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Research Article
Effect of Caraway, Fennel and Melissa addition on *in vitro* Rumen Fermentation and Gas Production

[1]H.A.F. Rahmy, [1]H.M. El Bana, [2]N.E. El-Bordeny, [1]Adel E.M. Mahmoud and [1]Wafaa M.A. Ghoneem

[1]Department of Animal Production, Faculty of Agriculture, Cairo University, Egypt
[2]Department of Animal Production, Faculty of Agriculture, Ain Shams University, Egypt

Abstract

Background and Objective: Medicinal herbs and aromatic plants could be used to manipulate rumen fermentation. This study aimed to evaluate the effect of adding herbal and aromatic plants at 1, 3, 5, 7% of total ratio DM supplementation of the incubation media of an *in vitro* rumen model. **Materials and Methods:** About 400±4 mg of feed sample (roughage and concentrate ratio of 45:55%) with each level, weighted into 125 mL glass bottles (6 bottles for each treatment), rumen fluid injected into these bottles and incubated at 39°C, after 24 h incubation digestibility of dry matter (IVDMD) and organic matter digestibility (IVOMD), total gas production (TG) and metabolic energy (ME) were studied. **Results:** The differences among plants, added at different levels, were significant. Significant differences were also observed between highest level added compared with control in ammonia (NH_3) and volatile fatty acids (VFA), IVDMD and IVOMD compared with control. Total gas (TG) was significantly higher at level 7%, especially with added Melissa compared with other plants. Metabolic energy (ME), was significantly higher in all treatments compared with control. **Conclusion:** It may be concluded that addition of different medicinal and aromatic herbal plants Caraway (*Carum carvi*), fennel (*Foeniculum vulgare*) and Melissa (*Melissa officinali*)), especially at highest levels tested has a great potential in manipulating rumen fermentation, which may be of benefit when applied in ruminant nutrition.

Key words: Caraway, fennel, Melissa, *in vitro*, digestibility, medicinal herbs and aromatic plants

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Corresponding Author: Adel E.M. Mahmoud, Department of Animal Production, Faculty of Agriculture, Cairo University, Egypt Tel: +2 01012592006

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

For decades, one of the goals of ruminant microbiologists and nutritionists has been to manipulate the ruminal microbial ecosystem to improve the efficiency of converting feeds to animal products edible to humans. As well as reducing methane (CH_4) emissions from domestic ruminants which contribute 16-25% of the global greenhouse gases (GHG) and about 33% to global anthropogenic CH_4 emissions. About 2-15% of ingested energy lost from CH_4emissions different according to level of feed intake and diet composition[1-4]. So, reducing this emission from ruminants has a benefit in nutrition and environment. Several studies in dietary strategies have been suggested to mitigate CH_4 emission from ruminants[5-7].

Ionophore has proved to be a useful tool in reducing energy as methane and nitrogen, ammonia losses[8].

There are also many ways to reduce this emission by using essential oils (plant extracts, phyto-factors, volatile or ethereal oils) appears to be one of the most natural new generation use in animal nutrition. The possibility of using biologically active plant compounds for modulating changes in the rumen was first reported in 1911. In response to the requirements of animal production, the animal feed industry has marketed animal feed additives containing mixtures of secondary plant metabolites

Herbs and spices have been introduced also to ruminant nutrition. Microbial ecosystem in the rumen is composed from complex anaerobic microbial population of bacteria, fungi, protozoa, *Methanogeneous* species and bacterifagi. Numerous metabolites produced in rumen during microbial fermentation affect the basic digestive and metabolic functions and productivity of the host. Researchers have been searching for new possibilities to modulate microbial fermentation in the rumen. The main goal of manipulating the rumen fermentation is to increase the effectiveness of digestion and metabolism of nutrients, to increase the productivity of the animals and to suppress the undesirable processes as methanogenesis. Some of numerous studies have evaluated using herbal and aromatic plants, including essential oils (EO) which used in reducing methane. EO can directly inhibit methanogenic archaea and/or indirectly decrease methane production by directly depressing some microbial metabolic processes contributing to methanogenesis[9].

The objective of this study aimed to investigate that the optimum level of incorporation of these plants *Carumcarvi* (Caraway), *Foeniculum vulgare* (fennel) and *Melissa officinali* (Melissa), as feed additive in complete feed by *in vitro* technique and their efficacy in improving the utilization of nutrient and improving rumen environment.

MATERIALS AND METHODS

Three species of aromatic herbs were used in this study (Caraway, fennel and Melissa). The percentages of EO were determined in the collected plant parts (Table 1), using 100 g samples for each cut per plant. Distillation of the EO were applied as described by Miller [10].

Experimental procedures: Two days before beginning of the experiment, 400±4 mg of feed sample for each level (contained clover hay as a roughage and concentrate ratio of 45:55%) was weighed into 125 mL glass bottles(6 bottles for each treatment). These bottles had a total volume of 125±2 mL. A buffer solution was prepared before addition of rumen fluid as described by McDougall[11] and flushed continuously with CO_2 at 39°C during sample inoculation. Rumen fluid was obtained from slaughter house and it was collected from cows. The collected rumen fluid was mixed into a bottle (1 L) with an O_2-free headspace and immediately transported to laboratory at 39°C. Upon arrival at the laboratory, the rumen fluid was filtered through four layers of cheesecloth to eliminate large feed particles. The buffer solution was added to rumen fluid at ratio 4:1. forty mL of this inoculum was added to each bottle, then the headspace of

Table 1: Percentage of Volatile oil in plant

Caraway (Oil, 054%)		Fennel (Oil, 0.67%)		Melissa (Oil, 0.47%)	
Essential oil	%	Essential oil	%	Essential oil	%
Limonene	24.9	α-pinene	0.31	b-pinene	0.951
Trance di-hydrocarvone	0.205	Myrcene	0.55	Myrcene	3.99
Trance Carveol	0.472	Limonene	6.14	Linalool	0.546
Carvone	74.17	1,8 Cineole	2.55	Neral (citral B)	32.02
B-Caryophyllene	0.166	Methylchavicol	88.9	Geranial (citral A)	53.74
		Anethole	1.42	Geranylacetate	3.638
				B-caryophyllene	2.383

each bottle was flushed with CO_2 and closed. The initial pH of the inoculums was from 6.8-6.9. Triplicates of each sample were used in two separate runs.

Chemical analyses of feeds and *in vitro* residuals: Dry matter (DM) was determined by drying samples at 105°C for 24 h and ash content, by combusting dried samples in a muffle furnace at 550°C for 8 h. Nitrogen (N) content was estimated using the Kjeldahl method[12]. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) determinations were based on the method using an ANKOM fiber analyzer[13]. Feed was analyzed for proximate analyses According to AOAC[14] and nitrogen free extract was calculated by difference. Non fiber carbohydrate (NFC) was calculated according to the following formula[15]:

$$NFC\ (\%) = 100-(ND\ (\%)+CP\ (\%)+Fat\ (\%)+Ash\ (\%))$$

Formulation and chemical analysis of basil ration presented in Table 2.

Degradability measurements: Dry matter degradability (% dDM) was calculated as the (difference between the sample DM content and that in the residual after 48 h incubation/sample DM content×100). NDF and ADF of the residuals after fermentation were analyzed with the same methods used for feed ingredient analysis. Degradability of NDF and ADF were calculated as difference between the content in the sample before and after incubation/content in the sample before incubation×100.

Gas production estimation: After 24 h of samples incubation, the total gas production was estimated by the displacement of syringe piston, which was connected to the serum flasks. The gas produced due to fermentation of substrate was calculated by subtracting gas produced in blank vessels (without substrate) from total gas produced in the vessels containing buffered rumen fluid and substrate. Where GP is net GP in mL from 200 mg of dry sample after 24 h of incubation, 2.2 mg mL^{-1} is a stoichiometric factor that expresses mg of C, H and O required for the short chain fatty acids (SCFA) gas associated with production of 1 mL of gas.

Rumen pH, ammonia and total volatile fatty acids: After 24 h of incubation, the filtrated rumen liquor for each sample was subjected for further investigation. The pH of rumen fluid was measured (pH meter) and quantitative analysis of ammonia concentration was carried out by Nessler method modified by Szumacher-Strabel and Cieslak[16]. Total volatile fatty acids (TVFA's)[1].

Calculations and statistical analysis: Metabolizable energy (ME, Mcal/kg DM), *in vitro* organic matter digestibility (OMD, g kg^{-1} OM) were estimated according to Menke and Steingass[17], Short Chain Fatty Acid (SCFA) concentrations were calculated according to Getachew *et al.*[18]:

$$ME\ (MJ/kg\ DM) = 2.20 + 0.136\ GP + 0.057\ CP\ (\%)$$

$$OMD = 14.88 + 0.889\ GP + 4.5\ CP\ (\%) + 0.0651\ ash\ (\%)$$

$$SCFA\ (mmol/200\ mg\ DM) = -0.00425 + 0.0222 \times GPMCP\ (mg\ g^{-1}\ DM)$$
$$= mg\ dDM - GP \times 2.2$$

Data were analyzed using the general liner model procedure[19]. The data of DM, OM, NDF, ADF degradability as well as gas production, metabolic energy and short chain fatty acids. Where analyze according to the following model:

$$Yij = \mu + Gij + Eij$$

where, μ is the overall mean of Yij is Gij is the group effect, Eij is the experimental random error. The differences among means were separate according to Duncan's New Multiple Range test[20].

Table 2: Formulation and chemical analysis of basil ration

Ration formulation		Chemical analysis	
Ingredientes	%	Items	%
Alfalfa hay	45	Dry matter (DM)	86.57
Yellow corn	20	Organic matter (OM)	89.16
Soya bean meal	15	Ash	5.45
Wheat bran	5	Crude protein (CP)	17.96
Cotton seed meal	10	Ether extract (CP)	2.85
Common salts	1	Crude fiber (CF)	22.57
Limestone	22.7	Neutral detergent fiber (NDF)	28.49
*Minerals and vitamins mix	0.3	Acid detergent fiber (ADF)	17.24

*Each 3 kg contained 7000000 IU vit. A,2000000 IU vit. D3, 25000 mg vit. E, 40 g zinc, 40 g manganese, 50 g iron, 15 g copper, 8 g iodine, 4 g cobalt, 3 g selenium and carrier $CaCo_3$ up to 3 kg

Table 3: Rumen liquor parameters, short chain fatty acids and metabolizable energy

Items	Level	pH	NH_3 (mg dL^{-1})	VFA (mequ mL^{-1})	ME/MJ	SCFA (mmol g^{-1} DM)
Control	0	7.30^{a}	13.89^{a}	6.35^{c}	15.10^{f}	0.45^{f}
T1	1%	6.92^{b}	9.79^{c}	8.51^{ab}	16.82^{de}	0.72^{de}
	3%	6.95^{b}	10.06^{b}	8.53^{ab}	17.08^{bcd}	0.76^{bcd}
	5%	6.92^{b}	9.36^{e}	7.43^{bc}	16.73^{e}	0.70^{e}
	7%	6.95^{b}	10.15^{b}	8.58^{ab}	16.76^{e}	0.71^{e}
T2	1%	6.94^{b}	9.16^{f}	8.00^{ab}	16.89^{de}	0.73^{de}
	3%	6.94^{b}	10.11^{b}	9.16^{a}	17.11^{bcd}	0.76^{bcd}
	5%	6.94^{b}	7.78^{i}	8.73^{ab}	16.95^{cde}	0.74^{cde}
	7%	6.92^{b}	9.22^{ef}	8.98^{a}	17.23^{abc}	0.78^{abc}
T3	1%	6.93^{b}	8.14^{h}	8.76^{ab}	17.11^{bcd}	0.76^{bcd}
	3%	6.92^{b}	9.42^{d}	9.35^{a}	17.43^{a}	0.80^{ab}
	5%	6.94^{b}	8.35^{g}	8.48^{ab}	17.43^{a}	0.81^{a}
	7%	6.92^{b}	7.69^{i}	8.96^{a}	17.31^{ab}	0.80^{ab}
SEM		0.18	0.24	0.16	0.54	0.02

C: Control; T1: Caraway; T2: Fennel; T3: Melissa; NH_3: Ammonia; VFA: Volatile fatty acids; ME/MJ: Metabolic energy per mega joule, SCFA: Short chain fatty acids and SEM: Standard error mean

RESULTS

Ruminal liquor parameters pH, ammonia (NH_3), volatile fatty acids (VFA) and short chain fatty acid (SCFA) were in comparable together. Whereas, media pH decreases significantly, volatile fatty acids and short chain fatty acids increase, in contrast ammonia concentration decrease, when treatments compared with control without addition of herbs. showed significant differences between treatments compared to control one (Table 3). Control one recorded higher values with pH in (7.30), ammonia (13.89 mg dL^{-1}) and lower values with VFA (6.35 mequ mL^{-1}) and SCFA (0.45 mmol/g DM). The highest level in VFA was found with Melissa (T3) in level 3%, it was 9.35 mg mL^{-1}. Also, Melissa appeared significant effect in short chain fatty acid (0.81 mmol/g DM) with all its levels comparing with other treatments. Metabolic energy among different treatment are showed in Table 3 fluctuated between 15.10 MJ to 17.43 MJ were Melissa showed the highest significant different compared with control.

Data in Table 4 cleared that dry matter digestibility was affected by three plants addition without significant differences among it comparing with control (43.70%). numerically Caraway in level 1% recorded higher value (57.00%) in compare with other levels in different treatments. The same trend was observed with *in vitro* organic matter digestibility (OM), neutral detergent fiber (NDF) and acid detergent fiber (ADF) where control recorded lower significant value being 41.30, 9.52 and 7.90% compared with other treatments.

Gas production Table 5 showed highly significant difference among Melissa treatment especially at level 7% compared with control group. Gas production per dry matter (GP/DM) and gas production per neutral detergent fiber (GP/NDF) showed highest values in Melissa at 7% compared

Table 4: *In vitro* digestibility

		Digestibility (%)			
Items	Levels	DM	OM	NDF	ADF
Control	0	43.70^{b}	41.30^{f}	9.52^{c}	7.90^{c}
T1	1%	57.00^{a}	52.00^{de}	28.64^{ab}	23.19^{ab}
	3%	55.50^{a}	53.70^{bcd}	24.46^{ab}	18.05^{abc}
	5%	55.20^{a}	51.30^{e}	25.56^{ab}	19.15^{abc}
	7%	54.20^{a}	51.50^{e}	23.24^{ab}	20.91^{ab}
T2	1%	54.80^{a}	52.40^{de}	24.75^{ab}	17.55^{abc}
	3%	55.60^{a}	53.80^{bcd}	27.46^{ab}	20.77^{ab}
	5%	54.10^{a}	52.80^{cde}	23.77^{ab}	17.06^{abc}
	7%	56.10^{a}	54.60^{abc}	29.62^{ab}	26.35^{a}
T3	1%	55.40^{a}	53.80^{bcd}	21.40^{ab}	13.51^{bc}
	3%	55.20^{a}	55.40^{ab}	19.50^{ab}	13.38^{bc}
	5%	54.60^{a}	55.90^{a}	23.40^{ab}	16.62^{abc}
	7%	54.70^{a}	55.20^{ab}	20.37^{ab}	13.00^{bc}
SEM		0.50	0.50	0.51	0.50

C: Control; T1: Caraway; T2: Fennel; T3: Melissa; ADF: Acid detergent fiber; NDF: Neutral detergent fiber; DOM: Digestible organic matter; DMD: Dry matter disappearance and SEM: Standard error mean

Table 5: *In vitro* gas production

		Gas production (mL g^{-1})			
Items	Levels	GP/DM	GP/OM	GP/NDF	GP/ADF
Control	0	101.1^{f}	98.2^{f}	295.9^{f}	491.9^{f}
T1	1%	161.3^{de}	156.6^{de}	472.1^{de}	784.7^{de}
	3%	170.8^{bcd}	165.9^{bcd}	500.09^{bcd}	831.2^{bcd}
	5%	157.9^{e}	153.3^{e}	462.1^{e}	768.1^{e}
	7%	158.9^{e}	154.36^{e}	465.2^{e}	773.2^{e}
T2	1%	163.7^{de}	159.03^{de}	479.3^{ed}	796.7^{de}
	3%	171.8^{bcd}	166.8^{bcd}	502.7^{bcd}	835.7^{bcd}
	5%	166.2^{cde}	161.3^{cde}	486.3^{cde}	808.4^{cde}
	7%	176.32^{abc}	171.2^{abc}	515.9^{abc}	857.6^{abc}
T3	1%	171.98^{bcd}	166.9^{bcd}	503.2^{bcd}	836.5^{bcd}
	3%	180.5^{ab}	175.3^{ab}	528.4^{ab}	878.3^{ab}
	5%	183.4^{a}	178.1^{a}	536.9^{a}	892.5^{a}
	7%	179.3^{ab}	174.1^{ab}	524.8^{ab}	872.4^{ab}
SEM		0.25	0.31	0.58	0.29

C: Control; T1: Caraway; T2: Fennel; T3: Melissa; GP/DM: Gas production per dry matter; GP/OM: Total Gas production per Organic matter; GP/NDF: Gas production per Neutral detergent fiber; GP/ADF: Gas production per Acid detergent fiber and SEM: Standard error mean

with other group, in contrast, the lowest value of 101.1, 295.9 mL g^{-1} in GP/DM and GP/NDF was noted in the control group.

DISCUSSION

The chemical composition is the first step to evaluate the nutritive value for any feedstuff. In the same time, ration has an important impact on ruminal fermentation reflected on all rumen parameters specially gas production[21]. So, *in vitro* digestibility, which represents the second evaluation parameter of any feed. Also, gas production is generally a good indicator of digestibility, fermentation and rumen microbial protein production[22].

The addition, of the plants used in this study was expected to be beneficial to rumen function, by increasing degradability of crude protein and increase microbial protein production. In general, rumen microbial activity has been shown to be affected using plant extracts and secondary plant metabolites that may provide an alternative to ruminal modifiers for their ability to improve energy or protein use in the rumen[3]. Patra *et al.*[23] found that *in vitro* GP increased significantly with plant extracts supplementation. The increase in gas production might be partly due to the addition of soluble sugars in the reaction mixture through inclusion of the extracts.

Phenolic acids such as p-coumaric, ferulic, cinnamic and phloretic acids and some monomeric phenolic have been found to decrease production of methane, acetate and propionate production[24]. The effects of different levels of added plant, dry and organic matter degradability, NH_3-N concentration and VFA count are presented in Table 2 and 3. Feed supplements with growth promoting activity increase stability of feed and beneficially influence the gastrointestinal ecosystem mostly through growth inhibition of pathogenic microorganism's growth. Due to improved health status of digestive system, animals are less exposed to the toxins of microbiological origin. Consequently, herbs and spices help to increase the resistance of the animals exposed to different stress situations and increase the absorption of essential nutrients, thus improving the growth of the animals[25].

Numerous secondary metabolites formed by plants serve as defense agents against physiological and environmental stressors, predators and pathogenic microorganisms. Several *in vitro* studies showed strong antimicrobial activity of certain plant extracts against Gram- and Gram+ bacteria, Di Pasqua *et al.*[26] found a change in long chain fatty acid profile in the membranes of *E. coli* grown in the presence of limonene or cinnamaldehyde. Similar observations were reported for *Salomonella enterice* grown in the presence of carvacrol or eugenol and with *Bronchotrix thermosphacta* grown in the presence of either limonene, cinnamaldehyde, carvacrol or eugenol. In the case of Pseudomonas fluorescens and *Staphylococcus aureus*, none of the tested phytochemicals changed the fatty acid profile in the media. The changes in fatty acid composition can affect survival of microorganisms.

Variation in the results of *in vitro* digestibility can be due to several factors such as processing of sample, difference in chemical composition and component of each plant, preparation of buffer solution or handling of equipment[27]. IVDMD showed a wide range of differences among the four levels in each plant (Table 3). The IVDMD is strongly influenced by the amount of fiber represented by the NDF, ADF and cellulose levels in the plant tissues[28].

CONCLUSION AND FUTURE RECOMMENDATION

The main goal of animal production is to ensure high productivity from healthy animals and quality of animal products, which are stable and appropriate for further processing for human consumption. In that respect, herbs and spices are not just appetite and digestion stimulants, but they also impact other physiological functions, help to sustain good health and welfare of the animals and improve their over all performance. Current studies show promising results regarding the use of phytochemicals as growth and production promoters. There is still a need for further research to clarify the phytochemical composition and the mechanisms of action for many herbs, spices and their extract. Furthermore, studied designed to assess the appropriate doses that are both safe and effective, when used under defined conditions for specific animal species, will greatly enhance their nutritional application in commercial animal production.

SIGNIFICANCE STATEMENT

This study confirmed that the supplementation of different level of Caraway, Fennel and Melissa has a potential effect on rumen fermentation. As well as this study contributes to the effective monitoring of reducing methane (CH_4) emissions from domestic ruminants.

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