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Effect of Garlic Powder on Methane Production, Rumen Fermentation and Milk Production of Buffaloes

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Authors' contributions

This work was carried out in collaboration between all authors. Author RZ designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author MM managed the analyses of the study and managed the literature searches. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: Use of natural feed additives to improve the milk production of buffalos.
Place and Duration of Study: National Dairy Research Institute, Karnal, India between May 2011 and July 2012.
Methodology: Garlic Powder (GRP) at 2, 4, and 6% of DMI was incubated for 24h in diluted ruminal fluid with a 50:50 roughage: concentrate wheat straw based diet. GRP at 2 and 6% DMI have resulted in decrease of molar proportion of acetate and butyrate but it has not effected on in vitro true dry matter digestibility and propionate production.
Results: Methane emission was significantly ($P<0.05$) decreased in presence of GRP. Further, GRP (at the rate 2% of DMI) was evaluated under in vivo conditions in Ten lactating Murrah buffaloes divided into two groups i.e. Control and Treatment group , and it shown very promising results on methane reduction. Methane was reduced up to 31% in case of GRP, without affecting digestibility of nutrients and milk composition in comparison to control group.
Conclusion: Overall milk production was remained similar in all groups but just after supplementation of GRP, and up to 12 weeks of supplementation, it remained significantly higher in treatment group as compared to control group.

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1. INTRODUCTION

Methane from agriculture arises primarily from enteric fermentation (59%) followed by rice paddy cultivation (23%), manure management (5%), burning of agriculture crop residue (1%) and soils (12%). Enteric fermentation results in the production of hydrogen during the digestion of nutrients, especially carbohydrates. Methanogenices utilize this excess H2 to reduce CO2 to CH4. Ruminant livestock are one of the largest single sources of methane emission with 80–115 million tons per year, equivalent to 15–20% of total anthropogenic methane [1].

Modification of rumen microbial fermentation to decrease methane using feed additives, such as antibiotics and ionophores already proved to improve production efficiency in dairy animals (McGuffey et al. 2001). Furthermore, the use of antibiotics as a feed additive has been banned in the European Union since 2003 [2]. The secondary plant products of Garlic are allicin, diallylsulfide, dialyldisulfide and allyl mercaptan among others [3]. These active compounds used in animal diets shown promising results in modifying rumen fermentation and as a result acetate decreased, propionate and butyrate production increased and have direct effect on methanogens which further decreased the ruminal methane production [4].

2. MATERIALS AND METHODS

The present study was divided in two experiments. The first experiment consisted of a short-term in vitro batch fermentation trial with different doses of Garlic Powder (2, 4, and 6% DMI) and the second experiment was conducted to confirm the hypothesis of the ability of Garlic Powder (2% of DMI) to decrease methane production under in vivo condition in lactating Murrah buffaloes.

2.1 *In Vitro* Study

The effect of GRP was evaluated using an in vitro batch culture of rumen fluid supplied with a 50:50 roughage: concentrate wheat straw based diet. The incubations were carried out in 100ml calibrated glass syringes (Haberle, Germany) as described by [5,6]. After 24h of incubation, volume of gas was withdrawn from the tip of the incubation syringe using Hamilton gas tight syringe and analyzed for methane with the help of gas chromatograph (Nucon 5700, India). In vitro true DM digestibility (IVDMD) was estimated as per the method outlined by [7]. Individual VFAs were determined using Gas Chromatograph (Nucon 5700, India).

After 24 hour of incubation, volume of gas was withdrawn from the tip of the incubation syringe using Hamilton gas tight syringe and analyzed for methane with help of gas chromatograph (Nucon 5700, India) fitted with stainless steel column packed with Porapak-Q (length 6';o.d.1/8" i.d. 2mm; mesh range 80-100) and Flame ionizing detector (FID). The temperature of injection port, column and detector was 40, 50 and 500°C respectively. Volume of gas taken for injecting was 200µl. The flow rate of carrier gas (nitrogen) through the column was 30ml/min and H2 was 30ml/min and air was 300ml/min. The standard gas for methane estimation (Spantech caliberation gas, Surrey, England) composed of 50% methane and 50% CO2. The peak of methane gas was identified on the basis of retention time of standard methane gas and the response factor obtained was used to calculate

methane percentage in the gas sample. The methane produced from substrate during 24h incubation was corrected for the blank values. The volume of methane produced was calculated as follows:

Methane production (ml) = Total gas produced (ml) X % methane in the sample

In vitro true DM digestibility (IVDMD) was estimated as per the method outlined [7]. After 24h incubation, the contents of the syringe were directly transferred in a 500ml spoutless beaker. The syringe was washed with 25ml of NDS and washings added to the beaker. The contents in the beaker were refluxed for 1h, filtered under vacuum through pre-weighed sintered (G1) crucibles. Content of the residue was determined. IVTDMD was calculated as follows:

$$\text{IVDMD} \quad (\%) = \frac{\text{Wt. of dried sample} - \text{Wt. of residue}}{\text{Wt. of dried sample}} \times 100$$

The final pH of each syringe after 24h of incubation and methane estimation was immediatlly measured with a pH meter (Model 507; Eutech Instruments, pH Tutor, Singapore), and samples were collected for determination of further analysis.

For protozoa counting, one milliliter of the fermentation fluid was diluted with 1ml of formalin (18.5% formaldehyde) and 3-4 drops of brilliant green and then incubated for 24h at room temperature. The stained protozoa were diluted (if needed) and counted by haemocytometer. The supernatant of each syringe including that of blank was used for NH3-N estimation. Supernatant (5ml) was mixed with 1 N NaOH (2ml) and steam passed by using KEL PLUS-N analyzer (Pelican, India) and the NH3 liberated was collected in 20% boric acid solution having mixed indicator and titrated against N/100 H2SO4.

Individual VFAs were determined using Gas chromatograph (Nucon 5700, India) equipped with flame ionization detector and glass column packed with chromosorb –101 (length 4'; o.d ¼"; i.d. 3mm; mesh range 80-100). The carrier gas was N and peaks were identified by comparison with a standard of known composition. The volume of IVFAs produced (millimoles) were corrected for standard conditions and the amount of IVFAs produced was calculated by multiplying the IVFAs produced by the concentration of IVFAs in the analyzed sample.

2.2 *In vivo* Study

Ten lactating Murrah Buffaloes (2^{nd} to 3^{rd} lactation) were selected from the buffalo herd of National Dairy Research Institute, Karnal, India and randomly distributed into two groups of five each according to their milk yield, live body weights and lactation stage. The animals were given concentrate and roughage diet which comprised of concentrate mixture, berseem and wheat straw. Nutritional requirement was as per CIRB feeding standard (2010) for a period of 120 days. Group I animals were fed control diet and Group II (Treatment I) animals were given GRP (2% of DMI) in addition to control diet. Drinking water was offered free choice thrice a day. Milking was done twice daily i.e. morning at 5:30 A.M and evening at 5:00 P.M and milk yield recorded was maintained throughout experimental period. Milk composition i.e. fat, protein, lactose and SNF was determined weekly by using of automatic milk analyzer. The animals were weighed before feeding and watering in the morning on two consecutive days at the start of experimental feeding and thereafter at fortnightly intervals during the experimental period. Dry matter intake was recorded fortnightly by subtracting the

residual DM from the quantity of DM offered. A digestion trial was conducted in mid of experimental period with 7 days collection period to determine the nutrients digestibility, as described above. Feeds and their respective residues were collected daily. Dry matter, organic matter, crude protein, ether extract and total ash were estimated as per AOAC [8] and cell wall constituent's viz., neutral detergent fiber and acid detergent fiber were measured as per the method [9].

In vivo methane emission from animals was measured using sulphur hexafluoride (SF6) tracer technique (Johnson et al., 1994), which has already been standardized in the Environment Laboratory, NDRI, Karnal. The methane emission rate (QCH4) was calculated from the ratio of methane CH4 to SF6 in collected gas and the known release rate of SF6 (QSF6). Background methane [{CH4} b] was subtracted from methane concentration in the PVC canister [{CH4} Y].

$$QCH_4 = \frac{QSF_6 \times [\{CH_4\}Y - \{CH_4\}b]}{[SF_6]}$$

2.3 Statistical Analysis

For all statistical procedures, analysis of variance (ANOVA), mean and standard error of the mean values were computed by using Statistical Package for Agricultural Workers (OPSTAT) in randomized block design with three (*in vitro* study) and five (*in vivo* study) replicates. Results are expressed as mean values ±SE of the mean values. Critical difference (CD value) was used to compare mean values. Statistical significance was accepted at $P < 0.05$.

3. RESULTS AND DISCUSSION

Relative to the control, addition of GRP at 2, 4, and 6% of DMI has not affected the DDM (Table 1). These findings are in agreement with [4], who reported that the supplemented Garlic Oil and its compounds at 30 and 300mg/l RL did not affect the true DM, OM, NDF and ADF digestibility in 50:50 forage: concentrate diet. In addition, [4] observed that supplementation of Garlic and berry essential oil did not affect total digestibility of DM, OM, fiber and starch, while ruminal DM and OM digestibility was increased. Acetate production and proportion of butyrate were decreased significantly by addition of GRP at 2 and 6% of DMI when compared with control. Acetate: Propionate ratio was decreased by addition of GRP at low dose but increased at higher dose (Table 1). These results were in accordance to results of [10].

In 50R:50C wheat straw based diet, by supplementation of GRP, methane production decreased significantly ($P<0.05$) in all of the doses as compared with control (Table 1). Similarly [11] investigated the effect of a commercially available aqueous allicin product in the rumen simulation technique (RUSTTEC), at 2 and 20mg/l allicin. They found no effect on daily total VFA, but a decrease (94% at 20 mg/l allicin addition) in methane production was observed.

Under in vivo condition, initial milk yield as well as final and overall milk yield was not affected by supplementation of GRA, but apparently remains higher in treatment group as compared to control group (Table 2). These results were in agreement with [12] who reported that milk production of lactating Holstein dairy cows were increased by about 1%

during of first month across the whole experiment with EO mixture (EOM) when added at 16mg L-1 in drinking water.

In whole of the experiment period, body weight and DMI of treatment group was remained unaffected as compared to the control group (Table 3). Our results were in confirmation of results of [12,2,3] who also did not found any effect on body weights when diets were supplemented with EOM.

Apparent digestibility of all the nutrients such as DM, OM, CP, EE, NDF, ADF, CHO and HC was found higher in experimental group than in control group; however, the differences among the groups were not significant (Table 4). These observations indicated that the dietary supplementation of GRP improved the digestibility of nutrients in dairy buffaloes and supplementation of GRP did not affect the nutrient digestibility adversely as recorded in vitro experiments in some cases. [2] also reported that essential oil supplementation in dairy cows had no effect on DM intake and digestibility of different nutrients. These results were confirmation with that of [3] who reported that DM and OM digestibility were not altered by Garlic oil supplementation.

Results showed significant ($P<0.05$) decrease in methane production in lactating buffaloes with addition of GRP as compared to control (Table 5). When it expressed as g/kg DDM and mM/kg DMI the similar trend was noticed. These observations indicated that the total methane emission (g) and methane emission g/kg DMI in experimental buffaloes reduced ($P<0.05$) by 31% in case of GRP supplemented in comparison to control group. Results were in confirmation of other studies in which different products of Garlic were evaluated and methane was significantly decreased in different diets [2,11,12,13,14].

Table 1. Effect of different doses of GRP on *in vitro* digestibility, IVFA, acetate: propionate ratio and methane production on wheat straw based diet (50R:50C)

Parameter	GRP (% of DMI)				
	Control	2	4	6	CD value
DDM (mg)	141.00±0.58	142.33±1.45	145.67±2.03	139.00±1.53	5.58
NH3-N (mg/100ml)	16.52±0.49	26.69±0.49	18.01±0.52	17.83±0.19	1.01
pH	7.01±0.02	6.99±0.02	6.96±0.01	6.98±0.02	0.05
Protozoa (x104/ml)	3.67±0.17	2.67±0.17	4.67±0.17	5.33±0.17	0.77
Acetate (mM/100ml)	8.31±0.12	2.72±0.31	8.00±0.12	7.73±0.50	0.71
Propionate (mM/100ml)	0.98±0.02	0.66±0.06	1.02±0.01	0.69±0.03	0.13
Butyrate (mM/100ml)	0.24±0.02	0.17±0.01	0.20±0.02	0.32±0.02	0.04
A:P	8.44±0.21	4.13±0.38	7.83±0.05	11.22±0.86	1.15
CH4 (ml/gDM)	30.25±1.01	24.07±0.27	24.21±0.13	27.58±0.52	1.48

Table 2. Effect garlic powder on milk production in lactating Murrah buffaloes

Treatment	Milk yield					
	Initial	1-3wks	4-7 wks	8-11 wks	Final	Overall average
Control	7.42±0.60	6.20±0.16	5.43±0.131	5.08±0.05	5.37±0.12	5.48±0.12
Garlic powder	7.90±0.90	6.87±0.09	6.20±0.122	5.48±0.12	5.10±0.21	5.90±0.19
CD Value	N.S.	0.47	0.42	0.27	N.S.	N.S.

Table 3. Effect of garlic powder on body weight change and dry matter intake in lactating Murrah Buffaloes

Treatment		Control	Garlic powder	CD value
Body weight	Initial	527.40±11.39	534.10±38.20	N.S.
	Final	528.00±12.73	542.90±35.44	N.S.
	Overall	530.4±01.62	543.28±4.07	N.S.
DMI (kg/d/a)	Initial	10.56±0.49	10.80±0.58	N.S.
	Final	10.60±0.26	10.64±1.25	N.S.
	Overall	10.66±0.24	11.60±0.62	N.S.

Table 4. Effect of garlic powder on total nutrient digestibility in lactating Murrah Buffaloes

Treatment	Control	Garlic Powder	CD Value
DMD	74.82±0.34	75.24±1.30	N.S.
OMD	77.13±0.33	76.97±1.17	N.S.
EED	80.60±0.79	78.59±2.16	N.S.
NDFD	63.42±1.09	65.14±1.82	N.S.
ADFD	58.24±1.22	60.04±1.43	N.S.
CPD	90.48±1.13	90.28±2.49	N.S.
CHOD	74.68±0.36	74.68±1.18	N.S.
HCD	72.74±1.17	74.20±2.63	N.S.

Table 5. Effect of garlic powder on methane production in lactating Murrah Buffaloes

Treatment	Control	Garlic Powder	CD Value
Body Wt. Average	528.3	538	
DMI (kg/d/a)	10.98±0.49	12.24±0.59	N.S.
Methane (g/kg DMI)	40.70±2.91	27.0 0±01.99	7.36
Methane (g/kg DDM)	54.03±3.75	36.37±2.11	8.95

4. CONCLUSION

On the basis of in vitro studies, it was concluded that GRP was effective in decreasing methane production in wheat straw based diets. Further it was concluded from in vivo study that methane was reduced up to 31% in case of GRP supplementation in wheat straw based diet without affecting digestibility of nutrients and milk composition in comparison to control group in lactating buffaloes. Overall milk production was remained similar but just after supplementation of GRP and up to 12 weeks; it remained significantly higher in treatment group as compared to control group.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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