Effect of yeast culture on in vitro fermentation of a high-concentrate or high-fiber diet using equine fecal inoculum in a Daisy II incubator[1]

J. M. Lattimer,* S. R. Cooper,†[2] D. W. Freeman,† and D. L. Lalman†

*Department of Applied Sciences, Black Hawk College-East Campus, Kewanee, IL; and †Department of Animal Science, Oklahoma State University, Stillwater, OK

ABSTRACT: Two experiments were conducted to evaluate the use of a closed system, fermentation apparatus (Daisy II incubator) and determine the effects of a yeast culture (YC) preparation (*Saccharomyces cerevisiae*) on in vitro microbial populations, diet digestion, and fermentation patterns in horses. In Exp. 1, 4 mature horses were fed a pelleted concentrate and alfalfa cubes in a 50:50 (%, as-fed) ratio. Fecal samples were taken from each horse to form the inoculum and placed in 4 separate incubation vessels. Twenty nylon bags (10 with 0.25 g and 10 with 0.50 g of the total mixed diet) were placed in each vessel, and in vitro fermentation was carried out for 48 h to determine DM, NDF, and ADF digestibility. In Exp. 2, fecal samples were taken from 4 mature horses consuming either a high-concentrate (HC) or high-fiber (HF) diet. Filter bags containing the HC or HF diet were added to the 4 incubation vessels along with their respective inoculums. Yeast culture was added to 2 of the vessels containing the HC or HF diet, whereas the other 2 vessels served as controls. Vessels were incubated as in Exp. 1 with samples taken at 24 and 48 h. Filter bags were used to determine DM, NDF, ADF, and OM digestibility, whereas vessel fluid was analyzed for lactate, ammonia, VFA, and microbial concentrations. Results of Exp. 1 indicated that DM, NDF, and ADF digestibility were greater ($P < 0.05$), whereas the corresponding CV was lower ($P < 0.05$) for the 0.25- vs. the 0.50-g sample size. In Exp. 2, YC tended ($P = 0.10$) to decrease ammonia concentrations in the HF diet and increased ($P < 0.05$) acetate production in the HC diet when compared with the control. There were no effects of YC on pH, lactate, or the measured microbial populations, as well as DM, NDF, or ADF digestibility. The results did, however, show that in vitro and in vivo DM digestibility estimates were similar within a diet. Data from Exp. 1 indicated that the 0.25-g sample size provides a more accurate estimate of DM digestibility with less variation. Although YC had little, if any, effect in Exp. 2, the results indicated that the Daisy II incubator does provide valid estimates of total tract DM digestibility in the horse. These data provide further evidence that this process would be an effective and practical means of approximating the digestibility of diets with varying concentrate to forage ratios.

Key words: in vitro fermentation, *Saccharomyces cerevisiae*, yeast culture

J. Anim. Sci. 2007. 85:2484–2491
doi:10.2527/jas.2006-655

INTRODUCTION

The use of in vitro fermentation procedures to study diet digestion and fermentative end products has become increasingly more popular in equine nutrition. Recent data have validated the use of equine feces as a source of inoculum for in vitro studies. Researchers have shown that grains and forages incubated with equine feces produced similar gas production profiles as known gas concentrations (Macheboeuf and Jestin, 1997; Lowman et al., 1999). Furthermore, Ringler et al. (2005a,b) reported that the combined use of equine fecal inoculum with a closed-system fermentation apparatus (Daisy II incubator) yielded valid in vitro estimates of DM, NDF, and ADF digestibility.

Research has shown that yeast culture (**YC**) can be beneficial to horses on both a high-concentrate and high-fiber diet. Moore et al. (1994) observed that mature ponies fed a 70:30 concentrate to roughage diet experienced an increase in lactate utilizing bacteria and a subsequent increase in cecal pH when supplemented with YC. This would be favorable to horses consuming large amounts of starch because these nonstructural polysaccharides would be easily converted to lactate inside the cecum and colon, thus potentially leading to gastric upset.

[1]Approved for publication by the Oklahoma Agric. Exp. Stn.
[2]Corresponding author: steven.cooper@okstate.edu
Received September 26, 2006.
Accepted May 17, 2007.

Table 1. Composition of experimental diet (Exp. 1)

Item	As-fed, %
Ingredient	
Alfalfa cubes	50.00
Ground shelled corn	21.00
Wheat midds	12.25
Cottonseed hulls	10.00
Dehydrated alfalfa meal	5.50
Limestone	1.00
Trace mineral salt	0.25
Nutrient	
DE,[1] Mcal/kg	2.72
CP, %	15.10
NDF, %	34.79
ADF, %	24.35
Ca, %	1.17
P, %	0.34

[1]Value calculated based on NRC (1989).

The DM, NDF, and ADF digestibilities were enhanced in mature horses fed a forage diet supplemented with YC (Glade, 1991). In support of these data, McDaniel et al. (1993), using cecal fluid from mature horses consuming a high-fiber diet, observed an increase in acetate:propionate ratio as well as in total VFA concentrations in vitro.

The objectives of this study were to evaluate in vitro fermentation of equine diets in the Daisy II incubator and determine the effects of YC on diets with varying concentrate to roughage ratios.

MATERIALS AND METHODS

All animal protocols were approved by the Oklahoma State University Institutional Animal Care and Use Committee.

Experiment 1

Initial in vitro fermentation procedures were conducted to evaluate the use of 2 sample sizes (0.25 and 0.50 g) in the Daisy II incubator (D200, Ankom Technology, Mecedon, NY) using equine feces as the source of inoculum. Four mature Quarter Horses (3 geldings and 1 mare) with a mean age of 10 yr (range 5 to 16 yr) and a mean initial weight of 558 kg (range 500 to 591 kg) were fed a pelleted concentrate (Table 1) in a 50:50 (%, as-fed) ratio with alfalfa cubes. The diet was fed on an individual basis twice daily (0700 and 1900) at the rate of 1.5% of initial BW. Daily refusals of the diet were weighed and recorded 12 h after feeding and were used to estimate daily intake. Horses had ad libitum access to water. Horses were allowed 4 to 5 h of free exercise in an outdoor arena throughout the 14-d acclimation period. Before the beginning of the experiment, all horses were dewormed, vaccinated, and their hooves were trimmed.

After the initial 14-d acclimation period, total fecal collections were conducted for 72 h. After each 24-h period, total fecal output was weighed and multiple subsamples were taken and frozen for subsequent analysis. Individual fecal samples were thawed at room temperature for 24 h, after which three 50-g subsamples per horse were placed into a drying oven at 50°C for 96 h to determine DM. Fecal output was calculated for each horse from which apparent DM digestibility was determined. Average in vivo, apparent DM digestibility was 62 ± 3%.

In vitro fermentation was carried out for 48 h using the Daisy II incubator. The complete unit consisted of 4 incubation vessels with a capacity of 2,000 mL each. Each vessel contained 1,600 mL of buffer solution, 400 mL of fecal inoculum, and 20 nylon bags. Grab samples were obtained from each bag of concentrate and hay cubes used throughout the acclimation period. These samples were ground to pass a 2-mm screen (Wiley Mill, Laboratory Mill Model 4, Thomas-Wiley, Philadelphia, PA) and mixed together by hand in a 50:50 (wt/wt) ratio. Twenty nylon filter bags (Ankom F57, Ankom Technology, Fairpost, NY) per incubation vessel were rinsed in acetone and allowed to air dry. Bags were then placed into a 100°C oven for 24 h, after which their weight was recorded. A 0.25-g and a 0.50-g sample of the total mixed diet were weighed into 10 bags each, then heat-sealed. Bags were then placed into a 50°C oven and dried for 24 h, after which the bag and dry sample were weighed and recorded.

The microbial inoculum was prepared by collecting fresh feces via a rectal grab sample using palpation sleeves. Fecal samples were placed into an air-tight freezer bag to maintain an anaerobic environment and transported to the laboratory in a cooler containing warm (39°C) water. Once in the laboratory, feces from each horse were prepared separately. A 40-g sample of feces was placed in a blender with 360 mL of warm, distilled water (10:1, vol/wt). Samples were blended for 2 min while being gassed with CO_2, then strained through a double layer of cheesecloth directly into the prewarmed incubation jars (Hayes et al., 2003).

Each fermentation jar contained 400 mL of fecal inoculum and 1,600 mL of buffer solution. Buffers consisted of 2 solutions that were combined in the incubation jars immediately before the fecal inoculum. Buffer solution A (KH_2PO_4, 10.0 g/L; $MgSO_4 \cdot 7\ H_2O$, 0.5 g/L; NaCl, 0.5 g/L; $CaCl_2 \cdot 2\ H_2O$, 0.1 g/L; and urea, 0.5 g/L) was added at 1,330 mL to 266 mL of buffer solution B (Na_2CO_3, 15.0 g/L and $Na_2S \cdot 7\ H_2O$, 1.0 g/L) to obtain a final pH of 6.8. Reagents for these solutions were obtained from Sigma-Aldrich, St. Louis, MO. The fecal inoculum was then added to each fermentation jar, after which the jars were purged with CO_2 for 30 s and then sealed. The sealed jars were placed into the prewarmed Daisy II incubator. The incubator maintained a constant temperature of 40°C throughout the incubation, and the jars were continuously agitated. The jars were removed after 48 h, and the filter bags were immediately rinsed for 30 min with cold water to stop microbial activity. Samples were analyzed for DM, NDF, and ADF digest-

Table 2. Composition of experimental diets, as-fed basis (Exp. 2)

	Diet[1]	
Item	HF	HC
Ingredient, %		
Alfalfa cubes	70.00	30.00
Ground shelled corn	12.60	31.57
Soybean meal	—	4.20
Wheat midds	7.35	21.90
Cottonseed hulls	6.00	—
Dehydrated alfalfa meal	3.30	10.50
Limestone	0.60	1.68
Trace mineral salt	0.15	0.15
Nutrient		
DE,[2] Mcal/kg	2.50	2.94
CP, %	15.17	16.62
NDF, %	33.14	21.03
ADF, %	24.38	13.64
Ca, %	1.22	1.21
P, %	0.27	0.29

[1]HC = high-concentrate; HF = high-fiber.
[2]Values calculated based on NRC (1989).

ibility using techniques developed by Goering and Van Soest (1970) and modified by Ankom Technology (1998a,b,c).

Data for DM, ADF, and NDF digestibilities were analyzed using the MIXED procedure (SAS Inst. Inc., Cary, NC) with jar and sample size as main effects. Least squares means were calculated, and the LSD procedure was used to detect differences between treatment means. The CV was used to describe the relative amount of variation within the population of feed sample sizes (0.25 and 0.50 g). Because the CV is a ratio of 2 averages having the same unit of measurement, it is independent of the unit employed and also allows the experimental samples to be compared despite the diversity of the variables measured. A 2-tailed *F*-test was utilized to test for differences between variances of DM, NDF, and ADF digestibilities (Snedecor and Cochran, 1967).

Experiment 2

Four mature Quarter Horses (from Exp. 1) were paired by weight and age and randomly allotted to the high-fiber (**HF**) or high-concentrate (**HC**) diet to obtain feces to produce the respective inoculums (Table 2). The pelleted concentrate primarily consisted of corn, wheat, soybean meal, and dehydrated alfalfa meal, and the forage component was alfalfa cubes. All horses were maintained on their respective diets without YC throughout the in vitro study to provide a continual source of inoculum for the fermentation process. After an initial 14-d acclimation period, total fecal collections were conducted for 72 h to determine the average in vivo DM digestibility for each horse.

The in vitro portion of the study was conducted as a 4 × 4 Latin square, utilizing 2 diets with varying concentrate:roughage ratios (70:30 and 30:70, % as-fed) with and without the inclusion of a YC preparation containing *Saccharomyces cerevisiae* (Diamond V "XP", Cedar Rapids, IA). After the 72-h collection period, in vitro fermentation was carried out for 48 h using the Daisy II incubator, as described in Exp. 1. Each of the 4 treatment diets were rotated through the 4 incubation vessels by utilizing 4 fermentation periods. The fecal inoculum was prepared as in Exp. 1. Filter bags containing the HC or HF diet were added to the 4 incubation vessels with their respective inoculums. A 2.2-mg aliquot of YC was added directly to 2 of the incubation vessels containing the HC or HF diet, and the other 2 vessels served as controls. The amount of YC added to the incubation vessels is equivalent to the recommended feeding level of 50 g/d, assuming a mature (500 kg) sedentary horse consuming 2% of BW per d of total diet.

Sixteen filter bags containing a 0.25-g sample of the total mixed diet were added to each incubation vessel and used to determine DM, NDF, and ADF digestibility. At 24 and 48 h, the vessels were removed from the incubator, opened, agitated by hand, and flushed with CO_2 while the samples were taken. Eight of the bags were removed at each time period with sterile tongs and placed into cold water to stop the fermentation process. The pH of each vessel was then measured electronically (Orion Model SA 720 pH meter, Orion Research Inc., Boston, MA) and recorded. Fluid for the microbial analysis was collected using a 35-mL sterile syringe and placed into a Ziploc bag (SC Johnson, Racine, WI). The bag was then flushed with CO_2, sealed, placed into a cooler of 39°C water, and transported to the laboratory for analysis. A 15-mL sample of fluid was then taken into a 20-mL syringe. A 5-mL sample was then placed into 3 separate plastic snap-top vials for VFA, ammonia, and lactate analysis.

After incubation, filter bags were placed in a 50°C oven and dried for 48 h. Bags were then weighed and DM digestibility was determined. After DM determination, 8 bags were analyzed for ADF and NDF using a fiber analyzer (A200, Ankom Technology). The other 8 bags were ashed in a 500°C oven for 10 h to determine OM digestibility.

Concentrations of cellulolytic bacteria were determined with a broth medium by the most probable number method. Lactic acid-utilizing bacteria were determined using an overlay method followed by a colony count. Concentrations of *streptococci* and *lactobacilli* were determined using an overlay method with an Enterococcosel Agar (DIFCO 212205, Becton, Dickinson and Co., Franklin Lakes, NJ) and a Rogosa SL Agar (DIFCO 248020), respectively.

Data were analyzed using the MIXED procedure (SAS Inst. Inc., Cary, NC), with jar, period, and treatment as main effects and time as the repeated variable. Least squares means were calculated, and the LSD procedure was used to test for differences between treatment means. Significance was declared at $P < 0.05$.

Table 3. Effect of sample size on in vitro DM, NDF, and ADF digestibility (%) and the CV (%) corresponding to each mean (Exp. 1)[1]

	DM		NDF		ADF	
Sample size, g	Mean	CV	Mean	CV	Mean	CV
0.25	55.90[a]	2.91[a]	18.68[a]	14.19[c]	13.28[a]	20.12[a]
0.50	52.23[b]	5.63[b]	15.26[b]	18.34[d]	9.15[b]	35.40[b]

[a,b]Means within a column with different superscripts differ ($P < 0.05$).
[c,d]Means within a column with different superscripts differ ($P = 0.10$).
[1]n = 40.

RESULTS AND DISCUSSION

Experiment 1

Data for DM, NDF, and ADF digestibility along with the corresponding CV for the 0.25- and 0.50-g sample sizes are presented in Table 3. Mean DM, NDF, and ADF digestibilities were greater ($P < 0.05$) when the 0.25-g samples were utilized. This is probably due to having a smaller sample of feed in an equivalent amount of fluid, thus resulting in greater substrate digestion. Although the mean apparent DM digestibility for the 0.25-g sample (56%) was lower than that obtained from the in vivo estimates (62%), it more closely approximates this value as compared with the 0.50-g sample (52%). These data agree with Patterson et al. (2002) who observed similar in vivo DM digestibility estimates (60%) when mature horses were fed a 50:50 concentrate to roughage diet. Furthermore, Murray et al. (2003) reported comparable in vitro DM digestibility estimates (60%) when a 50:50 concentrate to roughage diet was incubated in equine fecal inoculum. When Ringler et al. (2005a) incubated 0.50-g samples of a 70:30 alfalfa and oat diet with equine fecal inoculum in a Daisy II incubator, mean in vitro DM digestibility values (55%) were lower than the in vivo estimates (60%) after 48 h of incubation. This difference in digestibility estimates could be due to the use of 0.50-g sample sizes, which allowed for greater variation and lower digestibilities, as demonstrated in the current study.

The current study also observed that the CV for DM and ADF digestibility was lower ($P < 0.05$) for the 0.25-g vs. the 0.50-g sample size. In addition, the CV for NDF digestibility tended ($P = 0.10$) to be lower for the 0.25-g vs. the 0.50-g sample size. Data from this study indicated that the smaller sample size not only yields digestibility values closer to in vivo estimates but also provides a more accurate estimate of digestibility with less variation.

The Tilley and Terry (1963) method has been used extensively to predict IVDMD of ruminant feeds; however, this method is time and labor consuming and requires each feedstuff be incubated separately. The Daisy II apparatus allows multiple feeds to be simultaneously analyzed for IVDMD, thus making it more efficient and possibly more accurate. Furthermore, the digestion inoculum in the Daisy II is continuously mixed and does not require an additional centrifugation step at the end of incubation.

Holden (1999) digested 10 different feeds (alfalfa hay, grass pasture, grass hay, mixed haylage, corn silage, total mixed diet, grain mixture, high moisture shelled corn, steam flaked corn, and dry ground corn) and observed no difference in IVDMD values between the Tilley and Terry method and the Daisy II. Other researchers (Mabjeesh et al., 2000; Brons and Plazier, 2005) have also reported no effect of digestion method on the IVDMD of selected forages, grains, and total mixed diets when comparing the Daisy II to Tilley and Terry method. Furthermore, the repeatability of IVDMD measurements in the Daisy II appears to be greater as indicated by the lower SD for all feeds measured (Mabjeesh et al., 2000). These data, and results from the current study, would indicate that the combined use of the Daisy II incubator with the smaller sample size yields valid IVDMD estimates and provides an alternative in vitro method for measuring the digestibility of various feedstuffs

Experiment 2

In vitro and in vivo apparent DM digestibility estimates were similar within a diet; however, digestibilities were greater ($P < 0.05$) for the HC vs. the HF diet (Table 4). These data agree with Ringler et al. (2005a) who found that, after 72 h of incubation, IVDMD (57%) of a 70:30 alfalfa-oat diet was not different from in vivo estimates (60%). Results from the current study would indicate that the Daisy II incubator may provide valid estimates of apparent DM digestibility and could be utilized when evaluating total mixed diets with varying concentrate to roughage ratios.

Yeast culture had no effect on DM, NDF, ADF, and OM digestibility; therefore, data are presented by diet and time (Table 5). The current study revealed the expected differences due to diet and time as mean digestibility values were greater ($P < 0.05$) at 48 vs. 24 h for all variables measured. Furthermore, DM, NDF, ADF, and OM digestibilities were greater ($P < 0.05$) for the HC vs. the HF diet. These data agree with Ringler et al. (2005b) who showed that DM, NDF, and ADF digestibility increased between 15 and 45 h when alfalfa

Table 4. Effect of diet on in vivo and in vitro DM digestibility estimates (Exp. 2)

Diet[1]	In vitro[2]	In vivo[3]	SEM[4]
HC	75.00[a]	77.30[a]	0.28
HF	64.10[b]	63.60[b]	0.28

[a,b]Means within a column with different superscripts differ ($P < 0.05$).
[1]HC = high-concentrate; and HF = high-fiber.
[2]Values are after 48 h of incubation; n = 16.
[3]n = 2.
[4]Values are pooled SEM.

hay was incubated with equine fecal inoculum in a Daisy II incubator.

The lack of effect of YC supplementation on DM, NDF, ADF, and OM digestibility coincides with McDaniel et al. (1993) who observed in vitro that YC supplementation had no effect on digestibility of alfalfa hay. Similar data were reported by Carro et al. (1992) who showed no effect of *S. cerevisiae* on DM, NDF, and filter paper cellulose degradabilities when medium (50:50 concentrate:forage) and low concentrate (30:70 concentrate:forage) diets were incubated in vitro with rumen fluid. Enjalberta et al. (1999) also reported that the in situ DM, NDF, and ADF disappearance of grass hay was not enhanced when YC was added to the diet. In contrast to these findings, yearling (Glade and Sist, 1988) and mature horses (Glade, 1991) had greater DM, NDF, and ADF digestibilities when YC was added to mixed hay-grain diets. Chademana and Offer (1990) also observed that YC supplementation increased DM digestibility for the first 24 h when sheep were fed low, medium, and high concentrate diets. However, DM digestibility values were not different at 48 h, thus indicating an increase only in the initial rate of degradation (Chademana and Offer, 1990).

Table 5. Effects of diet and time on in vitro DM, NDF, ADF, and OM digestibility (Exp. 2)

Item	HF diet[1]	HC diet[1]	SEM[2]
DM			
24 h	48.63[ac]	64.84[bc]	0.91
48 h	64.10[ad]	75.00[bd]	0.91
NDF			
24 h	8.82[ac]	24.39[bc]	1.38
48 h	24.31[ad]	31.75[bd]	1.38
ADF			
24 h	3.76[ac]	16.50[bc]	1.28
48 h	21.83[ad]	25.45[bd]	1.28
OM			
24 h	46.44[ac]	64.55[bc]	1.16
48 h	61.62[ad]	75.28[bd]	1.16

[a,b]Means within a row with different superscripts differ ($P < 0.05$).
[c,d]Means for a specific item and within a column with different superscripts differ ($P < 0.05$).
[1]HC = high-concentrate; and HF = high-fiber; n = 16/diet.
[2]Values are pooled SEM.

Table 6. Effect of diet and time on in vitro microbial populations (Exp. 2)

Item[1]	HF diet[2]	HC diet[2]	SEM[3]
Cellulolytic, $\log_{10}$ MPN/mL			
24 h	6.05[a]	6.33[c]	0.31
48 h	5.35[b]	5.41[d]	0.31
Lactate utilizers, $\log_{10}$ cfu/mL			
24 h	8.06[c]	7.90[c]	0.14
48 h	7.56[d]	7.49[d]	0.14
Lactobacilli, $\log_{10}$ cfu/mL			
24 h	6.72[c]	6.86[c]	0.23
48 h	5.63[d]	5.86[d]	0.23
Streptococci, $\log_{10}$ cfu/mL			
24 h	6.50[c]	6.35[c]	0.24
48 h	5.35[d]	5.00[d]	0.24

[a,b]Means for a specific item and within a column with different superscripts differ ($P = 0.10$).
[c,d]Means for a specific item and within a column with different superscripts differ ($P < 0.05$).
[1]MPN = most probable number; and cfu = colony forming units.
[2]HC = high-concentrate; and HF = high-fiber; n = 8.
[3]Values are pooled SEM.

There was no effect of YC on the concentrations of streptococci, lactobacilli, lactate utilizers, or cellulolytic bacteria (data not shown). This concurs with Medina et al. (2002) who observed no change in cellulolytic, streptococci, and lactobacilli bacteria in the cecum or colon of mature horses fed a high-starch or high-fiber diet and supplemented with YC. However, Moore et al. (1994) observed that horses consuming a 50:50 and 70:30 concentrate to roughage diets supplemented with YC had increased numbers of cellulolytic and lactate utilizing bacteria, respectively, in the cecum. Kumar et al. (1997) also showed that YC added to a high roughage diet and fed to buffalo calves increased rumen concentrations of cellulolytic bacteria.

There were no changes in the concentration of streptococci, lactobacilli, lactate utilizers, or cellulolytic bacteria due to diet (Table 6). The lack of difference in cellulolytic bacteria between diets is unexplained because the HF diet would be expected to have an increased population of these bacteria when compared with the HC diet. This might be a result of the incubation process because it was a closed system as opposed to the continuous culture system, which more closely simulates the fermentation environment. Medina et al. (2002) observed that horses fed a high-starch diet had reduced concentrations of cellulolytic bacteria compared with those consuming a high-fiber diet. This is most likely due to the decrease in cecum and colon pH that occurred in horses consuming the diet high in soluble carbohydrates. Furthermore, Medina et al. (2002) observed an increase in streptococci, lactobacilli, and lactate-utilizing bacteria when horses were fed a high-starch diet as compared with a high-fiber diet. These bacteria would therefore be expected to adapt and increase their concentrations because the fermentation of nonstructural polysaccharides and simple sug-

Table 7. Effect of YC and diet on in vitro ammonia, lactate, acetate, and propionate concentrations (Exp. 2)

Item[1]	YC[2,3]	Control[3]	SEM[4]
Ammonia, mmol/mL			
HC diet	5.57	4.88[e]	0.46
HF diet	5.45[a]	6.34[bf]	0.46
Lactate, ppm			
HC diet	6.12	5.75	0.38
HF diet	5.00	5.38	0.38
Acetate, ppm			
HC diet	325.31[c]	270.19[d]	15.53
HF diet	285.75	266.13	15.53
Propionate, ppm			
HC diet	202.19[e]	196.25[e]	22.13
HF diet	126.00[f]	124.88[f]	22.13

[a,b]Means within a row with different superscripts differ ($P = 0.10$).
[c,d]Means within a row with different superscripts differ ($P < 0.05$).
[e,f]Means for a specific item and within a column with different superscripts differ ($P < 0.05$).
[1]HC = high-concentrate; and HF = high-fiber.
[2]YC = yeast culture.
[3]n = 8.
[4]Values are pooled SEM.

ars in a high-starch diet produces primarily lactic acid. The lack of change in the current study could be due to the amount of substrate because only 0.25 g of diet sample was placed in each filter bag. These bacteria may not have been able to increase their concentration or energetics due to a low amount of substrate, which was incubated in a closed environment. Additionally, this closed in vitro environment does not simulate the continual infusion and passage of feed as would occur in the live animal. Consequently, this method of in vitro incubation is not recommended when attempting to evaluate dietary effects on bacterial populations.

Concentrations of each bacterium (cellulolytic, lactate utilizers, streptococci, and lactobacilli) decreased ($P < 0.05$) over time between 24 and 48 h (Table 6). This is probably due to the decreasing substrate level because, on average, 81% of the total DM was digested in the first 24 h. This substantial decline in substrate most likely caused the microbial population to decrease because additional substrate was not added to the incubation vessels to maintain the microbial population once the fermentation process began.

Data for the effect of YC and diet on ammonia concentrations are given in Table 7. Yeast culture tended ($P = 0.10$) to decrease ammonia concentrations when supplemented to the HF diet. Medina et al. (2002) also demonstrated that horses supplemented with YC had decreased cecal ammonia concentrations. Also, Carro et al. (1992) observed a 10% decrease in ammonia concentrations when YC was added to incubation vessels containing a high-concentrate (70:30) diet and inoculated with rumen fluid. The previous study also demonstrated that those vessels containing YC had a greater ($P < 0.05$) proportion of microbial N derived from ammonia. This might be explained by an increased ability of the microflora to capture ammonia and convert it to microbial cell protein. The current study further observed lower ($P < 0.05$) ammonia concentrations in control vessels containing the HC as compared with the HF diet. Because the HC diet is higher in energy, it would enhance the ability of the microbial cells to convert the ammonia to microbial cell protein and use it for their own protein requirements.

There was no effect of YC or diet on vessel fluid lactate concentrations (Table 7). In contrast, Medina et al. (2002) observed that YC supplemented to mature horses consuming a high-starch diet decreased cecal lactate concentrations. The expected increase in lactate production due to anaerobic fermentation of soluble carbohydrates was not observed in the current study. Lactate concentration for the HC diet was not different from the HF.

The addition of YC increased ($P < 0.05$) the in vitro concentration of acetate on the HC diet. This is in agreement with McDaniel et al. (1993), who observed that the addition *Aspergillus oryzae* to vessels inoculated with cecal fluid and fermented with starch or Bermudagrass increased acetate concentrations. Medina et al. (2002) also observed in vivo that YC added to the high-starch or high-fiber diets of mature horses resulted in an increased concentration of acetate in the cecum and colon. Subsequently, the acetate to propionate ratio was increased in the YC group (1.86) vs. the control (1.67). The current study showed no change in the concentration of acetate due to diet. In contrast, Hintz et al. (1971) and Medina et al. (2002) observed that horses consuming a high-fiber diet had greater concentrations of cecal acetate when compared with a high concentration diet. The lack of difference observed in the current study may be related to the population of cellulolytic bacteria, which was also showed to be similar between diets. Consequently, the production of their fermentation end products was not altered with changes in diet substrate.

There was no effect of YC supplementation on propionate production. These data coincide with Medina et al. (2002) who observed no change in cecal or colonic propionate concentrations of mature horses due to the addition of YC. Carro et al. (1992) also showed no change in the molar proportions of propionate when a high, medium, or low concentrate diet was supplemented with YC and incubated in vitro with rumen fluid. In contrast, McDaniel et al. (1993) observed an increase in propionate production when *Aspergillus oryzae* was added to vessels inoculated with cecal fluid and fermented with starch or Bermudagrass. There was an effect of diet in the current study because propionate concentrations were greater in horses receiving the HC diet vs. the HF. This would be expected because the HC diet had a greater starch content, thus producing more propionate through the dicarboxylic acid pathway or the acrylate pathway. These data are in agreement with Medina et al. (2002) who showed that horses consuming a high-concentrate diet had greater cecal and

Table 8. Effect of incubation time and diet on in vitro pH (Exp. 2)

Time, h	HC diet[1]	HF diet[1]	SEM[2]
0	6.52[a]	6.55[b]	0.03
24	6.50[a]	6.53[b]	0.03
48	6.46[a]	6.50[b]	0.05

[a,b]Means within a row with different superscripts differ ($P < 0.05$).
[1]HC = high-concentrate; HF = high-fiber; linear decrease ($P < 0.05$) in pH over time; n = 8.
[2]Values are pooled SEM.

colonic propionate concentrations. McDaniel et al. (1993) observed that incubation vessels containing starch had a greater propionate production as compared with Bermudagrass.

There was no effect of YC on vessel pH; therefore, data are presented by diet over time (Table 8). Vessel pH was lower ($P < 0.05$) for the HC vs. the HF diet at all measured intervals, while decreasing ($P < 0.05$) linearly over time in both diets. The lower pH of the HC diet is most likely due to fermentation of greater amounts of soluble carbohydrates, resulting in the increased production of H^+, which lowered pH. The lack of effect of YC supplementation on pH agrees with Carro et al. (1992) who found no effect of *S. cerevisiae* on the pH of vessels containing a high, medium, or low concentrate diet. The lack of difference observed in the current study could be due to the very high buffering capacity of the in vitro fermentation processes because 4 parts buffer solution (pH = 6.8) were added to 1 part diluted fecal fluid.

Results from the current study showed that the overall effects of *S. cerevisiae* on in vitro diet digestion and measures of fermentation are relatively insignificant. The lack of difference observed in certain variables may be related to the type of fermentation process used because the Daisy II incubator is a closed system and therefore does not allow for a continuous flow of microflora and nutrients. The current study did, however, demonstrate that the use of 0.25-g sample sizes may yield more accurate estimates of DM digestibility with less variation. Furthermore, DM digestibility values were similar between in vitro and in vivo estimates. These data would, therefore, indicate that the combined use of the Daisy II incubator with the smaller sample size incubated in equine fecal inoculum provides a valid and accurate estimate of total tract DM digestibility in the horse.

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