BMC Biotechnology

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Comparison of lipidome profiles in serum from lactating dairy cows supplemented with *Acremonium terrestris culture* based on UPLC-QTRAP-MS/MS

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Abstract

This study evaluated the effects of supplementing the diet of lactating cows with *Acremonium terrestris culture* (ATC) on milk production, serum antioxidant capacity, inflammatory indices, and serum lipid metabolomics. Over 90 days, 24 multiparous Chinese Holstein cows in mid-lactation (108 ± 10.4 days in milk, 637 ± 25 kg body weight, 30.23 ± 3.7 kg/d milk yield) were divided into either a control diet (CON) or a diet supplemented with 30 g of ATC daily. All the data were analyzed using Student's *t* test with SPSS 20.0 software. The results showed that compared with CON feeding, ATC feeding significantly increased milk yield, antioxidant capacity, and immune function. Lipidome screening identified 143 lipid metabolites that differed between the two groups. Further analysis using "random forest" machine learning revealed three glycerophospholipid serum metabolites that could serve as lipid markers with a predictive accuracy of 91.67%. This study suggests that ATC can be a useful dietary supplement for improving lactational performance in dairy cows and provides valuable insights into developing nutritional strategies to maintain metabolic homeostasis in ruminants.

Keywords *Acremonium terrestris* culture, Glycerophospholipids, Sphingolipids, Lipidomics, Biotransformation

Introduction

Animal products such as milk and dairy are considered a primary food source providing consumers with nutrients, energy, protein, calcium, essential fatty acids (EFAs) and vitamins [1]. Through further understanding of lactation biology and milk biosynthesis, we have been able to improve management practices and use phytogenic feed additives. However, long-term production from high-yield dairy cows can lead to a decline in immune system function and an increase in metabolic disease. This poses a serious challenge to breeding healthy dairy cows globally [2, 3]. Consequently, we need to improve feeding management practices and use natural green feed additives to enhance the health of dairy cows and ensure a steady supply of safe, high-quality human food.

Acremonium terrestris culture (ATC) is a bioactive substance produced through the artificial fermentation of *Acremonium* sp., derived from *Cordyceps sinensis* found in Gougnies, Belgium. ATC serves as an alternative to Cordyceps products due to its content of cordycepin, polysaccharides, and other functional components, which exhibit growth-promoting, antioxidant, and

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immunomodulatory properties [4–6]. Previous studies have shown that the artificial fermentation process of *Acremonium terrestris* culture involves first, growth of slant cultures, the liquid culture, and lastly, large-scale culture in a fermenter. Dietary supplementation with 50 mg of ATC per kg of body weight significantly increased the total serum antioxidant capacity and immunoglobulin A and M levels in weaned calves [7]. Addition of 30 g/day of ATC to the diet improved performance, rumen fermentation, antioxidant capacity, and immune functioning in dairy cows [8]. ATC also significantly inhibited the expression of inflammatory cytokines in an LPS-induced rat mastitis model [9]. Recent research revealed that adding 3 g/kg of ATC to the basal diet of Hoertobagi geese significantly increased total antioxidant capacity, reduced serum malondialdehyde (MDA) concentration, and improved growth [10]. Therefore, it is hypothesized that dietary supplementation with ATC is a potentially effective strategy for enhancing the health and performance of dairy cows.

Milk fat is a crucial component that serves as the primary criterion for assessing milk quality and is one of the main target traits of dairy herd improvement breeding programs [11]. It is a key ingredient for desirable the physical and chemical properties, flavor, and quality of dairy products [12]. Proper lipid metabolism is essential to meet the increasing demand for premium milk and dairy products globally. A study conducted by Zhou et al. showed that disturbances in lipid metabolism led to hepatic triglyceride deposition and cholesterol reduction, which can lead to ketosis in dairy cows [13]. Research has revealed a connection between lipid metabolism and the immune response. Sun et al. suggested that changes in the gut microbiota and their functions related to secondary bile acid synthesis suppressed monocyte functions during excessive lipolysis in transition dairy cows [14]. However, it is still uncertain whether ATC can affect lipid metabolism and facilitate immune function in dairy cows.

Our hypothesis was that supplementing the feed of lactating dairy cows with ATC could improve their lipid metabolism and boost their immune system by altering their serum metabolites. These changes would have a positive impact on the productivity of dairy cows. Therefore, our objectives were to assess the effects of ATC on milk composition, antioxidant activity, and anti-inflammatory function and to investigate its contribution to lipid metabolism.

Materials and methods

The Ethics Committee on Animal Use of the Beijing University of Agriculture approved all animal care and handling protocols mentioned in this study (BUA-2022056). The dairy cows used in this study were from Sunlon Dairy Farm Center, the graduate co-culture teaching practice base of the Beijing University of Agriculture in the Yanqing district of Beijing.

Experimental design

This study involved 24 multiparous Chinese Holstein cows in mid-lactation (108 ± 10.4 days in milk, 637 ± 25 kg body weight, 30.23 ± 3.7 kg/d milk yield) that were divided into two groups. The first group was fed TMR supplemented with 30 g/d *Acremonium terrestris* culture (ATC), while the second group served as a control (CON) and was not given any ATC. The functional components of ATC included cordyceps acid (84.50 g/kg), cordyceps polysaccharide (44.60 g/kg), cordycepin (0.432 g/kg), sterols (0.597 g/kg), and total amino acids (218.1 g/kg) [7]. The experimental run lasted for 90 days, which included 15 days of acclimation and 75 days of sampling.

During the experiment, the cows were housed in a tie-barn and had free access to water. Before the morning feeding, 100 g of TMR mixed with ATC was given to the cows to ensure that they ingested the entire supplement. Additional TMR was fed to individual animals as per their requirements.

Milk composition analysis

Milking was conducted thrice daily, at 5:30 am, 2 pm, and 10 pm. The milk production of each cow was recorded daily via an automatic system (Afimilk animal monitoring system, Israel). The milk composition was analyzed at the end of the experiment, and 50 mL samples were collected in the morning, mid-day, and evening at ratios of 4:3:3. These samples were mixed with a preservative (2-bromo-2-nitropropane-1,3-diol) and stored at 4 °C for subsequent analysis. The fat, protein, and lactose contents, as well as the somatic cell count (SCC), were determined using a Milkoscan-6000 FT (from Foss Electric, Hillerød, Denmark).

Serum biochemical characteristics

At the end of the trial, blood was drawn from the tail vein, allowed to clot, and then centrifuged at 2000 × g for 10 min. The serum was collected and stored at -20 °C for subsequent analysis. The levels of catalase (CAT)(#A007-1-1), superoxide dismutase (SOD)(#A001-1-2), glutathione peroxidase (GSH-Px)(#A005-1-2), and malondialdehyde (MDA) (#A003-1-2) in serum samples, as well as interleukins-1β(ELISA, #H002-1-2), IL-2 (ELISA, #H003-1-2), and IL-6 (ELISA, #H007-1-2), and tumor necrosis factor-α (ELISA, #H052-1-2) were determined using kits according to the instructions from Nanjing Jian Cheng Bioengineering Institute, Nanjing, China.

Serum lipidomics analysis

After collecting the serum samples, we added 200 μL of methanol and 800 μL of MTBE following the methods of Xia et al. [15]. Briefly, the mixture was sonicated for 30 min at 40 kHz in a 4 °C water bath and then held at -20 °C for 20 min. Next, we centrifuged the mixture at 13,000 × g for 15 min at 4 °C to obtain the organic phase. We transferred 350 μL of the supernatant to a new centrifuge tube and dried it with nitrogen gas. Afterward, we reconstituted the dried samples with 100 μL of isopropanol/acetonitrile (1:1, v/v), vortexed them for 30 s, sonicated them for 10 min, and centrifuged them at 13,000 × g for 15 min at 10 °C. Finally, the supernatants were collected and assayed by LC–MS.

2.5 UHPLC–QTOF–MS analysis and data preprocessing.

LC–MS analysis of the UHPLC–Q Exactive HF–X system and Accucore C30 column (2.1×100 mm with 2.6 μm particles). The sample injection volume was 2 μL. Quality control (QC) standards were injected into the six samples to assess their stability. MS was run in either positive or negative ion mode. The secondary MS data were obtained from molecular ions with strengths > 100. The MS raw data files were converted to mzXML format using ProteoWizard and processed using XCMS to obtain retention times, mass-to-charge ratio (m/z) values, and peak intensities, which were used for identifying metabolites. A data matrix was created, including metabolite classes, retention times, mass-to-charge ratios, and peak areas. Only analytes with relative SDs < 30% were chosen for data processing, and the peak intensities were normalized. Multivariate statistical analyses of peaks, types of metabolites, changes in metabolite expression after treatment (OPLS-DA), and screening of differentially abundant metabolites were performed using MetaboAnalyst 5.0 (https://www.metaboanalyst.ca/). The metabolites with *P* values < 0.05 according to Student's *t* test, a fold change (FC) > 1.2 or < 0.83, and a variable importance in projection (VIP) > 1 according to the OPLS-DA were considered differentially abundant.

The principal metabolic pathways were identified by searching the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (https://www.kegg.jp/kegg/) and the Human Metabolome Database (HMDB, https://www.hmdb.ca).

Table 1 Effect of ATC supplementation on milk yield, composition, and SCC in dairy cows

Variable	CON	ATC	Mean ± SD	*P* value
Milk production (kg/d)	32.10	33.34	0.30	< 0.01
ECM[1]	32.01	34.50	0.56	< 0.01
Fat (g/kg)	33.31	35.51	1.58	< 0.01
Protein (g/kg)	30.69	31.11	0.66	0.65
Lactose (g/kg)	46.28	46.02	0.51	0.69
SCC (×10^{4}cells/ml)	12.73	10.06	0.77	< 0.01
Milk yield (kg/d)				
Fat	1.07	1.18	0.16	0.03
Protein	0.99	1.04	0.21	0.34
Lactose	1.49	1.53	0.04	0.16

[1]ECM (kg/d) = 0.3246 × milk yield (kg/d) + 13.86 × fat yield (kg/d) + 7.04 × protein yield (kg/d) [16]

Statistical analyses

The milk composition and SCC data were analyzed using SPSS 20.0 software. Continuous variables are expressed as the means ± SD. To identify significant differences between groups, Student's *t* test was used. Principal component analysis (PCoA) and orthogonal partial least squares discriminant analysis (OPLS-DA) were performed to screen additional compounds and identify differentially expressed metabolites. Following metabolite difference screening, hierarchical clustering analysis, KEGG annotation, and differentially abundant metabolite pathway analysis were conducted. Pearson's rank correlation test was used to estimate the relationships between different metabolites, milk composition, and serum antioxidant capacity.

Results

Milk yield and composition

ATC supplementation did not affect milk protein or lactose content ($P > 0.05$). Compared with that in the control group, the milk yield significantly increased in the ATC group ($P < 0.01$). ATC supplementation also significantly increased milk fat ($P < 0.01$) and decreased the somatic cell count (SCC) ($P < 0.01$; Table 1).

Serum biochemical parameters

ATC supplementation significantly increased serum SOD ($P < 0.01$), CAT ($P < 0.01$), and GSH-Px ($P < 0.01$) activity, whereas serum MDA ($P < 0.01$) was significantly decreased compared with that in the CON group (Fig. 1).

As shown in Fig. 2, compared with CON, supplementation with ATC decreased the serum concentrations of IL-1β ($P < 0.05$) and TNF-α ($P < 0.05$). The contents of IL-2 and IL-6 were similar between the treatments ($P > 0.10$).

Serum metabolite profiles

We used LC–MS in both positive and negative ion modes for better coverage of serum metabolites. A total of 589 cation peaks and 384 anion peaks were detected by metabolomics, and 973 metabolites were identified. As shown in Fig. 3A-B, the PLS-DA plot for supervised multivariate analysis of the differences in metabolites between CON and ATC showed good ion segregation, and all samples fell within a Hotelling T2 ellipse. The classification parameters of cations and anions are

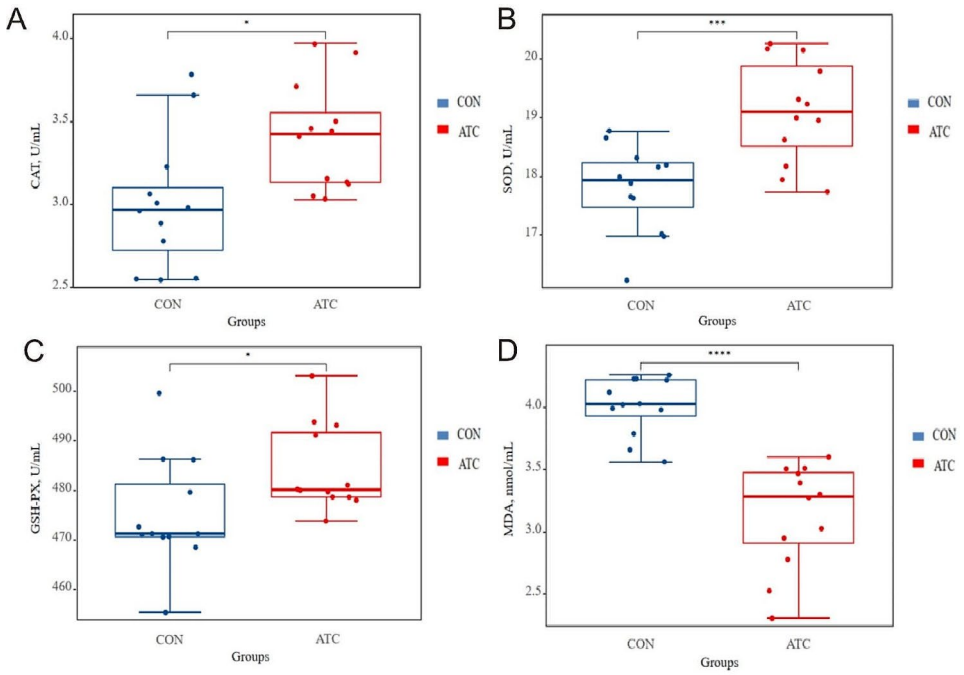

Fig. 1 Effects of ATC on serum antioxidant parameters: (**A**) catalase, CAT (**B**) superoxide dismutase, SOD (**C**) glutathione peroxidase, GSH-Px, and (**D**) malondialdehyde, MDA. *$P < 0.05$, **$P < 0.01$. CON, control. ATC, *Acremonium terrestris* culture. Data are presented as the mean ± SD

R2Y = 0.863 and 0.667, respectively, indicating good fitting and prediction ability. At the same time, the low Q2 intercept also confirmed the stability of the model (Fig. 3C-D).

Mutually differentially abundant metabolites and pathways between CON and ATC

By differentially abundant metabolite analysis, 143 distinct metabolites ($P<0.05$) were detected, of which 61 were upregulated, 82 were downregulated (Fig. 4A), and the top 30 were identified by VIP analysis (Fig. 4B). The profiles of potential biomarkers were significantly different between the groups but similar within groups. The results indicate that the metabolites are the main factors causing differences between the two groups. Table 2 shows 30 significantly different serum metabolites between the ACT and CON groups according to VIP ≥ 1 and $P<0.05$. These metabolites are broadly classified into glycerophospholipids, glycerolipids, and sphingolipids.

The principal biomarkers were classified and enumerated by comparison of their structures in the HMDB dbase (Fig. 5). The main categories included glycerophospholipids (63.91%), glycerolipids (9.41%), and sphingolipids (2.86%), followed by FAs (23.39%), PCs (21.69%), CLs (17.85%), and BisMePA (15.69%). We mapped the differentially expressed metabolites according to the pathways in the KEGG database to determine how ATC influences metabolic functions, as reflected in the blood. The *P* values and pathway impacts determined by pathway topology are given in the metabolomics map. Figure 6 shows the enrichment of differentially abundant metabolites in the central metabolic pathways.

Correlation analysis

Nine hundred and seventy-three metabolites identified in the serum samples were subjected to Pearson correlation analyses with the FCR phenotype to screen for milk-related metabolites. Thirty metabolites were considered milk-related and were used for predicting milk ingredients via the random-forest method. Four milk-related metabolites, PC (10:0/22:3), PC (16:1/8:0), PC (16:1e/22:6) and ZyE (18:3), were selected with an MDA > 4 (Fig. 7A). The area under the curve (AUC) values for PC (10:0/22:3), PC (16:1/8:0), PC (16:1e/22:6) and ZyE (18:3) were 68.8%, 71.2%, 72.2% and 62.5%, respectively (Fig. 7B). The correlations between serum lipid metabolites, inflammatory factors and milk composition are presented in Fig. 7C. IL-1β was shown to have a positive correlation with ZyE (18:3), PC (16:1/8:0), and PC (14:1e/18:2). However, there was a positive correlation between TNF-α and PC (18:1/18:1) and a negative correlation between IL-2 and PC (14:1e/18:2). Milk fat and dMePE (20:0/20:4), which is a glycerophospholipid, were positively correlated.

Discussion

Our data indicated that incorporating ATCs into animal feed can increase milk production and improve serum immune function by altering lipid metabolites such as

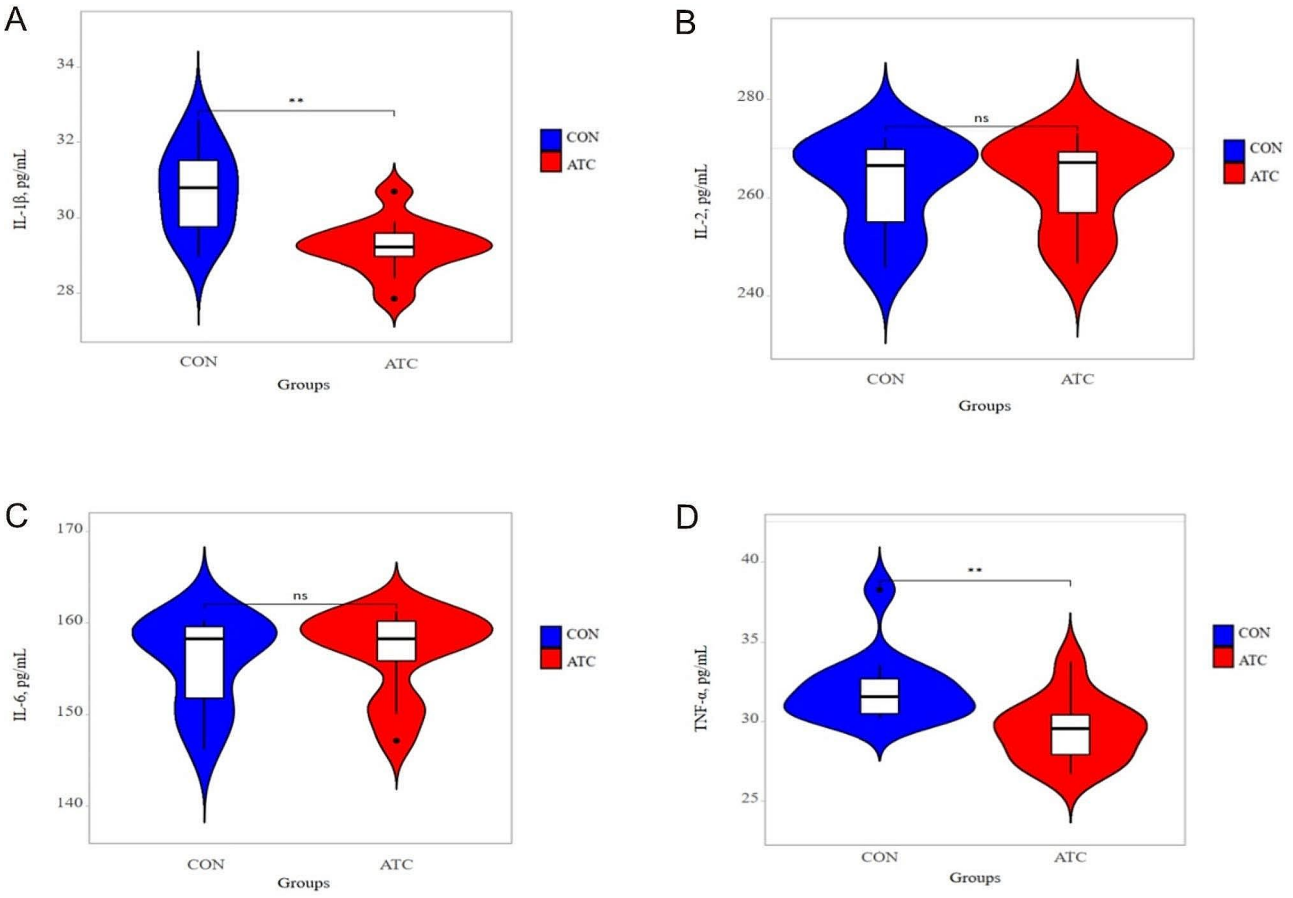

Fig. 2 Effect of ATC on the serum inflammatory factors IL-1β (**A**), IL-2 (**B**), IL-6 (**C**), and TNF-α (**D**). *$P < 0.05$, **$P < 0.01$. CON, control; ATC, *Acremonium terrestris* culture. Data are presented as the means ± SD; ns = not significant

GPs, FAs, GLs, and SPs. These polar lipids are vital components of milk and have been shown to possess biological properties that regulate inflammatory responses and reduce the risk of cardiovascular disease in humans [17]. Previous studies have demonstrated that ATC can enhance animal performance, boost immunity, and promote mammary gland health in dairy cows [18]. In line with these findings, this investigation revealed that dietary ATCs led to a decrease in serum interleukin-1β, tumor necrosis factor-α, and other inflammatory factors, indicating that ATCs positively influence metabolism and immune function.

Numerous studies have revealed that feeding ATC to dairy cows can significantly enhance milk production and improve its composition [7–10, 19] to meet the current demands of consumers for high-quality dairy products. In this study, we observed that supplementary feeding with ATC significantly increased the milk and fat production of dairy cows. Milk is the primary source of protein for human neonates and provides essential amino acids and nitrogen for growth and development [20, 21]. Milk fat is essential for producing high-quality dairy products that have high economic value [20]. The amount of fat in milk is also indicative of the cattle's physiological status and the quality of their environment and feed [22]. Studies by Li et al. [7] and Deng et al. [23] have confirmed that ATC and its active ingredients are beneficial for improving feeding efficiency and health. ATC can significantly reduce somatic cell count (SCC) by improving both antioxidant and immune functions [8]. These findings demonstrate that ATC has positive regulatory effects on milk quality and immune function in dairy cows.

Changes in specific blood indices can reflect an animal's health status, which ultimately affects production performance [24, 25]. In this study, dietary supplementation with ATC increased the serum levels of SOD, CAT, and GSH-Px while decreasing MDA levels. This indicates that ATC can enhance the antioxidant capacity of dairy cows. Additionally, the levels of serum proinflammatory factors, such as IL-1β and TNF-α, were also reduced. These findings are consistent with earlier studies on dairy cows, which showed that ATC can boost antioxidant and immunological activities [26–28]. This effect can be attributed to cordyceps polysaccharide, a natural antioxidant that can eliminate free radicals and regulate inflammation [29–31].

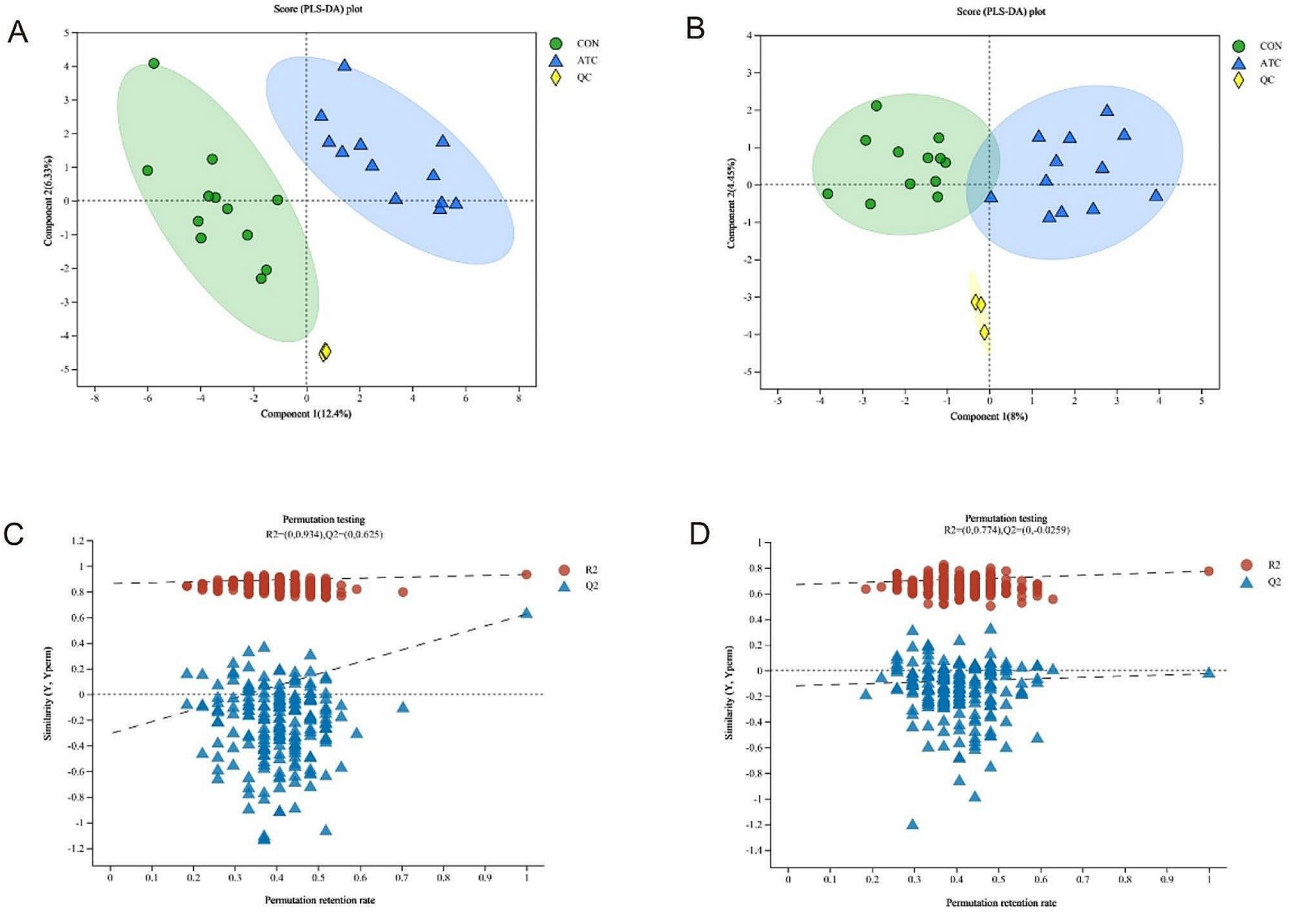

Fig. 3 Differential serum metabolite profiles between CON and ATC. PLS-DA score plots (**A**, **B**) and OPLS-DA permutation test (**C**, **D**) based on serum metabolite data from CON and ATC in positive and negative ion modes

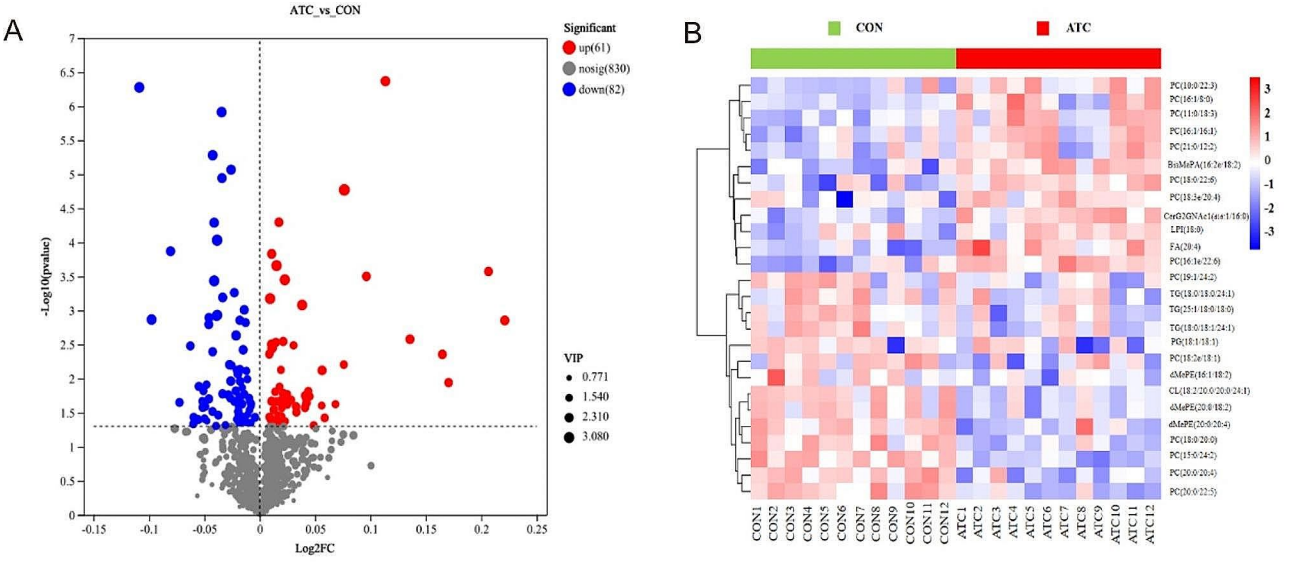

Fig. 4 (**A**) Volcano plot of differentially expressed metabolites (ATC vs. CON). Blue symbols are downregulated and red symbols are upregulated. Gray symbols (ns) are metabolites with no significant differences between ATC and CON. (**B**) Clustered heatmaps of metabolites in serum from cows fed the CON diet compared to those fed the ATC diet

Table 2 Differentially expressed metabolites in serum from cows fed CON or ATC diets

Metabolite	VIP value	FC	*P* value	Tendency
Glycerophospholipids				
PC(18:3e/20:4)	2.33	1.04	0.04	↑
PC(10:0/22:3)	3.67	1.12	0.00	↑
PC(16:1/8:0)	3.44	1.13	0.01	
PC(16:1/16:1)	2.37	1.04	0.01	
PC(21:0/12:2)	2.25	1.03	0.02	
PC(11:0/18:3)	3.18	1.10	0.00	
PC(18:0/22:6)	2.31	1.05	0.01	↑
PC(16:1e/22:6)	2.74	1.07	0.00	↑
PC(18:0/20:0)	2.60	0.97	0.01	↓
PC(20:0/20:4)	2.78	0.95	0.00	↓
PC(20:0/22:5)	2.21	0.97	0.00	↓
PC(14:1e/18:2)	2.22	0.96	0.00	
PC(18:2e/18:1)	2.62	0.96	0.04	
PC(15:0/24:2)	3.60	0.93	0.00	
PC(19:1/24:2)	2.23	0.96	0.01	
BisMePA(16:2e/18:2)	4.08	1.15	0.00	↑
dMePE(20:0/20:4)	2.27	0.97	0.00	↓
dMePE(20:0/18:2)	2.40	0.97	0.00	↓
dMePE(16:1/18:2)	2.34	0.97	0.03	
PG(18:1/18:1)	2.32	0.96	0.05	
CL(18:2/20:0/20:0/24:1)	2.58	0.97	0.00	
CL(18:2/16:1/18:0/24:1)	3.57	0.93	0.00	
LPI(18:0)	2.16	1.03	0.05	↑
Glycerolipids				
FA(20:4)	3.96	1.05	0.01	↑
TG(25:1/18:0/18:0)	2.70	0.95	0.02	↓
TG(18:0/18:0/24:1)	2.46	0.96	0.04	
TG(18:0/18:1/24:1)	2.19	0.97	0.02	
Sphingolipids				
CerG2GNAc1(d16:1/16:0)	2.52	1.03	0.00	↑
Cer(d16:0/18:2)	3.32	1.08	0.00	
Sterol Lipids				
ZyE(18:3)	4.42	1.17	0.00	

Dyslipidemia can lead to elevated levels of proinflammatory cytokines such as IL-6 and TNF-α [32–34]. *Ophiocordyceps sinensis*, which has the same bioactive components as ATC, can affect the expression levels of genes associated with lipid biosynthesis [35]. However, the exact mechanism of regulation remains to be elucidated. Previous studies have also shown that ATC can improve the immunoregulatory functions and antioxidant activity of dairy cows, which can lead to reduced SCC and increased milk production [8, 10]. These findings support our hypothesis that adding ATCs to the diet can promote animal health.

It has been reported that mammary gland cells can utilize phosphatidylcholine (PC) in the blood as an energy source, which promotes triglyceride production by increasing choline glycerophosphate and free fatty acids [36]. Milk fat is predominantly composed of triglycerides (96–98%) [38]. In this study, compared with those in the control group, the serum PC content in the cows fed ATC increased to 21.69%. This increase could be attributed to the activity of alkaloids present in ATC, which promote the production of phosphatidylserine decarboxylase, a regulator of phosphatidylethanolamine (PE) synthesis. Continuous methylation of PE enables its conversion into PC [38]. This finding was also supported by previous research showing that natural isoquinoline alkaloids alleviate glucose and lipid metabolism disorders by regulating mitochondrial energy balance [39, 40]. Plasmodia rely on phosphatidylethanolamine N-methyltransferase (PEMT) to methylate PE at three positions to synthesize PC, which sustains their life cycle. GPs constituted 63.91% of the serum GPs in the current study after the addition of ATC. GPs regulate various biological pathways, such as oxidative stress, inflammation, cell proliferation, and lipoprotein function [41, 42]. Therefore, ATC and its bioactive components may play a crucial role in regulating lipid metabolism.

In addition to the glycerophospholipids, glycerolipids, and sphingolipids, we considered other metabolites that contribute to the observed effects on lipid metabolism, inflammation, and immune responses in dairy cows supplemented with *Acremonium terrestris* culture (ATC). Compounds such as fatty acids, particularly conjugated linoleic acid (CLA) and omega-3 fatty acids enhance milk fat content and improve milk quality by modulating lipid metabolism and reducing inflammation [12]. Triglycerides, the main form of stored fat, are directly involved in milk fat synthesis, with higher serum levels correlating with increased milk fat percentage [37]. Phosphatidylserine, crucial for cell function and signaling, promotes the conversion of phosphatidylethanolamine to phosphatidylcholine, enhancing lipid metabolism [43]. Ceramides act as second messengers in inflammatory pathways, such as NF-κB, regulating oxidative stress and inflammation to maintain cellular homeostasis [44]. Lysophospholipids, including lysophosphatidylcholine (LPC), modulate inflammatory responses and improve lipid metabolism, influencing milk fat composition and quality [45]. These metabolites are integral to lipid metabolism and significantly affect milk production, fat content, and immune responses in dairy cows. Future studies should investigate these metabolites to further elucidate their roles and the mechanisms through which ATC supplementation exerts its beneficial effects.

There was a significant positive correlation between milk fat and dMePE (20:0/20:4), which is consistent with previous studies showing that the GP dMePE accounts for a substantial part of the milk fat spherosome membranes (MFGM) [44, 46]. GPs can also form

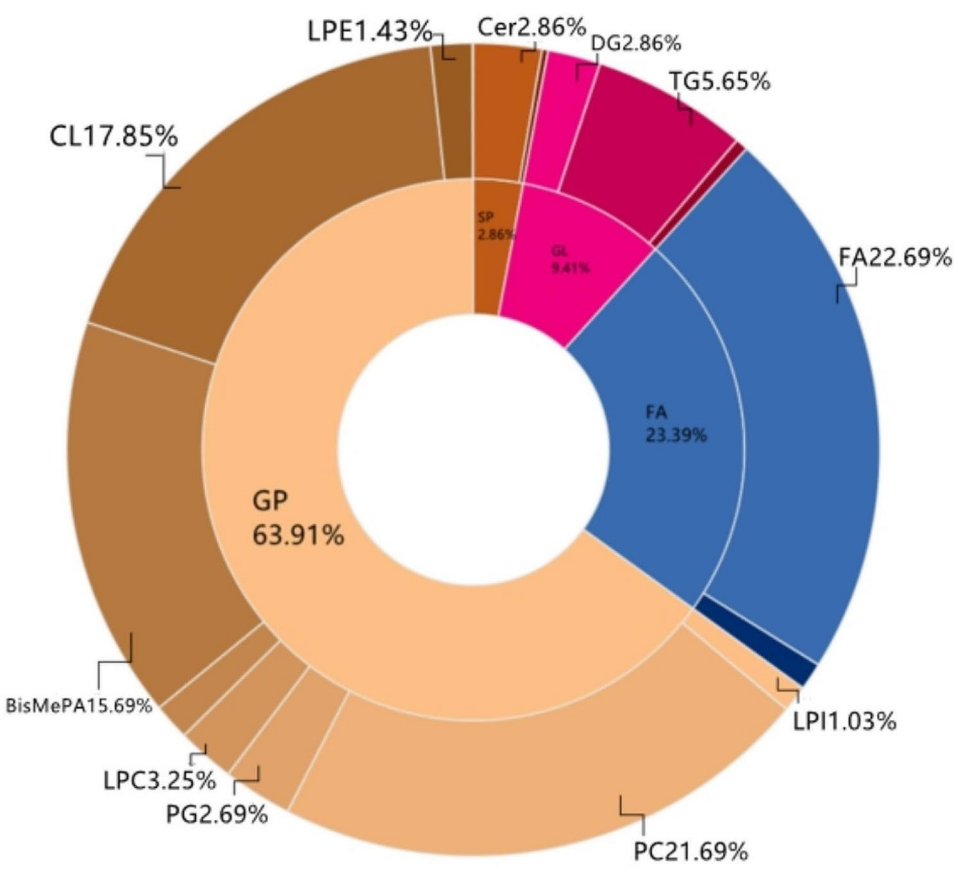

Fig. 5 Percentages of the main lipid classes determined by LC–MS in the serum of dairy cows. Glycerophospholipids (yellow), fatty acids (blue), glycerolipids (pink), and sphingolipids (brown)

a lipid bilayer in the cell membrane and release signal molecules such as lysophospholipids and diacylglycerides [41]. We also observed a significant negative correlation between IL-2 and phosphatidylcholine (PC) (14:1e/18:2), which is similar to the finding of Eurtivong et al. [44] that PC is associated with the progression of many pathological conditions, including inflammation and cancer. This could be because PC can reduce leukocyte recruitment, improve inflammation, and directly or indirectly impact ROS production [50]. In conclusion, dietary ATCs may induce changes in milk composition, immune function, and lipid metabolism, which could benefit dairy farms. Although this study covered numerous lipids, further research is required to precisely quantify and comprehend how dietary ATCs cause these lipid changes.

Conclusions

Supplementation with dietary *Acremonium terrestris* culture (ATC) can increase milk production and improve milk quality in dairy cows. It can also regulate inflammatory reactions, which reduces the somatic cell count (SCC) and improves milk production. This study revealed that the levels of ATC supplements were correlated with the levels of metabolites that affect milk composition and immune function. Additionally, ATC supplementation was found to be related to glycerophospholipids, which are important for lipid metabolism. This study provides valuable insights into developing nutritional strategies that can enhance milk quality and immunity in the dairy cow industry. Furthermore, this study suggested that using ATC supplements to modulate host glycerophospholipids could be an effective way to maintain metabolic homeostasis in ruminants.

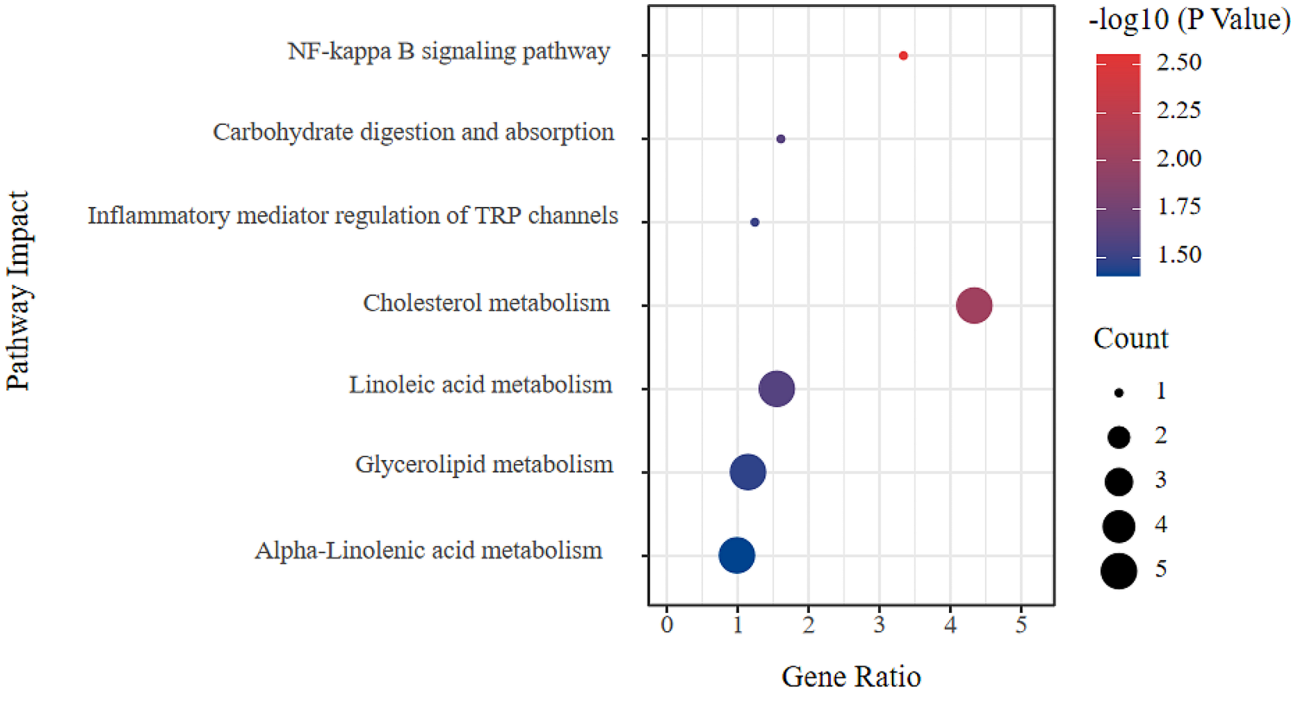

Fig. 6 Main pathways associated with differentially expressed lipids in serum from cows fed the CON or ATC diet

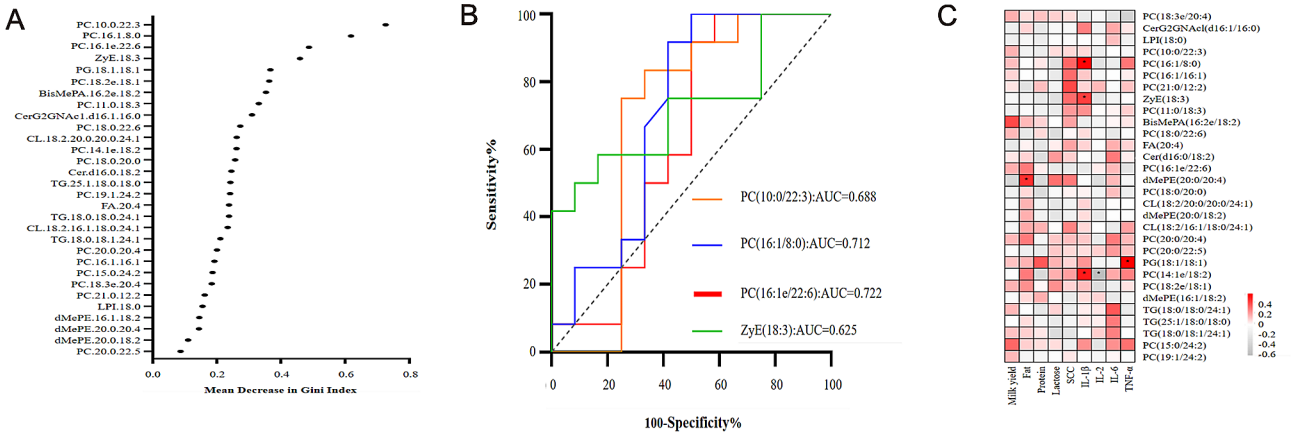

Fig. 7 (**A**) The confusion matrix reflects the random forest model performance. (**B**) Receiver operating characteristic (ROC) curve. (**C**) Pathway correlations of the differentially abundant serum lipids between CON and ATC

Author contributions

JT, FL, and ZH proposed the hypotheses, generated the project, and were responsible for all the data, figures, and text. JT, SZ, and CZ conducted the experiments, while FL and XG analyzed the data. FL drafted the paper, and JT and LJ revised it. All authors contributed to the article and approved the submitted version.

Funding

This work was financially supported by the National Natural Science Foundation of China (Grant No. 32272904).

Data availability

Sequence data supporting this study's findings have been deposited in the MetaboLights datasets. Data will be made available on request.

Declarations

Institutional review board statement

All animal use protocols and handling procedures were approved by the Ethics Committee on Animal Use at the Beijing University of Agriculture.

Conflict of interest

The authors declare no conflicts of interest.

Received: 16 May 2024 / Accepted: 23 July 2024
Published online: 12 August 2024

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