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Effects of Oregano Essential Oil on Reduction of Weaning Age and Increasing Economic Efficiency in Holstein Friesian Calves

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Authors' Contribution
IT designed the study and carried out statistically analysis, HBO and MHS performed experimental work and laboratory analysis. NT analyzed the economical data. MA edited the paper.

Key words
Dairy calf, Thyme oil, Economic efficiency, Growth, Weaning

ABSTRACT

This study examined the potential use of oregano essentail oil as a milk additive for purposes of reducing weaning age; increasing economic efficiency; promoting calf growth as well as for improving blood parameters and general health status of Holstein Friesian calves. Twenty-eight Holstein Friesian calves were allocated into three groups: (a) Control calves were fed with whole milk (n=10), (b) OreganoLow (n=9), and (c) OreganoHigh (n=9) calves were fed with whole milk, plus 100 and 150 mg/l oregano essential oil per kg milk respectively. The amount of the milk offered on daily basis was calculated as 10% of each calf live weight and was given in two meal times. All calves were kept in individual pens during experimental period. Calves got weaned following the consumption of daily 900 g of concentrate feed over three consecutive days. Results demonstrated that OreganoLow calves marked improved growth performance, earlier weaning age and lower farm costs ($P < 0.05$) compared to Control and OreganoHigh calves. To conclude, dietary oregano essential oil seems to be a potential liquid feed additive that improves feed efficiency, health status, growth performance, and that reduces diarrhea incidents, hastens weaning age and minimizes dairy farm costs when used at the level of 100 mg/l.

INTRODUCTION

Information on calf growth and the cost of calf rearing is important for cattle production. The sustainability of any cattle enterprise is directly correlated with the successful raising of calves for replacement stock. Replacement dairy calves are susceptible to many pathogens that cause preweaning diseases during the milk drinking period. Acute scours usually occur in newborn calves aged 15 days and earlier and are clinically characterized by watery diarrhea, progressive dehydration, and death within a few days of its onset (Blood *et al*., 1992; Izzaddeen and Kaygısız, 2018). Hastening weaning age, with the intention of reducing feed and labor costs, has attracted special attention among researchers during the last decades (Owen and Larson, 1982; Kehoe *et al*., 2007; Ozkaya *et al*., 2015). Antibiotics were used as feed additives to shorten the period of milk drinking. However, these substances have been completely prohibited as feed additive in the European Union and Turkey since 2006 as they are suspected of contributing substantially to the increasing resistance among animal and human pathogens (regulation 1831/2003/EC and 26056/2006 TR). Due to these restrictions, companies have resorted to essential oils (EOs) as feed additives in order to increase the palatability of feed; stimulate appetite, saliva production and secretion of digestive enzymes as well as to promote longer villi lengths. EOs dietary supplementation also appears as a natural and eco-friendly alternative (Jouany and Morgavi, 2007). The improved appetite, digestion and absorption of nutrients can result in maximum growth potential in a shorter period of milk drinking period with reduced costs (Jouany and Morgavi, 2007; Mohammed *et al*., 2013). During last two decades a substantial increase in the use of aromatic herbs and essential oils as feed additives in animal nutrition was recorded. Essential oils are extracted from various aromatic plants generally grown in warm areas such

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as the Mediterranean, and tropical countries. They are liquid, volatile, rarely coloured and dissolvable in organic solvents with a generally lower density than that of water (Bakkali *et al*., 2008). Essential oils, known for their antimicrobial effects, have been proposed as modulators of rumen fermentation. One of the most important essential oils is that of oregano. Oregano essential oil has antifungal and antibacterial effects on numerous microrganisms such as *Erwinia amylovar*, *Bacillus subtilis*, *Escherichia coli*, *Hafnia alvei, Micrococcus luteus, Proteus vulgaris, Staphylococcus aureus* and *Streptococcus faecalis* mainly due to its basic components involving carvacrol, thymol, α-terpinene and p-cymene (Azzouz and Bullerman, 1982; Lawrence and Reynolds, 1984; Deans and Svoboda, 1990; Ezzeddine *et al*., 2001; Marino *et al*., 2001; Burt *et al*., 2007). During the neonatal life of calves, some pathogens such as *Escherichia coli* and *Salmonella* spp. can be found in their digestive tracts from first day of their lives. These bacteria can cause enteritis, enterotoxemia or much more severe septicemia in the small intestine of calves (Scrag, 1982; Webster, 1986; Thickett *et al*., 1990). However, limited information regarding the effects of oregano essential oil doses on reducing the weaning age and farm costs of dairy calves is available.

The main objective of this study was hence to investigate the possible use of oregano essential oil as a milk additive to reduce weaning age, increase economic efficiency, promote calf growth, improve blood parameters and minimize production costs of Holstein Friesian calves.

MATERIALS AND METHODS

Calves, diets and handling

The study was conducted on a private dairy farm in Hatay, Turkey. Twenty-eight Holstein Friesian calves were divided into 3 equal groups based on their birth weight and sex: (a) Control group calves fed with whole milk (n= 10), (b) OreganoLow calves (n= 9) and OreganoHigh calves (n= 9) fed with whole milk plus 100 and 150 mg oregano oil per kg milk respectively. Calves were weighed right after birth; taken away from their mothers within three hours; placed into individual pens and allowed to drink colostrum for three days. The calves started to be fed with whole milk or whole milk plus oregano oil on day 4 postpartum. The amount of the milk offered daily was calculated as 10% of each calf live weight and was given in two meal times. Calf starter feed, alfalfa hay and fresh water were available ad libitum. Calves were weaned after consuming 900 g of concentrate feed daily over three consecutive days. The commercial calf starter comprised of dry matter, 900g/kg; metabolic energy, 2800 Kcal/kg; crude protein, 198 g/kg; crude fiber, 55, g/kg; vitamin A, 12.000 IU/kg; vitamin D3, 3600 IU/kg; vitamin E, 25mg/kg; calcium, 1.5%; phosphorus, 0.5%. The calf starter ingredients were barley, 30; maize, 18; wheat bran, 10; soybean meal, 25; cotton seed meal, 5; sunflower meal, 5; molasses, 5; mineral and vitamin mixture, 2 in % and alfalfa hay nutrient content were dry matter, 850 g/kg; metabolic energy, 1530 Kcal/kg; crude protein, 150 g/kg; crude fiber, 290 g/kg.

The oregano (*Origanum onites* L.) plants were collected from the the farm of Mustafa Kemal University, Turkey in 2012 growing season. The stems, leaves and flowers of oregano plants were air-dried in the shade. Essential oil was isolated by hydrodistillation in a clevenger type apparatus for 3 h. Analysis of the essential oil was carried out using a Thermo Scientific Focus gas choromatograph equipped with mass spectrometry (GC-MS) using DB-5MS fused silica column. Helium was used as the carrier gas at a flow rate of 1 ml/min. Mass range m/z 50–650 amu. Mass spectrometry transfer line temperature 250 °C. MS ionization source temperature and injection port temperature were 220 °C. The samples were injected with a 250 split ratio. The injection volume was 1 µL. Oven temperature was programmed from 50 °C to 220 °C at 3 °C /min. The structure of each compound was identified by comparison of their mass spectrum with the Wiley Registry, 9th edition.

The data got gathered Xcalibur software program. The retention indices (RIs) were calculated for all volatile compontents using a homologous series of n-alkane standard solutions C_8–C_{20} (Fluka, product No. 04070) and C_{21}–C_{40} (Fluka, product No. 04071). The chemical composition of the oregano essential oil is shown in Table I.

Calf performance characteristics such as weaning body weight, total body weight gain, daily live weight gain, weaning age, initial hay and concentrate feed consumption ages, fecal scores, total milk, hay, concentrate, oregano essential oil consumption, dry matter intake, feed conversion ratio, daily milk, hay and concentarate feed consumption were recorded during the experimental period. The number of calves with diarrhea and the severity of the diarrhea were determined daily. Faeces scores and health status were recorded each morning at feeding time.

Consistency of faeces was scored on a four-point scale. Score 1: Faeces were normal (soft solid consistency, no fluid); Score 2: Faeces were soft (semisolid consistency); Score 3: Faeces were loose (mostly solid, runny, but stays on top of bedding, and Score 4: Faeces were watery diarrhea (fluid, no solid materials which sifts through bedding (Larson *et al*., 1977).

Economic efficiency measurements

Cost parameters were classified according to the

methods implied by Debertin (1986). Total fixed costs (TFC) were considered the same for each group, e.g. labor, vaccination etc.

Total variable costs (TVC) during the weaning period included the cost of hay, concentrate feed, milk, oregano essential oil and veterinary services.

Total cost (TC) was calculated as the sum of the total fixed and variable costs.

Partial measures of efficiency were calculated according to Debertin (1986) as essential oil cost/total cost (EOC/TC), essential oil cost/total variable cost (EOC/TVC) and essential oil cost/feed cost (EOC/FC)

Table I. Chemical composition of oregano essential oil.

No	Compound name	RT	KI	Area (%)
1	Carvacrol	44.46	2237	65.2
2	Terpinen-4-ol	21.78	1588	6.12
3	β-Terpinene	8.86	1275	5.49
4	p-Cymene	9.68	1285	4.89
5	trans-Sabinene hydrate	19.72	1546	4.54
6	α-Terpinene	6.91	1183	2.17
7	Isoborneol	25.35	1597	2.12
8	α-Myrcene	6.51	1169	1.22
9	trans-Sabinene hydrate	16.58	1508	1.00
10	α-Bisabolene	26.36	1604	0.87
11	Caryophyllene	21.38	1575	0.73
12	1-Terpıneol	20.28	1560	0.55
13	α-Terpınolene	10.05	1453	0.50
14	Sum of the other 21 compounds	------	------	4.60
	Total			100,00

Components with levels equal or over 0.5% are shown. RT, Retention time; KI, Kovats index.

Statistical analysis

The data got tested via one-way ANOVA procedure of SPSS (Windows version of SPSS, release 16.00) and means were compared by using Duncan Multiple Range Test of the same software. Birth weight of calves was used as a covariant factor in the analysis of the live weight, daily weight gain and weaning weight. The data were presented as mean ± SE and significance was declared at ($P < 0.05$).

RESULTS

Chemical composition of oregano oil

According to the gas chromatography (GC-MS) analysis, oregano essential oil involved 34 components (Table I). The seven main ingredients, representing more than 2% of the total oil content, were carvacrol (65.2%), terpinen-4-ol (6.12%), β-terpinene (5.49%), p-cymene (4.89%), trans-sabinene hydrate (4.54%), α-terpinene (2.17%) and isoborneol (2.12%). According to these reports, carvacrol and β-terpinene are the main compounds of oregano essential oil. From this point of view, our results obtained from the GC-MS analysis are highly consistent with the previous studies.

Growth performance

Means ± standard error, and results of variance analysis for growth performance traits are shown in Table II and an economic analysis is provided in Table III. Birth weight differences were not significant among the calf groups ($P > 0.05$). The calves were assigned to treatments based on their birth weight. Thus, there should not be a significant difference in birth weight among different groups. Although the tracked weaning weight differences among the calves were not significant ($P > 0.05$), the weaning weights of the OreganoLow calves were 2.2 and 1.3 kg heavier than that of the Control and the OreganoHigh calves respectively. The non-significant weaning weight differences among three groups could be attributed to earlier weaning age of the OreganoLow calves (Table II).

The tracked differences for the total body weight and daily live weight gains among calves were significant ($P < 0.05$). The OreganoLow calves had 3.3 and 2.7 kg higher total body weight and 0.108 and 0.063 kg greater daily live weight gain compared with that of the Control and the OreganoHigh calves respectively (Table II). Faecal scores (Table II) among three groups were significantly different ($P < 0.05$) since the OreganoLow calves had lower faecal scores than the Control and OreganoHigh calves by 1.77 and 1.07 point respectively. As indicated, the OreganoLow calves had less frequent diarrhea which could be attributed to the diarrhea prevention characteristic of oregano oil.

The differences tracked for weaning age among calf groups were significant ($P < 0.05$). OreganoLow and OreganoHigh calves fed with whole milk supplemented with oregano essential oil at the level of 100 and 150 mg/l respectively had earlier weaning ages than that of the Control group calves, and weaning ages for the OreganoLow calves were 7.2 and 1.7 days earlier than that of the Control and the OreganoHigh calves respectively (Table II).

Initial hay and concentrate feed consumption ages were significantly different among three groups ($P < 0.05$). The OreganoLow calves had 2.9 and 1.3 days

Table II. The effects of dietary oregano oil on calf growth performance (mean ± SE).

Growth parameters	Control (n=10)	Oregano low (n=9)	Oregano high (n=9)	p values
Initial BW (kg)	38.8 ± 3.16	37.7 ± 2.82	39.1 ± 2.95	0.910
Weaning BW (kg)	59.4 ± 8.51	61.6 ± 9.28	60.3 ± 7.63	0.165
Total BW gain (kg)	20.6 ± 2.48^{a}	23.9 ± 3.01^{b}	21.2 ± 2.89^{ab}	0.021
Daily live weight gain (kg)	0.34 ± 0.01^{a}	0.44 ± 0.01^{b}	0.38 ± 0.01^{c}	0.002
Weaning age (d)	60.6 ± 2.8^{a}	53.4 ± 2.2^{b}	55.1 ± 2.5^{c}	0.004
Initial hay consumption age (d)	13.6 ± 1.1^{a}	10.7 ± 1.5^{b}	12.0 ± 1.2^{c}	0.038
Initial concentrate consumption age (d)	11.0 ± 1.4^{a}	9.1 ± 2.0^{b}	10.3 ± 1.2^{c}	0.042
Average fecal score (p)	2.85 ± 0.079^{a}	1.08 ± 0.031^{b}	2.15 ± 0.053^{c}	0.002
Total milk consumption (kg)	252.5 ± 10.1^{a}	225.5 ± 9.1^{b}	232.3 ± 8.2^{c}	0.001
Total hay consumption (kg)	6.16 ± 0.30	6.23 ± 0.32	6.14 ± 0.37	0.074
Total concentrate consumption (kg)	30.94 ± 2.73^{a}5^{a}	28.91 ± 2.53^{b}	28.70 ± 2.47^{b}	0.001
Total dry matter intake (kg)	64.7 ± 5.23^{b}	59.5 ± 3.98^{a}	60.1 ± 4.09^{ab}	0.047
Feed conversion ratio	3.14 ± 0.05^{a}	2.49 ± 0.08^{b}	2.83 ± 0.04^{c}	0.032
Daily milk consumption (kg)	4.17 ± 0.07	4.23 ± 0.04	4.22 ± 0.05	0.072
Daily hay consumption (kg)	0.102 ± 0.018^{a}	0.117 ± 0.019^{b}	0.112 ± 0.011^{c}	0.023
Daily concentrate consumption (kg)	0.51 ± 0.06^{a}	0.54 ± 0.07^{b}	0.52 ± 0.09^{c}	0.024

Mean values within a row having different superscripts are different (P < 0.05); Control calves, fed whole milk; OreganoLow Calves, whole milk plus 100 mg oregano oil per kg milk; OreganoHigh Calves, whole milk plus 150 mg oregano oil per kg milk.

Table III. The economy of dietary oregano oil in calf feeding (mean ± SE).

Economical parameters	Control (n=10)	Oregano low (n=9)	Oregano high (n=9)	p values
Total fixed cost per calf (USD)	273.4±14.21	273.4±14.21	273.4±14.21	-
Total variable cost per calf (USD)	263.8±20.1^{c}	167.5±17.4^{a}	185.7±19.5^{b}	<0.001
Milk cost (USD)	123.4±14.2^{c}	110.2±12.9^{a}	113.6±13.0^{b}	0.020
Hay cost (USD)	1.64±0.09^{c}	1.52±0.01^{b}	1.50±0.05^{a}	0.013
Concentrate feed (USD)	16.5±2.5^{b}	15.42±3.5^{a}	15.31±2.7^{a}	0.015
Essential oil (USD)	0	2.5±0.06	4.2±0.08	0.003
Veterinary drugs and services (USD)	122.2±7.1^{c}	37.8±6.8^{a}	51.1±9.5^{b}	0.002
Total costs per calf (USD)	537. 2±19.5^{c}	440.9±15.6^{a}	459.1±19.1^{b}	0.024
Total cost per kg live weight gain (USD)	26.1±3.8^{c}	18.4±2.2^{a}	21.7±3.0^{b}	0.013
Total cost per kg DM (USD)	8.3±1.1^{b}	7.4±0.9^{a}	7.6±1.2^{a}	0.044
Essential oil cost/total cost (%)	0	0.57±0.01	0.91±0.07	<0.001
Essential oil cost/total variable cost (%)	0	1.49±0.04	2.26±0.09	0.003
Essential oil cost/feed cost (%)	0	14.76±1.8	24.99±2.4	<0.001

Mean values within a row having different superscripts are different (P < 0.05); Control Calves: fed whole milk, OreganoLow Calves: whole milk plus 100 mg oregano oil per kg milk; OreganoHigh Calves: whole milk plus 150 mg oregano oil per kg milk.

earlier initial hay consumption age and 1.9 and 1.2 days earlier initial concentrate feed consumption age compared with the Control and the OreganoHigh calves respectively. The lower initial hay and concenrate feed consumption ages of the Oregano Low calves resulted from the increased saliva secretion, increased appetite and

the promotion of rumen development. Apart from total hay consumption, there were significant differences in total milk and concentrate feed consumption among three goups ($P < 0.05$). The Oregano Low calves consumed 27 and 6.8 kg less milk compared with the Control and the OreganoHigh calves respectively. Although there was no total hay consumption difference among calf groups, the OreganoLow calves consumed 0.076 and 0.087 kg more hay compared with the Control and the OreganoHigh calves. Compared with the Control group, the OreganoLow and the OreganoHigh calves consumed 2.032 and 2.242 kg less concentrate feed respectively (Table II). Total dry matter intake differences were significant among three groups ($P < 0.05$). There were also significant ($P < 0.05$) differences among the calves in terms of feed conversion ratio. The OreganoLow calves had 0.65 and 0.34 greater feed conversion compared with the Control and OreganoHigh calves respectively. Also, the OreganoLow calves consumed more daily hay (0.015 and 0.005 kg) and concentrate (0.031 and 0.021 kg) compared with the Control and OreganoHigh calves respectively. This fact resulted in the earlier weaning age of the OreganoLow calves. Although there was no difference in daily milk consumption among calf groups, total milk consumption was significantly different ($P < 0.05$) among all groups (Table II).

Economical analysis

Total variable cost per calf was significantly different ($P < 0.05$) among calf groups in terms of milk, hay, concentrate feed, oregano oil, veterinary drugs as well as service costs. The OreganoLow calves had 96.3 and 18.2 USD lower total variable cost per calf than that of the Control and the OreganoHigh calves respectively. There was a significant ($P < 0.05$) difference among three groups with respect to total cost per kg live weight gain. The OreganoLow calves had 7.7 and 3.3 USD lower total cost per kg live weight gain compared with the Control and the OreganoHigh calves respectively. Total cost per kg dry matter varied significantly among groups ($P < 0.05$). The OreganoLow calves had 0.9 and 0.2 USD lower total costs per kg dry matter compared with Control and OreganoHigh calves. The ratio of oregano oil cost within the total costs, total variable costs, and total feed costs were significantly different between the OreganoLow and the OreganoHigh calves. The OreganoLow calves had 0.34%, 0.77% and 10.23% better cost ratio compared with the OreganoHigh calves ($P < 0.05$) (Table III).

The milk cost had the highest ratio in the total variable costs followed by the veterinary drugs and services costs. Total cost per kg live weight gain and total cost per dry matter intake were, also, the lowest in the OreganoLow calves. The OreganoLow treatment had better esential oil cost/total cost (%), essential oil cost/total variable cost (%) and essential oil cost/feed cost (%) than the Oregano High calves ($P < 0.05$) (Table III).

DISCUSSION

Oregano essential oil has been known to possess antifungal and antibacterial activities against many different types of microorganisms, including milk-born pathogens (Bampidis *et al*., 2006; Benchaar *et al*., 2008; Arslan and Dervis, 2010; Shafaghat, 2011; Rahbar *et al*., 2012). The antifungal and antibacterial activities of essential oils are mainly due to the oxygenated terpenoids in essential oils while some hydrocarbons also display antifungal and antibacterial effects.

Feeding efficiency, diarrhea reduction, weaning age, growth performance, and general health profiles of Holstein Friesian calves were improved by the use of oregano essentail oil as a milk additive. Similar results were reported by Bampidis *et al*. (2006), Castillejos *et al*. (2008), Simitzis *et al*. (2008), Hernandez *et al*. (2009), Unlu and Erkek (2013), Vakili *et al*. (2013), Van der Vliet and Cardozo (2013), Ozkaya *et al*. (2015) and Seifzadeh *et al*. (2017). Bampidis *et al*. (2006) reported that dried oregano leaves administered as an oral solution to calves with diarrhoea may be as effective in the treatment of colibacillosis. In contrast, Chaves *et al*. (2008) reported that oregano essential oil did not improve growth or feed efficiency of lambs. In addition, Santos *et al*. (2015) reported that oregano essential oil reduces amount of milk replacer consumption and did not improve growth of calves. It can be claimed that these differences were derived from the different animals species used and/or oregano oil forms. Experimental design was different in that some animals were fed with oregano oil sprayed onto the diet, some were fed with dried oregano, and some swallowed capsulated oregano oil. The results of the current study demostrated that the OreganoLow group of calves was weaned earlier than that of the Control or OreganoHigh groups. In addition to this, oregano essential oil improved calves' appetite and accelerated the stimulation of the digestive tract for food consumption and prolongs the due to antibacterial activities against many different types of bacteria. Wenk (2000), Simitzis *et al*. (2008) and Hernandez *et al*. (2009) indicated similar results, whereas contrasting results were published by Chaves *et al*. (2008). These discrepancies were derived from different doses and forms of essential oil. Therefore, the smell and/or overdose of essential oil can have a negative effect on rumen microflora and fermentation. A threshold profile, characterized by a virtual stop of fermentation when doses are higher than the

threshold level, occurs for thymol/carvacrol based essential oil. Effective dose could help determine which compound would be the most appropriate for a particular production objective, such as decreasing protein degradation. This also, highlights the toxic nature of oregano essential oil on rumen microbes, and that there is a narrow dose window for the successful adoption (Macheboeuf *et al*., 2008). Essential oils at high doses reduced the number of rumen proteolytic bacteria, decreased rumen deamination rate and decreased hay consumption (Garcia *et al*., 2007). Effects among essential oil components lead to antagonistic and synergistic interactions which are considered crucial for microorganisms. Some studies have demonstrated that whole or partially purified essential oil components are considered to have more intense antibacterial activity than single isolated components (Lachowicz *et al*., 1998; Hammer *et al*., 1999; D'Antuono *et al*., 2000; Ezzeddine *et al*., 2001; Mourey and Canillac, 2002).

The use of oregano oil in dairy calves improves economic efficiency. Our results overlap with the findings of Mohammed *et al*. (2013).

CONCLUSION

Essential oils are becoming increasingly popular for a wide variety of purposes including flavouring substances for food products, food preservation, alcoholic beverages, complementary medicine as well as cosmetic industries due to its antimicrobial, antioxidant and other biological activities. The results showed that oregano essential oil seems to be an ideal liquid feed additive to improve feed efficiency, health status, and growth performance of calves and to reduce diarrhea incidents and hastened weaning age. It also decreases farm costs when used in effective doses. However, further studies should be performed in large scale dairy farms under semi-commercial and commercial farm conditions.

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Statement of conflict of interest

Authors have declared no conflict of interest.

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