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Ruminal Fermentation, Degradability and Gas Production Response to Supplementing Diets with Marjoram or Basil Leaves *In-vitro*

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Abstract

Eleven experimental treatments were used by in-vitro batch culture technique to study the effect of marjoram (*Origanummajorana L.*) or basil (*Ocimumbasilicum L.*) leaves supplementation to diets on gas production, ruminal fermentation, ammonia nitrogen concentration, dry matter (DM) and cell wall contents (NDF and ADF) degradability. The basal diet consisted of a total mixed ration (TMR), the experimental treatments were used as follow: 50% CFM, 50% alfalfa hay (control), control diet plus 5, 10, 15, 20 and 25 grams of marjoram or basil leaves / kg DM for G1, G2, G3, G4, G5, G6, G7, G8, G9 and G10, respectively. No differences ($p>0.05$) were observed with marjoram or basil leaves supplementation in pH, DM, NDF and ADF degradability at all different levels compared with the control diet. Ammonia nitrogen and short chain fatty acid (SCFAs) concentrations revealed significant ($p<0.05$) decreased for the experimental treatments compared with the control. Also, the experimental treatments recorded the lowest values ($p<0.05$) in total gas production (TGP), gas production per each gram of dry matter, NDF andADF at the different levels of marjoram or basil leaves compared with control group. It could be concluded that the addition of marjoram or basil leaves to diets had a positive impact on total gas production without adverse effect on dry matter, and cell wall contents degradability.

Keywords: Marjoram, Basil, additives, gas production, rumen fermentation and *in-vitro*.

1. Introduction

Nowadays, the greenhouse gases have received a lot of attention in several scientific studies aimed to decreasing it by using different mitigation options [1, 2]. Livestock production system contributes about 18 percent of to the global anthropogenic greenhouse gas (GHG) emissions. The enteric methane emission is contributed around 30 percent, mostly from feeds fermentation in the rumen [3]. More than 95 percent of methane emission during enteric fermentation of feeds in the rumen is lost via the mouth to the atmosphere, while 2 to 3 percent is lost via rectal emissions [4]. Estimated data of produced methane from mature cattle ranging between 330 to 600 litres, which it represents a significant feed energy loss 2 to 12 percent, depending upon the diet composition, dry matter intake, size, type, addition of lipids or ionophores and animal productivity [5,6].

Since,European Union banned using synthetic and medicinal chemistry as feed additives, the phytogenic was used as alternative of antibiotics in modifying rumen fermentation [7,8]. Herbal plants have varied secondary metabolites such as essential oils, tannins and saponins, which used to inhibit growth of some gram-positive bacteria that produce hydrogen, which used by archaea bacteria to produce methane [7].

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Several herbal plantssuch as marjoram, basil, coriander, cinnamomum, rosemary, galangal, lemongrass, thyme and celery have been investigated on manipulating rumen fermentation and inhibition of deaminationprocess and methanogenesis bacteria, resulting in lower ammonia, methane emission and acetate concentration, and in higher propionate and butyrate concentrations, also decreased ruminal bio-hydrogenation [9-15].

Marjoram (*Origanummajorana L.*) or basil (*Ocimumbasilicum L.*) contain several active components such as saponins, thymol, carvone, eugenolγ-terpinene, linalool and p-cymenedepending on the origin and species of plants [16-18]. Thesecomponents have antimicrobial activity against gram-positive and negative bacteria [19-21]. Also, it has been able to increase propionate production, reduce methane production or acetate, and modify proteolysis, deamination or peptidolysis in the rumen [14, 22]**.** Therefore, the aim of the current study was to evaluate the effect of using different levels of marjoram (*Origanummajorana L.*) or basil (*Ocimumbasilicum L.*) leaves as a natural feed additive on ruminal fermentation, total gas production, ammonia nitrogen concentration, and dry matter, NDF and ADF degradability.

2. Materials and methods

2.1. Study location

The experiment was carried out at the laboratory of dairy production, dairy sciencedepartment, Food Industries and Nutrition Research Institute, National Research Centre (Egypt).

2.2. Experimental treatments

In vitro incubation procedures were carried out as described by Khattab et al. [10] rumen fluid was obtained from slaughterhouse and it was collected from goats, mixed and squeezed through 4-layers cheesecloth under continuous flushing with CO_2 and immediately transported to laboratory at 39°C (used as a source of inoculum). A total mixed ration (TMR) was used as the control diet in all experiments, treatments were: 50% CFM, 50% alfalfa hay (control), control diet +5 g marjoram leaves / kg DM (G1), control diet + 10 g marjoram leaves / kg DM (G2), control diet + 15 g marjoram leaves / kg DM (G3), control diet + 20 g marjoram leaves / kg DM (G4), control diet + 25 g marjoram leaves / kg DM (G5), control diet + 5 g basil leaves / kg DM (G6), control diet + 10 g basil leaves / kg DM (G7), control diet + 15 g basil leaves / kg DM (G8), control diet + 20 g basil leaves / kg DM (G9), control diet + 25 g basil leaves / kg DM (G10). The chemical composition of feed ingredients are shown in Table (1)[15]**.** Each treatment was tested in three replicates accompanied by blank bottles (no substrate). Substrate (400 mg) was added to the incubation bottles of 125 mL capacity.Each bottle was filled with 40 mL of the incubation medium which consists of a buffer solution was prepared by (292 mg K2HPO4, 240 mg KH2PO4, 480 mg (NH4)2SO4, 480 mg NaCl, 100 mg MgSO4.7H2O, 64 mg CaCl2.2H2O, 4 mg Na2CO3 and 600 mg cysteine hydrochloride) per 1 liter of double distilled water (ddH2O) and added to rumen fluid by 4:1 (v/v) ratio, then the headspace of each bottle was flushed with CO2. The pH was adjusted to 6.8-6.9 prior to inoculum injection. Then the bootles were incubated at 39°C for 24h.

Table (1), Chemical composition of feed ingredients (g/kg DM basis)

Item	CFM	Alfalfa hay	TMR
Dry matter	912.7	926.0	919.4
Organic matter	956.7	939.0	947.9
Neutral detergent fibre	213.3	438.8	326.1
Acid detergent fibre	63.2	326.6	194.9
Crude protein	149.0	175.0	162.0
Ether extract	53.0	29.0	41.0
Ash	43.3	61.0	52.2
Non-fibre carbohydrate	541.4	296.2	418.8

CFM: concentrate feed mixture; consisted of 57% yellow corn, 25% wheat bran, 15%soybean meal, 0.6% sodium bicarbonate, 1% limestone, 0.8% NaCl, 0.3% vitamins and 0.3%minerals. TMR: total mixed ration

2.3. Samples analysis

After 24 h of incubation, gas production (GP), pH value and NH3-N concentration were analyzed according to Khattab and Abd El Tawab [23]. the amount of dry matter degradability (DMD) was determined according to AOAC [24]. Short chain fatty acids (SCFA) were calculated using the equation of Makkar[25]. Fiber fraction NDF and ADF were analyzed according to Van Soest et al. [26]. Gas production per gram DM, NDF and ADF were calculated according to Khattab et al. [10].

2.4. Statistical analysis

Data were statistically analyzed using GLM procedure of Statistical Analysis System [27], version 9.2. Significant differences between means of

treatments were carried out by the Tukey's test, and the significance threshold was set at $p < 0.05$.

3. Results and discussion

3.1. Ruminal fermentation parameters

Data in Table (2) showed that the effect of fed a diet supplemented with marjoram or basil leaves onsome rumen fermentation parameters. No differences were recorded (P = 0.9323) in pH values with adding marjoram or basil leaves to diets. pH values are a good indicator for investigating the inclusive herbal plants effect on the rumen microbial population, ruminal fermentation and environmental [28]. These results are confirmed by the results from the previous studies that noted no significant impact on rumen pH, when fed diets with oregano leaf for lactating dairy cows [29,30]. Also, Zhou et al. [31] observed that decreasing in ruminal protozoa population but the pH values not affected in sheep fed oregano essential oil. Moreover, an *in-vitro* batch culture fermentation system technique the pH values not affected when used different levels of basil or marjoram of essential oils [15]. While, Kouazounde et al.[32] found that increasing in pH values in the rumen simulation technique (RUSITEC) with addition of African basil (*Ocimumgratissimum*) essential oils to diets.A significant decrease in rumen ammonia nitrogen (P = 0.0181) with addition marjoram or basil leaves to diets.

Table (2): Effect of a diet supplemented with marjoram or basil leaves on pH values, ammonia nitrogen and SCFA concentrations

Item	Control	Marjoram leaves					Basil leaves					*SEM*	*P value*
		G1	G2	G3	G4	G5	G6	G7	G8	G9	G10		
pH	6.75	6.68	6.87	6.86	6.89	6.88	6.77	6.82	6.85	6.87	6.85	0.028	0.9323
Ammonia nitrogen (mg/dl)	35.5a	33.5ab	33.1ab	29.2b	31.9ab	34.2ab	33.3ab	32.7ab	32.7ab	30.7ab	29.2b	0.444	0.0181
SCFA (mmol)	1.42a	1.20b	1.25b	1.20b	1.23b	1.26b	1.22b	1.24b	1.26b	1.25b	1.27b	0.011	<0.0001

Control: 50% CFM, 50% alfalfa hay,G1: control diet +5 g marjoram leaves / kg DM, G2: control diet + 10 g marjoram leaves / kg DM, G3: control diet + 15 g marjoram leaves / kg DM,G4: control diet + 20 g marjoram leaves / kg DM, G5: control diet + 25 g marjoram leaves / kg DM, G6: control diet + 5 g basil leaves / kg DM, G7: control diet + 10 g basil leaves / kg DM, G8: control diet + 15 g basil leaves / kg DM, G9: control diet + 20 g basil leaves / kg DM, G10: control diet + 25 g basil leaves / kg DM.
SCFA: short chain fatty acids.
Different superscripts a and b at the same row differ significantly ($p<0.05$).
SEM: standard error of the means.

A decrease in ammonia nitrogen may attributed to adverse effect of additives on ammonia producing bacteria(*Peptostreptecoccusanaerobius*and *Clostridium sticklandii)*in addition to their adverse effect on deamination [33-35]. This could lead to increase the efficiency of protein utilization by reduce the rate of ruminal ammonia nitrogen yield in the rumen [36].

Short chain fatty acids (SCFA) concentration was recorded the highest (P < 0.0001) values in control group compared with other groups. A reduction of SCFA in marjoram or basil leaves treatments could be a good indicator of simultaneous with methane emission reduction in the rumen tract [37]. These results are agreement with Khattab et al. [12] who found that a reduction in total volatile fatty acids (TVFAs) production, when essential oils supplementation to diets. Also, Kouazounde et al. [32] found that TVFAs production was linearly decreased by the essential oils of African basil *(Ocimumgratissimum)* supplementation to diets with a shift inthe VFA profile towards less propionate and more acetate and butyrate. Moreover, Evans and Martin [38] observed that the decreasing in TSCFAs, acetate and propionate concentrations, while acetate to propionate ratio was increased, when tested high level of thymol (400 mg/L).

3.2. Gas production

Effect of a diet supplemented with different levels of marjoram or basil leaves on *In-vitro* ruminal gas production are revealed in Table (3).Generally,the experimental treatments were recorded the lowest values in total gas production (TGP) (P < 0.0001), gas production per each gram of dry matter (GP/gram

DM) (P < 0.0001), gas production per each gram of NDF (GP/gram NDF) (P < 0.0001) and gas production per each gram of ADF (GP/gram ADF) (P < 0.0001) at the different levels of marjoram or basil leaves compared with control treatment, which recorded the highest value. Several *in-vitro* and *in-vivo* studies suggested that the essential oils supplementation have caused modifying rumen fermentation and positively affect gas production [6,8,12,39,40]. These findings were synchronized with Kouazounde et al. [32] who found that the total gas production and methane emission were decreased, when African basil (*Ocimumgratissimum*) addition to diets in the rumen simulation technique (RUSITEC). Also, Selim et al [15] found that the TGP was decreased, when used different

Table (3): Effect of a diet supplemented with marjoram or basil leaves on ruminal gas production (ml)

Item	Control	Marjoram leaves					Basil leaves					*SEM*	*P value*
		G1	G2	G3	G4	G5	G6	G7	G8	G9	G10		
TGP	128a	108b	113b	109b	111b	114b	111b	112b	114b	113b	115b	1.024	<.0001
GP/gram DM	346a	288b	303b	293b	300b	308b	300b	302b	306b	304b	310b	2.809	<.0001
GP/gram NDF	1057a	883b	927b	896b	917b	941b	917b	924b	936b	930b	947b	8.555	<.0001
GP/gram ADF	1763a	1476b	1549b	1497b	1532b	1570b	1531b	1544b	1565b	1552b	1581b	14.184	<.0001

Control: 50% CFM, 50% alfalfa hay,G1: control diet +5 g marjoram leaves / kg DM, G2: control diet + 10 g marjoram leaves / kg DM, G3: control diet + 15 g marjoram leaves / kg DM,G4: control diet + 20 g marjoram leaves / kg DM, G5: control diet + 25 g marjoram leaves / kg DM, G6: control diet + 5 g basil leaves / kg DM, G7: control diet + 10 g basil leaves / kg DM, G8: control diet + 15 g basil leaves / kg DM, G9: control diet + 20 g basil leaves / kg DM, G10: control diet + 25 g basil leaves / kg DM.
TGP; total gas production after 24hours., DM; dry matter, NDF; neutral detergent fibre, ADF; acid detergent fibre.
Different superscripts a and b at the same row differ significantly (*p<0.05*).
SEM: standard error of the means.

levels of basil or marjoram essential oils an *in-vitro*. Moreover, Rezaei and Pour [41] reported that the gasproduction was reduced with thyme methanolic extracts supplementation to diets. Chaudhry and Khan [42] noted that the methane emission was decreased by 40% when tested five curry spices such as coriander, cumin, turmeric, clove, and cinnamon by using an *in-vitro* gas production technique. It is observed that the ADF content was negatively correlated with gas emission which tends to inhibit the microbial activity [23].

3.3. Nutrients degradability

In-vitro nutrients degradability are presented in Table (4). No significant difference in dry matter (P = 0.2809), NDF (P = 0.9874) and ADF (P = 0.2089) degradability among groups. These results indicated that the concentrations of active components in marjoram or basil leaves insufficient to effect on the microbial activity in the rumen, and sequentially nutrients digestion [10,11,12,43].These results were agreement with Selim et al. [15]who found no

Table (4): Effect of a diet supplemented with marjoram or basil leaves on DM, NDF and ADF degradability (%)

Item	Control	Marjoram leaves					Basil leaves					*SEM*	*P value*
		G1	G2	G3	G4	G5	G6	G7	G8	G9	G10		
DMD	37.6	38.9	41.6	39.3	39.5	39.2	38.2	40.1	41.9	39.8	39	0.355	0.2809
NDFD	36.1	33.7	34.8	33.8	34.8	35.9	33.7	34.3	33.4	33	33.4	0.55	0.9874
ADFD	31.7	25.8	32.7	27.7	29.5	32.6	29	28.7	27	30.3	26.6	0.634	0.2089

Control: 50% CFM, 50% alfalfa hay,G1: control diet +5 g marjoram leaves / kg DM, G2: control diet + 10 g marjoram leaves / kg DM, G3: control diet + 15 g marjoram leaves / kg DM,G4: control diet + 20 g marjoram leaves / kg DM, G5: control diet + 25 g marjoram leaves / kg DM, G6: control diet + 5 g basil leaves / kg DM, G7: control diet + 10 g basil leaves / kg DM, G8: control diet + 15 g basil leaves / kg DM, G9: control diet + 20 g basil leaves / kg DM, G10: control diet + 25 g basil leaves / kg DM.
DMD; dry matter degradability, NDFD; neutral detergent fibre degradability, ADFD; acid detergent fibre degradability
SEM: standard error of the means.

(*p>0.05*) variances in cellwall contents (NDF and ADF) degradability by marjoram or basil oils supplementations *in-vitro*. Also, Khattab *et al.* [12] found the similar results when they used thyme and celery in *in-vitro* study. No negative impact of growth and activity of the major cellulolytic bacterial population and NDF, and ADF degradability by

0.005, 0.05, and 0.5 g/L of eugenol supplementation in a continuous-culture fermenter [44].

4. Conclusion

Based on the condition of the present *in-vitro* study. It could be concluded that the addition of marjoram or basil leaves to diets had a positive impact on total gas production without adverse effect on dry matter, and cell wall contents (NDF and ADF) degradability.

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6. Conflicts of interest

No potential conflict of interest was reported by the authors

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