Effects of Quercetin Supplementation in a High-Concentrate Diet on Rumen Fermentation, Rumen Microbial Population, and Serum Parameters in Goats (Postprint)

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Abstract

This experiment aimed to investigate the effects of quercetin supplementation in a high-concentrate diet on rumen fermentation, microbial populations, and serum parameters in goats. Using a randomized block design, 12 Boer crossbred goats (Boer × Yangtze River Delta white goat) fitted with permanent rumen fistulas were randomly divided into a control group (no quercetin supplementation) and a treatment group (quercetin supplementation at 100 mg/kg BW), with 6 goats per group. The experimental period lasted 42 days; rumen fluid was collected at 0 (08:00), 2, 4, and 8 h after morning feeding on days 35, 38, and 41; jugular vein blood was collected at 0 and 6 h after morning feeding on days 36, 39, and 42. The results showed that, compared with the control group, quercetin supplementation in the high-concentrate diet significantly increased rumen fluid pH (P=0.047) and the concentrations of isobutyrate (P=0.001) and valerate (P=0.034); significantly increased serum total antioxidant capacity (P=0.031), reduced glutathione content (P=0.002), and blood urea nitrogen concentration (P=0.006), while significantly decreasing serum potassium concentration (P=0.042); and had no significant effect on the quantities of Firmicutes and Bacteroidetes bacteria in rumen fluid (P>0.10). These results suggest that under high-concentrate diet conditions, quercetin supplementation can increase rumen fluid pH in goats, enhance systemic antioxidant capacity, and has potential protective effects on goat health.

Full Text

Effects of Quercetin Supplementation in High-Concentrate Diet on Rumen Fermentation, Rumen Bacterial Counts, and Serum Indices in Goats

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Abstract: This study investigated the effects of quercetin supplementation in a high-concentrate diet on rumen fermentation, rumen bacterial counts, and serum indices in goats. Twelve Boer crossbred goats (Boer × Yangtze River Delta White goat) fitted with permanent rumen fistulas were randomly allocated to two groups (n = 6) using a randomized block design. The control group received no quercetin supplementation, while the treatment group received quercetin at 100 mg/kg body weight. The experiment lasted 42 days. Rumen fluid samples were collected at 0 (08:00), 2, 4, and 8 hours after morning feeding on days 35, 38, and 41. Blood samples were collected via jugular venipuncture at 0 and 6 hours after morning feeding on days 36, 39, and 42. Compared with the control group, quercetin supplementation significantly increased rumen fluid pH ($P = 0.047$) and the concentrations of isobutyrate ($P = 0.001$) and valerate ($P = 0.034$). Quercetin also significantly elevated serum total antioxidant capacity ($P = 0.031$), reduced glutathione content ($P = 0.002$), and urea nitrogen concentration ($P = 0.006$), while significantly decreasing serum potassium ion concentration ($P = 0.042$). However, quercetin had no significant effect on the counts of Firmicutes and Bacteroidetes in rumen fluid ($P > 0.10$). These results suggest that under high-concentrate feeding conditions, quercetin supplementation can increase rumen fluid pH, enhance antioxidant capacity, and may exert protective effects on goat health.

Keywords: high-concentrate diet; subacute ruminal acidosis; quercetin; antioxidant; bacterial flora

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High-concentrate diets are widely used in modern ruminant production to meet the energy demands of high-yielding animals. However, such diets can disrupt rumen fermentation, leading to subacute ruminal acidosis, which compromises animal health and performance. Nutritional strategies to mitigate these negative effects and oxidative stress are therefore crucial for improving ruminant health. Quercetin is a polyhydroxy flavonoid compound abundant in plant flowers, leaves, and fruits that exhibits antioxidant, anticancer, anti-inflammatory, and vasodilatory properties. Additionally, Shu et al. reported that quercetin inhibits the growth of *Streptococcus mutans*, *Streptococcus sanguis*, *Lactobacillus*

acidophilus, and *Streptococcus sobrinus*. These findings suggest that quercetin may potentially improve rumen fermentation, exert anti-inflammatory effects, and prevent oxidative stress in animals fed high-concentrate diets. Based on the detrimental effects of high-concentrate diets on rumen and systemic health and the therapeutic potential of quercetin, this study examined the effects of quercetin supplementation on rumen fermentation, bacterial counts, and serum indices in goats fed a high-concentrate diet, aiming to provide a theoretical basis for quercetin application in ruminant production.

1.1 Experimental Design

The experiment was conducted at the animal facility of Nanjing Agricultural University from April to May 2015. Quercetin dihydrate (purity $\geq$ 98%) was purchased from Adamas and added to the diet in this form. Twelve healthy Boer crossbred goats (Boer $\times$ Yangtze River Delta White goat) with permanent rumen fistulas and similar body weight [(28.4 $\pm$ 3.0) kg] were individually housed with ad libitum access to water. Using a randomized block design, the goats were randomly divided into a control group (no quercetin) and a treatment group (quercetin supplementation at 100 mg/kg body weight), with six goats per group. The experiment lasted 42 days. The basal diet was provided at 3.5% of body weight with a concentrate-to-forage ratio of 65:35. Concentrate and forage were fed separately twice daily (08:00 and 17:00) in equal amounts. The composition and nutrient levels of the basal diet are presented in Table 1 .

1.2 Sample Collection

Rumen fluid samples were collected on days 35, 38, and 41 at 0 (08:00), 2, 4, and 8 hours after morning feeding. Samples were filtered through four layers of cheesecloth, and pH was measured immediately. Additional samples were frozen at -20 °C for subsequent analysis of rumen fermentation parameters. Blood samples were collected via jugular venipuncture on days 36, 39, and 42 at 0 and 6 hours after morning feeding. Serum was separated by conventional methods, aliquoted, and stored at -20 °C until analysis.

1.3 Index Determination

Rumen volatile fatty acid (VFA) concentrations were determined using the method of Qin Weilin, and ammonia nitrogen (NH_3-N) concentration was measured using the colorimetric method of Feng Zongci et al. Serum antioxidant indices were determined using assay kits purchased from Nanjing Jiancheng Bioengineering Institute. Serum biochemical indices were measured at Jiangsu Provincial Hospital of Integrated Chinese and Western Medicine.

1.4 Bacterial DNA Extraction and Real-Time PCR Quantification

Total rumen bacterial DNA was extracted from 1 mL of rumen fluid using a bead-beating method based on Murray et al. An ABI 7500 real-time PCR sys-

tem (Applied Biosystems) was used to quantify Bacteroidetes and Firmicutes in rumen fluid. *Bacteroides pyogenus* and Firmicutes 16S rRNA genes were used as templates at concentrations of 3.29×10^9 and 7.48×10^9 CFU/ L, respectively. Templates were serially diluted tenfold, and five points on the standard curve were analyzed in triplicate to generate standard curves for bacterial quantification. The real-time PCR reaction mixture (20 L) contained 10.4 L SYBR Green Supermix (TOYOBO), 0.4 L each of 10 mol/L forward and reverse primers, 2 L of sample DNA, and 6.8 L sterile water. Primer information is provided in Table 2 . Primers Bact934F/Bact1060R and Firm934F/Firm1060R were used to quantify Bacteroidetes and Firmicutes, respectively. The PCR program consisted of initial denaturation at 95 °C for 10 min, followed by 40 cycles of 95 °C for 30 s and annealing/extension at 60 °C for 1 min.

1.5 Data Processing and Statistical Analysis

Data were initially processed using Excel 2010 and analyzed using the GLM procedure in SPSS 18.0. Differences were considered significant at $P < 0.05$ and trends at $0.05 \leq P < 0.10$.

2.1 Rumen Fermentation Parameters

As shown in Table 3 , quercetin supplementation significantly increased rumen fluid pH ($P = 0.047$) and the concentrations of isobutyrate ($P = 0.001$) and valerate ($P = 0.034$) compared with the control group. Butyrate concentration tended to increase ($P = 0.074$). Quercetin did not significantly affect acetate, propionate, isovalerate, total VFA, acetate/propionate ratio, or ammonia nitrogen concentrations ($P > 0.10$). However, sampling time significantly influenced rumen fluid pH ($P = 0.024$) and the concentrations of acetate ($P = 0.003$), isobutyrate ($P = 0.001$), butyrate ($P = 0.023$), isovalerate ($P = 0.015$), valerate ($P = 0.016$), total VFA ($P = 0.008$), acetate/propionate ratio ($P < 0.001$), and ammonia nitrogen ($P < 0.001$). Significant interactions between sampling time and quercetin supplementation were observed for rumen fluid pH ($P = 0.001$), propionate ($P = 0.021$), and ammonia nitrogen ($P = 0.004$).

2.2 Rumen Firmicutes and Bacteroidetes Counts

Table 4 shows that quercetin supplementation, sampling time, and their interaction had no significant effects on the counts of Firmicutes and Bacteroidetes or the Firmicutes/Bacteroidetes ratio ($P > 0.10$).

2.3 Serum Antioxidant Indices

As presented in Table 5 , the treatment group exhibited significantly higher total antioxidant capacity ($P = 0.031$) and reduced glutathione content ($P = 0.002$) than the control group, with no significant differences in malondialdehyde content or superoxide dismutase activity ($P > 0.10$). Sampling time significantly affected malondialdehyde content ($P < 0.001$), superoxide dismutase activity (P

< 0.001), and reduced glutathione content ($P < 0.001$). No significant interactions between sampling time and quercetin supplementation were observed for these indices ($P > 0.10$).

2.4 Serum Biochemical Indices

Table 6 shows that serum potassium concentration was significantly lower ($P = 0.042$) while serum urea nitrogen concentration was significantly higher ($P = 0.006$) in the treatment group compared with the control group. Quercetin supplementation did not significantly affect serum sodium, chloride, calcium, or glucose concentrations ($P > 0.10$). However, sampling time significantly influenced serum sodium ($P = 0.001$), chloride ($P < 0.001$), and glucose concentrations ($P = 0.008$). No significant interactions between sampling time and quercetin supplementation were detected for these parameters ($P > 0.10$).

2.5 Feed Intake

As shown in Table 7 , quercetin supplementation had no significant effect on feed intake ($P > 0.10$).

Rumen fluid pH is a critical indicator of rumen metabolism and health, influenced by total VFA concentration, saliva secretion, VFA absorption by rumen epithelium, and digesta outflow. The present study demonstrated that quercetin supplementation significantly increased rumen fluid pH, suggesting its potential to alleviate ruminal acidosis in ruminants. However, total VFA concentration did not differ significantly between groups. We hypothesize that the increased pH may be related to enhanced saliva secretion, though this mechanism requires further investigation as saliva production was not measured in this study.

Sodium, potassium, and chloride ions are primary regulators of acid-base balance and osmotic pressure in body fluids and are commonly used to assess water-salt metabolism and acid-base status. Serum sodium is the most important extracellular cation, with normal concentrations ranging from 135–145 mmol/L. Serum potassium is the primary intracellular cation. Research has confirmed that when rumen VFA concentrations increase, hydrogen ions from VFAs diffuse into cells while intracellular potassium ions move extracellularly to maintain rumen pH homeostasis. Studies have shown that acidosis is often accompanied by hyperkalemia. The significant reduction in serum potassium concentration observed in the treatment group suggests that quercetin may enhance hydrogen ion absorption, thereby stabilizing rumen fluid pH.

Blood urea nitrogen is a waste product of protein metabolism and serves as an accurate indicator of protein metabolism and dietary amino acid balance in animals. In ruminants, blood urea nitrogen originates from ammonia nitrogen absorbed across the rumen wall and tissue protein catabolism, correlating with rumen ammonia nitrogen concentration. The significant increase in serum urea nitrogen concentration in response to quercetin supplementation indicates that quercetin may influence nitrogen metabolism in goats.

Isoacids, comprising isobutyrate, 2-methylbutyrate, isovalerate, and valerate, have been shown to increase milk yield in dairy cows when elevated in the rumen. This study found that quercetin significantly increased rumen fluid isobutyrate and valerate concentrations. Cui et al. demonstrated that quercetin supplementation significantly increased milk yield in dairy cows, suggesting that the positive effect of quercetin on milk production may be mediated through increased isoacid concentrations.

Both in vitro and in vivo studies have demonstrated the antioxidant properties of quercetin. The present study showed that quercetin supplementation significantly increased serum total antioxidant capacity and reduced glutathione content, indicating that quercetin can mitigate oxidative stress induced by high-concentrate feeding. Although in vitro studies have reported that quercetin inhibits the growth of microorganisms such as *Streptococcus mutans*, *Streptococcus sanguis*, *Lactobacillus acidophilus*, and *Streptococcus sobrinus*, this study found no significant effect on the two dominant bacterial phyla in the rumen, suggesting that quercetin may not substantially alter rumen microbial populations.

In conclusion, under high-concentrate feeding conditions, quercetin supplementation can increase rumen fluid pH and enhance antioxidant capacity in goats, potentially exerting protective effects on animal health.

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