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Study of the influence of high levels of sulfate on the rumen microbial synthesis of thiamin in a semi-continuous fermentor (Rusitec)

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The cerebrocortical necrosis (CCN) of ruminants is a nervous syndrome usually linked to thiamin metabolism. Recently, cases of CCN have also been described in animals receiving high dietary sulfate levels (Rousseaux *et al*, 1991, J Vet Med, A38, 229-239). The Rusitec system was used in the present experiment to study the effect of high sulfate levels on the rumen microbial synthesis of thiamin.

Four one litter vessels received daily 14 g of a thiamin free purified substrate composed of pure cellulose (3.5 g), pectin (2 g), xylan (1.5 g) and starch (7 g). The substrate was enclosed in a nylon bag remaining 48 h in the fermentors. All nitrogen (N) was provided by 0.94 g/d of urea. The composition of artificial saliva was derived from Durand *et al* (1988, Anim Feed Sci Technol, 21, 197-204) by adding trace elements, vitamins and growth factors. A control fermentor received 24 mg/d of sulfur (S) in the form of sodium sulfate (Na_2SO_4) in order to satisfy microbial requirements (3 g S/kg fermented organic matter). The 3 other fermentors received increasing amounts of Na_2SO_4 corresponding to 33, 42 and 51 mg S/d. N and S were introduced in the artificial saliva which was continuously perfused at a rate of 1 l/d. The first week of experiment was an equilibration period and during the 13 subsequent days, microbial protein synthesis (estimated as non ammonia nitrogen) and thiamin (measured by fluorimetry) were estimated on effluents and solid undegraded substrate of fermentation. Sulfide concentration was measured in fermentors 6 and 24 h after solid substrate introduction by the method of Cline (1969, Limnol Oceanogr, 14, 454-458). Means were compared by a covariance analysis realized according to the GLM procedure of SAS® STAT.

Sulfate reduction was very active in all fermentors and no residual sulfate was detected in fermentors or effluents. Free sulfide concentration increases with the amount of sulfate perfused. Sulfide concentrations in the control fermentor were in the range corresponding to value found in the rumen of sheep fed a normosulfur purified diet (2 g S kg/DM in the form of Na_2SO_4). In all other fermentors, sulfide concentration reached values corresponding to *in vivo* rumen concentrations obtained with a high sulfur (6 g S kg/DM) diet (Jean-Blain, unpublished data). High levels of perfused sulfate induced a significant but moderate (-14 %) decrease in thiamin production. This reduction is within the same range as obtained *in vivo* when comparing normo and high sulfur purified diets in sheep (Jean-Blain *et al*, 1994, Proc Soc Nutr Physiol, 3, 199). Nevertheless this experiment showed that the decrease in thiamin production was not related to a reduced microbial pool since the amount of nitrogen incorporated (Ni) by microbes was not modified by high sulfate levels.

Sulfate level (mg S/d)	24	33	42	51	SEM
Daily range (6 h-24 h) of fermentor free sulfide concentration (mg S/l)	2.9-5.8[a]	5.1-9.6[b]	12.9-15.6[c]	15.5-22.9[d]	1.37
Protein synthesis (mg Ni/d)	306[a]	295[a]	288[a]	293[a]	9.66
Net thiamin production (µg B_1/d)	96[a]	99[a]	89[b]	82[b]	3.59

Different letters in a row indicate a significant difference ($P<0.05$)