EFFECTS OF DIETARY INCLUSION OF GARLIC OIL ON RUMEN FERMENTATION IN HOLSTEIN COWS

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ABSTRACT
This study was conducted to investigate the influence of garlic oil on rumen fermentation in lactating Holstein cows. Twenty (20) lactating Holstein cows with 52.35 (± 7.23 SD) days in milking (DIM) were randomly divided into two (2) groups of 10 cows each; Control group with no additive (CN), and Garlic oil group (GO) supplemented with garlic oil (1.2 ml/kg DM of diet). Rumen content was collected via oral ruminal probe at the beginning and end of the 8 week feeding trial for analysis of rumen volatile fatty acids (VFA). The results obtained for volatile fatty acid concentrations were analyzed with the Student's T- test in IBM SPSS (v20). The results showed that supplementation of garlic oil increased the proportions of acetate, propionate, and total VFA at week 8. The study found that garlic oil was able to increase the ruminal propionate concentration and unsaturated fatty acids, both of which could trap hydrogen thereby resulting in decreased ruminal methanogenesis. Further studies investigate the effects of garlic oil on the population of rumen microbiota and enteric methane emssion in cows.
Keywords: Garlic oil, Holstein, Cows, Volatile fatty acids, Rumen

INTRODUCTION

The anaerobic fermentation in ruminants produces methane gas which is an important greenhouse gas (GHG) (Bodas *et al*., 2012). Cattle contributes significantly to GHG emissions as compared to other livestock species (about 35%) and majority of these gases are in the form of methane (Bach *et al*., 2023).. Hence, methane emission from dairy cow industry constitute up to 17 % of global methane production (Olijhoek *et al*., 2019). Enteric methane production in ruminant results in energy loss, which can be as high as 12% of the gross energy intake thereby causing a reduction in animal performance and production (Lettat *et al.*,2013 and Pitta *et al*., 2022).

Hence, it is imperative to develop strategies of decreasing enteric methane emission from dairy cows and other ruminants. This will aid in reducing the contribution of ruminants to global methane production while increasing the production performance of the animals (Martin *et al*., 2016).The supplementation of essential oils in diet has proven to be an effective approach to reduce rumen methane production in animals (Olijhoek *et al*., 2019). Essential oils are natural metabolites and volatile compounds with antimicrobial properties against various species of bacteria, protozoa, fungi, and viruses (Blanch *et al*., 2016). The mechanism through which essential oils mitigate enteric methane production linked to its ability to starve methanogens of hydrogen or decrease the ruminal proportions of methanogens and protozoa. Essential oils could also alter rumen fermentation to support an increased propionate production (Beauchemin *et al*., 2022; Silvestre et al., 2023). Other benefits of essential oils in ruminants include improved lactation performance and feed efficiency (Silvestre *et al*., 2023). Garlic oil has been reported to possess the ability to alter rumen fermentation, improve animal performance, and decrease rumen methane production (Ding et al., 2023). In an in-vitro study (Busquet *et al*., 2005), garlic oil caused a decreased methane production while increasing concentration of propionate and butyrate. Research on the use on garlic oil in dairy cow are very few and the studies were mainly in-vitro. Hence, this study is aimed at investigating the efficacy of garlic oil in modifying the rumen fermentation of Holstein cows.

MATERIALS AND METHODS

Experimental Animals and experimental design

Twenty (20) lactating cows with 52.35 (± 7.23 SD) days in milking (DIM) were randomly selected at Jiangyin Baoxiang Animal husbandry company Ltd, China. The cows were divided into two (2) groups; Control group with no additive (CN), and Garlic oil group (GO) supplemented with GO at 1.2 ml/kg DM of diet (Canbolat *et al*., 2021) The garlic oil was purchased commercially and included in the TMR fed to the cows in the GO group. The cows were fed the experimental diet for two (2) weeks prior to the commencement of the experiment to enable the animals adapt to the diet. The cows were housed in tie stalls and were physically healthy at the commencement of the feeding trial. The cows had unlimited access to clean drinking water throughout the study period. The composition of experimental diet is displayed in Table 1.

Table 1: Composition of experimental diet (dry matter basis)

Feed ingredients	Percentage
Corn	26.87
Soybean meal	14.98
Cotton seed	6.86
Alfalfa hay	6.17
Oat hay	5.14
Alfalfa silage	3.50
Corn silage	25.70
Molasses	3.12
Yeast culture	0.12
Palm oil fat powder	0.12
Lysine	1.17
Methionine	0.04
Coated urea	0.31
Premix[1]	2.50
Total	100.00
Analyzed nutrient composition	
Net energy of Lactation (MJ/Kg)	1.70
Crude protein, %	17.5
Neutral detergent fiber, %	30.9
Acid detergent fiber, %	18.9
Ash, %	9.3
Calcium, %	1.24
Phosphorus, %	0.71

Premix contains 7 mg/kg Selenium yeast, 6 mg/kg selenium, 20 mg/kg iodine, 4 mg/kg cobalt, 700 mg/kg Zinc methionine, 10 g/kg Methionine hydroxy analogue, 150 mg/kg Copper methionine, 11.18% sodium chloride, 1 % Total phosphorus, 5.1% calcium, and 4 % magnesium. 180 KIU/kg Vit A, 1400 KIU/kg Vit E, 45 KIU/kg Vit D3, 360 mg/kg copper, 170 mg/kg iron, 910 mg/kg manganese, and 680 mg/kg zinc

Sampling procedure, data collection and chemical analysis

Rumen content were collected from each cow with a 50-mL tube twice (on the 1st and 8th week of the experimental period). A digital pH meter (Woonsocket, Rhode Island, USA) was used to measure the rumen fluid pH. The rumen fluid samples for the determination of NH_3-N were preserved with sulfuric acid (50%, vol/vol) stored in liquid nitrogen until required for further analysis. The VFAs were detected with a gas chromatography (GC-2014B, Shimadzu, Kyoto, Japan) equipped with a capillary column (thickness of the film is 30 m × 0.32 mm × 0.25 µm) at 110 °C, while the concentration of NH_3-N was determined using the phenol-hypochlorite reaction (Weatherburn, 1967).

Statistical Analysis

The results obtained for rumen fermentation paramters were analyzed with the Student's T- test in IBM SPSS (v20). Significant differences between treatments means were declared at $p < 0.05$.

RESULTS AND DISCUSSION

The rumen fermentation parameters of cows in the CN and GO groups for week 1 and 8 are given in Table 1 and 2 respectively. At the first week of the feeding trial, all the fermentation parameters were similar ($p > 0.05$) between the 2 groups except propionate, total VFA and NH_3-N (mg/dl) which were found to be significantly higher ($p < 0.05$) in the group supplemented with garlic oil (GO group). Rumen ammonia nitrogen (NH_3-N) is a central component of nitrogen metabolism in dairy cows. It is primarily derived from the deamination of dietary protein by rumen microbes, as well as from the hydrolysis of urea recycled from the blood (Tan *et al*., 2021). Ammonia serves as a key nitrogen source for microbial protein synthesis, which is essential for providing amino acids to the host animal (Pengpeng and Tan, 2013). At the end of the feeding trial (week 8), the proportions of acetate, propionate, and total VFA were significantly increased ($p < 0.05$) in the GO group. Other VFAs, rumen pH, and NH_3-N (mg/dl) were not affected ($p > 0.05$) by the supplementation of garlic oil. Ruminal propionate is derived from succinate and acrylate metabolic pathways and is a major precursor for gluconeogenesis. As a result, Propionate plays a key role in lactose synthesis in the mammary gland, which is the main stimulator of milk production (Pereira *et al*., 2022).

Table 1 Rumen fermentation parameters of cows for Week 1

Items	CN	GO	SEM	P value
Acetate (mmol/L)	66.37	72.01	1.498	0.057
Propionate (mmol/L)	22.44	25.40	0.732	0.040
Isobutyrate (mmol/L)	0.88	0.87	0.026	0.901
Butyrate (mmol/L)	13.30	13.96	0.375	0.394
Isovalerate (mmol/L)	1.60	1.62	0.056	0.821
Valerate (mmol/L)	1.65	1.58	0.082	0.710
Total VFA (mmol/L)	106.24	115.44	2.250	0.038
A:P	2.96	2.88	0.072	0.632
Rumen Ph	6.44	6.51	0.033	0.297
NH_3-N (mg/dl)	13.28	18.36	1.156	0.023

A:P Acetate to Propionate ratio, CN: Control group, SEM: Standard Error of Mean and GO: Garlic oil group

Table 2 Rumen fermentation parameters of cows for Week 8

Item	CN	GO	SEM	P value
Acetate (mmol/L)	66.49	86.70	2.944	<0.001
Propionate (mmol/L)	22.25	29.96	1.102	<0.001
Isobutyrate (mmol/L)	0.88	0.89	0.026	0.980
Butyrate (mmol/L)	13.50	14.61	0.430	0.205
Isovalerate (mmol/L)	1.59	1.58	0.061	0.933
Valerate (mmol/L)	1.60	1.52	0.078	0.680
Total VFA (mmol/L)	106.31	135.26	4.102	<0.001
A:P	2.99	2.92	0.079	0.658
Rumen Ph	6.53	6.50	0.039	0.776
NH_3-N (mg/dl)	13.59	18.87	1.449	0.067

A:P Acetate to Propionate ratio, CN: Control group, SEM: Standard Error of Mean and GO: Garlic oil group

CONCLUSION

The inclusion of garlic oil into the diets of Holstein cows improved acetate and propionate % as well as total VFA concentration in the rumen of cows. Further studies should study the effects of garlic oil on the population of rumen microbiota and enteric methane emission in cows.

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