Effects of cottonseed meal supplementation and wheat pasture maturity on forage intake and digestion characteristics of cows grazing winter wheat pasture

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INTRODUCTION

Winter wheat pasture (WWP; *triticum aestivum*) is commonly used in the Southern Great Plains of the United States to develop cattle. Cattle grazing WWP develop frame and muscle fairly fast while fat deposition is restricted at moderate cost (Torell et al., 1999; Hersom et al., 2004). The nutritional value of WWP is high because it contains above 20% crude protein (CP), and over 70% digestible dry matter (DM) (Mader and Horn, 1986; Branine and Galyean, 1990; Torell et al., 1999). However, cattle grazing WWP might be deficient in metabolizable protein because its protein is highly soluble (Beever, 1984; Vogel et al., 1989; Chabot et al., 2008), and most of it can be excreted in urine (Poos et al., 1979). The protein absorbed by ruminants (metabolizable protein) is supplied by both undigested intake protein and microbial protein (NRC, 2000). Furthermore, microbial protein synthesis requires N-containing compounds and organic matter (OM) for fermentation (Hespell, 1979). Although cattle grazing WWP frequently meet the recommended level of digestible intake protein; [DIP; 13% of total digestible nutrients (TDN); NRC, 2000], some ruminal microbes require other nitrogenous compounds such as peptides and amino acids (Zinn and Owens, 1983; Garrett et al., 1987; Russell et al., 1992). Thus, supplementation of feedstuffs like cottonseed meal (CSM) that provides peptides and amino acids in addition to ammonia (NRC, 2000), could improve microbial growth and (or) digestion. Hence, objectives of this experiment were to determine effects of CSM supplementation on forage intake, digestion characteristics, and metabolizable protein of cattle grazing WWP.

MATERIALS AND METHODS

All procedures and experimental protocols were approved by the New Mexico State University Institutional Animal Care and Use Committee.

Eight mixed-breed mature cows [736 ± 32.6 kg of body weight (BW)] fitted with cannulas in the rumen and proximal duodenum were used in a split-plot design. Supplemental treatment was the main plot, and experimental period was the subplot.

Experimental Design and Treatments

Cows were randomly assigned to 1 of 2 supplemental treatments: 1) Control (CON; un-supplemented), and 2) CSM supplementation [41.9% CP; 12.0% acid detergent fiber (ADF), and 23.5% neutral detergent fiber (NDF), DM basis] offered at 106 g/d to provide 25 g of DIP daily. The experiment consisted of two 14-d experimental periods; the first 10 d were used for adaptation to wheat pasture grazing and supplement, the last 4

d for sample collection. Experimental period occurred during late February and late March. Cows grazed a single wheat pasture (Weathermaster, West Gaines Seed, Inc., Seminole, TX; beardless wheat; *Triticum aestivum*). Supplemental CSM was placed into their rumens through the rumen cannula at 0700 h daily. Gelatin capsules containing chromic oxide (8 g) were dosed ruminally twice daily (at 0700 and 1900 h) on d 6 to 14 of the experimental periods. Chromic oxide was used as digesta marker. Because cows received the supplement individually, cow was considered the experimental unit. For each supplementation, chromic oxide dosing or sample collection, cows were gathered into a holding pen and secured to a fence post with 1-m long halter.

Sample Collection

Eight fecal samples from rectal grabs and eight duodenal samples from duodenal cannula were collected on d 11, 0700 and 1300 h; d 12, 0100 and 1900 h; d 13, 1000, 1600 and 2200 h; and d 14, 0400 h. The 8 collection times represent 1 collection each 3 h in a 24-h cycle. Individual samples consisted of approximately 100 mL of duodenal chyme and 200 g of fecal matter. Samples from each cow and within each collection period were composited for analysis.

Ruminal fluid samples were collected directly from the rumen cannula with a suction strainer on d 12 at 0700 (before supplementation), 0900, 1100, 1300, 1500, 1700 and 1900 h. Ruminal fluid pH was determined immediately after collection, and the samples were then acidified with 7.2 N H_2SO_4 at a rate of 1 mL/25 mL of rumen fluid and frozen (−10 °C) in 50-mL polypropylene conical tubes (VWR International, Radnor, PA) for later analysis of VFA and ammonia.

On d 14 of each period at 1200 h after the last duodenal and fecal sample collection, one randomly selected cow from each treatment was ruminally evacuated. Digesta was placed in plastic bags lining 133-L plastic containers. A 2-kg subsample of ruminal content was obtained and mixed with 1 L of saline solution (0.9% NaCl; wt/vol) for isolation of bacterial cells (Zinn and Owens, 1986). Ruminal content samples were frozen (−10 °C) for bacterial isolation at a later time. After evacuations, cows returned to pastures and were allowed to graze for 60 min. Masticate samples were subsequently collected and 10% subsample was retained to estimate in vitro digestibility and forage quality. Masticate samples were dried in a forced-air oven (50 °C) to a constant weight, and ground in a Wiley

mill (2-mm screen; Thomas Scientific, Swedesboro, NJ), and composited on an equal, dry weight basis.

Laboratory Analysis

Duodenal samples were lyophilized (VirTis LyoTroll; SP Scienctific, Garnier, NY), and ground with a microgrinder (Model CM4; Salton/Maxim Houseware Inc., Mt. Prospect, IL). Masticate, supplemental CSM, duodenal and fecal samples were analyzed for DM, OM, and CP (Methods 930.15, 942.05, and 990.02, respectively; AOAC, 1997). Also, NDF (with heat stable amylase addition) and ADF analysis were performed according to Robertson and Van Soest (1991) using an ANKOM 200 fiber analyzer (ANKOM Technology, Macedon, NY), sequentially. Lyophilized duodenal samples were analyzed for purines (Zinn and Owens, 1986), and ammonia (Broderick and Kang, 1980). Fecal and duodenal samples were analyzed for Cr with an air-plus acetylene flame using atomic absorption spectroscopy Cr (Hill and Anderson, 1958).

In vitro OM digestibility of masticate and supplemental CSM samples were determined according to the procedure described by Tilley and Terry (1963) adapted to a Daisy incubator (ANKOM Technology). Composited inoculate from two ruminally cannulated cows fed a grass hay diet was used for in vitro incubation.

Ruminal bacteria were isolated from saline-treated ruminal contents (2 kg). Ruminal contents were blended on high speed in a food processor for 1 min, and the mixture was strained through four layers of cheesecloth. Feed particles and protozoa in ruminal samples were removed via centrifugation at 1,000 × g for 10 min. Bacteria were separated from supernatant by centrifugation at 27,000 × g for 20 min. Isolated bacteria were lyophilized (VirTis Lyotroll) and analyzed for DM, ash, CP, and purines (as previously described).

Acidified rumen fluid samples were centrifugated at 20,000 × g for 20 min and analyzed for ammonia concentration by the phenol-hypochlorite method (Broderick and Kang, 1980). Ruminal VFA concentrations were determined using 2-ethyl butyric acid as internal standard (Erwin et al., 1961).

Calculations

Fecal output and duodenal DM flow were calculated using fecal and duodenal Cr concentration, respectively. Fecal DM output was calculated by dividing Cr dose by fecal Cr concentration. Similarly, DM flowing to the duodenum

was calculated by dividing Cr dose by Cr concentration in duodenal chime. Forage fecal output on DM basis was determined by subtracting the indigestible fraction of the supplement from total fecal output of cows using the in vitro indigestibility of CSM.

Forage DMI was estimated by dividing forage fecal DM output by forage in vitro DM indigestibility. Microbial OM and N flowing to duodenum were calculated using purines as microbial marker ([Zinn and Owens, 1986](#)). Organic matter truly fermented

Table 1. Effects of cottonseed meal supplementation[1] and forage maturity[2] on DM and OM intake and characteristics of digestion of beef cows grazing wheat pasture

	Supplementation			Forage maturity			P- value[3]		
Item	CON	CSM	SE	FEB	MAR	SE	SUP	FM	S×F
DMI, kg/d									
Forage	14.2	18.8	1.35	16.6	16.4	1.27	0.03	0.89	0.14
CSM	—	0.1	—	0.1	0.1	—	—	—	—
Total	14.2	18.9	1.35	16.7	16.4	1.26	0.03	0.89	0.14
OMI, kg/d									
Forage	11.3	15.1	1.08	13.2	13.2	1.01	0.03	0.99	0.13
CSM	—	0.01	—	0.05	0.1	—	—	—	—
Total	11.3	15.1	1.08	13.2	13.2	1.01	0.03	0.99	0.13
CP intake, kg/d	2.2	2.9	0.20	2.6	2.4	0.19	0.02	0.48	0.15
NDF intake, kg/d	8.1	10.8	0.80	8.8	10.0	0.75	0.03	0.29	0.11
ADF intake, kg/d	4.5	6.0	0.45	5.0	5.6	0.42	0.03	0.30	0.11
OMI, g/kg of BW[4]	15.4	21.1	2.05	18.2	18.2	1.92	0.06	0.99	0.26
Flow to duodenum, kg/d									
DM	7.1	9.4	0.70	7.1	9.3	0.61	0.03	0.04	0.38
OM	4.5	6.1	0.65	4.9	5.6	0.61	0.10	0.43	0.90
Microbial OM	1.6	2.3	0.25	1.9	2.0	0.24	0.07	0.70	0.73
Feed OM	2.9	3.8	0.42	3.0	3.6	0.40	0.15	0.33	0.99
CP	1.6	2.2	0.23	1.8	2.0	0.21	0.09	0.47	0.74
Microbial	1.0	1.5	0.16	1.2	1.3	0.15	0.07	0.70	0.73
Feed	0.6	0.7	0.07	0.6	0.7	0.07	0.21	0.24	0.80
NDF	2.0	2.4	0.20	2.0	2.4	0.19	0.20	0.13	0.65
ADF	1.3	1.4	0.17	1.1	1.6	0.16	0.54	0.08	0.40
True ruminal digestion, %									
DM	78.9	81.8	1.89	89.5	71.2	1.77	0.28	0.01	0.10
FEB	90.3	88.6	2.68	—	—	—	0.66	—	—
MAR	67.5	74.9	2.68	—	—	—	0.06	—	—
OM	74.3	74.6	2.72	77.4	71.5	2.55	0.92	0.13	0.26
Fecal excretion, kg/d									
DM	4.3	4.9	0.36	3.9	5.3	0.37	0.27	0.04	0.30
OM	2.4	2.7	0.33	2.4	2.7	0.31	0.50	0.43	0.90
Total tract digestion, %									
DM	69.2	74.4	—	76.9	66.7	—	—	—	—
OM	79.0	81.8	2.05	82.3	78.5	1.91	0.33	0.19	0.34
Total tract digestion, kg/d									
DM	9.9	14.0	0.97	12.8	11.1	0.91	0.01	0.21	0.11
OM	8.9	12.4	0.94	10.8	10.5	0.88	0.02	0.77	0.10
FEB	10.3	11.4	1.34	—	—	—	0.53	—	—
MAR	7.6	13.3	1.34	—	—	—	0.01	—	—
CP	1.6	2.3	0.18	2.1	1.9	0.17	0.02	0.39	0.13
NDF	6.0	8.4	0.64	7.1	7.4	0.60	0.02	0.70	0.14
ADF	3.2	4.6	0.34	3.9	3.9	0.32	0.31	0.92	0.15

[1]Supplemental treatment were supplemented control (CON), and supplemented cows that received 106 g/d of cottonseed meal intraruminally to provide 60 g of DIP daily (CSM).

[2]Forage stage of maturity consisted of allowing beef cows to graze winter wheat pasture during late February (FEB), and late March (MAR).

[3]Probability values associated with CSM supplementation (SUP), forage maturity (FM), and supplementation × forage maturity interaction (S×F).

[4]Total OM intake (forage + supplement), g/kg of BW.

in the rumen was calculated as OM intake minus the difference between the total OM leaving the abomasum and the microbial OM leaving the abomasum. Feed N escape to the small intestine was considered equal to total N leaving the abomasum minus NH_3-N and microbial-N and, thus includes endogenous N additions. Microbial efficiency was calculated as g of microbial N divided by kg of OM truly fermented in the rumen.

Statistical Analysis

Data collected as a single point collection were analyzed as a split-plot design using the mixed procedures of SAS (SAS Inst. Inc., Cary, NC). The statistical model included CSM supplementation in the main plot, and forage stage of maturity and CSM supplementation × forage stage of maturity interaction in the subplot. Forage stage of maturity was considered repeated effect, and cow within CSM supplementation was used to test supplementation effects. The covariance structure used was compound symmetry. When CSM supplementation × forage stage of maturity interaction was significant ($P < 0.10$), the effects (LSD; $P < 0.10$) of CSM supplementation were tested within each forage stage of maturity. When such interaction was not significant ($P > 0.10$), only main effects were reported. When significant ($P < 0.10$) F-statistics were noted, means were separated using LSD.

The mixed procedure of SAS was also used to analyze the ruminal fermentation data (pH, NH_3-N, and VFA) using a split-split-plot design. The statistical model included CSM supplementation, forage stage of maturity, and forage stage of maturity × CSM supplementation interaction. Time of sample collection was considered repeated effect, and cow within forage stage of maturity × supplementation was used as the error term for split-split-plot. Individual cow was the experimental unit in all analyses. A compound symmetry covariance structure was used.

RESULTS AND DISCUSSION

Supplementation of CSM increased ($P \leq 0.10$) forage and total intake of DM, OM, CP, NDF, and ADF, increased ($P \leq 0.10$) the flow of nutrients to the small intestine (DM, OM, CP, NDF, and ADF), increased ($P \leq 0.03$) total tract digestibility of DM, CP, NDF, and ADF (kg/d). On the other hand, forage maturity did not affect nutrient intake ($P \leq 0.29$), nutrient flow to the small intestine ($P \leq 0.29$), or total tract digestibility of nutrients ($P \leq 0.39$). The CP flowing to the small intestine from microbial origin

increased ($P < 0.07$) with CSM supplementation and was not affected ($P = 0.70$) by stage of forage maturity. Supplementation of CSM decreased ($P = 0.08$) propionate (20.58 and 18.74 ± 0.71 mol/100 mol), increased ($P = 0.01$) butyrate (11.48 and 15.45 ± 0.97 mol/100 mol), while butyrate decreased ($P = 0.0.09$) with advancing stage of maturity (14.65 and 12.27 ± 0.91 mol/100 mol). Improvements on microbial synthesis and digestibility were not expected with CSM supplementation because WWP provided more than the recommended DIP required for optimal rumen fermentation (NRC, 2000). The reason for the positive response to CSM is not certain. A possibility is that CSM provided amino acids and peptides required by ruminal microorganisms. Some ruminal microbes require other nitrogenous compounds such as peptides and amino acids (Zinn and Owens, 1983; Garrett et al., 1987; Russell et al., 1992). Solubility of WWP protein is very rapidly, 50% to 75% disappears at rates of 16% to %/h (Vogel et al., 1989), and 25% to 37% of the N content on WWP is the form of nonprotein N (Horn et al., 1977). Therefore, WWP might not provide the appropriate peptides and amino acids required by ruminal microbes for appropriate microbial growth and digestibility. Another possibility is that CSM improved microbial synthesis by providing energy (75% TDN; NRC, 2000). Because WWP is low in fiber content, it is considered deficient in energy (Mader and Horn, 1986; Branine and Galyean, 1990). However, CSM supplementation was only 106 g, which only explains 10 g of microbial protein synthesis if used as energy source (NRC, 2000). In the present experiment microbial protein synthesis increased approximately 700 g with CSM supplementation.

IMPLICATIONS

Results from this experiment imply that the DIP present in WWP is deficient in amino acids in peptides required by some ruminal microbes, and supplementation of feedstuffs like CSM provides such peptides and amino acids in addition to ammonia to optimize microbial protein synthesis and digestibility.

Conflict of interest statement. None declared.

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