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Original Research

Effects of High-Sugar and High-Starch Diets on Postprandial Inflammatory Protein Concentrations in Horses

Jessica K. Suagee [a,*], Rebecca K. Splan [b], Kelcey L. Swyers [c], Raymond J. Geor [d], Benjamin A. Corl [e]

[a] Agricultural Technical Institute, The Ohio State University, Wooster, OH
[b] Department of Animal and Poultry Sciences, Virginia Tech, Middleburg, VA
[c] Ranch Way Feeds, Fort Collins, CO
[d] Department of Large Animal Clinical Sciences, Michigan State University, East Lansing, MI
[e] Department of Dairy Science, Virginia Tech, Blacksburg, VA

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ABSTRACT

Mature, nonpregnant, Thoroughbred mares were used to determine the influence of high-starch and high-sugar diets on postprandial inflammation. Plasma samples were obtained hourly from mares (n = 12) consuming one of two treatment diets, either a diet high starch and sugar (STR) or the control (CON) diet that was low in starch and sugar. Plasma was analyzed for concentrations of lipopolysaccharide (LPS) and the inflammatory cytokines interleukin (IL)-1β and IL-6. Hour 0 was included as a covariate in the statistical model, and where interactions between the covariate and other model variables existed, simple effect means were separated at three levels of the covariate: lower 95% confidence limit (CL), mean, and upper 95% CL. For horses with low ($P = .016$) and average ($P = .065$) initial LPS concentrations, LPS was greater or tended to be greater in STR compared with CON at hour 2 after feeding. No other differences were detected for LPS concentrations. For horses with low ($P = .037$), average ($P = .006$), and high ($P = .001$) initial IL-1β concentrations, plasma IL-1β was greater in STR than CON at hour 2 after feeding. For horses with high initial IL-1β concentrations, IL-1β also tended to be greater at hour 3 ($P = .077$). For horses with low ($P = .022$) or average ($P = .063$) initial IL-6 concentrations, IL-6 was greater or tended to be greater at hour 1 than 0. No effect of diet was detected for horses that started with high initial IL-6 concentrations. High-starch and high-sugar diets increase postprandial IL-1β concentrations, and it is likely that this effect is independent of LPS.

1. Introduction

In horses, obesity leads to an increased risk of insulin resistance, and it is possible that consumption of high-glycemic diets (those high in starch and sugar, HSS) exacerbate the onset or degree of this dysfunction [1,2]. Recent research on 300 horses in Virginia indicated that more than half of the studied population was overweight or obese [3]. Furthermore, 70% of the studied population was offered a grain-based concentrate meal every day. Because insulin resistance increases the risk of laminitis [4], an excruciatingly painful disease of the equine hoof, it is important to determine how HSS diets could specifically influence insulin resistance.

It is possible that HSS diets induce insulin resistance by promoting increased plasma concentrations of proinflammatory cytokines. Cytokines such as interleukin 1β (IL-1β) [5], IL-6 [6], and tumor necrosis factor (TNF)-α [7]

* Corresponding author at: Jessica K. Suagee, Agricultural Technical Institute, The Ohio State University, Wooster, OH 44880.
E-mail address: Suagee.2@osu.edu (J.K. Suagee).

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are known to reduce insulin sensitivity in a variety of species and tissue types. Additionally, the acute-phase protein, serum amyloid A (SAA), correlates with obesity and insulin concentration in horses [8] and alters insulin sensitivity in vitro in adipocytes [9]. In ruminants, there is evidence that starch fermentation, particularly after consumption of high-starch diets, promotes increased postprandial inflammation [10]. Similarly, fermentation of starch in the gastrointestinal tract could link HSS diets to increased inflammation in horse, which could be a factor relating HSS diets to insulin resistance.

Although starch is primarily digested in the small intestine, it overwhelms the digestive capacity of the small intestine when ingested in large enough concentrations and enters the cecum and large intestine (hindgut) where it is fermented by bacteria [11,12]. Starch is one of several carbohydrates that on reaching the hindgut are rapidly fermented. Although different from starch in its structure, oligofructose is a rapidly fermented carbohydrate that may have similar effects on the hindgut when it is consumed in large amounts. As soon as 4 hours after consuming a large quantity of oligofructose, cecal concentrations of organic acids (such as lactate) are altered, cecal pH is lowered [13], and blood concentrations of lipopolysaccharide (LPS) are increased [14]. In horses, IV infusion of LPS induces insulin resistance and increases the concentrations of several proinflammatory cytokines in plasma [15–18]. The ability of starch to influence plasma LPS and inflammation has only been shown after experimental overfeeding of starch, but we hypothesize that routine consumption of HSS diets generates a whole-body state of low-grade chronic inflammation that in turn facilitates and promotes insulin resistance and, further, that this inflammation occurs due to routine exposure to increased blood LPS.

2. Materials and Methods

All procedures were approved by Virginia Tech's Institutional Animal Care and Use Committee. Methods and results of a companion study were previously published [19] and are briefly described here.

2.1. Horses

Twelve mature (9–18 years), nonpregnant, Thoroughbred mares were used for this experiment. Mares were housed in drylots for 30 days before the beginning of the study to allow for acclimation to study conditions, including consuming concentrate twice daily in individual stalls. As previously described, mares were blocked by fasting insulin (0.4–16.6 mIU/L), age, and body condition (5–7; [20]), and two horses from each block were randomly assigned to each treatment (n = 6 to a low-starch control [CON] and n = 6 to a high-starch [STR] treatment diet) [19]. Horses were housed in three separate drylots, with two horses from each treatment per drylot. At all times throughout the study, horses had ad libitum water and iodized salt. Horses were brought into stalls for concentrate feeding at 8 AM and 2 PM and then returned to the drylots where they were group-fed hay. Daily observations showed that hay was consumed in entirety within a few hours, and thus horses were likely in a fasted state before consuming concentrate. Horses were not glucose intolerant nor did they have fasting hyperinsulinemia (CON = 2.1 mIU/L; STR = 5.6 mIU/L) before the start of the study [19]. Further, neither treatment induced fasting hyperinsulinemia (CON = 2.7 mIU/L; STR = 6.9 mIU/L) or glucose intolerance after 90 days of continuous feeding.

2.2. Treatments and Experimental Design

The experiment was a randomized complete block design with repeated measures, whereby drylots had staggered start dates of the study to enable intensive blood collections. Daily digestible energy (DE) requirements were estimated using the 2007 National Research Council (NRC) recommendations for horses at maintenance [21]. Treatment diets were formulated to contain either 10% (CON) nonstructural carbohydrates (NSC; sum of starch and ethanol soluble carbohydrate fractions) or 60% NSC (STR). Please see our previous article for the dietary ingredients and analysis [19]. All horses received the CON diet during the 30-day adaptation period, and those assigned to STR were abruptly switched on day 1 of the study. Horses were then fed their treatment diets for the duration of the 90-day study. Concentrate provision was estimated to provide 20% of daily DE requirements, with the remainder of energy coming from group-fed hay.

2.3. Blood Sampling

On days 1 and 90 of the study, blood samples were collected at −1, 60, 120, 180, 240, and 300 minutes relative to concentrate offering. On the day before testing, horses were fitted with indwelling 14-ga jugular venous catheters (Abbocath; Abbott Corp, Abbott Park, IL), using aseptic technique, and after sterilization and desensitization (2% lidocaine) of the overlying skin. Horses were maintained in individual stalls overnight with feed withheld for at least 10 hours. Blood samples were collected into 10-mL heparin-coated evacuated tubes (Vacutainer; Becton-Dickson, Franklin Lakes, NJ) and immediately centrifuged at 2,000*g* for 10 minutes and at 4°C. Plasma was stored in 1.5-mL tubes at −20°C until analysis.

2.4. Plasma Analysis

Plasma samples were analyzed using commercially available kits and previously published methods for concentrations of LPS (Pierce LAL Chromogenic Endotoxin Quantitation Kit; Thermo Scientific, Rockford, IL) [22], SAA (Phase SAA Assay; Tridelta Development Ltd, Maynooth, County Kildare, Ireland) [17], TNF (Equine TNF alpha ELISA Reagent Kit; Thermo Scientific) [23], and IL-1β (Equine IL-1 beta ELISA VetSet; Kingfisher Biotech, Inc, Saint Paul, MN) [8]. Plasma IL-6 concentrations were analyzed using previously published methods [24,25].

2.5. Statistical Analysis

The effects and interactions of day and diet on body condition scores (BCSs) were analyzed by mixed-models analysis of variance (ANOVA) using the mixed models procedure of SAS (SAS version 9.3; SAS Institute, Cary, NC). The effects and interactions of treatment, hour, and day on plasma concentrations of LPS, IL-1β, TNF, IL-6, and SAA were determined by repeated measures ANOVA. The repeated term was hour, and the subject was horse within treatment × day. The 96-well plate number that each sample was run on was included as a random effect. For each variable, residuals were analyzed for normality and homogeneity of variance by visually assessing plots of studentized residuals. All variables were transformed to improve normality and homogeneity of variance of residuals (SAA, IL-6, and LPS were $\log_{10}$ transformed; TNF and IL-1β were square root transformed). Covariance structures for repeated measures were selected based on having the lowest Corrected Akaike Information Criterion (AICC) index. Hour 0 values were included as a covariate after appropriate model reduction methods to determine the correct model for each variable. As is required for statistical models including covariates, significant interactions between the covariate and fixed effects were tested by sequential removal of nonsignificant effects. If found to be significant, the covariate interaction was left in the model, and simple effect means were separated at three levels of the covariate—lower 95% confidence limit (CL), mean, upper 95% CL. Where appropriate, pairwise comparison simple effect means were separated with a Tukey test, Dunnett test, or with specific contrast statements. Correlations were run between initial concentrations of inflammatory markers to determine whether horses with low starting concentrations of one marker also had low concentrations of other markers.

3. Results

Horses on the CON treatment averaged 11.7 ± 0.9 years, 610.4 ± 24.3 kg, and a BCS of 6.7 ± 0.4 initially, whereas horses on STR averaged 12.5 ± 0.9 years ($P > .5$), 595.7 ± 24.3 kg ($P > .6$), and a BCS of 6.7 ± 0.4 ($P = 1.0$) initially. Neither body weight nor BCS was influenced by the day × diet interaction ($P > .6$), or the main effects of day ($P > .4$) or diet ($P > .4$; data not shown). Horses were not tested for insulin sensitivity status before the start of the study; however, fasting insulin concentrations on day 1 of the study ranged from 0.4 to 16.6 mIU/L, which are concentrations below the suggested limit for determining insulin resistance (data not shown).

Initial LPS concentration was a covariate in the LPS statistical model and interacted with the hour × diet interaction ($P < .001$). Because of this interaction, simple effect means of the hour × diet interaction were separated at three levels of the covariate. For horses with low initial LPS concentrations, LPS was greater in STR compared with CON at hour 2 after feeding ($P = .016$; Fig. 1A). Similarly, horses with average initial LPS concentrations, LPS tended to be higher in STR than CON at hour 2 after feeding ($P = .065$; Fig. 1B). No differences were detected between

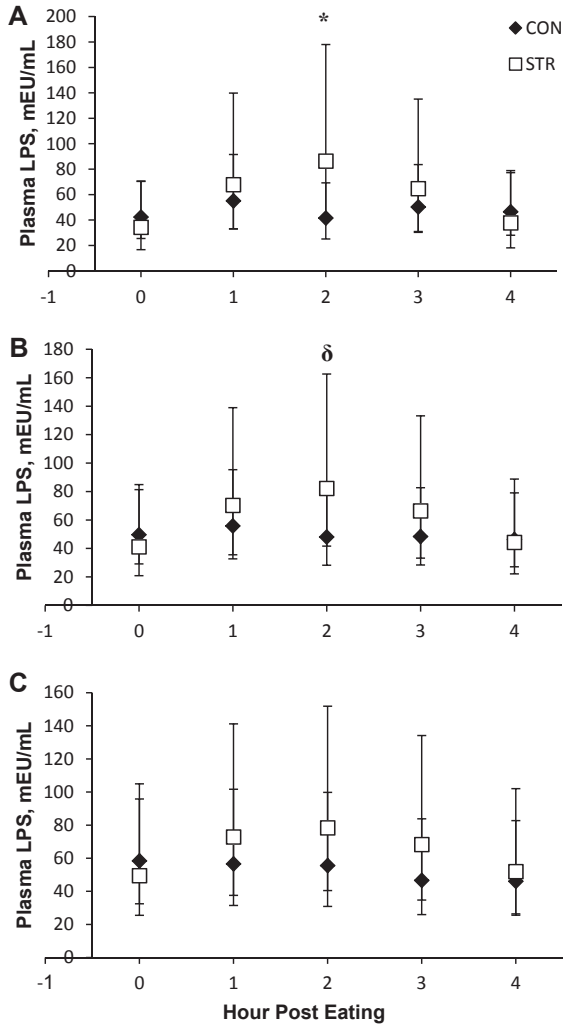

Fig. 1. Geometric mean (±95% confidence intervals) plasma lipopolysaccharide (LPS) concentrations in horses after consuming a control (CON) diet designed to be low (10%) in starch and sugar or a treatment diet designed to be high in starch (STR) and sugar (60%) in nonstructural carbohydrates. Initial LPS concentration was a covariate in the statistical model and was significantly interacted with the hour × diet interaction ($P < .001$). Data are presented at the lower 95% confidence limit (A), mean (B), and upper 95% confidence limit (C) of the initial LPS concentrations. *Geometric means within hour are different $P < .05$. δGeometric means within hour are different $P < .1$.

treatments for horses with high initial LPS concentrations ($P > .1$; Fig. 1C). There was no effect of day on LPS concentrations ($P > .4$).

In the IL-1β statistical model, initial IL-1β concentration interacted as a covariate with the hour × diet interaction ($P = .009$). Because of this interaction, simple effect means of the hour × diet interaction were separated at three levels of the covariate. For horses with low initial IL-1β concentrations, plasma IL-1β was greater in STR than CON at hour 1 after feeding ($P = .037$; Fig. 2A). Similarly, horses that started with average initial IL-1β concentrations, postfeeding IL-1β concentrations were higher in STR than CON at hour 2 ($P = .006$; Fig. 2B). For horses with high initial

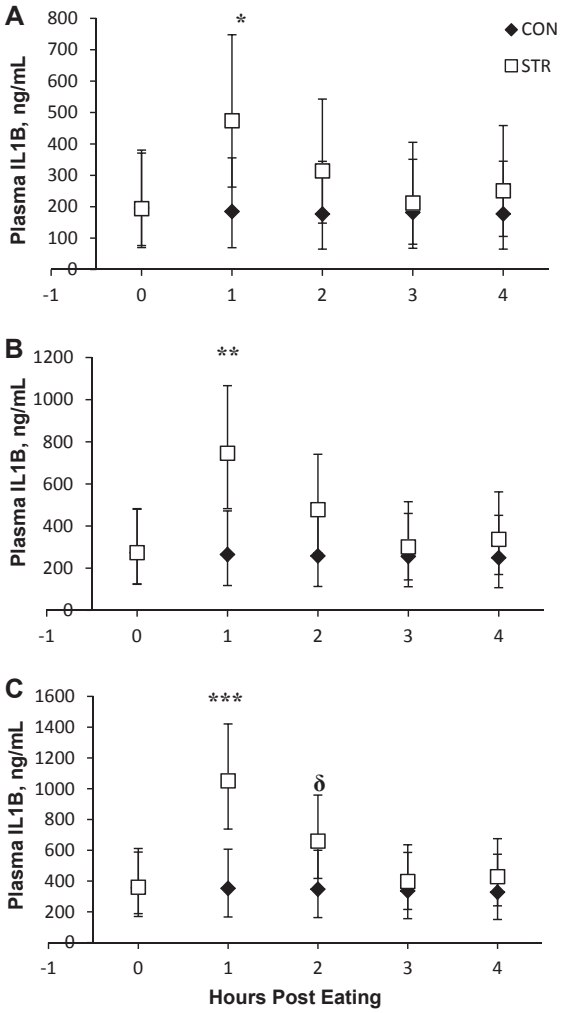

Fig. 2. Geometric mean (±95% confidence intervals) plasma interleukin 1β (IL-1β) concentrations in horses after consuming a control (CON) diet designed to be low (10%) in starch and sugar or a treatment diet designed to be high in starch (STR) and sugar (60%) in nonstructural carbohydrates. Initial IL-1β concentration was a covariate in the statistical model and was significantly interacted with the hour × diet interaction ($P = .009$). Data are presented at the lower 95% confidence limit (A), mean (B), and upper 95% confidence limit (C) of the initial IL-1β concentrations. ***Geometric means within hour are different $P < .001$. **Geometric means within hour are different $P < .01$. *Geometric means within hour are different $P < .05$. $^{\delta}$Geometric means within hour are different $P < .1$.

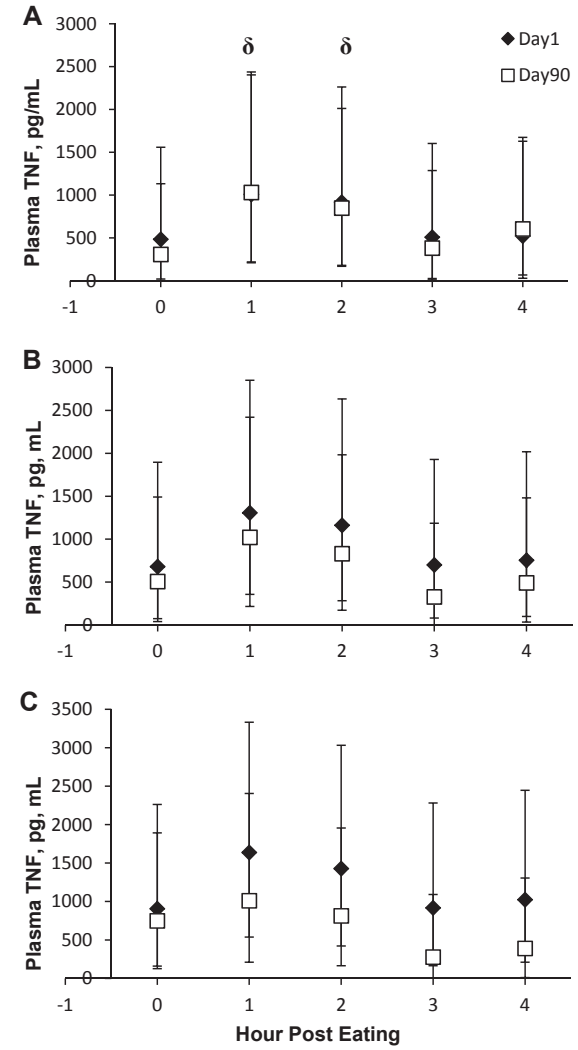

Fig. 3. Geometric mean (±95% confidence intervals) tumor necrosis factor (TNF) α concentrations in horses after consuming concentrate feed on day 1 and day 90. Initial TNF concentration was a covariate in the statistical model and was significantly interacted with the hour × day interaction ($P < .001$). Data are presented at the lower 95% confidence limit (A), mean (B), and upper 95% confidence limit (C) of the initial TNF concentrations. $^{\delta}$Geometric means are different from hour 0 (prefeeding) $P < .1$.

IL-1β concentrations, IL-1β concentrations were higher in STR than CON at hour 2 ($P = .001$) after feeding and tended to be higher at hour 3 ($P = .077$; Fig. 2C). There was no effect of day on IL-1β concentrations ($P > .9$).

Initial TNF concentration was a covariate in the TNF statistical model and was interacted with the day × hour interaction ($P < .001$). Because of this interaction, simple effect means of the day × hour interaction were separated at three levels of the covariate. For horses with low initial TNF concentrations, postfeeding TNF concentrations tended to be higher at hours 1 ($P = .072$) and 2 ($P = .076$) after feeding than concentrations measured at hour 0 (Fig. 3A). No differences were detected between measurements made on day 1 versus day 90 ($P > .5$). No differences were detected for hour after feeding or day of study for horses with initial average or high TNF concentrations ($P > .1$; Figs. 3B, 3C). There was no effect of diet on TNF concentrations ($P > .2$).

Initial IL-6 concentration was included as a covariate in the IL-6 statistical model and was interacted with hour ($P = .001$). Neither the interaction nor effects of day and diet were significant ($P > .5$). Simple effect means of hour after feeding were separated at three levels of the covariate. For horses with low initial IL-6 concentrations, IL-6 was higher at hour 1 than 0 ($P = .022$, Fig. 4), whereas in horses with average initial IL-6 concentrations, IL-6

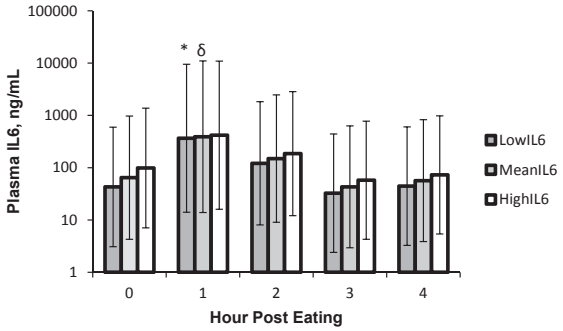

Fig. 4. Geometric mean (±95% confidence intervals) interleukin 6 (IL-6) concentrations in horses after consuming concentrate feed. Initial IL-6 concentration was a covariate in the statistical model and was significantly interacted with hour ($P = .001$). Data are presented at the lower 95% confidence limit (LowIL6), mean (MeanIL6), and upper 95% confidence limit (HighIL6) of the initial IL-6 concentrations. *Geometric means within initial IL-6 concentration group are different $P < .05$. [δ]Geometric means are different from hour 0 (prefeeding) within initial il-6 concentration group $P < .1$.

tended to be higher at hour 1 than 0 ($P = .063$). No effect of feeding was detected for horses that started with high initial IL-6 concentrations.

Initial SAA concentrations were included as a covariate in the SAA statistical model ($P < .001$). No effect of diet, day, or hour post feeding, or the interactions of these variables was detected for SAA concentrations ($P > .1$, Fig. 5).

Initial TNF concentration tended to positively correlate with initial IL-1β concentration ($r = 0.56$; $P = .059$), whereas other initial inflammatory protein concentrations were not correlated: LPS versus TNF ($r = 0.54$; $P = .070$) and LPS versus IL-1β ($r = 0.47$; $P = .122$).

4. Discussion

It is likely that horses evolved consuming diets that were high in indigestible and slowly fermentable carbohydrates and low in starches and sugars. However, modern feeding management practices commonly include a grain-based concentrate meal [3], which would likely be much higher in starches and sugars. The higher level of

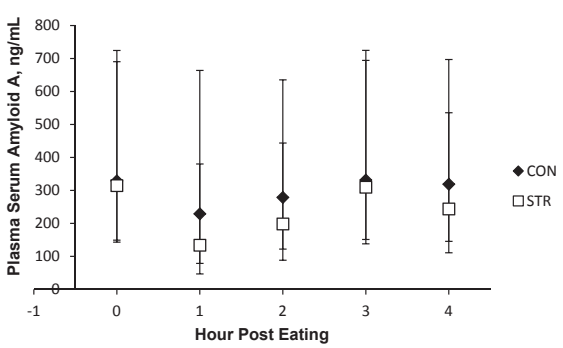

Fig. 5. Geometric mean (±95% confidence intervals) serum amyloid A (SAA) concentrations in horses after consuming a control (CON) diet designed to be low (10%) in starch and sugar or a treatment diet designed to be high in starch (STR) and sugar (60%) in nonstructural carbohydrates. Initial SAA concentration was a covariate in the statistical model ($P < .001$).

starch and sugar in grains such as barley, corn, and oats increases plasma glucose and insulin levels after consumption [26–28]. For instance, as previously reported, STR consumption was associated with peak glucose and insulin concentrations of 170.5 mg/dL and 120.8 mIU/L, respectively, whereas CON consumption only induced peak glucose and insulin concentrations of 120.8 mg/dL and 33.9 mIU/L, respectively. Prolonged intake of HSS diets has been shown to induce insulin resistance [1,2], although, in the present study, horses consumed STR for 90 days and were not found to have altered glucose tolerance [19]. The STR horses did however have reduced suppression of nonesterified fatty acid concentrations after eating, as reported previously [19]. Given that insulin is responsible for suppressing fatty acid release from adipose tissue, we hypothesize that horses consuming STR were experiencing some level of reduced insulin function after 90 days. Given the high proportion of horses fed grain-based concentrate meals, it is of high importance to uncover the potential mechanisms linking high-starch diets with insulin resistance.

Although the equine digestive tract is primarily designed for digestion of starch in the small intestine, hindgut fermentation can occur if starch intake is greater than 2–4 g/kg of body weight [11,12]. Because of rapid fermentation of starch in the hindgut, lactic acid production outpaces use, leading to elevated lactic acid concentrations in the digestive tract [29]. This lactic acid accumulation can lower the pH of the intestinal contents [30]. Further, foregut fermentation also occurs in the stomach and small intestine of horses [31], with starch fermentation presumably contributing to elevated lactic acid concentrations and having similar pH effects on digesta in the foregut. In ruminants, a lowered pH has been shown to damage the intestinal epithelia, leading to the potential for bacterial by-products, such as LPS, to translocate out of the digesta and into the blood stream [32]. Potentially, this increase in blood LPS could trigger a low-grade inflammatory state. For instance, IV-infused LPS will induce short-term insulin resistance and increases concentrations of proinflammatory cytokines in horses [15–17]. Therefore, we hypothesized that consumption of STR would result in low-grade chronic inflammation as a result of routine exposure to increased blood LPS.

Our first objective was to determine if feeding a high-starch and high-sugar diet would increase plasma LPS concentrations. Nasogastric tube administration of high levels of carbohydrates has increased LPS concentrations in experimental settings; however, the influence of moderate starch doses on LPS has not, to our knowledge, been previously investigated. For instance, starch administration at 15 g/kg of body weight increased plasma LPS concentrations in horses [33]. Similarly, oligofructose, a carbohydrate that also results in lactate accumulation [13], increased plasma LPS concentrations and did so in as little as 4 hours after consumption [14]. Both of those studies used much higher doses of fermentable carbohydrate, at 10–15 g/kg of body weight, whereas in the present study, horses offered STR consumed 1.14 g of NSC/kg of body weight per meal. The amount of NSC consumed per meal in the present study was designed to be lower than the 2–4 g/kg body

weight previously shown to cause starch bypass of the small intestine and fermentation in the large intestine [11,12]. This was done to reduce the risk of illness and more closely align with industry-standard feeding practices. Therefore, we hypothesized that LPS concentrations would rise above baseline at 4 hours after consumption of STR. As such, our finding of the increase at 2 hours in STR was surprising, and further so because it only occurred in horses with very low initial plasma LPS levels. It is likely that this LPS was derived from either the stomach or small intestine, as the probably earliest occurrence of digesta reaching the hindgut is 3 hours after feeding [34]. Furthermore, the equine foregut contains large populations of bacteria capable of fermenting carbohydrates [35,36], and hydrogen gas concentrations in breath, suggestive of prececal starch fermentation, are increased at 2 hours after consumption of a meal high in starch [31]. Thus, it is possible that prececal starch fermentation occurred in our STR-fed horses and contributed to the rise in LPS concentrations. As previously mentioned, this rise was only observed in horses with low initial LPS concentrations. None of the horses on the study exhibited clinical signs of illness, and it is unclear what factors contribute to the variation in initial plasma LPS concentrations.

Our second objective was to determine the effects of STR on postprandial inflammatory cytokine concentrations. We originally hypothesized that increased plasma LPS would drive changes in cytokine concentrations; however, given the minimal changes in plasma LPS concentrations after feeding, our finding of increased plasma IL-1β in STR was most likely not because of LPS stimulation. It is possible that IL-1β was stimulated by glucose or insulin, as previous research in horses showed that skeletal muscle and white blood cell messenger RNA (mRNA) expression of IL-1β was increased after 6 hours of simultaneous insulin and glucose infusions used to induce hyperinsulinemia [25]. Interestingly, glucose stimulates IL-1β synthesis in goat liver [37], mouse adipose tissue [38], and the pancreas of humans [39,40] and rats [41]. In the pancreatic β cells, high glucose concentrations stimulate IL-1β production, which enhances insulin production and secretion, as well as proliferation of β cells [42]. However, exposure to IL-1β for longer than 4–8 hours suppresses the insulin stimulating effects, and eventually IL-1β induces nitric oxide production and β-cell damage [43]. Thus, it appears that IL-1β has multiple effects. In the short term, it may help β cells adapt and respond to hyperglycemia by promoting insulin production; however, if exposure to IL-1β is prolonged, it can damage β cells and contribute to pancreatic dysfunction. In fact, IL-1β is implicated in the development of type II diabetes in humans [44], whereby administration of the IL-1β receptor antagonist (IL-1ra) is protective against β-cell destruction in vitro [45]. Our finding of elevated IL-1β concentrations in horses after consuming a high-starch meal is consistent with results demonstrated in other species, and it is possible that high postprandial glucose concentrations stimulated the increased plasma IL-1β concentrations.

In horses, IL-1β has been implicated in the development of laminitis, as mRNA expression was increased in laminar tissue during the early stages of black walnut extract–induced laminitis, but was not detected in hoof sections from nonlaminitic horses [46]. Furthermore, blood mRNA expression of IL-1β was correlated to obesity in a population of Thoroughbred mares [47]. Although none of those mares were laminitic, obesity is a predisposing factor for laminitis. In contrast, circulating IL-1β protein concentrations was not correlated to insulin, glucose, or BCS in a mixed-breed population of horses [8].

The influence of STR on postprandial inflammation was limited to changes in plasma IL-1β concentration. It was surprising that there was no effect of diet and that either treatment, whether CON or STR, increased TNF and IL-6 concentrations in horses with low initial levels of inflammation. Previous research has documented the wide range of plasma values of both TNF and IL-6 [8], but it is unclear why such a wide variation in plasma concentrations of TNF and IL-6 exists. It is also unclear why horses with divergent starting concentrations of cytokines responded differently to feed consumption. Furthermore, both cytokines exhibited increased plasma concentrations in response to 6 hours of sustained hyperinsulinemia [25]. It is possible that high levels of insulin drove the increase in TNF and IL-6 concentrations in the previous study and that sustained hyperinsulinemia is a component of their regulation. Also interesting is the lack of change in SAA concentrations, which was previously noted to correlate with BCS and insulin in a large population of mixed-breed horses [8] and to increase significantly, as expected, after IV LPS administration [17]. It is possible that the minimal change in LPS was not great enough to stimulate SAA in the present study or that it is more closely tied to increased BCS than insulin.

5. Conclusions

Feeding high-starch diets to horses may promote mild increases in IL-1β and LPS. However, it is unlikely that LPS causes the rise in IL-1β, as the peak of LPS occurred later than that of IL-1β. The potential ability of glucose to stimulate IL-1β secretion should be investigated, and the role of IL-1β in etiologies of insulin resistance should be explored.

Acknowledgments

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