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The effect of oregano essential oil and *Bacillus subtilis* supplements on winter fur growth in raccoon dogs (*Nyctereutesprocyonoides*)

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Abstract

A total of 160 healthy raccoon dogs with no significant difference in body weight were selected and randomly divided into 10 groups, with each group having 4 replicates and each replicate consisting of 4 individuals. The pre-test period lasted for 7 days, followed by a formal test period of 82 days. The raccoon dogs in the 10 groups were fed basal diet supplemented with 50 mg/kg chlortetracycline and 100 mg/kg quinocetone (control group), 100 mg/kg OEO and 150 mg/kg BS (group I), 100 mg/kg OEO and 300 mg/kg BS (group II), 100 mg/kg OEO and 450 mg/kg BS (group III), 200 mg/kg OEO and 150 mg/kg BS (group IV), 200 mg/kg OEO and 300 mg/kg BS (group V), 200 mg/kg OEO and 450 mg/kg BS (group VI), 300 mg/kg OEO and 150 mg/kg BS (group VII), 300 mg/kg OEO and 300 mg/kg BS (group VIII), 300 mg/kg OEO and 450 mg/kg BS (group IX). The results showed that there were no significant difference in final weight and average daily gain among all groups ($P > 0.05$). The ADFI of group IV was the least, which was significantly lower than that of the control group ($P < 0.05$). The F/G in group IV was significantly higher than that in groups I, II, III, V, VII and VIII ($P < 0.05$). There were no significant differences in DM output, DM digestibility, protein digestibility and fat digestibility among groups ($P > 0.05$). There were no significant differences in nitrogen intake, fecal nitrogen, urine nitrogen, nitrogen retention, net protein utilization rate and protein biological value among the ten groups ($P > 0.05$). There was no significant difference in serum SOD activity, GSH-PX activity, MDA content and T-AOC activity between the groups supplemented with OEO and BS compared with the antibiotic group ($P > 0.05$). There was no significant difference in body length, skin length, skin weight, guard hair length and underfur length between each level group and the control group ($P > 0.05$). The feed input cost of the other experimental groups was lower than that of the control group except for the experimental groups II, VIII and IX. The cost of feed additives in each experimental group was higher than that in the control group. The income of group III, VII and IX was higher than that of the control group, and the income of group III was the highest. In conclusion, under the experimental conditions, dietary supplementation of OEO and BS to the diet can improve the growth performance, fur quality as well as absorption of nutrients and antioxidant level

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by the body of raccoon dogs in winter fur period. The optimum supplemental levels of OEO and BS in the diet of raccoon dog in winter fur period were 100 mg/kg and 450 mg/kg.

Keywords Raccoon dogs, Growth performance, Nutrients digestibility, Metabolism, Antioxidant capacity, Fur quality

Introduction

Antibiotics have been widely used for more than 70 years since they were discovered in the 1940s [1]. It is mainly added to the feed to reduce the disease rates and mortality of animals and increase productivity [2]. However, the increased utilization of antibiotics had led to the emergence of resistant bacteria and drug residues in animal products [3, 4]. In 1986, based on food safety considerations, Sweden banned the use of antibiotics in feed. Since then, Norway, the European Union, South Korea, the United States, China and other countries have also promulgated relevant regulations to implement a total ban on the use of antibiotics in feed [5–8]. China is the origin of raccoon dog (*Nyctereutes procyonoides*), which is a fur animal that can bring huge benefits every year [9]. With the prohibition of antibiotics, many different types of antibiotic substitutes have been used in livestock and poultry industry, whereas the raccoon dog diets of alternative antibiotic products are rarely studied. Therefore, it is urgent to search for the alternatives.

In animal production, a study had showed that probiotics, prebiotics, synbiotics, plant essential oils, etc. can be used as alternatives to antibiotics [10]. Oregano essential oil (OEO) is a volatile essential oil extracted from the plant oregano. The main components of OEO are thymol and carvacrol [11], which have strong antibacterial effect. The antibacterial mechanism of thymol and carvacrol is mainly to make the plasma membrane permeability and depolarization [12, 13], inhibit the formation of bacterial biofilm [14], affect the synthesis of bacterial proteins [15], and the bacterial communication system-quorum sensing [16–18]. Whysner [19] found that OEO played an important role in stabilizing lipid peroxidation and inhibiting oxidase activity, and produced superoxide free radicals, thereby improving antioxidant activity. By producing various digestive enzymes, such as protease, amylase, lipase, etc [20]., *Bacillus subtilis* (BS) promoted the production of nutrients that can be directly absorbed by the body and improved the feed conversion rate. BS can produce organic acids, thereby promoting the absorption of vitamin D, calcium and iron in animal bodies. BS can synthesize some nutrients and vitamins that can be directly absorbed by animals, such as amino acids, short-chain fatty acids, vitamin K, B vitamins, etc., further to promote the body's nutritional metabolism [21]. Phuoc [22] showed that BS could significantly increase the body weight of New Zealand white rabbits, and the feed conversion rate was higher. Bai [23] added BS *fmbJ* and antibiotics to the broiler diet, respectively and the results showed that the concentrations of IgA and IgG in the serum of broilers in the BS group were significantly increased, and the activities of GSH, glutathione reductase (GR), GSH-Px and SOD were increased. The content of MDA in serum and liver was significantly lower than that in the antibiotic group. Therefore, OEO and BS are considered to be good alternatives to antibiotics. Notably, the effect of single additive is limited and the experimental results of these two additives are very different. In recent years, studies have showed that the combined addition of organic acids, probiotics and essential oils in animal diets could achieve better results in some aspects [24]. At present, there are relatively few studies on the combination of plant essential oil and probiotics. Alfaig [25] added 0.05% thyme essential oil and 0.05% BS to the broiler diet had a positive effect on the amino acid content in chicken. Liu [26] found that adding a mixture of 900 mg/kg BS and plant essential oil to the diet can enhance the antioxidant capacity, inflammatory response and plasma hormone concentration of broiler breeders, and significantly improve the reproductive performance of broiler breeders in the late laying period. Saliu [27] showed that the efficiency of adding different mixtures of Lactobacillus strains and plant essential oil to broiler diets in reducing the prevalence of β-lactamase and plasmid transfer ability was different, but it had a positive effect. Tan [28] showed that adding antibiotics or essential oil + probiotics to the diet of weaned piglets could improve their growth performance and significantly reduce the diarrhea rate of weaned piglets. The diet supplemented with 300 g/t essential oil + 1000 g/t probiotics significantly increased the serum IgG concentration and immunity of piglets, and the fecal ammonia emission of piglets was lower than that of antibiotic group. In summary, OEO and BS need to reach a certain dosage to be effective. However, it is as yet unclear if the combination of OEO and BS have a positive effect on fur animals. Therefore, the purpose of this study was to investigate the effects of combined use of OEO and BS on the growth performance, nutrient digestion and metabolism, antioxidant capacity and fur quality of raccoon dogs. Due to the limited research on fur-bearing animals, a gradient incremental design will be implemented starting from a relatively low baseline. These results will provide a theoretical reference for farms.

Table 1 Ingredients composition and nutrients level of the basal diet (%, as-fed basis)

Ingredients	Content (%)	Nutrient levels[b]	Content (%)
Expanded corn	46.0	Metabolizable energy(ME)/(MJ/kg)[b]	15.38
Soybean meal	10.0	Moisture	10.12
Fishmeal	10.0	Crude protein	25.91
Corn protein powder	3.0	Coarse fiber	2.542
Expanded soybean	12.5	Crude ash	6.07
Beef bone meal	9.5	Calcium	1.51
Chicken oil	4.0	Total phosphorus	1.13
Premix[a]	5.0	Methionine	0.83
Total	100.0	Lysine	1.65

[a]The premix provided the following for per kg diet: VA 10,500 IU; VD 1650 IU; VE 250 IU; VK 31.5 mg; VC 250 mg; $VB_1$15 mg; $VB_2$15 mg; $VB_6$4 mg; nicotinic acid 30 mg; pantothenic acid 3.0 mg; choline 750 mg; Fe 80 mg; Zn 60 mg; Mn 45 mg; I 0.5 mg; Se 0.3 mg; Cr 0.35 mg.

[b]ME was a calculated value, while the others were measured values

Table 2 Levels of additives used in various experimental groups

Items	group I	group II	group III	group IV	group V	group VI	group VII	group VIII	group IX	control group
quinocetone(mg/kg)	0	0	0	0	0	0	0	0	0	100
chlortetracycline(mg/kg)	0	0	0	0	0	0	0	0	0	500
oregano essential oil(mg/kg)	100	100	100	200	200	200	300	300	300	0
Bacillus subtilis(mg/kg)	150	300	450	150	300	450	150	300	450	0

Materials and methods

All the experimental procedures were approved by the Animal Welfare and Ethics Committee of Hebei University of Engineering. And strictly follow the AVMA policies on euthanizing raccoon dogs using electrocution, minimizing animal suffering and ensuring animal welfare as much as possible.(AVMA Guidelines for the Euthanasia of Animals: 2020 Edition).

Experimental additives

The content of OEO used in this experiment was 10% (provided by Shandong Luxi Veterinary Medicine Co., Ltd., the recommended dosage by the company: Add 125–350 g of this product per 1000 kg of compound feed); BS (provided by Xindayang Neiqiu Biotechnology Co., Ltd., viable count $\geq 2.0 \times 10^{10}$ CFU/g); the effective component content of chlortetracycline was 10% (provided by Jinhe Biotechnology Co., Ltd.); the effective component content of quinocetone is 50% (provided by Jinhe Biotechnology Co., Ltd.).

Experimental animal

All experimental animals were provided by Zhengdan Farm, which cooperated by Shijiazhuang Academy of Agriculture and Forestry Sciences. A total of 160 healthy raccoon dogs with no significant difference in body weight at 70 ± 5 days of age were selected and randomly divided into 10 groups, with each group having 4 replicates and each replicate consisting of 4 individuals. And were housed in the raccoon dog breeding base in Jinzhang Village, Daiwu Township, Pingshan, Hebei Province.

Instrument and equipment

Electrothermal blast drying oven (DHG-9070 A), automatic kjeldahl apparatus (RK-9870), soxhlet fat analyzer (SOX606).

Experimental basal diet

The experimental basal diet was provided by the feed branch of Beijing Sanyuan Seed Technology Co., Ltd., and the formula was designed according to the reference NRC(1982) [29] and related references [30, 31] about the nutrient requirements of raccoon dogs during the breeding period. The nutrient contents of calcium, total phosphorus, methionine, and lysine were calculated, the composition and nutrition level of the diets were shown in Table 1. Feeds have been tested regularly to ensure that aflatoxin levels remain below the prescribed animal safety limits. The pre-feeding test was carried out on October 1, 2022, with a pre-feeding period of 7 days. The test was officially started on October 8, and the test period was 60 days.

Experimental design

The selected 160 raccoon dogs were randomly divided into 10 groups, 16 in each group, 4 replicates in each group, 4 in each replicate, and the ratio of male to female was 1:1. There was no significant difference in weight among the groups ($P > 0.05$). Based on the results of our previous studies [28], our current study reduced the amount of OEO and BS added to raccoon dogs according to body weight. The 3×3 factorial design was used in the experiment, and the addition levels of each group were as follows in Table 2.

Feeding and management

Before the start of the experiment, all raccoon dogs have been vaccinated. During the period of the test, single cage feeding was used. The animals were feed twice a day (8:00, 17:00), and drink ad libitum. Feeding management was carried out according to the daily management procedures of the farm, and the food boxes were cleaned and disinfected daily.

Determination index and method

Growth performance test

At the end of study, animals were sacrificed after overnight fasting. The initial body weight and final body weight were recorded. The average daily dry matter intake (ADFI), average daily gain (ADG) and feed to gain ratio (Feed/Gain, F/G) were calculated. Nitrogen, contents of crude fiber, crude protein, crude fat (ether extract) contents of the samples were determined according to AOAC [32].

The calculation method was as follows:

Average daily feed intake (ADFI) = total feed intake/(test days×number of test pups).

Average daily gain (ADG)=(final weight-initial weight)/test days.

Feed - gain weight ratio (F/G) = average daily dry matter intake/average daily gain.

Digestion and metabolism trial

The digestion and metabolism trials were carried out for 3 days. Feces and urine were collected for 3 consecutive days using the total collection method. Three male and female raccoon dogs were selected for sample collection in each group by total collection of feces and urine. Digestion and metabolism tests were performed using a fecal collection plate separated from feces and urine, and daily feed intake was recorded. The amount of feces in each replicate was weighed and recorded. The samples were taken at 10% of fresh weight, dried in an oven at 65 °C, crushed through a 40-mesh sieve, and the nutrient content was detected. After the total volume of all urine samples was weighed evenly, the total volume was recorded and filtered through 4 layers of gauze to sample at 10%, and stored at -20 °C for inspection. The dry matter content was determined according to GB/T 6435 – 2014, the crude ash content was determined according to GB/T 6438 – 2007, the crude fat content was determined according to GB/T 6433 – 2006, and the crude protein content was determined by Kjeldahl method.

The relevant calculation formula is as follows:

Nutrients digestibility (%)= (daily feed intake×nutrient content in feed-daily feces output×nutrient content in feces)/(daily feed intake×nutrient content in feed)×100%.

Nitrogen deposition (g/d) = ingested nitrogen-fecal nitrogen - urinary nitrogen.

Net protein utilization rate (NPU) (%) = nitrogen deposition/nitrogen intake×100%.

Protein biological value (PBV) (%) = nitrogen deposition/(ingestion nitrogen-fecal nitrogen)×100%.

Preparation and determination of serum samples

At the end of the study, 3 male raccoon dogs were randomly selected from each group, and 3 female raccoon dogs were randomly selected from each group. Blood was taken from the hind limb vein of the three female raccoon dogs under fasting conditions, and 5 mL of blood was taken using a coagulation tube. After the blood was coagulated, it was centrifuged in a centrifuge at 3000r/min for 20 min. The isolated serum was packaged in a 1.5mL centrifuge tube and stored at-20 °C. The serum samples were sent to Nanjing Jiancheng Biological Engineering Research Institute Co., Ltd.to determine antioxidant indicators, including GSH-Px, SOD, MDA, and total antioxidant capacity (T-AOC).

Preparation of fur sample

At the end of the study, all raccoon dogs were euthanized by electrocution according to the animal welfare organization for fur production [33], and were pelted immediately after death by skilled workers. The length of the fur was measured from the base of the tail to the tip of the nose with an accuracy of ± 0.1 cm. The needle hair length and villus length were measured with an improved micrometer. The skin of raccoon dogs was collected for further analysis.

Data processing and analysis

The statistical analysis was performed using IBM SPSS Statistics 23. The original data were screened by box plot. In addition, the Shapiro-Wilk test and Levene's test were performed to test the data for normality and homoscedasticity, respectively. The data were analyzed by two-way analysis of variance. The results were expressed as' mean ± standard deviation '. Duncan method was used for multiple comparisons. $P < 0.05$ indicates that the difference is significant, and $P > 0.05$ indicates that there is no the difference.

Results

Growth performance

The main indicators for measuring animal growth performance are ADG and feed-to-gain ratio F/G, while ADFI also indirectly affects the increase in average daily weight gain. The effects of dietary OEO and BS levels on the growth performance of raccoon dogs were shown in Table 3. There was no significant difference in final weight and average daily gain among all groups($P > 0.05$). And the final weight of group III was the highest in all the comparison groups. Except for group IV, the ADG of the

Table 3 Effects of dietary OEO and BS on growth performance of raccoon dog

Items	Initial weight/kg	Final weight/kg	Average daily gain/(g/d)	Average daily feed intake /(g/d)	Feed/Gain
control group	8.73 ± 0.42	8.46 ± 0.19	15.34 ± 1.72	244.95 ± 1.67^{a}	18.67 ± 2.62^{ab}
group I	8.80 ± 0.18	8.64 ± 0.28	21.21 ± 2.95	228.76 ± 2.10^{cd}	14.72 ± 2.63^{b}
group II	9.31 ± 0.30	8.79 ± 0.24	20.59 ± 2.09	250.21 ± 1.18^{a}	14.73 ± 2.26^{b}
group III	8.18 ± 0.30	**9.08 ± 0.23**	20.95 ± 2.99	219.76 ± 9.00^{de}	13.63 ± 2.78^{b}
group IV	8.38 ± 0.39	7.92 ± 0.22	**10.34 ± 1.51**	**216.54 ± 1.79^{e}**	**25.95 ± 3.02^{a}**
group V	8.51 ± 0.45	8.38 ± 0.25	18.75 ± 2.30	232.89 ± 3.65^{bc}	15.24 ± 2.20^{b}
group VI	8.34 ± 0.29	8.21 ± 0.24	22.27 ± 7.74	230.87 ± 2.78^{bcd}	20.31 ± 4.46^{ab}
group VII	8.64 ± 0.36	8.16 ± 0.20	19.22 ± 2.54	241.62 ± 1.06^{ab}	15.67 ± 2.94^{b}
group VIII	8.71 ± 0.21	8.28 ± 0.28	16.23 ± 2.74	240.93 ± 4.45^{ab}	20.62 ± 3.10^{ab}
group IX	8.73 ± 0.42	8.53 ± 0.23	20.97 ± 1.66	**251.21 ± 2.88^{a}**	13.34 ± 1.40^{b}
P-value	0.370	0.06	0.16	< 0.001	0.03

In the same column and the same item, values with no letter or the same letter superscripts mean no significant difference (P > 0.05), while with different small letter superscripts mean significant difference (P < 0.05). The same as below in Tables 4, 5 and 6

Table 4 Effects of dietary OEO and BS on nutrients digestibility of raccoon dog

Items	DM output/g	DM digestibility/%	Protein digestibility/%	Fat digestibility/%
control group	63.27 ± 6.79	73.05 ± 2.12	74.48 ± 2.25	87.12 ± 1.93
group I	59.10 ± 6.45	72.56 ± 1.18	73.86 ± 1.05	87.37 ± 0.78
group II	69.33 ± 6.17	66.82 ± 7.59	66.86 ± 8.62	85.46 ± 3.78
group III	69.45 ± 3.73	72.40 ± 1.58	73.68 ± 1.52	85.89 ± 1.62
group IV	68.02 ± 9.17	69.82 ± 3.30	71.78 ± 2.83	81.21 ± 2.34
group V	60.27 ± 2.54	75.47 ± 0.85	76.89 ± 1.02	85.97 ± 1.63
group VI	71.41 ± 3.02	70.31 ± 1.87	71.75 ± 2.03	85.28 ± 1.44
group VII	71.11 ± 3.80	72.83 ± 1.16	73.47 ± 1.64	84.38 ± 1.66
group VIII	67.84 ± 6.65	68.29 ± 2.81	70.25 ± 2.72	81.57 ± 2.38
group IX	60.13 ± 4.70	73.75 ± 0.95	75.51 ± 0.98	87.48 ± 1.07
P-value	0.690	0.540	0.550	0.240

other groups was higher than that of the control group. The ADFI of group IV was the least, which was significantly lower than that of the control group($P < 0.05$). The ADFI of group IX was the highest, but there was no significant different with the control group($P > 0.05$). There was no significant difference in F/G between each level group and control group($P > 0.05$), but F/G in group IV was significantly higher than that in groups I, II, III, V, VII and VIII($P < 0.05$).

Nutrient digestibility

By calculating the animal's DM output, DM digestibility, protein digestibility, and fat digestibility, one can intuitively assess the animal's ability to digest essential nutrients.

The effects of dietary OEO and BS levels on nutrient digestibility were shown in Table 4. There were no significant differences in DM output, DM digestibility, protein digestibility and fat digestibility among groups ($P > 0.05$).

Nitrogen metabolism

The effects of dietary OEO and BS levels on nutrient digestibility were shown in Table 5. There were no significant differences in nitrogen intake, fecal nitrogen, urine nitrogen, nitrogen retention, NPU and PBV among the ten groups ($P > 0.05$).

Serum antioxidant indexes

SOD activity, GSH-PX activity, MDA content, and T-AOC activity are conventional important indicators for measuring the antioxidant capacity of animals. As shown in Fig. 1, compared with the antibiotic group, there was no significant difference in serum SOD activity, GSH-P_X activity, MDA content and T-AOC activity between the groups supplemented with OEO and BS ($P > 0.05$). Importantly, most of the experimental groups had higher GSH-Px activity than the control group, and the SOD activity of groups I and VIII was higher than that of the control group. Except that the MDA level in group V was lower than that in the control group, the other levels were increased. The T-AOC level in group VII was slightly higher than that in the control group, and the other levels were almost the same.

Fur quality

As shown in Table 6, There was no significant difference in body length, skin length, skin weight, guard hair length and underfur length between each level group and the

Table 5 Effects of dietary OEO and BS on nitrogen metabolism of raccoon dog

Items	Nitrogen intake/(g/d)	Fecal nitrogen/(g/d)	Urine nitrogen/(g/d)	Nitrogen retention/(g/d)	Net protein utilization rate/%	Protein biological value/%
control group	9.22 ± 0.72	2.35 ± 0.26	3.07 ± 0.69	3.80 ± 1.08	40.52 ± 9.38	52.82 ± 10.73
group I	8.53 ± 0.80	2.24 ± 0.25	2.92 ± 0.48	3.37 ± 0.46	39.71 ± 4.38	53.64 ± 5.53
group II	9.70 ± 0.00	2.40 ± 0.25	4.02 ± 0.52	3.28 ± 0.48	33.80 ± 4.90	45.04 ± 6.49
group III	9.70 ± 0.00	2.55 ± 0.15	3.78 ± 0.58	3.36 ± 0.68	34.69 ± 7.02	46.55 ± 8.97
group IV	9.07 ± 0.72	2.54 ± 0.32	3.43 ± 0.28	3.10 ± 0.55	33.28 ± 4.66	45.65 ± 5.01
group V	9.83 ± 0.00	2.27 ± 0.10	3.71 ± 0.25	3.86 ± 0.32	39.21 ± 3.20	50.84 ± 3.65
group VI	9.58 ± 0.25	2.69 ± 0.16	3.40 ± 0.56	3.49 ± 0.69	35.79 ± 6.85	49.57 ± 9.75
group VII	9.77 ± 0.00	2.59 ± 0.16	4.51 ± 0.43	2.67 ± 0.44	27.29 ± 4.46	37.10 ± 6.14
group VIII	8.70 ± 0.78	2.54 ± 0.26	3.46 ± 0.66	2.69 ± 0.67	30.54 ± 6.62	43.16 ± 9.98
group IX	9.15 ± 0.62	2.23 ± 0.16	3.66 ± 0.48	3.27 ± 0.52	35.41 ± 4.56	46.83 ± 6.03
P-value	0.69	0.80	0.61	0.92	0.86	0.91

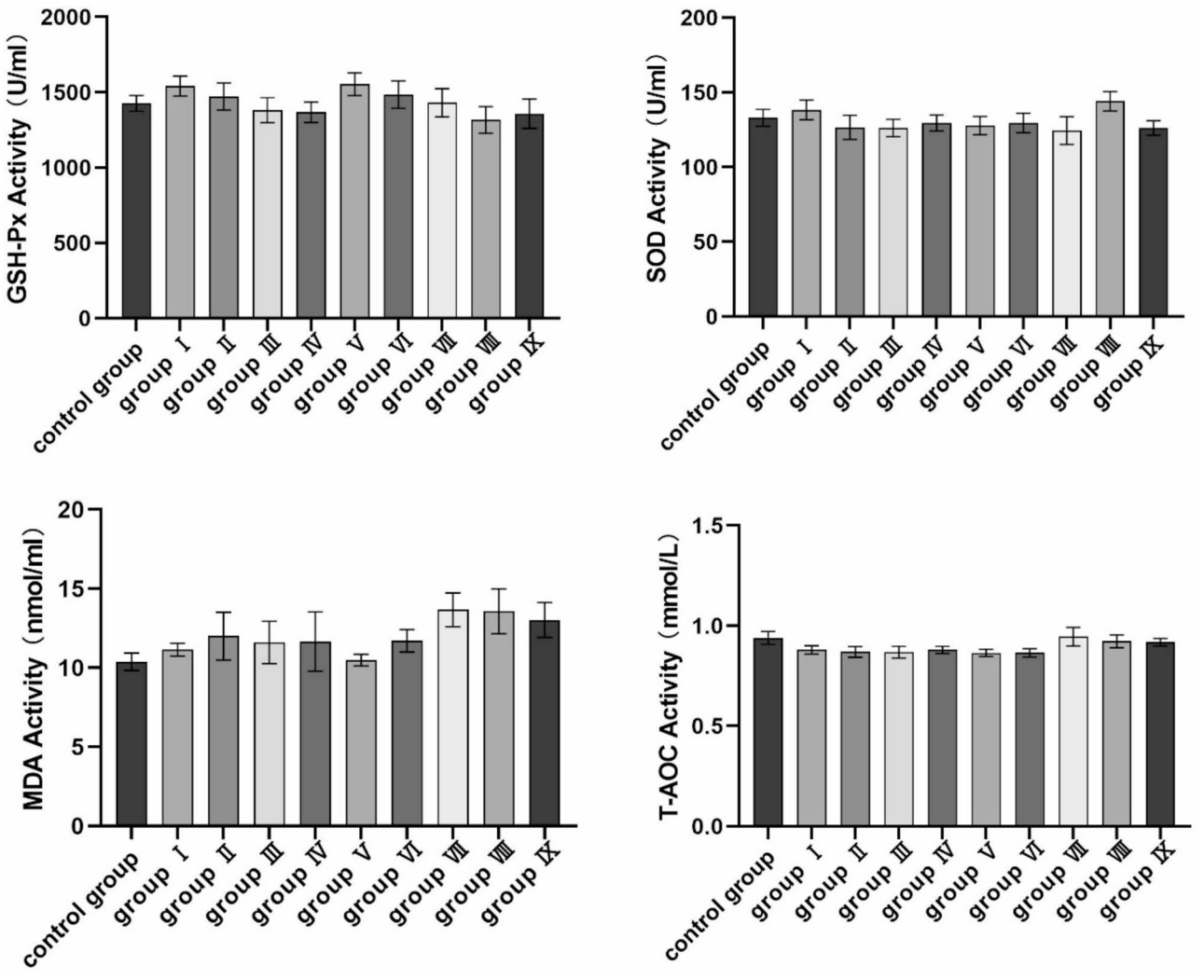

Fig. 1 Effects of dietary OEO and BS on serum antioxidant indexes of raccoon dog

Table 6 Effects of dietary OEO and BS on fur quality of raccoon dog

Items	Body length (cm)	Skin length (cm)	Skin weight (g)	Guard hair length (mm)	Underfur length (mm)
control group	66.47 ± 0.79	108.56 ± 1.72	548.22 ± 18.11	88.00 ± 1.94	63.00 ± 2.17
group I	67.00 ± 0.86	101.14 ± 1.81	474.57 ± 32.84	92.19 ± 2.46	66.88 ± 2.58
group II	67.00 ± 1.10	107.43 ± 2.22	535.57 ± 24.20	90.00 ± 2.24	63.18 ± 1.82
group III	**68.38 ± 0.38**	**112.75 ± 1.32**	**575.88 ± 29.08**	**90.00 ± 3.00**	**66.54 ± 3.73**
group IV	66.00 ± 0.53	100.67 ± 8.20	502.92 ± 11.15	85.38 ± 2.56	59.23 ± 2.18
group V	**68.27 ± 0.73**	108.83 ± 1.04	**549.67 ± 19.50**	**89.67 ± 1.86**	63.00 ± 1.53
group VI	68.00 ± 0.70	108.38 ± 1.32	534.69 ± 15.23	87.14 ± 2.95	62.50 ± 2.55
group VII	65.50 ± 0.72	107.00 ± 1.54	534.00 ± 18.53	87.50 ± 2.44	65.36 ± 2.37
group VIII	67.64 ± 0.95	107.33 ± 1.74	524.89 ± 18.34	85.45 ± 2.07	66.09 ± 3.86
group IX	67.88 ± 0.55	108.42 ± 1.17	560.08 ± 14.58	87.81 ± 2.46	63.13 ± 2.18
P-value	0.078	0.424	0.057	0.644	0.586

control group($P > 0.05$). The body length of all the level groups was better than that of the control group. The skin length and skin weight of group III and group V were better than those of the control group.

Discussion

Growth performance

In the past few decades, OEO and BS have been used as effective alternatives to improve animal growth performance. Previous studies have shown that OEO can improve the growth performance of ducks and chickens and improve egg quality [34–36]. BS can improve animal feed conversion rate, promote growth, and have a certain inhibitory effect on pathogenic Escherichia coli and Salmonella [37]. In this study, the weight of group III was the highest, and the weight of group IV was the lowest in all the comparison groups, but there was no significant difference with the control group ($P > 0.05$). At the same time, although there was no significant difference in the average daily gain between the experimental group and the antibiotic group ($P > 0.05$), the results indicate that most of the experimental groups showed an increasing trend compared to the antibiotic group, which also showed that the addition of OEO and BS to the feed can achieve the same effect as the use of antibiotics. Raccoon dogs choose to sleep in the coldest season of the year, which is related to their physiological changes and melatonin cycle. Before hibernation, raccoon dogs will overeat in the fall to gain weight, and store a lot of fat to withstand the cold winter [38]. Therefore, the weight gain of raccoon dog was less and the feed weight ratio was larger during the whole period of the study. Except that the F/G of groups IV, VI and VIII was higher than that of the control group, the F/G of the other experimental groups decreased, but there was no significant difference. Thus, the results of our current study showed that the effects of OEO and BS on animals are similar to those of antibiotics, and had lower F/G than antibiotics. Considering the fur animal breeding environment is outdoor cage breeding, the breeding conditions are relatively poor compared with other livestock and poultry. Under the condition of no antibiotics or alternative anti-additives, raccoon dogs are easily infected by pathogens, with a high mortality rate, which seriously affects the breeding income and is an unacceptable risk for farms. In addition, comprehensive analysis and consideration of other fur animal alternative resistance test studies, the basal diet group with antibiotics was used as the control group. This is also the reason why most of the results of this experiment are not significantly different. Therefore, in order to ensure the sustainable development of the test and find the appropriate level of alternative resistance additives, the basal diet group without antibiotics or alternative resistance additives was not designed in the course of this experiment. However, this also limits the current study, as it cannot be compared with raccoon dogs that were not given antibiotics. Instead, the antibiotic group was used as the control, and different concentrations of OEO and BS were designed to explore the optimal addition amount of alternative antibiotics in the raccoon dog diet.

Nutrient digestibility

Addition of OEO and BS to the feed can improve the digestion and absorption of nutrients by animals. The main reason is that the bacteriostatic effect of oregano extract can improve the intestinal flora structure and small intestinal morphology of animals, protect the intestinal barrier function, and improve the digestion and utilization of nutrients [39–41]. The special smell of OEO can stimulate the appetite of animals. Ding [36] showed that adding 100 mg/kg oregano essential oil to the diet of Peking ducks had a positive effect on its dry matter digestibility. Su [42] showed that the addition of oregano essential oil to the broiler diet could increase the digestibility of dry matter and crude protein, and the activity of sucrase in the jejunum was significantly increased. BS has good secretion of protease, cellulase, amylase, lipase and other active substances, and has a strong degradation

effect on dietary plant-derived antigen protein. In production, it is often used as one of the compound probiotics to ferment full-price feed or a ingredient raw material to reduce the anti-nutritional factors and increase the digestibility of the diet [43, 44]. The content of soluble protein and total amino acid increased and the content of anti-nutritional factors decreased in feed and protein raw materials fermented by BS. Meanwhile, studies have shown that BS can reduce the content of soybean antigen, phytic acid and neutral detergent fiber in corn-soybean meal diet, and increase the content of free amino acids and small peptides [45]. Shi [45] showed that BS could significantly reduce the content of soybean antigen protein (β-conglycinin and soybean globulin) in mixed feed, and the content of small peptides and free amino acids (AA) increased exponentially. In this study, there was no significant difference in protein digestibility, DM digestibility and fat digestibility among groups. The DM digestibility and protein digestibility of group V were higher than those of other groups, and the fat digestibility of group IX was higher than that of other groups. This also indicates that OEO and BS have similar effects on the digestibility of nutrients in raccoon dogs compared to antibiotics. Under the conditions of this experiment, the apparent digestibility of nutrients in the diet of Wusuli raccoon dogs was found to be similar to that of antibiotics maybe since OEO and BS improved their intestinal morphology and regulated the intestinal flora. However, the specific mechanism of action needs to be further verified by molecular biology methods.

Nitrogen metabolism

The main source of nitrogen intake of raccoon dogs is protein in feed, and nitrogen metabolism can reflect the metabolism of protein in the body. BS can reduce the discharge of nitrogen and the concentration of ammonia in the breeding environment by improving the utilization rate of nitrogen in animals. Previous studies have shown that adding 60 or 80 mg / kg Fe and 5×10^9 CFU / kg BS to the diet of breeding geese can significantly reduce serum urea nitrogen levels [46]. Adding BS to broiler diets can significantly reduce NH_3 emissions in feces [47]. BS can significantly increase the number of lactic acid bacteria in piglet feces, significantly reduce blood urea nitrogen concentration, and reduce NH_3 emissions [48]. The results of our study showed that the nitrogen deposition in group V was the highest, but there was no significant difference with the control group. The net protein utilization rate of group I and group V was close to the control group, but the nitrogen intake of group I was the least. The biological value of the protein in group I was higher than that in the control group, but there was no significant difference, followed by group V. There was no significant difference in nitrogen metabolism between all levels and the control group. Under the conditions of this experiment, both OEO and BS can partially replace the effect of antibiotics on the nitrogen intake of raccoon dogs, reflecting that the combination of the two influences the animals' feeding amount.

Serum antioxidant indexes

OEO has the characteristics of anti-oxidation, which mainly depends on its rich phenolic components. The action pathways mainly include scavenging free radicals, chelating with metal ions, inhibiting cell membrane lipid peroxidation and regulating antioxidant enzymes [49]. Studies have shown that the addition of OEO to broiler feed can achieve the same effect as antibiotics, which can increase the concentration of GSH-Px and SOD and reduce the level of MDA [50]. Xu [51] showed that adding BS to the diet could improve the antioxidant capacity of broilers. It was found that BS could significantly increase the antioxidant enzyme activity of ducks contaminated with aflatoxin-contaminated moldy corn and reduce the accumulation of aflatoxin in duck liver [52]. Under the conditions of this experiment, when the addition level of OEO was 200 mg/kg and the addition level of Bacillus subtilis was 300 mg/kg, the activity of GSH-Px in the serum of raccoon dogs was the highest, and the content of MDA was the lowest. In addition, OEO and BS on raccoon dog. serum T-AOC also had a positive effect ($P > 0.05$). The above results showed that an appropriate amount of OEO and BS can achieve the same antioxidant capacity as the addition of antibiotics.

Fur quality

Raccoon dog is a fur animal, and its fur quality determines its economic value. The fur quality index of raccoon dog is related to fur quality, fur size, fur flatness and fur softness [53]. OEO and probiotics can improve the quality of animal fur. Adding 200 mg / kg thymol to the diet of raccoon dogs in winter fur period can increase the skin length and Guard hair length of blue foxes and increase the ductility of the skin [54]. Adding 250 mg / kg oregano extract and 300 mg / kg BS to the diet of Arctic foxes in winter fur period can significantly increase dry skin weight, skin length, skin plate thickness and villus length [55]. In this study, the skin weight, length of guard hair and villi in the experimental group showed no significant differences compared to the antibiotic group. This means that the addition of OEO and BS to the feed can achieve the same effect on maintaining the fur quality of raccoons as using antibiotics, without the need for antibiotic use. Of course, whether to ultimately use OEO and BS in the diets of raccoon dogs will also depend on a comprehensive consideration of purchasing costs and economic benefits.

Conclusion

For most indicators in the experiment, the addition of a certain level of OEO and BS showed no significant difference compared to antibiotics, indicating that the inclusion of OEO and BS in the diet could potentially serve as an alternative to antibiotics. Additionally, there was a trend of improvement in ADG, F/G, and body length in the experimental group. The sale of the pelts from this experiment took place around March 2023. The results indicated that the highest selling price and maximum profit for the pelts were achieved at addition levels of 100 mg/kg and 450 mg/kg of OEO and BS, respectively. This is consistent with the improved growth performance of the Ussuri raccoon, suggesting that both additives can enhance body length, resulting in greater skin length and increased pelt area, thereby boosting the profits for farmers. The optimum supplemental levels of OEO and BS in the diet of raccoon dog in winter fur period were 100 mg/kg and 450 mg/kg, respectively.

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Author contributions
CP, YL, JL: Software, formal analysis, writing original draft. WL, XL, LD, WZ: collected the sample and conducted the experiments. ER: Project administration, funding acquisition; HW: Supervision; Investigation. All authors contributed to the article and approved the submitted version.

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Data availability
The original contributions presented in the study are included in the article/supplementary materials, further inquiries can be directed to the corresponding author/s.

Declarations

Ethics approval and consent to participate
Prior to perform any experimental procedures, all the experimental procedures were approved by the Animal Welfare and Ethics Committee of Hebei University of Engineering. Strictly carry out euthanasia on mink in accordance with AVMA policies. (AVMA Guidelines for the Euthanasia of Animals: 2020 Edition). Faculty of College of Life Science and Food Engineering, Hebei University of Engineering. Consent to participate is not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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