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ORIGINAL ARTICLE

Effects of juniper essential oil on growth performance, some rumen protozoa, rumen fermentation and antioxidant blood enzyme parameters of growing Saanen kids

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Summary

This study aimed to evaluate the effects of juniper essential oil on the growth performance, rumen fermentation parameters, rumen protozoa population, blood antioxidant enzyme parameters and faecal content in growing Saanen kids. Thirty-six male Saanen kids (36 ± 14 days of age) were used in the study. Each group consisted of 9 kids. The control group (G1) was fed with a diet that consisted of the above concentrated feed and oat hay, whereas the experimental groups consumed the same diet but with the concentrated feed uniformly sprayed with juniper essential oil 0.4 ml/kg (G2), 0.8 ml/kg (G3) or 2 ml/kg (G4). There were no differences ($p > 0.05$) in live weight, live weight gain or feed consumption between the control and experimental groups. There was a significant improvement ($p < 0.05$) in feed efficiency in the G3 group. There were no differences in the rumen pH, rumen volatile fatty acid (VFA) profile or faecal pH of the control and experimental groups. The rumen NH_3N values were similar at the middle and end of the experiment, but at the start of the experiment, the rumen NH_3N values differed between the control and experimental groups ($p < 0.05$). The faecal score value was significantly ($p < 0.05$) decreased in the experimental groups. The addition of juniper essential oil supplementation to the rations caused significant effects on the kids' antioxidant blood parameters. Although the superoxide dismutase (SOD) activity, total antioxidant capacity (TAC) and catalase values were significantly ($p < 0.05$) increased in the experimental groups (G2, G3 and G4), especially group G4, the blood glutathione peroxidase (GPX) value significantly decreased in the experimental groups. The results of this study suggest that supplementation of juniper oil is more effective on antioxidant parameters than on performance parameters and may be used as a natural antioxidant product.

Keywords antioxidant, enzyme, growth parameters, juniper, rumen ecology

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Introduction

In recent years, the use of plant bioactive components as a natural feed additive for animal nutrition has seen increased research interest. This rise in interest has been stimulated by the search for alternatives to growth-promoting antibiotics in livestock production. Essential oils have been reported to possess strong antimicrobial properties related to a number of small terpenoid and phenolic compounds that have been shown to possess high levels of antimicrobial activity (Fraser et al., 2007). Therefore, the studies have concentrated on the antimicrobial properties of essential oils to regulate rumen microbial fermentation to improve nutrient utilization and reduce the environmental impact of livestock production systems. According to data in the literature, rumen microbial populations may adapt when continually exposed to essential oils. Therefore, more *in vivo* research is required to fully assess the potential use of essential oils as feed additives in ruminant nutrition. However, there is very limited information about the effects of essential oils on the blood values and antioxidative properties of ruminants.

Juniperus is a coniferous plant in the family *Cupressaseae* (Bakkour et al., 2013). Alpha-pinene is the dominant monoterpene in the Juniperus genus. The antioxidant effects of aromatic plants occur due to

the presence of hydroxyl groups in their phenolic compounds (Shahidi and Wanasundara, 1992). Based on study results, juniper extracts have significant antioxidant activity against various antioxidant systems *in vitro*; moreover, juniper fruit can be used as an easily accessible source of natural antioxidants (Bakkour et al., 2013). The objective of this study was to determine the effects of juniper essential oil on the performance parameters, ruminal fermentation, antioxidative blood parameters, faecal score, faecal pH and rumen microflora of growing Saanen kids.

Materials and methods

Experimental area, animals and diets

The study was carried out at Uludag University Applied Research Center for Veterinary Faculty Unit in Bursa, located within the North West Turkey, 40° north latitude, 29° east longitude and at an altitude of 120 m above sea level. 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. In addition, this study was conducted under a protocol approved by the Animal Care and Use Committee of University of Uludag.

Thirty-six male Saanen kids were used in this study. Each group consisted of 9 kids with an average initial body weight of 15.0 kg. There were 2 kids born as twins, 6 kids born as triplets and 1 kid born as a single birth in both control and experimental groups. The groups were similar with regard to body weight (kg) at the beginning of the study (G1-control: 15.1 ± 0.681; G2: 14.9 ± 0.574; G3: 15.2 ± 0.791; G4: 14.8 ± 0.60). The diets were calculated to have similar concentrations of metabolizable energy, starch and CP based on published values from the NRC (1994). The four diets contained approximately 90% concentrate and 10% forage, which was oat hay. The diets were prepared as a total mix ration, divided into equal portions. Particle size distributions of TMR samples were determined using the Penn State Particle Separator (PSPS) containing 3 sieves (19.8 and 1.18 mm) and a pan as described by Kononoff et al. (2003).

Management and experimental design

The kids were housed in a paddock equipped with feeders and waterers, with straw bedding. All 36 paddocks were identical, with the same direction, orientation and covered area (2 m^2/paddock). Water, pelleted concentrate feed (Matli Feed Industry, Karacabey, Turkey) and oat hay were supplied *ad libitum*. The nutrient compositions of pelleted concentrate feed and oat hay are presented in Table 1. The control group (G1) was fed with a diet that consisted of the above concentrated feed and oat hay, whereas the experimental groups consumed the same diet with the concentrated feed uniformly sprayed with juniper essential oil at 0.4 ml/kg (G2), 0.8 ml/kg (G3) or 2 ml/kg (G4). Feed mixtures were prepared and the essential oils were added on a weekly basis. Uniform feeding and management standards were applied in the control and experimental groups. The experimental period lasted for 60 days.

Feed intake and gain

In the study, the quantity of feed offered was recorded daily and refusals were weighed fortnightly, on an individual basis, for the determination of feed intake (FI). Samples of the offered and refused feeds were collected fortnightly. Animals were weighed on days 15, 30, 45 and 60 of the trial, and the average daily gain was determined by dividing weight gain (initial live weight ILW - final live weight FLW) by the number of days (i.e. 14 days). Feed conversion rate (FCR) was calculated as the ratio between dry matter intake and average daily gain (g of live weight gain-LWG/g of dry matter intake).

Faecal score and faecal pH

Faecal samples were collected from each kid by retrieval from the rectum on days 15, 30 and 60 of

Table 1 Nutrient compositions of starter concentrate and oat hay on a dry matter basis*

Item	Starter concentrate †,‡	Oat hay
Dry matter, %	90.7	94.7
CP, %	21.3	6.37
Ether extract, %	4.04	2.2
NDF, %	25.7	60.1
ADF, %	15.5	38.6
ADL, %	4.09	8.06
NFC§, %	20.2	22.5
Ash, %	8.62	8.81
Ca, %	1.29	3.7
P, %	0.63	1.4

*Nutrient analyses of the feeds were performed according to AOAC (1990).
†Matli Feed Industry, Bursa, Turkey.
‡Contained the main ingredients: ground corn grain, ground barley grain, wheat bran, soya bean oil, soya bean meal, corn gluten meal, sunflower meal, molasses, mineral and vitamin mix, limestone, salt.
§NFC (non-fibre carbohydrate): 100 − (% NDF + % CP + % Ether extract + % Ash).

the trial. Faecal samples were scored with regard to consistency by the same researcher on all collection days according to the following system: 1- watery, diarrhoea; 2- soft, unformed; 3- soft, formed; 4- hard, formed and 5- hard, dry pellets. Faecal pH was measured immediately following the collections. An electronic pH meter (PT-10 Sartorius AG, Goettingen, Germany) fitted with a glass electrode was used to determine faecal pH. Each faecal sample was placed in a 50-ml glass beaker and diluted 10-fold with distilled water as described by Verlinden et al. (2006). The mixture of faecal sample and distilled water was homogenized and pH was measured.

Rumen pH, ammonia-N and VFA concentrations

After an adaptation period followed by the beginning, middle and end of the study, rumen fluid was taken by rumen cannula. On days 15, 30 and 60 of the trial, rumen fluid samples were taken 2 h after feeding. Ruminal fluid was obtained as grab samples from the ventral of the rumen, and the pH was then immediately measured using the pH meter (Inolab pH, serial no: 00200018, pH Electrode SenTix 41, D-82362, Weilheim, Germany). Ruminal fluid was collected during each period from the rumen for volatile fatty acids (VFA – mmol/l) and ammonia content (NH_3-N – mg/dl) determination. Samples were immediately squeezed through 4 layers of cheesecloth with a mesh size of 250 μm. Samples of 10 ml were acidified with 0.5 ml of 50% H_2SO_4 and used to determine VFA, and 5 ml of filtrate was preserved by adding 1 ml of 1% H_2SO_4 and used to determine NH_3-N. The samples were subsequently stored frozen at −20 °C until analysed. The ammonia content of the ruminal samples was determined using the method described by Annino (1964).

Number and identification of protozoa in rumen fluid

The number of protozoa was counted and identified in the rumen fluid using a Fuchs-Rosenthal haemocytometer under a light microscope according to the method described by Ogimoto and Imai (1981). A volume of 0.5 ml of rumen fluid was mixed with 4.5 ml MFS (methylgreen-formalin-salt) solution and kept at room temperature for counting. Then, it was transferred into a counting chamber, and the protozoa were counted in 4 microscopic fields. The counts were performed four times, and thus, 16 microscopic fields were counted for each sample.

Antioxidant blood parameters

Bio Vision-Superoxide Dismutase Activity Assay Kit used according to the manufacturer's instructions to determine SOD activity (Biovision, Mountain View, CA, USA). Therefore, the inhibition activity of SOD can be determined using a colorimetric method. The results were expressed as the inhibition rate (%). Bio-Vision developed the TAC (total antioxidant capacity) colorimetric Assay Kit, which can measure the combination of both small molecule antioxidants and proteins or small molecules alone in the presence of our proprietary protein mask. In the assay, catalase first reacts with H_2O_2 to produce water and oxygen; the unconverted H_2O_2 reacts with the OxiRed™ probe to produce a product, which can be measured at 570 nm (Colorimetric method – μmol/min/ml). In BioVision's Glutathione Peroxidase Activity Assay, GPX reduces cumene hydroperoxide while oxidizing glutathione (GSH) to oxidized glutathione (GSSG). The generated GSSG is reduced to GSH with consumption of NADPH by glutathione reductase (GR). The decrease of NADPH (easily measured at 340 nm) is proportional to GPX activity (μmol/min/ml).

Statistical analysis

All statistical analyses were conducted using Statistical Package for the Social Sciences software (version 20.0, SPSS, 2004, USA). All data (except performance data) were analysed by the Kruskal–Wallis H-test, and the Mann–Whitney *U*-test was used for paired comparisons; the exact test was chosen to be only asymptotic for both tests. Performance data were tested for a normal distribution by an F-test, and one-way ANOVA was used for compare data according to groups. Homogeneity of variances was tested, and the Tukey HSD test was chosen as the *post hoc* multiple comparison test. Differences between groups were considered significant when p-values were <0.05.

Results

The ingredients and chemical composition of the diets are presented in Table 1. The volatile oil composition of juniper is shown in Table 2. The α-pinene (89.75%), myrcene (2.12%) and β-pinene (1.20%) were determined to be the main active components for juniper oil. At the end of the study, ILW, FLW, LWG and FI did not differ ($p > 0.05$) between groups, whereas the FCR value tended to be higher ($p < 0.05$) in G3 than in the control and other experimental groups (Table 3). The rumen pH value did not differ

Table 2 Volatile oil components of the Juniper Plant (*Juniperus communis*)

Components	(%)
α-Pinene	89.7
Mirsene	2.12
β-pinene	1.20
Terpinolen	0.851
Limonene	0.833
Ý-Terpinene	0.624
α-sedrene	0.452

between groups, but there were differences in the rumen NH_3N levels between the experimental groups at the starting period (Table 4). The amount of total VFA; the molar proportions of acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, n-valeric acid; and the acetic/propionic acid ratio in the rumen did not differ ($p > 0.05$) between the control and experimental groups (Table 5). Differences in the rumen protozoa populations of *Entodinium* and *Epidinium* were determined and compared between the experimental groups in period 1; the number of total protozoa populations differed between groups in period 2 (Table 6), and this was attributed to the decrease in the number of total rumen protozoa. The effects of juniper oil supplementation on various antioxidant blood enzyme parameters (SOD, GPX, TAC and Catalase) are shown Table 7. The blood antioxidant enzyme values were significantly different ($p < 0.05$) between the control and experimental groups. The results of faecal pH and faecal score values are presented in Table 8. Supplementing with juniper essential 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 juniper oil ration than in the control group.

Discussion

Effect of juniper oil on performance parameters

Juniper oil is a natural feed additive. The studies related to supplementation of growing goat rations with juniper oil are limited. The literature is very scarce regarding the effect of essential oils or their components on the performance parameters of ruminants. In this experiment, feed consumption and live weight gain were not affected by juniper oil addition at the level of 0.4, 0.8 or 2 ml/kg. However, supplementation of juniper oil affected FCR, and supplementation of juniper oil at the level of 0.8 ml/kg led to a significant improvement in FCR. Recent research with lactating Holstein cows reported no changes in feed intake when cows were supplemented with a mixture of essential oil (EO) compounds at doses of 0.75 or 2 g/day (Benchaar et al., 2006, 2007). Similarly, Beauchemin and McGinn (2006) observed no effect on feed intake in beef cattle fed a diet consisting of 75% whole-crop barley silage, 19% steamrolled barley and 6% supplement (DM basis) with 1 g/day of the same EO compounds mixture. Bampidis et al. (2005) observed no change in DM intake, ADG or FCR when growing lambs were fed diets supplemented with oregano leaves (*Origanum vulgare* L.) providing 144 or 288 mg of oregano oil (850 mg/g of carvacrol) per kilogram of diet DM. Chaves et al. (2008b) determined that there was no effect on the DMI or growth performance of lambs when supplemented with 200 mg/kg of DM of either cinnamaldehyde, garlic oil or juniper berry oil. Consequently, herbal essential oil activities on the performance parameters can lead to different results depending on the kind of aromatic herb used, the characteristic structure and the dosage used.

Table 3 The effects of juniper oil supplementation on performance parameters of Saanen kids

Group	Initial live weight (kg) Mean ± SE	Finish live weight (kg) Mean ± SE	Live weight gain (kg) Mean ± SE	Feed intake (kg) Mean ± SE	FCR (g/g) Mean ± SE
1	15.1 ± 0.68	16.8 ± 0.72	1.69 ± 0.22	4.81 ± 0.33	0.28^{ab} ± 0.34
2	14.9 ± 0.57	16.6 ± 0.68	1.67 ± 0.20	5.41 ± 0.21	0.26^{b} ± 0.41
3	15.2 ± 0.79	17.4 ± 0.92	2.27 ± 0.25	5.27 ± 0.42	0.40^{a} ± 0.51
4	14.8 ± 0.60	16.6 ± 0.51	1.80 ± 0.23	5.28 ± 0.18	0.30^{ab} ± 0.32^{ab}

Different letters in the same column indicate statistical differences ($p < 0.05$).
Data were analysed by one-way ANOVA, and Tukey HSD test was chosen for post-test.
FCR: live weight gain/feed intake.
Different letters (lowercase) within the same column indicate significant differences among treatment groups.

Table 4 The effects of Juniper oil supplementation on rumen pH and rumen NH_3N levels

Group	Experiment Period (days)	Rumen pH Mean ± SE	NH_3N (mg/dl) Mean ± SE
1	1st	6.25 ± 0.15	29.19^{A} ± 2. 46
	28th	6.43 ± 0.08	22.80 ± 3.48
	56th	6.18 ± 0.09	30.02 ± 6.53
2	1st	6.07^{b} ± 0.11	26.05^{AB} ± 1.92
	28th	6.62^{a} ± 0.06	25.14 ± 1.81
	56th	6.02^{b} ± 0.12	29.88 ± 2.65
3	1st	6.12^{b} ± 0.12	19.84^{B} ± 2.54
	28th	6.40^{a} ± 0.07	21.22 ± 1.75
	56th	6.11^{b} ± 0.09	23.33 ± 2.37
4	1st	6.13 ± 0.09	21.39^{abB} ± 2.17
	28th	6.30 ± 0.12	16.94^{b} ± 2.31
	56th	6.25 ± 0.15	26.94^{a} ± 3.24

Different letters in the same group in each column indicate statistical differences ($p < 0.05$).
Data were analysed by Kruskal–Wallis test, and Mann–Whitney *U*-test was used for paired comparisons.
Different letters (uppercase) within the same column indicate significant differences among treatment groups.
Different letters (lowercase) within the same column indicate significant differences among treatment period.

The effects of Juniper oil supplementation on rumen pH and rumen NH_3N levels

Various results have been reported for the effects of essential oil on ruminal pH. The findings of the present study for ruminal pH were in line with those of previous studies in sheep (Chaves et al., 2008a,b), dairy cows (Kung et al., 2008; Tager and Krause, 2011) and beef cattle (Cardozo et al., 2006; Meyer et al., 2009) that found no effect of different essential oils on rumen pH. However, Benchaar et al. (2006 and 2007) reported an increase in ruminal pH when dairy cows received essential oil. The results from *in vitro* studies indicated that pH could be influenced by the dose of essential oil delivered. For instance, Evans and Martin (2000) observed that the addition of 400 mg/l of thymol increased the pH *in vitro*; however, lower doses had no effect on pH. A higher pH at a high dose of essential oil could be related to a reduction in volatile oil concentrations, suggesting that the rumen microbial fermentation has been inhibited. Therefore, longer-term *in vivo* studies are needed to obviously establish the effects of the essential oil. In summary, the differences in effects of essential oil supplementation on pH could be described in part by the capacity of rumen microbes to adapt or degrade essential oil components (Benchaar and Greathead, 2011).

Table 5 The effects of juniper oil supplementation on rumen VFA profile

Group	Experiment period (days)	TVFA (mmol/l) Mean ± SE	Acetic acid (A) Mean ± SE	Propionic acid (P) Mean ± SE	Isobutyric acid Mean ± SE	Butyric acid Mean ± SE	Isovaleric acid Mean ± SE	n-Valeric acid Mean ± SE	A/P Mean ± SE
1	1st	28.2 ± 8.39	10.5 ± 2.63	8.49 ± 3.04	0.23 ± 0.04	8.00 ± 2.61	0.49 ± 0.03	0.44 ± 0.13	1.49 ± 0.18
	28th	25.6 ± 2.58	10.9 ± 1.44	6.12 ± 0.47	0.28 ± 0.02	7.39 ± 1.07	0.51 ± 0.05	0.37 ± 0.09	1.83 ± 0.21
	56th	34.7 ± 5.22	14.3 ± 2.25	8.66 ± 1.44	0.33 ± 0.04	10.2 ± 1.80	0.53 ± 0.05	0.58 ± 0.13	1.74 ± 0.17
2	1st	34.7^{a} ± 2.98	13.2^{b} ± 0.83	10.8^{a} ± 3.00	0.29 ± 0.01	9.26 ± 1.16	1.08 ± 0.62	1.87 ± 1.25	1.47^{b} ± 0.23
	28th	23.5^{b} ± 1.98	9.9^{a} ± 0.86	5.12^{b} ± 0.52	0.32 ± 0.02	7.24 ± 0.82	1.35 ± 0.73	0.64 ± 0.31	1.99^{a} ± 0.16
	56th	33.2^{ab} ± 3.84	14.1^{b} ± 1.32	9.28^{ab} ± 1.74	0.51 ± 0.21	9.20 ± 1.21	0.48 ± 0.07	0.62 ± 0.10	1.68^{ab} ± 0.18
3	1st	34.7 ± 3.86	13.4 ± 1.53	10.7^{a} ± 1.76	0.30 ± 0.04	9.16 ± 0.67	0.46^{b} ± 0.04	0.61^{a} ± 0.13	1.33^{b} ± 0.12
	28th	24.4 ± 2.77	10.3 ± 1.29	5.29^{b} ± 0.52	0.33 ± 0.02	7.54 ± 1.30	0.65^{a} ± 0.02	0.32^{b} ± 0.03	2.02^{a} ± 0.23
	56th	29.3 ± 4.70	11.4 ± 1.56	7.04^{ab} ± 1.29	0.33 ± 0.05	9.41 ± 1.85	0.58^{ab} ± 0.05	0.57^{ab} ± 0.13	1.82^{ab} ± 0.18
4	1st	39.3 ± 4.71	15.5^{a} ± 2.11	9.24 ± 1.57	0.35 ± 0.03	12.9 ± 1.41	0.61 ± 0.08	0.54^{a} ± 0.05	1.80 ± 0.20
	28th	28.3 ± 3.22	11.8^{b} ± 1.29	6.34 ± 1.13	0.32 ± 0.01	8.90 ± 1.15	0.57 ± 0.04	0.36^{b} ± 0.04	2.05 ± 0.17
	56th	28.3 ± 6.54	11.9^{ab} ± 2.01	5.94 ± 1.54	0.29 ± 0.04	9.16 ± 2.91	0.51 ± 0.08	0.44^{ab} ± 0.16	2.25 ± 0.23

TVFA, total volatile fatty acid
Different letters in the same group in each column indicate statistical differences ($p < 0.05$). Data were analysed by Kruskal–Wallis test, and Mann–Whitney *U*-test was used for paired comparisons.
Different letters (lowercase) within the same column indicate significant differences among treatment period.

Table 6 The effects of Juniper oil supplementation on rumen protozoa population (0.5 ml rumen content)

	Experiment period day (1st)			Experiment period day (56th)		
Protozoa	Group	Mean	Std. Error	Group	Mean	Std. Error
Entodinium	1	80.1^{A}	2.71	1	86.2	1.97
	2	65.8^{bB}	3.97	2	84.6^{a}	3.32
	3	75.3^{bAB}	4.82	3	88.6^{a}	1.86
	4	75.2^{bAB}	3.48	4	86.7^{a}	2.86
	Total	73.9	2.08	Total	86.5	1.24
Eudiplodi	1	2.84	1.52	1	0.66	0.23
	2	5.23^{a}	1.54	2	1.15^{b}	0.76
	3	2.68	0.81	3	1.13	0.60
	4	1.43	0.61	4	1.47	0.42
	Total	3.18	0.65	Total	1.08	0.27
Epidinium	1	17.2^{B}	2.24	1	12.2	1.70
	2	27.1^{aA}	2.56	2	10.1^{b}	2.06
	3	24.5^{aAB}	6.06	3	9.32^{b}	1.40
	4	23.0^{aAB}	3.88	4	9.73^{b}	1.71
	Total	22.9	1.96	Total	10.3	0.86
Ophryoscolex	1	0.39	0.24	1	0.40	0.17
	2	1.75	1.05	2	0.55	0.45
	3	0.02	0.02	3	0.38	0.25
	4	0.23	0.11	4	1.74	1.70
	Total	0.66	0.33	Total	0.75	0.41
Total	1	488355.56	121766.78	1	912000.00^{A}	102016.13
	2	320177.78	100134.82	2	650800.00^{AB}	100273.34
	3	433800.00	119156.44	3	487400.00^{B}	123425.74
	4	406400.00^{b}	86281.40	4	454800.00^{aB}	93833.33
	Total	412050.00	54442.53	Total	607200.00	60041.77

Different letters (uppercase) within the same column indicate significant differences among treatment groups.
Different letters (lowercase) within the same column indicate significant differences among treatment period.

Essential oils had variable impacts on ruminal NH_3-N concentration in the different studies. The results from different *in vitro* studies showed that the effects of EO and their main components on rumen NH_3-N concentration are dose dependent and that these compounds are more effective when used at high doses than at low doses. Essential oils had variable impacts on ruminal NH_3-N concentration in different studies. Most *in vivo* studies have shown that essential oils often do not influence the concentration of ruminal NH_3-N (Benchaar et al., 2007; Yang et al., 2010). Castillejos et al. (2006) evaluated the effects of increasing doses (0, 5, 50, 500 and 5000 mg/l) of thymol and eugenol on rumen fermentation in 24-h *in vitro* batch culture fermentations and reported these compounds decreased the ruminal concentration of NH_3-N that at the highest dose (i.e. 5000 mg/l) but that no effects were observed at lower doses.

The effects of Juniper oil supplementation on rumen VFA profile

The addition of essential oils or their main active components has caused either a reduction (Macheboeuf et al., 2008; Kumar et al., 2009) or no alteration (Yang et al., 2010; Vakili et al., 2013) in total VFA concentration in many *in vitro* and *in vivo* studies. There are some studies showing increased concentrations of total VFA in the rumen due to supplementation with cinnamaldehyde at 0.2 g/kg (Chaves et al., 2008a), and EO extract from oregano at 0.25 g/kg (Wang et al., 2009) increased total VFA without affecting nitrogen metabolism in dual flow continuous culture fermenters. Evans and Martin (2000) noted that 400 mg/l of thymol decreased the total VFA concentration and the proportion of acetate and propionate, whereas the acetate-to-propionate ratio increased. Chaves et al. (2008a) evaluated the effects of carvacrol and cinnamaldehyde in growing lambs and reported an increase in the total VFA concentration and that the molar proportions of acetate, propionate and the acetate-to-propionate ratio were not affected. The current study found no effect of juniper oil on total and individual VFA concentrations among the treatment groups but indicated significant differences among treatment periods. Responses of EO on total VFA concentrations may depend upon the types

Table 7 The effects of juniper oil supplementation on some antioxidant blood parameters

Group	Experiment period (days)	SOD (%) Mean ± SE	GPx (μmol/min/ml) Mean ± SE	TAC (mM) Mean ± SE	Catalase (μmol/min/ml) Mean ± SE
1	1st	57.7 ± 2.07	1.32 ± 0.15	0.49 ± 0.05	109.2 ± 16.36
	56th	57.6^{C} ± 2.61	1.16^{A} ± 0.19	0.36^{AB} ± 0.08	90.9^{AB} ± 15,27
2	1st	58.0 ± 2.85	1.35^{a} ± 0.24	0.49^{a} ± 0.07	75.4 ± 20.14
	56th	59.7^{B} ± 2.71	0.74^{bB} ± 0.05	0.28^{bB} ± 0.05	93.1^{AB} ± 5.86
3	1st	62.4 ± 2.50	1.05^{a} ± 0.13	0.41 ± 0.06	114.4 ± 14.85
	56th	66.3^{AB} ± 1.75	0.72^{bB} ± 0.08	0.42^{A} ± 0.05	87.9^{B} ± 9.58
4	1st	60.7^{a} ± 2.18	0.97 ± 0.10	0.44 ± 0.08	78.9 ± 12.51
	56th	68.2^{bA} ± 1.47	0.76^{B} ± 0.07	0.41^{A} ± 0.04	102.7^{A} ± 15.19

SOD, superoxide dismutase (%); GPx, glutathione peroxidase (μmol/min/ml).
TAC, total antioxidant capacity (mM) catalase (μmol/min/ml).
Different letters in the same group in each column indicate statistical differences ($p < 0.05$).
Data were analysed by Mann–Whitney *U*-test was used for paired comparisons.
Different letters (uppercase) within the same column indicate significant differences among treatment groups.
Different letters (lowercase) within the same column indicate significant differences among treatment period.

of substrate fed to the ruminants, and the effects of EO may depend upon the pH of the ruminal fluid.

The effects of Juniper oil supplementation on rumen protozoa population

The structure of rations consumed determines the number of bacteria and protozoa in rumen and depending on that, ratio of volatile fatty acids can vary. In most of the studies conducted, it was reported that the plant extracts have an impact on the number of protozoa. In a previously published study, it was shown that bioactive components from juniper essential oil, especially α-pinene and sabinene, possess a high inhibitory effect against many micro-organisms with small minimum inhibitory concentration (MIC) values (Glišić et al., 2007). Essential oils, which are known for their antimicrobial activity, have been proposed as modulators of rumen fermentation. There are mixed reports on the effects on rumen protozoa. McIntosh et al. (2003) found no effect on protozoal activity by feeding a 1000 mg/d blend of essential oils to dairy cattle. Similarly, Newbold et al. (2004) found that feeding a 110 mg/d mixture of essential oils had no effect on the number of ruminal protozoa in cannulated sheep at either 2 or 6 h post-feeding. However, Ando et al. (2003) have shown that feeding 200 g/d of dried peppermint to cannulated steers significantly decreased protozoa numbers by approximately 50%. However, in this study, juniper oil supplementation had no effects on individual protozoa population concentrations, only on the total protozoa population. Clearly, the effect of essential oils on rumen protozoa varies with the oil being tested, although relatively high concentrations seem to be required to have an effect.

Table 8 The effects of Juniper oil supplementation on faecal pH and faecal scores

Group	Experiment period (days)	Faecal pH Mean ± SE	Faecal Score* Mean ± SE
1	1st	7.64 ± 0.05	3.20^{A} ± 0.20
	56th	7.53 ± 0.10	2.89 ± 0.35
2	1st	7.58 ± 0.12	2.50^{B} ± 0.22
	56th	7.70 ± 0.08	2.56 ± 0.34
3	1st	7.62 ± 0.09	2.75^{AB} ± 0.25
	56th	7.61 ± 0.09	2.33 ± 0.29
4	1st	7.69 ± 0.08	3.17^{aA} ± 0.17
	56th	7.45 ± 0.10	2.33^{b} ± 0.29

Different letters in the same group in each column indicate statistical differences ($p < 0.05$).
Data were analysed by Kruskal–Wallis test, and Mann–Whitney *U*-test was used for paired comparisons.
Different letters (uppercase) within the same column indicate significant differences among treatment groups.
Different letters (lowercase) within the same column indicate significant differences among treatment period.
*Faecal sample was scored in respect to consistency by the same researcher on all collection days according to the following system:
1 = watery, diarrhoea 2 = soft, unformed 3 = soft, formed 4 = hard, formed 5 = hard, dry pellets.

The effects of Juniper oil supplementation on antioxidant blood enzyme parameters

Halliwell and Gutteridge (1999) described several lines of defence against reactive oxygen species in animals.

Enzymes with important antioxidant functions include superoxide dismutase (SOD), which catalyses the dismutation of superoxide radical to hydrogen peroxide and water; catalase (CAT), which catalyses the breakdown of hydrogen peroxide to oxygen and water; and glutathione peroxidase (GPX), which facilitates the destruction of both hydrogen peroxide and organic peroxides. There is little information concerning the antioxidant enzymes in goats. The values of SOD, TAC, GPX and CAT of goats are shown in Table 8. The mean value of serum SOD was significantly higher in the experimental groups than in the control group. Moyo et al. (2012) observed that the phenolic content of *M. oleifera* leaves may contribute significantly to the ability of the extracts to adsorb and neutralize free radicals or decompose peroxides. Catalase is another antioxidant enzyme widely distributed in animal tissues (Oyedemi et al., 2010). The enzyme is reported to protect the system from highly reactive hydroxyl radicals through hydrogen peroxide decomposition. In this study, the highest CAT value was observed in group 4. The reduction of this enzyme activity may promote cellular damage caused by the assimilation of superoxide and hydrogen peroxide. However, in this study, the ingestion of *M. oleifera* leaves by the goats had a higher inhibition of catalase activity. Glutathione (GSH) is the most abundant intracellular thiol-based antioxidant present in millimolar concentrations. GSH plays an important role in maintaining the primary defence capacity of cells. Under normal physiological conditions, the GSH concentration is mainly controlled by glutathione peroxidase (GPX), glutathione reductase (GR) and glutathione S-transferases (GST) (Cnubben et al., 2001). The activity of GPX decreased the groups fed Juniper essential oil. In this study, the highest TAC values were determined in the groups fed high dosages of juniper volatile oil (Group 2 and Group 3).

The effects of Juniper oil supplementation on faecal pH and faecal score

There is little information on the effect of essential oil on faecal pH and faecal structure. The faecal pH levels were similar between the control and experimental groups in the current study (Table 8). Huang et al. (2010) reported that blended essential oil supplementation lowered the faecal pH of weanling pigs. Nardi et al. (2014) determined that the addition of a polyphenol-essential oil mixture did not affected faecal pH. In this study, faecal scores were significantly different among the groups at the start of the experiment but were not different at the end of the study. Faecal score parameters were lower in the experimental groups than in the control group. However, the decrease in faecal score had no clinical importance because it remained in an acceptable range and was not associated with diarrhoea. Unlü and Erkek (2013) determined that there were no effects of oregano or garlic essential oil supplementation on faecal score. Softened faeces can be postulated to be caused by irritation of the intestine by juniper essential oil.

Conclusion

The main goals of animal production are high productivity, healthy animals and quality animal products that are stable and appropriate for further processing. Accordingly, herbs and spices are not just appetite and digestion stimulants but can, with impact on other physiological functions, help sustain good health and welfare in animals and improve animal performance. In the current study, the addition of Juniper essential oil positively affected live weight gain, especially at the level of 0.8 ml/kg addition. Juniper essential oil added to kids at the studied levels (0.4 ml/kg, 0.8 ml/kg and 2.0 ml/kg) did not affect the rumen pH, TVFA, other VFA, rumen protozoa population or faecal pH. A number of studies have shown that the monoterpene components also contained in juniper essential oil enhance the oxidative stress resistance of living organisms through their antioxidant activity. In this study, the most important enzymes responsible for the neutralization of ROS (SOD, catalase and total antioxidant activities) were determined to significantly increase in the experimental groups. Additionally, the addition of juniper essential oils to the kid diets softened faeces without inducing diarrhoea.

In summary, the effects of EO supplementation are varied and often contradictory; this difference is most likely because of differences in the plant extracts used (the concentration and nature of an active ingredient can vary even within the same species grown in different locations and even more widely between even relatively closely related plant species), concentrations used and possibly also the basal diet. There is still a need to clarify the phytochemical composition and the mechanisms of action of many herbs, spices and their extracts and to further assess the appropriate and safe dose for specific circumstances and animal species.

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