ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Effect of Supplementing Diet with Herbal Plants on Ruminal Fiber Digestibility and Gas Production.

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ABSTRACT

This study was carried out to investigate addition of lemongrass or galangal to diet and its effect on the ruminal nutrients digestibility and gas production. Treatments were: control diet consist of 50: 50 Concentrate : roughage; Lemongrass : control diet plus 4.5 g powdered lemongrass/Kg DM which equal 100 mg essential oil/ kg DM; galangal: control diet plus 4 g powdered galangal /Kg DM which equal 100 mg essential oil/ kg DM. The results showed that adding lemongrass or galangal decreased gas production per each gram of NDF, ADF, ADL, hemicellulose and cellulose ($p<0.05$) as compared with control; while, there were no differences ($p>0.05$) between treatments in total gas production. Ammonia concentration was lower ($p<0.05$) in lemongrass and galangal compared with control (7.29, 8.76 and 13.15 mM for lemongrass, galangal and control, respectively). Dry matter, organic matter, NDF and ADF digestibility were not significantly impacted ($p>0.05$) by adding lemongrass or galangal to the diets as compared with control. It could be concluded that adding lemongrass or galangal the diet could enhance ruminal fermentation and reducing gas production without adverse effect on nutrients digestibility.

Keywords: lemongrass, galangal, nutrients digestibility, gas production, ammonia

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ISSN: 0975-8585

INTRODUCTION

Feed entering the rumen is primarily digested by rumen microorganisms such as bacteria, fungi and protozoa, then produce final end-products such as volatile fatty acids (VFA) hydrogen (H_2) and CO_2. Methane is produced as secondary product from the feed fermentation in the rumen by Archaea to get energy for growth [1]. More than 95% of the CH_4 produced during enteric fermentation in the rumen is lost via the mouth to the atmosphere, whereas rectal emissions account for only 2-3% [2]. Estimated data of produced CH_4 from adult cattle ranging between 330 – 600 liter which equal 2 – 12 % of gross energy intake, depending on the diet composition, type, size, dry matter intake, addition of ionophores or lipids and animal productivity [3].

Ionophores mode of action concerned at inhibiting growth of gram-positive bacteria that produce H_2 which used by archaea bacteria to produce CH_4 [4]. Since EU banned using antibiotics as feed additive, researchers interested on using essential oils as alternative in manipulating rumen fermentation [4- 6].

Plant secondary metabolites (saponins, tannins and essential oils) are recently getting more interest as natural rumen modifier [4, 6, 7 and 8]. Mode of action of essential oils regards to its contents of monoterpene hydrocarbons and/or phenolic compounds, the phenolic compounds of essential oils have antimicrobial activity by inactivation of some microbial enzymes [9; 10].

Many of herbs contains essential oils have been investigated on modifying rumen fermentation and positivity affected volatile fatty acids, methane production, starch, protein degradation [11] and decrease ruminal bio-hydrogenation [4]. While, these positive effect accompanied with another negative effect on fiber degradation [12].

Therefore the aim of the present study was to evaluate the effect of two herbs (Lemongrass and Galangal) on rumen fermentation and gas production, dry matter and fiber degradation and ammonia production *in-vitro*.

MATERIALS AND METHODS

Diet and In-vitro procedures

In-vitro incubation were carried out according to Menke and Steingass [13]. Experimental diets were : control diet consist of 50: 50 Concentrate : roughage (table, 1); Lemongrass : control diet plus 4.5 g powdered lemongrass/Kg DM which equal 100 mg essential oil/ kg DM; galangal: control diet plus 4 g powdered galangal /Kg DM which equal 100 mg essential oil/ kg DM.

Table (1) Ration ingredient and chemical composition

Ingredients	% (DM)
Clover hay	50.0
Corn	19.3
Barley grain	5.8
Soyabean meal	11.5
Wheat bran	12.2
Di-calcium phosphate	0.15
Minerals &Vitamins	0.15
NaCl	0.4
Limestone	0.5
Ration chemical composition, % (DM)	
Dry matter	90.81
Organic matter	90.46
Neutral detergent fiber	32.83

Acid detergent fiber	16.39
Acid detergent lignin	3.67
Crude protein	17.13
Ether Extract	4.21
Ash	9.54
Non-fiber Carbohydrate	36.29
Hemicellulose	16.44
Cellulose	12.72

Rumen fluid was collected from 3 ruminally cannulated holestein dairy cows (mean weight 680±30 kg). The collected rumen fluid (before morning feeding) was mixed and squeezed through a 4-layers cheesecloth under continuous flushing with CO_2 and immediately transported to laboratory at 39°C where it was used as a source of inoculum. Each treatment was tested in eight replicates accompanied by blank vessels (no substrate). 400 mg of milled substrate was added to the incubation vessels of 100mL capacity. Each vessel was filled with 40 mL of the incubation medium (292 mg K_2HPO_4, 240 mg KH_2PO_4, 480 mg $(NH_4)_2SO_4$, 480 mg NaCl, 100 mg $MgSO_4x7H_2O$, 64 mg $CaCl_2.2H_2O$, 4 mg Na_2CO_3 and 600 mg cysteine hydrochloride) per 1 liter of double distilled water (ddH_2O) and dispensed anaerobically in the 1:4 (v/v) ratio. Then the treatments were incubated at 39°C for 48h.

The average of the volume of gas produced from the blanks was deducted from the volume of gas produced per sample. After 48 h digestion, the samples were transferred into test tubes and centrifuge for 1h in order to obtain the residues which was then filtered using Whatman No 4 filter paper temperature ranges between 30°C and 42°C being the by gravity and the residues placed in for drying at 65°C for 24 h. The dry residues were weighed and digestibility calculated using the equation as follows:

IVDMD (%) = [(initial DM input – DM residue – Blank) / initial DM input] *100

Samples analysis

Samples of fermenter fluid were analyzed for pH and NH_3-N. Substrates and substrate residues after 48 h of incubation were dried at 70°C and analyzed for the amount of DM (DM digestibility) according to AOAC [14]. The NH_3-N concentration was determined as described by Khattab et al. [15]. Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) and Acid Detergent Lignin (ADL) were analyzed by Ankom200 Fiber Analyzer (Ankom Technology Corporation, Fairport, NY) according to Van Soest *et al*. [16]. Microbial protein production was calculated as 19.3 g microbial nitrogen per kg OMD according to Czerkawski [17].

Statistical analysis

Data were statistically analysed using GLM procedure of SAS software (Version 9.2). Significant differences between means of treatments were carried out by the Duncan's test, and the significance threshold was set at $P<0.05$.

RESULTS AND DISSCUSSION

Gas production

Gas production results are presented in table (2). The results showed that supplementing diet with either lemongrass or galangal slightly ($p>0.05$) decreased gas production, the lower value for gas production was recorded for diet supplemented with lemongrass (123.3 ml) followed by diet supplemented with galangal (125.3 ml) compared with control diet (131.8 ml). While, data showed that the amount of gas production per each gram of dry matter were lower in galangal diet (333.83 ml) then lemongrass diet (351.73 ml) compared with control (373.91 ml).

ISSN: 0975-8585

Table (2) effect of experimental diets on Gas production

	Control	Lemongrass	Galangal	*Pr. > F*
Total GP	131.8 ± 4.57	123.3 ± 1.60	125.3 ± 2.72	0.1815
GP/g DM	373.19 ± 12.6	351.73 ± 3.38	333.83 ± 7.23	0.2747
GP/g NDF	439.64 [a] ± 44.1	312.37 [b] ± 15.9	301.04 [b] ± 32.8	0.0453
GP/g ADF	883.86 [a] ± 88.7	405.92 [b] ± 20.7	385.60 [b] ± 41.99	0.0014
GP/g ADL	3932.82 [a] ± 395.1	1803.49 [b] ± 92.1	1730.38 [b] ± 188.44	0.0015
GP/g Hemicellulose	877.95 [a] ± 88.2	627.08 [b] ± 32.02	604.02 [b] ± 65.78	0.0477
GP/g Cellulose	728.25 ± 68.8	719.30 ± 69.3	719.03 ± 98.87	0.5280

The gas production pattern relative to fiber fraction showed significant reduction ($p<0.05$) by supplementing diet with lemongrass or galangal, the results showed that galangal had positive effect in reducing gas production per every gram of NDF , ADF , ADL Hemicellulose and Cellulose by 31.5, 56.37, 56, 31.2 and 1.27 %, respectively as compared with control diet. Also results showed reduction in gas production when diet supplemented with lemongrass by 28.94, 54.07, 54.14, 28.57 and 1.23%, respectively for each gram of NDF, ADF, ADL , Hemicellulose and cellulose as compared with control diet. It well known that there is negative correlation between gas production and cell wall contents (NDF and ADF) which tends to reduce the microbial activity [18].

Rumen pH and NH_3-N concentrations

The effect of supplementing diet with lemongrass or galangal on rumen pH and NH_3-N are presented in table (3). The results showed that adding lemongrass or galangal to diet significantly ($p<0.05$) decreased rumen pH. NH_3-N concentrations were significantly reduced ($p<0.05$) by supplementing diet with lemongrass or galangal, lemongrass diet recorded the lowest value of NH_3-N (7.29 mM) followed by galangal diet (8.76 mM) compared with control diet (13.15 mM). These reduction of NH_3-N concentrations inagreement with Wanapat et al. [19]; Macheboeuf et al. [20] and Cobellis et al. [6] who reported that essential oils especially cinnamaldehyde and cinnamon decreased ammonia concentrations in the rumen, Wallace et al. [21] suggested that the mode of action of essential oils was inhibiting bacterial attachment to feed particles, and deamination of amino acids. McEwan et al. [22] noted that adding essential oils to diet resulted a decrease in the number and diversity of hyper-NH_3-producing bacteria, which reflect as a decrease in NH_3 production from amino acids. it was propsed that essential oil inhibit proteolysis, peptidolysis and deamination of amino acids [11]. different studies reported that the effective dose of essential oils which needed to decrease ammonia production was lower than that needed to decrease gas production [6, 12, 20, 23].

Table (3) effect of experimental diets on pH value and Ammonia concentration

	Control	Lemongrass	Galangal	*Pr. > F*
pH	6.53 [a] ± 0.04	6.27 [b] ± 0.03	6.25 [b] ± 0.016	0.0011
NH_3-N (mM)	13.15 [a] ± 0.39	7.29 [b] ± 0.48	8.76 [b] ± 0.69	0.0006

Nutrients digestibility

Table (4) effect of experimental diets on nutrients digestibility

	Control	Lemongrass	Galangal	*Pr. > F*
DM digestibility	58.31 ± 3.18	63.61 ± 2.96	63.64 ± 1.19	0.4997
OM digestibility	55.71± 2.35	54.13 ±2.94	54.50 ±2.48	0.597
NDF digestibility	46.07 ± 2.52	45.85 ± 4.13	50.27 ± 0.76	0.50
ADF digestibility	54.03 ± 2.01	55.64 ± 3.16	59.13 ± 1.06	0.40
Microbial protein (mg/g DM)	10.75 ± 1.03	10.45 ± 0.99	10.52 ± 1.01	0.65

Supplementing diet with lemongrass or galangal on nutrients digestibility are shown in table (4). Adding lemongrass or galangal to diet had no effect ($P>0.05$) on dry matter, organic matter, NDF and ADF digestibility. The results of dry matter and organic matter digestibility matched with previous studies [4, 19, 24, 26; 27; 28]. Patra [11] suggested that essential oil have no negative effect on fiber degradation. Also, Cobellis et al. [6] reported that adding some herbal plants to diet had no negative effect on growth and activity of the major cellulolytic bacterial species.

Results showed that experimental diet had no significant (P>0.05) effect on microbial protein. Essential oil could decrease archaeal community and gas production while cellulytic bacteria have not markedly affected [29] and the reduction in some bacterial population affected by essential oil reflect as increase in another bacterial population.

CONCLUSION

The present study showed that adding herbal plants (lemongrass or galangal) to ruminant diet had positive effect on gas production per each component of fiber components and reduced NH_3-N concentration in the rumen, and had no negative effect on DM, OM, NDF and ADF digestibility.

ACKNOWLDGEMENT

This work was supported by a National Research Center, grant No. (P100401)

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