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Effect of salinomycin and vitamin B_6 on *in vitro* production of phenylalanine and tyrosine by rumen micro-organisms

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Ionophores, such as salinomycin (SL) and monensin, have been shown to alter favourably ruminal fermentation characteristics and metabolism (Bergen and Bates, 1984, J Anim Sci, 58, 1465-1483 ; Schelling, 1984, J Anim Sci, 58, 1518-1527). The curative effect of SL on coccidiosis (Merchen and Berger, 1985, J Anim Sci, 60, 1338-1346) and feedlot bloat, negative effect on the *in vivo* total counts of Gram-positive cocci (Hoshino *et al*, 1986, Jap J Zootech Sci, 57, 833-841), effectiveness in preventing experimentally induced lactic acidosis (Nagaraja *et al*, 1985, Am J Vet Res, 46, 2444-2452), improved rate of body weight gain (Merchen and Berger, 1985, J Anim Sci, 60, 1338-1346) and feed efficiency (McClure *et al*, 1980, J Anim Sci, 51, Suppl, 1, 380) have been described. SL has been shown to decrease amino acid brake down (Hoshino *et al*, 1992, J Anim Sci Technol, 63, 308-309), and this may be due to the inhibitory effect of deamination as has been observed with other ionophores (Newbold *et al*, 1990, J Anim Sci, 68, 1103-1109). It is well established that vitamin B_6 (B_6) acts as a component of different enzymes that are involved in the metabolism of amino acids, including transamination, decarboxylation, deamination, desulfhydration and in the synthesis of amino acids (McDowell, 1989, Vitamins in Animal Nutrition : Comparative Aspects to Human Nutrition, Academic press, INC, London, 236-255).

In our previous study, we presented reductive carboxylation of phenylacetic acid (PAA) followed by transamination to produce phenylalanine (Phe) and hydroxylation of Phe to produce Tyrosine (Tyr) by rumen microorganisms. The present study was undertaken to investigate the effect of salinomycin and vitamin B_6 on the production of phenylalanine (Phe) and Tyrosine (Tyr) by rumen micro-organisms.

Rumen microorganisms were collected from three fistulated goats (Japanese native breed) and the suspensions of mixed bacteria (B), mixed protozoa (P) and B plus P (BP) were prepared (Onodera *et al*, 1992, Anim Sci Technol, 63, 23-31) and anaerobically incubated with and without (1mM) PAA and phenylpyruvic acid (PPY) as a substrate with and without SL (5 µg/ml) and vitamin B_6 (10 µg/ml) for 12 h at 39°C. Samples were collected at 0, 6 and 12 h, deproteinized with sulfosalicylic acid, and centrifuged at 27,000 x *g* for 20 min. Pellets were hydrolysed with 6 M HCl and used for the analyses of Phe and Tyr by HPLC (Amin *et al*, 1994, J Chromatogr, Accepted). Supernatants were also used for the analyses of Phe and Tyr.

PAA was converted mainly into Phe and Tyr during 12 h incubation and were found to be 160 and 117, 160 and 115 and 113 and 93 µmol/g microbial N by B, P and BP, respectively, in the sum of supernate and hydrolysate (S+H). SL and B_6 were effective on the *in vitro* production of Phe and Tyr. Phe and Tyr production were increased with SL in both B, P and BP. With B_6, Phe production was increased (3-8 %) in B, P and BP and Tyr production was inhibited (5 %) in P, but not changed in B in BP.

PPY was completely converted mainly into Phe, Tyr and PAA during 12 h incubation and were found to be 394, 256 and 221 ; 251, 194 and 330 ; 357, 109 and 118 µmol/g microbial N by B, P and BP, respectively, in S+H. Phe and Tyr production were increased, but PAA production was inhibited by SL in B, P and BP. On the other hand, Phe and PAA production were stimulated by 5-13 % and 16-25 %, respectively, but Tyr production was inhibited (11-19 %) by B_6 in B, P and BP during 12 h incubation.