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**Authors**

Johnson, Alexa CB

Rossow, Heidi A

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# Effects of two equine digestive aid supplements on hindgut health<sup>1</sup>

Alexa C. B. Johnson and Heidi A. Rossow<sup>2,•</sup>

Department of Population Health and Reproduction, SVM VMTRC University of California–Davis,  
Tulare, CA 93274

**ABSTRACT:** Gastrointestinal disease is the number one killer of horses. Little is known about the maintenance of microbes in the equine hindgut and how to distinguish a healthy gut in a live horse. Utilization of internal and external digestibility markers and starch fermentation has been extensively studied in ruminants and is the basis for research conducted on horses. The aims of this study were to investigate the effects of two equine feed digestive aid supplements on hindgut health (HGH) as reflected in fecal pH and digestibility and to compare and validate DM digestibility measurements through the use of internal and external markers such as chromium oxide (CR), lignin (Lig), indigestible ADF (iADF), indigestible NDF (iNDF), and indigestible lignin (iLig). Nine mature Quarter horses (six geldings, three mares) were used in a crossover design, three feeding periods of 17 d (51 d total), using three treatments: control, no feed additive (CON), Smartpak (SP; Plymouth, MA), or Platinum Performance (PP; Buellton, CA). Both SP and PP contained a strain of *Lactobacillus*, whereas SP further supplied mannanoligosaccharides (MOS) and fructooligosaccharides (FOS) and PP supplied *Saccharomyces*

*boulardii*. Within the 17-d period, horses were offered orchard grass hay and sweet cob grain and the assigned treatment daily and four CR cookies to deliver 8 g/d of CR for the last 7 d of each period. Total feces were collected from 15 to 17 d. Feed and fecal samples were dried, ground, and sent to ANALAB (Fulton, IL) for nutrient analysis. Duplicate samples of feed and feces were placed in ruminally cannulated cows for in situ determination of iADF, iNDF, and iLig to estimate digestibility. Estimated CR fecal output, CR DMI, and DM digestibilities were evaluated using the root mean square prediction error percentage of the observed mean (RMSPE), concordance correlation coefficient (CCC), and Nash–Sutcliffe efficiency methods. Marker predictive ability tests showed iADF to have the least amount of bias with the smallest RMSPE (4%), largest CCC (0.43), and the largest amount of random bias (error of dispersion = 0.45). Supplementation of PP decreased CR DM digestibility ( $P < 0.02$ ). Smartpak increased fecal pH ( $P < 0.09$ ), but PP had no effect on fecal pH. Therefore, SP had a beneficial effect on HGH that is believed to be due to MOS and FOS.

**Key words:** apparent digestibility, equine supplement, fecal pH, hindgut health

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<sup>2</sup>Corresponding author: [heidi.rossow@gmail.com](mailto:heidi.rossow@gmail.com)

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## INTRODUCTION

Modern feeding practices of two to three meals per day of starch-based concentrates disrupt normal digestion and are associated with

gastrointestinal (GI) disorders such as colic, laminitis, and lower gut acidosis (Biddle et al., 2013). Overfeeding starchy concentrates could induce these disorders. To combat the effects of starch overload and prevent a GI disorder, 84% of owners feed dietary supplements to improve health (Murray et al., 2015). Most digestive aid supplements are multiple bacterial strain preparations, but the majority of research has evaluated single-strain preparations (Stratton-Phelps, 2008). Multistrain supplements typically deliver yeast, bacteria, mannanoligosaccharides (MOS), and fructooligosaccharides (FOS). They also improve hindgut health (HGH) by decreasing the number of lactic acid producing bacteria and promoting lactate-utilizing bacteria (Medina, 2003; Respondek et al., 2008). Multiple strain supplements more efficiently stabilize the entire hindgut compared with a single-strain supplement by targeting different aspects of HGH such as fecal pH, which then affects digestibility. A surplus of lactic acid causes the hindgut to reach subclinically acidic levels (fecal pH < 6.2) that results in disrupted digestion (Richards et al., 2006).

To assess the impact of supplements on HGH, fecal pH, and apparent digestibility measurements can be made. Total fecal collections (TFC) to determine digestibility can be expensive and labor intensive. An alternative method to evaluate digestibility is to measure indigestible internal and external markers in the feed and feces.

The aims of this study were to investigate the effects of two digestive aid supplements Smartpak

(SP; Plymouth, MA) and Platinum Performance (PP; Buellton, CA) on HGH as reflected in fecal pH and digestibility as well as to compare and validate digestibility measurements of nutrients through the use of internal and external markers such as TFC, chromium oxide (CR), lignin (Lig), indigestible ADF (iADF), indigestible NDF (iNDF), and indigestible lignin (iLig).

## MATERIALS AND METHODS

This experiment was approved by the University of California, Davis Animal Care and Use Committee.

Nine mature Quarter horses (six geldings, three mares) were used in a crossover design. Horses were randomly assigned the following treatments: control, no feed additive (CON), SP, or PP. Smartpak is a mixture of MOS, FOS, and *Lactobacillus acidophilus* and delivered in a pelleted form. Platinum Performance is a mixture of *Saccharomyces boulardii* and *Lactobacillus delbrueckii* and delivered in a powder form. Horses were fed orchard grass hay split evenly into two feedings twice daily at 0700 and 1700 h (Table 1). Water and salt blocks were provided ad libitum. Horses were also fed sweet cob grain with hay and assigned supplement for 17 d per treatment (Table 2). Hay and grain offered and refused was weighed and recorded daily to determine total DMI.

The first 14 d of each feeding period served as an acclimation/washout period. Beginning at 10 to

**Table 1.** Description of horse subjects

HID <sup>1</sup>	Sex <sup>2</sup>	Age, y	Order <sup>3</sup>	Initial BW, kg	Initial BCS	Final BW, kg	Final BCS
1	G	6	1 <sup>4</sup>	538	7	513	6.5
2	G	14	1	611	7	589	5
3	G	12	2 <sup>5</sup>	601	8	538	6
4	G	24	2	468	6.5	460	5
5	G	14	3 <sup>6</sup>	546	5	533	4.5
6	G	17	3	532	6	505	5
7	M	10	4 <sup>7</sup>	576	5.5	537	5
8	M	7	5 <sup>8</sup>	516	5.5	461	5
9	M	10	6 <sup>9</sup>	434	5.5	454	5

<sup>1</sup>Horse identification number.

<sup>2</sup>Sex is defined as gelding (G) or mare (M).

<sup>3</sup>Order describes the order that horses were rotated between treatments between feeding periods: Smartpak (SP), Platinum Performance (PP), and Control (CON).

<sup>4</sup>SP, CON, PP.

<sup>5</sup>PP, SP, CON.

<sup>6</sup>CON, PP, SP.

<sup>7</sup>PP, CON, SP.

<sup>8</sup>CON, SP, PP.

<sup>9</sup>SP, PP, CON.

**Table 2.** Nutrient composition of hay and grain

DM, %	Orchard grass hay <sup>1</sup>	Grain <sup>2</sup>
DM	90.68	95.61
CP	11.61	9.37
ADF	36.79	3.32
NDF	58.95	11.42
Fat	2.11	3.5
Lignin	3.99	—
Ash	8.08	0.95
Ca	0.42	0.09
P	0.24	0.21
Mg	0.17	0.11
K	2.97	0.69
S	0.26	0.15
Na	0.07	0.01
Cl	0.81	0.32
Fe, ppm	69	49
Cu, ppm	6	2
Mn, ppm	64	17
Zn, ppm	32	20

Horses were fed a fixed rate of 4 kg ( $\pm$  2 kg) of orchard grass hay sweet cob grain (corn, oats, and barley) fed at 0.4 kg ( $\pm$  0.2 kg) to administer treatments (Farmers Best, Keyes, CA).

17 d, horses were dosed with four CR cookies to deliver 8 g/d of chromium oxide (Cr<sub>2</sub>O<sub>3</sub>). The CR cookies were made by creating a batter of 0.35 L of unsweetened applesauce with 128 g of oat bran cereal and 64 g of all-purpose flour. The batter was then pressed into a mini muffin tin, and 2 g of CR was placed into the center of the batter, and more batter pressed on top to seal CR powder inside the cookie and baked at 375 °C for 30 min.

### Animal Housing

The study was conducted at the College of the Sequoias Equine Science Facility in Tulare, CA, from July to August 2017. Horses were housed in individual stalls (average size of 5 m  $\times$  7 m) with fir shavings (Superior Soils, Hanford, CA). Horses, three at a time, were provided 2 h of socialization in a dirt arena three times a week. Prior to the study, horses were in consistent light work and housed in irrigated pasture with twice daily feedings of alfalfa hay.

### Data Collection

Fecal scores were recorded daily on a scale of 1 to 5 with 1 defined as osmotic liquid diarrhea; 2 as mild diarrhea, feces are semiliquid but have some solid component; 3 as normal manure with soft consistency; 4 as normal feces in well-formed fecal balls; and 5 as very dry small fecal balls (procedure

modified from [Rodrigues et al., 2013](#)). The daily high temperature and ambient barn temperature were recorded at 0700 and 1700 h. Body weights were monitored on a weekly basis using the average of two weight-monitoring methods: the Coburn Horse weigh tape (Coburn Co., Whitewater, WI) and a formula by [Carroll and Huntington \(1988\)](#), which uses heart girth and body length measurements in centimeters to estimate weight in kilo-

$$\text{grams: weight (kg)} = \frac{\text{girth}^2(\text{cm}) \times \text{length}(\text{cm})}{11,877}$$

Body condition scores were recorded on a weekly basis following the Henneke scoring system ([Henneke et al., 1983](#)).

### Total Fecal Collections

Stalls were stripped of all shavings, and horses were blocked off from outside runs at 1700 h for 14 d for TFC from 15 to 17 d. A 3-day collection period was used based on data from [Swyers et al. \(2008\)](#) and [Van Weyenberg et al. \(2005\)](#) who claim that 95% of digesta passes by 65 h post-feeding. Total feces were collected and weighed twice a day for 3 days. Urine and hair contamination was unavoidable, but a 10% subsample of noncontaminated feces was collected from each fecal collection in a gallon bag per horse. Sample DM was determined by drying the subsample in a forced-air oven at 60 °C for 48 h. Feces were ground through a 1-mm screen (Wiley mill, Arthur Gill Thomas Co., Swedesboro, NJ) and pooled by horse and period.

### Nutrient Analyses

Fecal and feed samples were analyzed for nutrient analysis by Analab (Fulton, IL) for DM, ADF, NDF, CP, fat, ash, and Lig (American Association for Analytical Chemists reference methods 935.29, 973.18, 2002.04, 990.03, 920.39, 942.05, and 973.18, respectively).

### Fecal pH

Fecal samples were collected rectally on 15 d at 0600 h. This time point was chosen based on results from [Swyers et al. \(2008\)](#) who found the lowest fecal pH at 14 h post-meal. The rectal sampling method was chosen because [Næsset and Austbø \(2010\)](#) discovered a difference in pH values of feces when collected from the ground and from the rectum and concluded the rectally obtained samples obtained a truer pH reading. The first rectal fecal grab was

discarded as contaminated, and the second grab was mixed with 20 mL of distilled water, and a pH reading was taken within 10 min of collection with a Hanna pH and ec combo meter (HI98129, Woonsocket, RI) calibrated at 0500 h with pH 4.0, 7.0, and 10.0 standard solutions.

### *In Situ Determination of Digestibility Using iADF, iNDF, and iLig*

Apparent DM digestibility was estimated using Lig, iADF, iNDF, and iLig concentrations in the diet and feces using the equations formulated for ruminants by [Huhtanen et al. \(1994\)](#) and [Bargo et al. \(2002\)](#) based on methodology presented in [Miraglia et al. \(1999\)](#) and [Smolders et al. \(1990\)](#). Ground feed and fecal samples (15 g) from TFC were placed in a rumen incubation bag (10 × 20 cm, 50 microporosity, ANKOM Technology, Macedon, NY), double sealed on each edge with an impulse sealer (AIE-200, Industry, CA), and ruminally incubated in two rumen fistulated dairy cows for 21 d. After incubation, bags were removed from the rumen, rinsed with cold tap water for 30 min, and dried in a forced-air oven at 60 °C for 24 h. Dry bags and residues were weighed, and DM disappearance was corrected for bag changes during digestion by subtracting weights of digested, blank bags ([Acetoze et al., 2018](#)). The incubated sample residues were pooled by horse and period and analyzed for ADF, NDF, and Lig to estimate iADF, iNDF, and iLig ([Huhtanen et al., 1994](#)). Fecal output (FO), DMI, and digestibility were then used to estimate individual horse feed intake according to the equations described in [Bargo et al. \(2002\)](#).

### *Determination of FO Using CR and Digestibility Using Lig, iADF, iNDF, and iLig*

Fecal output was predicted using the indigestible fecal marker CR and was calculated based on the assumption that CR intake had equilibrated with CR excretion after 7 d using the following equation ([Galyean et al., 1988](#)):

$$FO (g) = \frac{Cr \text{ consumed (g/d)}}{Cr \text{ in feces (g/g·DM)}}$$

DM digestibility was calculated for CR, Lig, iADF, iNDF, and iLig using the following equation:

$$DM \text{ digestibility \%} = 100 - 100 \times \left[ \frac{\% \text{ marker in feed}}{\% \text{ marker in feces}} \times \frac{\% \text{ DM in feces}}{\% \text{ DM in feed}} \right]$$

### *Statistical Analysis*

Individual horse was the experimental unit of interest because all horses were fed and housed separately. All digestibility statistical analyses were performed using R statistical software (R Core Group, Vienna, Austria). All treatment statistical analyses were performed using the MIXED procedure of SAS (v 9.4; SAS Inst., Inc., Cary, NC). The model used for the analyses was  $Y_{pnrs} = \mu + B_p + T_n$  or  $T_n \times A_r$  or  $T_n \times G_s + e_{pnrs}$  where  $Y_{pnrs}$  = response variables fecal pH, fecal score, fecal output, DM digestibility for each marker (CR, Lig, iADF, iNDF, and iLig);  $\mu$  = overall mean;  $B_p$  = horse where p = horse ID (HID) 1 to 9;  $T_n$  = effect of treatment where n = CON, SP, or PP;  $T_n \times A_r$  is the interaction term used for fecal pH with treatment by age (r = age in years);  $T_n \times G_s$  is the interaction term for fecal pH with treatment by sex (s = mare or gelding);  $e_{pnrs}$  = residual error. For fecal pH, the interaction terms  $T_n \times A_r$  and  $T_n \times G_s$  were used in separate analyses and were not used for any other response variables because age and sex did not have an impact ( $P > 0.05$ ). Treatment order, BW, and BCS had no effect on results ([Table 1](#)). A  $P$  value of  $\leq 0.05$  was considered significant, and a  $P$  value of  $> 0.05$  but  $\leq 0.10$  was considered as a trend.

### *Marker Prediction Evaluations Using Root Mean Square Prediction Error, Nash–Sutcliffe Efficiency, and Concordance Correlation Coefficient*

Digestibility analytical methods were performed via methods utilized by [Johnson et al. \(2016\)](#). Three methods were used to evaluate the markers: the root mean square prediction error (RMSPE), the Nash–Sutcliffe efficiency (NSE), and the concordance correlation coefficient (CCC).

The RMSPE was decomposed into the error of central tendency (ECT), the error due to regression, and the error of dispersion (ED) as described by [Bibby and Toutenburg \(1977\)](#). Guidelines proposed by [Reed et al. \(2015\)](#) were utilized when interpreting prediction error values. [Reed et al. \(2015\)](#) states that an RMSPE as a percentage of the mean observed value below 25% is “acceptable” and an RMSPE below 10% of the mean is considered “good.” An ED bias larger than 5% of the total error is considered a deviation from the desired random error.

The NSE is a normalized statistic that determines the relative magnitude of residual variance compared with the measured data variance ([Nash and Sutcliffe, 1970](#)). Nash–Sutcliffe efficiencies range from negative infinity to 1. When the NSE is

less than zero, the observed mean is a more accurate predictor than the model; when NSE closer to 1, the predictor is a perfect match modeled to the observed data.

The CCC was calculated using the *epi.ccc* function in the *epiR* package (Stevenson et al., 2014) in R statistical software (R Core Group). The CCC combines measures of both precision and accuracy to determine how far the observed data deviate from the line of perfect concordance (a line at 45° on a square scatter plot) with values close to 1 indicating agreement between observed and predicted (Lin, 1989, 2000). Like the RMSPE, the CCC can be decomposed to estimate a scale or slope shift ( $v$ ), location bias ( $u$ ) relative to scale shift, and a bias correction factor ( $C_b$ ) where values closer to 0 indicate less bias for  $v$  and  $u$  and values close to 1 indicate little to no deviation of the best fit line from the 45° line.

## RESULTS AND DISCUSSION

Horses are most susceptible to lower GI diseases during a sudden change of feed and routine. Accordingly, the horses on the current trial were probably stressed during enrollment to the trial when they were transitioned from irrigated pasture to orchard grass hay and exercise was stopped. Supplementation of SP and PP are expected to maintain HGH by buffering the GI system, stabilize the GI microflora populations, and subsequently maintain normal digestion. Horse ID 9 experienced a mild colic episode during the adaptation period of CON treatment, and HID 3 exhibited mild sounds of founder during the adaptation period of SP treatment (Table 1). Both horses appeared healthy and normal prior to the start of and during the TFC and were kept in the study.

Furthermore, during the course of the trial, horses lost weight due to the reduced workload and so lost muscle. The removal of exercise and subsequent weight loss may affect digestibility results in the present study. Weight loss was managed by offering varying levels of hay and grain (Table 1). Only horse HID 9 gained weight due to reduced competition for feed.

### *Effects of Supplements on Fecal pH*

The highest fecal pH was observed on the SP treatment, and the lowest fecal pH was observed on the CON treatment. These results show that SP and PP supplementation do increase fecal pH. Fecal pH probably has a tendency to be higher with SP

supplementation due to the presence of MOS and FOS that PP and CON do not contain (Table 3). In a dog study, MOS increased fecal pH ( $P = 0.088$ ), whereas FOS did not (Swanson et al., 2002). When MOS and FOS were supplemented to horses, humans (Finnie et al., 1995), and swine (Tsukahara et al., 2002), it was shown to regulate microbial dysbiosis. Von Engelhardt et al. (1989) speculates that naturally occurring short-chain fatty acids that make up FOS cannot affect fecal pH due to rapid absorption by microbes in the stomach before it may reach the hindgut and affect fecal pH. Platinum Performance contains yeast that has the ability to reduce cecal and colonic lactic acid bacteria and lead to more near-normal pH values in the hindgut, but PP was not able to increase fecal pH enough to impact HGH (Table 3; Morgan et al., 2007). Due to the small particle size of PP and top dressing of the grain, PP fines could have been sifted and altered the amount of treatment ingested by the animal even though no refusals were observed.

When horses were on the CON treatment, younger animals had a more neutral fecal pH than older animals ( $P < 0.02$ ). However, when horses were treated with PP, the opposite was observed. As age increased, fecal pH increased. There was no clear association between age and SP probably due to differing equine microbiome profiles between animals and differences in utilization of SP ingredients by different microbial profiles. Age has not been shown to have an effect on fecal pH in other studies such as Williamson et al. (2007) that observed horses 4 to 14 y, whereas this study observed horses 6 to 24 y.

Fecal pH was different by sex ( $P < 0.02$ ) with geldings (7.05) having a higher pH than mares (6.8). Mare fecal pH was observed to be highest on CON and decreased with SP and PP supplementation with the exception of HID 9. Deviation from the pattern shown by HID 9 could be due to the mild colic episode and being the only horse to gain weight throughout the trial. A significant relationship between sex and fecal pH has not been previously observed by other studies such as Williamson et al. (2007) who found the average fecal pH for males to be 6.41 and 6.46 for females ( $P < 0.4$ ). Fecal scores and total FO were not affected by treatment, age or sex.

### *HGH Assessment Using Fecal pH*

This study found, in agreement with Van den Berg et al. (2013), that measuring fecal pH can accurately assess HGH and is best used within horse but

**Table 3.** Treatment effects on fecal attributes and digestibility

	CON <sup>1</sup>	SP <sup>2</sup>	PP <sup>3</sup>	SEM	Treatment	Age × Trt <sup>4</sup>	Sex × Trt <sup>5</sup>
					<i>P</i> value	<i>P</i> value	<i>P</i> value
Fecal attributes							
Fecal pH	6.89 <sup>b</sup>	7.03 <sup>a</sup>	6.99 <sup>ab</sup>	0.08	0.2	0.02	0.02
Fecal score <sup>6</sup>	2.94	2.97	2.97	0.07	0.9	—	—
FO <sup>7</sup> , kg/d	2.61	2.75	2.64	0.10	0.6	—	—
DM digestibility, % <sup>8</sup>							
TFC	44.7	41.0	43.6	2.6	0.4	—	—
CR	84.6 <sup>a</sup>	84.0 <sup>a</sup>	81.4 <sup>b</sup>	0.9	0.04	—	—
Lig	91.5	91.1	91.1	0.52	0.64	—	—
iADF	81.0	80.6	80.1	1.17	0.7	—	—
iNDF	76.7	76.2	75.7	1.3	0.7	—	—
iLig	71.8	69.8	69.9	2.5	0.7	—	—

Superscripts within row indicates least square means differences of treatments  $P < 0.05$ .

<sup>1</sup>Control, no feed additive.

<sup>2</sup>Smartpak.

<sup>3</sup>Platinum Performance.

<sup>4</sup>The interaction of age and treatment.

<sup>5</sup>The interaction of sex and treatment.

<sup>6</sup>Fecal scores were recorded daily on a scale of 1 to 5 with 1 = osmotic liquid diarrhea; 2 = mild diarrhea, feces are semiliquid but have some solid component; 3 = normal manure with soft consistency; 4 = normal feces in well-formed fecal balls; and 5 = very dry small fecal balls.

<sup>7</sup>Total fecal output.

<sup>8</sup>DM digestibility measurements were made on total fecal collections (TFC), chromium oxide (CR), lignin (Lig), indigestible ADF (iADF), indigestible NDF (iNDF), and indigestible lignin (iLig).

may not be sufficient between horses. This conclusion is further confirmed due to large interanimal differences in fecal pH that appeared to be affected by both sex and age.

Due to experimental design, hindgut acidosis as indicated by fecal pH, was not observed in this study because of the small portion of commercially processed concentrates as well as twice daily feeding of hay. Commercial feeds typically contain processed grains that have been ground, rolled, or pelleted to facilitate quicker and easier digestion by the gastrointestinal tract (GIT) microbes (Julliand et al., 2006). When long-stem forages are offered twice daily rather than once daily, mastication and saliva production will be increased to provide a buffering effect in the GIT (Nicol et al., 2002). Treatment could not be evaluated during an acidotic event, but we were able to assess the supplements' ability to affect DM digestibility to establish a healthier hindgut environment.

### ***Effects of Supplements on Apparent Digestibility***

Apparent CR DM digestibility was decreased with both SP and PP supplementation, but PP supplementation decreased digestibility the most ( $P < 0.02$ ) when compared with CON (Table 3). Digestibility was expected to decrease with SP supplementation because studies by Gürbüz et al. (2010)

and Swanson et al. (2002) found that MOS and FOS supplementation decreased DM, ADF, and NDF digestibility. However, in this study, digestibility was increased with MOS and FOS (Table 3). In addition, it was expected that PP would result in increased DM, iADF, and iNDF digestibilities due to the presence of yeast, but this was also not observed in the present study. In fact, digestibility was decreased (Table 1). Increased ADF digestion after yeast supplementation has been observed by Jouany et al. (2008) who suggested this may be due to the stimulation of microbial cellulolytic activity in the hindgut. They also speculated a possible mechanism of ingested live yeast that survives transit to the hindgut and results in increased fiber digestibility that has been reported to be 7% (Agazzi et al., 2011).

According to research conducted by Weese and Martin (2011), digestive aid supplements are considered to be nutraceuticals, not pharmaceuticals, so there is minimal quality control. They also evaluated commercial digestive aid supplements in both human and veterinary medicine and found that the products often misrepresent the contents by not containing claimed organisms, the presence of additional unlabeled organisms, or markedly lower concentrations of organisms than stated. Lower concentrations of organisms that reported could be a function of either poor processing or poor survival after processing.

Alternatively, PP may have not produced results as expected due to the use of *S. boulardii* when the majority of studies examining the effects of yeast on digestion have primarily been conducted using *Saccharomyces cerevisiae* (Hossain et al., 2014). It is plausible that *S. boulardii* does not have the same digestibility properties as seen in *S. cerevisiae*. Findings from newer metabolomics tools show that *S. boulardii* has a unique clustering of metabolomic and genetic characteristics compared with *S. cerevisiae*. These differences have spurred a debate on whether *S. boulardii* should be reclassified as a separate species or stay as a subspecies of *S. cerevisiae* (McFarland, 2010). Further research will need to be conducted to elucidate the relationship between *S. boulardii* and *S. cerevisiae*.

### ***HGH Assessment Using Apparent Digestibility***

Although supplementation of SP and PP decreased digestibility, PP had a larger negative effect on digestibility than SP. The decreased digestibility observed from PP did not affect the animals on this study. However, it may affect other animals more severely based on age, sex, or health state of the animal. Disrupted feed digestion and fermentation is harmful to the horse because they depend on the fermentation of feedstuffs by microbes in the cecum and colon to produce short-chain VFA that can contribute up to 80% of the horse's energy requirements (Al Jassim and Andrews, 2009; Milinovich et al., 2006). Furthermore, disrupted normal feed fermentation may cause the chain reaction of increased microbial death that could decrease fiber digestion and cell lysis (Garner et al., 1975; Richards et al., 2006; Swyers et al., 2008).

### ***Assessing Chromium Oxide Digestibility Predictive Ability***

The average TFC digestibility for this study was 43% ( $\pm 1.24$ ) and aligns with TFC DM digestibility values reported by multiple other authors (Karlsson et al., 2000; Bush et al., 2001; Swyers et al., 2008), but others have also reported apparent DM digestibility 20% units higher (Moore and Dehority, 1993; Jouany et al., 2008). The large range of DM digestibility values reported by literature could indicate variations in sampling methods or reflect different diets. In this study, all apparent DM digestibility marker predictions were compared with CR values. Marker DM values may be better compared with CR because King and Moore (1957) suggested that CR is adsorbed to roughage particles and moves

with the fibrous portion in the GIT, which may have been enhanced by our method of incubating samples in fistulated cows. It was believed that these equations could be used for measuring apparent DM digestibility using equine feces because the ratio of indigestible marker fraction in feed and feces will not change regardless of what animal the markers came from or where they are incubated. Furthermore, Kern et al. (1973, 1974) determined that the bacterial populations of the rumen and the equine cecum are similar; therefore, sample digestion would have been similar regardless if digested in rumen fluid or cecal fluid.

This study set the threshold for an "acceptable" RMSPE of the observed mean is <25%, and CR FO is RMSPE of the observed mean was 26%. The majority of the bias was found in the ECT showing on average an overprediction of FO via CR. Haenlein et al. (1966) observed the same pattern in his study that CR overpredicted FO and thought this may be due to diurnal excretions and mean recovery rates. Diurnal excretion of CR in horses and ruminants can cause the mean recovery of CR to range between 59.8% and 134.8% (Raymond and Minson, 1955; Haenlein et al., 1966). This study had an 89% mean recovery of CR, which is adequate. In other studies, when fecal samples were collected twice a day, CR recovery was 81.5% (Knapka et al., 1967), and when samples were collected 12 times a day, CR recovery was 115% (Holland et al., 1998).

### ***Assessing Internal and External Marker Predictive Abilities***

The intake marker with the highest mean recovery and the best predictor of apparent DM digestibility was iADF. Indigestible ADF had the lowest RMSPE, as a percentage of the observed mean (4%), with majority of the bias in the ED (0.45) and the largest CCC (0.43; Table 4). Studies conducted by Bargo et al. (2002), Acetoze et al. (2018), and Maulfair et al. (2011) state that iNDF should be the more accurate marker to determine apparent digestibility due to higher levels of NDF present in the diet. However, none of these studies assessed iNDF predictive ability. When iNDF is evaluated by our methods, it follows closely behind iADF as a good predictor. The majority of bias lies in the ECT, which reduces confidence that iNDF, is a good predictor of apparent DM digestibility. All internal markers showed the same trend in digestibility values as seen in CR DM digestibility, which is that PP had the lowest digestibility regardless of what marker was used.



**Table 4.** Evaluation of DM digestibility when compared with CR DM digestibility results using the root mean square prediction error (RMSPE)<sup>1</sup>, concordance correlation coefficient (CCC)<sup>2</sup>, and the Nash–Sutcliffe efficiency (NSE) method and their decompositions

	$R^2$	Mean recovery	RMSPE, g	RMSPE % of	ECT	ER	ED	CCC	$\nu$	$u$	$C_b$	NSE
		%		observations mean								
Lig <sup>3</sup>	0.59	187.3	8.40	0.10	0.89	0.005	0.10	0.07	0.42	3.64	0.12	-5.28
iADF	0.61	83.8	3.93	0.04	0.50	0.04	0.45	0.43	0.85	-0.9	0.71	-0.4
iNDF	0.64	68.2	7.68	0.09	0.87	0.02	0.11	0.19	0.95	-2.2	0.29	-4.24
iLig	0.59	56.9	13.7	0.16	0.87	0.09	0.04	0.11	1.79	-2.9	0.19	-15.7

<sup>1</sup>RMSPE decomposes into the error of central tendency (ECT), the error due to regression (ER), and the dispersion error (ED).

<sup>2</sup>The CCC decomposes into the scale shift ( $\nu$ ), location shift ( $u$ ), and the bias correction factor ( $C_b$ ).

<sup>3</sup>Lig = lignin; iADF = indigestible ADF; iNDF = indigestible NDF; iLig = indigestible lignin.

## CONCLUSIONS

This study found that SP increased fecal pH and did not affect digestibility values due to the presence of MOS and FOS. Supplementation with PP, which contained a yeast strain, resulted in decreased CR DM digestibility and had little effect on fecal pH. The presence of MOS and FOS in SP optimized HGH by increasing the amount of feed available to GI microbiota. Supplementation with PP could be interpreted as more harmful to the horse due to decreased digestibility and reduced fecal pH. Yeast supplementation was expected to have a favorable effect on HGH, which was not observed in the present study with PP. Internal and external markers evaluated for predictive ability of apparent TFCDM digestibility could accurately predict apparent CR DM digestibility but did not reflect TFC. When markers were evaluated against CR DM digestibility values, iADF was the least biased marker.

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