Effects of Oregano Oil on Rumen Microbial Protein Production, Milk Production Performance, and Nitrogen Excretion in Dairy Cows: Postprint

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Abstract

This experiment was conducted to investigate the effects of oregano oil on rumen microbial protein production, milk performance, and nitrogen excretion in dairy cows. Forty healthy Holstein dairy cows with similar age, body weight, parity, milk yield, milk composition, and lactation period [(90±15) d] were selected and randomly divided into 4 groups with 10 cows per group. Cows in the control group, experimental group 1, experimental group 2, and experimental group 3 were supplemented with 0, 10, 13, and 16 g/(d · head) of oregano oil in their diets, respectively. The preliminary period was 15 days, and the formal experimental period was 60 days. The results showed: 1) Rumen microbial protein production in experimental groups 1, 2, and 3 increased by 9.77% ($P<0.01$), 16.14% ($P<0.01$), and 6.99% ($P<0.05$) compared with the control group, respectively. 2) In terms of milk yield, experimental groups 1, 2, and 3 increased by 5.10% ($P>0.05$), 8.36% ($P<0.05$), and 5.00% ($P>0.05$) compared with the control group, respectively; in terms of milk fat percentage, experimental group 2 was significantly higher than the control group ($P<0.05$); in terms of milk somatic cell count, all experimental groups were extremely significantly lower than the control group ($P<0.01$), with experimental group 2 being the lowest. 3) In terms of total nitrogen excretion, experimental groups 1, 2, and 3 decreased by 6.84% ($P<0.01$), 11.14% ($P<0.01$), and 4.72% ($P<0.05$) compared with the control group, respectively. The results of this experiment indicate that adding oregano oil to dairy cow diets has the effects of increasing rumen microbial protein production, improving milk performance, and reducing nitrogen excretion. Based on comprehensive consideration of these indicators, the optimal supplementation level of oregano oil in dairy cow diets is 13 g/(d · head).

Full Text

Abstract

This experiment was conducted to investigate the effects of oregano oil on ruminal microbial protein production, milk performance, and nitrogen excretion in dairy cows. Forty healthy Holstein cows with similar age, body weight, parity, milk yield, milk composition, and lactation stage [(90±15) d] were randomly allocated into four groups of ten cows each. The control group and experimental groups 1, 2, and 3 received dietary supplementation of oregano oil at 0, 10, 13, and 16 g/(d • head), respectively. The study consisted of a 15-day preliminary period followed by a 60-day formal experimental period. The results showed: (1) Ruminal microbial protein production in experimental groups 1, 2, and 3 increased by 9.77% ($P<0.01$), 16.14% ($P<0.01$), and 6.99% ($P<0.05$) compared to the control group, respectively. (2) For milk yield, experimental groups 1, 2, and 3 showed increases of 5.10% ($P>0.05$), 8.36% ($P<0.05$), and 5.00% ($P>0.05$) relative to the control. Milk fat percentage was significantly higher in experimental group 2 ($P<0.05$), while milk somatic cell count was extremely significantly lower in all experimental groups ($P<0.01$), with experimental group 2 showing the lowest values. (3) Total nitrogen excretion decreased by 6.84% ($P<0.01$), 11.14% ($P<0.01$), and 4.72% ($P<0.05$) in experimental groups 1, 2, and 3, respectively, compared to the control. These findings indicate that dietary oregano oil supplementation can increase ruminal microbial protein production, improve milk performance, and reduce nitrogen excretion in dairy cows. Based on comprehensive evaluation of these parameters, the optimal supplementation level of oregano oil in dairy cow diets is 13 g/(d • head).

Keywords: oregano oil; ruminal microbial protein; milk performance; nitrogen excretion

Introduction

With the rapid development and expanding scale of dairy farming in China, substantial amounts of unused nitrogen from dairy manure and urine are discharged directly into the environment, causing both waste of protein resources and environmental pollution. In recent years, national requirements for environmental protection in livestock development have become increasingly stringent. Therefore, in practical production, it is critically important to implement nutritional regulation techniques that improve dietary protein utilization and reduce nitrogen excretion without compromising milk performance. Oregano oil, also known as oregano phenol or origanum oil, represents a novel green feed additive that can enhance animal production and immune performance. It possesses antimicrobial, bacteriostatic, and bactericidal properties, promotes growth, improves intestinal microflora, and features zero withdrawal period, no drug resistance development, and no compatibility contraindications [1-2].

Yao et al. [3] found that adding oregano essential oil to total mixed ration (TMR) significantly improved dairy cow production performance. Wang and Li [4] reported that oregano oil supplementation in piglet diets significantly increased the digestibility and utilization of dietary protein and dry matter while reducing nitrogen excretion. Han [5] demonstrated that dietary oregano oil in laying hens significantly improved production performance and egg quality, regulated intestinal microflora balance, enhanced digestive enzyme activity, and improved nutrient absorption. Current research on oregano oil in animal production has primarily focused on poultry and swine, with limited studies in ruminants concentrating mainly on production performance. Simultaneous investigation of oregano oil' s effects on ruminal microbial protein (MCP) production, milk performance, and nitrogen excretion in dairy cows remains relatively scarce. This study examined the effects of different dietary oregano oil levels on these parameters to determine the optimal supplementation level, aiming to enhance production performance, feed protein utilization, and MCP production while reducing nitrogen excretion, thereby providing a reference for sustainable development of China' s dairy industry.

Materials and Methods

1.1 Experimental Design

The oregano oil used in this experiment was provided by Qingdao Runbot Biological Technology Co., Ltd. as a white powder containing 10% oregano oil and 12% moisture. A single-factor randomized design was employed. Forty healthy Holstein cows with similar body condition, age, body weight, parity, milk yield, milk composition, and lactation stage [(90±15) d] were selected from Yantai Hemuyuan Animal Husbandry Co., Ltd. and randomly divided into four groups of ten cows each. The control group and experimental groups 1, 2, and 3 received dietary supplementation of oregano oil at 0, 10, 13, and 16 g/(d · head), respectively. The supplementation method involved reserving 0.5 kg wheat bran from each cow' s daily ration, mixing it thoroughly with the oregano oil, dividing the mixture into two equal portions, and feeding twice daily with TMR. The composition and nutrient levels of the TMR are presented in Table 1 .

1.2 Feeding Management

Cows were fed in separate pens throughout the 75-day trial, comprising a 15-day preliminary period and a 60-day formal experimental period. Cows were milked three times daily (04:00, 12:00, and 18:00) using imported Dutch SAC automatic milking machines and fed TMR twice daily (04:30 and 18:30), ensuring access to TMR for over 20 hours per day. After feeding, cows had free access to exercise areas and water, with routine deworming, lighting, and management practices applied.

1.3 Sample Collection

1.3.1 Feed Samples During days 1-3, 28-30, and 58-60 of the formal period, TMR samples were collected three times using the quartering method. Samples were dried at 65°C in a constant-temperature oven to produce air-dried samples, then ground and mixed for subsequent analysis.

1.3.2 Urine Samples Urine samples were collected during days 1-3 of the preliminary period and days 28-30 and 58-60 of the formal period. Following the spot urine collection method described by Zhu [7], manual collection combined with bladder catheterization was performed twice daily at 12-hour intervals for three consecutive days, with collection times delayed by 4 hours each day relative to the previous day. Ten percent sulfuric acid was added to each sample to adjust pH below 3, and samples were stored at -20°C.

1.3.3 Fecal Samples Fecal samples were collected during days 1-3 of the preliminary period and days 28-30 and 58-60 of the formal period through 24-hour total fecal collection for three consecutive days. Before each collection, cow beds were thoroughly cleaned. Daily fecal output was collected in buckets, mixed uniformly, and weighed. Using the quartering method during weighing, 25 mL of 10% sulfuric acid was added per 100 g of feces for nitrogen fixation, and samples were frozen at -20°C. After the final sampling day, the three-day fecal samples were mixed proportionally by weight, oven-dried at 65°C to constant weight, and stored as air-dried samples for analysis.

1.3.4 Milk Samples During days 1-3 of the preliminary period and days 13-15, 28-30, 43-45, and 58-60 of the formal period, milk samples (50 mL) were collected at a morning:afternoon:evening ratio of 4:3:3. Potassium dichromate (30 mg) was added as a preservative, and samples were mixed and refrigerated at 4°C for milk composition analysis.

1.4 Measurements and Calculations

1.4.1 Feed Intake Cows were fed in separate pens with individual intake recorded. During the preliminary period, feed offered and refusals were weighed every two days for six total recordings, allowing calculation of average intake. During the formal period, intake was recorded and calculated every ten days for six total recordings, with three consecutive days measured each time. Feed allocation for subsequent periods was adjusted based on the previous period’ s average intake. Final average intake during the formal period was calculated from the six recordings and used to determine nutrient intake based on TMR composition.

1.4.2 Nutrient Content in Diets and Feces Dry matter (DM) content was determined after measuring moisture according to GB/T 6435-2006 [8]. Crude protein (CP) was measured by the Kjeldahl method per GB/T 6432-1994 [9].

Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined following GB/T 20806-2006 [10] and NY/T 1459-2007 [11], respectively. Calcium (Ca) and phosphorus (P) were measured by the potassium permanganate method (GB/T 6436-2002 [12]) and spectrophotometry (GB/T 6437-2002 [13]), respectively.

1.4.3 Ruminal Microbial Protein (MCP) Production Purine derivatives (PD) excreted in urine primarily originate from rumen microbial purines, enabling estimation of MCP production through urinary PD measurement. Uric acid and allantoin concentrations were determined by colorimetric methods, with their sum representing total PD content [14].

The amount of exogenous purines absorbed by the small intestine (X) was calculated as:

$$Y = 0.85X + 0.385BW^{0.75}$$

where Y is urinary PD excretion (mmol/d), 0.85 is the recovery rate of absorbed purines converted to urinary PD in cattle, 0.385 is endogenous PD excretion when purine absorption is zero, and BW^0.75 is metabolic body weight (kg).

MCP production was calculated as:

$$\text{Microbial protein production (g/d)} = \frac{6.25 \times 70X}{0.83 \times 0.116 \times 1000} = 6.25 \times 0.727X$$

where X is the amount of exogenous purines absorbed by the small intestine (mmol/d), 70 is the nitrogen content per mole of purines (mg/mol), 0.83 is the digestibility of microbial nucleic acid purines, 0.116 is the proportion of purine nitrogen in total microbial nitrogen, and 6.25 is the conversion factor from nitrogen to protein. MCP production during the formal period was calculated as the average of values obtained on days 30 and 60.

1.4.4 Milk Yield and Composition Milk yield was recorded automatically during milking using the Dutch SAC system. During both preliminary and formal periods, milk yield was recorded every five days for three consecutive days, with the three-day average used for analysis. Milk fat percentage, protein percentage, lactose percentage, and somatic cell count were determined using a Combi Foss FT+ automatic analyzer (Foss, Denmark) at the Dairy Performance Testing Laboratory of Shandong Academy of Agricultural Sciences.

1.4.5 Nitrogen Metabolism Parameters Urinary urea nitrogen was measured by the urease method [15], and urinary creatinine by the picric acid colorimetric method [16]; both assay kits were purchased from Nanjing Jiancheng Bioengineering Institute. Following Valadares et al. [16], urinary volume was estimated using creatinine as a marker (approximately 29 mg creatinine excreted per kg body weight daily).

Nitrogen metabolism parameters were calculated as: - Fecal nitrogen (g/d) = daily nitrogen output × crude protein content in feces × 0.16 - Milk nitrogen (g/d) = milk yield × milk protein percentage × 0.16 - Digestible nitrogen (g/d) = nitrogen intake - fecal nitrogen - Total nitrogen excretion (g/d) = fecal nitrogen + urinary nitrogen - Nitrogen apparent digestibility (%) = [(nitrogen intake - fecal nitrogen) / nitrogen intake] × 100

1.5 Data Processing and Analysis

Data were initially processed using Excel 2010 and analyzed by one-way ANOVA using SPSS 20.0. Duncan' s multiple comparison test was used to examine differences among groups. Significance was declared at $P<0.05$ and extreme significance at $P<0.01$. Results are expressed as means ± standard error.

Results

2.1 Effects of Oregano Oil on Main Nutrient Intake

As shown in Table 2 , dry matter and main nutrient intake in all experimental groups showed an increasing trend compared to the control group, but differences were not significant ($P>0.05$).

2.2 Effects of Oregano Oil on Ruminal Microbial Protein Production

Table 3 shows that urinary uric acid excretion in experimental group 2 was extremely significantly higher than in the control group ($P<0.01$), while experimental group 3 was significantly higher ($P<0.05$); experimental group 2 was also significantly higher than experimental group 1 ($P<0.05$). Allantoin excretion in experimental groups 1 and 2 was extremely significantly higher than in the control ($P<0.01$), while group 3 was significantly higher ($P<0.05$); experimental group 2 was significantly higher than group 3 ($P<0.05$). Purine derivative excretion in groups 1 and 2 was extremely significantly higher than the control ($P<0.01$), while group 3 was significantly higher ($P<0.05$); experimental group 2 was significantly higher than both groups 1 and 3 ($P<0.05$). MCP production in groups 1 and 2 was extremely significantly higher than the control ($P<0.01$), while group 3 was significantly higher ($P<0.05$); experimental group 2 was significantly higher than group 1 ($P<0.05$) and extremely significantly higher than group 3 ($P<0.01$). Compared to the control, MCP production increased by 9.77%, 16.14%, and 6.99% in experimental groups 1, 2, and 3, respectively.

2.3 Effects of Oregano Oil on Milk Yield and Composition

Table 4 indicates that milk yield in experimental group 2 was significantly higher than the control ($P<0.05$), while groups 1 and 3 showed no significant difference ($P>0.05$). Compared to the control, milk yield increased by 5.10%, 8.36%, and 5.00% in groups 1, 2, and 3, respectively. Milk fat percentage was significantly higher in experimental group 2 ($P<0.05$), while groups 1 and 3 did not differ significantly from the control. No significant differences were observed in milk protein or lactose percentages among groups ($P>0.05$). However, milk somatic cell count was extremely significantly lower in all experimental groups compared to the control ($P<0.01$).

2.4 Effects of Oregano Oil on Nitrogen Excretion and Apparent Digestibility

Table 5 reveals no significant differences in nitrogen intake among groups ($P>0.05$). Fecal nitrogen was extremely significantly lower in experimental groups 1 and 2 ($P<0.01$) and significantly lower in group 3 ($P<0.05$) compared to the control; experimental group 2 was significantly lower than group 3 ($P<0.05$). Urinary nitrogen was extremely significantly lower in groups 1 and 2 ($P<0.01$) and significantly lower in group 3 ($P<0.05$); experimental group 2 was extremely significantly lower than both groups 1 and 3 ($P<0.01$). Milk nitrogen was significantly higher in experimental group 2 ($P<0.05$) compared to the control. Digestible nitrogen was extremely significantly higher in groups 1 and 2 ($P<0.01$) and significantly higher in group 3 ($P<0.05$); experimental group 2 was significantly higher than group 3 ($P<0.05$). Total nitrogen excretion was extremely significantly lower in all experimental groups ($P<0.01$), with group 2 being extremely significantly lower than groups 1 and 3 ($P<0.01$). Compared to the control, total nitrogen excretion decreased by 6.84%, 11.14%, and 4.72% in groups 1, 2, and 3, respectively. Nitrogen apparent digestibility was extremely significantly higher in groups 1 and 2 ($P<0.01$) and significantly higher in group 3 ($P<0.05$); experimental group 2 was extremely significantly higher than group 3 ($P<0.01$).

Discussion

3.1 Effects of Oregano Oil on Main Nutrient Intake

Zhang [17] observed that dietary oregano oil could alleviate reduced feed intake caused by heat stress and improve intake in beef cattle. Wang et al. [18] found that oregano oil extract tended to increase feed intake in weaned piglets. Zhou et al. [19] reported that oregano oil improved daily feed intake and weight gain in growing pigs while significantly increasing dry matter digestibility, though it did not significantly affect apparent digestibility of crude protein, calcium, or phosphorus. In the present study, oregano oil supplementation did not significantly affect main nutrient intake in Holstein cows, consistent with these previous findings. Some studies suggesting improved dry matter intake may attribute this effect to oregano oil' s distinctive aroma stimulating appetite and activating digestive enzymes, thereby promoting feed consumption and digestion.

3.2 Effects of Oregano Oil on Ruminal Microbial Protein Production

Urinary purine derivatives primarily originate from rumen microbial purines, enabling estimation of MCP production through PD measurement. In this study, all three experimental groups showed significant or extremely significant increases in uric acid, allantoin, and PD excretion, resulting in significantly enhanced MCP production. Shi [20] found that dietary oregano oil increased uric acid and allantoin content while tending to elevate PD and microbial nitrogen content. As the primary nitrogen source for ruminants, MCP provides 60-70% of dietary protein [6]. Ruminal ammonia nitrogen (NH3-N) concentration serves as a crucial indicator of rumen nitrogen metabolism, reflecting the balance between protein degradation and MCP synthesis [21]. Wang et al. [22] reported that adding 200 mg/L oregano oil to rumen fermentation fluid reduced NH3-N concentration while increasing MCP production. These results suggest that oregano oil improves apparent digestibility of main nutrients [19], enhances rumen microbial utilization of crude protein, and strengthens the rate of NH3-N incorporation into MCP, ultimately increasing MCP production. Our findings align with these previous studies.

3.3 Effects of Oregano Oil on Milk Yield and Composition

Chen et al. [23] reported that adding 15 g of 10% oregano oil premix to dairy cow diets significantly increased milk fat percentage and tended to improve milk yield and protein percentage. Shi [20] observed positive effects of oregano oil on milk yield and protein percentage in lactating cows. Chen et al. [24] found that dietary oregano essential oil reduced the incidence of mastitis in individual mammary quarters. Yuan et al. [25] reported that combining oregano oil with flavomycin or monensin significantly improved milk fat and protein percentages. Guo [26] demonstrated that oregano oil supplementation significantly reduced milk somatic cell count when initial counts were high. In our study, supplementation at 13 g/(d · head) significantly increased milk yield and fat percentage while extremely significantly reducing somatic cell count, consistent with previous research. These results indicate that oregano oil can regulate ruminal pH, improve the rumen microbial environment [27], promote beneficial microbial growth, enhance nutrient digestion and absorption, and boost immunity through its antimicrobial effects, thereby improving milk performance and quality while reducing somatic cell counts.

3.4 Effects of Oregano Oil on Nitrogen Excretion and Apparent Digestibility

Manzanilla et al. [28] reported that oregano oil inhibits harmful bacteria while promoting normal microflora development, increasing rumen content retention time and enhancing protein digestion and absorption, thereby improving digestible nitrogen, nitrogen retention, and apparent digestibility. Shi [20] found that oregano oil reduced urinary and fecal nitrogen, consequently decreasing total nitrogen excretion. Guo et al. [29] demonstrated that oregano oil enhanced small intestinal protease activity, improved protein digestibility, reduced fecal nitrogen, and increased nitrogen retention in chickens. In our study, oregano oil supplementation significantly or extremely significantly reduced fecal and urinary nitrogen while increasing digestible nitrogen and nitrogen apparent digestibility. These results suggest that oregano oil inhibits harmful rumen bacteria, improves the rumen environment, slows protein degradation, prolongs nutrient retention time in the digestive tract [30], enhances protein utilization, increases PD excretion, boosts MCP production, and reduces nitrogen excretion by accelerating NH3-N utilization by rumen microbes.

Conclusion

Dietary oregano oil supplementation can increase ruminal microbial protein production, improve milk performance, and reduce nitrogen excretion in dairy cows. Based on comprehensive evaluation of these parameters under our experimental conditions, the optimal supplementation level of oregano oil in dairy cow diets is 13 g/(d · head).

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