

# Influence of diet fortification on body composition and apparent digestion in mature horses consuming a low-quality forage

Mattea L. Much,<sup>\*,1,◉</sup> Jessica L. Leatherwood,<sup>\*</sup> Jennifer L. Zoller,<sup>\*</sup> Amanda N. Bradbery,<sup>\*</sup> Rafael E. Martinez,<sup>\*</sup> Abby D. Keegan,<sup>†</sup> Emily D. Lamprecht,<sup>†</sup> and Tryon A. Wickersham<sup>\*,◉</sup>

<sup>\*</sup>Department of Animal Science, Texas A&M University, College Station, TX 77843; and <sup>†</sup>Cargill Incorporated, Elk River, MN 55330

**ABSTRACT:** Stock-type mares ( $498 \pm 9$  kg BW;  $12 \pm 7$  yr) were used in a completely randomized design for 56 d to test the hypothesis that concentrate fortification improves apparent digestion and enhances lean mass over the topline. Horses were stratified by age, BW, and BCS and randomly assigned to either a custom pelleted concentrate (CON;  $n = 13$ ), or an iso-caloric, iso-nitrogenous pellet that included amino acid fortification, complexed trace minerals, and fermentation metabolites (FORT;  $n = 10$ ). Concentrate was offered at a total 0.75% BW/d (as-fed) twice daily, and diets were designed to meet or exceed maintenance requirements for mature horses. Horses had ad libitum access to Coastal bermudagrass hay (7.4% CP, 67% NDF, and 40% ADF). Every 14 d BW and BCS were recorded, and ultrasound images were captured every 28 d. *longissimus dorsi* area (LDA) and subcutaneous fat thickness (FT) were measured between the 12th and 13th ribs (12th/13th) and 17th and 18th ribs (17th/18th). Intramuscular fat at the 17th/18th ribs and rump fat-thickness were also obtained. Horses were dosed with 10 g/d of titanium dioxide ( $\text{TiO}_2$ ) for 14 d to estimate forage dry matter intake (DMI). To account for

diurnal variation, fecal samples were collected twice daily at 12-h intervals during the last 4 days, advancing by 3 h each day to represent a 24-h period. Fecal samples were composited by horse and analyzed for  $\text{TiO}_2$  to estimate fecal output and acid detergent insoluble ash was used to calculate forage DMI. To evaluate body composition, horses were infused with a 0.12 g/kg BW deuterium oxide ( $\text{D}_2\text{O}$ ) on d 0 and 56. Body fat percentage (BF) was determined by quantifying  $\text{D}_2\text{O}$  in plasma samples collected at pre- and 4-h postinfusion via mass spectrometry. All data were analyzed using PROC MIXED (SAS v9.4). The model contained a fixed effect of diet; horse (diet) was a random effect. Horses receiving FORT gained 17th/18th FT ( $P < 0.01$ ) and increased 17th/18th LDA from d 0 to 56 ( $P < 0.01$ ) while 17th/18th FT and LDA were unchanged in CON. Regardless of diet, BF estimated by  $\text{D}_2\text{O}$  infusion increased in all horses from d 0 to 56 ( $P < 0.01$ ). Average hay DMI was 2.1% BW, but did not differ between diets. In this study, concentrate fortification did not significantly ( $P \geq 0.27$ ) affect apparent digestion. In conclusion, concentrate fortification may promote greater muscle development along the posterior topline.

**Key words:** deuterium oxide, digestibility, equine, forage, titanium dioxide, ultrasonography

© The Author(s) 2019. Published by Oxford University Press on behalf of the American Society of Animal Science.

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact [journals.permissions@oup.com](mailto:journals.permissions@oup.com)

Transl. Anim. Sci. 2020.4:1–9  
doi: 10.1093/tas/txz137

<sup>1</sup>Corresponding author: [matteasendgraff@tamu.edu](mailto:matteasendgraff@tamu.edu)

Received April 22, 2019.

Accepted August 16, 2019.

## INTRODUCTION

Forage is the primary component of the equine diet; however, variation in quality and consistency of available stored forages provides a challenge when developing a nutritional program. Enhancing the diet with key nutrients and feed additives may provide a layer of protection against forage low in nutritive value and improve the health and performance of mature horses. Adding lactic acid-producing bacteria (*Lactobacillus*, *Bifidobacterium*, and *Enterococcus*) to a diet benefits the horse by improving mineral digestibility (Swyers et al., 2008) and tends to improve NDF and ADF digestion (Coverdale et al., 2013). When forage low in nutritive value was offered to mature horses (8.1% CP, 75.3% NDF, and 37.6% ADF), Morgan et al. (2007) determined the dietary fermentation aid *Saccharomyces cerevisiae* enhanced digestion of DM, CP, and NDF. Although dietary fermentation aids may increase microbial utilization of structural carbohydrates, forage alone may not fully address the nutrient demands of the horse. Therefore, commercially formulated concentrates are used to offset this deficit by supplying the horse with dietary additives to promote maintenance of body condition.

Morphometric analysis is necessary to understand change in tissue development, which may outwardly reflect the role of dietary intervention. A tactile body condition scoring (BCS) system (1 = extremely thin; 9 = extremely obese) was established by Henneke et al. (1983) to visually quantify overall fatness of a horse. Ultrasonography provides a more objective method to monitor changes in tissue development. Additionally, rump fat-thickness (RF) measured ultrasonically may be utilized in an equation to estimate total body fat (BF; Westervelt et al., 1976). This equation assumes a specific location over the rump is representative of the animal's entire BF. Furthermore, the equation indicates that a horse with no rump fat would still have 8.64% BF. In contrast, a more precise BF measurement may be obtained through deuterium oxide (D<sub>2</sub>O) infusion and subsequent plasma analysis (Dugdale et al., 2011). The D<sub>2</sub>O method accounts for total BF, including intramuscular fat and deeper adiposity that is not detected by the aforementioned methods.

Limited information has been gathered to compare performance and digestibility characteristics of mature horses maintained on forage low in nutritive value. Therefore, the objective of the current

study was to determine the effect of concentrate fortification on apparent digestion and body composition in mature horses consuming a forage low in nutritive value.

## MATERIALS AND METHODS

The experimental protocol was approved by the Institutional Animal Care and Use Committee at Texas A&M University (AUP# 2016-0304).

### Horses and Dietary Treatments

Twenty-three mature stock-type mares (498 ± 9 kg BW; 12 ± 7 yr) were used in a completely randomized design for a 56-d trial. Horses were stratified by body weight (BW), age, and BCS and assigned within strata across two dietary treatments. Investigators were blinded to dietary treatments which consisted of either a control diet (CON, *n* = 13) offered at 0.75% BW daily and formulated to meet or exceed nutritional requirements for mature horses at maintenance (NRC, 2007), or a fortified pelleted concentrate (FORT; *n* = 10) which included the following enhancements: amino acid fortification, fermentation metabolite additives, and complexed trace minerals. Dietary treatments were formulated to be isocaloric and isonitrogenous (Table 1; Table 2). Horses had ad libitum access to Coastal bermudagrass (*Cynodon dactylon*) hay. Every 14 d, BW was recorded and rations were adjusted accordingly.

**Table 1.** Energy and nutrient composition of pelleted concentrate and Coastal bermudagrass (*Cynodon dactylon*) hay fed to stock-type mares

Item*	Concentrate <sup>†</sup>		Hay
	CON	FORT	
DM, %	93.40	92.90	94.90
Energy and nutrient (DM basis)			
NDF, %	41.50	40.70	77.50
ADF, %	20.20	21.10	46.00
ADIA, %	1.57	1.95	3.55
Starch, <sup>‡</sup> %	11.50	10.80	0.68
DE, <sup>§</sup> Mcal/kg	2.76	2.79	1.85

\*Determined from laboratory analyses conducted at Texas A&M University.

<sup>†</sup>Concentrate consisted of either a control (CON) pellet or the same pelleted concentrate fortified (FORT) with an additional amino acid package, fermentation metabolite additives, and complexed trace minerals concentrate that was offered at 0.75% BW (as-fed basis).

<sup>‡</sup>Values were obtained from laboratory analyses conducted at a commercial feed testing laboratory (SDK Laboratories, Hutchinson, KS).

<sup>§</sup>Calculated from equations in NRC (2007).

**Table 2.** Amino acid composition of the control (CON) pellet and fortified (FORT) pellet that contained an additional amino acid package, fermentation metabolite additives, and complexed trace minerals concentrate offered at 0.75% BW (as-fed basis)

Item (W/W%)*	CON	FORT
Glutamic acid	2.22	2.14
Aspartic acid	1.25	1.20
Leucine	1.08	1.07
Proline	0.88	0.86
Arginine	0.83	0.79
Alanine	0.74	0.73
Glycine	0.73	0.71
Valine	0.72	0.70
Lysine	0.68	1.02
Phenylalanine	0.64	0.62
Serine	0.56	0.53
Isoleucine	0.56	0.54
Threonine	0.52	0.53
Tyrosine	0.41	0.40
Histidine	0.36	0.35
Cysteine	0.27	0.25
Methionine	0.24	0.33
Tryptophan	0.15	0.13
Hydroxyproline	0.14	0.14
Lanthionine	0.08	0.09
Taurine	0.07	0.07
Hydroxylysine	0.03	0.03
Ornithine	0.01	0.01
Total	13.17	13.24

\*(W/W%) indicates percent mass of item per 100 g of solution.

### Morphometric Variables

Mares were evaluated every 14 d for BW and BCS, and on d 0, 28, and 56 for fat thickness (FT), *longissimus dorsi* area (LDA), and intramuscular fat (IMF) using an ultrasound machine (Aloka SSD-500V, Aloka, Inc., Tokyo, Japan). The musculoskeletal probe was fitted with a contoured pad and corn oil was applied to the ultrasound site to maximize echogenicity and minimize image artifacts. Measurements included the FT and LDA over the intercostal space between the 12th/13th ribs and the 17th/18th ribs, and FT over the rump. All FT and LDA measurements were taken with the transducer oriented perpendicular to the horse's spine. Estimates of IMF were determined by capturing four independent images over the 17th/18th ribs with the transducer positioned parallel to the horse's spine. In accordance with Westervelt et al. (1976), BF percentage was estimated from an equation using rump FT measurements taken 5 cm from the midline and centered between the tuber

coxae and tuber ischiadicum [% BF =  $4.70 \times$  fat depth (cm) + 8.64]. All ultrasound measurements were obtained and interpreted by a single certified technician blind to treatments (Designer Genes Technologies, Inc., Harrison, AR).

### Body Composition

On d 0 and 56 of the trial, horses underwent D<sub>2</sub>O (Aldrich Chemistry, 99.9% atom) infusion. On the morning of D<sub>2</sub>O infusion, BW was measured on a digital platform scale, and a jugular intravenous catheter (MILA International, Inc., 14 Ga. × 13 cm) was inserted and secured with adhesive bandaging tape. The jugular catheter site was prepared by clipping the coat to a sanitary length (blade size 0.25 mm), and sterilized using Chloradine scrub 4% and isopropyl alcohol 70%. Lidocaine was used as a local anesthetic and injected subcutaneously at the site of catheter insertion. Two 10 mL blood samples were obtained for baseline determination (Lithium Heparin 15 mL BD Vacutainer, 158 U.S.P. units). Horses were infused with D<sub>2</sub>O (D<sub>2</sub>O; 0.12 g/kg BW) over a time period of 60 s, followed by a 100 mL saline flush to ensure all D<sub>2</sub>O was delivered. The dose of D<sub>2</sub>O was determined using previously described methods in the horse (Dugdale et al., 2011; Ferjak et al., 2017). Feed and water were withheld during the 4 h D<sub>2</sub>O equilibration period. At 4 h postinfusion, two 10 mL blood samples were obtained contralateral to the site of catheter insertion. All blood samples were stored on ice then centrifuged at 2,000 × g for 12 min. Plasma was aliquoted and immediately stored at -80 °C in 2 mL air-tight tubes (VWR, 2 mL micro-centrifuge tubes). Samples were analyzed by a commercial laboratory (Stable Isotopes Lab, University of Arkansas).

In brief, plasma samples were thawed then filtered before analysis in triplicate by gas isotope ratio mass spectrometry. Samples underwent zinc reduction of the isotope-marked water. The deuterium abundance in the plasma samples was determined relative to a reference standardized tap water which had been normalized against the internal standards. Subsequent values were used to calculate the subject's total BF with a 4% correction factor to account for the exchange of nonwater related hydrogen atoms within the body. A body lean hydration factor of 0.732 was used as previously described (Speakman, 1997). The following calculations were used to estimate total body water (TBW), BF, and fat-free mass FFM: corrected body water (actual) (BW<sub>a</sub>) (kg) = BW/1.04; %TBW = BW<sub>a</sub> / BW; %BF = 100 - (%TBW/0.732); and %FFM = %TBW/0.732.

## Digestibility

Nutrient digestibility was assessed through the use of internal (acid detergent insoluble ash, ADIA) and external (Titanium IV Dioxide, rutile; Sigma–Aldrich) markers. On d 27, baseline fecal samples were collected. From d 28 to 41, 5 g of titanium dioxide ( $\text{TiO}_2$ ) was mixed into each concentrate meal. After a 10-d supplementation period, feces were collected from d 38 to 41 of the study. To account for diurnal variation of marker concentration, feces were collected twice daily at 12-h intervals, advancing by 3 h each subsequent day, which resulted in 8 fecal samples per mare over a 4-d collection period. After each collection time point, a 200 to 400 g sample of feces was stored at  $-20^\circ\text{C}$  before analysis.

To prepare samples for analysis, feces, hay, and grain were dried in a forced air oven at  $55^\circ\text{C}$  for 96 h and allowed to equilibrate to room temperature for 24 h. All samples were weighed before and after the drying process to determine partial dry matter (DM) percentage. Samples were ground in a Wiley mill through a 1 mm screen. To determine overall DM percentage, samples were exposed to a  $105^\circ\text{C}$  drying oven for 24 h. Similarly, to determine organic matter (OM) percentage, difference in sample weight was measured before and after exposure to a combustion oven at  $450^\circ\text{C}$  for 8 h. The NDF and ADF values were determined by the Ankom Fiber Analyzer with sodium sulfite and  $\alpha$ -amylase admitted and without correction for residual ash (Ankom Technology Corp., Macedon, NY). A Parr 6300 Calorimeter (Parr Instrument Company, Moline, IL) was used to measure gross heat energy (GE) for hay, grain, and fecal samples.

Forage consumption was estimated by  $\text{TiO}_2$  analysis following a previously established protocol (Myers et al., 2004). Concentrate, hay, and fecal samples were ashed at  $450^\circ\text{C}$  for 12 h then exposed to concentrated sulfuric acid (95% to 98% w/w) for 2 h before the addition of 30% hydrogen peroxide solution. Samples were digested at  $350^\circ\text{C}$  for 45 min in a SCP digester. Absorbance at 410 nm was measured and compared with standards of 10, 8, 6, 4, or 2 mg  $\text{TiO}_2$  per 50 g solution. The following calculations were used to estimate voluntary DMI of nutrients in the diet:  $\text{mg TiO}_2 / \text{g} = (\text{mg TiO}_{2\text{ sample}} / \text{g sample}) - (\text{mg TiO}_{2\text{ baseline}} / \text{g sample})$ ; fecal output (kg/d) =  $(10 \text{ g/d TiO}_2) / ([\text{TiO}_2] \text{ feces (g/kg)})$ , where  $[\text{TiO}_2]$  indicates concentration of  $\text{TiO}_2$  in the feces; and  $\text{DMI (kg/d)} = \text{fecal output (kg/d)} \times ([\text{ADIA}] \text{ feces} / [\text{ADIA}] \text{ feed})$ , where  $[\text{ADIA}]$  indicates concentration of ADIA.

## Statistical Analysis

Intake, digestion,  $\text{D}_2\text{O}$ , and morphometric variables were analyzed using the MIXED procedure in SAS (SAS Inst., Inc., Cary, NC). The model contained the main effect of treatment and horse within diet was included as a random effect. Pearson correlation coefficients were determined using the PROC CORR procedure in SAS for BCS and BF percentages determined by either a prediction equation (Westervelt et al., 1976) or  $\text{D}_2\text{O}$  infusion. Main effects were considered significant when  $P \leq 0.05$  and a trend toward significance when  $P \leq 0.10$ .

## RESULTS

### Nutrient Intake

There was no effect of dietary treatment on total DMI ( $P = 0.41$ ), with mares on the CON diet consuming 2.86% BW compared to 2.74% BW in mares in the treatment group (Table 3). Concentrate DMI, when expressed as a percentage of BW, was not affected ( $P = 0.24$ ) by dietary treatment. Similarly, dietary treatment did not affect hay DMI ( $P \leq 0.54$ ), with CON and FORT horses consuming 2.13% and 2.03% BW, respectively. Using measures of nutritive value and calculations based on the NRC (2007), the CON and FORT concentrates were calculated to contain 2.76 and 2.79 Mcal/kg, and the hay contained 1.85 Mcal/kg. Estimates based on the use of  $\text{TiO}_2$  in a dual marker system, CON mares consumed an average of 29.50 Mcal DE/d and FORT mares consumed

**Table 3.** Dry matter intake (DMI) of control (CON) or iso-caloric, iso-nitrogenous fortified (FORT) pelleted concentrate and Coastal bermudagrass (*Cynodon dactylon*) hay fed to stock-type mares (least square means)

Item	Treatment*		SEM	P value†
	CON	FORT		
Intake, % BW				
DMI Grain	0.73	0.71	0.01	0.24
DMI Hay‡	2.13	2.03	0.03	0.54
DMI Total	2.86	2.74	0.04	0.41

\*Treatment consisted of either a control (CON;  $n = 10$ ) concentrate or the same pelleted concentrate fortified (FORT;  $n = 12$ ) with an additional amino acid package, fermentation metabolite additives, and complexed trace minerals concentrate that was offered at 0.75% BW (as-fed basis).

†Main effect of dietary treatment.

‡Hay intake determined using a dual-marker system with  $\text{TiO}_2$  as the external marker and ADIA as the internal marker (Titgemeyer et al., 2001).

29.05 Mcal DE/d. Horses consumed an average of 638.5 and 654.6 g CP and 21.32 and 31.97 g Lys per d from the CON and FORT concentrates, respectively (Table 3). Based on NRC (2007) estimated nutrient composition values of Coastal bermudagrass hay, horses consumed a maximum of 0.36 g Lys/kg, or an average of 4.46 and 4.44 g Lys for the CON and FORT dietary treatments, respectively.

### Morphometric Variables

When evaluating the change over the feeding period (d 56 minus the d 0 measurement obtained at the beginning of the trial), BW and BCS were unaffected by dietary treatment ( $P > 0.63$ ). The LDA and FT over the 12th/13th ribs ( $P \geq 0.74$ ) and IMF ( $P = 0.57$ ) were not affected by diet. Interestingly, IMF values decreased numerically in both the CON and FORT groups while subcutaneous fat values increased, but these values did not achieve significance. Horses maintained on the FORT diet gained FT over the 17th/18th ribs ( $P \leq 0.02$ ) and tended to gain in LDA over the 17th/18th ribs from d 0 to 56 of the trial ( $P \leq 0.09$ ; Table 4). No differences in RF or BF estimation ( $P = 0.38$  and  $P = 0.58$ , respectively) were detected. Utilizing a D<sub>2</sub>O infusion, BF was not affected on d 56 by diet fortification ( $P \leq 0.58$ ; Table 5). Pearson correlation coefficients were determined for comparison between BCS and BF estimation through the Westervelt et al. (1976) RF equation and D<sub>2</sub>O infusion (Table 6). Overall, the correlations were moderate between D<sub>2</sub>O and BCS ( $r = 0.52$ ;  $P \leq 0.01$ ), and slightly weaker between D<sub>2</sub>O and RF ( $r = 0.43$ ;  $P \leq 0.01$ ) and RF and BCS ( $r = 0.42$ ;  $P \leq 0.01$ ).

### Apparent Digestion

All digestibility coefficients were calculated based on total tract apparent digestibility. Average starch digestibility coefficients were similar, 0.98 and 0.96, for horses receiving the CON or FORT diets, respectively ( $P = 0.16$ ; Table 7). Values for ADF and NDF did not differ ( $P \geq 0.93$ ) between dietary treatments. Similarly, DM and OM were not affected by diet ( $P \geq 0.96$ ). Despite the addition of fermentation aids in the FORT diet, apparent digestion did not differ between treatments in this time period.

## DISCUSSION

### Morphometric Variables

In the current study, BW and BCS of horses were not affected by 56 d of diet fortification. Lucia et al. (2014) observed a similar response

**Table 4.** Mean change in ultrasonography measurements ( $\pm$ SEM) of fat thickness (FT) and *longissimus dorsi* area (LDA) over the 12th/13th and 17th/18th ribs and intramuscular fat (IMF) in response to feeding either a CON or FORT pelleted concentrate and Coastal bermudagrass hay

Variable	Treatment*		SEM	P value <sup>†</sup>
	CON	FORT		
LDA 12th/13th ribs, cm <sup>2</sup>				
Initial	72.07	69.89	2.71	0.55
Final	77.28	73.32	2.71	0.35
Change	5.21	3.43	2.84	0.74
FT 12th/13th ribs, cm				
Initial	0.16	0.16	0.01	0.98
Final	0.17	0.17	0.01	0.99
Change	0.01	0.01	0.01	0.98
LDA 17th/18th ribs, cm <sup>2</sup>				
Initial	84.95	80.97	2.78	0.29
Final	89.43	92.45	2.71	0.41
Change	4.48 <sup>a</sup>	11.48 <sup>b</sup>	3.03	0.09
FT 17th/18th ribs, cm				
Initial	0.25	0.27	0.03	0.67
Final	0.28 <sup>a</sup>	0.41 <sup>b</sup>	0.03	$\leq 0.01$
Change	0.03 <sup>a</sup>	0.14 <sup>b</sup>	0.03	0.02
Intramuscular fat, % <sup>‡</sup>				
Initial	3.92	4.07	0.16	0.47
Final	3.13	3.45	0.23	0.37
Change	-0.79	-0.62	0.22	0.57

<sup>a,b</sup>Within item, means that do not have a common superscript differ by  $P \leq 0.10$ .

\*Treatments consisted of a controlled (CON) or fortified (FORT) pelleted concentrate offered at 0.75% BW (as-fed basis) and allowed ad libitum access to Coastal bermudagrass (*Cynodon dactylon*) hay.

<sup>†</sup>Main effect of dietary treatment.

<sup>‡</sup>Intramuscular fat percentage measurements obtained on the left hip determined by capturing four independent images over the 17th/18th ribs with the transducer positioned parallel to the horse's spine (Aloka SSD-500V, Aloka, Inc., Tokyo, Japan).

in a group of mature, sedentary horses housed in comparable climatic conditions that were fed a fortified pellet (complexed trace minerals, pre- and pro-biotics, enhanced amino acids, and elevated vitamin E) or a nonfortified control diet. Numeric increases in BW and BCS were observed over the 154-d study; however, similar to the current study, these variables were not affected by dietary treatment. Lucia et al. (2014) observed LDA, LDFT, and RF ultrasonography measurements increased ( $P \leq 0.01$ ) in response to diet fortification. In the current study, FT over the 17th/18th ribs increased in response to dietary fortification. A longer study duration may have yielded further development in the horses' topline over the 12th/13th ribs and a greater change in BW and BCS. In horses and

**Table 5.** Mean BW ( $\pm$  SEM), BCS, and BF change in response to feeding either a CON or FORT pelleted concentrate and Coastal bermudagrass

Variable	Treatment*		SEM	P value <sup>†</sup>
	CON	FORT		
<b>BW, kg</b>				
Initial	494.35	500.01	12.74	0.74
Final	505.44	508.34	13.54	0.87
Change	11.09	8.33	4.32	0.63
<b>Body condition score<sup>‡</sup></b>				
Initial	5.60	5.57	0.27	0.92
Final	5.74	5.78	0.29	0.92
Change	0.14	0.21	0.15	0.72
<b>Rump fat, cm<sup>  </sup></b>				
Initial	0.81	0.86	0.10	0.90
Final	1.04	0.86	0.10	0.31
Change	0.23	0.00	0.15	0.38
<b>Body fat estimation,<sup>§</sup> %</b>				
Initial	12.65	12.55	0.59	0.90
Final	12.74	13.51	0.57	0.31
Change	0.09	0.96	0.72	0.38
<b>Body fat estimation,<sup>¶</sup> %</b>				
Initial	7.71	7.57	0.73	0.89
Final	9.00	8.45	0.81	0.62
Change	1.29	0.88	0.56	0.58

\*Treatments consisted of a controlled (CON) or fortified (FORT) pelleted concentrate offered at 0.75% BW (as-fed basis) and allowed ad libitum access to Coastal bermudagrass (*Cynodon dactylon*) hay.

<sup>†</sup>Main effect of dietary treatment.

<sup>‡</sup>Average of three evaluators using a 1 to 9 scoring system (Henneke et al., 1983).

<sup>||</sup>Rump fat measurements obtained on the left hip at a point 5 cm dorsal of half way between the tuber coxae and tuber ischiadicum (Westervelt et al., 1976) using an ultrasound instrument (Aloka SSD-500V, Aloka, Inc., Tokyo, Japan).

<sup>§</sup>Body fat estimated by using prediction equation, % body fat =  $4.70 \times \text{fat depth (cm)} + 8.64$  (Westervelt et al., 1976).

<sup>¶</sup>Body fat estimated by using 0.12 g/kg BW of deuterium oxide.

other livestock species, fat-free mass remains relatively consistent throughout a mature animal's lifespan and is more difficult to change compared with fat tissue, which responds more sensitively to diet. Safely implementing change in an animal's morphometric parameters through diet intervention requires consistency and time. Using a mathematical model to predict alteration in body fat in nonlactating mares, Cordero et al. (2013) quantified an increase or decrease in one unit of BCS during 30 d to be reflected by 1.054% change in BF ( $r = 0.798$ ). Another study which modified the model from the aforementioned study to exercising horses observed a change of 1.4 BCS units over a 60 d period in the nonexercised control horses, and each BCS unit was reflected by an increase or decrease of 16.47 kg (Zoller et al., 2019).

**Table 6.** Pearson correlation coefficients of body condition score (BCS), deuterium oxide percent body fat (BF) estimation, and rump fat BF based on ultrasonography values and a prediction equation (Westervelt, 1976)

Variable	BCS*		D <sub>2</sub> O <sup>†</sup>		RF <sup>‡</sup>	
	r	P value	r	P value	r	P value
BCS	1.00	—	0.52	0.02	0.42	0.03
D <sub>2</sub> O%BF	0.52	0.01	1.00	—	0.43	0.02
RF BF	0.42	0.01	0.43	0.01	1.00	—

\*Body condition score determined by the average scores of three trained personnel.

<sup>†</sup>Percent body fat determined from analysis of plasma immediately before and 4 h post infusion of 0.12 g/kg BW deuterium oxide.

<sup>‡</sup>Rump fat measurements obtained on the left hip at a point 5 cm dorsal of half way between the tuber coxae and tuber ischiadicum (Westervelt et al., 1976) using an ultrasound instrument (Aloka SSD-500V, Aloka, Inc., Tokyo, Japan).

**Table 7.** Apparent digestion of nutrients in response to feeding either a CON or FORT pelleted concentrate and Coastal bermudagrass ha

Item	Treatment*		SEM	P value <sup>†</sup>
	CON	FORT		
<b>Apparent digestion, %</b>				
DM	0.67	0.65	0.04	0.96
Starch	0.98	0.96	0.01	0.16
NDF	0.66	0.63	0.06	0.95
ADF	0.61	0.58	0.06	0.93
GE	0.69	0.68	0.02	0.71

\*Treatments consisted of a control (CON) or fortified (FORT) pelleted concentrate offered at 0.75% BW (as-fed basis) and allowed ad libitum access to Coastal bermudagrass (*Cynodon dactylon*) hay.

<sup>†</sup>Main effect of dietary treatment.

The horse's topline is largely comprised of the *longissimus dorsi* muscle, making this location largely important for targeting and monitoring change. This location is also commonly assessed in beef cattle to determine meat quality. On a similar BW comparison, cattle have reliable ultrasonography measurements compared to horses. Perkins et al. (1992) assessed the average LDA in 646 choice or better quality grade cross-bred heifers to be, on average, 75.4 cm<sup>2</sup>. In the current trial, the greater increase following 56 d of supplementation in LDA over the 17th/18th ribs (5.27% CON and 14.18% FORT, respectively) for horses in the treatment group is likely due to concentrate fortification, which increased the bioavailability of Lys in the diet. Throughout the trial, mean LDA over the 17th/18th ribs ranged from 81.0 to 92.6 cm<sup>2</sup> for all horses, comparatively larger than the same measurement over 12th/13th ribs (7.23% CON and 4.91% FORT, respectively), which measured 69.0 to 73.9 cm<sup>2</sup> for all horses. Miller et al. (2018) reported

similar measures in mature Quarter horses ( $n = 10$ ; 2 to 6 yr). For instance, the LDA over the 17th/18th ribs ranged from 90.0 to 106.9 cm<sup>2</sup>, and over the 12th/13th ribs ranged from 77 to 92 cm<sup>2</sup>. Similarly, another study monitoring change in LDA in Arabian horses throughout an exercise program observed measurements from 69.6 to 89.8 cm<sup>2</sup> between baseline and 90 d into the program (D'Angelis, 2007).

### ***Dietary Supply of Amino Acids***

Lysine is the only amino acid with an established dietary requirement in the horse (NRC, 2007). Comparatively, Lys differed the greatest between diets (0.68 CON and 1.02 FORT W/W%). If amino acid requirements are not met, or are not bioavailable to the animal, then anabolism of muscle tissue will be diminished (Mastellar et al., 2016). In the current study, the following equations were utilized to estimate Lys requirements according to intake and digestibility parameters assessed through the dual-marker technique: daily CP requirement for an average horse at maintenance = BW (kg)  $\times$  1.26 g CP/kg BW/d; and Lys required for a horse at maintenance (g/d) = CP requirement  $\times$  4.3% (NRC, 2007).

Based on prior research that estimated the prececal digestion of Coastal bermudagrass hay to be 37% (Gibbs et al., 1988), the average digestible Lys intake from forage consumed by CON and FORT horses was calculated to be 1.41 and 1.37 g, respectively. Prececal digestion of protein and amino acids changes with diet composition (van Niekerk and van Niekerk, 1997; de Almeida et al., 1998a, 1998b), and the values calculated are likely an overestimation because the CP of hay in the present study was significantly lower (8% vs. 10%). Differences in other regions of the horses' topline may not have been detected in the current study due to the short 56 d duration. Winsco et al. (2011) assessed growth measurements and RF over 56 d in weanling horses receiving one of four isonitrogenous diets with varying methionine concentrations. The researchers did not observe a difference between treatments in BW or RF throughout the trial in horses undergoing rapid growth. Lucia et al. (2014) observed changes in RF beginning at d 56 in mature horses supplemented with a fortified diet similar to the one used in the current study. In one experiment involving ponies fed a pelleted concentrate at either 1.5% BW or ad libitum, Westervelt et al. (1976) noted a significant difference between fat cover in the ponies after 126 d. Therefore, changes in topline in the horse may take a minimum of 56 d to observe any significant difference from baseline.

### ***Intake and Digestibility***

In situations where both DMI and fecal output are not known, such as in the current group-housed setting, dual-marker systems may be used to estimate intake (Winsco et al., 2013). In the current study, DMI of hay did not differ between diets. All horses were group-housed in a dry lot with access to a round bale of hay. The dual marker system of TiO<sub>2</sub> and ADIA indicated no difference in the apparent digestion of starch, DM, OM, NDF, or ADF.

Yeast supplemented to the horse can have a positive effect on digestibility of structural carbohydrates when the concentration of cellulolytic bacteria is increased. For example, when *S. cerevisiae* is added to the diet, the prevalence of lactate in the hindgut decreases, reducing acidity and creating a more favorable environment for cellulolytic bacteria (Jouany et al., 2008). In a recent study, Agazzi et al. (2011) fed horses a grass/legume mixed forage (9.8% CP, 64.2% NDF, and 46.2% ADF) for two weeks, followed by 18 d of live yeast (*S. cerevisiae*) supplementation offered twice daily. No difference was observed in DMI ( $P \geq 0.48$ ); however, apparent digestion of DM, OM, NDF, and ADF increased ( $P \leq 0.04$ ) in supplemented horses compared with the control group, and apparent digestion of CP tended to improve ( $P \leq 0.08$ ). Morgan et al. (2007) used total fecal collection to assess apparent digestibility of hay that was either high or low in nutritive value, and fed to horses receiving a yeast culture supplement. Results from the study indicated that the higher quality forage (13.1% CP, 73.1% NDF, and 35.3% ADF) was more digestible in every component than the lower quality forage (8.1% CP, 75.3% NDF, and 37.6% ADF). In addition, the yeast culture supplement increased digestibility of NDF, hemicellulose, and CP. Although multiple studies have addressed the potential digestive benefit of dietary yeast supplements, comparison between trials is challenging due to the wide variation in the source and quantity of active microbes. For this reason, the unaltered digestibility observed in horses fed the FORT diet in the current study may be attributed to less numerous and active population of microbes which can be altered to enhance potential benefits of the supplement.

### ***Body Composition***

Deuterium oxide infusion is an objective, minimally invasive procedure, which has been demonstrated as a more accurate method for estimating body fat values in the live horse than utilizing rump

fat as a predictor (Ferjak et al., 2017). Using infusion of the stable isotope, total body water was quantitatively assessed in mature horses and percent BF was calculated for mares assigned to either the CON or FORT treatment diet. Percentage of BF increased across treatments over the 56-d feeding period; however, differences were not detected between dietary treatments. No change in percent BF relative to treatment correlates with no significant change observed in BW, BCS, or BF estimated from RF. In this experiment, percent BF ( $D_2O$ ) was quantified at 8.72% in mares with an average BCS of 5.58 and 5.76 on d 0 and 56, respectively. These values were comparable to those obtained in similar trials. Dugdale et al. (2011) estimated BF using  $D_2O$  at 13.1%, in mature horses that were between a BCS of 1 to 7. Ferjak et al. (2017) estimated BF to be 5.99% and 10.28% in horses with a BCS of 5 or 6, respectively. Body fat estimations from both previous trials were similar to values calculated in the current study in horses with a similar BCS, which ranged from 7.57% to 9.00% BF, and further supports the use of  $D_2O$  to evaluate live morphometrics in the horse.

When data sets were averaged across dietary treatments for BCS and BF according to both Westervelt et al. (1976) and  $D_2O$  evaluations, moderately positive correlations were observed. Values obtained in this study indicated a lower correlation than another study conducted on stock-type horses undergoing moderate exercise, which found  $r = 0.50$  between RF and BCS (Pritchett et al., 2016). Prior research which compared these morphometric measurements with postmortem fat assessment determined that  $D_2O$  estimates are the optimal method of assessing actual BF ( $r = 0.86$  to  $0.98$ ; Ferjak et al., 2017). The Westervelt equation used to calculate percent BF is as follows:  $BF = 8.64 + 4.70 \times RF$  (cm; Westervelt et al., 1976). Assuming that the horse measured had no RF, this equation would still estimate the total BF of the animal at 8.64%. Thus, this equation can present a source of error by estimating the horse's total BF based on one measured location, and may explain the current study's low correlation between the two variables calculating percent BF.

In summary, concentrate fortification has the potential to improve lean muscle development over the topline in the horse. This project investigated tissue change through objective means in the mature, sedentary horse and determined the region over the 17th/18th ribs was the most sensitive to lean muscle and fat development. In the current study, analysis of BF using a  $D_2O$  infusion provided

an objective means to quantify change in morphometric values, in addition to BCS and ultrasonography. Supplying dietary fermentation metabolites when feeding forage low in nutritive value did not significantly improve nutrient digestibility in the horses during this time frame; however, it should be considered that a longer trial may yield further positive results.

*Conflict of interest statement.* The authors affirmatively acknowledge that they were free from influence by Cargill, Inc. and its employees that would result in any conflict of interest.

## LITERATURE CITED

- Agazzi, A., M. Ferroni, A. Fanellie, S. Marocco, G. Invernizzi, V. Dell'Orto, and G. Savoini. 2011. Evaluation of the effects of live yeast supplementation on apparent digestibility of high-fiber diet in mature horses using the acid insoluble ash marker modified method. *J. Equine Vet. Sci.* 31(1):13–18. doi:10.1016/j.jevs.2010.11.012
- de Almeida, F. Q., S. de Campos Valdares Filo, J. L. Donzele, J. F. C. da Silva, M. I. Leao, P. R. Cecon, and C. de Queiroz. 1998a. Apparent and true prececal and total digestibility of protein in diets with different protein levels in equines. *R. Bras. Zootec.* 27:521–529.
- de Almeida, F. Q., S. de Campos Valdares Filo, J. L. Donzele, J. F. C. da Silva, M. I. Leao, P. R. Cecon, and C. de Queiroz. 1998b. Endogenous amino acid composition and true prececal apparent and true digestibility of amino acids in diets for equines. *R. Bras. Zootec.* 27:546–555.
- Cordero, V. V., C. A. Cavinder, L. O. Tedeschi, D. H. Sigler, M. M. Vogelsang, and C. E. Arnold. 2013. The development and evaluation of a mathematical nutrition model to predict digestible energy intake of broodmares based on body condition changes. *J. Anim. Sci.* 91:2169–2177. doi:10.2527/jas.2011-4659
- Coverdale, J. A., E. D. Lamprecht, P. Kropp, I. Yoon, J. L. Lucia, K. N. Winsco, A. E. Hanson, and C. M. Warzecha. 2013. Influence of diet fortification on mature horses at maintenance: performance characteristics. *J. Anim. Sci.* 91 (E-Suppl. 2):565. (Abstr.).
- D'Angelis, F. H., M. D. Mota, E. V. Freitas, G. C. Ferraz, A. R. Abrahão, J. C. Lacerda-Neto, and A. Queiroz-Neto. 2007. Aerobic training, but not creatine, modifies longissimus dorsi muscle composition. *J. Equine Vet. Sci.* 27(3):118–122. doi:10.2527/2005.833579x
- Dugdale, A. H., G. C. Curtis, P. A. Harris, and C. M. Argo. 2011. Assessment of body fat in the pony: part I. Relationships between the anatomical distribution of adipose tissue, body composition and body condition. *Equine Vet. J.* 43:552–561. doi:10.1111/j.2042-3306.2010.00330.x
- Ferjak, E. N. 2017. Body fat determination of stock-type horses predicted by rump fat thickness and deuterium oxide dilution and validated by near-infrared spectroscopy of dissected tissues. *J. Anim. Sci.* 95(10):4344–4351. doi:10.2527/jas2017.1676
- Gibbs, P. G., G. D. Potter, G. T. Schelling, J. L. Kreider, and C. L. Boyd. 1988. Digestion of hay protein in different segments of the equine digestive tract. *J. Anim. Sci.* 66:400–406. doi:10.2527/jas1988.662400x

- Henneke, D. R., G. D. Potter, J. L. Kreider, and B. F. Yeates. 1983. Relationship between condition score, physical measurements and body fat percentage in mares. *Equine Vet. J.* 15:371–372. doi:10.1111/j.2042-3306.1983.tb01826.x
- Jouany, J. P., J. Gobert, B. Medina, G. Bertin, and V. Julliand. 2008. Effect of live yeast culture supplementation on apparent digestibility and rate of passage in horses fed a high-fiber or high-starch diet. *J. Anim. Sci.* 86:339–347. doi:10.2527/jas.2006-796
- Lucia, J. L., E. D. Lamprecht, M. J. Anderson, K. J. Stutts, M. M. Beverly, S. F. Kelley, and D. L. Parker. 2014. Influence of diet fortification on mature horses at maintenance: performance characteristics. *J. Anim. Sci.* 92 (E-Suppl. 2):194. (Abstr.).
- Mastellar, S. L., R. J. Coleman, and K. L. Urschel. 2016. Controlled trial of whole body protein synthesis and plasma amino acid concentrations in yearling horses fed graded amounts of lysine. *Vet. J.* 216:93–100. doi:10.1016/j.tvjl.2016.07.007
- Miller, E. F., J. L. Leatherwood, M. J. Anderson, and M. M. Beverly. 2018. The effect of conjugated linoleic acid (CLA) supplementation on equine body composition. *Approach. Poultry Dairy Vet. Sci.* 3(3):1–5.
- Morgan, L., J. Coverdale, M. Froetschel, and I. Yoon. 2007. Effect of yeast culture supplementation on digestibility of varying forage quality in mature horses. *J. Equine Vet. Sci.* 27(6):260–265. doi:10.1016/j.jevs.2007.04.009
- Myers, W. D., P. A. Ludden, V. Nayigihugu, and B. W. Hess. 2004. Technical note: a procedure for the preparation and quantitative analysis of samples for titanium dioxide. *J. Anim. Sci.* 82:179–183. doi:10.2527/2004.821179x
- van Niekerk, F. E., and C. H. van Niekerk. 1997. The effect of dietary protein on reproduction in the mare. I. The composition and evaluation of the digestibility of dietary protein from different sources. *J. S. Afr. Vet. Assoc.* 68:78–80. doi:10.4102/jsava.v68i3.880
- NRC. 2007. Nutrient requirements of horses. 6th rev. ed. Washington (DC): National Academies Press.
- Perkins, T. L., R. D. Green, and K. E. Hamlin. 1992. Evaluation of ultrasonic estimates of carcass fat thickness and longissimus muscle area in beef cattle. *J. Anim. Sci.* 70:1002–1010. doi:10.2527/1992.7041002x
- Pritchett, K. B., J. L. Leatherwood, K. J. Stutts, and M. J. Anderson. 2016. Use of ultrasonography to evaluate the accuracy of objective and subjective measures of body composition in horses. *J. Anim. Sci.* 94(1):16. doi:10.2527/ssasas2015-033
- Speakman, J. R. 1997. Double labelled water: theory and practice. Springer Science & Business Media.
- Swyers, K. L., A. O. Burk, T. G. Hartsock, E. M. Ungerfeld, and J. L. Shelton. 2008. Effects of direct-fed microbial supplementation on digestibility and fermentation end-products in horses fed low- and high-starch concentrates. *J. Anim. Sci.* 86:2596–2608. doi:10.2527/jas.2007-0608
- Titgemeyer, E. C., C. K. Armendariz, D. J. Bindel, R. H. Greenwood, and C. A. Löest. 2001. Evaluation of titanium dioxide as a digestibility marker for cattle. *J. Anim. Sci.* 79:1059–1063. doi:10.2527/2001.7941059x
- Westervelt, R., J. Stouffer, H. Hintz, and H. Schryver. 1976. Estimating fatness in horses and ponies. *J. Anim. Sci.* 43(4):781–785. doi:10.2527/jas1976.434781x
- Winsco, K. N., J. A. Coverdale, T. A. Wickersham, and J. L. Shelton. 2011. Influence of dietary methionine concentration on growth and nitrogen balance in weanling quarter horses. *J. Anim. Sci.* 89:2132–2138. doi:10.2527/jas.2010-3380
- Winsco, K. N., J. A. Coverdale, T. A. Wickersham, J. L. Lucia, and C. J. Hammer. 2013. Influence of maternal plane of nutrition on mares and their foals: determination of mare performance and voluntary dry matter intake during late pregnancy using a dual-marker system. *J. Anim. Sci.* 91(9):4208–4215. doi:10.2527/jas.2013-6373
- Zoller, J. L., C. A. Cavinder, D. Sigler, L. O. Tedeschi, and J. Harlin. 2019. Development of a mathematical model for predicting digestible energy intake to meet desired body condition parameters in exercising horses. *J. Anim. Sci.* 97:1945–1955. doi:10.1093/jas/skz041