4 and 6.3%, respectively.

2.5 Data analysis

Statistics were run on Minitab Software 19.0 (Minitab LLC, Pennsylvania, USA). To evaluate differences in T-1 vs. T0 insulin in Study A, a T-test was performed. Prior to running all general linear models (GLM), normal homoscedasticity and residuals were confirmed. If GLM assumptions did not pass, data were logged transformed. Study A response variable was area under the curve for insulin (AUCi) with explanatory variable as metabolic status (ID vs. NID). With a significant difference in AUCi between ID and NID groups, metabolic groups' AUCi (response variable) were separated with dietary treatment set as the explanatory factor. Significant differences between diets were determined with a Tukey post-hoc analysis. For study B, data were not normal even after log transformation. Insulin responses from each group had drastically different distributions. Therefore, Moon's Median test was run with response variables of basal (T0), postprandial insulin (T60), and delta insulin and metabolic group as the explanatory variable. Nonparametric test confirmed that ID (median = 3.9) was different from NID (median = 2.6). GLM assumptions were met when metabolic groups were separated. The response variables were T0, T60 and delta insulinaemic responses and explanatory variable was dietary treatment. Differences in dietary treatment were determined by Tukey post-hoc analysis. For all analyses, statistical significance was considered at $p<0.05$ and trends at $p<0.10$.

3. Results

All horses remained healthy throughout both studies.

3.1 Study A

There was no difference in insulin concentrations between T-1 and T0, (data not shown) and no negative behavioural patterns were seen in response to catheter placement. Age, BW, BCS were not different between ID and NID horses ($p>0.5$); however, CNS and basal and 60-minute post-OST (T60) insulin were different between ID and NID horses ($p<0.001$; Table 1). Pasture and grass hay analysis did not change significantly throughout the study ($p<0.5$). CP in pasture and hay was 20.1 ± 0.5% and 12.7 ± 1.8% on a DM basis, respectively. NSC in pasture and hay was 9.4 ± 1% and 6.7 ± 0.5% on a DM basis, respectively. Basal (T0) insulin concentrations were different between ID (59.75 ± 39.64 µIU/mL) and NID (17.55 ± 5.98 µIU/mL) horses ($p<0.001$) for all diets; however, basal insulin concentrations were not different between diets within the ID ($p=0.7$; CC 67.4 ± 50.2 µIU/mL; RB-HP 68.2 ± 53.8 µIU/mL; RB-LP 48.97 ± 22.83 µIU/mL; MIX-P 54.42 ± 26.63 µIU/mL) and NID ($p=0.4$; CC 16.28 ± 3.94 µIU/mL; RB-HP 19.65 ± 6.13 µIU/mL; RB-LP 15.39 ± 3.31 µIU/mL; MIX-P 18.89 ± 8.94 µIU/mL) horses. The AUCi were significantly higher for all treatment diets in ID compared to NID horses ($p<0.001$; Table 3). The AUCi were not affected by dietary treatment in the NID horses ($p=0.2$; Figure 1a) but for the ID horses the AUCi for CC was significantly higher than for RB-LP ($p=0.01$). In addition, there was a trend for CC to be higher than MIX-P ($p=0.06$). The insulin concentrations were significantly higher in response to feeding the CC than RB-LP in the ID horses at 90, 105, 120, 150, 180, 210, and 240-minutes ($p<0.05$ Figure 1b). The concentrations in response to feeding the CC were also significantly higher than the MIX-P in ID horses at 120 and 180-minutes ($p<0.05$) and there was a trend to be higher at 90, 105, 150, and 210 minutes ($p<0.1$). Starch and NSC (g/kg BW) were not different between any diet ($p=0.06$; Table 4a).

3.2 Study B

Similar to study A, there were no differences in age, BW and BCS between NID and ID horses ($p>0.5$); however, there were differences in CNS, basal and T60 insulin concentrations between NID and ID horses ($p<0.001$; Table 1). Basal insulin concentrations were

higher in ID horses compared to NID ($p<0.001$; Table 4b); however, there were no differences in basal insulin concentrations within ID or NID horses ($p=0.5$ and $p>0.9$, respectively; Table 4b). Sixty-minute postprandial and delta insulinaemic responses (60 min minus basal insulin concentrations) were significantly higher in ID than the NID horses for all diets ($p<0.001$; Figure 2 and 3; Table 4b). There was a trend for a greater insulin response in the NID to OG compared to L-NSC ($p=0.06$). Delta insulin responses for NID horses were significantly higher for OG vs. CC ($p=0.038$) and there was a trend for them to be higher in response to feeding SFC compared to CC ($p=0.081$). The ID horses' postprandial (T60) responses were significantly lower for L-NSC compared to OG, SFC, and CC ($p<0.02$); however, the response to the L-NSC was not different from the response to RB ($p=0.1$) and there was no significant difference between OG, CC and SFC ($p>0.4$). Delta insulinaemic response values for ID horses were significantly lower for the L-NSC dietary treatment compared to all other diets ($p<0.001$). Starch and NSC (g/kg BW) were not different between any diet ($p=0.06$; Table 4b).

4. Discussion

Although there are multiple studies that have recorded both glycaemic and insulinaemic responses to varying feedstuffs in the healthy, NID horse[9, 15-18], very little has been reported specifically in the ID animal[10,19]. This study however, confirmed that ID horses' insulinaemic responses to feedstuffs varying in NSC and CP are significantly different from NID horses and therefore, data obtained from NID horses cannot be automatically transferred to the ID animal.

Several factors influence insulinaemic responses in the horse, for example composition of the diet[9], the rate of gastric emptying determined by meal size[15], the rate of consumption[21], as well as differences in the methodology used to measure insulin[22]. Previous work also suggested that there might be a threshold for NID horses above which significant insulin responses would be found. Vervuert *et al*. (2009), for example, fed increasing amounts of starch and found that feeding ≥1.1g starch/kg BW produced disproportionate peak insulin responses (1.1g starch/kg BW: 162 ± 32 μIU/mL at 188 ± 105 minutes vs. 0.8g starch/kg BW: 88 ± 69 μIU/mL at 225 ± 39 minutes) in healthy horses[9]. Similarly, Zeyner *et al*. (2017) found that insulin responses were highest with a meal containing ≥1 g starch/kg BW[20]. Study A: therefore, confirmed that feeding

small amounts (~1g/kg) of restricted NSC (~9-17% NSC) containing feedstuffs to NID horses (which all provided <1g starch/kg BW) produces little insulin response (Table 4a).

Recently a study provided evidence that the insulin responses of ID horses are different to those of healthy individuals when fed the same forages[19] and supported the current recommendations to feed forage with less than 10-12% NSC (on a dry matter [DM] basis) to laminitis prone horses. The insulinaemic responses to the feeding of forage[8], however, may not be directly applicable to the feeding of complementary feeds, which are typically ingested more quickly than forages[23], can provide greater NSC intakes and, due to likely increased NSC availability within the foregut, are more likely to induce enhanced circulating glucose and thereby insulin concentrations[24,25]. This present, therefore, importantly showed that even feeding the small amounts of the various diets in study A produced 1.7 – 3.4-fold greater postprandial insulinaemic responses in the ID horses compared to the NID horses. It has been suggested that insulin dysregulation may have at least in part a gastrointestinal etiology[26]. The enhanced metabolic response by the ID horse could be due to more glucose being absorbed from the feedstuff, a lack of metabolised insulin through the first pass of the liver, abnormal intestinal glucose transport, or the pancreatic insulin response being augmented by increased incretin secretions[26]. Bamford *et al*. (2015) showed a positive association between post prandial insulin concentrations and GLP-1 and in future studies concurrent evaluation of the incretin response may be beneficial to help understand the drivers of this enhanced insulin response in ID horses[27]. Study A also suggested that, at least for the range of concentrations provided, the main nutritional factor driving the insulin response was NSC rather than protein content in that it evaluated 4 treatment diets with 4 different CP (36.9, 16.8, 26.3, and 9.3% DM) and 2 NSC (14.3, 14.9, 15.3, and 74.4% DM respectively) concentrations. The CC (74% NSC) dietary treatment produced the highest AUCi and there were no significant differences in the responses between RB-HP, RB-LP, and MIX-P

As starch in ground corn is not very pre-caecally available for digestion by mammalian enzymes in the small intestine, alternative grains with more pre-caecally available starch (i.e. oat groats and steam-flaked corn)[28] were included in study B to further evaluate differences. Delta insulinaemic responses ([T60 insulin] – [T0 insulin]) provide information as to the responsiveness of that individual to the specific diet especially in those with high resting insulin

concentrations. The delta response refers to the increase in insulin concentration after the consumption of a meal (i.e. the metabolic response)[26]. In the NID horses there was a trend for a greater insulin response with the oats and steam-flaked corn, but the differences were not marked (and all increases were <30μIU/mL in all NID horses across all diets). However, in the ID horses the delta insulinaemic response increase was significantly higher (Mean ± SD; CC:111.2 ± 64.4 μIU/mL, DO: 95.4 ± 67.3 μIU/mL, RB: 63.1 ± 38.6 μIU/mL, SFC: 117.3 ± 75.1 μIU/mL) for all feeds other than the L-NSC (~6%NSC: 10.32 ± 16.11μIU/mL), although the actual T60 values reached were not significantly different between the L-NSC and the RB-HP diet (~14.3%NSC; Table 4a and 4b).

Whilst evaluating AUCi provides information with respect to the overall response to a diet, this is obviously not practical to undertake in a field situation; therefore, Study B evaluated the response to the feeds in a similar way to the OST. This enabled not only the value at 60-minutes to be compared between ID and NID but, more importantly, the extent of any individual dietary response i.e. the delta response as discussed above. If the L-NSC diet is excluded from the evaluation (as it produced a significantly lower response even in the ID animal) and using the same time points in both study A and B: the overall delta responses for NID horses in study A and B were 17.6 ± 12.3μIU/mL (1.2 – 35.3 μIU/mL) and 12.3 ± 5.4 μIU/mL (15.8 – 21.6 μIU/mL), respectively and for the ID horses were 59.0 ± 33.7μIU/mL (15.0 – 110.6μIU/mL) and 97.4 ± 23.3μIU/mL (28.7 – 205.5μIU/mL), respectively. This suggests that a normal delta response to meals fed at ~1 g/kg BW with a NSC content >14% (DM) might typically be < 35μIU/mL in NID horses. In the ID horses however, there was considerable variability in the delta insulin levels. For example, some horses showed a larger delta value after consuming the oat groats feed (>100 μIU/mL) than with the oral sugar test, while others showed a greater response at 60 mins with the OST (data not shown). Therefore, different horses may respond differently to certain diets, which could partly be related to the time taken to digest the feed (differences in T_{max}), and/or their individual ability to digest that sugar/starch composition/intake level, processing type of cereal grains[32, 33, 34], as well as differences in cereal grains[15]. This does suggest that the individual response to a feedstuff or even the ranking of responses within a group cannot automatically be presumed from the OST results. Furthermore, significant variability in insulin responses to the OST have been noted in previous studies[29-31].

Study B confirmed that meal feeding itself does not induce a marked insulin response in ID horses as feeding small quantities of starch (0.024 g/kg BW) and NSC (0.08 g/kg BW) in a meal provided at ~1g/kg BW produced little insulin response. However, feeding just over twice this at 0.054g starch/kg BW and 0.191g NSC/kg BW resulted in an increased insulinaemic (delta) response. This suggests that if a very low insulin postprandial response is required to minimise endocrinopathic laminitis risks[5], ID horses may have a threshold of intake for starch somewhere between 0.024-0.054g/kg BW and for NSC between 0.076-0.191g/kg BW, and a dietary NSC content somewhere between 6 and ~ 14%NSC (DM basis). This does support the current recommendations of feeding diets with an NSC less than 10-12% DM[35] even if only feeding small amounts per meal - although from the current study it is not possible to confirm where exactly between 6-14% the threshold may be. There was a large individual variability in the insulin response when the ID horses were fed all the diets apart from the L-NSC. Given that previous work has suggested that the extent of any increase may reflect laminitis risk[5], the threshold of NSC that can be fed and not produce a significant insulin response may vary with the severity of the ID or other currently unknown factors.

In addition to whether there is a threshold of NSC that can be fed to ID horses before a significant insulin response is produced, there is also a question whether there is a specific insulin threshold with respect to laminitis risk. Possible suggestions for a threshold around ~ 200 µIU/mL have been extrapolated from a few studies[4-5]; although it is not known whether such values have to be maintained for a certain period of time or repeated a certain number of times for laminitis to occur or what if any other contributory factors are required. In one recent experimental study in Standardbred horses, insulin concentrations sustained at a level between 300-500 µIU/mL caused them to develop laminitis within 24-30 hours[36]. The T60 insulin values when the ID horses were fed steam flaked corn were 187.9 ± 107.0µIU/mL with a range of 67.7 – 377.0 µIU/mL. Three ID horses had insulin concentrations >250 µIU/mL; therefore, at least at this one time point it is not possible to say how long any individual had such values or what the actual peak values might have been in any animal. No adverse clinical signs were seen during the study or in the weeks afterwards. One individual horse also had insulin values that ranged from 223.3 µIU/mL to 354.8 µIU/mL between 30 and 150-minutes post CC ingestion and yet did not show any signs of laminitis during the study or at least 3 months after the completion of the study.

Limitations in the current study were the relatively small number of horses used and the absence of ponies. Given that ponies and horses have different glucose and insulin dynamics[37, 38, 39], it would be prudent to identify the threshold for NSC in the pony. In the future, other hormones, like glucagon-like peptide 1 and 2, could also be measured to better understand the metabolic response of the ID horse. Until further information on the factors that link insulin response to laminitis risk is available, it would seem sensible to limit post prandial insulin responses especially in those horses prone to laminitis. Given the variability in individual responses to diet, it would seem to be a practical managemental tool, in the meantime, to recommend that the insulin responses pre- and 60-minutes post ingestion of at least an individual's normal complementary feed would be worth monitoring especially in those identified to be ID or at increased risk of laminitis. Appropriate dietary and managemental changes could then be instigated. With this in mind, it is important to note that in this study the whole daily ration of the balancer was given as one meal rather than in divided doses as commonly recommended. Other pilot work has suggested that even in severe ID horses, low insulin responses may be produced when fed a high protein low-NSC-providing balancer in smaller amounts i.e. < 0.5g/kg BW/meal (unpublished data). Further work, however, is required to determine what is the upper limit of NSC (taking size of meal as well as %NSC into consideration) for the ID horse in order to minimise any increases in insulin postprandially. In conclusion, the current threshold for a NSC intake in the ID horse that will not consistently result in a clinically relevant insulinemic response is unknown and further work is needed in this area.

Authors' declarations of interest

P. Harris is employed by the funder. Other authors declared no competing interests.

Ethical animal research

All procedures for both studies followed the US National Research Council's Guide for Care and Use of Laboratory Animals and had individual approval for both study A (#2014-1224) and B (#2018-2886).

Informed consent

Not applicable.

Data accessibility statement

All data will be archived in both a written and online format. The online format can be found on the laboratories' Dropbox Account and can be shared with EVJ.

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Authorship

All authors meet requirements and standards to be authors on this publication. The studies were designed by E. Macon, A. Adams, P. Harris and S. Bailey. All authors contributed to the data interpretation and manuscript preparation. E. Macon had access to all data in the study and takes responsibility for the integrity of the data and accuracy of the data analysis.

Table 1: ID and NID demographics. Values presented are means ± standard deviations. Age, bodyweight (BW), body condition score (BCS), cresty neck score (CNS), and insulin responses to the oral sugar test (OST) for NID and ID horses in study A and B. Within a row for study A, significant differences ($P<0.05$) are represented by different $^{a, b}$ superscripts. Within a row for study B, significant differences ($P<0.05$) are represented by different $^{\#, *}$ superscripts.

Parameter	Study A		Study B	
	NID	ID	NID	ID
Age (years)	12.6 ± 1.4 ^{a}	14.3 ± 2.6 ^{a}	13.8 ± 1.8 $^{\#}$	14.6 ± 2.8 $^{\#}$
BW (kg)	613.6 ± 79.3 ^{a}	618.8 ± 92.7 ^{a}	564.6 ± 79 $^{\#}$	612.4 ± 84 $^{\#}$
BCS	6.3 ±1.1 ^{a}	7.1 ± 0.6 ^{a}	7.1 ± 0.6 $^{\#}$	6.3 ± 1.1 $^{\#}$
CNS	1.5 ± 0.4 ^{a}	2.7 ± 0.7 ^{b}	1.3 ± 0.3 $^{\#}$	2.5 ± 1.2 *
OST – T0	19.7 ± 6.4 ^{a}	86.6 ± 53.0 ^{b}	13.6 ± 4.8 $^{\#}$	57.7 ± 22.1 *

Insulin (µIU/mL) OST – T60 Insulin (µIU/mL)	39.4 ± 15.6 [a]	157.6 ± 60.4 [b]	19.6 ± 8.2 [#]	126.6 ± 48.3 [*]

Abbreviations: BW = bodyweight; BCS = body condition score; CNS = cresty neck score; OST = oral sugar test.

Table 2: Wet chemistry analysis of diets for Study A and B. All values are presented on a DM basis; Mean ± SD. Crude protein (CP), starch, water-soluble carbohydrates (WSC), and non-structural carbohydrates (NSC) content of the feedstuffs are shown for study A and B.

Feedstuff	CP%	Starch%	WSC%	NSC%
Study A				
Ration Balancer-High Protein	36.9 ± 0.1	4.8 ± 0.7	9.3 ± 0.3	14.1 ± 0.8
Ration Balancer-Low Protein	16.8 ± 1.4	9.5 ± 1.2	5.4 ± 0.3	14.9 ± 1.5
Mixed Ration Balancer – Moderate Protein	26.3 ± 0.7	7.3 ± 0.1	7.4 ± 1.2	14.7 ± 1.1
Cracked Corn w/ Molasses	9.3 ± 0.0	65.5 ± 1.6	8.95 ± 0.2	74.4 ± 1.5
Study B				
Ration Balancer-High Protein	36 ± 1.0	4.5 ± 0.3	10.3 ± 1.0	14.8 ± 0.7
Cracked Corn w/ Molasses	9.6 ± 0.1	63.8 ± 1.6	8.1 ± 0.3	71.9 ± 1.3
Steam-Flaked	9.8 ± 0.6	68.1 ± 2.6	4.9 ± 2.8	73.0 ± 0.2

Corn w/ Molasses				
Oat Groats	13.9 ± 0.1	61.2 ± 0.3	3.1 ± 1.0	64.3 ± 0.7
Low NSC	12.0 ± 0.1	1.7 ± 0.4	4.5 ± 0.4	6.1 ± 0.1

Abbreviations: CP = crude protein; WSC = water soluble carbohydrates; NSC = non-structural carbohydrates; LOW = formulated low NSC pellet.

Table 3: AUCi for NID and ID horses for Study A. Values presented are mean ± standard deviations. AUCi for all dietary treatments for study A for NID and ID horses.

Dietary Treatment	NID AUCi (µIU/mL · min)	ID AUCi (µIU/mL · min)
CC	7,329 ± 2,017 $^{A, a}$	32,000 ± 13,960 ^{Ab}
RB-HP	5,966 ± 1,753 $^{A, a}$	22,069 ± 7,142 $^{AC, b}$
RB-LP	5,379 ± 1,416 $^{A, a}$	18,977 ± 6,731 $^{BC, b}$
MIX-P	5,906 ± 2,198 $^{A, a}$	16,403 ± 4,304 $^{AC, b}$

Within a row, significance ($P<0.05$) is represented by different lowercase superscripts. Within a column, significance ($P<0.05$) is represented by different uppercase superscripts.

Abbreviations: AUCi = Area under the curve for insulin; CC = cracked corn with molasses; RB-HP = ration balancer with high protein; RB-LP = ration balancer with low protein; MIX-P = mixed ration balancer with moderate protein.

Table 4a: Peak insulin concentrations for NID and ID horses with starch and NSC intakes in Study A. Values presented are means ± standard deviations.

			Basal Insulin (µIU/mL)		Peak Insulin (µIU/mL)	
Dietary Treatment	**Starch g/kg BW**	**NSC g/kg BW**	**NID**	**ID**	**NID**	**ID**
RB-HP	0.052 ^{A}	0.146 ^{B}	19.7 ± 6.1	68.2 ± 53.8	39.0 ± 16.0	127.6 ± 35.7
RB-LP	0.097 ^{A}	0.152 ^{B}	18.9 ± 8.9	49.0 ± 22.8	34.1 ± 14.2	112.2 ± 34.8
MIX-P	0.075 ^{A}	0.156 ^{B}	15.4 ± 3.3	54.4 ± 26.6	30.6 ± 8.6	111.0 ± 24.1
CC	0.668 ^{A}	0.759 ^{B}	16.3 ± 3.9	67.4 ± 50.2	39.7 ± 14.9	173.1 ± 61.6

Within a row, significance ($P<0.05$) is represented by different lowercase superscripts. Within a column, significance ($P<0.05$) is represented by different uppercase superscripts.

Abbreviations: CC = cracked corn with molasses; RB-HP = ration balancer with high protein; RB-LP = ration balancer with low protein; MIX-P = mixed ration balancer with moderate protein.

Table 4b: Insulin concentrations for NID and ID horses with starch and NSC intakes in Study B. Values presented are means ± standard deviations.

			Basal Insulin (µIU/mL)		T60 (µIU/mL)	
Dietary Treatment	**Starch g/kg BW**	**NSC g/kg BW**	**NID**	**ID**	**NID**	**ID**
RB-HP	0.054 ^{A}	0.191 ^{B}	14.3 ± 4.6	57.2 ± 33.4	21.5 ± 6.8	125.7 ± 62.6
CC	0.783 ^{A}	0.886 ^{B}	14.1 ± 3.8	48.5 ± 15.1	20.8 ± 6.8	159.7 ± 78.1
SFC	0.874 ^{A}	0.910 ^{B}	15.1 ± 2.6	70.6 ± 48.6	30.5 ± 12.2	187.9 ± 107.0
OG	0.761 ^{A}	0.799 ^{B}	13.9 ± 3.6	74.3 ± 51.7	32.1 ± 10.1	169.7 ± 111.7
L-NSC	0.024 ^{A}	0.076 ^{B}	14.5 ± 4.4	44.4 ± 16.0	17.2 ± 4.4	57.8 ± 18.5

Within a row, significance ($P<0.05$) is represented by different lowercase superscripts. Within a column, significance ($P<0.05$) is represented by different uppercase superscripts.

Abbreviations: CC = cracked corn with molasses; RB-HP = ration balancer with high protein; RP = research pellet; SFC = steam-flaked corn with molasses; OG = oat groats; L-NSC = low NSC pellet.

Figure legends:

Figure 1: NID insulin responses, Study A. Mean insulin concentrations ± SEM prior to (T-1 min) and following for 240 mins responses to four dietary treatments of ration balancer (RB), cracked corn (CC), research pellet (RP) and Mix treatments. No significant differences in area under the curve for insulin for NID horses ($p=0.215$).

Figure 1b: ID insulin responses, Study A. Mean insulin concentrations ± SEM prior to (T-1 min) and following for 240 mins responses to four dietary treatments of ration balancer (RB), cracked corn (CC), research pellet (RP) and Mix treatments. CC was significantly higher than RB-LP ($p=0.011$).

Figure 2: Postprandial insulin concentrations for NID and ID horses in Study A. Mean insulin concentrations ± SEM for postprandial insulin (T60) for five dietary treatments of cracked corn with molasses (CC), oat groats (OG), low NSC pelleted feed (L-NSC), ration balancer with high protein (RB-HP), steam-flaked corn (SFC). All ID horses' T60 responses were different than NID horses ($p<0.001$). Significance is denoted by difference of superscript.

Figure 3: Delta insulin concentrations for NID and ID horses in Study A. Mean insulin concentrations ± SEM for delta insulin for five dietary treatments of cracked corn with molasses (CC), oat groats (OG), low NSC pelleted feed (L-NSC), ration balancer with high protein (RB-HP), steam-flaked corn (SFC). All ID horses' DI responses were different than NID horses ($p<0.001$). Significance is denoted by difference of superscript.

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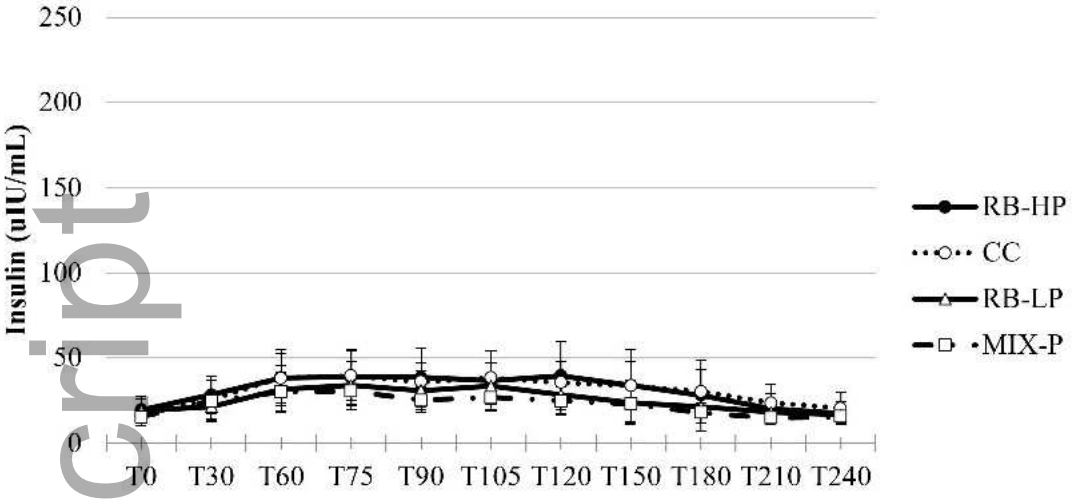

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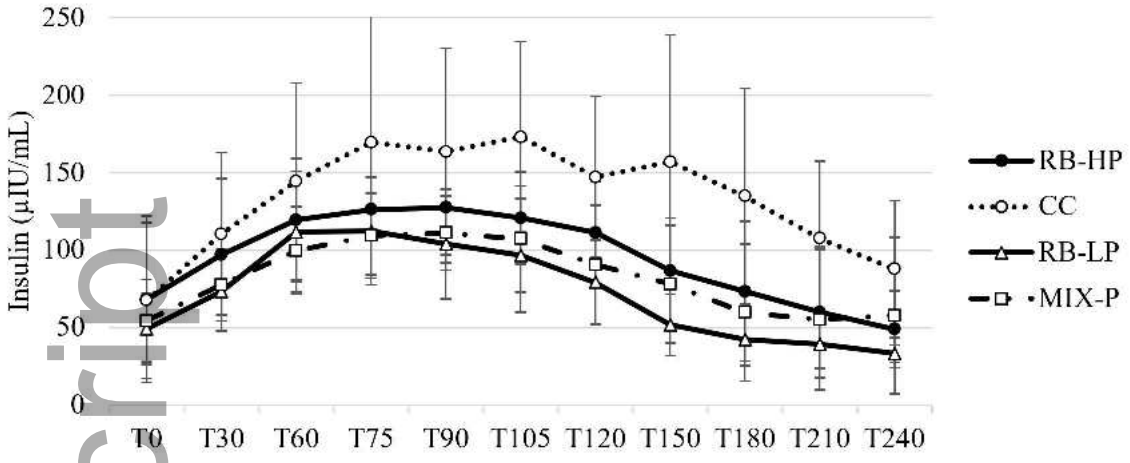

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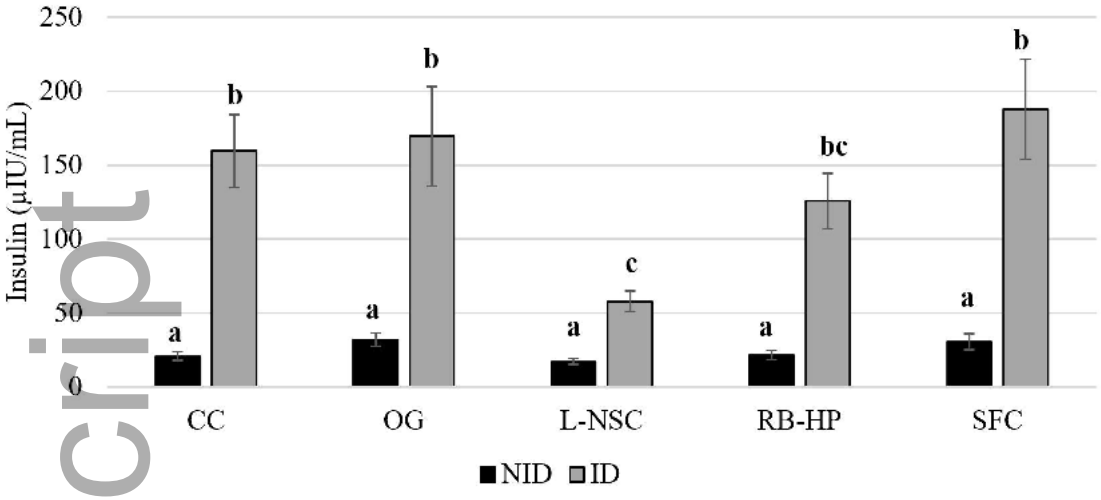

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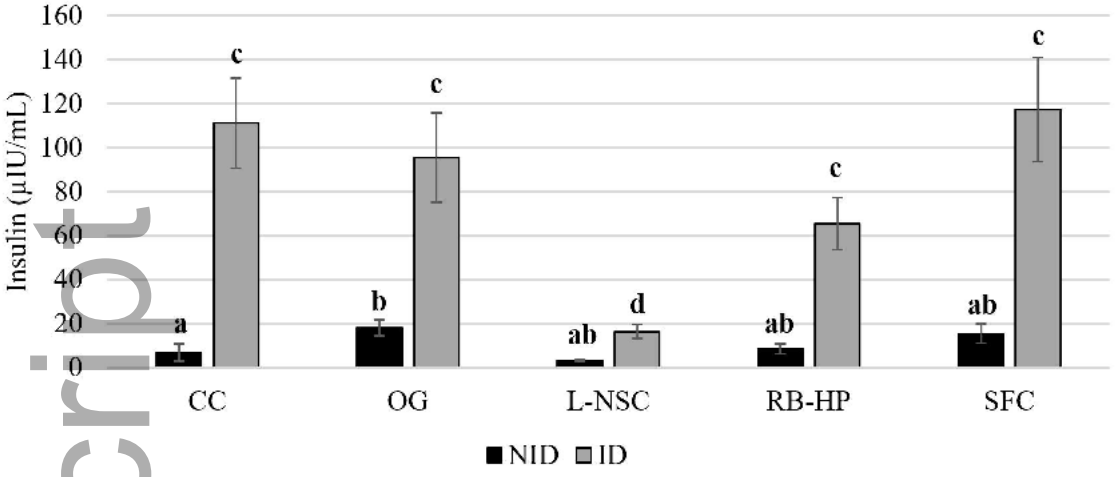

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