

Effects of crude protein content on intake and digestion of coastal bermudagrass hay by horses

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ABSTRACT: This study was conducted to determine the effects of forage crude protein (CP) level on intake and digestion of Coastal bermudagrass hay by horses. Four caecally fistulated geldings were used in a 4 × 4 Latin square design with four treatments and four periods. Horses were fed one of four Coastal bermudagrass hays consisting of 7%, 10%, 13%, or 16% CP during each of the four 15-d periods. Intake and apparent digestibility were determined for each horse at the end of each period by total fecal collection. In addition, cecal fluid and blood samples were collected on the last day of each period for the determination of cecal ammonia, cecal pH, plasma urea nitrogen (PUN), and plasma glucose concentrations. Data were analyzed using PROC MIXED of SAS. CP concentration of Coastal bermudagrass hay influenced equine intake and digestion. Increasing CP concentration linearly increased digestible organic matter intake (DOMI) from 3.79 to 5.98 kg/d for 7% and 16% CP hay, respectively ($P = 0.04$). Furthermore, as the forage CP level increased, CP intake increased linearly ($P < 0.01$). The forage CP

level had no effect on forage dry matter intake. Quadratic effects ($P \leq 0.05$) were observed for forage OM, neutral detergent fiber, acid detergent fiber, and digestible energy. Overall digestibility was lowest for the 7% CP hay and highest for the 10% CP hay. Cecal pH remained above 6.62 irrespective of treatment and time, indicating that cecal pH was suitable for microbial growth. As the forage CP level increased, cecal ammonia concentration increased linearly from 0.03 mM for the 7% to 1.74 mM for the 16% CP hay ($P < 0.01$). Concentration of plasma glucose also linearly increased ($P = 0.04$) from 68.77 to 73.68 mg/dL as CP concentration increased from 7% to 16% CP. PUN exhibited a quadratic effect as concentration increased ($P < 0.01$) from 4.34 to 5.61 mM for the 7% and 16% CP hays, respectively. Overall, the 10% CP hay had the highest digestibility due to its higher OM digestion. As forage OMI increased, digestible OM increased until physiological capacity for digestion is exceeded. At that point, digestion will decline with the decrease in OMI, explaining the lower digestion for other forages fed.

Key words: bermudagrass, digestibility, equine, intake

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INTRODUCTION

Coastal bermudagrass (*Cynodon dactylon*) hay is one of the most commonly fed hays in the southeastern United States. Like most grass hays, the nutrient content depends on fertilization, maturity, and environmental factors (Van Soest et al., 1978). Forage quality specifically refers to the potential of a forage to meet an animal's nutritional requirements and corresponding production goals. The relationship between nutritive value and forage quality is well characterized in other species; however, there is a dearth of information describing this relationship in equines.

Nutritional content alone is insufficient to determine nutritive value and forage quality. Intake and digestibility are important considerations to maximize forage utilization to meet nutrient requirements and performance goals. Furthermore, multiple factors influence intake and digestibility including crude protein (CP), acid detergent fiber (ADF), and neutral detergent fiber (NDF) concentrations. Higher CP concentrations have been associated with increased intake and digestibility but have not been well documented within forage species in horses (Moore et al., 1999).

While forages are typically selected based on maturity and CP content, not much is known about intake and digestibility of Coastal bermudagrass hay of varying CP levels in horses. Therefore, the objective of this study was to look at the effects of feeding Coastal bermudagrass hay of various levels of CP to provide insight on the ability of horses to utilize this forage to meet their dietary requirements.

MATERIALS AND METHODS

All care, handling, and sampling of horses were approved by the Texas A&M University Institutional Animal Care and Use Committee.

Horses and Treatments

Four previously cecally fistulated geldings [average initial body weight (BW) 548.2 ± 23.3 kg; average age 7.5 ± 2 yr] were used in a replicated 4 × 4 Latin square experiment. Geldings were housed in individual dry lots (6 m × 14 m) and provided ad libitum access to fresh water and a trace mineral/salt block (composition: 96.0% NaCl, 0.16% Fe, 0.40% Zn, 0.32% Mn, 0.01% I, 0.04% Cu, and 0.004% Co; Producers Co-op, Bryan, TX). All horses received dental care, vaccinations, and deworming according to the standard farm protocol. Dietary treatments were four qualities of Coastal bermudagrass hay

(*Cynodon dactylon*; 7%, 10%, 13%, and 16% CP). All hays were produced on an established stand of Coastal bermudagrass in College Station, TX and harvested at the same maturity. Hay (Table 1) was chopped through at 75 cm × 75 cm screen and fed at approximately 120% of the previous 4-d average intake to ensure that access to forage did not constrain intake. Hay was offered in two equal feedings at 06:00 and 18:00 each day. During period 3, a horse was removed from the study for reasons unrelated to treatment and returned to complete period 4.

Experimental Periods

The experiment was divided into four periods of 15 d with each horse receiving each treatment exactly one time. Each experimental period was divided into three phases: Phase I (d 1–10) for adaptation dietary to treatments; Phase II (d 11–14) for measurement of hay intake and digestion; and Phase III (d 15) cecal sampling. During the 10 d adaptation, horses were housed in individual dry lot pens (6 m × 14 m). During the 4 d collection phase, horses were housed in individual stalls (3 m × 3 m) with concrete floors covered with rubber mats to facilitate the determination of intake and total collection of feces. Horses were allowed 1 h of walking exercise daily at 10:00 using a free stall walker.

Sample Collections

Calculations of intake and digestion were made from observations in Phase II. Feed and ort samples were collected on d 10–13 to correspond with fecal samples collected on d 11–14. Hay was sampled as it was being fed, 400 g of each hay type was retained by grab sample daily and immediately dried for subsequent analysis. Orts were removed at 06:00 and approximately 200 g was retained for

Table 1. Forage composition of the different CP concentrations in bermudagrass hay for the different dietary treatments

Item ¹	7	10	13	16
	% of DM			
OM	92.7	93.0	92.7	90.5
CP	6.9	9.8	12.7	15.6
NDF	74.0	69.2	67.8	66.0
ADF	43.3	35.3	33.3	32.3
Gross energy, Mcal/kg	3.6	3.7	3.7	3.6

¹Crude protein levels of Coastal Bermudagrass hay.

analysis. Fecal bags (Bun-Bag, Inc., Sagle, ID) were removed and contents weighed at 06:00, 12:00, 18:00, and 24:00 daily. Feces collected over each 6-h period was thoroughly homogenized, and approximately 400 g from each horse was obtained by grab sample and immediately dried for later analysis.

Cecal fluid samples were collected on d 15 of each period just before feeding (0 h) and 4, 8, and 12 h after feeding. In order to facilitate fluid collection, the fistula was opened and cecal fluid was collected by a suction strainer (Raun and Burroughs, 1962; 19 mm diameter, 1.5 mm mesh). Immediately after sampling, cecal pH was measured using a hand-held pH meter (VMR symphony SP21; VWR International, Inc., Westchester, PA) with a Beckman 3-in-1 combination electrode (Beckman Coulter, Inc., Fullerton, CA). Following collection, 8 mL of sample fluid was transferred into an empty vial containing 2 mL meta-phosphoric acid and frozen at -20°C . Approximately 10 mL blood was collected via jugular venipuncture into an evacuated tube containing 15% EDTA (Tyco Healthcare Group LP, Mansfield, MA) and into a heparinized Vacutainer blood collection tube containing a minimum of 120 USP units of sodium-heparin (BD Vacutainer, Franklin Lakes, NJ) prior to feeding (0 h) and 4, 8, and 12 h after feeding. Samples were placed on ice immediately after collection and centrifuged at $2700 \times g$ for 20 min within 1 h after collection. Plasma was frozen for subsequent determination of plasma urea N and glucose concentrations.

Sample Analysis

Partial dry matter (DM) of hay, orts, and fecal samples was performed by drying at 55°C in a forced-air oven for 96 h. All dried samples were then ground with a Wiley mill to pass a 1-mm screen (Thomas Scientific, Swedesboro, NJ). Hay samples collected during the measurement period were pooled across days on an equal weight basis. Ort and fecal samples were each composited in proportion to their daily refusal or output, respectively, by horse across days. Hay, ort, and fecal samples were dried for 24 h at 105°C in a forced-air oven to determine DM and then combusted for 8 h at 450°C in a muffle furnace for percent ash determination. Percent organic matter (OM) was determined by subtracting percent ash from 100. Nitrogen content of hay was determined by total combustion (Rapid N-Cube, Elementar Americas, Inc., Mt Laurel, NJ). Gross energy (GE) of hay and fecal samples was determined using an oxygen bomb calorimeter

(Parr 6300; Parr Instrument Company, Moline, IL). Digestibility of energy was calculated as the remainder of consumed minus fecal excreted energy expressed as a percentage of intake.

CP was calculated as $N \times 6.25$. The ANKOM-Fiber Analyzer was used to determine NDF and ADF of all hay, ort, and fecal samples (ANDOM-Technology, Fairport WY). For determination of acid detergent insoluble ash (ADIA) of hay, ort, and fecal samples, the bags containing the ADF residues were combusted for 8 h at 450°C in a muffle furnace. Total tract digestion coefficients for DM, OM, and NDF were determined using total collection protocol, as described by Cochran and Galyean (1994). Colorimetric determination of cecal ammonia (Broderick and Kang, 1980), plasma glucose (Sigma-Aldrich Inc., St Louis, MO), and plasma urea nitrogen (PUN; Marsh et al., 1965) were made using an UV/VIS DU730 UV/VIS Spectrometer (Beckman Coulter, Inc., Fullerton, CA).

Statistical Analysis

Intake, digestion, and PUN concentration were analyzed using the PROC MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Terms in the model were treatment and period with horse included as a random effect. Fermentation profile variables were analyzed using the PROC MIXED procedure of SAS. Terms in the model were treatment, period, hour, and hour \times treatment with horse and treatment \times period \times horse included as random terms. The repeated term was hour with treatment \times horse as the subject. Compound symmetry was used for the covariance structure. The LSMEANS option was used to calculate individual treatment means. Orthogonal polynomial contrast (linear, quadratic, and cubic) was used to partition treatment sums of squares. Statistical significance was determined as $P \leq 0.05$, and trends toward significance were determined as $P \leq 0.10$.

RESULTS AND DISCUSSION

Increasing the CP concentration of Coastal bermudagrass resulted in a linear increase in digestible OM intake (DOMI) from 3.7 to 5.35 kg/d for 7% and 16% CP treatments, respectively ($P = 0.04$; Table 2). As expected, increasing forage CP content also resulted in a linear increase in CP intake ($P < 0.01$). In contrast to DOMI and CP intake, Coastal bermudagrass CP concentration did not affect any other measures of intake ($P \geq 0.11$). Increasing hay CP concentration resulted

Table 2. Effect of forage CP concentrations on intake by horses

Item	Forage crude protein, % of DM				SEM	Contrast <i>P</i> -value		
	7	10	13	16		Linear	Quadratic	Cubic
No. of Observations	3	4	4	4				
intake, kg/d								
Forage DM	9.58	9.19	10.25	12.08	1.38	0.16	0.37	0.90
Digestible DM	3.79	4.71	4.93	5.98	0.51	0.02	0.88	0.44
Forage OM	8.97	8.60	9.53	10.98	1.30	0.21	0.43	0.87
Digestible OM	3.70	4.47	4.61	5.35	0.49	0.04	0.98	0.50
NDF	7.14	6.50	7.07	8.14	1.02	0.39	0.35	0.85
Digestible NDF	2.76	3.22	3.27	3.73	0.39	0.11	1.00	0.58
ADF	4.05	3.28	3.43	3.91	0.54	0.89	0.20	0.75
Digestible ADF	1.31	1.34	1.17	1.20	0.17	0.46	1.00	0.54
CP intake	0.67	0.91	1.30	1.88	0.15	< 0.01	0.25	0.92
Energy intake, Mcal/d								
Gross	40.98	40.00	44.97	45.08	7.99	0.61	0.94	0.73
Digestible	14.86	19.37	20.36	20.47	3.04	0.21	0.45	0.82

Table 3. Effect of forage CP concentration on apparent total tract digestibility by horses

Item	Forage crude protein, % of DM				SEM	Contrast <i>P</i> -value		
	7	10	13	16		Linear	Quadratic	Cubic
No. of observations	3	4	4	4				
Total tract digestion, %								
DM	39.63	54.23	48.67	49.23	3.02	0.09	0.03	0.04
OM	41.11	55.01	48.85	48.52	2.92	0.22	0.03	0.05
NDF	38.84	52.76	46.62	45.55	2.99	0.28	0.03	0.06
ADF	33.06	43.95	34.72	30.29	3.53	0.23	0.04	0.09
Gross energy	36.04	51.65	45.70	45.33	3.69	0.19	0.05	0.10

in quadratic effects ($P \leq 0.05$) on digestion of OM, NDF, ADF, and GE (Table 3). In general, the lowest digestibility was observed with the 7% CP hay, with the exception of ADF digestibility. Digestibility was greatest with the 10% CP hay but declined for the 13% and 16% CP forages.

There were no treatment \times time interactions; thus, only overall treatment means are presented (Table 4). Cecal pH remained above 6.62 irrespective of treatment and time, indicating that cecal pH was suitable for microbial growth. While there was a cubic effect ($P < 0.01$) of CP content on cecal pH, the biological significance of these differences is negligible. There were no significant postprandial peaks or troughs that would indicate an effect of treatment on cecal pH. Concentration of cecal ammonia linearly increased ($P < 0.01$) from 0.30 mM for the 7% to 1.74 mM for the 16% CP hay. Plasma glucose linearly increased ($P = 0.04$) from 68.77 to 73.6 mg/dL as forage CP concentration increased and PUN exhibited a quadratic effect ($P < 0.01$) from 4.34 to 5.61 mM for 7% and 16% CP hays, respectively.

Voluntary dry matter intake (DMI) for grass forages is estimated around 2% BW/d in horses (Aiken et al., 1989). In the current study, intakes were 1.67, 1.64, 1.83, and 2.16% BW DMI for 7%, 10%, 13%, and 16% CP hays, respectively. Previous studies have similarly reported between 1.60% and 2.09% BW DMI (Harbers et al., 1981; Aiken et al., 1989; LaCasha et al., 1999; Edouard et al., 2008; Oliveira et al., 2015). Variations in CP, ADF, NDF, and GE are all contributing factors to DMI. In the present study, CP concentrations were the basis for dietary treatments and consisted of 7%, 10%, 13%, and 16%. Corresponding NDF concentrations were 74%, 69.2%, 67.8%, and 66.0% DM, and ADF concentrations were 43.3%, 35.3%, 33.3%, and 32.3% DM, respectively. Hays fed in the current study were higher in ADF than that which was reported by Aiken et al. (1989) and Edouard et al. (2008), in which horses were fed similar grass forages of 10.7% and 11.6% CP, respectively. Interestingly, Edouard et al. (2008) reported a 1.66% BW DMI which more closely agrees with what was observed in the present study (1.64% BW DMI for 11% CP

Table 4. Effect of forage CP concentration on cecal fermentation and plasma metabolic concentration in horses

Item	Forage crude protein, % of DM				SEM ¹	Contrast <i>P</i> -value		
	7	10	13	16		Linear	Quadratic	Cubic
No. of Observations	3	4	4	4				
cecal								
pH	6.98	6.81	6.84	7.00	0.07	0.11	0.66	< 0.01
Ammonia, mM	0.30	0.55	0.82	1.74	0.38	< 0.01	0.32	0.65
plasma								
Glucose mg/dL	68.77	70.65	72.85	73.68	0.29	0.04	0.76	0.82
Urea Nitrogen, mM	4.34	4.12	4.03	5.61	0.32	0.01	< 0.01	0.21

¹Standard error of the mean.

hay) compared to Aiken et al. (1989) and LaCasha et al. (1999) who reported intakes of 2.0–2.09% BW. Decrease in intake in the present study compared with what was reported may be due to higher NDF and ADF, which added indigestible bulk and decreased available energy for digestion in the hindgut.

Digestibility of dry matter (DMD) in the present study was 39.6%, 54.2%, 48.7%, and 49.2% for 7%, 10%, 13%, and 16% CP hays, respectively. In two previous studies in which horses were fed 11.3% and 11.6% CP grass hays, DMD was estimated at 46% and 50% (LaCasha et al., 1999; Edouard et al., 2008). Digestibility reported by LaCasha et al. (1999) is lower than that which was reported in the present study with the exception of the 7% CP diet, but Edouard et al. (2008) were most similar to the present study's observed digestibility.

Organic matter digestibility (OMD) was lower (41.1%, 55.0%, 48.8%, 48.5%) for all hays than what has been previously reported in similar studies (LaCasha et al., 1999; Schaafstra et al., 2015). In contrast, the 55% OMD for the 10% CP hay in the present study is similar (53%) to that which was observed by Eckert et al. (2010) when horses were fed a similar 10% CP Coastal bermudagrass hay. The 52.8% NDF digestibility of the 10% CP forage is similar to apparent NDF digestibilities of 52.0%, 51.7%, and 50% reported by LaCasha et al. (1999), Sturgeon et al. (2000), and Eckert et al. (2010) for horses consuming 11.3%, 8.28%, and 10% CP Coastal bermudagrass hay, respectively. The 33.1%, 43.9%, 34.7%, and 30.3% ADF digestibilities of the 7%, 10%, 13%, and 16% CP hays were lower than the 47.32% reported by Harbers et al. (1981) for warm-season forage and higher than the 22–30% reported by LaCasha et al. (1999) and Oliveira et al. (2015).

The lowest DMD observed for the 7% CP forage is likely due to the greater NDF (Darlington and Hershberger, 1968; Hinz et al., 1971; Glade,

1984) and ADF (Reid, 1988; Van Soest, 1993) contents of this hay. Additionally, the 7% CP forage did not meet the 15.2 Mcal/d digestible energy (DE) requirement for a 500 kg mature horse at rest (NRC, 2007). DMI of the 10%, 13%, and 16% CP forages exceeded the DE requirement at 19.4, 20.4, and 20.5 Mcal/day, respectively. Unexpectedly, the hay highest in CP and lowest in fiber content was intermediate in apparent digestibility. This was possibly due to a greater rate of passage and therefore less time for digestion of this forage. Furthermore, the forage highest in digestibility was intermediate in CP (10% CP). In our study, as forage OM intake increased, digestible OM intake also increased. However, a point is reached at which intake will exceed the physiologic capacity for digestion, at which time OMD will decline with increasing intake. Consequently, apparent OMD was lower for the 7%, 13%, and 16% CP forage, 41.1%, 48.9%, and 48.5%, respectively, than for the 10% CP forage, 55.0%.

Plasma glucose levels in our study showed no consistent postprandial peaks or troughs in plasma glucose (data not shown). This is consistent with the results of Stull and Rodiek (1988) who reported that horses consuming an alfalfa hay diet had postprandial plasma glucose levels that were within 5% of resting plasma glucose levels. All-forage diets produce smaller changes in plasma glucose due to limited foregut digestion of available polysaccharides.

PUN increased post feeding for all treatments except the 10% CP hay. The 16% CP hay produced a much higher increase in PUN after feeding than other treatments and higher h 0 PUN, indicating an intake of protein above dietary requirements (data not shown). Previous equine studies have also reported a corresponding increase in PUN with increasing dietary CP concentrations (Reitnour and Treece, 1971; Prior et al., 1974; Graham-Theirs and Bowen, 2011).

Graham-Theirs and Bowen (2011) reported PUN levels between 5.7 and 6.6 mM in horses fed a hay-only diet with CP concentrations of 9.98%, which is greater than that which was observed in the present study. The PUN concentration in horse fed the 7% CP forage, 3.65 mM, was lowest, but was only slightly lower than the 13% CP forage, 4.08 mM, indicating that the quality of protein in even the lowest CP hay was satisfactory to prevent an increase in PUN resulting from insufficient or excess protein in the diet.

The average cecal pH for all treatments is similar to the average cecal pH reported in other studies in which horses were fed hay-only diets (Willard et al., 1977; Brokner et al., 2012; Warzecha et al., 2017). However, results from Willard et al. (1977) showed a 3% decrease in cecal pH 4 h after feeding, whereas our results only showed a decrease in cecal pH 4 h after feeding for the 10% CP hay which was only 1% lower than the 0 h reading. Similarly, Warzecha et al. (2017) and Brokner et al. (2012) observed no significant postprandial change in cecal pH in horses fed a hay-only diet. The results for Willard et al. (1977) were likely due to the fact that horses in that study were fed at 12-h intervals, which may have resulted in restricted intake due to limited forage access. Horses in the present study were allowed ad libitum access to hay in order to provide intake that would not be limited by forage availability. Ad libitum access to hay would result in decreased differences between h 0 and h 4 cecal pH readings due to horses actively digesting hay in the hindgut up until the subsequent feeding at h 0, assuming that horses are eating the forage they are exposed to, which results in a higher initial cecal pH. Medina et al. (2002) reported an average cecal pH of 7.15 for horses consuming a diet of high fiber pellets and straw which were fed at two equal feedings 08:00 and 18:00 daily. This is slightly higher than the average cecal pH of 6.98, 6.81, 6.84, and 7.00 for horses on our study consuming 7%, 10%, 13%, and 16% CP hay. Medina et al. (2002) reported an average cecal pH at h 0 of approximately 7.8, much higher than those reported in our study, likely due to the feeding intervals of the study. This allowed for an extended amount of time to pass between feedings, and consequently, for active hindgut digestion to decrease and cecal pH to increase prior to the h 0 measurement. Cecal pH measured 5–7 h after feeding averaged approximately 6.9, which is similar to those reported in our study for horses consuming forage ad libitum. Cecal ammonia concentration increased as dietary CP concentration

increased. Increasing dietary CP concentration increases the amount of substrate available for ammonia production in the hindgut.

In summary, forage utilization has been defined as the product of intake and digestion and in ruminants it is, in part, driven by forage CP concentration (Moore et al., 1999). In contrast to the described relationship in ruminants, data in horses are limited in the literature, especially for warm-season forages. Therefore, the primary objective of this project was to determine the effect of forage CP content on the utilization of Coastal bermudagrass hay by horses. As previously described, the forages used in this project were taken from the same location and harvested at the same maturity; the only difference was the level of nitrogen fertilization provided. Ideally, forage CP concentrations would have been more divergent, allowing data to be collected on low CP bermudagrass hay (less than 6%). In this study, the lowest CP hay used was 7% CP which is the breakpoint reported by Moore et al. (1999) to be the point at which forage intake decreases in ruminants as CP concentration is reduced. In accordance with the observations in ruminants, forage intake did not significantly increase with increasing CP content. However, intake of the 16% CP was 22% greater than the intake of the 7% CP hay, indicating at least some benefit to increased CP content.

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