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Effects of Sulfur Containing Supplements on Ruminal Fermentation and Microbial Protein Synthesis

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Abstract: Sulfur (S) is a very important element for the formation of many S containing compounds in the body. These compounds include the S containing amino acids (methionine, cysteine, cystine, homocysteine, cystathionine, taurine, cysteic acid), thiamin, biotin and lipoic acid. Rumen microbial flora has an ability to convert inorganic S to organic S in the form of methionine, cysteine, and cystine. Therefore both organic and inorganic S sources are reduced to sulfide by ruminal bacteria. Then fate of the sulfide can be either microbial protein synthesis or absorption and oxidation to sulfate in liver. Not only efficient microbial protein synthesis but also cellulose digestion can be altered depending upon the S supplementation. Although the S requirement of lactating dairy cattle is 0.2% according to NRC[16], the estimation of this amount can be adjusted in certain levels for different lactation periods and diets. There might be also some possible interactions among milk yield, milk protein yield and various S sources. Ruminal fiber degradation might be improved with supplemental S if low quality forage sources are fed. Under these circumstances, effectiveness of S sources and the amount of these sources should be reevaluated for microbial protein synthesis and greater animal production.

Key words: Sulfur, microbial protein, ruminal fermentation

INTRODUCTION

There is current interest in focusing on the greater microbial protein synthesis and higher milk protein yield in forage based dairy rations. Although forage based rations provide variable energy sources for efficiency, production responses such as milk and milk component yields, need to be considered carefully in ruminant case. Thus the most important investigation in ruminant nutrition is to induce microbial protein synthesis greatly. It might be emphasized that highly fibrous plant materials may not provide greater microbial protein efficiency depending upon the protein content of those forage sources. So that the concept of inducible rumen microbial efficiency needs to be taken into account in terms of greater protein synthesis as well as efficient ruminal fermentation. Although there is little information explaining how the rumen bacteria and fermentation are influenced by S supplementation, it has been shown that the supplementation of poor quality fiber diets with S improved ruminal fiber degradation as well as apparent organic matter digestibility[3,14].

MATERIALS AND METHODS

Once the exogenous S sources, particularly inorganic sources, are taken by animals, sulfate reduction occurs. Then S amino acids are synthesized from reduced form of S, called sulfide. Although it has been shown that some rumen bacteria need S amino acids for growth, sulfide has been shown to be a major source of S for bacterial protein synthesis. Gawthorne and Nader[8] found in sheep that only 53 to 57% of the S in microbial protein came from the sulfide pool. They[8] concluded that other half of the S amino acid content resulted from the direct incorporation of amino acids from digested plant and saliva.

RESULTS AND DISCUSSIONS

Relationship between sulfur and bacterial protein synthesis: Sulfate recycling becomes so critical when the exogenous S sources are not included in the diet in terms of fermentation and bacterial protein synthesis. So that when the rumen dietary N to rumen fermentable organic matter ratio declines in the case of low quality forage and starch, it is need to be improved either sulfate recycling or exogenous S supplementation for protein synthesis. Therefore exogenous S sources become more critical for greater protein synthesis. Kennedy and Milligan[13] used ^{35}S-sodium sulfate as a marker for measurement of microbial protein and found that bacteria derived 52 to

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67% of organic S from ruminal sulfide in sheep given brome grass.

The role of S utilization (either organic or inorganic) in N-based diets has been considered for bacterial protein synthesis in terms of N:S ratio. It has been reported that a mean value of 18.5:1 N:S ratio for mixed rumen bacteria should be adequate to meet the S requirements of the rumen bacteria[9]. However they[9] suggested that this N:S ratio may not be adequate to determine the requirement of bacteria due to the N and S escape. These losses was calculated using sheep, growing steers and lactating cows and concluded that these losses represent 85, 72 and 50% of the amount required for microbial protein synthesis, respectively[17]. So that it is difficult to estimate how much ruminal sulfide is required for maximal microbial growth. It was also calculated that the requirements for available S in the rumen should be equivalent to 1.6-1.9 g/kg of digestible organic matter after S losses and recycling[17].

Although it is difficult to estimate S requirement for bacterial protein yield, improving S and N retention might have an effect for greater bacterial protein efficiency. Bray[2] showed the close relationship between bacterial N and S using most of the published data for sheep and found a positive linear relationship (r= 0.95) between N and S retention. In addition, it has been shown that an increase of S supply from 0.9 to 3.5 g/kg of rumen fermentable organic matter increased microbial N yield from 15 to 21 g/kg of rumen fermentable organic matter in semicontinious culture[6]. Bird[1] found that supplemental sodium sulfate (0.25% of diet DM) increased the daily flow of protein to the omasum by 2.1 g/d, and changed the N balance from -2.38 g/d to 0.15 g/d for basal (0.023% S) and sulfate supplemented diets, respectively. It was also found that the apparent digestibility of organic matter of the diet was increased from 43.8 to 65.7% when sulfate was added to the basal diet[1].

Bioavailability of the each S source (organic vs. inorganic) for bacterial protein synthesis has been evaluated in many experiments. Kahlon *et al*.[11] found that relative availabilities of calcium sulfate (94.1%) and ammonium sulfate (93%) had greatest sources for the in vitro bacterial protein synthesis (2.08 and 2.04 mg/ml of inoculum at 12 h, respectively). Sodium sulfate (55.4%) sodium sulfide (42.6%) and elemental S (35.8%) had lower capacity for in vitro protein synthesis (1.77, 1.69 and 1.62 mg/ml of inoculum at 12 h, respectively). They[11] also tested the in vivo availability of chemical forms of S in growing lambs. Although there were no significant difference across the various forms (calcium sulfate, ammonium sulfate, sodium sulfate, sodium sulfide and elemental S), lambs fed each of the S sources gained faster and consumed more dry matter than control group. Fron *et al*[7] examined the different forms of supplemental S (elemental, sodium sulfate and methionine) in response to N metabolism in heifers. Basal diet contained 0.21% S along with three supplemented diets each with 0.36% S. They[7] measured the ruminal ammonia-N concentration and found that overall 72 h adaptation for ammonia-N was highest in sodium sulfate and lowest in methionine (4.6, 3.14, 5.63 and 2.96 mg/dl for control, elemental S, sodium sulfate and methionine, respectively). Although there is a close relationship between N intake and the amount of protein produced by the rumen bacteria, it has been suggested that further protein synthesis can be improved by S supplementation.

Another major aspect for influencing the utilization of ruminal S in microbial growth and protein synthesis is the availability of carbohydrate sources. Kandylis and Bray[12] examined the flow of S and microbial protein synthesis in the rumen by changing the available energy to the microorganism. Both diets (15 and 30% starch) had the same amount of sodium sulfate (0.3%, DM basis). They[12] found that sulfide absorption was significantly higher in 30% starch diet as well as daily flow of microbial protein from the rumen (92.1 vs. 80.2 g of microbial protein/d for 30 and 15% starch containing diets, respectively). They[12] also found that microbial protein synthesized in the rumen was significantly higher in 30% starch containing diet (20.1 vs. 18.1 g of microbial protein/100 g OM digested in the rumen for 30 and 15% starch containing diets, respectively).

Relationship between sulfur, fiber degradation and ruminal fermentation: It is important to consider quality and quantity of fiber offered to animals along with S supplementation in terms of microbial efficiency of the rumen. Bull and Vandersall[5] compared the different S sources and amounts (sodium sulfate 0.12, 0.24 and 0.32%; calcium sulfate 0.12 and 0.24%; and methionine 0.32%) on the extent or rate of cellulose digestion in vitro. In first experiment, there was a higher cellulose digestion in calcium sulfate supplement at 0.12% level (93.2 vs. 85.6% for calcium and sodium sulfate, respectively). Based on the S level, 0.24% S supplement had higher cellulose digestion than 0.12% S supplement regardless of the S source. There was no significant difference for cellulose digestion at 0.32% S level for both sodium sulfate and methionine. However increasing the level of S from 0.20 to 0.32% increased the ADF digestibility from 33.7% to 42.3%. They[5] concluded that the rate of availability in supplemental S appears to be more conducive to metabolic response than diet originated S, suggesting a rate limiting step at the rumen bacteria level for S. Morrison *et al*[15] examined the nutrient metabolism of sheep fed poor-quality spear grass hay and supplemented with sodium sulfate. They[15] found a higher apparent OM digestibility in sodium sulfate supplemented spear grass diet compared to control diet. In situ DM disappearance was also significantly higher in sodium sulfate supplemented diet. There was also a higher ruminal N retention in sodium sulfate supplemented diet. They[15] also found that S supplementation increased the concentration of all three microbial groups but the most dramatic increase was observed with the number of sporangial forms of rumen anaerobic fungi. Weston *et al*[18] also examined the digestibility of wheat straw diet of low S content (0.71 g S/kg of OM) fed without additional S (low S diet) or containing sodium sulfate (high S diet; 1.84 g S/kg of OM). They (18) found higher OM (55.2 vs. 60% for low and high S diets) and ADF (23.3 vs.26.4% for low and high S diets) digestibilities in high S containing diet.

As previously indicated, N:S ratio also becomes important in S supplemented diets for ruminal fiber degradation as well. Brondani *et al*[4] tested the additional effect of N and S together using high fiber diets for ruminal fermentation. They[4] tried to achieve two different S level as sulfide (4 and 8 µg sulfide/ml of rumen fluid) using basal diet (0.2% S, DM basis) or supplemented with elemental S at 0.2% of DM, and two different ammonia-N, supplemented as urea (5 and 15 mg ammonia-N/dl of rumen fluid) in the rumen. They[4] set up four N:S treatment diets containing 3:1, 5:1, 8:1 and 12:1 using sugarcane bagasse and corn stover. They[4] considered DMI and ruminal acetate as response elements. They[4] found that DMI did not differ among treatment groups. In both forage types, acetate production was not changed by either urea or S when fed separately. However when both urea and S were supplemented together, acetate production was 44% higher in sugarcane bagasse diet. There was 63% higher acetate production in corn stover diet. They[4] concluded that S supplementation needs to be adjusted based on the N:S ratio and percentage of S in DM may not be adequate when high fiber diets containing low N are fed. Because the first consideration is maximal protein synthesis and this can be attained only if precursors for protein synthesis are made available to the bacteria. That is production of VFA from carbohydrates and closely related to maximal microbial protein yield. Therefore they[4] suggested that high fiber diets low in N are utilized better when N and S are adequate.

Another aspect of S bioavailability has been considered in fauna free animals in terms of microbial rumen fermentation. It has been hypothesized that the absence of rumen protozoa may reduce the supply of nutrients for absorption in ruminants consuming low S diets[10]. They[10] offered two levels of sodium sulfate (1 and 8 g S/d) in diet containing wheat straw. They[10] found that high S supplementation increased the H_2S concentration (5.77 vs. 4.12 mg S/l) but tended to reduce NH_3 concentration (78 vs. 88 mg N/l) in the rumen fluid of fauna free sheep. They[10] also found that fauna free animals had a reduced in situ fiber digestion (30 vs. 48% DM/24h) and total VFA concentration (43.8 vs. 78%) in low S containing diet compared to high S containing diet. Therefore they[10] concluded that the increase in rumen S bioavailability between fauna-free and faunated animals must be attributed to microbial change in the rumen, not because of the change in body S reserves.

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