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Effect of feeding range protein supplement on ruminal methylglyoxal production

S. L. Lodge-Ivey, R. L. Endecott, K. D. Richardson, and M. K. Petersen
Department of Animal and Range Sciences, New Mexico State University, Las Cruces, NM 88003

ABSTRACT: When beef cows are grazing dormant winter range nitrogen is typically the first limiting nutrient creating an imbalance with fermentable carbohydrate. Under these conditions nitrogen is often supplemented to remedy this deficiency. Methylglyoxal is a highly reactive electrophile produced by bacteria in response to an imbalance of carbohydrate (excess) to nitrogen (limitation) in growth medium. Therefore, presence of methylglyoxal in ruminal contents could be used as a tool to assess the effectiveness of protein supplementation strategies. An experiment was conducted at the Corona Range and Livestock Research Center to determine the influence of protein supplementation on ruminal production of methylglyoxal, ammonia, pH and blood ketones by cows grazing dormant winter range. Treatments consisted of no supplementation (n=4) or 36% CP cottonseed meal based supplement (n=3) fed at 0.45 $kg \cdot cow^{-1} \cdot d^{-1}$ three times weekly. Supplementation regimen had no effect on ruminal ammonia ($P = 0.13$), pH ($P = 0.99$), methylglyoxal ($P = 0.63$) or blood ketone ($P = 0.42$). Blood ketone concentrations were different depending on day, ($P < 0.01$) with detectable ketones increasing as the trial progressed. Ruminal pH decreased ($P < 0.01$) over day during the length of the experiment. Ruminal ammonia tended to decrease by day ($P = 0.06$) with lower values at the completion of the experiment compared to the start. Methylglyoxal increased ($P = 0.05$) during the duration of the experiment being highest on d 5 and undetectable on d 1. This experiment is the first attempt to quantify ruminal methylglyoxal in cows under winter range conditions. Ruminal methyglyoxal concentration may be a more sensitive to ruminal nitrogen and carbohydrate imbalances than the measurement of ruminal ammonia.

Key Words: methylglyoxal, protein, ruminal bacteria

Introduction

The use of the compound methylglyoxal as a marker to asses the nitrogen status of ruminants is a new concept that deserves deeper exploration. In mammals, methylglyoxal is synthesized by the host animal or microorganisms in the digestive tract. Methylglyoxal-producing bacteria use a variety of pathways such as glycolytic bypass, glycerol degradation, and amino acid catabolism to produce methylglyoxal.

The microorganisms that predominate in the rumen are saccharolytic. Carbohydrates, such as cellulose and other polysaccharides, make up most of the ruminant diet and constitute the main substrate available for fermentation. *Prevotella ruminicola* $B_1$4 accounts for as much as 19% of the cultivable bacteria from the rumen (Russel, 1993). When there is a loss of balance between nitrogen and carbon metabolism in the rumen bacterium *Prevotella ruminicola* $B_1$4 produces methylglyoxal *in vitro* (Russell, 1993). Therefore the hypothesis for this experiment is that methylglyoxal production by ruminal bacteria would indicate a loss of balance between carbon and nitrogen metabolism in the rumen.

When ruminants are consuming dormant range, the ruminal microbes may experience growth conditions where carbohydrate is supplied in excess of microbial needs and nitrogen is limiting to fermentation. Under these conditions ruminal bacteria have developed strategies involving futile cycles to spill energy until the carbon to nitrogen ratio favors the synthesis of microbial crude protein (Russell and Cook, 1994).

To overcome dietary inadequacies, cattle are supplemented with protein supplements formulated to meet their nutrient requirements (NRC, 1996). These protein supplements may contain ruminally degradable protein, undegradable protein, or combinations of both. However, prediction of the effectiveness of the supplementation program, in regards to the degradable protein requirement of the microbial population, is based partially on ammonia (NH_3) concentration in ruminal contents.

Ammonia in the rumen is a pool of several inputs and outputs. Ammonia is derived from degradation of dietary protein and dietary NPN, from the hydrolysis of urea recycled to the rumen, and from the degradation of microbial crude protein (NRC, 1996). Ammonia disappears from the rumen pool due to uptake by the microbes, absorption by the microbes, absorption through the rumen wall, and flushing to the omasum. Changes in any of these factors will alter NH_3 concentration in the rumen (Ørskov, 1982). Thus, NH_3 concentration is too dynamic to be a good indicator of the nitrogen status of the ruminal environment.

An *in vivo* method has been developed to test the feasibility of detecting methyglyoxal under extreme nutrient limitations (Lodge-Ivey et al. 2002). However, the idea of using methylglyoxal as a marker for effectiveness of protein supplementation under a typical range supplementation protocol has not been investigated. Therefore, an experiment was conducted at the Corona Range and Livestock Research Center to determine the influence of protein supplementation on ruminal production of methylglyoxal, ammonia, pH and blood ketones by cows grazing dormant winter range.

Materials and Methods

Seven ruminally fistulated English crossbred cows (BW = 590 ± 15 kg) were used in a completely randomized design. Surgical procedures and post-surgical care had been were reviewed and accepted by the New Mexico State University Institutional Animal Care and Use Committee. Treatments consisted of two feeding regimes a positive control (CON) representative of production practices in this region and a strategic supplementation designed to vary with cow demands (VAR). The CON treatment (n=3) consisted of a 36% CP cottonseed meal based pelleted supplement delivered at 0.45 $kg \cdot cow^{-1} \cdot d^{-1}$, prorated to 3 times per week delivery. The VAR treatment (n=4) consisted of provision of the 36% supplement used in CON on an as needed basis. Need was determined by visual assessment of cow body condition change and forage conditions. Due to favorable weather and range conditions during the course of this experiment, animals on the VAR treatment did not receive supplement. Cows on both treatments were assigned to separate pastures and were allowed *ad libitum* access to range forage and water. Additionally, free choice salt and mineral mix was available in the pastures.

Samples of ruminal contents were collected at three week intervals beginning in November, 2004 and ending February, 2005 via a suction strainer (Diamond V, Parma, ID). Three aliquots were collected during each sampling event for analysis of ammonia, methylglyoxal, and VFA (data not reported). Aliquots for analysis of methyglyoxal and ammonia were acidified with 1 mL 6N HCl. All ruminal samples were stored on ice until sampling was complete, transported to the laboratory and frozen (–20^{o} C). An additional aliquot of ruminal fluid was collected for pH determination. A blood sample was collected via coccygeal venipucture into evacuated serum tubes and tested ketone content with a handheld ketone meter. Acidified samples of ruminal fluid were thawed, centrifuged (1,500 x *g*) for 15 minutes, and analyzed for ammonia by the phenol-hypochlorite method (Broderick and Kang, 1980) using 96-well microtiter plate (MRX HD, Dynex Laboratories, Chantilly, VA.). Methylglyoxal concentration was determined on acidified ruminal samples by the method of Lodge Ivey et al., (2004).

Data were analyzed using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC) for repeated measures with cow as the experimental unit and compound symmetry covariance structure. The model included effects of sampling day, treatment and their interaction.

Results and Discussion

Supplementation regimen had no effect on ruminal ammonia (P = 0.13), pH (P = 0.99), methylglyoxal (P = 0.63) or blood ketone (P = 0.42). Blood ketone concentrations were different depending on day, (P < 0.01) with detectable ketones increasing as the experiment progressed. Ruminal pH decreased (P < 0.01) over day during the length of the experiment. Ruminal ammonia tended to decrease by day (P = 0.06) with lower values at the completion of the experiment compared to the start. Methylglyoxal increased (P = 0.05) during the duration of the experiment being highest on d 5 and undetectable on d 1. By the end of this experiment the cows were mobilizing body condition indicated by the increase in blood ketone

Mean ruminal ammonia levels for both CON and VAR were low (4.02 vs 2.3 mg/dL). Ruminal NH_3 levels are indicative of the balance between fermentable organic matter and DIP (Horn and McCollum, 1987; Owens et al., 1991). An imbalance of NH_3 and fermentable carbohydrate in the rumen could have a detrimental effect on the microbial population. Satter and Slyter (1974) suggested that 3.6 mM NH_3 N supported maximal microbial growth but that limiting concentrations were perhaps closer to 1.5mM. Russell (1993) demonstrated effects of this phenomenon with *Prevotella rumincola* $B_1$4. When exposed to high concentrations of glucose (50 mM) and low NH_3 (3.6 mM), *P. rumincola* $B_1$4 viable cell number decreased by at least 1,000 fold. This decrease in viability was correlated with an accumulation of methylglyoxal in the supernatant (3 to 4 mM).

In the current study mean levels of methlyglyoxal for the experiment were (0.8 and 1.4 mM ± 0.83 SE) for CON and VAR, respectively did not get as high as levels observed *in vitro* by Russell (1993). However by d 5 mean levels were comparable to those observed *in vitro* (3.7 and 6.9 mM ± 0.12 SE) for CON and VAR respectively. These values indicate that there was an imbalance in fermentable organic matter to ammonia nitrogen in the rumen. It is interesting to note that both treatments had detectable methylglyoxal in the ruminal fluid by d 4 indicating that although the cows on CON treatment were receiving extra DIP it was not enough or was not in the most utilizable form to balance the nutrient content of the rumen. This would support and validate the hypothesis for this study which was when ruminal bacteria experience an imbalance of nitrogen to carbohydrate ratio in the rumen methylglyoxal will be produced to dissipate the imbalance. These data support methylglyoxal concentrations in the rumen as being tool assess nitrogen status of the rumen. This is the first reported attempt to measure methylglyoxal in whole ruminal contents in cows under a commercial management setting. These results indicate that the ruminal microbial population experienced nutrient stress and responded by producing methylglyoxal. These data indicate that methylglyoxal may be a useful tool to assess ruminal nitrogen status. Further research is needed under different dietary conditions to determine the true usefulness of methylglyoxal to assess the effectiveness of protein supplementation strategies.

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