# Influence of housing type on the cecal environment of horses

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**ABSTRACT:** Eight previously cecally cannulated Quarter Horse geldings were utilized in a crossover design with two 28-d periods with a 21-d washout period between to evaluate the influence of housing on the cecal environment and dry matter intake (DMI). Horses were adapted to diet and housing from day 1 to 19, DMI was determined from day 20 to 24, and cecal fluid was collected on day 28. Horses were paired by age and body weight (BW) and randomly assigned to treatment. Treatments consisted of housing horses individually in stalls or group housed in a pen. Regardless of treatment, all horses were individually fed a pelleted concentrate at 1% BW (as fed) offered twice daily 12 h apart. All horses had ad libitum access to coastal bermudagrass hay (*Cynodon dactylon*). Hay was offered to stalled horses initially at 2% BW (as fed) and then adjusted based on 120% of a previous 3-d average of voluntary intake. A dual marker system was used to estimate forage consumption in all horses, using titanium dioxide ($TiO_2$) as the external marker and acid detergent insoluble ash (ADIA) as the internal marker. $TiO_2$ was offered at 10 g/d for 10 d with fecal samples collected on the final 4 d at 12-h intervals advancing by 3 h each day to account for diurnal variation. Cecal samples were collected on day 28, 4 h after the morning meal and immediately analyzed for pH, total anaerobic and lactic acid bacteria populations, methane and ammonia concentrations, as well as volatile fatty acid (VFA) concentrations. Data were analyzed using the PROC GLM procedure of SAS with the model containing effects for horse, period, and treatment. Cecal pH was affected by housing ($P = 0.02$) with group-housed horses having lower cecal pH values compared with stalled horses (6.52 vs. 6.69, respectively). There was no influence of housing on populations of total anaerobic or lactic acid bacteria. Furthermore, housing did not influence cecal concentrations of VFA or methane and ammonia concentrations. Estimates of voluntary forage DMI were greater for group-housed horses ($P = 0.04$) than stalled (8.47 and 5.17 ± 0.89 kg DM/d, respectively). In conclusion, confinement housing did not, with the exception of pH, alter cecal environment of a horse when similar diets were offered but did affect forage consumption.

**Key words:** cecum, confinement, forage intake, horse, housing

## INTRODUCTION

Common management practices in the equine industry present multiple challenges to the horse

that include feeding high-grain diets and confinement housing. Performance horses require a large amount of digestible energy (DE) and to meet this requirement, horses are supplemented with a high-grain diet that include a greater percentage of nonstructural carbohydrates (NSC), often requiring feeding horses two to three large meals per day. As NSC consumption increases, the proportion of NSC digested in the cecum increases. Rapid fermentation of starch in the cecum results in changes in cecal pH, volatile fatty acid (VFA) concentrations, and bacterial populations (Medina et al., 2002). As a result, the cecal environment can be altered based upon the diet fed.

Confinement of horses may induce stress and alter feeding behavior. Often, horses are housed in stalls at facilities with limited pasture turnout, or horses are housed on small acreage farms that provide inadequate grazing. These two management challenges pose potential problems to the gastrointestinal health of the horse due to the horse's unique digestive tract. Horses have a small stomach relative to their body size and are designed to eat many small meals throughout the day that is in contrast to the two to three meals typically fed. Feral horse meals primarily consist of forage with limited amounts of NSC.

Even when forage is provided ad libitum to stalled horses, they may spend less time eating (Buchanan and Andrews, 2003). There is limited information regarding dry matter intake (DMI) by horses in confinement, particularly voluntary forage intake. In many studies, effects of diet and housing are confounded by changing both factors simultaneously. Therefore, the objectives of this study were to determine influence of housing on cecal environment and voluntary forage consumption of horses fed a high-grain diet.

## MATERIALS AND METHODS

All procedures were approved by the Texas A&M University Institutional Animal Care and Use Committee.

### Horses, Diet, and Housing Treatments

Eight previously cecally cannulated Quarter Horse geldings (7–10 yr; 510–666 kg) were used in a crossover design with two treatment periods and consisted of housing horses individually in stalls (3.9 × 4.4 m) or group housed in a dry lot paddock (97.5 × 27.1 m). Prior to the start of the study, horses were paired by age and body weight (BW) and randomly assigned to treatment. Treatment periods were 28 d with a 21-d washout period between. Days 1–19 allowed for adaptation to dietary treatments, days 20–24 were used for determination of dry matter intake, and on day 28, cecal fluid was collected for enumeration of hindgut bacteria, determination of pH, and VFA concentrations.

All horses were individually fed a pelleted concentrate at 1% BW (as fed) that was offered twice daily and forage was provided in the form of coastal bermudagrass (*Cynodon dactylon*) hay (Table 1). Group-housed horses had ad libitum access to hay as round bales and stalled horses were offered hay individually that was collected daily from the same round bale. Hay was offered to stalled horses initially at 2% BW (as fed) and then adjusted based on 120% of a previous 3-d average of voluntary intake. Bodyweights were recorded on day 0 of each treatment period and amount of feed offered adjusted accordingly. Horses were fed twice daily, at 0600 and 1800 hours, in individual stalls or tied and observed individually when group housed. All horses had ad libitum access to water. Stalled horses were allotted 1 h of free exercise per day.

Grain and hay refusals were weighed and recorded daily. Grain samples were obtained weekly, and hay samples were obtained through grab samples from each round bale offered.

### Sample Collection and Preparation

A dual marker system was utilized for estimation of voluntary intake in both stalled and dry lot horses using titanium dioxide ($TiO_2$) as the external marker and acid detergent insoluble ash (ADIA) as the internal marker. Voluntary forage intake was measured directly and indirectly in stalled horses, whereas in dry lot horses it was only measured indirectly. $TiO_2$ was top-dressed onto the concentrate

**Table 1.** Nutrient components of concentrate and forage (DM basis) fed to mature Quarter Horse geldings

| Item | Concentrate[1] | Hay[2] |
|---|---|---|
| ADF, % | 15.74 | 37.18 |
| ADIA, % | 1.61 | 2.03 |
| NDF, % | 28.43 | 68.00 |
| CP, % | 16.79 | 9.10 |
| Starch, % | 18.70 | 2.70 |
| Ca, % | 1.29 | 0.33 |
| P, % | 0.87 | 0.17 |

[1]Diets consisted of 1% BW (as fed) per day in commercially pelleted concentrate.

[2]Coastal bermudagrass (*Cynodon dactylon*).

beginning on day 13 and was offered at 10 g/d separated in two 5 g doses offered prior to each grain meal. Fecal samples were collected twice daily at 12-h intervals on days 20–24 with collection times advancing by 3 h each day to account for diurnal variation and provide a composite sample for a 24-h period. Fecal samples were dried at 60 °C for 72 h and ground through a 1-mm Wiley Mill screen and composited into a representative 24-h sample. All fecal, hay, grain, and concentrate-based dosing device samples were analyzed for $TiO_2$ marker recovery using a UV Vis spectrophotometer by colorimetric assay and ADIA was analyzed using an ANKOM Fiber Analyzer and an ashing furnace (Short et al., 1996; Llewellyn et al., 2006).

Cecal samples were taken on day 28 of each treatment period 4 h after the morning meal, when cecal pH begins to decline (Willard et al., 1977). Cecal cannulas were opened and 350-mL insulated containers were filled with approximately 100–200 g of cecal contents (liquid and solid phase). Cecal fluid (50 mL) was collected through a ruminal strainer and immediately analyzed for pH with a handheld pH meter. Portions of the freshly collected cecal contents were then frozen at −20 °C for later analysis of VFA concentrations and other portions were immediately subjected to microbiological cultivation and in vitro activity assay as described below.

Samples were prepared for microbial analysis within 45 min of collection. Samples were prepared in a series of 10-fold serial dilutions in an anaerobic mineral solution (Bryant and Burkey, 1953) from $10^{-1}$ to $10^{-9}$. Dilutions were inoculated onto one of three specific media for bacteria enumeration. Anaerobic Brucella Blood agar plates were utilized to enumerate total culturable anaerobes (Mangels and Douglas, 1989). Previous research indicated a serial dilution of $10^{-4}$ to $10^{-10}$ provided an ideal range for enumeration on this media (Wilson et al., 2009). Plates were inoculated with 0.1 mL/plate in a Bactron Chamber maintained with an internal atmosphere of 90% $N_2$; 5% $CO_2$; 5% $H_2$. Lactobacilli were determined using two different selective media: Rogosa agar (Rogosa et al., 1951) and DeMan Rogosa Sharpe (MRS) agar (deMan et al., 1960). Previous research indicated that a dilution set of $10^{-1}$ to $10^{-8}$ was sufficient for enumeration on this media (Wilson et al., 2009). Difco Rogosa SL agar was prepared in petri dishes prior to sample collection. Both media were inoculated with serial dilutions $10^{-1}$ to $10^{-8}$ with 0.1 mL/plate and Rogosa was incubated anaerobically in the Bactron Chamber and MRS was incubated aerobically. All inoculated plates were incubated at 37 °C for 96 h, after which bacterial colonies were counted and recorded.

Methane concentration was determined using a procedure described by Anderson et al. (2006) through an in vitro incubation of cecal fluid. Each sample was performed in triplicate with two sets prepared: 3-h sample and 24-h sample. Samples were incubated for the respective time at 39 °C. Rates of ammonia accumulation were determined by measuring ammonia concentrations in fluid samples collected from these in vitro incubations at 0, 3, and 24 h using a colorimetric assay that uses a catalyzed indophenol reaction (Chaney and Marbach, 1962).

VFA concentrations in cecal contents that had been frozen were measured after thawing using gas chromatography with a flame ionization detector, a procedure described by Vanzant and Cochran (1994). Stoichiometric calculations based on VFA-measured accumulations were used to estimate the amount of hexose fermented according to Demeyer (1991). These equations were also used to estimate the amount of methane and carbon dioxide produced using the results from VFA analysis according to a series of fermentation balance equations from Wolin (1960). The amount of reducing equivalents generated and consumed can also be calculated based upon equations described by Demeyer (1991) along with the fermentative efficiency as described by Chapula (1997).

### Statistical Analysis

Data were analyzed using the PROC GLM procedure of SAS. The model contained effects for horse, period, and treatment with $P \leq 0.05$ considered significant and $P \leq 0.10$ considered a trend toward significance.

## RESULTS AND DISCUSSION

### Concentrate Intake

Concentrate intake was not affected by housing ($P > 0.17$). Concentrate consisted of 18.7% starch (Table 1), which provided an average of 0.86 g starch/kg BW/meal. According to previous research, intake of 2–4 g starch/kg BW/meal results in starch overload to the hindgut and causes significant alterations to cecal fermentation (Radicke et al., 1991; Potter et al., 1992; Kiensle, 1994). It is unlikely that starch overload occurred in the current study based upon the calculated starch intake of the diet. However, the current diet consisting of 1% BW (as fed) in concentrate per day is similar to the

requirement for horses performing light-to-moderate work (NRC, 2007).

### Forage Intake

Forage intake was affected by treatment with group-housed horses consuming greater amounts of forage ($P = 0.04$) compared with stalled horses despite similar concentrate consumption (Figure 1). The dual marker estimated forage intake averaged 8.46 kg DM/d (1.41% BW) when horses were group housed in a dry lot and 5.17 kg DM/d (0.90% BW) when horses were stalled individually ($P = 0.05$). Despite differences in forage intake, total dry matter digestibility did not differ ($P = 0.48$) between stalled or group-housed horses (57.72% and 56.79%, respectively).

In a previous voluntary forage intake study, mature horses fed only coastal bermudagrass hay consumed 2.0% BW (Aiken et al., 1989). Horses in the current study were fed concentrate at 1% BW (as fed) with minimal refusals, total forage and concentrate intake averaged 2% BW/d in stalls and 2.4% BW/d when group housed. Reductions in intake of forage by individually housed horses in the current study agrees with previous research in beef cattle (Tayler and Wilkinson, 1974). Decreased forage consumption in confinement can relate to a horse's natural behaviors, primarily the social interactions within a herd. Being herd animals, horses follow other conspecifics whether that is foraging, drinking, or sleeping; so when other horses are near, foraging might be a more constant activity compared with when confined with limited contact (visual or physical) with other horses (Sweeting et al., 1985). Group-housed horses could have possessed a more competitive behavior while consuming fo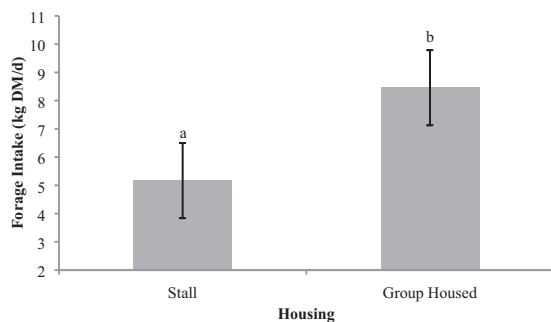rages than stalled since the group-housed horses were consuming forage from one round bale; whereas, stalled horses had ad libitum access to forage with no competition.

### Cecal pH

Cecal pH was affected by housing with group-housed horses having lower cecal pH values when compared with stalled horses ($P = 0.02$; Figure 2). Cecal pH values of this study are similar to those reported previously when horses were fed similar diets (Kern et al., 1973; Goodson et al., 1988). The pH values in the current study are more similar to the hay-only diets; based upon forage intake in the current study, horses were consuming a higher proportion of forage in their diet.

Gut motility is directly linked to feeding activity (Ruckebusch, 1984). The difference in cecal pH between treatments in the current study could be attributed to alteration in rate of concentrate meal intake caused by group-housed horses consuming the concentrate portion of their diet in a defined time period. Group-housed horses were given access to concentrate until the entire meal was consumed or 1 h had passed; in contrast, stalled horses had free access to concentrate for 12 h between meals. Offering concentrate to group-housed horses in this manner likely increased their rate of intake and potentially increased rate of passage. Rate of passage could be increased due to the fact that group-housed horses had been conditioned to consume their portion of concentrate quickly based upon previous conditioning when all horses were group fed in the same paddock before the current study began. If the group-housed horses consumed their concentrate quicker than stalled horses did, the substrates from the concentrate meal would reach the cecum quicker causing the pH to be lower at 4 h after the morning meal.



**Figure 1.** Estimated voluntary forage intake in mature Quarter Horse geldings that were group housed in a dry lot or individually in a stall. Titanium dioxide and ADIA were utilized together in a dual marker system to estimate intake. All horses were fed a diet consisting of 1% BW (as fed) pelleted concentrate with ad libitum coastal bermudagrass hay. Values are means ± SE ($n = 8$). Means without a common letter (a, b) are different ($P < 0.05$).
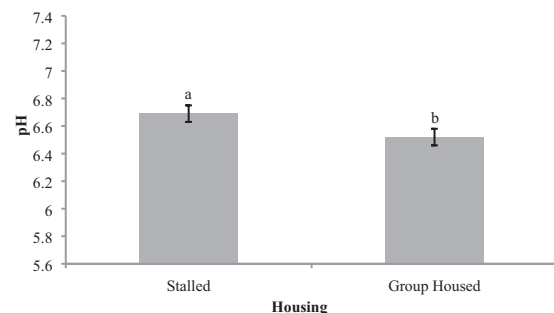


**Figure 2.** Cecal pH obtained 4 h after meal in mature Quarter Horse geldings that were group housed in a dry lot or individually in a stall. All horses were fed a diet consisting of 1% BW (as fed) pelleted concentrate with ad libitum coastal bermudagrass hay. Values are means ± SE ($n = 8$). Means without a common letter (a, b) are different ($P < 0.05$).

It is important to note that even though there was a decline in cecal pH, the average cecal pH remained above 6.13, regardless of treatment. A pH value lower than 6.0 is indicative of subclinical lactic acidosis and has been link to increased incidences of colic and laminitis (Radicke et al., 1991).

### Microbial Populations

Cecal microbial populations of total anaerobic bacteria and *Lactobacillus* spp. were not affected by housing ($P \geq 0.12$) despite the alterations in cecal pH (Table 2). Even though microbial populations did not differ among treatments, total anaerobic bacteria values in the current study were similar to values reported in previous studies utilizing similar diets (Kern et al., 1974; Julliand et al., 2001). This suggests that housing does not alter total bacterial populations when a concentrate and forage diet is offered.

*Lactobacillus* spp. counts were similar to values previously reported (deFombelle et al., 2001) along with previous data from our laboratory (Wilson et al., 2009). Limited data exists for the use of MRS agar with equine cecal fluid. However, previous data generated in our laboratory, presented a range of 7.04–8.00 $\log_{10}$ c.f.u./mL of *Lactobacillus* spp. counts with MRS agar when horses were fed varying concentrations of dietary starch (Wilson et al., 2009). Researchers have previously utilized Rogosa agar medium to enumerate equine cecal fluid for *Lactobacillus* spp. and observed bacterial counts of 6.2 $\log_{10}$ c.f.u./mL when feeding a pelleted diet rich in fiber and 6.3 $\log_{10}$ c.f.u./mL when feeding a pelleted diet rich in cereals (deFombelle

et al., 2003). Another study (Medina et al., 2002) determined *Lactobacillus* spp. counts to be 6.4 $\log_{10}$ c.f.u./mL when fed a high-fiber diet compared with a high-starch diet at 7.7 $\log_{10}$ c.f.u./mL when utilizing Rogosa agar. Values in the current study are lower than these reported values with the same agar. Given the lower starch content in the concentrate portion of the current diet, it is expected, as Amylolytic bacteria proliferate at an optimum pH range of 5.5–6.6, which is lower than the cecal pH values observed in the current study (Leek, 2004).

### VFA Concentrations

Cecal VFA concentrations were not significantly affected by treatment or period ($P \geq 0.15$; Table 3). Housing treatments had no effect on cecal acetate concentration ($P = 0.33$; Table 3). Cecal concentrations of acetate in the current study are similar to values reported by Kern et al. (1973) when ponies were fed timothy hay without oats producing acetate concentrations of 39.2 mM. Cecal propionate concentration was not affected by treatment ($P = 0.15$; Table 3). Concentrations from the current study are similar to concentrations reported by Kern et al. (1973) when ponies were fed timothy hay and oats. Propionate concentrations of the current study are lower than previous studies when starch content in the diet increased. Although starch was not fed in excess in the current study, the additional substrates from the concentrate portion of the diet did not significantly increase the propionate concentration to values closer to 12.8 or 19.7 that were reported by others using mixed diets (Medina et al., 2002; deFombelle et al., 2003). Production of butyrate was not affected by treatment ($P = 0.20$; Table 3). Concentrations of butyrate in the current study are similar to concentrations ranging from

**Table 2.** Microbial populations in the cecum of mature Quarter Horse geldings based on housing[1]

| Item | Housing | | | P-values |
| | Stall | Group housed | SEM | Treatment |
|---|---|---|---|---|
| Total anaerobes, $\log_{10}$ c.f.u./mL | 7.56 | 7.86 | 0.15 | 0.21 |
| *Lactobacillus*, $\log_{10}$ c.f.u./mL | | | | |
| MRS[2] | 7.40 | 7.80 | 0.24 | 0.29 |
| Rogosa[3] | 6.05 | 5.77 | 0.12 | 0.16 |

[1]Horses were group housed in a dry lot or individually in a stall. All horses were fed a diet consisting of 1% BW (as fed) pelleted concentrate with ad libitum coastal bermudagrass hay. Cecal contents were collected 4 h after morning meal. Values are means (*n* = 8).

[2]Difco Lactobacilli MRS Agar (Becton Dickson and Company, Sparks, MD) requires a carbon dioxide atmosphere to limit the amount of oxygen available allowing only strict anaerobes to grow.

[3]Difco Rogosa SL agar (Becton Dickson and Company, Sparks, MD) is incubated aerobically.

**Table 3.** Volatile fatty acid concentration in the cecum of mature Quarter Horse geldings based on housing[1]

| Item | Housing | | | P-values |
| | Stall | Group housed | SEM | Treatment |
|---|---|---|---|---|
| Acetate, mM | 37.93 | 40.16 | 1.47 | 0.33 |
| Propionate, mM | 9.97 | 11.03 | 0.45 | 0.15 |
| Butyrate, mM | 3.03 | 3.34 | 0.15 | 0.20 |
| Isobutyrate, mM | 0.12 | 0.12 | 0.01 | 0.88 |
| Isovalerate, mM | 0.10 | 0.12 | 0.01 | 0.74 |
| Valerate, mM | 0.23 | 0.27 | 0.01 | 0.07 |

[1]Horses were either group housed in a dry lot or individually in a stall and fed a diet consisting of 1% BW (as fed) pelleted concentrate with ad libitum coastal bermudagrass hay. Cecal contents were collected 4 h after morning meal. Values are means (*n* = 8).

3.50 and 3.64 reported by Kern et al. (1973) and Medina et al. (2002), respectively, when fed 100% timothy hay or a high-fiber diet.

Cecal valerate concentrations tended to be affected by treatment ($P = 0.07$; Table 3) with group-housed horses having a higher concentration than stalled horses. Increased valerate concentration, 4 h after the morning meal in group-housed horses could have been caused by a higher forage intake when compared with stalled horses. Branched chain VFAs, isobutyrate and isovalerate, were not affected by treatment ($P \geq 0.10$) and values are similar to those reported previously (Kern et al., 1973).

### Cecal Methane- and Ammonia-Producing Activity

Rates of cecal methane-producing activity were not affected by treatment ($P \geq 0.55$; Table 4). Although previously reported values are higher than current values, methane production was not affected by housing, which could be related to the minimal changes observed in the bacterial populations (Crutzen et al., 1986; McDaniel et al., 1993). Rates of ammonia accumulations were not affected by treatment ($P = 0.46$; Table 4). The lack of differences between treatments could be related to the minimal changes observed with bacterial populations as seen with methane concentration.

### Stoichiometric Calculations

Stoichiometric calculations have been utilized in ruminant research; however, there is limited research utilizing equine cecal VFA data to estimate reactants and products. In the current study, the amount of hexose fermented was not influenced by housing treatment ($P \geq 0.24$; Table 5). A previous study conducted in our laboratory performed stoichiometric calculations with equine cecal VFAs

and concluded similar results to the current study (Wilson et al., 2009). These values are proportional to the VFA concentrations at that sample time.

Fermentative efficiency was not influenced by treatment or period ($P \geq 0.15$); however, it was affected by individual horse variation ($P = 0.02$). The fermentative efficiency for stalled and group-housed horses was 72.18% and 72.45%, respectively. In ruminants, the fermentative efficiency has been reported as 76.9% (Anderson et al., 2010) and 73.4% to 77.4% (Chapula, 1997). Although horses are fermenting less hexose, they are still able to be as efficient as ruminants in relation to VFA concentrations. This also suggests that utilizing the fermentation balance equations to estimate fermentation products in horses could be useful. Thus, further research in this area is needed.

In conclusion, confinement housing did not significantly affect the cecal environment measured as cecal pH, enumeration of microbial populations, and fermentation products in horses offered similar diets. However, confinement housing decreased voluntary forage intake as estimated by a dual marker system. This study was designed to ensure that there were no confounding variables, such as diet, to determine the influence of housing, and the results concluded that housing does not alter microbial populations in the cecum. Further research is needed to determine any long-term effects of a reduced forage intake in stalled horses. Additionally, further research is required to more accurately describe behavioral modification that occurs in horses adapting to individual housing when all other variables remain constant.

**Table 5.** Stoichiometric calculations estimating fermentative products using equine cecal VFA concentrations in mature Quarter Horse geldings based on housing[1]

| | Housing | | | P-values |
|---|---|---|---|---|
| Item | Stall | Group housed | SEM | Treatment |
| Carbon dioxide, mM | 30.00 | 32.27 | 1.21 | 0.24 |
| Methane, mM | 13.97 | 14.56 | 0.53 | 0.47 |
| Hexose fermented, mM | 27.21 | 29.20 | 1.08 | 0.24 |
| Fermentative efficiency, % | 72.18 | 72.45 | 0.11 | 0.15 |
| Reducing equivalents consumed, mM | 82.14 | 87.27 | 3.19 | 0.30 |
| Reducing equivalents generated, mM | 98.39 | 105.25 | 3.89 | 0.26 |

[1]Horses were either group housed in a dry lot or individually in a stall and fed a diet consisting of 1% BW (as fed) pelleted concentrate with ad libitum coastal bermudagrass hay. Cecal contents were collected 4 h after morning meal. Values are means ($n = 8$).

**Table 4.** In vitro production using cecal fluid collected from Quarter Horse geldings based on housing[1]

| | Housing | | | P-values |
|---|---|---|---|---|
| Item | Stall | Group housed | SEM | Treatment |
| Methane, μmol/mL/h | | | | |
| 3 h | 0.0007 | 0.0006 | 0.0002 | 0.67 |
| 24h | 0.0007 | 0.0008 | 0.0001 | 0.67 |
| Ammonia, μmol/mL/h | | | | |
| 24 h | 0.170 | 0.160 | 0.007 | 0.46 |

[1]Horses were either group housed in a dry lot or individually in a stall and fed a diet consisting of 1% BW (as fed) pelleted concentrate with *ad libitum* coastal bermudagrass hay. Cecal contents were collected 4 h after morning meal. Values are means (n = 8).

*Conflict of interest statement.* None declared.

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