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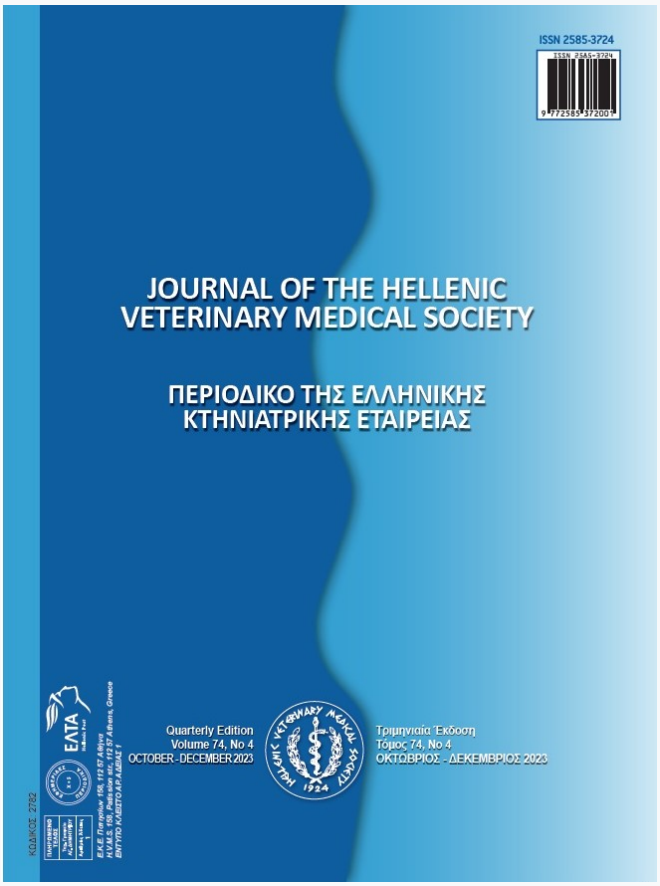

Addition of Japanese mint volatile oil (Menthaarvensis) to post weaning Saanen Kids: I. The effects on growth performance, rumen parameters, fecal characteristics and fecal microbiology

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Research article
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Addition of Japanese mint volatile oil (*Menthaarvensis*) to post weaning Saanen Kids: I. The effects on growth performance, rumen parameters, fecal characteristics and fecal microbiology

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ABSTRACT: The aim of this study was to evaluate the effect of diatery addition or oral intake of Japanese mint volatile oil (*M. arvensis*) on growth performance, rumen parameters, fecal characteristics and fecal microbiology of Saanen kids. Twenty-four male and female kids60 ± 5 d age and11.43kg body weight (BW) were randomly divided into three groups (n = 8). Japanese mint volatile oil was not added to the control group. In the research, Japanese mint volatile oil was either added to the feed at 200 mg/kg level (Group I) or orally given (Group II) to the Saanen kids while no treatment was applied to control group. The animals were weighed at the begining and the endof the study. Rumen and fecal samples were collected at the end of the study. While pH, NH_3N, volatile fatty acid (VFA) and protozoa were examined in rumen content, pH and microorganism population were examined in fecal content. There were no significant differences (P>0.05) in live weight (LW), live weight gain (LWG), average daily gain (ADG), daily dry matter intake (DMI) and feed conversion ratio (FCR) between the control and experimental groups. There were no differences in the rumen pH, faecal pH, rumen NH_3N and faecal score of the control and experimental groups.In the study, there were no significant differences between the control and experimental groups detected in terms of faecal microorganism population, while salmonella was detected in one of the control group faecal sample. Addition of Japanese mint volatile oil to kids diet caused significant decreases in rumen isobutyric acid (P<0.05), butyric acid (P=0.003), isovaleric acid (P=0.005) and total VFA (P< 0.05) values. It was determined that the use of Japanese mint by adding to the diet in kids diet caused a significant decrease (P=0.004) in the distribution of *Entodinium* and a significant increase (P≤0.001) in the distribution of *Epidinium* from rumen protozoa. The use of mint oil by drinking and adding to the feed caused a significant difference in the total number of protozoa. A significant increase (P≤0.05) in the total number of protozoa was detected with the addition of Japanese mint volatile oil by drinking (oral administration).In this study, it was determined that the addition of Japanese mint oil to the kids showed a positive trend on the ADG parameter. Also, the decrease in the number of pathogenic microorganisms *E.coli* and Salmonella in this study supports the antimicrobial effect of of Japanese mint volatile oil. In summary, the results of this study show that Japanese mint, which is natural and does not carry residual risk, can contribute to performance and support health criteria in kids after weaning.

***Keywords*:** feces; mint oil; protozoa; rumen; Saanen kids

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INTRODUCTION

In livestock production, antibiotics at sub-therapeutic levels were commonly used to enhance the efficiency of converting feeds to gain (e.g., milk and meat) and/or prevent metabolic disorders and health problems (Dibner and Richards,2005). However, the restriction and the ban on the use of antibiotics have prompted scientists and the feed industry to search for alternative products. Recently some researchers have suggested that some of phytogenics have potential to manipulate rumen fermentation since volatile oils or their mixture have antimicrobial activities against a wide range of rumen micro-organisms. Phytogenics offer a unique opportunity in this regard as many plants produce secondary metabolites, such as volatile oils that, when extracted and concentrated, may exert antimicrobial activities against a wide variety of rumen microorganisms(Mirzaei-Alamout.et al., 2016).In addition to the antimicrobial effects of these additives, they also have many functions such as antioxidant, anti-inflammatory, immune-modulating, stimulating digestive enzymes and pharmacological activities, and thus have been evaluated as natural feed additives in farm animals (Atanasov et al.,2015; Patra and Saxena, 2010).Studies have shown that phytobiotic additives positively affect rumen fermentation and provide advantages in terms of production performance, health status, welfare and environmental factors in ruminants.

One of the important phytogenic plants used in animal nutrition is mint. Mints belong to the genus *Mentha,* in the family *Labiatae (Lamiaceae)* which includes other commonly grown essential oil-yielding plants such as basil, sage, rosemary, marjoram, lavender, pennyroyal and thyme. The four most commonly cultivated species are: Japanese Mint/Menthol Mint (*M.arvensis*), Peppermint (*M.piperita*), Spearmint (*M.spicata*), Bergamot mint (*M. citrata*). The chemical components of mint leave and oilvary with plant maturity, variety, geographical region and processing conditions. Japanese mint (*Mentha arvensis*) popularly known as menthol mint is a source of natural menthol which is widely used in pharmaceutical and flavour industries (Zachariah and Leela, 2006). Souza et al. (2014) reported menthol (70.00%) as the main component of menthol mint essential oil followed by menthyl acetate (7.00-12.00%) which is an indicator of maturity. A total of 12 compounds were reported including menthol, pulegone and piperitone. Padalia et al. (2013) analysed the oil from 9 cultivars of *M. arvensis* and reported 33 constituents were menthol (73.70-85.80%), menthone (1.50-11.00%), menthyl acetate (0.50-5.30%), isomenthone (2.10-3.90%), limonene (1.20-3.30%) and neomenthol (1.90-2.50%).In particular, in this study, unlike other mint species, the extraction product of Japanese mint with high menthol content was used and Japanese mint volatile oil was applied to kids in different ways (by adding to the feed and oral administration). In addition, the effects of giving Japanese mint volatile oil, which is known to have antioxidant and antimicrobial effects and positive effects on digestive enzymes, to kids under stress after weaning, on performance parameters, changes in rumen and fecal contents were investigated. In the present study, we aimed to investigate to the potential use of mint volatile oil of Saanen kids on growth performance, rumen parameters and rumen protozoa population and faecal microbiology.

MATERIAL AND METHODS

Saanen Kids, management and experimental design

All experimental procedures used in this study were approved by Bursa Uludag University Animal Experiments Local Ethics Committee (Protocol number: 2020-02/06). All animals were handled according to the European Union directive number 86/609/EEC concerning the protection of animals used for experimental and other scientific purposes. The study was conducted from July to June, 2020, at Bursa Uludag University Applied Research Center for Veterinary Faculty Unite in Bursa, located within North West Turkey, 40^{o} north latitude, 29^{o} east longitude and an altitude of 120 m above sea level (Gorukle/ Bursa).

The kids were hosted in individual paddocks with approximately 2.0 m^2 with wheat straw bedding. All twenty-four paddocks were in the same condition. Twenty-four female and male Saanen kids were used in this study. Each group consisted of 8 kids. There were 3 kids born as twins, 1 kid born as triplets and 4kids born as single birth in both control and experimental groups. The groups were similar with regard to body weight (kg) at the beginning of the study (60 ± 5 d of age, Control:11.74;drinking group:11.25 and feed group: 11.30).The nutrient compositions of concentrate feed and alfalfa hay are presented in Table 1. The control group was fed with concentrate and alfalfa hay as indicated in Table 1 and essential oil was not added to this group. The animals in the experimental group (Group I), to which mint volatile oil was added to the feed, took the essential oil together with the feed

Table 1: Nutrient composition of grower concentrate and alfalfa hay (dry matter basis,g/kg)[1]

Item	Starter concentrate[2,3]	Alfalfa hay
Dry matter, %	919.0	921.6
CP, %	180.0	184.7
Ether extract, %	42.1	27.0
NDF, %	215.4	322.8
ADF, %	87.1	264.1
ADL, %	26.3	61.8
NFC[4], %	500.2	359.3
Ash, %	62.3	106.2
Ca, %	17.0	14.6
P, %	5.8	3.3

[1]Nutrient analyses of the feeds were performed according to AOAC (1990) and AOAC (2000)
[2]C.P. Feed Industry, Tekirdag, Turkey.
[3]Contained the main ingredients: wheat brain, ground corn grain, corn gluten meal, sunflower meal, palm meal, canola meal, ground barley grain, corn meal, molasses, calcium carbonate, vinasse, soya bean meal, vegetable oil, ammonium chloride, vitamin and mineral mix, salt.
[4]NFC (Non-Fibre Carbonhydrate): 100 - (% NDF + % CP + % Ether extract + % Ash).

and mixed with the mixed feed (TMR) by spraying at 200mg/kg daily. Mint volatile oil was administered orally to the animals in group II at a daily level of 200 mg/day/kids with a 10 ml syringe with a serum rubber attached. During the experiment, kids were given Japanese mint volatile oil before being fed at 09:00. The animals consumed peppermint essential oil without rejecting it in both applications. The volatile oils were extracted by hydrodistillation. Hydrodistillation is a simple form of steam distillation. High pressure steam is forced through crushed plant, picking up the oil. The vapor containing the oil is then condensed, producing a liquid containing a mixture of water and the plant oil. The oil is then separated from the water. Then the composition of peppermint oil was determined by GS-MS. The active ingredient components obtained from *M. arvensis* by steam distillation are shown in Table 2. The Japanese mint volatile oil was obtained from a local company (Semieterik®) that extracts aromatic plants in the Aegean region.

Uniform feeding and management standards were applied in the control and experimental groups. The trial lasted for a total of 8 weeks period, 2 weeks of adaptation and 6 weeks of trial. The diets were calculated to have similar concentrations of metabolizable energy, starch and CP based on published values from the NRC (2001). The basal diets contained approximately 90% concentrate and 10% forage, which was

Table 2: Volatile oil components of *M.Arvensis* used in this study

Components	%
Alpha pinen	*0,78*
Beta myrcene	*0,61*
Beta pinen	*0,77*
Germacren D	*1,25*
Isomenthon	*3,84*
Limonen	*3,69*
Menthol	***71,88***
Menthone	*12,15*
Menthyl acetate	*0,57*
Sabinen	*0,33*
Cis-3-Hexenyl valerate	*0,38*
Isopulegol	*0,79*
Neoisomenthol	*2,58*
Neoisopulegol	*0,39*

alfalfa hay. The diets were prepared as a total mix ration, divided into equal portions. TMR and freshwater were available ad libitum.

Chemical analysis

Dry matter (method 934.01), crude protein (method 984.13), ether extract (method 920.39), and ash (method 942.05) analyzes were made from calf starter and alfalfa hay using the methods specified in AOAC (2000). Calcium (method 927.02) and (method 965.17) phosphorus amounts were determined using the methods specified in AOAC (1990). Neutral detergent fiber (aNDF) and acid detergent fiber (ADF) analyzes were determined with a fiber analyzer-Ankom200 (Ankom Technology, USA) as described by Van Soest et al. (1991) with ash correction. Sodium-sulfite and heat-stable α-amylase were used in NDF analysis.

Body weight and feed intake

Total mix ration (concentrate feed + alfalfa hay) was weighed and recorded every day. The refusal feed was collected and weighed and the daily consumed TMR was calculated. Daily dry matter intake (DMI) was calculated based on daily consumed concentrate and alfalfa hay. Weighings to determine the live weight gain (LWG) of the kids were made only at the beginning (average initial weight-AIW) and end (average final weight-AFW) of the experiment in order to minimize the stress formation. Average daily gain (ADG) was calculated by dividing the difference two consecutive weighing by the number of days passed. Feed efficiency was calculated by dividing daily DMI by the ADG.

Rumen parameters

At the end of the study, rumen fluid was collected from all kids with an esophageal tube 4 h after morning feeding. Rumen pH was immediately assessed with a previously calibrated portable pH meter (Metler-Toledo, Switzerland). Ruminal fluid was immediately filtered using 4 layers of cheesecloth with a mesh size of 250 µm. 5 ml of filtrate sample was preserved by adding 1 ml of 1% H_2SO_4 for NH_3-N and 2 ml of filtrate was acidified with 0.04 ml of 50% H_2SO_4 for volatile fatty acid (VFA) concentrations. These samples were stored at -20°C for further analysis of nitrogen ammonia and VFA. The NH_3-N concentration was quantified using the method proposed by Annino (1964). Samples were prepared for VFA analysis and transferred into gas chromatography (GC) sample vial for analysis by GC (Hewlett Packard Agilent Technologies 6890N Network GC System, Serial CN10447002, China) with GPx 10% SP-1200/1% H_3PO_4 on 80/100 Chromosorb and using a 6'∞2 mm ID glass column (Supelco, Bellefonte, PA, USA).Protozoa were counted and identified in the rumen fluid using a Fuchs-Rosenthal hematocytometer under the light microscope, according to the method described by Ogimoto and Imai (1981).

Feces collection and analyses

Faecal samples were collected from each kids by retrieval from the rectum at the end of the study. Faecal pH was measured immediately using the electronic pH meter (Metler-Toledo, Switzerland). According to the method described by Verlinden et al., (2006) faecal samples were collected in glass beakers and 10-fold diluted with distilled water. Then the obtained mixture was homogenized and the pH was determined .Fecal scores were established as 0) normal, 1) semi-formed and/or pasty, 2) loose but stays on top of bedding, and 3) watery and/orsifts through bedding (Swedzinski et al., 2019).

For the isolation and enumeration of faecal microflora, 5 g of faecal content from each kids was aseptically transferred into a sterile stomacher bag and homogenised with 45 ml of saline peptone water (MRD, Merck, 1.12535.0500) in a Seward Stomacher 80 Lab System for 2 min. Serial 10-fold dilutions were made in sterile saline peptone water and plated in duplicate onto relevant selective media. Lactobacillus spp. were grown on de Man Rogosa and Sharpe (MRS, Merck, 1.10660) agar and were enumerated after 3 d of incubation at 35°C under 5% CO2 (Anaerobik Jar, Merck, 1.16387 and Anaerocult C, Merck, 1.16275). Bifidobacterium spp. was grown on Bifidobacterium Selective Medium agar (BSM agar, SIGMA, 88517). The plates were incubated at 37°C for 48 to 72 h in anaerobic jar with gas generating sachet (Anaerobik Jar, Merck, 1.16387 and Anaerocult C, Merck, 1.16275). For coliforms and E. coli isolation and enumeration, serial 10-fold dilutions of samples were made in saline peptone water and plated onto relevant selective media. Total coliform was grown on Violet Red Bile (VRB, Merck, 1.01406) agar using the "pour" plate technique and plates with 30-300 colonies were used for enumeration after 24-48 h of incubation at 37 °C. After incubation, typical colonies (red colonies with halos) were inoculated in Lactose Broth (LB, Merck, 1.07661) in a Durham tube at 44°C for 24 h. After incubation, acid and gas formation positive colonies were confirmed to be E. coli using the IMVC series of tests (indole, methyl red, Voges-Proskauer, citrate). Indol (+), Methyl red (+), Voges-Proskauer (−) and Citrate (−) indicated the presence of E. coli type-1.

Faecal samples was analyzed as indicated in ISO 6579/A1: 2007 (ISO, 2007). For pre-enrichment 25 g sample was homogenized in 225 ml of BPW for 2 min at 230 rpm, and incubated at 37°C. After pre-enrichment, 0.1 ml was transferred onto modified semi-solid Rappaport-Vassiliadis (MSRV, Oxoid, CM1112) agar and incubated at 41.5°C between 18-42 hour until a defined zone was observed. Selective plating was performed from MSRV agar onto XLD agar, xylose lysine tergitol-4 (XLT-4, Oxoid, CM1061) agar, and incubated at 37°C for 24 hours. After incubation, 1-5 suspected Salmonella colonies were selected and streaked onto MacConkey (MC, Oxoid, CM0115) agar to obtain pure culture to biochemical identification. For initial biochemical identification, pure MC agar culture was initially transferred into brain heart infusion (BHI, Oxoid, CM1135) broth and incubated at 37°C for 18-20 hour. This culture was then used for determining unease activity (Urea Agar Base, Oxoid, CM0053), triple sugar utilization and H_2S formation (Triple Sugar Iron Agar, Oxoid, CM0277), and lysine decarboxylase activity (Lysine Iron Agar, Oxoid, CM0381). Further identification was performed using API20E (Biomerieux, 20100), and profile results were evaluated accordingly.

Statistical analysis

All data were first tested for normality of distribution using the UNIVARIATE procedure in SAS. The data relating to growth performance and other parameters were analyzed using the PROC GLIMMIX

procedure of SAS 9.4 (SAS Institute Inc.; Cary, NC, USA). The model for parameters included only treatment as a fixed factor. In all models, kid within treatment was considered as a random effect in the models just to take the individual differences into account. Statistical differences were considered significant for $P < 0.05$. All results are reported as least squares means and corresponding standard errors.

RESULTS

Volatile oil components and growth performance

The ingredients and chemical composition of the concentrate feed and alfalfa hay are presented in Table 1. The control and experimental diets contained approximately 90% concentrate and 10% forage, which was alfalfa hay. The volatile oil composition of Japanese mint is shown in Table 2. The menthol (71.88%), menthone (12.15%), isomenthon (3.84%) and limonen (3.69%) were determined to be the main active components for Japanese mint oil. At the end of the study, AIW, AFW, LWG, ADG, DFI and FCR did not differ ($P > 0.05$) between groups, whereas the ADG value tended to be higher in the addition of Japanese mint oil by oral administration than in the control and other experimental group (Table 3).

Rumen pH, Rumen NH_3 N, rumen VFA profile and rumen protozoa population

The rumen pH and NH_3 N values did not differ ($P>0.05$) between groups (Table 4). In the study, these values remained within the limits that should be.The molar proportions of acetic acid, propionic acid, and the acetic/propionic acid ratio in the rumen did not differ ($P > 0.05$) between the control and experimental groups (Table 4). But total VFA ($P<0.05$), isobutyric acid ($P<0.05$), butyric acid ($P=0.003$) and isovaleric acid ($P=0.005$) concentrations were affected by treatment. These parameters were measured as the lowest in the rumen samples taken from the group in which mint volatile oil was added to the feed. In the study, differences were determined in the entodinium and epidinium populations in terms of rumen protozoa distribution, and in the total amount of protozoa in the control and experimental group samples (Table 5). Addition of mint volatile oil to the diet caused a significant decrease in entodinium number ($P=0.004$) and total protozoa number ($P\leq0.001$) and a significant increase in epidinium number ($P\leq0.05$) in the rumen.

Table 3. The effect of different applications of *M. arvensis* on performance parameters in Saanen kids

Parameters	*The way Japanese mint oil is given*				
	n	Control	Oral Administration	Adding to feed	*P*
Birth weight (kg)	8	3.67±0.12	3.42±0.12	3.57±0.13	0.398
Average initial weight (kg)	8	11.74±1.14	11.25±0.90	11.30±0.80	0.925
Average final weight (kg)	8	14.70±1.62	15.61±1.72	14.41±0.64	0.810
Live Weight Gain (kg)	8	2.96±0.71	4.36±1.04	3.11±0.39	0.371
Average daily gain (g)	8	87.43±17.97	115.69±25.66	86.39±10.93	0.483
Daily feed intake (g)	8	347.86±36.91	375.86±33.00	261.91±28.42	0.059
Daily DM intake (g)	8	319.93±33.94	345.68±30.35	240.88±26.14	0.059
Feed Conversion Ratio	8	4.31±0.66	3.69±0.31	3.58±0.93	0.747

FCR:Daily DM intake/Average daily gain

Table 4. The effect of different applications of *M. arvensis* on rumen parametersin Saanen kids

Parameters		*The way Japanese mint oil is given*			
	n	Control	Oral Administration	Adding to feed	*P*
Rumen pH	8	6.44±0.11	6.64±0.14	6.58±0.10	0.493
Rumen NH_3N (mg/dl)	8	17.73±0.82	17.43±0.46	15.87±0.64	0.523
Rumen VFA profile					
Acetic acid (A), (mmol/L)	8	43.72±2.77	35.13±7.34	30.26±3.24	0.144
Propionic acid (P), (mmol/L)	8	18.35±2.42	14.52±4.06	11.88±2.40	0.319
Isobutyric acid, (mmol/L)	8	**1.16±0.06^{a}**	**0.97±0.09^{ab}**	**0.77±0.104^{b}**	0.032
Butyric acid, (mmol/L)	8	**28.72±2.86^{a}**	**21.72±5.46^{a}**	**8.30±2.56^{b}**	**0.003**
Isovaleric acid, (mmol/L)	8	**5.08±0.18^{a}**	**4.04±0.42^{ab}**	**2.73±0.47^{b}**	**0.005**
A/P	8	2.50±0.21	2.71±0.28	2.93±0.28	0.545
Total VFA, (mmol/L)	8	**102.30±7.84^{a}**	**80.18±17.13^{ab}**	**56.62±8.79^{b}**	0.035

Table 5. The effect of different applications of *M. arvensis* on rumen protozoa population in Saanen kids

Parameters	n	*The way Japanese mint oil is given*			
		Control	Oral Administration	Adding to feed	*P*
Entodinium, (%)	8	**99.04±0.45^{a}**	**99.17±0.24^{a}**	**95.41±1.16^{b}**	**0.004**
Diplodinium, (%)	8	0.32±0.30	0.39±0.22	1.97±0.88	0.103
Epidinium, (%)	8	**0.64±0.30^{b}**	0.39±0.14^{b}	2.60±0.54^{a}	0.001
Total protozoa count/ml rumen fluid, (log_{10})	8	**5.91±0.15^{ab}**	**6.17±0.17^{a}**	**5.59±0.14^{b}**	**0.05**

Table 6. The effect of different applications of *M. arvensis* on faecal parameters in Saanen kids

Parameters	n	*The way Japanese mint oil is given*			
		Control	Oral Administration	Adding to feed	*P*
Faecal pH	8	8.20±0.07	7.95±0.17	8.17±0.08	0.317
Faecal score (initially)	8	1	2	2	-
Faecal score (average)	8	2	2	3	-
Faecal microbiology					
Coliform, (cfu Log_{10}/g)	8	3.38±0.73	3.17±0.64	1.97±0.43	0.219
Lactobacillus spp. (cfu Log_{10}/g)	8	3.20±0.56	2.70±0.24	3.17±0.50	0.706
Bifidobacteria spp. (cfu Log_{10}/g)	8	2.50±0.28	3.12±0.47	2.90±0.31	0.505
E.coli positive (fresh feces)	8	8/8	8/8	8/3	-
Salmonella positive (fresh feces)	8	8/1	8/0	8/0	-

Fecal scores were established as 0) normal, 1) semi-formed and/or pasty, 2) loose but stays on top of bedding, and 3) watery and/or sifts through bedding

Faecal pH, faecal score and faecal microorganism population.

The results of faecal pH and faecal score values are presented in Table 6. Supplementing with mint volatile oil did not alter the faecal pH from that of the control group, but the faecal score value was lower in the kids fed the mint volatile oil than in the control group. In the study, no difference was detected in the faecal samples of the control and experimental groups in terms of faecal microbiology. However, *E. coli*, one of the pathogenic microorganisms in the feces, was found less positive (8/3) in the feces samples of the group to which mint volatile oil was added to the feed. Salmonella was found only in control group stool samples (8/1).

DISCUSSION

In recent years, a variety of plant bioactive compounds, including saponins, essential oils-volatile oils, tannins, and flavonoids have been evaluated for their ability to modulate rumen microbial fermentation processes to improve feed utilization efficiency while decreasing methane emission and nitrogen excretion (Patra and Saxena, 2009, 2010). In the present study, Saanen kids were fed a different applications of Japanese mint volatile oil, which contains high levels of menthol as the main active ingredient. Japanese mint volatile oil extracted from *M. arvensis* leaves which is rich in menthol (71.88%) used in this study. In the study, the application of mint volatile oil with feed and orally was accepted by the animals and consumed comfortably.

No significant differences were found between the control and experimental groups in terms of performance data. The present study, we observed that 200 mg/day/kids Japanese mint volatile oil markedly increased ADG (115.69 g) and AFW (15.61 kg)in oral administration group. The reason for this increase in ADG and AFW may be related to the decrease of pathogen bacteria count and the efficiency of dietary energy utilization for maintenance and weight gain in goats. Similar studies supplementing with volatile oils reported varying results. Similar to our results, Moura et al. (2017) reported that adding 500 mg copaiba essential oils/kg DM had no effect on DMI but increased ADG and gain efficiency compared to a non-adding-group. On the other hand, in a meta-analysis report, Dorantes-Iturbide et al. (2022) concluded that in small ruminants, the use of essential oils increased the dry matter intake, daily live weight gain and feed efficiency. In their study, Simitzis et al. (2014) found no effect in growth performance in lambs supplemented with cinnamon oil (1 ml/kg of concentrated feed) for 35 days.When the studies conducted with aromatic plants belonging to the Lamiaceae family and the Mentha genus are examined, results have been obtained indicating that there are no significant

changes in performance parameters or that they affect performance positively. Mohamadi et al. (2017) reported that pennyroyal had no effect on performance parameters in sheep, likewise Olmez et al. (2022) determined that pennyroyal added at different levels did not affect the growth performance of Tuj lambs. Khamisabadi et al. (2016) stated that there was no change in FCR in the groups in which 3% mint and 3% thyme were added to lamb rations. The same researchers declared that the addition of 3% mint from the Mentha family increased the daily BWG. As a result, reasons such as aromatic plants, ration formulation, interaction between feeds, compatibility with animal species and rumen microbial fermentation may cause aromatic plants to give different results.

In the present study, ruminal pH and rumen NH_3-N was not affected by the dietary inclusion of Japanese mint volatile oil. A meta-analysis conducted by Orzuna-Orzuna et al. (2022) reported that, dietary addition with EOs reduced rumen NH_3-N levels without affecting rumen pH in beef cattle. On the other hand, Benchaar et al. (2008) stated that it is possible for rumen microorganisms to adapt the effects of EOs when used for a long time, and this may reduce their positive effects. The results observed for ruminal pH in our study suggest that rumen functions of ruminants are performed under stable conditions and mint volatile oil added to the ration does not affect this situation. The molar ratios of acetate, propionate and A/P, which are important volatile fatty acidsare products of carbohydrate fermentation in the rumen, were not affected by the addition of Japanese mint oil, but caused significant changes in the amount of other volatile fatty acids and total volatile fatty acids. Jahani-Azizabadi et al. (2022) fed a mixture including peppermint oil to calves and determined that total VFA and molar proportions of propionate, butyrate, and isovalerate, as well as the acetate-propionate ratio in the rumen were not affected. Poudel et al. (2019) also observed an increase in propionate concentration in dairy calves fed a diet supplemented with a mixture of EO (thymol, caryophyllene, p-cymene, cineole, terpinene, and carvacrol). Inconsistent results from different studies on the effects of EO on rumen fermentation depend on diet composition, environment, major bioactive compounds, and doses.

Factors such as the use of feed additives, the physiological condition of the animal and the ration may affect the rumen microbial population. Many of these factors may not eliminate bacteria, protozoa, or fungi, but are typically observed as fluctuations in microbial populations (Naseri et al.2022).Similarly, there were fluctuations in the distribution of rumen protozoa in our study. On the other hand, Naseri et al. (2022) observed that supplementation with 1.2 ml/sheep/day *Pistacia atlantica* gum essential oil did not affect the protozoa population in sheep.

It has been concluded that fecal bacterial counts such as Bifidobacterium and Lactobacillus were similar between control and experimental groups. Similar to our results, Swedzinski et al. (2019) noted that addition of a 1,25 g/d essential oils mix and a prebiotic did not affect the fecal bacteria counts of the pathogens (*Clostridia, Escherichia* and *Salmonella*) and benefical bacteria (*Bifidobacter* and *Lactobacillus*) between calves. Fecal microbiology in animals is a guiding data for interpreting the microorganisms in the digestive system and their health (Celi et al., 2017). The decrease in faecal coliformcounts and the number of feces positive for *E.coli*may indicate that the supplementation of Japanese mint volatile oil added to the feed at the 200 mg/kg level prevented the growth of harmful microorganisms and that Japanese mint oil was better utilized by beneficial bacteria in the intestines of growing Saanen kids.

CONCLUSIONS

The results of the study showed that supplementing Japanese mint volatile oil by drinking or adding to the diet did not make a significant difference in performance and rumen fermentation parameters compared to the control group. However, it was concluded that the application of mint volatile oil by drinking affects the ADG parameter and performance positively. In addition, the number of *E.coli* in the feces was found to be less in the group added to the feed, and Salmonella was determined only in the control group. In conclusion, in this study, it was observed that Japanese mint volatile oil at 200 mg/day/kids level was able to reduce the fecal pathogenic load without causing a significant effect on rumen parameters in terms of both applications (feed or drinking). Considering these results, it can be said that Japanese mint volatile oil which has a high menthol content, can be beneficial for kids post weaning period by reducing the pathogenic microorganism load with its antimicrobial effect.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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