INTRODUCTION

Winter wheat pasture (WWP; *Triticum aestivum*) is an important source of forage in the U.S. southern Great Plains (Epplin et al., 2001). Usually, WWP is used to develop young cattle, grazing WWP allows muscle and frame development at low feeding cost (Torell et al., 1999). Exceptional forage quality (20% to 30% crude protein (CP) and 70% digestibility and seasonal availability are just some of characteristics of this crop that has promoted the popularity of use in cattle development (Branine and Galyean, 1990). Protein in WWP is characterized by a high proportion of soluble protein (59%; Vogel et al., 1989), in situ effective degradability of 90% (Chabot et al., 2008), and nonprotein nitrogen of 25% to 37% of total CP (Horn et al., 1977). Although the requirements of digestible intake protein (13%, total digestible nutrients (TDN); NRC, 1996) are usually met by cattle grazing WWP, specific amino acids and peptides are required by some ruminal microbes (Zinn and Owens, 1986; Russell et al., 1992). Previous research demonstrated that cottonseed meal supplementation as a source of rumen available peptides and amino acids increases microbial protein synthesis of cattle grazing WWP (Urias et al., 2019). However, research related to supplementation of rumen available amino acids to cattle grazing WWP is limited. It is hypothesized that supplementation of rumen available methionine can improve the characteristics of rumen fermentation of cattle grazing WWP. Therefore, the objective of this experiment was to evaluate the effect of methionine supplementation and forage quality on forage intake, rumen fermentation characteristics, and microbial protein synthesis of cows grazing WWP.

MATERIALS AND METHODS

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

Seven mature Angus-Hereford crossbred cows (665.90 ± 15.14 kg of body weight [BW]) fitted with ruminal and duodenal cannulas grazing WWP were used in a split-plot design to evaluate the effects of methionine supplementation and WWP stage of maturity on forage intake, ruminal fermentation, and metabolizable protein. Treatments were the main plot, and stage of forage maturity was the subplot. Treatment was methionine (Rhodimet NP99; Addiseo, Alpharetta, GA) supplementation offered at 8 g/d. Stages of forage maturity were early April (Ear) and late April (Lat).

During each grazing period, animals were allowed 10 d to acclimate to WWP and methionine supplementation. After that, 4 d were utilized for sample collection. Cattle grazed a single wheat pasture per period. Cows were gathered...
into a holding pen and secured to a fence post with a halter, and supplemented with gelatin capsules containing their respective treatment directly into the rumen daily at 0700. Chromic oxide (8 g) placed in gelatin capsules was dosed intraruminally on days 6 to 14 at 0700 and 1900 to be use as a digesta flow marker.

**Sample Collection**

Duodenal and fecal samples were collected during each collection period from all cows as follows: day 11: 0700 and 1300; day 12: 0100 and 1900; day 13: 1000, 1600 and 2200; and day 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 compiled independently for analysis.

Rumen fluid samples were collected via rumen cannula using a suction strainer at 0, 3, 6, 9, 12, 15, and 21 h after the day 12. Ruminal pH was assessed (accurmet AP72) immediately after collection, samples were acidified with 7.2 H₂SO₄ at a rate of 1 mL/25 mL of rumen fluid, and stored at −20°C for further analysis of NH₃-N and volatile fatty acid (VFA). At 0900 on day 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 dry matter (DM), ash, N, and purines. Samples were stored at −20°C until analysis.

For each period, two cows were placed and secured at a holding pen and cows were ruminally evacuated at 1,000 on day 14. 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 without access to water. Afterwards, masticate samples were 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 combine 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 neutral detergent fiber using an Ankom 200 fiber analyzer (Ankom Co., Fairport, NY), sequentially. Duodenal samples were analyzed for purines (Zinn and Owens, 1986). Masticates were analyzed for in vitro digestibility using the procedures described by Tilley and Terry (1963). 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 2 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. Forage DM intake was calculated as forage fecal output divided by forage in vitro indigestibility. Microbial organic matter (OM) and N leaving the abomasum were calculated using purines as a microbial marker (Zinn and Owens, 1986). Feed N reaching the small intestine was considered equal to total N leaving abomasum minus NH₃-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.

**Statistical Analysis**

Data were analyzed as a split-plot design using the mixed procedures of SAS (SAS Inst. Inc., Cary, NC). For intake, digesta flow, and digestibility data, the model included methionine supplementation, stage of maturity, and methionine supplementation × maturity. The repeated effect was stage of maturity, and cow within methionine supplementation was used to test the effects of methionine 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,
Translate basic science to industry innovation

VFA) using a split-plot design. Effects in the model included methionine supplementation, stage of maturity, and methionine supplementation × stage of maturity. The repeated measurement was time of rumen fluid sample collection, and cow within methionine supplementation × stage of maturity was used as the error term to test the effects of methionine supplementation. Individual cow was the experimental unit in all analysis.

**RESULTS**

Effects of methionine supplementation, and stage of WWP forage maturity on intake, and digestion are shown in Table 1. Methionine supplementation did not affect \( (P \geq 0.47) \) nutrient intake, whereas forage maturity reduced \( (P \leq 0.02) \) DM intake. The flow of DM, CP, RUP to duodenum and microbial protein efficiency were not affected \( (P \geq 0.23) \) by methionine supplementation or forage maturity. A tendency for methionine supplementation × forage maturity interaction was observed \( (P = 0.08) \) for microbial protein. However, methionine supplementation did not affect microbial protein synthesis during early \( (P = 0.39) \) or late \( (P = 0.72) \) April grazing period. True ruminal digestion (% of intake) of DM was not affected \( (P = 0.93) \) by methionine supplementation and decreased \( (P = 0.01) \), with advancing stage of forage maturity. Methionine supplementation or forage maturity did not affect \( (P \geq 0.22) \) DM fecal excretion. Total tract digestion (g/d) of nutrients was not affected \( (P = 0.47) \) by methionine supplementation, but total tract digestion of DM decreased \( (P = 0.01) \) with advancing forage maturity. Methionine supplementation did not affect \( (P \geq 0.14) \) ruminal pH, ammonia concentration, total VFA, and molar proportions of acetate propionate and butyrate. Ruminal pH and butyrate decreased \( (P \geq 0.05) \), acetate increased \( (P = 0.004) \), and

### Table 1. Effects of methionine supplementation\(^1\), and forage quality\(^2\) on forage intake and digestion characteristics of beef cows

| Item                               | Methionine, g/d | Maturity | \( P\)-value\(^3\) |
|------------------------------------|-----------------|----------|---------------------|
| DMI, kg/d                          |                 |          |                     |
| Flow to duodenum, kg/d             |                 |          |                     |
| DM                                 | 3.16            | 3.05     | 0.47                |
| CP                                 | 0.79            | 0.84     | 0.97                |
| Microbial protein                  | 0.47            | 0.50     | 0.70                |
| Early                              | 0.41            | 0.50     | 0.39                |
| Late                               | 0.46            | 0.50     | 0.72                |
| RUP                                | 0.35            | 0.33     | 0.58                |
| Microbial protein efficiency\(^4\) | 19.28           | 19.39    | 0.65                |
| True ruminal digestion, % of intake|                 |          |                     |
| DM                                 | 59.59           | 64.19    | 0.93                |
| Fecal excretion, kg/d              |                 |          |                     |
| DM                                 | 2.64            | 2.56     | 0.47                |
| Total tract digestion, kg/d        |                 |          |                     |
| DM                                 | 3.91            | 4.36     | 0.47                |
| Rumen fluid                        |                 |          |                     |
| pH                                 | 6.51            | 6.63     | 0.82                |
| Ammonia, mM                        | 2.18            | 1.75     | 0.98                |
| Total VFA, mM                      | 76.56           | 81.87    | 0.14                |
| VFA, mol/100 mol                   |                 |          |                     |
| Acetate                            | 64.3            | 58.23    | 0.66                |
| Propionate                         | 21.52           | 20.68    | 0.24                |
| Butyrate                           | 16.68           | 18.95    | 0.68                |
| Acetate:propionate ratio           | 3.00            | 2.83     | 0.01                |
| Early                              | 2.85            | 2.83     | 0.53                |
| Late                               | 3.15            | 3.48     | 0.008               |

\(^1\)Supplementation type: 0 = 0 g of methionine supplementation; 8 = 8 g of methionine supplementation.

\(^2\)Forage maturity: Early = grazing occurred during early April; late = grazing occurred during late April.

\(^3\)Probability value associated with methionine supplementation (TRT), stage of forage maturity (MT), and methionine supplementation × stage of maturity (TRT × MT).

\(^4\)Grams of microbial N per kilogram of OM truly fermented. Truly fermented OM = OM intake minus apparent feed OM flow at the duodenum.
ammonia concentration and propionate were not affected \((P \geq 0.39)\) with advancing stage of maturity.

**DISCUSSION**

Microbial protein synthesis depends on ruminal concentration of protein and OM fermented in the rumen as a source of energy (Hespell, 1979). Although the ruminal requirements of protein are typically presented on a CP or N basis, some of the ruminal microbes require other nitrogenous compounds for optimal microbial growth and fiber digestion such as peptides and amino acids (Zinn and Owens, 1986). The protein in WWP is highly soluble (Chabot et al., 2008), with high proportion of non-protein nitrogen (Johnson et al., 1973). Therefore, deficiencies of specific amino acids might exist. In previous research, it was observed that microbial protein synthesis increased when cottonseed meal was supplemented as a source of rumen available peptides and amino acids to cattle grazing WWP (Urias et al., 2019). Methionine is an essential amino acid that often limits growth (Richardson and Hatfield, 1978). Therefore, it was expected that the supplementation of rumen available methionine to cattle grazing WWP would have increased microbial protein synthesis as well as characteristics of ruminal digestion. However, supplementation of rumen available methionine failed to improve microbial protein synthesis and rumen digestion in the present study.

In conclusion, methionine is not limiting microbial protein synthesis of cattle grazing wheat pasture. Results from this study suggest that performance of cattle grazing wheat pasture should not improve by supplementing rumen available methionine.

**ACKNOWLEDGMENTS**

This research was supported by the New Mexico Agricultural Experiment Station.

*Conflict of interest statement.* None declared.

**LITERATURE CITED**

AOAC. 1997. Official Method of Analysis. 16th ed. Arlington (VA): Association of Official Analytical Chemists.

Branine, M. E., and M. L. Galyean. 1990. Influence of grain and monensin supplementation on ruminal fermentation, intake, digesta kinetics and incidence and severity of frothy bloat in steers grazing winter wheat pastures. J. Anim. Sci. 68:1139–1150. doi:10.2527/1990.6841139x

Broderick, G. A., and J. H. Kang. 1980. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and in vitro media. J. Dairy Sci. 63:64–75. doi:10.3168/jds.S0022-0302(80)82888-8

Chabot, D. A., C. D. Chabot, L. K. Conway, and S. A. Soto-Navarro. 2008. Effect of fat supplementation and wheat pasture maturity on forage intake and digestion. J. Anim. Sci. 86:1263–1270. doi:10.2527/jas.2007-0388

Epplin, F. M., E. G. Krenzer Jr., and G. Horn. 2001. Net returns from dual-purpose wheat and grain-only wheat. J. ASFMRA 64: 8–14.

Goetsch, A. L., and M. L. Galyean. 1983. Influence of feeding frequency on passage of fluid and particle markers in steers fed a concentrate diet. Can. J. Anim. Sci. 63:727–730. doi:10.4141/cjas83-084

Hespell, R. B. 1979. Efficiency of growth by ruminal bacteria. Fed. Proc. 38:2707–2712.

Hill, F. W., and D. L. Anderson. 1958. Comparison of metabolizable energy and productive energy determinations with growing chicks. J. Nutr. 64:587–603. doi:10.1093/jn/64.4.587

Horn, G. W., B. R. Clay, and L. I. Croy. 1977. Wheat pasture bloat of stockers. Animal Science Research Report. Oklahoma Agric. Exp. Stn. MP-101. p. 27–31.

Johnson, R. R., M. McGeehan, E. Williams, and H. C. Young Jr. 1973. Studies on the nutritive value of wheat pasture. Page 13 in Oklahoma State Univ. Res. Rep., MP-90. Stillwater: Oklahoma Agric. Exp. Stn.

NRC. 1996. Nutrient requirements of beef cattle. 7th rev. ed. Washington (DC): Natl. Acad. Press.

Richardson, C. R., and E. E. Hatfield. 1978. The limiting amino acids in growing cattle. J. Anim. Sci. 46:740–745. doi:10.2527/jas1978.463740x

Russell, J. B., J. D. O’Connor, D. G. Fox, P. J. Van Soest, and C. J. Sniffen. 1992. A net carbohydrate and protein system for evaluating cattle diets. I. Ruminal fermentation. J. Anim. Sci. 70:3551–3561. doi:10.2527/jas1992.70113551x

Tilley, J. M. A., and R. A. Terry. 1963. A two-stage technique for the in vitro digestion of forages. J. Br. Grass. Soc. 18:104–111.

Torell, R., W. Riggs, D. Bruce, and B. Kuasnick. 1999. How good is wheat pasture for winter grazing lightweight calves? Nevada Cooperative Extension Fact Sheet. p. 99–40.

Urias, S., U. A. Sánchez-Sandoval, J. J. Figueroa-Zamudio, J. A. Rodela, and S. A. Soto-Navarro. 2019. Effects of cottonseed meal supplementation and wheat pasture maturity on forage intake and digestion characteristics of cows grazing winter wheat pasture. Transl. Anim. Sci. 3(Suppl. 1):1664–1668. doi:10.1093/tas/txz051

Vogel, G. J., G. W. Horn, W. A. Phillips, and C. A. Strasia, and J. J. Martin. 1989. Effects of supplemental protein on performance of stocker cattle grazing wheat pasture. Anim. Sci. Res. Report No. MP-127:208–216. Stillwater (OK): Oklahoma Agricultural Experiment Station.

Zinn, R. A., and F. N. Owens. 1986. A rapid procedure for purine measurement and its use for estimating net ruminal protein synthesis. Can. J. Anim. Sci. 66: 157–166. doi:10.4141/cjas86-017