Animal Feed Science and Technology 240 (2018) 40–51

Contents lists available at ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci

Effects of *Acremonium terricola* culture on performance, milk composition, rumen fermentation and immune functions in dairy cows

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ARTICLE INFO

Keywords:
Acremonium terricola culture
Antioxidant and immune functions
Dairy cow
Performance
Rumen fermentation and microbiota

ABSTRACT

The effects of *Acremonium terricola* culture (ATC) on performance, rumen fermentation and microbiota, and antioxidant and immune functions in dairy cows were investigated in two experiments. In Experiment 1, thirty multiparous Holstein dairy cows were blocked for day in milk, somatic cell counts and milk production and were randomly assigned to 1 of 3 treatments in an 8-week trial: a basal diet plus 0, 15 or 30 g/d ATC. The result of supplementing dairy cows with ATC was a linear increase in the apparent digestibility of dry matter, crude protein, acid detergent fiber and neutral detergent fiber, milk production, 4% fat-corrected milk, energy-corrected milk, feed efficiency, milk fat and lactose yields, and milk fat percentage, but a linear decrease in the milk somatic cell count. In Experiment 2, five late-lactating dairy cows were fed the basal diet from d 1 to 14 (pre-trial period). From d 15 to 35 (trial period), the same cows were fed 30 g/d ATC. Supplementation with ATC was stopped from d 36 to 42 (post-trial period). Feed, rumen and blood samples were collected on d 13, 14, 34, 35, 41 and 42. Compared with the pre- and post-periods, the ruminal pH and molar proportion of butyrate decreased during the trial period. Additionally, the ammonia nitrogen and total volatile fatty acid concentrations as well as the molar proportion of acetate were increased. For most of the rumen bacteria that were measured, the relative expression level increased during the trial period, but that of *Fibrobacter succinogenes* decreased. Moreover, ATC significantly increased the total antioxidant capacity as well as the activities of glutathione peroxidase and total superoxide dismutase during the trial period. The concentrations of blood glucose and immunoglobulins A, M, and G also increased in the plasma. By contrast, ATC resulted in a significant reduction in the plasma concentrations of aspartate transaminase, non-esterified fatty acids, beta-hydroxybutyric acid, interleukin-1, interleukin-6, tumor necrosis factor-α and malondialdehyde. These results show that feeding ATC

Abbreviations: ADF, acid detergent fiber; ALB, albumin; ALT, alanine transaminase; AST, aspartate transaminase; ATC, *Acremonium terricola* culture; BHBA, beta-hydroxybutyric acid; BUN, blood urea nitrogen; BW, body weight; CF, crude fiber; CHOL., cholesterol; CP, crude protein; DIM, day in milk; DM, dry matter; DMI, dry matter intake; DNA, deoxyribonucleic acid; EE, ether extract; ELISA, enzyme-linked immunosorbent assay; GLB, globulin; GLM, general linear model; GLU, glucose; GSH-Px, glutathione peroxidase; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; IL-1, interleukin-1; IL-2, interleukin-2; IL-4, interleukin-4; IL-6, interleukin-6; iNDF, indigestible neutral detergent fiber; MDA, malondialdehyde; NDF, neutral detergent fiber; NEFA, non-esterified fatty acids; NFE, nitrogen-free extract; NH_3-N, ammonia; ROS, reactive oxygen species; SCC, somatic cell counts; T-AOC, total antioxidant capacity; TG, triglyceride; TMR, total mixed ration; TNF-α, tumour necrosis factor-alpha; TP, total protein; T-SOD, total superoxide dismutase; VFA, volatile fatty acid

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https://doi.org/10.1016/j.anifeedsci.2018.03.015
Received 4 January 2018; Received in revised form 25 March 2018; Accepted 26 March 2018

can increase performance, improve rumen fermentation, and increase antioxidant and immune functions in dairy cows, when fed up to 30 g/d.

1. Introduction

Dairy cattle experience an increased incidence of diseases during their lactation period, when host defense mechanisms are compromised. Some additives have been studied for their capacity to alter the rumen microbial ecosystem and fermentation characteristics as a way to improve animal performance and feed efficiency as well as to prevent disease (Seo et al., 2010). Among these agents, Chinese herb additives have been demonstrated to have positive effects on cattle health (Liu et al., 2013).

Cordyceps gunnii is a major entomogenous fungus that belongs to the Ascomycota, Pyrenomycetes, Sphaeriales, and Clavicipitaceae and parasitizes the larvae of Hepialidae. The fungus contains natural active substances that have therapeutic effects such as cordycepin, cordycepic acid and *Cordyceps* polysaccharide (Zhu et al., 2012). However, the expensive price of *Cordyceps gunnii* limits the application and development for livestock production. Therefore, artificial culture could be used as a substitute for *Cordyceps gunnii* to meet livestock production demands. *Acremonium terricola* is a parasite of *Cordyceps gunnii* that has been isolated and identified (Fan et al., 1999). *Acremonium terricola* culture (ATC) is obtained in an inactive solid-state as a fermentation product, through processing the strain by artificial solid fermentation.

Acremonium terricola cultures have functional components similar to those of *Cordyceps gunnii*, such as cordycepin, cordycepic acid, *Cordyceps* polysaccharide and ergosterol (Sun et al., 2011). Some research shows that ATC supplementation improves growth performance in piglets, ducks and rats (Wei et al., 2009; Sun et al., 2011; Li et al., 2016a), and increase the antioxidant and immune functions of calves (Li et al., 2016b). The effects of ATC in dairy cows have not been studied. However, the mycelia of *Cordyceps militaris* have a composition similar to that of ATC and alter *in vitro* rumen microbial fermentation, causing increased production of gas and volatile fatty acid (VFA) (Yeo et al., 2009), improved cellulose digestion and cellulolytic enzymes's activities (Yeo et al., 2011), and inhibited methane production *in vitro* (Kim et al., 2014). Moreover, the extract of *Cordyceps sinensis* alters the ratio of beneficial to pathogenic bacteria in the small intestine of broiler chicks and can be used as a substitute for antibiotic growth promoters (Koh et al., 2003). Cordycepin and polysaccharides in ATC are used as prebiotics in animal nutrition (Scholzahrens et al., 2007) and have great potential as replacements for antibiotics in poultry production (Han et al., 2015). Additionally, *Cordyceps sinensis* and bioactive substances have the effect of adjusting the levels of alanine transaminase and aspartate transaminase and contents of total cholesterol and urea nitrogen in patients and mice (Li et al., 2009; Choi et al., 2014). All these results indicate that ATC possesses great potential to modulate rumen fermentation, apparent digestibility of nutrients and blood metabolism in dairy cows.

With the growing problem of antibiotic resistance, researchers are attempting to identify alternatives for antibiotics and new immunopotentiators. Therefore, the objectives of the present study were to determine the effects of ATC on performance, milk composition, rumen fermentation and microbiota, and antioxidant and immune functions in dairy cows.

2. Materials and methods

This study was performed in strict accordance with the recommendations of the National Research Council Guide, and all animal experimental procedures were approved by the Northeast Agricultural University Animal Science and Technology College Animal Care and Use Committee (Protocol number: NEAU-[2011]-9). The experiment did not involve any endangered or protected species, and the owner of KEDONG farm (Kedong County, Qiqihar City, China; east longitude: 126°01′; north latitude: 47°43′) permitted the conduction of this study.

2.1. Culture of solid-state fermentation products of Acremonium terricola

Acremonium terricola used in the present study is a recorded species for China isolated from the sclerotium of *Cordyceps gunnii* (Fan et al., 1999). The *Acremonium terricola* preservation number is CGMCC NO. 0346 in the China General Microbiological Culture Collection Center. *Acremonium terricola* culture was obtained through processing of the strain by artificial solid fermentation. Briefly, *Acremonium terricola* was inoculated on sterile solid medium, which contained 200 g/kg soybean meal, 100 g/kg wheat bran, 699 g/kg corn, 0.8 g/kg KH_2PO_4 and 0.2 g/kg $MgSO_4$, and incubated at 25 °C, with 800–900 g/kg relative humidity for 76 h. The medium was dried at 80 °C for 1 h, and passed through a 0.15 mm mesh screen.

Acremonium terricola culture was provided by Hefei Micro Biological Engineering Co. Ltd. (Hefei, China) and had the following functional composition: cordycepic acid (D-mannitol; 84.50 g/kg of dry matter (DM)), *Cordyceps* polysaccharide (Galactomannan; 44.60 g/kg of DM), cordycepin (3′-Deoxyadenosine; 0.432 g/kg of DM), ergosterol (0.597 g/kg of DM), and total amino acid content (218.10 g/kg of DM). It contained 56 g/kg moisture, 50 g/kg crude fiber (CF), 40.4 g/kg crude ash, 30.6 g/kg ether extract (EE), 245.3 g/kg crude protein (CP), and 633.7 g/kg nitrogen-free extract (NFE).

The contents of cordycepic acid, *Cordyceps* polysaccharide, cordycepin and ergosterol in ATC were determined using high performance liquid chromatography (Chang et al., 2005). Total amino acid content was determined using a HITACHI L-8800 automatic amino acid analyzer (HITACHI Co., Tokyo, Japan). *Acremonium terricola* culture was analyzed for DM (method 930.15), crude fiber