



Sulfur sources in protein supplements for ruminants

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ABSTRACT - The present study evaluates the efficiency of different sulfur sources for ruminant nutrition. The fiber digestibility and the amino acid profile were analyzed in the duodenal digesta of crossbred steers fed *Brachiaria dictyoneura* hay. The sources utilized were elemental sulfur (ES70S), elemental sulfur (ES98S); calcium sulfate in hydrated (HCS), $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$, and anhydrous (ACS), CaSO_4 , forms; and ammonium sulfate (AS), $(\text{NH}_4)_2\text{SO}_4$, keeping a nitrogen:sulfur ratio of 11:1. The iso-protein supplements had 50% of protein in the total dry matter (DM). Five Holstein \times Zebu steers, which were fistulated in the rumen and abomasum, were distributed in a 5×5 Latin square. The different sulfur sources in the supplement did not affect any of the evaluated nutritional factors, such as intake of hay dry matter and protein supplement, crude protein (CP), neutral detergent fiber corrected for ash and protein (NDFap), organic matter (OM), non-fibrous carbohydrate (NFC), ether extract (EE), total digestible nutrients (TDN), NDFap and CP digestibility coefficients, ruminal pH, and ruminal ammonia concentration. The concentrations of amino acids available in the abomasal digesta did not differ significantly in the tested diets. The sulfur sources evaluated in the present study are suitable as supplement for cattle, and their employment may be important to avoid environmental contaminations.

Key Words: *in vitro* microbial growth, sources of sulfur, sulfur-containing amino acids

Introduction

The supply of sulfur can be achieved from various sources, such as synthetic amino acids, sodium sulfate, ammonium sulfate, calcium sulfate and elemental sulfur. However, the main source of sulfur absorbed in the digestive system is associated with the ingestion of sulfur-containing amino acids, which occurs subsequently to the incorporation of sulfur in the microbial mass. Nevertheless, a small fraction can be absorbed in the form of hydrogen sulfide, together with the sulfur originated from the oxidation of methionine and cysteine, which generate sulfate anions and participate in the acid-base balance of the animal organism.

Feed intake can be influenced directly by the presence of sulfur in the diet, because the synthesis of sulfur-containing amino acids is essential for the maximum microbial growth and, consequently, dry matter digestibility (Underwood and Suttle, 1999).

The nitrogen:sulfur ratio in the microbial proteins is 14.5:1, and a sulfur deficiency in the diet causes changes in the microbial fermentation. In this context, it is interesting to note that the utilization of non-protein nitrogen (NPN) is decreased by low levels of sulfur in the rumen fluid, implying that the microbial growth is attenuated. Such a deficiency also reduces the utilization of lactate by the rumen bacteria, resulting in its accumulation. Consequently, there is an appreciable reduction in the digestion of cellulose, possibly due to the reduction of the microbial growth. For this reason, sulfur improves the microbial digestion of cellulose, contributing to the synthesis of amino acids, especially methionine and cysteine.

Many bacteria of the rumen need sulfur. In fact, this element can be obtained by several ways. Some microorganisms are able to degrade inorganic sources of sulfur into sulfide, incorporating this compound to the amino acids, while others only utilize organic sulfur (Durand and Komisarczuk, 1988). In previous work elaborated by Kennedy and Milligan (1978), approximately 50% of the bacterial organic sulfur encountered in sheep was obtained from sulfate. Sulfur supplements in diets are complemented by sulfur recycled in saliva, in a mixture of this element that includes organic and inorganic forms (Bird, 1974).

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Thus, considering the availability of sources originated in industrial processes for the production of phosphoric acid as well as the need for a viable destination for the excess of sulfur produced in these processes, the objective of this research was to evaluate different sulfur inorganic sources in protein supplements.

Material and Methods

The experiment was elaborated with five rumen-and-abomasum-fistulated crossbred Holstein × Zebu steers, with initial average body weight of approximately 280 kg. These animals were distributed in a 5 × 5 Latin square design with 21-day periods; the first fourteen days were employed to the adaptation of the animals to the diet and the subsequent days were used for collection of data.

Animals were maintained in individual covered pens, with troughs for roughage, supplement feeding, and water. Feed was supplied *ad libitum* twice daily (at 08.00 h and 15.00 h). The amount (weight) of diet supplied to each animal as well as leftovers from each animal were measured on a daily basis in order to estimate the real intake. The intake, was monitored daily in order to keep the leftovers in approximately 10% of the offered diet, on a dry matter basis. The quantitative determination of diets and leftovers was made in the moment of feeding, during the experimental procedure.

Animals were subjected to five treatments, varying the sulfur source in the protein supplement, as follows: 70S elemental sulfur (ES70S); 98S elemental sulfur (ES98S); hydrated calcium sulfate (HCS); anhydrous calcium sulfate (ACS), and ammonium sulfate (AS). The roughage supplied to the animals was *Brachiaria dictyoneura* hay. In all treatments, a nitrogen:sulfur ratio of 11:1 was maintained. Diets were isoprotein, with 50% crude protein (CP) in the mineral mixtures, formulated to meet the nutritional requirements, according to recommendations of NRC (1996) for an average daily gain (ADG) of 0.5/day body weight (BW) (Tables 1 and 2).

Samples of ruminal fluid were obtained to determine pH and to analyze the ruminal ammonia (N-NH₃) concentrations. The analyses were made at 0, 4, 8 and 12 h after the morning feeding, on the 15th day of each experimental period. For pH measurements, approximately 100 mL ruminal fluid were collected and pH was read immediately, employing a digital potentiometer. Subsequently, 1 mL of sulfuric acid (H₂SO₄) at 50% was added to each sample, aiming to cease the microbial activity. Then, samples were stored in a freezer at -20 °C for further analysis of rumen N-NH₃ concentrations. Ammonia analysis was performed

Table 1 - Percentage of ingredients present in protein salt, expressed on a dry matter basis

Item	ES70S	ES98S	HCS	ACS	AS
	(%)				
Calcium carbonate	2.18	2.18	0.24	0.61	2.18
Cobalt sulfate	0.015	0.015	0.015	0.015	0.015
Copper sulfate	0.12	0.12	0.12	0.12	0.12
Elemental sulfur 70 S	0.81	-	-	-	-
Flowers of sulfur ES98S	-	0.61	-	-	-
Hydrated calcium sulfate	-	-	3.1	-	-
Anhydrous calcium sulfate	-	-	-	2.7	-
Ammonium sulfate	-	-	-	-	2.5
Dicalcium phosphate	13.89	13.89	13.89	13.89	13.89
Calcium iodate	0.004	0.004	0.004	0.004	0.004
Manganese sulfate	0.11	0.11	0.11	0.11	0.11
Sodium selenite	0.0011	0.0011	0.0011	0.0011	0.0011
Sodium chloride	27.03	27.03	27.03	27.03	26.83
Zinc sulfate	0.172	0.172	0.172	0.172	0.172
Corn meal	25.45	25.45	25.45	25.45	25.45
Soybean meal	15.00	15.00	15.00	15.00	15.00
Urea	14.90	14.90	14.90	14.90	13.74
Excipient q.s.	0.33	0.53	-	-	-
Total	100	100	100	100	100
	(%)				
Total digestible nutrients	30.94	30.94	30.94	30.53	30.94
Crude protein	50.91	50.91	50.91	49.52	50.91
	(g/kg)				
Equivalent NPN protein	418.69	418.69	418.69	418.69	418.69
NPN	67.05	67.05	67.05	67.05	67.05
ME (Kcal/kg)	1118.67	1118.67	1118.67	1103.70	1118.67
N:S ratio	11.18	11.18	11.18	11.18	11.18

ES70S - elemental sulfur; ES98S - elemental sulfur ("flower of sulfur"); HCS - hydrated calcium sulfate; ACS - anhydrous calcium sulfate; AS - ammonium sulfate. NPN - non-protein nitrogen; ME - metabolizable energy.

Table 2 - Chemical composition and macro and micro minerals of hay utilized in each period (P)

Item	Roughages				
	P1	P2	P3	P4	P5
% DM	90.43	90.65	90.57	90.97	92.00
	(g/kg DM)				
Organic matter	84.32	84.44	85.10	75.93	84.00
Crude protein	7.62	6.25	4.40	3.64	4.39
Ether extract	1.60	1.15	1.16	1.01	1.52
Neutral detergent fiber	79.32	80.29	81.14	80.83	77.90
Non-fibrous carbohydrates	5.62	8.68	10.43	12.58	10.14
Acid detergent fiber	50.70	51.36	49.40	47.11	49.20
Lignin	6.02	6.00	4.75	4.67	5.15
NDIP	3.47	2.10	1.42	1.80	2.54
	(g/kg DM)				
Mg	2.38	2.59	2.73	3.13	2.76
Ca	1.47	1.50	1.52	1.72	1.57
K	0.18	0.01	0.01	0.00	0.03
S	2.62	2.76	2.58	2.63	2.60
Na	0.38	0.39	0.30	0.34	0.30
Mn	0.21	0.21	0.13	0.21	0.18
Zn	0.018	0.017	0.010	0.03	0.014
Cu	0.05	0.01	0.01	0.03	0.01
Fe	0.18	0.14	0.14	0.18	0.13

NDIP - neutral detergent insoluble protein.

by the colorimetric method, in agreement with Chaney and Marbach (1962).

The sulfur content in the feces was determined according to Williams et al. (1962), with an atomic absorption spectrometer.

Chromic oxide (Cr_2O_3), with 57% of chrome, was used as external marker in a quantity of 10 g/animal/day. This compound was infused directly via ruminal fistula to estimate the fecal production. Feces were collected directly from the rectum between the 11th and 15th days of each experimental period. The feces were disposed on a properly identified aluminum tray, and then dried in oven at 65 °C. Afterwards, samples were ground in a Wiley mill with 1-mm sieve and stored as samples elaborated per animal and period. These samples were subjected to analyses of chromium concentration, dry matter (DM), mineral matter (MM), crude protein (CP), neutral detergent fiber corrected for ash and protein (NDFap), neutral detergent insoluble protein, neutral detergent insoluble ash, and ether extract (EE) for determination of total digestibility.

Non-fibrous carbohydrate (NFC) contents were estimated according to Sniffen et al. (1992):

$$\text{NFC} = 100 - [\% \text{CP} + \% \text{EE} + \% \text{MM} + \% \text{NDFap}].$$

The chromium content in the feces and abomasal digesta was determined in agreement with Williams et al. (1962), by utilizing atomic absorption spectrometry.

The equation utilized for the calculation of TDN was:

$$\text{TDN} = \text{DCP} + 2.25 \times \text{DEE} + \text{NDFap} + \text{DNFC},$$

in which DCP, DEE, NDFap and DNFC correspond to digestible crude protein, digestible ether extract, digestible neutral detergent fiber corrected for ash and protein, and digestible non-fibrous carbohydrates, respectively.

During the period of six days, in which the marker was supplied, 500 mL of abomasal digesta were collected and stored in a freezer at -20 °C, to determine the partial digestibility. Subsequently, the material collected was thawed and dried in oven at 65 °C, producing composite samples per animal and period, in which contents of chromium, DM, MM, CP, NDFap, EE and NFC were determined by analyses concerning partial digestibility.

In the final part of the experiment, samples of abomasal digesta were thawed to form a composite sample per treatment. Subsequently, the samples were frozen in an ultra-freezer at -80 °C, and after 24 hours they were taken to a lyophilizer for removal of total moisture. This procedure was adopted to determine the profile of amino acids by digestion with acid hydrolysis and reading by high-performance liquid chromatography (HPLC).

The experimental design adopted was a Latin square with five time periods and five treatments, described by the following mathematical model:

$$y_{ijk} = \mu + \alpha_i + \beta_j + c_k + e_{ijk},$$

in which y_{ijk} is the observation related to the variable measured in the k -th cow fed the i -th treatment during the l -th period. The fixed effects are the mean (μ), treatment (α_i), and the periods for the two simultaneous balanced Latin squares (β_j). The random effects are cow (c_k) and the usual error term (e_{ijk}).

The data collected were analyzed using the MIXED procedure of the SAS statistical software (Statistical Analyses System, version 9.0). This procedure defines the fixed and random variables of the model, employing the method of restricted maximum likelihood to estimate the variance components (Perri and Iemma, 1999), considering 5% as significance level.

For the analysis of pH and N-NH_3 , PROC MIXED was used through several measurements. Statistical differences of the parameters over time were determined through the following mathematical model:

$$Y_{ikl} = \mu + \alpha_i + C_k + \beta_l + \alpha\beta_{il} + e_{ikl},$$

in which Y_{ikl} is the observation related to the variable measured in the k -th cow fed the i -th treatment during the l -th period. The fixed effects are the mean (μ), treatment (α_i), the periods for the two simultaneous balanced Latin squares (β_l), and the treatment by period interaction $\alpha\beta_{il}$. The random effects are cow (C_k) and the usual error term (e_{ikl}). The statistical model was fitted using the PROC MIXED procedure of SAS (version 9) with restricted maximum likelihood (REML) as the estimation method. The repeated command was used with (C_k) as subjects.

Results

The results obtained in the present work allow us to infer that the average daily intake of hay dry matter and protein supplement did not differ statistically between treatments employed herein. The same occurred with the intakes of organic matter, crude protein, ether extract, neutral detergent fiber, non-fibrous carbohydrates and total digestible nutrients (Table 3).

The average intake of NDFap for experimental diets of $1.40 \pm 0.35\%$ BW was higher than the 1.2% BW suggested by Mertens (1992); DM intake was developed by filling. Thus, DM intake was controlled by filling, which indicates that the drawbacks caused by fistulas and collection procedures did not affect significantly the NDFap intake.

Absence of effect ($P > 0.05$) generated by sulfur source on the total apparent digestibility of nutrients indicates that,

Table 5 - Amino acid profile in the abomasal digesta according to the different treatments and respective coefficients of variation (CV)

Item	ES70S	ES98S	HCS	ACS	AS	CV (%)
mg/kg of DM						
Essential amino acid						
Arginine	18.52	18.04	18.80	18.78	19.16	10.28
Phenylalanine	541.54	541.76	529.08	552.12	590.66	14.16
Histidine	7.22	7.02	7.12	7.42	7.56	13.24
Isoleucine	18.16	18.38	18.72	18.58	20.22	17.22
Leucine	32.04	30.68	31.86	31.74	32.46	13.68
Lysine	34.92	33.72	34.04	35.28	36.22	14.83
Methionine	5.46	5.60	5.68	5.52	5.70	18.03
Threonine	19.52	18.80	18.64	18.96	19.40	10.65
Valine	20.40	19.82	19.70	19.94	20.30	11.36
mg/kg of DM						
Non-essential amino acid						
Aspartic acid	45.34	44.06	44.30	43.80	44.96	12.08
Glutamic acid	45.82	46.38	45.64	45.30	46.98	15.72
Alanine	26.66	25.06	25.18	24.98	26.02	12.45
Cystine	5.58	5.44	5.00	5.24	5.54	12.66
Glycine	21.06	19.98	20.68	19.64	19.86	15.15
Proline	20.30	19.74	19.74	20.20	20.76	13.34
Serine	100.42	101.24	107.62	149.18	114.10	32.64

ES70S - elemental sulfur; ES98S - elemental sulfur ("flower of sulfur"); HCS - hydrated calcium sulfate; ACS - anhydrous calcium sulfate; AS - ammonium sulfate.

Discussion

The absence of effect of the treatments on the dry matter intake as well as the similar intakes of the other nutrients in the different diets can be explained by the specific characteristics and chemical composition of the diets. The different sulfur sources utilized in the diets were enough to meet the specific requirements of the microorganisms (similarly to the usual sulfur source, which is ammonium sulfate). In fact, the digestibility was not affected and no symptoms of deficiency of this nutrient were observed.

However, according to Mertens (1992), intake is not only related to feed, but is also associated with the specific animal characteristics, as well as weather and feeding conditions.

The positive coefficients related to ruminal digestibility of CP indicate that there is ammonia absorption in the rumen, and that diets probably had excess of degradable protein in the rumen, in relation to the available energy.

The concentration of the N-NH₃ in the rumen is a consequence of the balance between its production, absorption and utilization by microorganisms. Hoover (1986) suggested that 3.3 to 8.0 mg N-NH₃/dL are necessary for maximization of the microbial growth and fiber digestion. The NRC (2001), on the other hand, considered the concentration 5 mg/100 mL an adequate condition for digestion of organic matter.

Souza et al. (2006) estimated maximum ruminal ammonia of 13.14 mg dL⁻¹, with 2.9 hours after feeding on diets with pre-dried Tifton 85 Bermuda grass and

sorghum silage. Pereira et al. (2007) estimated maximum ruminal ammonia concentration of 14.89 mg dL⁻¹ with 2.39 hours after feeding by cattle fed sorghum silage with 40% concentrate.

The mean concentration of N-NH₃ varied from 8.43 to 13.53 mg dL⁻¹, which is sufficient to promote bacterial growth. This fact corroborates the data obtained by Pereira et al. (2007). However, these values are relatively high, which may have caused ammonia loss and consequent energy consumption via the urea cycle, in the process of urea elimination. The higher values of ruminal ammonia may have been caused by the fast hydrolysis of urea in the rumen associated with the lower fiber degradation and, as a consequence, low carbohydrate utilization. It is possible that these processes decreased the nutritional gain from the intake of the respective compounds. This lower efficiency would be associated with a significant lack of physiological synchrony in the utilization of these nutrients.

It is known that, in cases of intoxication for excessive sulfur, it is possible to observe the lack of ruminal motility and loss of appetite. Preston and Leng (1987) claimed that higher levels of intake can provoke the generation of great amounts of hydrogen sulfide gas (H₂S), which, when belched, may penetrate the lungs, causing nervous and respiratory distress. Nevertheless, no such symptoms were observed in the present study, which shows that the values found for fecal sulfur may be considered in an adequate range for intake, in spite of the absence of reference values in the literature.

In this study, the urea was the main protein source to the animals. On the other hand, Rossi Júnior et al. (2007) worked with animals that received diet containing cottonseed meal as protein source, with respective levels of asparagine, proline, alanine, valine, lysine and arginine significantly higher than those obtained in the diets with urea. In this same study, the amount of amino acids absorbed in the intestine was very similar to all amino acids, and there were no significant differences between diets, including the evaluation of threonine, serine, methionine, leucine, tyrosine, phenylalanine, histidine and non-essential amino acids. Therefore, these data corroborate the results found in the present research. It should be noted that the alteration in the composition of microbial protein is not a trivial task, since its obtainment can be altered by several factors.

It well known that the contents of digestible lysine and methionine are 6.82 and 2.19% of the metabolizable protein, respectively, or that the digestible lysine:digestible methionine ratio is 3.1:1. In the case of the diets utilized in this analysis, this ratio is 7:1, approximately, which shows that the diets provided excess lysine to the animals. Thus, it is expected that, in a near future, all rations for beef cattle, especially for dairy cattle, can be formulated aiming to meet the requirements for digestible amino acids. Recent feeding procedures have recommended diets containing less than 18% CP for cows in the beginning of lactation. It is noteworthy that the optimization of the balance related to the amino acids of the diet is more important than the amount of crude protein in the diet, when the objective is to increase the presence of protein in milk. The excess of certain amino acids, such as leucine, decreases the absorption of others, e.g. lysine, which is important for milk production.

Silva et al. (2002), evaluating the requirements for metabolizable amino acids for maintenance and gain of 1 kg in Nellore cattle, found dietary lysine:methionine ratios from 4.6 to 5.6:1. These results were close to those found for available amino acids in this study, considering all sulfur sources utilized in the respective evaluation.

McDowell (1992) reported that Na_2SO_4 (sodium sulfate), CaSO_4 (calcium sulfate), and a mixture of K_2SO_4 (potassium sulfate) and MgSO_4 (magnesium sulfate) were similar with respect to the sulfur supplementation to cows in lactation, with a recommended level of 0.16 to 0.20% of sulfur in the dry matter (DM). For lambs, sulfur in the elemental form was required by approximately three times more than the sulfur in the organic form (Johnson et al., 1971). Bull and Vandersall (1973) observed an increase from 68% to 81% in the apparent absorption of sulfur utilizing DL-methionine,

when compared with elemental sulfur. Ammerman et al. (1995) identified ^{35}S in the amino acids of wool, milk, blood plasma and tissues, after feeding animals with ^{35}S originated from sulfate.

Sulfur is widely known as a component of the essential amino acids methionine and cysteine and it is basically found in the animal organism in the form of sulfates. The ruminal microorganisms need sulfur to develop their normal activities, which are necessary to avoid the decrease in the feed digestibility as well as to increase the nitrogen retention. They can incorporate inorganic sulfur (from forages and mineralized salts) in organic compounds, used for the synthesis of sulfur-containing amino acids that are incorporated in the microbial protein. Thus, there is a ratio between nitrogen and sulfur (N:S) considered optimal, which is 15:1 (varying, according to the literature, between 9:1 and 16:1). Indeed, the ruminal microorganisms can synthesize the sulfur-containing amino acids from sources of non-protein nitrogen.

In ruminants, the exact determination of the contribution of sulfur to the amino acid synthesis remains a difficult task, due to the influence of the endogenous proteins and the non-degradable proteins in the rumen.

Conclusions

The sulfur sources evaluated in the present study (70S elemental sulfur (ES70S), 98S elemental sulfur (ES98S), hydrated calcium sulfate (HCS), anhydrous calcium sulfate, and ammonium sulfate) are suitable as supplement to cattle, and their employment may be important to avoid environmental contaminations.

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