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The effect of ajowan (*Carum copticum* L.) essential oils on eukaryotic ruminal microorganisms of Mehraban sheep

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ABSTRACT

Background and Objectives: Essential oils may improve the utilization of nutrients by ruminal microorganisms. The aim of this study was to assess the effect of different doses of ajowan essential oils (AEO) on growth and fibrolytic activity of anaerobic fungi, and generic distribution of ciliated protozoa (*in vitro*).
Material and Methods: Different doses of AEO (0, 150, 300, 450 and 600 ppm) were added to experimental tubes. The effect of AEO was evaluated on growth and fibrolytic enzyme activity of an anaerobic fungus (*Neocalimastix spp.*). Generic distribution of ciliated protozoa were evaluated in response to different doses of AEO.
Results and Conclusion: The growth of fungus (*Neocalimastix* spp.) were inhibited and activity of fibrolytic enzymes of fungus were reduced by adding AEO. Also, an inhibitory effect was seen in concentration of ciliated protozoa and some genus were completely disappeared at the doses of 300 ppm and higher. The doses used in this study reduced the fibrolytic activity of the studied rumen microorganisms which is undesirable in practical animal nutrition. Further research is needed to assess the effects of AEO at lower doses on these parameters and also proteolysis and methanogenesis.

Keywords: Rumen, *Carum copticum*, anaerobic fungi, ciliated protozoa, ajowan

INTRODUCTION

Using antibiotics (i.e., ionophores) as feed additives are banned in some countries due to emergence of antibiotic resistance strains of bacteria. An alternative to ionophores is secondary plant metabolites such as essential oils which their effects have been extensively studied on rumen fermentation (1). Since the digestion of dietary organic matter in rumen occurs as synergic cooperation of all microorganisms, studying the responses of other microorganisms along with bacteria would be beneficial in terms of interpreting the EO effects on ruminal fermentation. The result of a study showed that the growth of *Neocalimastics frontalis* RE1was inhibited by adding a commercial blend of EO to the culture media (2). In another study protozoa number decreased after inclusion of *Zataria multiflora* EO (3). As the activity and metabolism of microorganisms varies in species of ruminants and different geographical location (4), then obtained results in published studies cannot be generalized to all microorganisms in ruminant species from different areas of world.

Ajowan (*Carum copticum* L.) essential oils (AEO) has antimicrobial activity against a broad spectrum of bacteria (5). Therefore, the aim of this study was to assess the effect of AEO on the growth and activity of some fibrolytic enzymes isolated anaerobic ruminal fungi, total number and generic distribution of ciliated protozoa.

MATERIAL AND METHODS

Essential oils. The AEO were obtained from horticultural science department of Shiraz University,

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and according to their laboratory reports thymol (50.07%), P-cymen (22.9%) and γ-terpinen (23.94%) were the main constituents of this oil. The experimental doses (0, 150, 300, 400 or 600 ppm AEO) were made by adding appropriate working solutions to the culture media (1% w/v).

Growth and fibrolytic activity of fungi. The fungi used in this trial were *Neocalimastix* spp. isolated by Talebzadeh *et al*. (3). Growth and enzyme activity of isolated fungi were studied as described by Wang *et al*. (6) with some modifications.

Avicelase (exo-β-1,4-glucanase) activity of the extracellular fluid and crude cell extract was determined with avicel (7). Extracellular fluid or crude cell extract (1 ml) was mixed with 1 ml of substrate solution containing 10 g/l avicel in 0.1 M sodium phosphate buffer (pH 6.8). After incubation of the mixtures at 39°C for 1 h, reducing sugars in the supernatants were assayed colorimetrically using the dinitrosalicylic acid method (7). The β-glucosidase activity in extracellular fluid and crude cell extract was determined using *p*-nitrophenyl-β-D-glucopyranoside as substrate and measuring the amount of *p*-nitrophenol released during incubation of substrate with the enzyme.

Ciliated protozoa. Two hours after morning feeding rumen fluid was obtained before morning feeding, from 3 ruminally fistulated Mehraban sheep. The incubation condition was the same as described by Talebzadeh *et al* (3). The substrate of incubation was composed of 61% barley grain, 17% dried alfalfa, 12% wheat straw, 9% soybean meal and 1% commercial vitamin-mineral premix. After 24 hours of incubation whole bottle contents were preserved by diluting with an equal volume of formalin solution (185 ml formaldehyde/l distilled water). Total numbers and generic composition of ciliate protozoa were determined according to the procedures described by Dehority (8).

Statistical analysis. Data were analyzed using the GLM procedure of SAS 8.1. For all analyses, specific orthogonal contrasts were used to test (1) control *vs*. the average of AEO doses and (2) linear (L), quadratic (Q) and cubic (C) effects of AEO doses on parameters with contrast coefficients generated using the Proc IML function of SAS. For protozoal count data, normality assumptions of residuals were tested using Proc Univariate (SAS 8.1) with the Kolmogorov–Smirnov test. Normality of the residuals allows statistical analysis without transformation of the data.

RESULTS

Inclusion of AEO to fungal culture media completely inhibited filter paper digestion (Fig. 1). Adding AEO reduced gas production by *Neocalimastix* spp. after seven days of incubation from 20.7 ml to 4.3, 4.9, 4.2 and 3.8 ml for control, 150, 300, 450 and 600 ppm, respectively. The effect of AEO on extracellular avicelase activity was in a dose dependent manner, and at the doses of 600 ppm minimum activity was observed (Table 1). The activity of this enzyme was also declined in cell debris by adding AEO. The extracellular β-glucosidase activity was lower than cell associated activity and less affected by inclusion of AEO (Table 1). During this experiment five groups of ciliated protozoa were observed (i.e., *Entodinium* spp., *Epidinium caudatum, Epidinium ecaudatum, Isotricha* ssp. and *Dasytricha* spp.). The results showed that AEO had a strong antiprotozoal activity (Table 2).

Table 1. Effects of different doses of AEO on the activity of fibrolytic enzymes produced by *Neocalimastix* ssp.

	Doses of AEO (µg/ml)						Contrasts		
Enzyme activity	0	150	300	450	600	SEM	Control *vs*. AEO	L	Q
Avicelase (exo- β-1,4-glucanase)									
Extracellular fluid	57.4	50.83	44.17	37.5	10.83	3.87	***	***	*
Cell associated	23.1	23.1	21.9	25.9	22.3	1.13	NS	NS	NS
β-1,4-glucosidase									
Extracellular fluid	2.458	2.498	2.493	2.724	5.67	0.1	***	***	***
Cell associated	800.2	786.2	750.5	686.1	677.1	13.03	**	***	NS

GP_{24}: volume of gas after 24 hours of incubation; TVFA: total volatile fatty acids; PF: partitioning factor; MB: microbial biomass; IVTOMD: true in *vitro* organic matter digestibility; L: linear; Q: quadratic; C: cubic; * P < 0.05;** P < 0.01 *** P < 0.001; NS: non-significant; SEM: Standard error of the means.

Table 2. Effects of different doses of AEO on the ruminal protozoa concentration (×10^4/ml).

	Doses of AEO (µg/ml)						Contrasts		
Genus	0	150	300	450	600	SEM	Control *vs.* AEO	L	Q
Entodinium spp	11.5	7.1	0.62	0.79	1.3	0.479	***	***	***
Epidinium caudatum	2.4	2.04	0.68	1.1	0.8	0.373	***	**	NS
Epidinium ecaudatum	0.65	1.04	0.11	0.074	0.099	0.141	***	***	NS
Isotricha spp	0.13	0.037	0	0	0	0.011	***	***	NS
Dasytricha spp	0.67	0.6	0	0	0	0.064	***	***	*
Total	15.35	10.72	1.41	1.96	2.19	0.512	***	***	***

L: linear; Q: quadratic; C: cubic; *: P < 0.05; ** P < 0.01; *** P < 0.001; NS: non-significant; SEM: Standard error of the means.

With increasing the doses of AEO, the concentrations of *Entodinium* spp., *Epidinium caudatum* and *Epidinium ecaudatum* were singnificantly decreased. At the doses higher than 150 ppm, *Isotricha* ssp. and *Dasytricha* spp. were completely disappeared.

DISCUSSION

After 24 hours of incubation, AEO supplementation decreased the total concentration of protozoa by more than 85 percent. The concentration of *Entodinium* spp, *Epidinium caudatum* and *Epidinium ecaudatum* were significantly affected by AEO supplementation. Similar to the results of this study, Talebzadeh *et al*. (3) found that different doses of *Z. Multiflora* EO decreased the total protozoal counts and generic distribution. The antiprotozal effect of AEO is most likely due to the phenolic structure of its main active compounds, namely thymol. Such a structure can lead to disruption of cell membrane, inactivation of enzymes and deprivation of substrates and metal ions which are essential for cell metabolism (9). A strong relationship between some genera of protozoa and methane production has been cited previously (10).

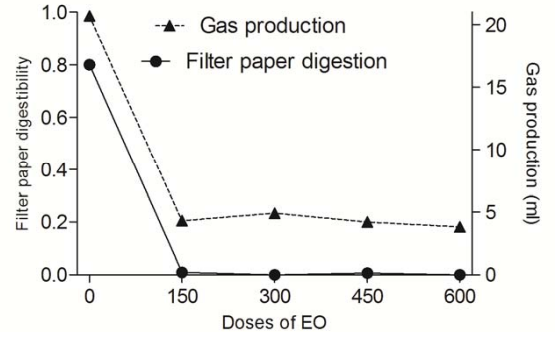

Fig. 1. The effect of different doses of AEO on gas production and filter paper digestion by isolated anaerobic fungus from Mehraban sheep.

They showed a high relationship between *Entodinium caudatum* and methane production in the rumen. We did not measure the methane production in this trial. However, defaunation property of AEO can lower methane production in the rumen.

The results of this study showed that AEO has an antimicrobial effect against rumen microorganisms. The results of tested *Neocalimastix* spp. showed a fungistatic activity. Although the effect of AEO on protozoa is undesirable in terms of fibre digestibility, its possible effects on methane production (by archaea live symbiotically on the surface of protozoa body) would be beneficial (9).

Successful application of EO in manipulation of rumen fermentation depends on decreasing nutrient loss of nutrients during inefficient routs of metabolism without adverse effects on feed digestibility. The doses used in this study had an inhibitory effect on rumen eukaryotic microorganisms. If these findings were expressed *in vivo,* AEO may not be beneficial for use in ruminant nutrition to improve efficiency of feed utilization. Further research is needed to assess the influence of lower doses of AEO on other parameters of rumen fermentation such as methane, ammonia and volatile fatty acids.

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