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► **To cite this version:**

Emilie Mercier, S Komisarczuk-Bony. In vitro study of the relationship between sulfur level and various parameters of rumen microbial activity. *Annales de zootechnie*, 1995, 44 (Suppl1), pp.144-144. hal-00889306

HAL Id: hal-00889306

<https://hal.science/hal-00889306>

Submitted on 11 May 2020

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***In vitro* study of the relationship between sulfur level and various parameters of rumen microbial activity**

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The positive effect of sulfur (S) supplementation on microbial protein synthesis, organic matter and cell wall degradation in the rumen is well documented. However, the assessment of the microbial requirement for S to optimize both microbial protein synthesis and carbohydrate degradation is still lacking of precision (Durand and Komisarczuk, 1988, *J Nutr*, 118, 249-260). The present experiment was designed to evaluate the minimum sulfur requirement to optimize various parameters of the rumen microbial activity using a substrate having a high cell wall carbohydrate content in a Rusitec type fermentor. Thirteen grams of delipided and partly delignified wheat straw according to Al Katreb *et al* (1988, *Holzforchung*, 42, 21-27) and 3 g of soluble starch were introduced daily into four 1 l capacity vessels. The treated straw was introduced in a nylon bag and the starch was poured directly into the vessel once a day. Four increasing levels (one per vessel) averaging : 8.2, 12.9, 20.2 and 31.5 mg S/d of available sulfur (sodium sulfate) were continuously infused with an artificial saliva (850 ml/d) derived from Durand *et al* (1988, *Anim Feed Sci Technol*, 21, 197-204). The straw treatment resulted in a marked reduction in the total nitrogen and sulfur content (2.4 g N and 0.1 g S/kg DM). The available S and N in treated straw were considered negligible. Additional nitrogen (urea) was provided by infusion of 0.3 g N/d/ vessel. Each bag remained 48 h in the vessel and residual treated straw DM, production of volatile fatty

acids (VFA), nitrogen incorporated by microbes (estimated as non ammonia N), and residual soluble starch in effluents were estimated during 9 subsequent days after one week of adaptation. Data were submitted to polynomial regression analysis to detect linear, quadratic and cubic effects of the sulfur level (X) on the parameters measured (Y) according to the GLM procedure of SAS[®] STAT.

Sulfate reduction was very active at all S levels and resultant sulfide concentration increased linearly (P = 0.001) with S perfused. The 3 g of soluble starch were entirely fermented in all vessels. A quadratic effect of S level was obtained for the dry matter degradation (DMD) of the treated straw, the amount of organic matter fermented (OMF) calculated according to Van Nevel and Demeyer (1977, *Br J Nutr*, 38, 101-114) and the amount of N incorporated by microbes. The total VFA production was related to the S level by a cubic polynomial regression. Regression equations were used to calculate the amount of S (X) needed to maximize the value of each parameter tested (Y_{max}). The amount of S required to optimize the microbial activity ranged from 24.56 mg S/d for the microbial nitrogen incorporation to 28.91 mg S/d for the amount of OMF. These results show that the fermentescibility of the diet is the first limiting factor in this type of diet rather than microbial protein synthesis as suggested by Durand and Komisarczuk (1988). The rumen microbial sulfur requirement can be expressed in our experiment as 4.95 mg S/g OMF.

Y	Y overall mean	regression (probability)	Y max	X for Y max (mg S/d)
VFA (mmol/d)	55.39	Cubic (0.035)	71.41	28.50
OMF (g/d)	4.83	Quadratic (0.006)	5.84	28.91
Treated straw DMD (%)	44.55	Quadratic (0.0001)	56.94	25.96
N incorporated (mg/d)	183.91	Quadratic (0.0111)	192.86	24.56