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Effects of abomasal supplementation of quercetin on performance, inflammatory cytokines, and matrix metalloproteinase genes expression in goats fed a high-grain diet

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ABSTRACT

Ten abomasally fistulated male goats fed twice daily at a constant rate of 4% of body weight were used to investigate the effects of abomasal supplementation of quercetin on rumen fermentation and the mRNA expression of inflammatory cytokines in the rumen epithelium, liver and hoof tissues, and the expression of matrix metalloproteinase genes in hoof tissues. The goats were placed in individual pens (1.2 × 1.2 m) with free access to water and randomly divided to two groups: one group fed an high grain diet (HG) without quercetin (control, n = 5) and the other group fed HG and treated with quercetin (treatment, n = 5) for 4 weeks. Results showed that abomasal supplementation of quercetin increased the average daily feed intake ($P < 0.001$) and body weight ($P < 0.01$). The concentration of free lipopolysaccharide (LPS) ($P < 0.05$) in rumen fluid was higher in goats supplemented with quercetin compared with the control group. Quercetin supplementation did not affect the mRNA expression of IL-1β, IL-6, TNF-α, IL-10, IL-12, and IFN-γ in the ruminal epithelial tissue ($P > 0.05$). Quercetin supplementation decreased the IL-1β mRNA expression ($P < 0.05$) and increased IL-10 mRNA expression ($P < 0.05$) in the liver tissues. Quercetin supplementation downregulated the mRNA expression of IL-1β ($P < 0.01$) and tended to downregulate the TNF-α mRNA expression ($P > 0.05$) in the hoof. Quercetin supplementation also lowered the mRNA expression levels of MMP-2 ($P < 0.05$) and MMP-9 ($P < 0.01$) in the hoof tissue. In conclusion, abomasal supplementation of quercetin improved the performance, decreased the mRNA expressions of inflammatory cytokines in the liver and hoof tissues, and reduced the risk of laminitis in goats.

1. Introduction

Feeding a high-grain diet (HG) is a commonly used strategy for meeting the nutritional demands of ruminants (dairy cattle, beef cattle, and goats), as well as for obtaining greater economic benefits from intensive breeding. However, an HG diet can stimulate rumen microbial fermentation, which in turn results in the accumulation of volatile fatty acids (VFAs). High VFA levels, in combination with a reduction in pH, can give rise to subacute ruminal acidosis (SARA) and its complications, including liver abscesses and hoof laminitis. Previous studies showed that SARA increased the levels of ruminal free lipopolysaccharide (LPS) and biogenic amines such as histamine, which caused damage to the rumen epithelium (Plaizier et al., 2008), and LPS and histamine can translocate through the damaged regions and further activate nuclear transcription factor (NF-κB) that triggers the release of inflammatory cytokines (Khafipour et al., 2009a), and even results in systemic immune response. One hypothesis is that the mechanism underlying laminitis involves inflammatory cytokines such as IL-1β and TNF-α induced by translocated LPS and histamine. In detail, these cytokines are potent activators of matrix metalloproteinases (MMPs) (Rodgerson et al., 2001). Activated MMPs can degrade the collagen fiber bundles of the suspensory apparatus of the third phalanx of the hoof, thereby leading to laminitis (Asplin et al., 2007).

Quercetin, a type of flavonoid commonly found in many plants, shows anti-oxidative and anti-inflammatory effects (Erlund, 2004) through its suppression of the pro-inflammatory cytokine production stimulated by LPS in macrophages (Cho et al., 2003). Quercetin also downregulates the expression of MMP-2 and MMP-9 proteins in prostate cancer cells (Vijayababu et al., 2006). These responses seen in cell cultures would be beneficial if they also occurred in ruminants, as similar anti-inflammatory responses would be needed to prevent the occurrence of laminitis. Unfortunately, the bioavailability of quercetin administered via ruminal supplementation is very low in cows because of degradation by the ruminal microbiota (Berger et al., 2012).

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However, post-ruminal administration significantly enhances quercetin bioavailability (Gohlke et al., 2013a).

Thus, the hypothesis of the present study was that post-ruminal supplementation of quercetin could provide anti-inflammatory benefits and have a positive effect on laminitis. To our knowledge, this is the first study to investigate the effects of abomasal supplementation of quercetin on inflammation and laminitis in goats fed a HG diet.

2. Materials and methods

The experimental procedures were conducted in accordance with the Animal Care and Use Committee of Nanjing Agricultural University.

2.1. Animals and diet

Ten abomasally fistulated male goats (Boer × Yangtze River Delta White) aged 2–3 years were used in the current study. The goats were fed on 4% body weight. After surgery, for 4 weeks before the trial, the goats were placed in individual pens (1.2 × 1.2 m) with free access to water but were only offered a hay diet to insure recovery from the surgery. The goats were randomly divided to two groups: one fed HG without quercetin (control, n = 5) and the other fed HG with quercetin (treatment, n = 5). The goats were supplied daily with 100 mg quercetin dehydrate (Adamas, Shanghai, China)/kg body weight for a total of 4 weeks. The amount was divided into two equal portions, suspended in a volume of 5 ml 0.9% NaCl solution (39 °C), and administered by one shot at 0800 h and 1700 h via the abomasal fistula. The control group received only an identical volume of NaCl solution. The diets (Table 1) were provided in equal amounts at 0800 h and 1700 h daily for 4 weeks. This amounted to a total of 4% body weight for 4 weeks. Dry matter intake was monitored daily, whereas body weight was measured weekly.

2.2. Sample collection

On day 28, jugular vein blood was collected at 0800 h before feeding. A pyrogen-free blood collection tube was used, containing 40 KIU Na-heparin/ml blood, for measuring free LPS concentration or IL-1β and IL-6 concentration. All blood samples were centrifuged at 3000 × *g* for 10 min. Blood samples for IL-1β, IL-6, and LPS measurements were stored at − 20 °C in pyrogen-free glass tubes. The goats were killed by exsanguination before the morning feeding. Rumen fluid was collected immediately and strained through 4 layers of cheesecloth to determine the pH value. Rumen fluid for measuring VFAs was centrifuged at 3000 × *g* for 15 min, and supernatants were preserved in 25% (w/v) metaphosphoric acid (5:1). Rumen fluid for measuring LPS was centrifuged at 13,000 × *g* at 4 °C for 40 min, and the supernatants were transferred to pyrogen-free glass tubes and boiled for 30 min. The two samples were stored at − 20 °C until analysis.

Within 5 min of slaughter, a representative tissue sample of the rumen epithelium was collected and washed three times in ice-cold PBS. The sample was then dissected into smaller pieces of about 0.5 × 0.5 cm. The lamellar epidermis and dermis samples of hoof were collected as described by Pollitt (1996), and a segment of the liver was collected. All the tissue samples were snap-frozen in liquid nitrogen and stored frozen until analysis.

Table 1
Ingredients and chemical composition of the diet.

Items	Amount
Ingredients (% of DM)	
Leymus chinensis	25.00
Corn	38.00
Wheat	23.50
Soybean meal	10.00
Limestone meal	1.00
Calcium phosphate dibasic	0.50
Salt	1.00
Premix[a]	1.00
Total	100.00
Nutrient levels (%)	
Net energy (MJ/kg)	11.56
CP	17.19
NDF	22.75
ADF	12.78
Calcium	0.82
Phosphorus	0.55

[a] Premix contained Na, 0.24%; K, 0.5%; S, 0.2%; Zn, 4000 mg/kg; Cu, 1000 mg/kg; Mn, 2500 mg/kg; I, 64 mg/kg; Co, 5 mg/kg; vitamin A, 16,000,000 IU/kg; vitamin D, 110,000 IU/kg, and vitamin E, 800,000 IU/kg.

2.3. Physiological parameter measurements

The VFA concentrations were determined using capillary column gas chromatography (GC-2014, Shimadzu; capillary column: 30 m × 0.32 mm × 0.25 μm film thickness). The pH of the rumen liquid was measured using a portable pH meter (HI 9125; HANNA Instruments). The levels of IL-1β and IL-6 in serum were analyzed via an enzyme-linked immunosorbent assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The concentrations of free LPS in the rumen fluid and blood plasma were determined with a chromogenic end-point Tachypleus amebocyte lysate assay kit (Chinese Horseshoe Crab Reagent Manufactory, Xiamen, China).

2.4. RNA extraction and real-time quantitative PCR

Total RNA was extracted from the tissues of the rumen epithelium, liver, and hoof using TRIzol kits (Takara Bio, Otsu, Japan), as described by Chomczynski and Sacchi (Chomczynski and Sacchi, 2006). The RNA concentration was determined by measuring the absorbance at 260 and 280 nm using a Nanodrop spectrophotometer (ND-1000UV–Vis; Thermo Fisher Scientific, Madison, WI). The absorption ratio (260/280 nm) of all samples was between 1.96 and 2.09, indicating high RNA purity. Aliquots of RNA samples were subjected to electrophoresis through a 1.4% agarose-formaldehyde gel to verify integrity. The concentration of RNA was adjusted to 1 μg/μl based on optical density and stored at − 80 °C. Total RNA (1 μg) was reverse transcribed carefully using a PrimeScript RT reagent kit with a gDNA eraser (Takara Bio, Otsu, Japan), according to the manufacturer's instructions. The primers used for IL-1β, IL-6, IL-10, TNF-α, IFN-γ, and GAPDH (used as a housekeeping gene) were those described by Liu et al. (2013). Primer sets were designed to recognize and amplify conserved nucleotide sequences encoding goat MMPs. The cDNA sequences and/or homologue (s) were identified using the BLAST (basic local alignment search tool) computer program (National Center for Biotechnology Information, Bethesda, MD, USA). Primers for MMP-2, MMP-9, MT1-MMP, and TIMP-1 (Table 2) were designed using the Primer 5 computer program (Whitehead Institute, Cambridge, MA, USA). All primers were synthesized by Invitrogen Life Technologies (Shanghai, China). Real-time quantitative PCR of target genes and GAPDH were performed using the ABI 7300 real-time PCR system (Applied Biosystems, Foster, City, CA) with fluorescence detection of SYBR Green dye. Amplification conditions were as follows: 95 °C for 30 s, followed by 40 cycles composed of 5 s at 95 °C, and 31 s at 57.5 °C (for GAPDH) or 60 °C (for others). Each sample contained 1–10 ng cDNA in 2 SYBR Green PCR master mix (Takara Bio), and 200 nmol/l of each primer in a final volume of 20 μl. All measurements were performed in triplicate. A reverse-transcription negative blank of each sample and a no-template blank served as negative controls. The relative amount of each studied mRNA was normalized to GAPDH mRNA levels, and the data were analyzed according to the $2^{-\triangle\triangle Ct}$ method.