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

Yang Li[a,1], Yu-Kun Sun[a,1], Xin Li[a], Guang-Ning Zhang[a], Hang-Shu Xin[a], Hong-Jian Xu[a], Li-Yang Zhang[a], Xiao-Xiang Li[b], Yong-Gen Zhang[a,*]

[a] College of Animal Sciences and Technology, Northeast Agriculture University, Harbin 150030, PR China
[b] Microbial Biological Engineering Company Limited, Hefei 230088, PR China

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

* Corresponding author.
E-mail address: zhangyonggen@sina.com (Y.-G. Zhang).
[1] These authors contributed equally to this study.

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
0377-8401/

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 (method 993.21), crude ash (method 942.05), ether extract (method 920.39) and crude protein (method 976.05) according to the methods of the Association of Official Analytical Chemists (AOAC, 2000).

2.2. Experiment 1

2.2.1. Cows, diets and experimental design

Thirty multiparous pregnant Holstein cows (day in milk (DIM) = 133 ± 6 d, somatic cell counts (SCC) = 3.61 ± 0.28 (1×10^5 cells/mL), milk yield = 25.06 ± 3.23 kg/d) were blocked into 10 groups based on DIM, SCC and milk production, and cows within a block were randomly allocated to 1 of 3 treatments. The treatments were: (1) Control: no additive; (2) ATCLOW: 15 g/d ATC; and (3) ATCHIGH: 30 g/d ATC. The composition and nutrient concentrations of total mixed ration (TMR) are presented in Table 1. This experiment consisted of a one-week adaptation period and an eight-week collection period. The animals were kept in a tie-stall barn and had free access to water. The cows were offered the diets for ad libitum intake (50 g refusals/kg) twice daily (0700 and 1900 h) and milked 3 times daily (0630, 1200, and 1830 h). Before the morning feeding, ATC was individually hand - mixed with 100 g of the TMR feed to ensure that all supplement was ingested by the cows, and additional TMR was fed to individual cows subsequently (Sun et al., 2013).

2.2.2. Sample collection and analysis

The amount of feed offered was recorded every day, and dry matter intake (DMI) was calculated based on the feed offered and the orts. The feed samples of each cow were collected weekly and measured for DM (method 930.15), CP (method 976.05), and calcium and phosphorous (method 935.13) (AOAC, 2000). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed using an Ankom 220 Fiber Analyzer (Ankom Technology, Macedon, NY) by the methods of Van Soest et al. (1991). Heat stable α-amylase and sodium sulfite were used in the NDF procedure. Starch content was determined using a colorimetric method (Weiss and Wyatt, 2000).

Fresh fecal samples (100 g per sample, wet weight) were collected from the rectum (Cheng et al., 2014) during the final three days of each week at 0900, 1500, and 2100 h (d 5); at 0300, 0600, and 1800 h (d 6); and at 0000 h (d 7). Samples were composited per animal for measurements of nutrient digestibility. During each three-day collection period, samples of TMR and orts were collected daily and stored at −20 °C for subsequent analysis. Apparent total-tract nutrient digestibility was calculated using indigestible NDF (iNDF) as an internal marker based on the concentration of iNDF in the diet and feces, where iNDF (12-d ruminal incubation in 25 μm-pore-size bags) was determined as described by (Lee and Hristov, 2013).

Milk production was measured for the final 2 consecutive days of each week. A 24 h composite sample (approximately 50 mL) was daily collected from three milkings of each cow, which was mixed according to the actual production by volume that reflected the milk yield of day and night. The milk samples, with added bronopol tablets (milk preservative, D & F Control Systems, San Ramon, CA), were stored at 4 °C for analysis of fat, crude protein and lactose content, using an infrared automatic milk analyzer (MilkoScan FT120; Foss, Hillerød, Denmark). Somatic cell count was measured using an optical somatic cell counting analyzer (Fossomatic 400, Foss Electric, Hillerød, Denmark).

2.3. Experiment 2

2.3.1. Cows, diets and design

Five pregnant Holstein cows (BW = 637 ± 25 kg, DIM = 132 ± 3 d, SCC = 3.26 ± 0.15 (1×10^5 cells/mL), milk yield = 25.74 ± 0.87 kg/d) were used with a switchback design (self-control) in this experiment to evaluate the effects of ATC supplementation on rumen fermentation and microbiota, and antioxidant and immune functions. The dose of 30 g/d ATC was selected because of linear improvement for apparent digestibility, performance and milk composition in experiment 1. Cows were individually housed in tie-stalls and had free access to water. The cows were offered the same diets as in experiment 1 for ad libitum (50 g refusals/kg) twice daily (0700 and 1900 h). Experiment 2 was conducted over 42 d and included three periods (Sun et al., 2013). On d 1–14 (pre-trial): cows were fed TMR only (Table 1). On d 15–35 (trial period): cows were fed TMR + 30 g/d ATC. On d 36 to 42 (post-trial): cows were fed TMR only. TMR and production management program were same to Experiment 1. The ATC supplements were fed to animals in a manner similarly to that used in Experiment 1.

2.3.2. Sample collection and analysis

Three hours after the morning feeding on d 13, 14, 34, 35, 41 and 42 of the experiment, rumen fluid samples were collected from each cow using a stomach vacuum pump (Lodge-Ivey et al., 2009). To minimize contamination with saliva, the first 100–200 mL of rumen fluid was discarded, and then, 200 mL of rumen fluid was collected from each cow.

Ruminal fluid samples were strained through 4 layers of cheesecloth. Thereafter, the pH was immediately measured using a portable pH meter (Sartorius Basic pH Meter, Germany). To prepare samples for analysis of ammonia (NH_3-N) and VFA concentrations, 1 mL of 250 g/kg metaphosphoric acid was added to 5 mL of rumen filtrate and stored at −20 °C until analysis. The VFA concentration was determined by gas chromatography (Stewart and Duncan, 1985). Ammonia-N was determined using the phenol/hypochlorite method (Broderick and Kang, 1980).

Feed samples were collected from individual cows on d 13, 14, 34, 35, 41 and 42 of the experiment. Dry matter intake was determined using the same method as in experiment 1.

Total nucleic acids were extracted from the rumen contents using the bead-beating procedure described previously (Yu and

Table 1
Composition and nutritional indicators (mean ± SD) of the total mixed ration.

Items	Content (g/kg DM)
Ingredients	
Corn silage[1]	250
Alfalfa[2]	170
Chinese wildrye[3]	127
Ground corn[4]	239
Soybean meal[5]	90
Soybean hull[6]	54
Wheat bran[7]	45
$CaHPO_4$	10
Limestone	5
NaCl	5
Premix[8]	5
Total	1000
Nutritional indicators[9]	
NE_L, MJ/Kg DM	6.40
DM	436 ± 25.0
CP, g/kg DM	132 ± 5.5
NDF, g/kg DM	475 ± 12.0
ADF, g/kg DM	269 ± 14.5
Starch, g/kg DM	294 ± 22.2
Ca, g/kg DM	10.1 ± 0.2
P, g/kg DM	5.4 ± 0.1

[1] Chemical composition (g/kg of DM unless otherwise noted; mean ± SD) of corn silage was 251 ± 3.2 g/kg DM, 90.3 ± 3.68 CP, 461 ± 9.8 NDF, 272 ± 14.6 ADF, 286 ± 11.4 starch, 33.0 ± 1.90 EE, 68.2 ± 1.27 as h, 347 ± 11.7 NFC. NFC = 100 - % NDF - % CP - % ether extract - % ash.

[2] Chemical composition (g/kg of DM unless otherwise noted; mean ± SD) of alfalfa was 900 ± 10.9 g/kg DM, 203 ± 7.1 CP, 414 ± 17.8 NDF, 322 ± 17.5 ADF, 25.9 ± 3.83 EE, 109 ± 12.1 ash, 249 ± 20.2 NFC.

[3] Chemical composition (g/kg of DM unless otherwise noted; mean ± SD) of Chinese wild rye hay was 904 ± 6.6 g/kg DM, 66.7 ± 2.29 CP, 626 ± 12.8 NDF, 338 ± 16.8 ADF, 24.2 ± 1.67 EE, 54.8 ± 2.52 ash, 228 ± 13.3 NFC.

[4] Chemical composition (g/kg of DM unless otherwise noted; mean ± SD) of ground corn was 823 ± 12.3 g/kg DM, 91.8 ± 1.42 CP, 76.7 ± 4.58 NDF, 30.0 ± 1.71 ADF, 737 ± 17.6 starch, 29.6 ± 1.05 EE, 54.3 ± 2.34 ash, 748 ± 4.0 NFC.

[5] Chemical composition (g/kg of DM unless otherwise noted; mean ± SD) of soybean meal was 907 ± 16.0 g/kg DM, 468 ± 18.4 CP, 223 ± 15.6 NDF, 116 ± 11.6 ADF, 90.9 ± 8.11 EE, 55.2 ± 5.08 ash, 163 ± 22.3 NFC.

[6] Chemical composition (g/kg of DM unless otherwise noted; mean ± SD) of soybean hull was 908 ± 16.2 g/kg DM, 158 ± 14.8 CP, 605 ± 41.0 NDF, 447 ± 23.2 ADF, 31.8 ± 5.50 EE, 49.5 ± 5.42 ash, 155 ± 49.0 NFC.

[7] Chemical composition (g/kg of DM unless otherwise noted; mean ± SD) of wheat bran was 892 ± 8.4 g/kg DM, 174 ± 6.3 CP, 435 ± 32.1 NDF, 156 ± 17.2 ADF, 219 ± 12.2 starch, 43.8 ± 6.03 EE, 66.3 ± 7.60 ash, 281 ± 34.3 NFC.

[8] Contained per kilogram of the premix: Mn 6000 mg, Fe 4800 mg, Zn 9000 mg, Cu 2600 mg, I 120 mg, Se 80 mg, Co 70 mg, Vitamin A 130000 IU, Vitamin D3 36000 IU, and Vitamin E 465 IU.

[9] Net energy for lactation was calculated according to the (NRC, 2001) based on actual dry matter intake, and the rest of the nutrition indicators were estimated using the actual measured values.

Morrison, 2004). The air-dried DNA pellet was redissolved in TE buffer and stored at −20 °C until analysis. Deoxyribonucleic acid concentration and purity were determined spectrophotometrically by measuring the A260/280 ratio using a Spectramax microplate spectrophotometer. The DNA samples were diluted to concentrations of 10 ng/μl and stored at 4 °C until real-time PCR amplification. Quantitative qPCR assays for the enumeration of *Ruminococcus flavefaciens*, *Ruminococcus albus*, *Fibrobacter succinogenes, Butyrivibrio fibrisolvens*, *Streptococcus bovis*, *Prevotella*, *Ruminobacter amylophilus*, *Succinimonas amylolytica*, *Selenomonas ruminantium*, and ciliate protozoa were performed according to the methods described previously (Denman and Mcsweeney, 2006; Denman et al., 2007) using a real-time PCR machine (ABI PRISM 7500 SDS thermal cycler) and an SYBR Green I RT-PCR Kit (TaKaRa Biotechnology Co., Ltd. Dalian, China). The PCR primer sets used are shown in Table 2 and are based on previously used primers. These included group-specific primers for total bacteria (reference genes), and species-specific primers for *R. flavefaciens, R. albus, F. succinogenes, B. fibrisolvens, S. bovis, Prevotella, R. amylophilus, S. amylolytica, S. ruminantium* and ciliate protozoa. Relative gene expression of microbes was calculated using the $2^{-\triangle\triangle Ct}$ method as follows: Relative quantification = $2^{-[(\text{Ct target gene-Ct reference gene}) \text{ treatment group-(Ct target gene-Ct reference gene) control group}]}$ (Kim et al., 2014), where Ct represents the threshold cycle.

Blood samples were collected from the tail vein of all cows before the morning feeding on d 13, 14, 34, 35, 41 and 42 of the experiment. Blood was centrifuged at 2000 × *g* for 10 min. Plasma was collected and stored at −20 °C before analysis for total protein (TP), albumin (ALB), globulin (GLB), blood urea nitrogen (BUN), alanine transaminase (ALT), aspartate transaminase (AST), cholesterol (CHOL), triglycerides (TG), glucose (GLU), non-esterified fatty acids (NEFA), and beta-hydroxybutyric acid (BHBA). These analyses were performed by a fully automatic biochemical analyzer using standard commercial kits supplied from Biosino Bio-tec (Beijing, China). Non-esterified fatty acids and BHBA were analyzed using kits from Randox (Crumlin, UK). The concentrations of immunoglobulin A (IgA), immunoglobulin M (IgM) and immunoglobulin G (IgG) in plasma were analyzed using commercial ELISA kits (R&D Systems, Minneapolis, MN, USA). The concentrations of cytokines [interleukin-1 [IL-1], interleukin-2 [IL-2], interleukin-4 [IL-4], interleukin-6 [IL-6], and tumor necrosis factor-α [TNF-α]] were determined using commercial kits according to their respective manufacturer's instructions (Pierce, Rockford, IL). The total antioxidant capacity (T-AOC) and malondialdehyde (MDA), glutathione peroxidase (GSH-Px) and total superoxide dismutase (T-SOD) concentrations in the plasma were determined by colorimetry, using standard commercial kits supplied by the Nanjing Jian Cheng Bioengineering Institute (Nanjing, China).

2.4. Statistical analysis

Data of DMI, apparent digestibility, lactation performance and SCC obtained from Experiment 1 were analyzed using the PROC MIXED of SAS software (version 9.1). A randomized block design with repeated measures was used, with week, treatment, and interaction of treatment × week as fixed effects, and cow within treatment as a random effect. The statistical model was as follows: $Y_{ijk} = \mu + B_i + T_j + W_k + TW_{jk} + E_{ijk}$, where, Y_{ijk} = dependent variable, μ = overall mean, B_i = block effect, T_j = treatment effect, W_k = week effect, TW_{ijk} = interaction of treatment and week, E_{ijk} = error term. Linear and quadratic polynomial contrasts were used to determine the dose-dependent effect of supplementation of ATC. The results from Experiment 2 were tested using the GLM procedure of SAS software system (version 9.1). The statistical model was as follows: $Y_{ij} = \mu + T_i + E_i$, where, Y_{ij} = dependent variable, μ = overall mean, T_i = treatment effect, E_i = error term. Duncan's multiple range tests was used for both experiments. Statistical significance was defined at $P < 0.05$, with highly significant values at $P < 0.01$; trends were declared at $0.05 < P < 0.10$.

3. Results

3.1. Effects of ATC on nutrient intake and digestibility, performance and milk composition

The results of nutrient intake and digestibility, performance and milk composition are listed in Table 3. No significant difference was found in nutrient intake with the supplementation of ATC in Experiment 1 ($P > 0.05$). However, yields of 4% fat-corrected milk (FCM), energy-corrected milk (ECM), fat and lactose showed a quadratic ($P < 0.01$) and linear ($P < 0.01$) increase, and supplementing dairy cows with ATC linearly increased in apparent digestibility of DM, CP, NDF and ADF, milk yield, feed efficiency, and milk fat content ($P < 0.01$) but quadratic ($P < 0.01$) and linearly ($P < 0.01$) decreased milk SCC.

3.2. Effects of ATC on DMI and ruminal fermentation

Table 4 shows the results for DMI, ruminal pH, and NH_3-N and VFA concentrations. No difference was observed in DMI with the supplementation of ATC. Compared with the pre- and post-trial periods, ruminal pH decreased with the supplementation of ATC during the trial period ($P < 0.01$). However, NH_3-N and total VFA concentrations were increased during the ATC feeding period ($P < 0.05$) compared with those in the pre- and post-trial periods.

The cows in the trial period had a greater ruminal acetic acid molar proportion of total VFA than that of the cows in the pre-trial period ($P = 0.02$), and no difference was observed in the molar proportion of propionic acid in Experiment 2. However, compared with the pre-trial period, the molar proportion of butyric acid was lower during the trial and post-trial periods ($P < 0.01$). Additionally, the acetate to propionate ratio tended to linearly decrease ($P = 0.07$) with ingestion of ATC during the trial period compared with that in the pre- and post- trial periods.

Table 2
Primers used for RT-PCR detection of microbial species.

Target species tested	Forward primer Reverse primer
Bacterial universal primers	GAAGAGTTTGATCATGGCTCAG CTGCTGCCTCCCGTAG
Prevotella	GCGAAAGTCGGATTAATGCTCTATG CCCATCCTATAGCGGTAAACCTTTG
R. flavefaciens	CGAACGGAGATAATTTGAGTTTACTTAGG CGGTCTCTGTATGTTATGAGGTATTACC
R. albus	CCCTAAAAGCAGTCTTAGTTCG CCTCCTTGCGGTTAGAACA
F. succinogenes	GTTCGGAATTACTGGGCGTAAA CGCCTGCCCCTGAACTATC
B. fibrisolvens	ACCGCATAAGCGCACGGA CGGGTCCATCTTGTACCGATAAAT
S. bovis	TTCCTAGAGATAGGAAGTTTCTTCGG ATGATGGCAACTAACAATAGGGGT
R. amylophilus	CTGGGGAGCTGCCTGAAT CATCTGAATGCGACTGGTTG
S. amylolytica	CGTTGGGCGGTCATTTGAAAC CCTGAGCGTCAGTTACTATCCAGA
S. ruminantium	GGCGGGAAGGCAAGTCAGTC CCTCTCCTGCACTCAAGAAAGACAG
Ciliate protozoa	GCTTTCGWTGGTAGTGTATT CTTGCCCTCYAATCGTWCT

Table 3
Effects of the *Acremonium terricola* culture on nutrient intake and digestibility, performance and milk composition during eight-week study (Experiment 1).

Item	Treatment[1]			SEM[2]	*P*-vlaue	
	CON	ATCLOW	ATCHIGH		Linear	quadratic
Intake, kg/d						
DM	19.8	19.9	19.6	0.22	0.67	0.45
CP	2.61	2.63	2.59	0.029	0.61	0.41
NDF	8.73	8.82	8.68	0.099	0.73	0.35
ADF	5.31	5.35	5.28	0.059	0.74	0.47
Digestibility (g/kg)						
DM	629^{b}	633^{b}	642^{a}	1.5	< 0.01	0.14
CP	630^{b}	633^{b}	641^{a}	1.6	< 0.01	0.11
NDF	378^{b}	379^{b}	384^{a}	1.7	0.02	0.39
ADF	231^{b}	231^{b}	236^{a}	1.5	0.01	0.33
Yield, kg/d						
Milk	22.5^{b}	22.7^{b}	24.0^{a}	0.29	< 0.01	0.09
4% FCM[3]	20.8^{b}	21.2^{b}	23.1^{a}	0.35	< 0.01	< 0.01
ECM[4]	22.9^{b}	23.2^{b}	25.0^{a}	0.39	< 0.01	< 0.01
Fat	0.785^{b}	0.807^{b}	0.896^{a}	0.0173	< 0.01	< 0.01
Protein	0.699	0.700	0.717	0.0148	0.62	0.39
Lactose	1.08^{b}	1.08^{b}	1.16^{a}	0.0154	< 0.01	< 0.01
Milk composition, g/kg						
Fat	34.9^{b}	35.6^{b}	37.3^{a}	0.54	< 0.01	0.48
Protein	31.1	30.8	29.9	0.52	0.10	0.62
Lactose	47.9	47.8	48.3	0.26	0.36	0.34
$\log_{10}$ (SCC / mL)	5.63^{a}	5.51^{b}	5.29^{c}	0.015	< 0.01	< 0.01
Efficiency						
ECM/DMI	1.16^{b}	1.18^{b}	1.27^{a}	0.023	< 0.01	0.10

[a–c]Means within a row with different superscripts differ ($P < 0.05$).
[1] Control: TMR; ATCLOW: TMR plus 15 g/d ATC; ATCHIGH: TMR plus 30 g/d ATC.
[2] SEM = Standard error of the mean.
[3] 4% FCM = 0.4 × actual milk yield (kg/d) + 15 × actual milk fat yield (kg/d) (NRC, 2001).
[4] ECM = 0.327 × milk (kg) + 12.95 × fat (kg) + 7.65 × protein (kg; Dairy Records Management Systems, 2011).

3.3. Effects of ATC on microbial profile

Supplementation of ATC increased ($P < 0.05$) the relative expression levels of *Prevotella* sp., *R. flavefaciens*, *R. albus*, *S. bovis*, *R. amylophilus*, *S. amylolytica*, *S. ruminantium* and ciliate protozoa in the trial period (Table 5). Although ciliate protozoa decreased in the post-trial period, expression levels remained greater than those in the pre-trial period. By contrast, the relative expression level of *F. succinogenes* decreased ($P < 0.01$) during the trial period compared with that during the pre-trial and post-trial periods. ATC had no effect ($P = 0.33$) on the relative expression levels of *B. fibrisolvens* during the trial or post-trial period.

3.4. Effects of ATC on blood metabolites

No significant effect of ATC supplementation was detected on plasma TP, ALB, GLB, ALT, BUN, TG or CHOL concentrations throughout the trial period (Table 6). The concentrations of AST, NEFA and BHBA decreased with ATC supplementation during the trial period ($P < 0.01$). By contrast, the GLU concentration increased with ATC supplementation during the trial and post-trial periods ($P = 0.03$). Additionally, although NEFA and BHBA concentrations decreased in the post-trial period, concentrations remained greater than those in the pre-trial period. Moreover, compared with the pre-trial period, GLU concentration in plasma was greater during the post-trial period. The results showed that ATC supplementation not only directly influenced energy metabolism, but also had potential effects for at least 7 d after ATC supplementation was terminated.

3.5. Effects of ATC on antioxidant function

As shown in Table 7, ATC supplementation significantly increased the T-AOC level and T-SOD and GSH-Px activities and decreased MDA content in plasma during the ATC feeding period compared with those in the pre-trial period ($P < 0.05$). Additionally, although GSH-Px activity decreased in the post-trial period, activity remained greater than that in the pre-trial period. After the supplementation of ATC was stopped, the effect of ATC on the activity of GSH-Px continued for at least seven days.

3.6. Effects of ATC on immune function

Immune parameters in the plasma are presented in Table 8. During the trial period, ATC supplementation significantly increased the contents of IgA, IgG and IgM, and decreased those of the IL-1, IL-6 and TNF-α in the plasma compared with the plasma contents during the pre-trial period ($P < 0.01$). During the post-trial period, IgA, IgG and IgM contents decreased to some extent, but IgA and IgG contents remained greater than those in the pre-trial period ($P < 0.01$). The IL-1 content showed a downward trend ($P < 0.01$) throughout the study. In the post-trial period, the IL-6 content remained similar to the content in the trial period, whereas the TNF-α content increased rapidly. The content of IL-2 and IL-4 was not affected by ATC supplementation ($P > 0.05$).

4. Discussion

4.1. Effects of ATC on nutrient intake and digestibility, milk performance and milk composition

In these studies, we demonstrated for the first time that ATC boosts the productivity and health of dairy cows. The positive effects of ATC on livestock production has been reported earlier across pigs (Wei et al., 2009) and poultry (Sun et al., 2011), whereas our previous research shows that ATC can increase weight gain and improve antioxidant and immune functions simultaneously in rats (Li et al., 2016a) and calves (Li et al., 2016b). The results of performance showed that ATC supplementation significantly increased milk production by 68.9 g/kg and caused a 94.8 g/kg improvement in feed efficiency. Although little other has been conducted on dairy

Table 4
Effects of the*Acremonium terricola* culture on DMI and ruminal fermentation in Holstein cows (Experiment 2).

Items	Period[1]			SEM[2]	*P*-value
	Pre-trial	Trial	Post-trial		
DMI, kg/d	20.1	19.1	19.3	0.84	0.68
pH	6.32[a]	6.21[b]	6.36[a]	0.030	0.01
NH_3-N, mg/L	92.0[b]	116.3[a]	92.8[b]	6.40	0.03
Total VFA, mmol/L	71.7[b]	102.4[a]	83.0[b]	4.72	< 0.01
Molar proportion of VFA, (mol/100 mol)					
Acetic acid	66.6[b]	69.3[a]	67.1[a]	0.66	0.02
Propionic acid	21.3	19.9	21.3	0.59	0.16
Butyric acid	9.59[a]	7.98[c]	8.87[b]	0.206	< 0.01
Acetic:propionic acid ratio	3.19	3.51	3.16	0.114	0.07

[a–c]Means within a row with different superscripts differ ($P < 0.05$).
[1] Period: pre-trial = no ATC (14 d), trial period = 30 g/d ATC (21 d), post-trial = no ATC (7 d).
[2] SEM = Standard error of the mean.

cow response to ATC, studies in a monogastric animal (Wei et al., 2009; Sun et al., 2011) and an aquatic animal (Deng et al., 2015) demonstrate that ATC and its functional components have a positive effect on improving feed efficiency and health status. These effects indicate excellent regulating effects of ATC on animal growth and antioxidative and antibacterial activities.

Based on the results of experiment 1, milk production increased and milk components were improved with elevated milk fat and lactose yield because of the improved rumen fermentation when dairy cows were fed ATC. It is well known that propionate is the major glucogenic precursor, which may increase the availability of blood glucose supply, caused by changes in rumen fermentation and subsequently increase milk production (Sauer et al., 1989). However, SCC decreased in the milk from ATC-treated cows compared with that in the control milk. The possible explanation for those results is that ATC improves immune function and antioxidant capacity of cows (as demonstrated in current study), thereby decreasing SCC and improving the milk production performance of dairy cows (Mukherjee, 2008; Sun et al., 2013). Many studies demonstrate that *Cordyceps* polysaccharide and cordycepin in ATC are natural antioxidants (Wang et al., 2012; Chen et al., 2013; Olatunji et al., 2016), which have strong capacity for eliminating radicals and modulating inflammation, and therefore exhibit immense potential to prevent mastitis and improve breast health. Tufarelli and Laudadio (2011) showed that the administration of antioxidants results in a reduction in SCC and an improvement in milk yield, and that VFA concentrations are a key factor that influences milk production and composition (Oba and Allen, 2003a).

According to our results in experiment 2, the feeding of 30 g/d of ATC provided continuously adequate levels of NH_3 for the persistent growth of rumen microorganisms, which therefore could more effectively utilize fiber and protein in feedstuff (Wanapat and Cherdthong, 2009; Cherdthong et al., 2011). Increases in the relative expression of rumen bacteria and protozoa in the rumen is one of the key factors for improved apparent digestibility of fiber and protein (Pino and Heinrichs, 2016). In experiment 2, for most of the rumen bacteria and protozoa that were measured, the relative expression level increased during the trial period. Moreover, the adhesive ability and amount of rumen microorganisms are positively associated with feed degradation (Paul et al., 2004). Protozoa are also an important component of the ruminal microorganism community and are well known to play an important role in increasing NH_3-N concentration and digestibility of fiber and protein in the rumen, thereby directly influencing the digestion and utilization of carbohydrates and nitrogenous material in feed. Faciola and Broderick (2013) also report that ruminal protozoa numbers decrease as apparent digestibility of fiber and protein decrease. Alternatively, cordycepic acid is verified as a potent extender to facilitate growth of probiotics to resolve protein and fiber, which may be one reason for augment of DM digestibility (Hanieh and Sakaguchi, 2009; Min et al., 2013).

4.2. Effects of ATC on ruminal fermentation and microbial profile

For most of the rumen bacteria measured, the relative quantity increased during the ingestion of ATC, but that of *F. succinogenes* decreased. According to previous studies, *Cordyceps militaris*, with the same active ingredients as ATC, and some plant secondary metabolites inhibit the growth of *F. succinogenes* (Wang et al., 2009; Kim et al., 2014), although the mechanism is not clear. These results are particularly significant because they demonstrate that the introduction of ATC into the rumen can change the numbers of certain cellulolytic, proteolytic and amylolytic bacteria in rumens of dairy cows. Results from previous research showed that addition of *Cordyceps militaris* mycelia to a culture of mixed ruminal microorganisms increases VFA production (Yeo et al., 2009) and the numbers of total and cellulolytic bacteria and activities of cellulolytic enzyme (Yeo et al., 2011). Therefore, the above results demonstrated that the supplementation of ATC had a positive effect on stimulating the growth of some rumen bacteria and protozoa, thereby improving the degradation of dietary proteins and carbohydrates and increasing VFA concentrations, subsequently boosting the concentration of GLU (as demonstrated in the current study), which results in improved lactation performance.

Table 5
Effects of the *Acremonium terricola* culture on relative expression of microbial gene in the rumen of Holstein cows (Experiment 2).

Items	Period[1]			SEM[2]	*P*-value
	Pre-trial	Trial	Post-trial		
Prevotella sp.	1.00[b]	2.66[a]	2.18[a,b]	0.450	0.04
R. flavefaciens	1.00[b]	1.63[a]	1.38[a,b]	0.156	0.03
R. albus	1.00[b]	1.30[a]	1.09[a,b]	0.077	0.03
F. succinogenes	1.00[a]	0.577[b]	0.734[b]	0.0790	< 0.01
B. fibrisolvens	1.00	1.10	1.06	0.048	0.33
S. bovis	1.00[b]	2.02[a]	1.44[a,b]	0.273	0.04
R. amylophilus	1.00[b]	9.18[a]	2.39[b]	1.621	< 0.01
S. amylolytica	1.00[b]	2.72[a]	1.25[b]	0.387	< 0.01
S. ruminantium	1.00[b]	1.57[a]	1.44[a,b]	0.162	0.047
Ciliate protozoa	1.00[c]	1.97[a]	1.59[b]	0.131	< 0.01

[a–c]Means within a row with different superscripts differ ($P < 0.05$).
[1] Period: pre-trial = no ATC (14 d), trial period = 30 g/d ATC (21 d), post-trial = no ATC (7 d).
[2] SEM = Standard error of the mean.

Table 6
Effects of the *Acremonium terricola* culture on plasma metabolic parameters (Experiment 2).

Items	Period[1]			SEM[2]	P-value
	Pre-trial	Trial	Post-trial		
TP, g/L	70.6	73.4	73.4	2.01	0.54
ALB, g/L	13.4	13.4	13.0	0.93	0.94
GLB, g/L	57.2	60.0	60.4	1.91	0.46
AST, U/L	108[a]	81.0[b]	117[a]	3.3	< 0.01
ALT, U/L	24.6	25.8	25.2	1.53	0.86
BUN, mmol/L	5.60	4.78	4.60	0.331	0.12
TG, mmol/L	0.182	0.164	0.172	0.019	0.80
CHOL, mmol/L	8.22	8.90	8.38	0.523	0.64
NEFA, μmol/L	661[a]	612[c]	644[b]	3.2	< 0.01
BHBA, mmol/L	0.480[a]	0.386[c]	0.424[b]	0.0111	< 0.01
GLU, mmol/L	3.86[c]	4.50[a]	4.20[b]	0.045	< 0.01

[a–c]Means within a row with different superscripts differ ($P < 0.05$).
[1] Period: pre-trial = no ATC (14 d), trial period = 30 g/d ATC (21 d), post-trial = no ATC (7 d).
[2] SEM = Standard error of the mean.

Table 7
Effects of the *Acremonium terricola* culture on antioxidant function (Experiment 2).

Items	Period[1]			SEM[2]	P-value
	Pre-trial	Trial	Post-trial		
T-AOC, U/mL	4.79[b]	7.34[a]	5.83[b,a]	0.641	< 0.05
MDA, nmol/mL	6.29[a]	4.08[b]	5.24[b,a]	0.466	0.02
T-SOD, U/mL	159[b]	175[a]	166[b,a]	3.4	0.02
GSH-Px, U/mL	164[c]	188[a]	175[b]	3.2	< 0.01

[a–c]Means within a row with different superscripts differ ($P < 0.05$).
[1] Period: pre-trial = no ATC (14 d), trial period = 30 g/d ATC (21 d), post-trial = no ATC (7 d).
[2] SEM = Standard error of the mean.

Table 8
Effects of the *Acremonium terricola* culture on immune function (Experiment 2).

Items	Period[1]			SEM[2]	P-value
	Pre-trial	Trial	Post-trial		
IgA, g/L	1.42[c]	1.87[a]	1.62[b]	0.044	< 0.01
IgG, g/L	9.12[c]	14.02[a]	11.83[b]	0.422	< 0.01
IgM, g/L	1.22[b]	2.17[a]	1.48[b]	0.107	< 0.01
IL-1, ng/L	177[a]	137[b]	95.8[c]	8.71	< 0.01
IL-2, ng/L	287	280	299	12.5	0.56
IL-4, ng/L	86.9	82.9	78.7	2.92	0.16
IL-6, ng/L	19.3[a]	14.9[b]	15.7[b]	0.72	< 0.01
TNF-α, ng/L	238[a]	205[b]	248[a]	10.4	0.02

[a–c]Means within a row with different superscripts differ ($P < 0.05$).
[1] Period: pre-trial = no ATC (14 d), trial period = 30 g/d ATC (21 d), post-trial = no ATC (7 d).
[2] SEM = Standard error of the mean.

4.3. Effects of ATC on blood metabolites

Several blood metabolites are important indexes that reflect physiological function and health condition of dairy cow. Aspartate transaminase and ALT are specific to the liver where it exists in the cytosol in much greater concentrations, when compares to other tissues (Adams, 2007). Two enzymes are normally found at low levels in blood; however, if the liver suffers pathological or chemical injury, the levels of AST and ALT in blood will be greater. Therefore, AST and ALT concentrations in the plasma are sensitive markers of liver damage, even when the damage is of a subclinical nature (Kauppinen et al., 1984). In our study, decreases in the AST levels suggested that ATC could have protective effects on hepatic cells. *Cordyceps sinensis* has been used to treat liver disease in traditional Chinese medicine for thousands of years (Peng et al., 2013). Peng et al. (2013) also reported that *Cordyceps* polysaccharide in ATC plays a certain role in the treatment of hepatic fibrosis through inhibiting hepatic stellate cell activation and transforming growth factor-β1/Smads signalling pathway, as well as matrix metalloproteinase 2, matrix metalloproteinase 9 activity and tissue inhibitor of metalloproteinase 2 expression. Glucose, NEFA, and BHBA can be used as indicators of energy balance and energy mobilization status. In the current study, the linearly reduced plasma NEFA, BHBA, and linearly increased plasma glucose suggest that energy availability was improved with ATC supplementation and the glucogenic capacity of this additive. Therefore, we think ATC has great potential in slowing the mobilization of body fat, and improving negative energy balance and milking capacity in dairy cows (Zheng et al., 2012). The lower NEFA and greater plasma glucose in the trial period may be related to greater propionate levels in the rumen. Propionate is likely the major glucogenic precursor, which may increase blood glucose availability via gluconeogenesis (Sauer et al., 1989). Previous studies report that plasma glucose levels increase and NEFA concentrations decrease linearly as propionate infusion increase (Oba and Allen, 2003b). Propionate can inhibit the oxidation of fatty acids by decreasing the activity of fatty acyl-CoA dehydrogenase (Shaw and Engel, 1985; Emery et al., 1992) or by decreasing fatty-acid transport to mitochondria (Jesse et al., 1986), which reduce lipolysis in adipose tissue.

4.4. Effects of ATC on antioxidant function

Oxidative stress induced by reactive oxygen species (ROS) is likely a primary factor that contributes to the incidence of various diseases in cattle (Sordillo, 2012). The positive influence of antioxidants on health is reflected in the increased immunological potential of dairy cows, and in the reduced risk of mastitis and SCC in milk (Umesh et al., 2013; Krzyżewski et al., 2014). To optimize performance, antioxidants are included in some rations to control oxidative stress in dairy cows. Some studies indicate that active ingredients of ATC possess potent antioxidant and anti-lipid peroxidation activities that can be effective in scavenging various types of oxygen free radicals and their products (Wang et al., 2012; Deng et al., 2015). Our previous study shows that ATC improves antioxidant enzyme activities and adjusts levels of inflammatory factors in weaned calves (Li et al., 2016b). The results of these studies indicate that functional components of substances extracted from *Cordyceps* may improve the antioxidant capacity in animals. The *Cordyceps* polysaccharide and other immunopolysaccharides improve activities of T-SOD and GSH-Px (Kobayashi et al., 1994; Li et al., 2012). Although the antioxidant mechanism of *Cordyceps* polysaccharide is not clear, the data presented here further suggests the biological function of effective components in ATC. Therefore, the effect of ATC as an antioxidant is comprehensive and worthy of further investigation. Based on the results of the current study, we speculate that ATC increases immune function, at least in part, by stimulating antioxidant endogenous enzyme activities and protecting against oxidative stress.

4.5. Effects of ATC on immune function

According to oxidation–inflammation theory, overproduction of oxidant compounds can induce an inflammatory response, since oxidants are inflammation effectors. Oxidative stress may generate an inflammatory immune response, resulting in decreased function of virtually all immune cells. Molecules involved in humoral immunity, such as IgA, IgG and IgM, and cytokines, such as IL-1, IL-2, IL-4, IL-6 and TNF-α, reflect the immune functions of the body. In this study, the results for the immune indexes showed that ATC had moderating effects on the immune system of dairy cows. Immunoglobulins are a type of globulin protein which either act as antibodies or have similar chemical structures to those of antibodies. Immunoglobulins function in antigen-specifc binding and play important roles in regulating immune responses (Li et al., 2016b). *Acremonium terricola* culture can increase the concentrations of immunoglobulins in plasma of dairy cows, which ATC have proven obvious progress in immune functions. Kuo et al. (2001) found that extracts of *Cordyceps sinensis* suppress the production of IL-6, TNF-α and other cytokines. Piao and Wang (2014) demonstrated that dietary supplementation with products extracted from *Cordyceps* increases the concentrations of IgG, IgM and IL-4 in mice. The above results are similar to our results. Previous study indicates that cordycepin significantly inhibits the excessive production of pro-inflammatory cytokines in a concentration-dependent manner without causing cytotoxicity (Jeong et al., 2010), and then plays a role in anti-inflammation. The reason why ATC supplementation can enhance immune function may be due to the presence of specific compounds within the supplemented feed. Various bioactive constituents from ATC have been reported, such as cordycepin, polysaccharides, antibacterial and antitumor adenosine derivatives (Cho et al., 2007; Paterson, 2008). Accumulated evidence strongly suggests that active components from ATC have a wide range of biological activities, particularly related to the immune system (Kuo et al., 1996; Jeong et al., 2010; Wang et al., 2012; Deng et al., 2015). *Acremonium terricola* culture originated from *Cordyceps gunnii*, and its active components and functions are similar to those of natural *Cordyceps*, which is regarded as a tonifying herb with both 'Yin-nourishing' and 'Yang-invigorating' actions. Pharmacological studies have shown that *Cordyceps* possesses a wide spectrum of biological activities including antioxidation (Yamaguchi et al., 2000), immunopotentiation (Gong et al., 1990), anti-inflammation (Kim et al., 2003) and anti-tumorigenesis (Shin et al., 2003). Therefore, according to results of previous research, we speculated that ATC stimulate mitochondrial adenosine triphosphate (ATP) generation, presumably through the intermediacy of reactive oxidant species, leading to the enhancement of cellular/mitochondrial antioxidant status (Ming and Yan, 2007). *Acremonium terricola* culture, besides possessing antioxidant properties, exerts mainly immunomodulatory functions that may boost a weak immune system and may suppress overreactive immune responses. However, further researches are required to elucidate the specific mechanism by which ATC enhances immune function.

5. Conclusion

The use of ATC improved the performance and milk quality, rumen fermentation, antioxidant status and immune function of dairy cows. The results of the present study show that ATC could be a new type of feed additive, with a recommended dose of 30 g/d. Future research is required to investigate the mechanism by which ATC decreases the SCC and improves immune function in dairy cows.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Acknowledgements

This study was financially supported by the Postdoctoral Foundation in Heilongjiang Province (LBH-Z17035, LBH-Z17013), the China Agriculture Research System (CARS-36) and China Postdoctoral Science Foundation Grant for the 62nd 312 batch (2017M621237). The authors also thank Hefei Micro Biological Engineering Co., Ltd. (Heifei, China) for donating the *Acremonium terricola* culture used in this study.

References

Adams, D.H., 2007. Sleisenger and Fordtran's gastrointestinal and liver disease. Gut 56, 1175.
AOAC, 2000. Official Methods of Analysis, 17th ed. Association of Official Analytical Chemist, Arlington, VA, USA.
Broderick, G.A., Kang, J.H., 1980. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and *in vitro* media. J. Dairy Sci. 63, 64–75.
Chang, C.Y., Lue, M.Y., Pan, T.M., 2005. Determination of adenosine, cordycepin and ergosterol contents in cultivated antrodia camphorata by HPLC method. J. Food Drug Anal. 13, 338–342.
Chen, X.L., Wu, G.H., Huang, Z.L., 2013. Structural analysis and antioxidant activities of polysaccharides from cultured cordyceps militaris. Int. J. Biol. Macromol. 58, 18–22.
Cheng, J.B., Bu, D.P., Wang, J.Q., Sun, X.Z., Pan, L., Zhou, L.Y., Liu, W., 2014. Effects of rumen-protected γ-aminobutyric acid on performance and nutrient digestibility in heat-stressed dairy cows. J. Dairy Sci. 97, 5599–5607.
Cherdthong, A., Wanapat, M., Wachirapakorn, C., 2011. Influence of urea-calcium mixtures as rumen slow-release feed on *in vitro* fermentation using a gas production technique. Arch. Anim. Nutr. 65, 242–254.
Cho, H.J., Cho, J.Y., Rhee, M.H., Park, H.J., 2007. Cordycepin (3'-deoxyadenosine) inhibits human platelet aggregation in a cyclic AMP- and cyclic GMP-dependent manner. Eur. J. Pharmacol. 558, 43–51.
Choi, H.N., Jang, Y.H., Kim, M.J., Min, J.S., Kang, B.W., Yong, K.J., Kim, J.I., 2014. *Cordyceps militaris* alleviates non-alcoholic fatty liver disease in ob/ob mice. Nutr. Res. Pract. 8, 172–176.
Deng, B., Wang, Z.P., Tao, W.J., Li, W.F., Wang, C., Wang, M.Q., Ye, S.S., Du, Y.J., Wu, X.X., Wu, D., 2015. Effects of polysaccharides from mycelia of *Cordyceps sinensis* on growth performance, immunity and antioxidant indicators of the white shrimp Litopenaeus vannamei. Aquacult. Nutr. 21, 173–179.
Denman, S., Tomkins, N., Mcsweeney, C., 2007. Quantitation and diversity analysis of ruminal methanogenic populations in response to the antimethanogenic compound bromochloromethane. Fems Microbiol. Ecol. 62, 313–322.
Denman, S.E., Mcsweeney, C.S., 2006. Development of a real-time PCR assay for monitoring anaerobic fungal and cellulolytic bacterial populations within the rumen. Fems Microbiol. Ecol. 58, 572–582.
Emery, R.S., Liesman, J.S., Herdt, T.H., 1992. Metabolism of long chain fatty acids by ruminant liver. J. Nutr. 122, 832–837.
Faciola, A.P., Broderick, G.A., 2013. Effects of feeding lauric acid on ruminal protozoa numbers, fermentation, and digestion and on milk production in dairy cows. J. Anim. Sci. 91, 2243–2253.
Fan, M.Z., Huang, B., Li, C.R., Li, Z.Z., 1999. A new record species of the genus *Acremonium* from China. Mycosystema 18 (449) 1999.
Gong, M.Zhu, Wang, Q., Wang, T., Ma, X.L., Zhang, J.X., Wei-Jie, 1990. Molecular structure and immunoactivity of the polysaccharide from *Cordyceps Sinensis* (Berk) Sacc. Chin. J. Biochem. Mol. Biol. 144–150.
Han, J.C., Qu, H.X., Wang, J.G., Yan, Y.F., Zhang, J.L., Yang, L., Zhang, M., Cheng, Y.H., 2015. Effects of fermentation products of *Cordyceps militaris* on growth performance and bone mineralization of broiler chicks. J. Appl. Anim. Res. 43, 236–241.
Hanieh, H., Sakaguchi, E., 2009. Effect of D-mannitol on feed digestion and cecotrophic system in rabbits. Anim. Sci. J. 80, 157–162.
Jeong, J.W., Jin, C.Y., Kim, G.Y., Lee, J.D., Park, C., Kim, G.D., Kim, W.J., Jung, W.K., Seo, S.K., Choi, I.W., 2010. Anti-inflammatory effects of cordycepin via suppression of inflammatory mediators in BV2 microglial cells. Int. Immunopharmacol. 10, 1580–1586.
Jesse, B.W., Emery, R.S., Thomas, J.W., 1986. Control of bovine hepatic fatty acid oxidation. J. Dairy Sci. 69, 2290–2297.
Kauppinen, K., 1984. ALAT, AP, ASAT, GGT, OCT activities and urea and total bilirubin concentrations in plasma of normal and ketotic dairy cows. Zentralblatt Für Veterin rmedizin Reihe A 31, 567–576.
Kim, K.M., Kwon, Y.G., Chung, H.T., Yun, Y.G., Pae, H.O., Han, J.A., Ha, K.S., Kim, T.W., Kim, Y.M., 2003. Methanol extract of *Cordyceps* pruinosa inhibits *in vitro* and *in vivo* inflammatory mediators by suppressing NF-kappaB activation. Toxicol. Appl. Pharmacol. 190, 1–8.
Kim, W.Y., Hanigan, M.D., Lee, S.J., Lee, S.M., Kim, D.H., Hyun, J.H., Yeo, J.M., Lee, S.S., 2014. Effects of *Cordyceps militaris* on the growth of rumen microorganisms and *in vitro* rumen fermentation with respect to methane emissions. J. Dairy Sci. 97, 7065–7075.
Kobayashi, Y., Kariya, K., Saigenji, K., Nakamura, K., 1994. Oxidative stress relief for cancer-bearing hosts by the protein-bound polysaccharide of Coriolus versicolor QUEL with SOD mimicking activity. Cancer Biother. 9, 55–62.
Koh, J.H., Suh, H.J., Ahn, T.S., 2003. Hot-water extract from mycelia of *Cordyceps sinensis* as a substitute for antibiotic growth promoters. Biotechnol. Lett. 25, 585–590.
Krzyżewski, J., Bagnicka, E., Horban Czuk, J.O., 2014. The effect of selenium supplementation to the diet of dairy cows and goats on production traits and animal health - a review. Anim. Sci. Pap. Rep. 32, 283–299.
Kuo, Y., Tsai, W., Shiao, M., Chen, C., Lin, C., 1996. *Cordyceps sinensis* as an immunomodulatory agent. Am. J. Chin. Med. 24, 111–125.
Kuo, Y.C., Tsai, W.J., Wang, J.Y., Chang, S.C., Lin, C.Y., Shiao, M.S., 2001. Regulation of bronchoalveolar lavage fluids cell function by the immunomodulatory agents from Cordyceps sinensis. Life Sci. 68, 1067–1082.
Lee, C., Hristov, A.N., 2013. Short communication: evaluation of acid-insoluble ash and indigestible neutral detergent fiber as total-tract digestibility markers in dairy cows fed corn silage-based diets. J. Dairy Sci. 96, 5295–5299.
Li, X.T., Li, H.C., Li, C.B., Dou, D.Q., Gao, M.B., 2012. Protective effects on mitochondria and anti-aging activity of polysaccharides from cultivated fruiting bodies of cordyceps militaris. Am. J. Chin. Med. 38, 1093–1106.
Li, Y., Lin, C., Xun, W.B., Zhang, X.Y., Wang, Y.Z., Gao, H., Dou, X.J., Hao, X.Y., Zhang, Y.G., 2016a. Effects of *Acremonium terricola* culture on growth performance, antioxidant and immunity function of blood and liver in Sprague Dawley rats (in Chinese). Chin. J. Anim. Nur. 28, 2657–2666.
Li, Y., Xue, W.J., Tian, P.X., Ding, X.M., Yan, H., Pan, X.M., Feng, X.S., 2009. Clinical application of *Cordyceps sinensis* on immunosuppressive therapy in renal transplantation. Transpl. P. 41, 1565–1569.
Li, Y., Wang, Y.Z., Ding, X., Zhang, Y.G., Xue, S.C., Lin, C., Xu, W.B., Dou, X.J., Zhang, L.Y., 2016b. Effects of *Acremonium terricola* culture on growth performance, antioxidant status and immune functions in weaned calves. Livest. Sci. 193, 66–70.
Liu, D.Y., He, S.J., Jin, E.H., Liu, S.Q., Tang, Y.G., Li, S.H., Zhong, L.T., 2013. Effect of daidzein on production performance and serum antioxidative function in late lactation cows under heat stress. Livest. Sci. 152, 16–20.
Lodge-Ivey, S.L., Browne-Silva, J., Horvath, M.B., 2009. Technical note: bacterial diversity and fermentation end products in rumen fluid samples collected via oral

lavage or rumen cannula. J. Anim. Sci. 87, 2333–2337.
Min, X., Li, X., Hiura, S., Kawasaki, K., Xiao, J., Sakaguchi, E., 2013. Effect of D-mannitol on nitrogen retention, fiber digestibility and digesta transit time in adult rabbits. Anim. Sci. J. 84, 551–555.
Ming, K.K., Yan, L.H., 2007. Enhancement of ATP generation capacity, antioxidant activity and immunomodulatory activities by Chinese Yang and Yin tonifying herbs. Chin. Med. 2, 1–10.
Mukherjee, R., 2008. Selenium and vitamin E increases polymorphonuclear cell phagocytosis and antioxidant levels during acute mastitis in riverine buffaloes. Vet. Res. Commun. 32, 305–313.
Oba, M., Allen, M.S., 2003a. Intraruminal infusion of propionate alters feeding behavior and decreases energy intake of lactating dairy cows. J. Nutr. 133, 1094–1099.
Oba, M., Allen, M.S., 2003b. Dose-response effects of intrauminal infusion of propionate on feeding behavior of lactating cows in early or midlactation. J. Dairy Sci. 86, 2922–2931.
Olatunji, O.J., Feng, Y., Olatunji, O.O., Tang, J., Ouyang, Z., Su, Z.L., 2016. Cordycepin protects PC12 cells against 6-hydroxydopamine induced neurotoxicity via its antioxidant properties. Biomed. Pharmacother. 81, 7–14.
Paterson, R.R., 2008. *Cordyceps*: a traditional Chinese medicine and another fungal therapeutic biofactory? Phytochemistry 69, 1469–1495.
Paul, S.S., Kamra, D.N., Sastry, V.R.B., Sahu, N.P., Agarwal, N., 2004. Effect of administration of an anaerobic gut fungus isolated from wild blue bull (Boselaphus tragocamelus) to buffaloes (Bubalus bubalis) on *in vivo* ruminal fermentation and digestion of nutrients. Anim. Feed Sci. Tech. 115, 143–157.
Peng, J.H., Li, X.M., Feng, Q., Chen, L., Xu, L.L., Hu, Y.Y., 2013. Anti-fibrotic effect of *Cordyceps sinensis* polysaccharide: inhibiting HSC activation, TGF-β1/Smad signalling, MMPs and TIMPs. Exp. Biol. Med. (Maywood) 238, 668–677.
Piao, M.Z., Wang, X.D., 2014. Effects of *Cordyceps militaris* soybeanon antioxidant and immune function in mice. J. Chin. Inst. Food Sci. Technol. 14, 32–37.
Pino, F., Heinrichs, A.J., 2016. Effect of trace minerals and starch on digestibility and rumen fermentation in diets for dairy heifers. J. Dairy Sci. 99, 2797–2810.
Sauer, F.D., Kramer, J.K.G., Cantwell, W.J., 1989. Antiketogenic effects of monensin in early lactation. J. Dairy Sci. 72, 436–442.
Scholzahrens, K.E., Ade, P., Marten, B., Weber, P., Timm, W., Aςil, Y., Glüer, C., Schrezenmeir, J., 2007. Prebiotics, probiotics, and synbiotics affect mineral absorption, bone mineral content, and bone structure. J. Nutr. 137, 838S–846S.
Seo, J.K., Kim, S.W., Kim, M.H., Upadhaya, S.D., Kam, D.K., Ha, J.K., 2010. Direct-fed microbials for ruminant animals. Asian-australas. J. Anim. Sci. 23, 1657–1667.
Shaw, L., Engel, P.C., 1985. The suicide inactivation of ox liver short-chain acyl-CoA dehydrogenase by propionyl-CoA. Formation of an FAD adduct. Biochem. J. 230, 723–731.
Shin, K.H., Lim, S.S., Lee, S., Lee, Y.S., Jung, S.H., Cho, S.Y., 2003. Anti-tumour and immuno-stimulating activities of the fruiting bodies of *Paecilomyces japonica*, a new type of *Cordyceps* spp. Phytother. Res. 17, 830–833.
Sordillo, L.M., 2012. Selenium-dependent regulation of oxidative stress and immunity in periparturient dairy cattle. Vet. Med. Int. 2013, 154045.
Stewart, C.S., Duncan, S.H., 1985. The effect of avoparcin on cellulolytic bacteria of the ovine rumen. Microbiology 131, 427–435.
Sun, J.H., Li, X.X., Ding, Q., Zhu, Y., Chen, Z., 2011. Effects of *Cordyceps* feed additive on production performance and egg quality of laying ducks (in Chinese). J. Anhui Agric. Sci. 39, 3618–3620 3626.
Sun, P., Wang, J.Q., Deng, L.F., 2013. Effects of *Bacillus subtilis natto* on milk production, rumen fermentation and ruminal microbiome of dairy cows. Animal 7, 216–222.
Tufarelli, V., Laudadio, V., 2011. Dietary supplementation with selenium and vitamin E improves milk yield, composition and rheological properties of dairy Jonica goats. J. Dairy Res. 78, 1–5.
Umesh, D., Mahesh Chandra, S., Singh, S.K., Pankaj, K., Ricky, J., Bishwambhar, S., Samiran, B., Med Ram, V., 2013. Amelioration of altered oxidant/antioxidant balance of Indian water buffaloes with subclinical mastitis by vitamins A, D_3, E, and H supplementation. Trop. Anim. Health Pro. 45, 971–978.
Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583–3597.
Wanapat, M., Cherdthong, A., 2009. Use of real-time PCR technique in studying rumen cellulolytic bacteria population as affected by level of roughage in swamp buffalo. Curr. Microbiol. 58, 294–299.
Wang, M., Meng, X.Y., Yang, R.L., Qin, T., Wang, X.Y., Zhang, K.Y., Fei, C.Z., Li, Y., Hu, Y.L., Xue, F.Q., 2012. *Cordyceps militaris* polysaccharides can enhance the immunity and antioxidation activity in immunosuppressed mice. Carbohydr. Polym. 89, 461–466.
Wang, Y., Alexander, T.W., Mcallister, T.A., 2009. *In vitro* effects of phlorotannins from Ascophyllum nodosum (brown seaweed) on rumen bacterial populations and fermentation. J. Sci. Food Agric. 89, 2252–2260.
Wei, J.Z., Zhang, W., Li, Y., Fan, M.Z., Wang, G.J., Yang, D.K., 2009. Effects of the culture of *Acremonium terricola* on performance and immunity of piglets (in Chinese). Chin. Anim. Hus. Vet. Med. 36, 33–35.
Weiss, W.P., Wyatt, D.J., 2000. Effect of oil content and kernel processing of corn silage on digestibility and milk production by dairy cows. J. Dairy Sci. 83, 351–358.
Yamaguchi, Y., Kagota, S., Nakamura, K., Shinozuka, K., Kunitomo, M., 2000. Antioxidant activity of the extracts from fruiting bodies of cultured Cordyceps sinensis. Phytother. Res. 14, 647–649.
Yeo, J.M., Lee, S.J., Shin, S.H., Lee, S.H., Ha, J.K., Kim, W.Y., Lee, S.S., Yeo, J.M., Lee, S.J., Shin, S.H., 2011. Effects of *Cordyceps militaris* mycelia on fibrolytic enzyme activities and microbial populations in vitro. Asian-Australas. J. Anim. 24, 364–368.
Yeo, J.M., Lee, S.J., Lee, S.M., Shin, S.H., Lee, S.H., Ha, J.K., Kim, W.Y., Lee, S.S., 2009. Effects of *Cordyceps militaris* mycelia on *in vitro* rumen microbial fermentation. Asian-Australas. J. Anim. 22, 201–205.
Yu, Z., Morrison, M., 2004. Improved extraction of PCR-quality community DNA from digesta and fecal samples. Biotechniques 36, 808–812.
Zheng, J.S., Xia, C., Zhang, H.Y., Chuang, X.U., 2012. Effect of rumen protected choline supplemented into diet on performance and energy metabolism of dairy cows in transition period. J. Chin. Agric. Univ. 114–120.
Zhu, Z.Y., Chen, J., Si, C.L., Liu, N., Lian, H.Y., Ding, L.N., Liu, Y., Zhang, Y.M., 2012. Immunomodulatory effect of polysaccharides from submerged cultured Cordyceps gunnii. Pharm. Biol. 50, 1103–1110.