



## Effect of Oregano aromatic water supplementation to rumen fluid on *in vitro* digestion and rumen parameters of starter in calves

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### ABSTRACT

Restriction on the use of antibiotics in animal nutrition has led researchers and consumers to use alternative antimicrobial agents. Aromatic plants and their essential oils are one of the alternatives. In the literature, there are studies on the effects of essential oils on *in vitro* digestion and rumen parameters; however, no studies on the effects of aromatic water, a by-product, have been found. Therefore, this study aims to determine the effects of Oregano aromatic water (OAW) supplemented in 0, 40, 60 and 80 ml/L on *in vitro* digestion and rumen parameters of starter in calves (n=6 for each doses). The rumen fluid used in the study was obtained from calves of 60 d-age. Total gas and methane gas production, metabolic energy and organic matter digestion were determined by the *in vitro* gas production technique and the effects on rumen essential oils were observed. The effect of supplemented OAW doses on *in vitro* gas production, methane production, organic matter digestion, and metabolic energy level was not found to be significant. However, total volatile acids (VFA), acetate (AA), propionate (PA) and butyrate (BA) values and proportions were affected by OAW doses. The findings of the study showed that supplementation of OAW might be useful in modifying rumen fermentation in ruminants.

**Keywords:** Calves, *In vitro* digestion, Oregano aromatic water, Rumen fluid, Rumen parameters

Medicinal aromatic plants have been used in the treatment of various diseases in animals and humans for many years. Medicinal aromatic plants include antimicrobial, anti-inflammatory and antioxidant effect, immune system stimulant, and appetizing and digestion stimulating factor (Patra 2011, Bodas *et al.* 2012). These plants contain secondary metabolites responsible for some effects which can control methanogenesis without affecting rumen microorganisms (Kamra *et al.* 2012). The studies have determined that methanogenesis which causes economic losses in ruminants can be prevented by using medicinal aromatic plants leaves and oils as feed additives (Baytok *et al.* 2013). Essential oils obtained from *Lippia turbinata* and *Tagetes minuta* plants did not affect *in vitro* methane gas production, total VFA concentration, and composition, but decreased ammonia concentration (Garcia *et al.* 2018). Pour *et al.* (2017) stated that thyme (*Thymus*) and *Mentha piperita* (Carvacrol) essential oils could reduce *in vitro* gas production and affect the energy and protein metabolic pathways. Therefore, researchers suggested incorporation of these essential oils to manipulate rumen fermentation. Boga and Kilic (2017) investigated the effects of the addition of different essential oils to different feedstuff and reported that oleaster, coriander and grape seed essential oils reduced *in vitro* gas production and

increased propionate, whereas mint and orange peel essential oils increased gas production and acetate while decreasing propionate. Joch *et al.* (2018) observed that the secondary metabolites of seven pure plants were capable of altering rumen fermentation and reduced methane production. In their study, Tekeli *et al.* (2017) indicated that different doses of *T. vulgaris*, *O. vulgare*, *S. aromaticum*, and *Z. officinale* essential oils decreased the production of methane and that the essential oils may be useful in ruminant nutrition.

No study could be found in the literature investigating the effects of the OAW supplementation to rumen fluid on *in vitro* digestion and rumen fermentation. Thus, the purpose of the present study was to examine the effect of oregano aromatic water supplementation to rumen fluid on *in vitro* digestion of starter feed and rumen fermentation in calves.

### MATERIALS AND METHODS

Suleyman Demirel University Animal Experiments Local Ethics Committee has approved the protocol number 2013–03.

*Chemical analysis of concentrated mix feed.* The starter was milled through a 1 mm sieve (IKA MF10.1, Germany) for use in chemical analysis and *in vitro* gas production. Dry matter (DM) (method 14.081), crude ash (method 942.05), crude protein (CP) (method 954.01), diethyl ether extract (EE) (method 920.39), and crude fibre (CF)

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(methods 7.066–7.070) compositions of the diet were analysed using the AOAC methods (AOAC, 1990). The neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents were analyzed by using a fibre analyzer (Velp FIWE3, Italy) according to the methods reported by Van Soest *et al.* (1991). The NDF was determined using sodium sulfite and thermostable  $\alpha$ -amylase (Megazyme, Ireland). Neither NDF nor ADF was inclusive of residual ash. Non-fibrous carbohydrate (NFC) levels were calculated using the following formula (NRC, 2001):

$$\text{NFC \%} = 100 - (\text{NDF \%} + \text{CP \%} + \text{EE \%} + \text{Ash \%}).$$

The chemical composition of the starter is presented in Table 1.

Table 1. The chemical composition of the starter

Ingredient	%
CP	19.67
NFC	52.42
NDF	18.84
ADF	7.87
CF	5.80
EE	3.11
Ash	5.96

*In vitro gas production technique.* The technique was performed using different doses of oregano aromatic water supplementation to calf starter. In the control group, there was no added oregano aromatic water; in the treatment groups, there was oregano aromatic water supplementation at 40, 60 and 80 mL/kg.

Rumen fluid, which is necessary for *in vitro* fermentation, was obtained from two Holstein calf fed with a starter. Rumen fluid was collected into a thermos with water at 39°C under CO<sub>2</sub> gas, and filtered with four layers of cheesecloth in the laboratory. The technique was carried out according to the procedures of Menke *et al.* (1979). The plant samples were incubated in rumen fluid and buffer mixture in 100 ml glass syringes (Model Fortuna, Germany). Dried samples (200±10 mg) and 30 ml of the rumen fluid + buffer mixture at a 1 : 2 (v/v) ratio were incubated into syringes as triplicate. Besides, three unused syringes (no template; rumen fluid + buffer mixture) were used to calculate the total gas production. The clips of the syringes were closed, the initial volume recorded and the syringes were incubated in a water bath at 39°C for up to 96 h.

*Determination of total gas and methane production.* In incubation, the total gas volume was recorded from the calibrated scale on the syringe for 24 h. After measuring the total gas volume at 24 h, the tubing of the plastic syringe outlet was inserted into the inlet of the methane analyzer (Sensor, Europe GmbH, Erkrath, Germany) and the piston was pushed to insert the accumulated gas into the analyzer. The methane as a per cent (%) of the total gas was displayed on a PC (Kara 2015, Kara *et al.* 2015).

*Estimation of metabolic energy (ME) and organic matter digestibility (OMD) levels.* The ME and OMD values of

feeds were calculated using the equations of Menke and Steingass (1987) for concentrate feed:

$$\text{ME (MJ/kg DM)} = 0.157 * \text{GP} + 0.0084 * \text{CP} + 0.022 * \text{EE} - 0.0081 * \text{CA} + 1.06$$

$$\text{OMD (\%)} = 0.9991 * \text{GP} + 0.0595 * \text{CP} + 0.0181 * \text{CA} + 9$$

where GP is 24-h net gas production (ml/200 mg DM), and CP, EE, CA are crude protein, ether extract, crude ash (g/kg DM), respectively.

*Rumen fluid characteristic.* The concentrations of rumen total VFA, acetic acid (AA), propionic acid (PA) and butyric acid (BA) were evaluated by GC (TRACE™ 1300 GC, Thermo Fisher Scientific, Waltham, MA, USA) equipped with flame ionization detector (Erwin *et al.* 1961). Through 4 layers of cheesecloth, the rumen fluid was centrifuged by mixing with 25% (w/v) metaphosphoric acid. The identification of components was made with the help of the retention time of the standard substances (Fluka-Sigma Aldrich, St. Louis, MO, USA). Data were evaluated with the calibur software program (Thermo Fisher Scientific, Waltham, MA, USA). The total VFA concentration was determined as mmol, and the acid rations in total VFA were calculated as the percentage.

## RESULTS AND DISCUSSION

The effect of oregano aromatic water supplementation on the rumen fluid obtained from calves (fed for 60 days) on the *in vitro* digestion was investigated by *in vitro* gas production technique. The results showed that oregano aromatic water used in 40, 60 and 80 ml/L rumen fluid dosage had no effect on *in vitro* gas production, *in vitro* methane production, organic matter digestion and *in vitro* metabolic energy level ( $P > 0.05$ ) (Table 2). However, it was found that the oregano aromatic water used in the starter at a dose of 40 ml/L increased *in vitro* gas production ( $P=0.133$ ), metabolic energy ( $P=0.134$ ) and organic matter digestion ( $P=0.133$ ) ( $P > 0.05$ ).

Molarities of total VFA, AA, PA, and BA in rumen fluids increased ( $P < 0.05$ ) in both doses (60 and 80 ml/L) OAW (Table 3). The individual ratio (%) of PA and BA also increased; however, ratio (%) AA decreased the dose of OAW.

Herbs and spices improve the effectiveness of digestion and the metabolism of nutrients, prevent energy loss through the undesired methanogenesis and provide an overall increase in animal productivity. The effect of herbs and spices varies depending on the dosage used. Low doses may have no effect, but high doses applied may be toxic to animals (Frankic *et al.* 2009). In our study, the gas production was not affected by supplementation of AOW. An *in vitro* study, Rezaei and Pour (2012), reported that the addition in the ratio of thyme reduced gas production. Benchaar *et al.* (2007) indicated that the dose of essential oils and essential oils components in the study did not have any beneficial effect. The supplementation of oregano oil reduced *in vitro* methane gas production, rumen VFA, ammonia, metabolic energy level and organic matter

Table 2. Effect of thyme supplementation to rumen fluid on *in vitro* digestion of starter concentrate feed in calves

	Thyme supplementation, ml/L rumen fluid				SEM	Contrasts		
	0	40	60	80		L	Q	C
Total Gas Production	51.87	53.91	51.76	52.08	0.46	0.712	0.362	0.133
ME	11.06	11.37	11.04	11.09	0.07	0.703	0.367	0.134
OMD	73.61	75.65	73.50	73.82	0.46	0.713	0.361	0.133
Methane production, %	12.15	11.75	11.60	12.10	0.14	0.822	0.157	0.764
Methane production, ml	6.30	6.33	6.00	6.30	0.07	0.617	0.381	0.157

SEM, Std. Error of means; L, Linear contrast; Q, Quadratic contrast; C, Cubic contrast. Total Gas Production, produced by 0.2 g DM at 24 h of incubation; ME, metabolic energy as MJ/kg DM; OMD, Organic matter digestibility as % DM; methane production (%), methane production (%) in total gas produced; methane production (ml), methane production ml/0.2 g DM.

Table 3. Effect of OAW on concentrations of organic acids in the calves rumen fluid

	Thyme supplementation, ml/L rumen fluid				P
	0	40	60	80	
<i>Molarities in rumen fluid, mmol/L</i>					
AA	43.13±0.57 <sup>b</sup>	43.55±0.26 <sup>b</sup>	45.59±0.29 <sup>a</sup>	45.55±0.14 <sup>a</sup>	0.00
PA	31.32±1.07 <sup>b</sup>	32.33±0.39 <sup>b</sup>	35.23±0.43 <sup>a</sup>	35.31±0.21 <sup>a</sup>	0.00
BA	11.09±0.40 <sup>b</sup>	11.61±0.19 <sup>b</sup>	12.94±0.21 <sup>a</sup>	12.98±0.08 <sup>a</sup>	0.00
VFA	85.54±2.04 <sup>b</sup>	87.49±0.83 <sup>b</sup>	93.77±0.92 <sup>a</sup>	93.84±0.43 <sup>a</sup>	0.00
<i>Individual ratios in total VFA, %</i>					
AA	50.47±0.56 <sup>a</sup>	49.78±0.18 <sup>a</sup>	48.63±0.18 <sup>b</sup>	48.55±0.07 <sup>b</sup>	0.00
PA	36.58±0.40 <sup>b</sup>	36.95±0.09 <sup>b</sup>	37.57±0.09 <sup>a</sup>	37.62±0.06 <sup>a</sup>	0.00
BA	12.95±0.17 <sup>b</sup>	13.27±0.09 <sup>b</sup>	13.80±0.09 <sup>a</sup>	13.83±0.02 <sup>a</sup>	0.00

AA, Acetic acid; PA, Propionic acid; BA, Butyric acid; VFA, Volatile fatty acid. a, b, Means in line with different lowercase letters differ significantly (P<0.05).

digestion, as reported by Canbolat *et al.* (2010). However, the supplementation of dried thyme pulp to silage did not affect organic matter digestion, metabolic energy level, and methane production, but increased AA and PA and in addition decreased ammonia and BA (Aksu *et al.* 2017). Our results showed that the supplementation of AOW did not affect methane gas production, metabolic energy and organic matter digestion. Different plants show different effects in rumen fermentation, while some plants reduce methane production; some plants have no affect (Boga and Kilic 2017, Wang *et al.* 2017). Essential oils obtained from *Lippia turbinata* and *Tagetes minuta* plants did not affect total VFA and methane gas production while they decreased ammonia concentration (Garcia *et al.* 2018). Tannins and saponins, which are secondary metabolites derived from plants by reducing the methane emission and increasing gas production, did not affect the organic digestion (Yulina *et al.* 2014). Pour *et al.* (2017) observed that the essential oils of thyme (*Thymus*) and *Mentha piperita* (Carvacrol) could reduce the *in vitro* gas production and that essential oils could be effective at different levels in the energy and protein metabolic pathways and could be useful in manipulating rumen fermentation. In a study of essential oils (EO) and components of these oils (EOC), Benchaar *et al.* (2007) observed that carvacrol, thymol, and eugenol affected rumen fermentation. They indicated that carvacrol

and eugenol increased butyrate ratio and decreased propionate ratio, at the same time thymol also decreased propionate ratio, and they observed a shift from propionate to butyrate in the total VFA components. They also suggested that the EO and EOC would not be useful for dairy cattle, and therefore should be used with caution in ruminant feeding. Plant extracts and secondary metabolites reduced total VFA, acetate, and propionate, while others increased propionate and butyrate. However, in our study, total VFA and its components increased with the supplementation of AOW.

Garlic oil and benzyl salicylate reduced acetate, increased propionate and butyrate, and inhibited methane production. The use of some plant extracts for dairy cows may not be useful, so it is important carefully to select the extracts and doses to be recommended as rumen modifiers (Bosquet *et al.* 2006). Joch *et al.* (2018) observed that the secondary metabolites of seven pure plants were capable of altering rumen fermentation and reduced methane production. Tekeli *et al.* (2017) indicated that different doses of *T. vulgaris*, *O. vulgare*, *S. aromaticum*, and *Z. officinale* essential oils decreased the production of methane and that the essential oils might be useful in ruminant nutrition. The study by Jahani-Azizabadi *et al.* (2011) on the effects of the application of 18 natural medicinal aromatic plant essential oil and garlic oil on the production of methane gas in semi-arid climate, observed that coriander, cinnamon, red basil, oregano, cumin, caraway and dill essential oils and garlic oils reduced the total methane gas production. In conclusion, our study revealed that supplemented oregano aromatic water doses are not effective in *in vitro* gas production, methane production, organic matter digestion and metabolic energy levels. However, oregano aromatic water doses have a positive impact on total VFA, acetate, propionate and butyrate levels. Therefore, supplementation of oregano aromatic water is suggested for modification of rumen fermentation. Further research is needed to determine the optimal dose for livestock.

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