Effects of Oregano Oil on Rumen Fermentation Characteristics and Rumen Degradation Rate of Dietary Nutrients in Sheep: Postprint

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Abstract

This experiment aimed to investigate the effects of oregano oil on rumen fermentation characteristics and ruminal degradation rate of dietary nutrients in sheep. Four 4-year-old Xinjiang fine-wool × Dorper crossbred sheep fitted with permanent rumen fistulas, with an average body weight of (40.83±4.11) kg, were used in a 4×4 Latin square experimental design. The control group was fed a basal diet (without oregano oil supplementation), while the experimental groups were fed test diets supplemented with 0.015%, 0.030%, and 0.045% oregano oil in the basal diet, respectively. The experiment consisted of 4 periods, each lasting 12 days, including a 10-day preliminary period and a 2-day formal experimental period. On day 1, rumen fluid was collected at 2, 4, 6, and 10 hours after morning feeding to determine rumen fluid pH, volatile fatty acids, total nitrogen, ammonia nitrogen, and urea nitrogen concentrations. On day 2, the nylon bag method was used to determine the ruminal degradation rate of nutrients in the basal diet. The results showed that: 1) oregano oil supplementation had no significant effect on ruminal degradation rates of dietary dry matter, neutral detergent fiber, and acid detergent fiber ($P>0.05$); 2) oregano oil supplementation had no significant effect on rumen fluid pH, total nitrogen, ammonia nitrogen, and urea nitrogen concentrations in each group at each time point ($P>0.05$); 3) the total volatile fatty acid concentration at 4 h in the 0.030% and 0.045% groups was significantly lower than that in the control group ($P<0.05$); the acetate proportion at 0, 2, and 6 h in the 0.015% group was significantly lower than that in the other groups ($P<0.05$), the acetate/propionate ratio at 6 h was significantly lower than that in the other groups ($P<0.05$), while the propionate proportion at 2 h was significantly higher than that in the other groups except the control group ($P<0.05$). It can be concluded that dietary oregano oil supplementation does not affect ruminal degradation rate of dietary nutrients and has no significant effect on rumen nitrogen concentration, but affects volatile

fatty acid concentration in the rumen, with 0.015% oregano oil supplementation showing the best effect.

Full Text

Effects of Oregano Essential Oil on Ruminal Fermentation Characteristics and Dietary Nutrient Degradability in Sheep

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Abstract

This study investigated the effects of oregano essential oil on ruminal fermentation characteristics and dietary nutrient degradability in sheep. Four crossbred wethers (Xinjiang Fine-wool × Dorper) fitted with permanent rumen fistulas, averaging (40.83 ± 4.11) kg body weight and four years of age, were used in a 4 × 4 Latin square design. The control group received a basal diet without supplementation, while experimental groups received the basal diet supplemented with 0.015%, 0.030%, and 0.045% oregano essential oil, respectively. The experiment comprised four periods, each lasting 12 days (10-day adaptation and 2-day sampling). On day 1, ruminal fluid was collected at 2, 4, 6, and 10 hours post-feeding to determine pH, volatile fatty acids (VFA), total nitrogen, ammonia nitrogen, and urea nitrogen concentrations. On day 2, ruminal degradability of dietary nutrients was measured using the nylon bag technique. The results showed: (1) oregano essential oil supplementation had no significant effect on ruminal degradability of dry matter, neutral detergent fiber, or acid detergent fiber ($P > 0.05$); (2) supplementation did not significantly affect ruminal pH or concentrations of total nitrogen, ammonia nitrogen, or urea nitrogen at any time point ($P > 0.05$); (3) the 0.030% and 0.045% groups exhibited significantly lower total VFA concentrations at 4 hours compared to the control ($P < 0.05$); the 0.015% group showed significantly lower acetate proportions at 0, 2, and 6 hours ($P < 0.05$), significantly lower acetate/propionate ratio at 6 hours ($P < 0.05$), and significantly higher propionate proportion at 2 hours compared to all other groups except the control ($P < 0.05$). These findings indicate that dietary oregano essential oil does not affect ruminal nutrient degradability or nitrogen concentration but does influence ruminal VFA profiles, with 0.015% supplementation showing the most favorable effects.

Keywords: oregano essential oil; in vivo trial; ruminal fermentation characteristics; degradability

Introduction

Oregano essential oil is a volatile oil extracted from the leaves and flowers of *Origanum* plants, with carvacrol (2-methyl-5-isopropylphenol) and thymol (5-methyl-2-isopropylphenol) as its primary active components [1]. These compounds exhibit broad-spectrum antimicrobial activity and offer significant advantages over antibiotics, including high efficiency, environmental friendliness, absence of residues, low toxicity, and no development of drug resistance. Approved by China' s Ministry of Agriculture as an antimicrobial growth promoter (Agricultural Law [2001] No. 20), oregano oil can replace antibiotics in feed additives. Its antibacterial mechanism involves phenolic compounds interacting with bacterial cell membranes, altering membrane structure, increasing fluidity and permeability, or reacting with membrane phospholipids to disrupt protein synthesis and inhibit microbial growth [2]. Additionally, phenolic acids and terpenoids in oregano oil exert antioxidant effects [3], with studies demonstrating increased serum superoxide dismutase and glutathione peroxidase activities and reduced malondialdehyde concentrations in animals fed oregano oil-supplemented diets. Oregano oil also enhances immunity and promotes immune organ development [4].

Plant essential oils selectively inhibit specific microorganisms such as methanogens, protozoa, and hyper-ammonia-producing bacteria, representing a primary mechanism for modulating ruminal fermentation [5-6]. The beneficial effects of oregano oil on ruminal fermentation are generally considered to include reduced acetate and ammonia nitrogen concentrations and methane production, increased propionate and butyrate concentrations, and decreased acetate/propionate ratios, thereby maintaining glucose metabolic balance in ruminants. Previous in vitro studies have demonstrated that oregano oil acts similarly to ionophores under culture conditions, altering methane production and concentrations of acetate, propionate, butyrate, and ammonia nitrogen, as well as the acetate/propionate ratio. However, these conclusions derive from simulated rumen environments, and while in vitro methods provide intuitive insights into fermentation modulation, the actual rumen environment in live animals is far more complex with numerous dynamic influencing factors, creating substantial differences between in vitro and in vivo conditions. This study investigated the effects of different oregano oil supplementation levels on ruminal fermentation characteristics and dietary nutrient degradability in fistulated sheep to determine optimal inclusion rates and validate in vitro findings, providing a reference for oregano oil application in sheep production.

1.1 Experimental Materials

Oregano essential oil was purchased from Hanle Biological Technology Co., Ltd. (Xinghua, Jiangsu) with a purity of 10%; the carrier component was silicon dioxide.

1.2 Experimental Design and Animal Groups

Four crossbred wethers (Xinjiang Fine-wool × Dorper) fitted with permanent rumen fistulas, averaging (40.83 ± 4.11) kg body weight and four years of age, were allocated to a 4 × 4 Latin square design. The control group received a basal diet, while experimental groups received the basal diet supplemented with 0.015%, 0.030%, and 0.045% oregano essential oil, respectively. The experiment comprised four periods, each lasting 12 days (10-day adaptation and 2-day sampling), with respective oregano oil levels introduced during the adaptation phase. Animal allocation is shown in Table 1 .

1.3 Basal Diet and Management

The basal diet was formulated according to nutrient requirements for growing ram lambs (40 kg body weight, 100 g daily gain) as recommended in the Feeding Standard of Meat-Producing Sheep and Goats (NY/T 816–2004), with a concentrate-to-forage ratio of 30:70. Diet composition and nutrient levels are presented in Table 2 . Animals were fed equal portions twice daily at 07:00 and 17:00 (forage first, then concentrate) with free access to water.

1.4 Sample Preparation and Nylon Bag Technique

Diet samples were ground to pass through a 40-mesh sieve. Nylon bags (12 cm × 8 cm, 0.045 mm pore size) were filled with 5 g of diet sample, with six bags placed in a larger mesh bag. Following the principle of simultaneous insertion and sequential removal, bags were introduced into the rumen before morning feeding on day 1 of the sampling period and retrieved after 6, 12, and 24 hours of incubation. Two bags were removed at each time point, providing two replicates per time point. Retrieved bags were immediately rinsed under tap water, soaked for 45 minutes, rinsed again under moderate water flow, and dried at 65°C for determination of dry matter (DM), neutral detergent fiber (NDF), acid detergent fiber (ADF), and crude protein (CP) content.

1.5 Rumen Fluid Collection

On day 2 of the sampling period, rumen fluid was collected from multiple sites within the rumen at 0 (pre-feeding, 07:00), 2 (09:00), 4 (11:00), 6 (13:00), and 10 hours (pre-evening feeding, 17:00). pH was measured immediately, and samples were stored at -20°C for subsequent analysis of total nitrogen (TN), ammonia nitrogen (NH -N), urea nitrogen (UN), and volatile fatty acids (VFA).

1.6 Analytical Methods

Rumen fluid pH: Measured using a pH meter (pHS-3C, Shanghai Leici Instrument Factory).

VFA concentration: Determined by gas chromatography (6890N, Agilent, USA) using an HP19091N-213 capillary column (Agilent, USA). Chromato-

graphic conditions: injector temperature 220°C, nitrogen flow rate 2.0 mL/min, split ratio 40:1, injection volume 0.6 L, temperature program (120°C for 3 min, then 10°C/min to 180°C, hold 1 min), flame ionization detector (FID) 250°C, FID air/hydrogen/nitrogen flow rates 450/40/45 mL/min.

Total nitrogen and dietary nutrient analysis: CP determined by Kjeldahl method; ether extract per GB/T 6433–2006; calcium and phosphorus per GB/T 6437–2002; other parameters analyzed according to *Feed Analysis and Feed Quality Detection Technology* [7].

Ammonia nitrogen: Determined by modified colorimetric method of Feng et al. [8].

Urea nitrogen: Determined by diacetyl monoxime method using assay kits from Nanjing Jiancheng Bioengineering Institute.

NDF and ADF: Determined according to Van Soest et al. [9].

Nutrient degradability calculation:

$$\text{Nutrient degradability (\%)} = \left[\frac{\text{Initial nutrient content} - \text{Residual nutrient content after incubation}}{\text{Initial nutrient content}}\right] \times 100$$

1.7 Statistical Analysis

Data were analyzed using Latin square ANOVA in SPSS 19.0 software. When significant differences were detected, multiple comparisons were performed using Tukey' s test (for homogeneous variances) or Tamhane' s test (for heterogeneous variances). Significance was declared at $P < 0.05$.

Results

2.1 Effects of Oregano Essential Oil on Ruminal Nutrient Degradability

As shown in Table 3 , oregano essential oil supplementation had no significant effect on ruminal degradability of any dietary nutrient ($P > 0.05$). Across all supplementation levels, nutrient degradability increased with longer ruminal incubation time.

2.2 Effects of Oregano Essential Oil on Rumen Fluid pH and VFA Profiles

Table 4 presents the effects on ruminal pH and VFA concentrations. Oregano oil supplementation did not significantly affect ruminal pH ($P > 0.05$). However, total VFA concentration at 4 hours was significantly lower in the 0.030% and 0.045% groups compared to the control ($P < 0.05$). The 0.015% group exhibited significantly lower acetate proportions at 0, 2, and 6 hours ($P < 0.05$), significantly lower acetate/propionate ratio at 6 hours ($P < 0.05$), and significantly

higher propionate proportion at 2 hours compared to all supplemented groups ($P < 0.05$), though not different from the control.

2.3 Effects of Oregano Essential Oil on Rumen Fluid Nitrogen Concentrations

Table 5 shows that oregano essential oil supplementation had no significant effect on concentrations of total nitrogen, ammonia nitrogen, or urea nitrogen in rumen fluid ($P > 0.05$).

Discussion

3.1 Effects on Ruminal Nutrient Degradability

The nylon bag technique provides accurate evaluation of dietary nutrient degradation in the rumen with simple operation, with DM, CP, NDF, and ADF serving as key indicators [10]. In this study, oregano oil did not significantly affect degradability of DM, CP, NDF, or ADF. This likely reflects that nutrient digestibility is primarily determined by physicochemical properties of the nutrients themselves; probiotics cannot alter these inherent characteristics but may affect fiber degradation by modifying bacterial populations [11]. Additionally, oregano oil supplementation may not reduce fungal populations that contribute to fiber degradation [12]. Benchaar et al. [13] conducted a Latin square trial with rumen-fistulated Holstein cows fed alfalfa and corn silage-based diets supplemented with 750 mg/d of mixed essential oils, reporting no significant effects on ruminal degradability of DM, CP, NDF, or ADF—consistent with our findings. However, some research suggests essential oil effects depend on diet type, potentially altering microbial attachment and colonization to affect solubilization of dietary components. When soluble rumen substrates are used, essential oils may inhibit amino acid deamination without affecting protein or peptide hydrolysis [14]. Molero et al. [14] added 700 mg/d of mixed essential oils to growing cattle diets and observed reduced CP degradability only under high-concentrate feeding conditions. Newbold et al. [15] found that mixed essential oils reduced soybean meal CP degradability in sheep fed a 40:60 concentrate-to-forage diet. Mcewan et al. [16] reported that essential oils affected rumen microbial capacity to colonize starch-rich grain diets and protein-rich meal diets, a conclusion supported by Duval et al. [17]. In contrast, most in vitro studies show effects on nutrient degradability. Righi et al. [18] demonstrated that 0.5 mg/L oregano oil significantly reduced in vitro DM degradability of soybean meal at 4 and 24 hours and of corn meal and total mixed ration at 24 hours. Kilic et al. [19] similarly reported reduced gas production with oregano oil supplementation in diets containing barley, soybean, and wheat straw. Bai [20] used in vitro batch culture to evaluate oregano oil at 0, 45, 450, and 4,500 mg/L, finding that 45 and 450 mg/L groups had significantly higher DM degradability than the control, while the 4,500 mg/L group showed no difference.

3.2 Effects on Rumen Fluid pH and VFA Profiles

Ruminal pH is a critical indicator of rumen metabolism, influenced by diet composition, saliva secretion, and organic acid accumulation, and determines microbial fermentation efficiency [21]. Volatile fatty acids, primarily derived from carbohydrate fermentation, supply 60–80% of energy requirements for ruminants, with acetate, propionate, and butyrate as the main products. In this study, oregano oil did not significantly affect ruminal pH, which ranged from 5.61 to 6.42 across groups—within the optimal range for microbial fermentation (5.5–7.5) [22]. The pH pattern followed typical diurnal variation: highest before feeding, declining 1–5 hours post-feeding, then gradually recovering. Total VFA concentration initially increased then decreased post-feeding, reflecting stimulated microbial growth and fermentation followed by absorption through the rumen wall, passage to lower digestive tract, or neutralization by salivary buffers. The finding that 0.030% and 0.045% supplementation reduced total VFA concentration at 4 hours, while 0.015% supplementation decreased acetate proportion and acetate/propionate ratio but increased propionate proportion, suggests dose-dependent modulation of fermentation pathways.

In vitro studies report inconsistent effects of oregano oil on VFA production, with some showing reduced total VFA [23-24] or no effect [25-26], while others demonstrate increased concentrations [27-28]. In vivo studies generally show minimal effects. Beauchemin and McGinn [25] reported that 1 g/d of mixed plant essential oils in beef cattle did not affect total or individual VFA concentrations. Benchaar et al. [13] found that 750 mg/d of mixed essential oils in lactating dairy cows had no significant impact on total VFA, individual VFA, or acetate/propionate ratio. Shi [29] used a 4×4 Latin square design with four rumen-fistulated dairy cows to evaluate oregano oil at 250, 500, and 750 g/d, observing no effects on pH, total VFA, or individual VFA proportions except for butyrate. Giannenas et al. [30] supplemented ewes with thymol-containing essential oil mixtures at 0, 50, 100, and 150 mg/kg, finding no significant effects on pH, total VFA, or individual VFA, though acetate/propionate ratio was reduced. Günal et al. [31] reported that thymol at 125, 250, and 500 mg/L in vitro did not affect pH or VFA proportions. The relatively modest effects in vivo compared to in vitro likely reflect the limiting strong aroma of oregano oil, which restricts supplementation levels and thus effective dosage, combined with the far greater complexity of the live animal rumen environment.

3.3 Effects on Rumen Fluid Nitrogen Concentrations

Oregano oil did not significantly affect total nitrogen, ammonia nitrogen, or urea nitrogen concentrations in this study. Ammonia nitrogen concentrations ranged from 11.14 to 20.34 mg/dL, within the optimal range for microbial growth (8.5–30 mg/L) [32]. Ammonia concentration depends on dietary protein degradation rate, microbial ammonia synthesis capacity, and availability of energy and carbon skeletons. Some researchers propose that essential oils inhibit hyper-ammonia-producing bacteria and deaminase activity, reducing amino acid deam-

ination [33-34]. Giannenas et al. [30] reported that while essential oils did not affect total viable bacteria, fiber-degrading bacteria, or protozoa, supplementation at 100 and 150 mg/kg significantly reduced hyper-ammonia-producing bacteria and decreased ammonia nitrogen at 150 mg/kg. Wang et al. [35] found that 250 mg/d oregano oil premix in sheep significantly reduced ruminal ammonia nitrogen after 15 days. Shi [29] observed dose-dependent reductions in ammonia nitrogen in dairy cows, with no effect on urea nitrogen. Castillejos et al. [24] reported that 500 and 5,000 mg/L thymol significantly reduced ammonia nitrogen in batch culture after 24 hours, but no effects were observed in continuous culture after 6 days, suggesting microbial adaptation or degradation of active compounds. In our study, the 12-day supplementation period per period may have allowed for microbial adaptation or inactivation of active components. Overall, effects on nitrogen metabolism vary with active components, experimental conditions, diet type, supplementation level, and main constituents.

Conclusion

Under the conditions of this study, oregano essential oil supplementation at 0.015% to 0.045% did not significantly affect ruminal nutrient degradability or nitrogen metabolism but did modulate volatile fatty acid profiles. Supplementation at 0.015% effectively reduced acetate proportion and represents the optimal inclusion level.

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Note: Figure translations are in progress. See original paper for figures.