Effect of salinomycin and vitamin B₆ on *in vitro* production of phenylalanine and tyrosine by rumen micro-organisms

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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 B₆ 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 (1 mM) PAA and phenylpyruvic acid (PPY) as a substrate with and without SL (5 µg/ml) and vitamin B₆ (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 pmol/g microbial N by B, P and BP, respectively, in the sum of supernate and hydrolysate (S+H). SL and B₆ were effective on the *in vitro* production of Phe and Tyr. 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 B₆ in B, P and BP during 12 h incubation.

PPY was completely converted mainly into Phe and Tyr 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. SL and B₆ 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 B₆, 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.