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Effect of Essential Oil on Growth Performance, Rumen Fermentation and Nutrient Digestibility of Hu Sheep

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ABSTRACT

Background: Plant essential oil is used as a feed additive instead of antibiotics, but there is little research on its application in ruminants. Therefore, it is very necessary to study the effects of essential oil on growth performance, rumen fermentation and nutrient digestibility of Hu sheep.

Methods: 96 weaned male Hu sheep, weighing 17.69±0.20 kg, were randomly divided into 3 groups (control, 300 mg/kg and 600 mg/kg essential oil), with 4 replicates per group and 8 sheep per replicate. The pre-feeding period was 10 days and the full trial period was 61 days.

Result: The results showed that the final weight of Hu sheep increased significantly (P<0.05) and the rumen liquid ammonia nitrogen decreased significantly (P<0.05) in the 600 mg/kg essential oil group when compared with the control group. The addition of essential oil to the diet had no significant effect on the rumen pH, volatile fatty acids and the digestibility of various nutrients. In conclusion, the diet addition of 600 mg/kg essential oil was better than 300 mg/kg for weaned Hu sheep performance, which can improve the growth performance and reduce the ammonia nitrogen of the rumen fluid. Essential oil can be added to Hu sheep feed as a good feed additive.

Key words: Antibiotic substitutes, Essential oil, Growth performance, Hu sheep, Nutrient digestibility, Rumen fermentation.

INTRODUCTION

Essential oils are complex mixtures of volatile lipophilic secondary metabolites of plants and are usually composed of terpenoids and phenylpropanoids (Kholif and Olafadehan, 2021). The representative compounds of terpenoids are limonene, thymol, carvacolol, linalool, while the representative compounds of phenylpropanoids are cassia bark aldehyde, eugenol, fennel brain. Essential oils, such as oregano oil, thymol, carvacolol, *etc*, have the functions of antibac-terial, antioxidant, enhancing immunity and antiparasitic (Mutlu-Ingok *et al.,* 2020; Razni *et al.,* 2019; Bhaisare *et al.,* 2015; Naik *et al.,* 2021). The consumer's tendency to buy more natural and drug-free animal products encouraged the livestock husbandry industry to use natural alternatives. In monogastric animal production, essential oils are one of the important antibiotic substitutes in feed (Zeng *et al.,* 2015). The application of essential oils (especially thyme and carvacrol) in ruminants are also studied. Researches have found that carvacrol and/or thymol have no positive effects on the performance and nutrition utilization efficiency in ruminants (Baruh and Kocabağli, 2017; Biricik *et al.,* 2016). However, a large part of the positive effects of essential oil on ruminal fermentation have been obtained from *in vitro* studies and using high doses (Joch *et al.,* 2016; Amin *et al.,* 2021). When applied *in vivo* studies in ruminants, it is likely to negatively affect feed intake and ruminal fermentation (Alemu *et al.,* 2019). In general, the optimal dietary dose and the effect of essential oil on ruminants performance were not consistent among the studies, it still needs to be explored. The objective of this study was to determine the effect of essential oil on the growth performance, rumen fluid index and nutrient digestibility of Hu sheep.

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MATERIALS AND METHODS

The experiment was conducted by the Research Institute of Anyou Group at the Donglin Ecological sheep farm in Taicang of China, from 2023.6.12 to 2023.8.12, lasted for 61 days. Essential oil product was purchased from Center Group Bio-Tech (wuhan) Co., Ltd., China, the active ingredients ware 11% cinnamaldehyde, 6% thymol and 3% carvacrol, carrier was silicon dioxide.

Experimental design, diets and management: 96 weaned male Hu sheep, weighing 17.69±0.20 kg, were randomly divided into 3 groups (control, 300 mg/kg and

600 mg/kg essential oil), with 4 replicates per group and 8 sheep per replicate. The basal diet (Table 1) was designed and fed twice a day (07:00 and 15:30), sheep were free-choice feeding and drinking, adapted to the experimental diets for 10 days and then fed for 61 days. All animal management procedures were carried out within the scope of the local approved technical guidelines for animal use and care.

Growth performance indicators

During the experiment, the animals were weighed in pens before feeding in the morning of the 1st day, 37th day and 62nd day, the daily feed intake was recorded, the average daily gain (ADG), dry matter intake (DMI) and feed conversion rate (FCR) were calculated. The following performance measures were taken at each assessment during the trial:

$$ADG = \frac{\text{Final body weight - Initial body weight}}{\text{Feeding days}} \quad ...(i)$$

$$FCR = \frac{\text{Dry matter intake (g)}}{\text{Average weight gain (g)}} \quad ...(ii)$$

Ruminal fermentation parameters

On the 61th day, two sheep were randomly selected from each replicate to collect the ruminal fluid. Within 4 hours after the sheep meal, a sample of rumen fluid (50 mL) was obtained by using an esophageal tube. Rumen liquor was filtered by using a double-layer gauze and pH was immediately measured with a portable potentiometer (HANNA® Instruments, Woonsocket, USA) which were previously calibrated with reference buffers at pH 4, 7 and 10. After determining the pH, an aliquot of the rumen liquid was centrifuged for 10 min at 2 500 × g (4°C). 15 mL of the filtrate was mixed with 3 mL of hydrochloric acid solution (0.2 N) and frozen (-20°C) to measure ammonia-N. Then, 4 mL of rumen liquor added with 1 mL of 25% (wt/vol) metaphosphoric acid were stored at -20°C for volatile fatty acid analysis by gas chromatography (Perkin Elmer, model Claurus 500, USA).

Feed intake and nutrient apparent digestibility

The digestibility trial was conducted using acid-insoluble ash as an internal indigestibility marker. During the last 3 days of the experiment, fecal samples were collected from two sheep randomly selected from each replicate. Samples of feed and faeces were dried at 60°C in a forced-air oven, ground by a pulverizer to pass through a 1-mm screen, then analyzed for the dry matter (DM), ash, organic matter (OM), crude protein (CP, calculated as N × 6.25) using standard methods (AOAC, 2016) and the crude fiber (CF), neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were measured using an Ankom 2200 fiber Analyzer (Ankom Technology, Macedon, NY) by placing the sample in an individual Ankom fiber bag (F57 Filter Bags; Ankom Technology, Macedon, NY) following Van Soest method (Van *et al*, 1991). Sodium sulphite (10 g/L of NDF solution) was added to the solution with heat-stable α-amylase. The acid insoluble ash (AIA) concentration was measured in feed and fecal samples as an internal marker to determine coefficient of apparent nutrients digestibility.

$$\text{Nutient digestibility} = 1 - \left(\frac{\%\text{ marker in diet}}{\%\text{ marker in feces}} \times \frac{\%\text{ nutrient in feces}}{\%\text{ nutrinent in diet}}\right)$$

Calculations and statistical analysis

The data were analyzed and sorted in Excel version 15 and one-way ANOVA was performed with SPSS version 22 (SPSS Inc., IL, USA). The pen was considered as the experimental unit. ANOVA was used for the statistical analysis and Duncan's multiple comparison test was used to analyse the results. The results were presented as the least square means with the standard error of the mean (SEM). The significance level for all analyses was set at $P<0.05$, with a trend of $0.05 \leq P \leq 0.1$.

RESULTS AND DISCUSSION

Growth performance

The 61-day body weight of the 600 g/t essential oil group was significantly higher than that of the control group ($P \leq 0.05$). The 37-day body weight ($P=0.069$) and the daily gain of 0-61 days ($P=0.094$) in the essential oil 600 g/t group were higher than those in the control group. Dry matter intake and feed conversion rate were not significantly different among the groups ($P>0.05$, Table 2).

Great attention should be paid to the added amount of plant essential oil in the ruminant diet. Too low dose has

Table 1: Composition and nutrient levels of the basal diet (DM basis, %).

Item	Content
Ingredients	
Corn	20.4
Wheat bran	18.3
Soybean meal	11.2
Soybean residue	7.1
Silage corn	27
Wheat straw	12.8
Premix[1]	3.2
Total	100
Nutrient levels[2]	
ME, (MJ/kg)	10.79
CP	15.00
NDF	34.62
ADF	20.63
Ca	0.78
P	0.42

[1]Premix provided the following per kilogram of the diet: VA 10 500 IU, VD 4 000 IU, VE 1000 IU, Cu 10 mg, Zn 85 mg, Fe 50 mg, Mn 50 mg, Se 0.3 mg, I 1 mg, Co 0.3 mg.

[2]ME metabolizable energy, was a calculated value according to NRC(2007), while the others were measured values.

no obvious effect while too high dose may have negative effects on growth performance (Torres *et al.,* 2020). Sun *et al.* (2022) reported that 300 mg/kg and 450 mg/kg of oregano oil (5.65% purity) in the fattening Sewa sheep diet, could significantly increase ADFI and ADG, the growth performance of sheep was improved by changing intestinal morphology and intestinal flora structure. Torres *et al.* (2020) found that essential oil has a negative impact on animal performance, it would reduce ADG (100 mg/kg of DM), slaughter and carcass weight (200 mg/kg of DM). Meanwhile, Muñoz-Cuautle *et al.* (2022) reported that 200 and 400 mg/kg of oregano essential oil had no effects on the growth performance of lamb. In our experiment, adding 600 mg/kg essential oil to the diet of Hu sheep significantly increased the final weight of sheep at 61st day of age. For the growth performance of Hu sheep, 600 mg/kg dietary supplementation is better than 300 mg/kg. This experiment was carried out in summer, heat stress was very serious (highest temperature were 35~38°C). The essential oils may moderate heat stress. In addition, the Hu sheep in this trial were just weaned recently, the function and development of their tissues, organs, gastrointestinal tract, were easily affected by nutritional factors. Belanche *et al.* (2020) reported that short-term studies showed minor and inconsistent effects while long-term studies (>4 weeks duration of feeding) revealed that essential oil (Agolin Ruminant®) supplementation increases milk yield and feed efficiency. Our trial lasted 61 days and had an obvious effect on improving the growth performance, which was consistent with the above study. Inconsistent studies on the dose and effect of plant essential oils in ruminant diets require further research.

Table 2: Effects of essential oil on the growth performance of Hu sheep.

Items[2]	Treatments[1]			SEM	*P*-value
	CON	EO 300	EO 600		
BW					
0 days, (kg)	17.68	17.68	17.70	0.19	0.999
37 days, (kg)	24.39	25.40	25.56	0.23	0.069
61 days, (kg)	27.59^{b}	28.85^{ab}	29.21^{a}	0.27	0.017
0~37 days					
ADG, (g/d)	181.25	209.47	212.42	6.80	0.110
DMI, (g/d)	683.89	685.32	691.69	2.54	0.451
FCR	3.84	3.29	3.27	0.14	0.151
37~61 days					
ADG, (g/d)	133.46	144.59	147.01	7.30	0.749
DMI, (g/d)	849.71	856.01	853.20	3.07	0.743
FCR	6.77	5.99	5.87	0.40	0.646
0~61 days					
ADG, (g/d)	162.45	178.50	186.85	4.78	0.094
DMI, (g/d)	749.12	751.84	755.20	1.33	0.180
FCR	4.66	4.22	4.05	0.12	0.110

$^{a-b}$ Means with different superscripts in each row are significantly different ($P < 0.05$).

[1]CON, control (basal diet); EO300, basal diet + 300 mg/kg of essential oil; EO 600, basal diet + 600 mg/kg of essential oil.

[2]BW: body weight, ADG: average daily gain, DMI: dry matter intake, FCR: feed conversion ratio.

Table 3: Effects of essential oils on ruminal fermentation index in Hu sheep.

Item	Treatments[1]			SEM	*P*-value
	CON	EO 300	EO 600		
Rumen pH	6.29	6.03	5.93	0.08	0.213
Ammonia N, (mg/dL)	15.27^{a}	14.32^{a}	9.07^{b}	0.99	0.005
Total volatile fatty acids, (mmol/L)	48.47	50.50	53.65	2.60	0.753
Acetic acid, (%)	47.79	46.85	44.83	0.86	0.396
Propionic acid, (%)	29.86	31.20	33.76	1.00	0.289
Butyric acid, (%)	15.28	15.49	15.56	0.40	0.965
Valeric acid, (%)	2.31	2.17	2.02	0.06	0.134
Isovaleric acid, (%)	1.91	1.66	1.71	0.20	0.891
Isobutyric acid, (%)	1.19	1.11	1.06	0.06	0.737
Acetate / Propionate	1.65	1.50	1.36	0.08	0.337

[1]CON, control (basal diet); EO300, basal diet + 300 mg/kg of essential oil; EO 600, basal diet + 600 mg/kg of essential oil.

Rumen fermentation index

Compared with the control group, the 600 g/t group of essential oil significantly reduced the ammonia nitrogen in rumen fluid (P<0.05). There were no significant differences among the sheep in terms of the ruminal pH and individual VFA proportions (Table 3). Ruminal liquid ammonia nitrogen content is an apparent indicator reflecting ruminal nitrogen degradation in ruminants. It is not only the final product of the degradation of nitrogencontaining substances in diet, but also the raw material for microbial synthesis of bacterial body proteins. The content of ammonia and nitrogen in the rumen fluid are related to the degradation rate of nitrogen in the diet and the ability of microorganisms to synthesize ammonia. Study showed that essential oil can inhibit protein hydrolysis in the rumen and selectively inhibit high yield bacteria such as *Prevotella ruminicola*, *Clostridium sticklandii* and *Peptostreptococcus anaerobius* in the rumen, which leads to a significant decline in rumen ammonia production (McIntosh *et al.,* 2003). In this experiment, adding 600 mg/kg essential oil (blend of cinnamaldehyde, thymol and carvacrol) to the diet significantly reduced the contents of ammonia and nitrogen in the rumen fluid, the results were consistent with Naseri *et al.* (2022). This is beneficial for protecting the environment by reducing nitrogen and ammonia excretion. Some reports suggest, the effect of essential oils on rumen nitrogen metabolism is more effective in short-term *in vitro* trials (Zhou *et al.,* 2022), while the results are less pronounced in longer-term trials, such as *in vitro* continuous culture, rumen nylon bag test and *in vivo* tests (Soltan *et al.,* 2018; Khorrami *et al.,* 2015). The reason may be that the long-term tolerance has changed the ruminal microbial flora, or some essential oil compounds are degraded by the ruminal bacteria. This may be related to the type of essential oil, the amount of addition.

Rumen pH is an important indicator to evaluate environmental stability of ruminal fluid. The pH for normal fermentation of sheep rumen fluid was 5.5~7.5. In this experiment, the rumen fluid pH was in the normal range, there were no significant differences, which may be related to the ability of essential oil to promote the production of volatile fatty acids in the rumen. Many studies showed that plant essential oils (such as mixture oil from clove, oregano, cinnamon and lemon; eucalyptus) have no significant effect on ruminal pH in ruminants (Lin *et al.,* 2013; Akbarian-Tefaghi *et al.,* 2018). However, Biricik *et al.* (2016) reported that the rumen fluid pH was raised by carvacrol and thymol in sheep while Yadeghari *et al.* (2015) reported lavender essential oil decreased ruminal pH *in vitro* experiments. The inconsistency in the literature may be related to the sampling error of rumen fluid, the type of essential oil and the amount of addition.

El-Essawy *et al.* (2021) confirmed that anise, clove and thyme essential can promote the production of volatile fatty acids, increase the yield of propionate and reduce the ratio of acetate to propionate. The content of total VFA decreased with the addition of high level of essential oil (1 g/day) and the propionate increased significantly with 0.5 g/day essential oil. The ratio of acetate to propionate decreased significantly with the addition of mixture essential oil (eugenol, carvacrol, citral and cinnamaldehyde) (Lin *et al.,* 2013). Cobellis *et al.* (2016) reported that low doses of essential oils do not seem to alter volatile fatty acids production in the rumen and ruminal microorganisms can also adapt to the presence of essential oils over time, thus suggest that essential oils can only affect VFA production in the short term. Our study also found that plant essential oils increased rumen volatile fatty acids but did not show a significant difference. It may be related to the fact that the amount of essential oil added is not very high.

Nutrient digestibility

Essential oil has no significant influence on the digestibility of various nutrients, including DM, OM, CP, CF, NDF and ADF (Table 4). The effects of plant essential oils on ruminant digestibility are inconsistent. Malekkhahi *et al.* (2015) reported that 400 mg/day mix essential oil (thymol, carvacrol, eugenol, limonene and cinnamaldehyde) could significantly increase the protein digestibility of Baluchi sheep. Lin *et al.* (2013) reported that addition of 0.5 g/day and 1 g/day essential oil did not influence digestibility of the nutrients in total or at different parts of the digestive tract but decreased ruminal protein digestibility. Our results showed that the essential oil has no obvious effect on the digestibility of various nutrients, the diet addition of 600 mg/kg essential

Table 4: Effects of essential oil on nutrient digestibility in Hu sheep.

Item[2]	Treatments[1]			SEM	P-value
	CON	EO 300	EO 600		
DM, (%)	72.17	71.92	71.96	0.26	0.926
OM, (%)	72.67	72.21	72.35	0.27	0.811
CP, (%)	67.43	67.95	66.49	0.51	0.537
CF, (%)	65.58	65.16	68.04	0.60	0.094
NDF, (%)	60.01	60.97	62.19	0.93	0.677
ADF, (%)	58.98	60.08	61.11	1.00	0.727

[1]CON: Control (basal diet); EO300, basal diet + 300 mg/kg of essential oil; EO 600, basal diet + 600 mg/kg of essential oil.

[2]DM: Dry matter, OM: organic matter, CP: crude protein, CF: crude fiber, NDF: neutral detergent fiber, ADF: acid detergent fiber.

oil reduced the crude protein digestibility and improved the NDF and ADF digestibility (P<0.05). Essential oils increase the relative abundance of fiber-degrading bacteria (such as *Fibrobacter succinogenes*, *Ruminococcus albus* and R. *flavefaciens*) in sheep rumen fluid, so it is beneficial for fiber digestion (Patra and Yu, 2015). But Soltan *et al.* (2018) reported that the addition of 200 and 400 mg/kg essential oil to sheep diet had no effect on nutrient digestibility. Rajkumar *et al.* (2023) also reported that there were no significant differences in daily feed intake, total digestible nutrients and digestible crud protein intake could be noted with supplementation of essential oils in indigenous dairy cattle. The inconsistent conclusions between the various studies may be related to essential oil composition, additive amount, coating process, the different animals' growth stage, duration of feeding and feeding environment.

CONCLUSION

Essential oils can be used in the feed of Hu sheep to improve growth performance and nitrogen metabolism, the dosage of 600 mg/kg was better than 300 mg/kg. In order to apply essential oils effectively in ruminants, it is necessary to further study the effects of different kinds and optimum amounts of essential oils on the growth performance and the action mechanism.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the Animal Welfare Committee of Anyou Group.

Conflict of interest

We declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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