

Effect of *Sapindus mukorossi* seed saponins on *in vitro* Rumen Fermentation and Methane Production

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Abstract—Reducing livestock methane emission and replacing antibiotic and other chemical feed additives with natural plant bioactives for improving the animal production are the priority areas of animal nutrition research. In line with the above objectives, a study was conducted to investigate the effect of *Sapindus mukorossi* seed coat saponins on ruminal fermentation and methane production by using Hohenheim *in vitro* gas production technique (IVGPT). Dried *Sapindus mukorossi* seed coat samples were defatted with hexane and extracted with ethanol: water. Solvent was removed from the ethanol water extract in vacuum and the resultant aqueous suspension was partitioned using *n*-butanol and the saponin residue obtained. The Saponin was subjected to foam test and erythrocyte hemolysis test to verify their chemical characteristics and after verification it was used to study the effect on rumen fermentation by using *in vitro* gas production method. The inoculum used for the *in vitro* studies was rumen fluids obtained from 2 fistulated steers fed on a maintenance diet consisting of wheat straw *ad lib* and concentrate mixture 2 kg per day. The rumen fluid was sampled just before morning feeding (0h) from these animals and transported in insulated flasks under anaerobic condition to the laboratory, pooled in equal proportions and used as a source of inoculum. The effect of *Sapindus mukorossi* seed coat was evaluated using different substrates like starch, concentrate, mulberry and white clover leaves in Hohenheim *in vitro* gas production method. Saponin was added at level of 1mg/ml. Thirty ml of rumen liquor buffer solution was added and the substrate incubated for 24hrs as per the procedure of Menke. After 24 h, the incubation was stopped and the inoculants were determined for total gas production, ammonia-N, total volatile fatty acid (TVFA), individual volatile fatty acids (VFAs), methane production and protozoal counts. Methane in the gas phase and VFA in the fermentation medium were estimated by Gas liquid chromatography. Addition of *Sapindus mukorossi* seed coat saponin reduced methane production (ml/200mg) by 5.80, 11.08, 23.60 and 18.74% in starch, white clover, concentrate and mulberry substrates, respectively. Saponin addition also reduced protozoal counts(10^4 /ml) by 53.21, 52.76, 66.50 and 35.58% and ammonia nitrogen by 10.84, 17.13, 23.83 and 25.46% in starch, white clover, concentrate and mulberry, respectively indicating the reduction in protein turn over in rumen. Though total volatile fatty acid production was not affected, due to increase in propionate production there was decrease in acetate: propionate ratio. The results suggest that the reduction in protozoal counts and shift in rumen fermentation towards more propionate production might have resulted in the reduction in methanogenesis. It is concluded that *Sapindus mukorossi* seed coat saponin has potential to decrease methane emission and improve rumen fermentation and the *in vitro* results needs to be validated through animal trials.