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Study of the effect of a combination containing some plant essential oils on growth performance, blood parameters, and health of Holstein suckling calves

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ABSTRACT

This study investigated the effects of supplementing a commercial phytogenic powder containing a blend of peppermint, coriander, cumin, and anise essential oils on growth performance, blood parameters, and health status of Holstein suckling calves. Thirty-six newborn female Holstein calves were randomly assigned to one of three dietary treatments: 1) a control diet (no experimental powder); 2) a diet supplemented with 2 kg of the essential oil blend powder per ton of starter (EO2); and 3) a diet supplemented with 4 kg of the essential oil blend powder per ton of starter (EO4). Calves were weaned at 79 days of age, and the trial continued for 21 days post-weaning. Average dry matter intake, feed efficiency, body weight, and average daily gain were significantly greater ($P<0.05$) for calves fed the EO2 diet compared to the other treatments. Similarly, average withers height and average daily height gain were also significantly improved ($P<0.05$) in the EO2 group. Mean blood urea nitrogen concentration was significantly higher ($P<0.05$) in the EO2 treatment, while mean serum glucose concentration was significantly lower ($P<0.05$). Regarding health, the EO2 treatment exhibited significantly better scores ($P<0.05$) for nasal discharge, cough, and eye discharge compared to the control and EO4 treatments. No significant differences were observed among treatments for fecal score or ear status. In conclusion, the addition of 2 kg of the tested essential oil blend powder per ton of starter improved growth performance and health status in Holstein suckling calves, primarily by increasing dry matter intake.

Keywords: essential oils, Holstein calves, growth performance, dry matter intake, blood parameters, health status

INTRODUCTION

Proper nutrition of dairy calves and their impact on the health and growth of these animals plays a decisive role in the future production and economic status of dairy herds. One of the most vital principles in raising dairy calves is to encourage them to consume starter (Ababakri et al., 2012). Antibiotics and plant essential oils have been considered as the main feed additives for improving the growth and health of calves in recent decades. Unfortunately, the use of growth-promoting antibiotics leads to the development of antibiotic resistance (Blair, 2017). Despite widespread recommendations against the use of synthetic chemical growth promoters in animal feed, previous studies show that about 300 chemical compounds are used in agriculture, which indirectly endanger human health (Blair, 2017). On the other hand, after the ban on the use of antibiotics by the European Union due to the possibility of resistance in the human body, as well as the popularity of healthy and organic goods, the use of aromatic plant essential oils has grown considerably (Franz et al., 2010). Some

studies have confirmed the use of plant essential oils as additives in animal feed to improve feed efficiency and maintain animal health (Benchaar et al., 2008; Salazar et al., 2019; Liu et al., 2020). It has been reported that essential oils derived from medicinal plants have low molecular weight and that the hydrophobic oils extracted from these plants as secondary metabolites can increase feed intake and lead to earlier weaning of calves (Burt, 2004; Jeshari et al., 2016). Salazar et al. (2019) showed that the use of plant essential oils in the diet of dairy calves had positive effects on digestibility, increased dry matter intake, reduced weaning age, reduced diarrhea, and improved their health. The use of a combination of plant essential oils (including p-cymene, thymol, carvacrol, caryophyllene, cineole, and terpinene) increased dry matter intake, height, and body weight of Holstein dairy calves from three days of age to four weeks after weaning (Liu et al., 2020). In studies on the use of plant essential oils, different results have been reported. Ebrahimi et al. (2017), using the essential oils of peppermint and Shiraz thyme, despite positive effects on feed intake and body weight before weaning, did not observe a positive effect on feed conversion rate after weaning as well as nutrient digestibility throughout the experiment. Hosoda et al. (2006) showed that the use of medicinal plants (peppermint, cloves) in the diet of Holstein steers leads to a significant increase in the concentration of urea nitrogen in plasma. To investigate the effect of plant essential oils, Swedzinski et al. (2020) added the essential oils of pennyroyal and thyme to the diet of Holstein dairy calves and did not observe a significant effect on the status of fecal score, ear status, cough rate, nasal discharge, and eye discharge. Other properties of plant essential oils include strengthening the immune system and the antioxidant defense system (Mishra et al. 2020; Pavlić et al., 2021). Some plant essential oils affect the pH and ammonia concentration of rumen fluid, the amount of methane produced, and fiber digestion (Williams, 2007). Gunal et al. (2017) reported that adding anise essential oil (500 mg/L) to a culture medium containing dairy cow rumen fluid reduces methane production. In other studies, the use of coriander essential oil (Jahani-Azizabadi et al., 2014) as well as cumin essential oil, in addition to reducing methane production in rumen fluid under laboratory conditions, has improved the energy and protein efficiency of nutrients. These findings suggest that these essential oils may be a suitable alternative to growth-promoting antibiotics (Jahani-Azizabadi et al., 2015).

Few studies have been conducted on the effect of medicinal plants on Holstein dairy calves; therefore, the objective of the present study was to evaluate the effects of different inclusion levels of a combination of peppermint, coriander, cumin, and anise essential oils on growth performance, nutrient digestibility, blood biochemical parameters, and health status of Holstein suckling calves.

MATERIALS AND METHODS

This experiment was conducted in Azadi Dairy and Livestock Company in Varamin County, Tehran Province.

Animals, feeding, and experimental diets

In this experiment, 36 newborn Holstein calves with an average initial weight of 38.45 ± 1.8 kg (12 calves in each treatment) were randomly placed in separate individual pens. Pasteurized milk was given in two meals, morning (6 o'clock) and evening (18 o'clock), with an average of 5 litres per day. Water was provided freely with an automatic drinking system. The calves had free access to the starter throughout the experimental period (the starter contained 5% in the first month, 10% in the second month, and 15% in the third month of chopped dry alfalfa). The composition of the starter is shown in Table 1. In order to select healthy calves and also low starter consumption at early ages, the calves were tested from 14 days of age to 21 days after weaning (100 days of age). Weaning was also done at the age of 79 days. The starter was provided to the calves from the third day, after the morning milking, and daily, according to the feed composition of the treatments. Throughout the 86-day experimental period (from 14 to 100 days of age), During the experiment, the remaining starter was collected, measured, and recorded daily.

Table 1. Igredients and chemical composition of basal starter (on a DM basis)

Ingredient, % of DM		Nutrients and chemical composition	
Corn grain	59	Metabolizable energy (ME), Mcal/kg of DM[2]	2.96
Soybean meal	30	Dry matter (DM), % of concentrate	91.4
Wheat bran	8.5	Crude protein (CP), % of DM	20.2
Calcium carbonate	1	Ether extract (EE), % of DM	3.17
Vitamin-mineral mixture, Selenium-free[1]	1	Natural detergent fiber (NDF), % of DM	19.38
Salt	0.5	Calcium, % of DM	0.83
		Phosphorus, % of DM	0.42

[1] Contained per kilogram of the supplement: Vitamin A: 500,000 IU, Vitamin D3: 100,000 IU, Vitamin E: 1000 IU, Calcium: 190 g, Magnesium: 19 g, Sodium: 50 g, Manganese: 2,000 mg, Ferrous: 3,000 mg, Copper:300 mg, Zinc: 3,000 mg, Cobalt: 100 mg, Iodine: 100 mg, Antioxidant: 3,000 mg.
[2] Calculated based on NRC (2001)

The powder of medicinal plants under study was a commercial combination from Pars Imen Darou Company (Bioherbal), including the essential oils of peppermint, coriander, cumin, and anise; the exact ratio of each essential oil in the tested powder is manufacturer-specific information. The main bioactive compounds found in these essential oils are menthol and menthone for peppermint, linalool for coriander, cumin aldehyde for cumin, and anethole for anise (Bakkali et al., 2008). The experimental diets included: 1) Control: starter without essential oil, 2) Second diet (EO2): containing 2 kg of plant essential oil powder per ton of starter, 3) Third diet (EO4): containing 4 kg of plant essential oil powder per ton of starter.

Blood parameters and health score

Blood samples were taken from the jugular vein of the calves by heparin-containing vacuum tubes to measure the concentration of glucose, total protein, blood urea nitrogen, triglycerides, and cholesterol after morning feeding at the beginning of the experiment (first stage), weaning (second stage), and the end of the experiment (third stage). The prepared blood samples were immediately placed in a centrifuge at 3000 rpm for 15 minutes. After separating the plasma, the samples were stored at -20 degrees Celsius until analysis. Blood parameters were measured using kits from Pars Azmoun Company (made in Iran), in the laboratory of the Department of Animal Science, Faculty of Agriculture, University of Tehran. To evaluate the health status of the calves, every day after the evening meal distribution, the condition of the eyes and nose in terms of secretions, the position of the ears, the amount of cough, and the consistency of the feces, based on the modified scoring system of the University of Wisconsin (Table 2), each calf was given a score for the mentioned factors (McGuirk, 2013).

Body weight, withers height and digestibility

The body weight and withers height of the calves were measured at the beginning of the experiment, at two months, at weaning (79 days), and at the end of the experiment (100 days of age), before the morning milk distribution. The apparent digestibility of dry matter and crude protein was measured based on the AOAC (1990) method, and the apparent digestibility of organic matter was calculated based on the information obtained from the apparent digestibility of dry matter. For this purpose, during the final five days of the experiment, both the remaining feed and the fecal matter of each calf were collected daily and separately. Subsequently, the five daily samples for each calf were pooled to create a single composite sample of both feed and feces. These samples were then stored at -20 °C until laboratory analyses were conducted.

Table 2. Calf health scoring criteria

Clinical Parameter[1]	0	1	2	3
Nasal Discharge	Normal, serous discharge	Small amount of unilateral, cloudy discharge	Bilateral, cloudy, or excessive mucus	Copious, bilateral mucopurulent nasal discharge
Ocular Discharge	Normal	Mild ocular discharge	Moderate bilateral ocular discharge	Heavy ocular discharge
Ear Position	Normal	Ear flicking	Slight unilateral ear drop	Severe head tilt or bilateral ear droop
Cough Score	No cough	Induce single cough	Induce repeated coughs or occasional spontaneous cough	Repeated spontaneous coughing
Feces	Normal	Semi-formed, pasty	Loose, but stays on top of the bedding	Watery, sifts through bedding

[1] McGuirk (2013)

Statistical analysis

The data of this experiment were analyzed in the form of a completely randomized design with three experimental diets and 12 replications (calves) in each diet.

The statistical model used was:

$$Y_{ijkl} = \mu + A_i + B_j + C_k + IBW_l + AB_{ij} + e_{ijkl}$$

where:

- Y_{ijkl} denotes the observed value of the dependent variable;
- μ denotes the overall mean;
- A_i denotes the fixed effect of diet (i = 1, 2, 3; three diet groups);
- B_j denotes the fixed effect of period (j = 1, 2; two periods: pre-weaning and post-weaning);
- C_k denotes the random effect of animal (k = 1 to 36; each individual calf);
- IBW_l denotes the covariate for initial body weight (l = 1 to 36; corresponding to each calf);
- AB_{ij} denotes the interaction effect between diet and period; and
- e_{ijkl} denotes the random residual error associated with each observation.

Data were statistically analyzed using SAS software (version 9.1; SAS Institute Inc., Cary, NC, USA), according to the methods of Steel and Torrie (1980). For traits measured repeatedly over time, the PROC MIXED procedure was used. For measured only once, the PROC GLM procedure was applied. Differences among treatment means were considered statistically significant at $P<0.05$.

RESULTS

Growth performance and dry matter intake

The effects of using the studied combination of medicinal plants in the starter of dairy calves on growth performance, feed conversion, and dry matter intake at the time of weaning, as well as the end of the experiment (100 days of age), are shown in Table 3. These results show a statistically significant difference in the increase in dry matter intake for the EO2 treatment compared to other treatments ($P<0.05$). Body weight at weaning was also significantly greater in the EO2 group than in the other treatments ($P<0.05$). Feed efficiency ratio was significantly improved in the EO2 treatment compared to the control and EO4 groups($P<0.05$). At the end of the experiment (100 days of age), although the EO2 treatment showed more weight gain than the other treatments, this increase was not statistically significant. Average daily gain during the experimental period was significantly higher in the EO2 group compared to other treatments. Withers' height at weaning and average daily height gain were also significantly greater in calves receiving the EO2 treatment than in the control and EO4 groups ($P<0.05$).

Table 3. Average dry matter intake, feed efficiency, and growth performance of suckling calves fed with different experimental diets

Performance	Treatment[1]			SEM	*P* value[2]		
	Control	EO2	EO4		T	P	T × P
Total DMI (kg/d)	0.96[b]	1.20[a]	0.97[b]	0.05	0.02	<.0001	0.29
FE (%)	0.67[b]	0.59[a]	0.65[b]	0.02	0.03	<.0001	0.41
BW at weaning (kg)	82.68[b]	87.53[a]	82.51[b]	1.45	0.03	<.0001	0.37
BW at 100 days of age (kg)	106.10	111.55	104.77	2.10	0.06	-	-
ADG (kg/d)	0.58[b]	0.66[a]	0.58[b]	0.02	0.03	<.0001	0.38
WH at weaning (cm)	95.82[b]	98.43[a]	96.25[b]	0.71	0.03	<.0001	0.14
WH at 100 days of age (cm)	109.86	111.43	108.16	1.53	0.33	-	-
ADH (cm)	0.26[b]	0.30[a]	0.27[b]	0.01	0.01	<.0001	0.37

[1] Treatments include: Control: diet without essential oils, EO2: diet with 2 kg of experimental powder per ton of starter, EO4: diet with 4 kg of experimental powder per ton of starter.

[2] P-values include: T: Treatment, P: Period, T×P: Treatment × Period.

Abbreviations: DMI, dry matter intake; FE, feed efficiency; BW, body weight; ADG, average daily gain; WH, withers height; ADH, average daily height.

* Different lowercase Latin letters in each row indicate significant differences between the presented means ($P<0.05$).

Digestibility

The results related to the apparent digestibility of dry matter, organic matter, and crude protein are shown in Table 4. The digestibility of dry matter, organic matter, and crude protein was investigated during the experimental period. The results showed that the EO2 treatment had the highest digestibility in all three nutrients. However, no significant difference was observed between the EO2 treatment and other treatments in the digestibility of any of the nutrients. In other words, although the EO2 treatment appeared to perform better in digesting nutrients, this superiority was not statistically significant.

Blood parameters

The results of blood parameters during the entire experimental period are shown in Table 5. The results showed that the concentration of blood urea nitrogen (BUN) in the EO2 treatment was significantly higher than in the control and EO4 treatments ($P<0.05$). In contrast, the glucose concentration in the EO2 treatment was significantly lower than in the control and EO4 treatments ($P<0.05$). Regarding other blood parameters, namely triglycerides, total protein, and cholesterol, no significant difference was observed between the treatments.

Table 4. Average nutrient digestibility of experimental diets

Performance	Treatment[1]			SEM	*P* value
	Control	EO2	EO4		
Dry Matter	74.92	77.40	75.64	1.82	0.62
Organic matter	77.17	78.64	76.88	1.70	0.74
CP	76.71	77.94	77.71	2.03	0.90

[1] Treatments include: Control: diet without essential oils, EO2: diet with 2 kg of experimental powder per ton of starter, EO4: diet with 4 kg of experimental powder per ton of starter

Abbreviations: CP, crude protein

Table 5. The average concentration of blood parameters of suckling calves fed with different experimental diets

Performance	Treatment[1]			SEM	*P* value[2]		
	Control	EO2	EO4		T	P	T × P
BUN (mmol/L)	4.66[b]	5.00[a]	4.60[b]	0.07	0.0013	<.0001	0.0005
Glucose (mmol/L)	3.99[b]	3.46[a]	3.66[b]	0.17	0.03	<.0001	0.46
Triglyceride (mmol/L)	0.57	0.56	0.58	0.03	0.89	<.0001	0.37
Total Protein(g/L)	82.54	84.84	74.06	4.05	0.15	<.0001	0.86
Cholesterol (mmol/L)	1.63	1.69	1.64	0.05	0.75	<.0001	0.77

[1] Treatments include: Control: diet without essential oils, EO2: diet with 2 kg of experimental powder per ton of starter, EO4: diet with 4 kg of experimental powder per ton of starter.

[2] P-values include: T: Treatment, P: Period, T×P: Treatment × Period.

Abbreviations: BUN, blood urea nitrogen.

* Different lowercase Latin letters in each row indicate significant differences between the presented means ($P < 0.05$).

In other words, although the EO2 treatment in some cases (such as triglycerides and cholesterol) showed higher values and in some other cases (such as total protein) showed lower values, these differences were not statistically significant.

Health score of calves

The results related to health status scoring during the entire experimental period are shown in Table 6. Regarding the status of nasal discharge, the results showed that the EO2 treatment significantly showed a greater number of days with a score of 0 (no discharge) compared to the control and EO4 treatments ($P<0.05$); in other words, the calves in the EO2 treatment had less nasal discharge. In contrast, the number of days with a score of 1 (mild discharge) in the EO2 treatment was significantly lower than in other treatments ($P<0.05$). Regarding the cough score, the results were similar to the nasal discharge score. The EO2 treatment significantly showed a greater number of days with a score of 0 (no cough) compared to the control and EO4 treatments ($P<0.05$). Also, the number of days with a score of 1 (mild cough) in the EO2 treatment was significantly lower than in other treatments ($P<0.05$). Regarding the eye discharge score, the EO2 treatment significantly showed a greater number of days with a score of 0 (no discharge) compared to the control treatment ($P<0.05$). Regarding the fecal and ear scores, no significant difference was observed between the treatments in any of the cases (scores 0 to 3). This means that the different treatments did not have a specific effect on the ear status and fecal score of the calves.

Table 6. Health scores for suckling calves fed with different experimental diets

Health scores	Treatment			SEM	*P* value
	Control	EO2	EO4		
Total days of fecal scores					
Score 0	90.33	93.16	91.66	1.23	0.28
Score 1	6.58	5.35	6.35	1.05	0.32
Score 2	2.83	1.41	1.66	0.82	0.43
Score 3	0.25	0.08	0.33	0.14	0.44

Continued. Table 6

Health scores	Treatment			SEM	*P* value
	Control	EO2	EO4		
Total days of nasal scores					
Score 0	87.83[b]	93.50[a]	91.41[b]	1.33	0.01
Score 1	11.08[b]	5.50[a]	7.33[b]	1.20	0.0083
Score 2	0.75	0.66	1	0.37	0.80
Score 3	0.33	0.33	0.25	0.17	0.92
Total days of cough scores					
Score 0	78.91[b]	92.58[a]	90.33[a]	1.45	0.0001
Score 1	17.83[b]	5.33[a]	7.16[a]	1.26	0.0001
Score 2	1.92	1.41	1.41	0.26	0.32
Score 3	1.33	0.66	1.08	0.21	0.10
Total days of eye scores					
Score 0	68.91[b]	83.41[a]	78.66[a]	3.85	0.03
Score 1	0.91	0.75	2	1.08	0.68
Score 2	29.33	15.41	19.33	4.07	0.05
Score 3	0.83	0.41	0	0.49	0.49
Total days of ear scores					
Score 0	93.33	90.08	90.25	2.21	0.51
Score 1	1	1.41	1.83	0.57	0.59
Score 2	5.50	8.33	7.66	2.27	0.65
Score 3	0.16	0.16	0.25	0.13	0.88

[1] Treatments include: Control: diet without essential oils, EO2: diet with 2 kg of experimental powder per ton of starter, EO4: diet with 4 kg of experimental powder per ton of starter.

* Different lowercase Latin letters in each row indicate significant differences between the presented means ($P<0.05$).

DISCUSSION

The results of this study showed that the use of a combination of the essential oils of peppermint, coriander, cumin, and anise at a rate of 2 kg per ton of starter for Holstein dairy calves (EO2) significantly increased starter intake and growth performance in calves. The lower feed intake and growth performance observed in calves fed the EO4 diet compared with the EO2 treatment may be explained by a dose-dependent sensory effect of essential oils. Moderate inclusion levels of essential oils can enhance feed palatability through their pleasant aroma, thereby stimulating feed intake. However, higher inclusion levels may intensify the aroma and flavor of the diet acceptance as previously reported by Franz et al. (2010) and Benchaar et al. (2007). In such cases, the strong sensory characteristics of essential oils may outweigh their positive effects, leading to a reduction in voluntary feed intake and consequently growth performance. These findings suggest that the beneficial effects of essential

oils on feed intake and performance are dependent on their inclusion level. These findings are consistent with the results of previous studies. For example, Franz et al. (2010) reported that essential oils, due to their pleasant aroma, increase palatability, and their antioxidant properties prevent spoilage and off-flavors in feed. In another study, Liu et al. (2020) showed that the use of a combination of plant essential oils (including p-cymene, thymol, carvacrol, caryophyllene, cineole, and terpinene) significantly increased dry matter intake, height, and body weight in Holstein dairy calves. Similarly, Salazar et al. (2019) showed that adding a combination of plant essential oils (including thymol and carvacrol) to the diet of Holstein calves, with its positive effect on improving gut health, likely leads to increased intestinal reabsorption of nutrients and increased feed intake. These results are consistent with the findings of other studies (Kung et al., 2008; Chavez et al., 2008; Kholif et al., 2012; Vakili et al., 2013; Jashari et al., 2016). The antimicrobial properties of plant essential oils can reduce methane production and create conditions for improving energy and protein utilization, although not all studies confirm this. For example, in the studies of Benchaar et al. (2007) as well as the study of Ebrahimi et al. (2017), which was conducted using a combination of peppermint and Shiraz thyme essential oils in the diet of Holstein dairy calves, despite the positive effect on increasing body weight and feed conversion before weaning, no significant effect was observed on the amount of dry matter intake, feed conversion and body weight gain after weaning. In this study, although the apparent digestibility of dry matter, organic matter, and crude protein in the two groups that used the combination of plant essential oils in this experiment, EO2 and EO4, was higher than that of the control group, no significant effect was observed on the digestibility of nutrients. These findings are consistent with the results of the experiments of Ebrahimi et al. (2017), Salazar et al. (2019), and Mayer et al. (2007). However, Soltan (2009) showed that the use of a combination of plant essential oils (menthol essential oil, peppermint oil, and eucalyptus oil) in the diet of young calves has no effect on the digestibility of crude protein, but the digestibility of dry matter is increased. Also, in the experiment of Liu et al. (2020), which was previously mentioned, a significant difference in the digestibility of nutrients was reported for the group that received feed containing plant essential oils.

The use of a combination of plant essential oils in this experiment led to an increase in the concentration of blood urea nitrogen and a decrease in blood glucose concentration in the calf, which probably indicates better rumen growth and development. Adding peppermint essential oil to the starter of dairy calves increased the amount of blood urea nitrogen (Ababakri et al., 2012; Ebrahimi et al., 2017) and decreased blood glucose concentration during the experiment (Ebrahimi et al., 2017). Different results have been reported in the concentration of blood parameters in different experiments. For example, Hosoda et al. (2006) showed that the use of medicinal plants (peppermint, cloves and lemon) in the diet of Holstein calves, in agreement with the results of this experiment, did not create a significant difference in the concentration of total blood protein in calves, while they reported a significant increase in the concentration of blood urea nitrogen. They also, contrary to the results of this experiment, reported a significant difference in triglyceride concentration and no significant difference in blood cholesterol concentration.

Based on the results of this experiment, the use of a combination of plant essential oil powder has reduced the amount of cough as one of the symptoms of pneumonia and also reduced eye discharge in calves, which shows that this combination can be used as a natural alternative to antibiotics in the prevention of complications and diseases. Despite the antimicrobial effects of plant essential oils that may improve gut health (Jashari et al., 2016), in the results of this experiment, no significant effect was observed on fecal score, ear status, and nasal discharge. Soltan (2009), using a combination of plant essential oils (menthol, peppermint oil, and eucalyptus oil), reported that increasing the amount of plant essential oils increases the risk of diarrhea. Ababakri et al. (2012) reported that adding peppermint essential oil to the starter of dairy calves significantly increased their

fecal consistency score in the pre-weaning period and also affected the fecal score in the post-weaning stage. Katsoulos et al. (2017), by adding peppermint essential oil to the starter of calves, observed a significant effect in improving fecal score and diarrhea. Adding plant essential oils (pennyroyal and thyme) in the diet of newborn Holstein calves, contrary to the results of this experiment, did not have a significant effect on the score of nasal and eye discharge, and also in agreement with the results of this experiment, did not have a significant difference in the score of feces, ear status and nasal discharge (Swedzinski et al., 2020).

CONCLUSION

The use of a combination of plant essential oil powder (peppermint, coriander, cumin, and anise) at a rate of 2 kg per ton of Holstein dairy calf starter as a feed additive, in addition to maintaining health, had a positive and significant effect on improving growth performance. It increased dry matter intake, body weight, and height from the withers. It increased daily weight gain and daily height gain. It did not affect the concentration of total protein, cholesterol, and triglycerides in the blood. But while reducing glucose concentration, it increased blood urea nitrogen concentration. Despite not having an effect on fecal score and ear status, it had a positive and significant effect on reducing the amount of cough, eye discharge, and nasal discharge in calves. Although the post-weaning period of the present study was relatively short, the observed responses in feed intake, growth performance, and health indicators provide meaningful evidence of the short-term effects of the essential oil blend. In the end, a limitation of this study is that the precise composition and concentration of individual essential oils in the commercial blend were not disclosed by the manufacturer. Therefore, the observed effect should be attributed to the synergistic action of the overall blend as provided. Future studies using defined mixtures could help elucidate the contribution of each component. On the other hand, it is necessary to mention that the use of plant essential oils, due to differences in the method of extraction of the essential oil from the plant, the type of essential oil, the prescribed dose, and the experimental conditions, may show different results after consumption, and it seems that more experiments are needed to overcome these limitations.

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