Summary of Doctoral Thesis

Enrollment year: 2014 y 04 m
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Title	Methane emission and plasma nutrients metabolism in sheep fed garlic diets

Background

The animal agriculture is a vital part of food industry but it raises environmental concerns due to its contribution on greenhouse gas. Manipulating the rumen ecosystem to enhance the digestibility of fibrous feeds, and reduce methane emission by ruminants to improve animal performance are the major goals for animal nutritionists. Feed additives such as organic acids, ionophores, halogen compound and other antibiotics were used to modify ruminal fermentation, affect ruminal methanogenesis and improve animal performance. However, the use of antibiotics as feed additives due to the concerns of wide spread antibiotic resistant bacteria and residues in dairy and meat products have shifted the research towards use of safe alternatives such as plants containing secondary metabolites. Garlic contains a complex mixture of many secondary metabolites which include allicin, diallyl sulfide, diallyl disulfide and allyl mercaptan. These secondary metabolites present in garlic have been tested for rumen fermentation and methane emission; but studies on use of garlic on plasma glucose, leucine and phenylalanine kinetics have not been studied, thus through a series a experiment, we tried to see the role of garlic on plasma glucose and amino acid kinetics along with rumen fermentation and nitrogen utilization in sheep.

Experiment 1

It is well discussed that rate of CH_4 emission are influenced by a range of diet and animal factors, such as feed intake, diet quality, and nutrient utilization efficiency. Thus, an experiment was conducted to see the methane emission in sheep fed iso-energetic diet (100kcal) of mixed hay (MH) (57 g/kg$^{0.75}$/d of feed, 7 g/kg$^{0.75}$/d CP) diet and mixed hay and concentrate (Conc.) diet (40:60 ratio, 45 g/kg$^{0.75}$/d of feed,) on methane emission, rumen fermentation characteristics, nitrogen utilization, microbial

protein synthesis and microbial diversity in rumen of sheep. Experiment was carried out in crossover design of 21 days period with 14 days of adaptation to diet. Conc. diet lowered rumen pH ($P<0.01$). Rumen ammonia, total volatile fatty acid, acetic acid and propionic acid did not differ between the diets however butyric acid was higher ($P<0.01$) in Conc. diet. Acetic to propionic acid ratio was lower ($P=0.01$) in Conc. diet. Total methane emission during the 24 h period did not differ between the diets. The nitrogen intake, fecal nitrogen excretion and urinary nitrogen were higher ($P<0.05$) in MH diet. Nitrogen digestibility was higher ($P<0.01$) in Conc. diet. Urinary derivatives namely allantoin, uric acid, and xanthine and hypoxanthine were higher ($P<0.05$) in Conc. diet and thus total microbial nitrogen supply was also higher ($P<0.01$) in Conc. diet. Microbial diversity measured using Shannon index, Dominance index and Evenness index by DGGE gels did not differ between the diets. Thus, from the present experiment, we can conclude that in iso-energetic diets, concentrate diet have higher nitrogen digestibility and higher microbial nitrogen supply to the animal thus might have positive impact on productivity. Although methane emission and rumen fermentation characteristics did not differ did not differ between the diets, concentrate diet might be beneficial from environmental aspect due to less loss of nitrogen into the environment as nitrogen retention from both the diets was comparable in an iso-energetic condition. Better utilization of nitrogen in diet might can be correlated to faster body growth and thus lesser emission of methane.

Experiment 2

In our second experiment, we tried to find if secondary metabolites present in garlic could influence the rumen fermentation, methane emission and dry matter digestibility. The experiment was carried out using Rumen Simulation Technique (RUSITEC) as invitro methods are easy, cheap and faster to compare multiple samples. Control (Con.) diet was 15 g DM of mixed hay (orchardgrass and reed canarygrass). Four levels of freeze dried garlic leaves (10, 20, 40% and 60% DM respectively added to Con. diet) and four levels of freeze dried garlic bulbs (3, 6, 12, 18% DM respectively added to Con. diet). Rumen pH did not differ between the diets. Total VFA was lower ($P<0.05$) in 40% and 60% garlic leaves added diets and 12 % and 18% garlic bulb added diets. Ammonia concentration did not differ between the diets except for 40% garlic leaves added diet tended to be higher ($P=0.07$). Dry matter digestibility lower ($P<0.05$) in 20%, 40%, 60% garlic leaves added diets and 6%, 12%, 18% garlic bulb added diets. Total methane collected was lower ($P<0.05$) in 20%, 40% and 60% garlic leaves added diets and 12% and 18% garlic bulb added diets. Methane collected per g DMD was lower ($P<0.05$) in 20%, 40% and 60% garlic leaves added diet and 12 % and 18 % garlic bulb added diets. In the present experiment, garlic leaves or bulb at higher concentration lowered methane emission

which was desirable but consequently lowered the dry matter digestibility also which was undesirable, thus garlic leaves (around 10 to 20%) or bulb (3% to 6%) concentration seems to be appropriate and more experiments using garlic at these lower concentration could give us appropriate dose to use as a diet additive based on this study on the parameters studied.

Experiment 3

Although in-vitro methods are cheap, easier and faster, but its significance cannot be validated until they are further verified on in-vivo conditions, thus in the third experiment we fed garlic leaves to sheep to see the effects on rumen fermentation, methane emission, plasma glucose kinetics and nitrogen utilization in sheep. Six sheep were fed freeze dried garlic leaves at 2.5 g/kg $BW^{0.75}$/d (about 5% DM of diet) (FDGL diet) added to control diet using a crossover design. Control diet (GLCon. diet) consisted of mixed hay and concentrate at 60:40 ratio. Plasma glucose turnover rate was measured using primed continuous infusion of [U-^{13}C]glucose. No significant differences in rumen fermentation parameters were noticed except for rumen ammonia tended to be higher for FDGL diet. Methane emission per animal per day did not differ between the diets but methane emission per kg dry matter ingested and methane emission per kg dry matter digested was lower ($P<0.05$) for FDGL diet. Plasma glucose concentration was similar between the diets but plasma glucose turnover rate tended ($P<0.10$) to be higher in FDGL diet. Nitrogen intake was higher ($P<0.01$) in FDGL diet due to extra N available due to addition of garlic leaves. Fecal nitrogen was lower ($P<0.05$) in FDGL diet and thus nitrogen absorption was higher ($P<0.01$). Nitrogen retention and nitrogen digestibility were also higher ($P<0.05$) in FDGL diet. Purine derivatives namely allantoin tended ($P<0.10$) to be higher in FDGL diet and thus total microbial nitrogen supply also tended ($P<0.10$) to be higher in FDGL diet. Inclusion of FDGL as feed supplement had no negative effects on ruminal fermentation characteristics and had positive N utilization. However, further research seems necessary to explain its potential on methane reduction as well as glucose turnover.

Experiment 4

In the fourth experiment, we examined the effect of feeding garlic oil to sheep on rumen fermentation characteristics, methane emission, nitrogen utilization, plasma glucose, leucine, phenylalanine and tyrosine kinetics. Primed continuous infusion of [U-^{13}C]glucose, [1-^{13}C]leucine, [$^{2}H_5$]phenylalanine and [$^{2}H_2$]tyrosine were carried out followed by hyperinsulinemic euglycemic clamp. Control (GOCon.) diet consisted of mixed hay and concentrate (70:30) and experimental (GO) diet consisted of garlic oil

added at 30 mg/kgBW$^{0.75}$/d to GOCon diet. Rumen pH, ammonia, VFA concentration and methane emission did not differ between the diets. Glucose concentration was lower ($P<0.05$) in GO diet and glucose turnover rate was higher ($P<0.05$) in GO diet. Nitrogen intake did not differ between the diets. Fecal nitrogen was lower ($P<0.05$) in GO diet and thus nitrogen absorption was higher ($P<0.05$) in GO diet. Urinary nitrogen excretion did not differ between the diets and thus nitrogen retention also was similar between the diets. The leucine, phenylalanine and tyrosine turnover rates did not differ within the diets. The whole body protein synthesis and degradation based on phenylalanine tyrosine model did not differ between the diets. The hyperinsulinemic euglycemic clamp did not differ between two diets in rate of glucose production and utilization and exoogenous glucose infusion despite the lower blood glucose concentration and tendency of increased glucose turnover rate. Based on these the effect of garlic oil on ruminant feedstuff use is still debatable and further studies are necessary.

Conclusion

Based on the above experimental findings we can thus conclude that garlic leaves, garlic bulb or garlic oil failed to mitigate methane emission; and positive effects on nitrogen utilization and glucose metabolism are still debatable. Feeding garlic leaves or garlic oil in ruminants could be a potential feed additive in future, however further research is recommended.

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