Effects of monensin supplementation and wheat pasture maturity on forage intake and digestion characteristics of cows grazing winter wheat pasture[1]

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INTRODUCTION

Developing cattle on winter wheat pasture (**WWP**) is a popular practice in the Southern Great Planes of the United States. Wheat pasture (*Triticum aestivum*) is a highly valued source of forage as it allows frame and muscle development while simultaneously restricting fat deposition at a moderate cost (Torell et al., 1999; Hersom et al., 2004). The forage contains above 20% CP, and over 70% digestible DM (Mader and Horn, 1986; Branine and Galyean, 1996; Torell et al., 1999). However, protein is highly soluble and as such, cattle grazing WWP might be deficient in metabolizable protein (Beever, 1984; Vogel et al., 1989; Chabot et al., 2008). Excess rumen soluble protein (DIP) increases the excretion of N into the environment (Poos et al., 1979). In ruminants, metabolizable protein is comprised of both undigested intake protein (UIP) and microbial protein (NRC, 2000). Furthermore, adequate microbial protein synthesis depends on DIP and ruminal fermentation of OM (Hespell, 1979). However, the OM content of WWP is limited during the vegetative state due to low fiber content (Mader and Horn, 1986), which limits N retention.

Monensin selectivity inhibits Gram-positive bacteria, which modifies rumen fermentation (Schelling, 1984). Among several effects of monensin on rumen fermentation, inhibition of proteolytic activity has been reported in beef cattle and lambs (Hanson and Klopfenstein, 1977; Poos et al., 1979). Although the effects of monensin on forage intake, weight gain, and digestive function have been evaluated (Horn et al., 1981, Branine and Galyean, 1990), the effects of monensin on microbial protein synthesis and UIP have not been studied. Therefore, it is hypothesized that monensin decreases ruminal proteolytic activity of cattle grazing WWP, which increases the amount of protein reaching the small intestine. The objective of this experiment was to evaluate the effects of monensin supplementation and WWP stage of maturity 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 Institute Animal Care and Use Committee.

Eight mature Angus mixed-breed cows (669 ± 23.2 kg of BW) fitted with duodenal and ruminal cannulas were used in a split-plot design. Cows were randomly allotted to one of

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two treatments: 1) Control (**CON**; 1.0 g of wheat middling only); or 2) monensin (**MON**; CON plus 200 mg of monensin).

Sample Collection

Two experimental periods were conducted from March 13 to March 26 (**MAR**), and March 27 to April 8 (**APR**) of 2017. During each period, animals were allowed 10 d to adapt to WWP and supplements and 4 d for sample collection. Cattle grazed a single wheat pasture (*T. aestivum*). Cows were gathered into a holding pen and secured to a fence post with a 1-m-long halter, and supplemented directly into the rumen daily at 0700.

Chromic oxide (8 g) placed in gelatin capsules (Torpac Inc., Fairfild, NJ) was dosed intraruminally on d 6 to 14 at 0700 and 1900 to be used as a digesta flow marker. Duodenal and fecal samples were collected during the collection period from all cows as follows: d 11, 0700 and 1300; d 12, 0100 and 1900; d 13, 1000, 1600 and 2200; and d 14, 0400. Individual samples consisted of 100 ml of duodenal chyme and 200 g (wet basis) of fecal material. Duodenal and fecal samples from each cow and within each collection period were composited independently for analysis.

Rumen fluid samples were taken via rumen cannula using a suction strainer (Precision Machine Co. Inc., Lincoln, NE) at 0, 3, 6, 9, 12, 15, and 21 h after the d 12 supplementation. Ruminal pH was assessed immediately after collection; samples were acidified with 7.2 N H_2SO_4 at a rate of 1 ml/25 ml of rumen fluid, and stored at −20 °C for subsequent analysis of NH_3-N and VFA. At 0900 on d 14 of each experimental period, a 2-kg sample of ruminal contents was obtained and mixed with 1 L of saline solution (0.9% NaCl, wt/vol) for isolation of bacterial cells (Zinn and Owens, 1986), and analysis of DM, ash, N, and purines. Samples were stored at −20 °C until analysis.

Two cows were ruminally evacuated in a holding pen at 1000 on d 14 of each period. Digesta was placed in plastic bags lining 133-L plastic containers. After evacuation, cows were returned to pasture and were allowed to graze for 60 min. Masticate samples were subsequently collected. Masticate samples were dried in a forced oven (50 °C) to a constant weight and ground in a Wiley mill (2-mm; Wiley mill model 4, Thomas Scientific, Swedesboro, NJ), and composited on an equal dry-weight basis.

Laboratory Analysis

Fecal, duodenal, and bacterial samples were dried in a freeze dryer at −50 °C, and were ground in a Wiley mill. Duodenal, masticate, and fecal samples were analyzed for DM, ash, CP (Methods 930.15, 942.05 and 990.02, respectively; AOAC, 1997), and NDF using an Ankom 200 fiber analyzer (Ankom CO, Fairport, NY). Duodenal samples were analyzed for purines (Zinn and Owens, 1986), and ammonia (Broderick and Kang, 1980). Masticate and supplement samples were analyzed were analyzed for in vitro DM digestibility using the procedures described by Tilley and Terry (1963) with adaptations for Ankom Daisy Incubator (Daisy II Incubator). Duodenal and fecal samples were analyzed for Cr (Hill and Anderson, 1958).

Ruminal bacteria cells were isolated from rumen contents/saline mixture. Ruminal contents were blended, and the mixture was strained through four layers of cheesecloth. The resulting fluid was centrifuged at 1,000 × *g* for 10 min to remove feed particles and protozoa. The supernatant was then centrifuged at 27,000 × *g* for 20 min to separate bacteria. Isolated bacteria were then mixed with a saline solution (0.9 NaCl; wt/vol) and centrifuged once more at 27,000 × *g* for 20 min. Isolated bacteria were frozen, lyophilized, and analyzed for DM, N, ash, and purines as described above. Acidified rumen fluid samples were centrifuged at a 27,000 × *g* for 20 min and supernatant was analyzed for ammonia (Broderick and Kang, 1980) and VFA (Goetsch and Galyean, 1983).

Calculations

Daily fecal DM output and duodenal chyme were calculated by dividing the Cr dose by fecal and duodenal Cr concentration, respectively. Supplement fecal DM output was calculated by multiplying supplement intake by supplement in vitro DM indigestibility. Forage fecal DM was calculated by subtracting supplement fecal DM output from fecal DM output. Forage DM intake was calculated as forage fecal output divided by forage in vitro indigestibility. Microbial OM and N leaving the abomasum were calculated using purines as a microbial marker (Zinn and Owens, 1986). Organic matter fermented in the rumen was considered equal to OM intake minus the difference between the amount of total OM reaching the duodenum and microbial OM reaching the duodenum. Feed N reaching the small intestine was considered equal to total N leaving abomasum minus NH_3-N, and microbial N and, thus includes endogenous N addition. Microbial N efficiency was calculated as g of duodenal microbial N per kg of OM fermented in the rumen.

Table 1. Effects of monensin supplementation, and forage stage of maturity on forage intake and digestion characteristics of beef cows grazing winter wheat pasture

	Monensin, mg/d			Stage of maturity			*P*-value[a]		
Item	0	200	SE	March	April	SE	TRT	PD	TRT × PD
DM intake, kg/d									
Forage	12.65	12.17	0.70	13.94	10.88	0.70	0.64	0.02	0.92
Wheat middling[b]	0.89	0.89	—	0.89	0.89	—	—	—	—
Total	13.54	13.06	0.70	14.83	11.77	0.70	0.64	0.09	0.92
OM intake, kg/d									
Forage	11.13	10.71	0.62	12.26	9.63	0.62	0.64	0.02	0.91
Wheat middling[b]	0.84	0.84	—	0.84	0.84	—	—	—	—
Total	11.97	11.55	0.62	13.05	10.48	0.62	0.64	0.02	0.91
Total OM intake, g/kg of BW	18.41	17.12	1.36	19.65	15.88	1.47	0.43	0.02	0.95
CP intake, kg/d									
Forage	1.48	1.42	0.07	1.73	1.16	0.07	0.63	0.02	0.94
Wheat middling[b]	0.16	0.16	—	0.16	0.16	—	—	—	—
Total	1.64	1.59	0.07	1.90	1.33	0.07	0.63	0.02	0.94
NDF intake, kg/d									
Forage	8.11	7.80	0.46	8.59	7.32	0.46	0.65	0.10	0.90
Wheat middling[b]	0.32	0.32	—	0.32	0.32	—	—	—	—
Total	8.43	8.12	0.46	8.91	7.64	0.46	0.65	0.10	0.90
Flow to duodenum, kg/d									
DM	8.91	8.26	0.83	9.06	8.11	0.64	0.60	0.12	0.52
OM	6.45	6.17	0.64	6.75	5.86	0.49	0.76	0.05	0.57
Microbial OM	1.74	1.37	0.56	1.97	1.13	0.08	0.03	0.01	0.11
Feed OM	4.70	4.79	0.57	4.78	4.72	0.43	0.91	0.84	0.89
NDF	3.06	2.79	0.25	2.76	3.09	0.19	0.47	0.10	0.72
CP	1.93	1.78	0.16	2.23	1.48	0.13	0.53	0.01	0.52
Microbial protein	1.11	0.87	0.63	1.26	0.78	0.55	0.03	0.01	0.11
Feed protein	0.82	0.91	0.12	0.98	0.76	0.10	0.66	0.04	0.81
Ammonia	0.08	0.07	0.05	0.08	0.07	0.05	0.53	0.29	0.13
Microbial protein efficiency[c]	27.3	23.5	2.0	30.7	20.1	1.6	0.27	0.01	0.35
True ruminal digestion, %									
DM	54.28	55.24	5.28	62.75	46.77	4.74	0.90	0.02	0.96
OM	59.12	58.13	4.47	63.40	53.84	3.75	0.88	0.05	0.96
CP	48.02	43.23	6.91	49.03	42.21	5.77	0.64	0.30	0.79
Fecal excretion, kg/d									
CP	0.55	0.55	0.02	0.66	0.48	0.02	0.86	0.01	0.62
OM	3.40	3.48	0.21	3.38	3.50	0.19	0.81	0.65	0.98
NDF	2.89	2.79	0.21	2.45	3.24	0.22	0.75	0.05	0.74
Total tract digestion, %									
DM	63.14	62.97	0.10	68.56	57.54	0.10	0.28	<0.01	0.01
OM	71.05	69.42	0.77	74.06	66.41	0.67	0.18	<0.01	0.60
CP	66.33	65.18	0.80	65.36	66.15	0.83	0.35	0.53	0.47
NDF	65.31	64.97	0.71	72.45	57.82	0.70	0.75	<0.01	0.57
Total tract digestion, kg/d									
DM	8.57	8.30	0.39	10.17	6.76	0.40	0.58	0.01	0.92
OM	8.57	8.07	0.44	9.66	6.97	0.45	0.45	0.01	0.89
CP	1.09	1.03	0.05	1.24	0.88	0.05	0.50	0.01	0.87
NDF	5.53	5.34	0.27	6.46	4.39	0.26	0.59	0.01	0.93

[a]Probability value associated with monensin supplementation (TRT), stage of forage maturity (PD), and monensin supplementation × stage of maturity (TRT × PD).

[b]Wheat middling was used as a carrier for monensin.

[c]Microbial protein efficiency = duodenal microbial N, g.kg^{-1} OM fermented in the rumen.

Statistical Analysis

Data were analyzed as a split-plot design using the mixed procedures of SAS (SAS Inst. Inc., Cary, NC). Supplemental monensin was included in the main plot and stage of WWP maturity was in the subplot. For intake, digesta flow, and digestibility data, the model included monensin supplementation, stage of maturity, and monensin × maturity. The repeated effect was stage of maturity, and cow within monensin supplementation was used to test the effects of monensin supplementation. When significant ($P < 0.05$) *F*-statistics were noted, means were separated using LSD.

The mixed procedure of SAS was also used to analyze the ruminal fermentation data (pH, NH3-N, VFA) using a split-split-plot design. Effects in the model included monensin supplementation, stage of maturity, and monensin supplementation × stage of maturity. The repeated measurement was time of rumen fluid sample collection and cow within monensin supplementation × stage maturity was used as the error term to test the effects of monensin supplementation. Individual cow was the experimental unit in all analysis.

RESULTS AND DISCUSSION

Effects of monensin supplementation, and stage of WWP forage maturity on intake and characteristics of digestion are shown in Table 1. There was no monensin supplementation × forage maturity interactions ($P \geq 0.11$) for any of the variables, except for total tract DM digestion (%; $P = 0.01$). Therefore, main effects are presented. Monensin supplementation did not affect forage intake ($P = 0.64$), nutrient flow to duodenum ($P \geq 0.47$) or total tract digestion expressed kg/d ($P \geq 0.45$). Although OM and CP flowing to duodenum were not affected by monensin supplementation ($P \geq 0.53$), microbial OM and microbial CP decreased ($P = 0.03$) with monensin supplementation. As stage of maturity of WWP advanced, forage intake ($P = 0.02$), ruminal digestibility of DM and OM ($P \leq 0.05$), and total tract digestion expressed as kg/d ($P = 0.01$) decreased. Also, the flow to duodenum of CP ($P = 0.01$), microbial protein ($P = 0.01$), and feed protein (bypass protein; $P = 0.03$) decreased with advancing WWP stage of maturity. The results are consistent with previous results (Horn et al., 1981; Branine and Galyean, 1990), which reported that supplementing monensin to cattle grazing WWP did not affect intake or digestibility (Horn et al., 1981). Although previous research reported reduction of proteolytic activity with monensin supplementation to cattle and lambs (Hanson and Klopfenstein, 1977; Poos et al., 1979), feed protein bypassing the rumen in the present experiment was unaltered by monensin. The decrease in microbial protein synthesis observed in this experiment was probably caused by the selective inhibition of Gram-positive bacteria (Schelling, 1984).

There were no monensin supplementation × stage of WWP maturity interactions for ruminal pH, ammonia, or VFA production ($P \geq 0.18$). Monensin supplementation increased ($P = 0.01$) propionate (18.38, and 20.79 ± 0.55 mol/100 mol), and decreased ($P = 0.01$) the acetate : propionate ratio (3.26, and 2.73 ± 0.13). Ruminal pH (6.03, and 6.31 ± 0.08), acetate (54.6, and 58.3 ± 0.98 mol/100 mol), and acetate propionate ratio (2.52, and 3.48 ± 0.13) increased ($P \leq 0.04$), and ammonia (8.39, and 2.40 ± 0.89 µM), and propionate (21.9, and 17.3 ± 0.56 mol/100 mol) decreased ($P \leq 0.05$) with advancing WWP stage of maturity.

Results from the present experiment agree with previous research that observed that monensin increased ruminal propionate and decreased ruminal acetate (Horn et al., 1981; Branine and Galyean, 1990). In disagreement with these results, those experiments reported increased pH values with monensin (Horn et al., 1981; Branine and Galyean, 1990).

In summary, monensin supplementation failed to improve supply of metabolizable protein of cattle grazing WWP. In fact, microbial protein synthesis decreased with monensin supplementation.

IMPLICATIONS

Results from this experiment imply that the improved performance observed in previously reported research for cattle grazing winter wheat pasture and supplemented with monensin is not due to improvements of metabolizable protein supply. Perhaps, improved weight gains are caused in part by the greater production of propionate by rumen fermentation when monensin is supplemented to cattle grazing winter wheat pasture.

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