

# Feeding DigestaWell Buffer to horses neutralizes the effects of high starch diets on blood pH and inflammation

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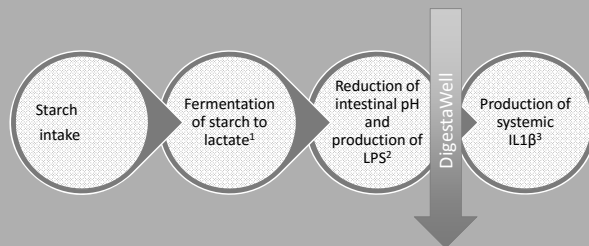
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## ABSTRACT

When fed to horses, high starch diets elevate plasma concentrations of lipopolysaccharide (LPS) at 2 hours post eating and IL-1 $\beta$  at 1 hour post eating. These changes are possibly due to rapid bacterial fermentation of starches and sugars in the digestive tract, which may alter the pH in the digestive tract and lead to inflammation. The purpose of this research was to investigate the efficacy of a dietary supplement containing bicarbonate, DigestaWell Buffer (DB), to mitigate these postprandial changes, as compared to control horses receiving the same concentrate but without the supplement (HS). Six mature light-breed geldings were used in a switchback design. Horses were fasted overnight prior to being offered a concentrate feed at 0800 hours that provided 1.2 g/kg bodyweight of nonstructural carbohydrates. The DB treatment supplied 150g of top dressed DB supplement. Plasma from blood was harvested at -30 minutes, 1, 2, 4, 6, and 8 hours post feeding. Whole blood was analyzed for pH and TCO<sub>2</sub>. Horses were offered *ad libitum* grass hay following completion of their concentrate meal. Concentrations of IL-1 $\beta$ , LPS, d-lactate, glucose, and insulin, and pH and TCO<sub>2</sub> values were analyzed by repeated measures ANOVA (SAS v. 9.3). Where necessary, values were log transformed and are presented as geometric means. Supplementation with DB reduced ( $p < 0.01$ ) postprandial concentrations of IL-1 $\beta$  at post-feeding hours 2 ( $18.4$  vs.  $23.5 \pm 1.6$  pg/mL), tended to decrease geometric mean d-lactate ( $p = 0.060$ ) concentrations at 8 hours post feeding ( $1215$  [1120-1318] vs.  $1457$  [1344-1581]  $\mu$ mol/L), and decreased mean LPS concentrations across all time points ( $p < 0.001$ ). Meal consumption reduced blood pH in both treatments, however pH was higher in DB than HS ( $7.414 \pm 0.003$  vs.  $7.398 \pm 0.003$ ) treated horses ( $p < 0.05$ ). Plasma glucose and insulin increased postprandially for both treatments ( $p < 0.001$ ) with no effect of DB treatment ( $p > 0.1$ ). Blood TCO<sub>2</sub> levels were below the upper limit of 37 mmol/L but tended to be higher in DB treated horses ( $31.4 \pm 0.3$  vs.  $30.6 \pm 0.3$  mmol/L,  $p = 0.068$ ). Given these findings, we believe that DB mitigates the negative effects of rapid starch and sugar fermentation in the equine digestive tract, as seen through reduced postprandial inflammation.

## HYPOTHESIS



## METHODS

- Six mature (6 – 16 years) geldings of light breeding (1 Thoroughbred, 1 Appaloosa, 1 Paint, 3 Quarter Horses) were used for this experiment.
- Horses weighed 453 – 491 kg and had a body condition score of 5 – 6.
- Beginning 24 hours prior to experimentation, horses were housed in individual 12'x12' box stalls with *ad libitum* access to water and hay.
- Hay was removed at 2000 hours on the day prior to testing and horses were fasted overnight.
- Horses assigned to the HB treatment received 150 g of DigestaWell Buffer top dressed onto a concentrate meal whereas horses assigned to the HS treatment received only concentrate.
- At 0800 hours on the day of testing, horses were offered a concentrate meal that provided 1.2 g nonstructural carbohydrates (NSC; where NSC = starch + ethanol soluble carbohydrates)/kg of bodyweight.
- Blood samples (10 mL into heparinized vacutainers) were collected at -0.5 (0730), 1 (0900), 2 (1000), 4 (1200), 6 (1400) and 8 (1600) hours post feeding. Blood was centrifuged for collection of plasma, and plasma was frozen at -20°C until analysis.
- Plasma glucose was analyzed using a colorimetric assay (Glucose Colorimetric Assay Kit, Caymen Chemical) according to the manufacturer's instructions.
- Plasma insulin was analyzed using an ELISA (Mercodia Equine Insulin ELISA, Mercodia) that has been previously validated for use in the horse.
- Plasma d-lactate was analyzed using a colorimetric assay (D-lactate assay kit, Eton Bioscience Inc.)
- Plasma IL-1 $\beta$  was analyzed using an ELISA (Equine IL-1 beta ELISA VetSet, Kingfisher Biotech, Inc.)
- Plasma LPS was analyzed using a previously validated assay (Pierce LAL Chromogenic Endotoxin Quantitation kit, Thermo Scientific).
- Whole blood pH and TCO<sub>2</sub> were analyzed using an auto-analyzer (VetStat® Electrolyte and Blood Gas Analyzer, IDEXX).
- Data were analyzed for repeated measures analysis of variance using the PROC MIXED procedure of SAS (v 9.3). The repeated term was horse within diet and the fixed effects were time and diet.
  - Glucose and insulin concentrations were log transformed to improve the normality and homogeneity of variance of the residuals. Means are represented as geometric means.

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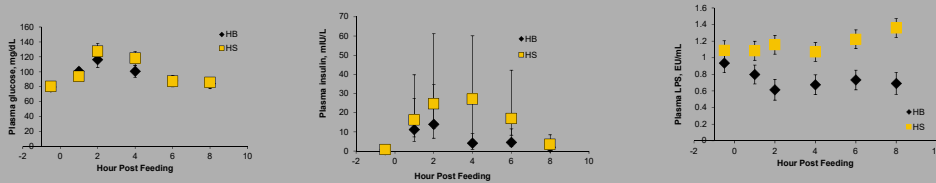
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## RESULTS AND CONCLUSIONS

- Plasma glucose was effected by time only ( $P < 0.001$ ), where both treatments had higher glucose at hours 1 ( $P = 0.01$ ), 2 ( $P < 0.001$ ), and 4 ( $P = 0.002$ ) post feeding.
- HB horses had lower insulin concentrations than HS horses at hours 1 ( $P = 0.004$ ), 2 ( $P = 0.010$ ), 4 ( $P = 0.069$ ), 6 ( $P = 0.012$ ), and 8 ( $P = 0.059$ ) post feeding (time x treatment interaction  $P < 0.001$ ).
- HB horses had lower LPS concentrations across the time points of the study ( $P < 0.001$ ).
- As expected the HB treatment did not influence the glycemic response to a consuming a high starch meal. However, horses did have a lower insulinemic response, and this is possibly related to the lower inflammatory response.<sup>4</sup>

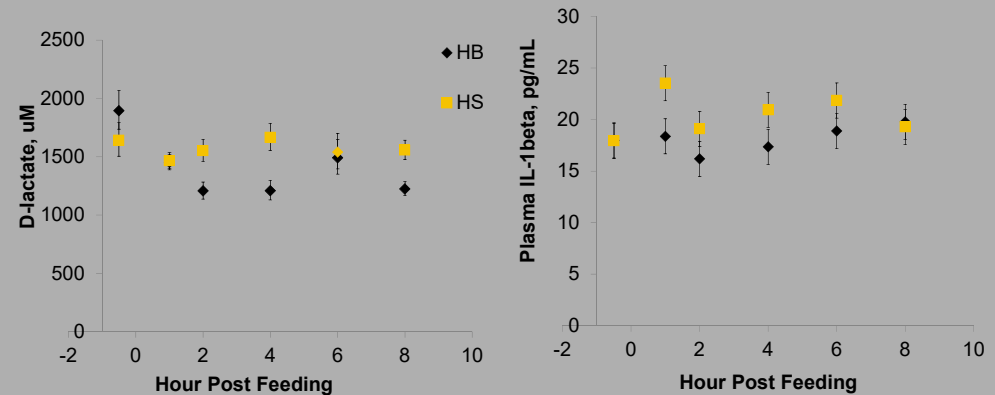


Mean blood pH and TCO <sub>2</sub> concentrations in horses fed DigestaWell (HB) buffer along with a high starch meal, or without the addition of buffer (HS)									
Treatment	Sampling Hour					SEM	TRT	P Values	
Treatment	-0.5	1	2	4	6			Hour	TRT*HOUR
Blood pH									
HS	7.43	7.41	7.38*	7.39	7.38*	0.001	0.012	0.011	0.240
HB	7.43	7.41	7.41	7.41	7.41				
TCO <sub>2</sub>									
HS	32.67	31.48	29.72	28.84**	30.53	0.600	0.068	0.056	0.081
HB	31.60	31.33	31.04	31.58	31.62				

\*  $P < 0.05$ , \*\*  $P < 0.01$

## RESULTS AND CONCLUSIONS

- HB horses had lower lactate concentrations than HS horses at 2 ( $P = 0.018$ ), 4 ( $P = 0.010$ ), and 8 ( $P < 0.001$ ) hours post feeding (time x treatment interaction  $P = 0.022$ ).
  - This indicates that DigestaWell buffer promoted a less acidic environment in the digestive tract.
- HS horses had higher IL1 $\beta$  concentrations than HB horses across all time points ( $P = 0.036$ ).
  - This finding suggests that DigestaWell buffer reduced post-prandial inflammation in horses, possibly through a more neutral digestive tract or physiological environment.



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