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Acremonium terricola culture supplementation in the diet of pregnant and lactating Ewes can improve the production performance of Ewes and lambs by regulating maternal metabolism and antibody delivery

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Abstract

Background The fungal culture of *Acremonium terricola* culture (ATC) has been extensively utilized in livestock farming systems due to its demonstrated efficacy in improving productivity and preventing disease outbreaks. However, the effects of dietary ATC supplementation on pregnant and lactating ewes and their offspring remain a critical knowledge gap requiring investigation. Therefore, this study was designed to address two primary objectives: (1) to evaluate the effects of dietary supplementation with ATC on production performance and hematological parameters in ewes; (2) to determine whether maternally ingested ATC can be transmitted to offspring via lactation and subsequently influence lamb growth performance. This study employed eighteen ewes randomly stratified into two groups: a basal diet control (CON, $n=9$) and an experimental group receiving basal diet supplementation with 9 g of ATC per ewe daily (ATC, $n=9$). The study design comprised a 115-day protocol consisting of a 10-day pre-experimental acclimatization phase with environmental parameter standardization, followed by a 105-day controlled experimental intervention period.

Results The findings demonstrated that administration of ATC supplemented diets throughout the gestational-lactational period significantly enhanced maternal dry matter intake (DMI) and late-gestation (day 145) body mass ($p<0.05$), concurrently attenuating gestational lipolysis compared with control group. Regarding lactation performance, ATC supplementation led to an increase in the average daily milk yield (0.90 vs. 0.78 kg/d), decelerated the decline rate of the lactation peak, and enhanced milk quality by boosting the percentages of milk fat, total solids (Ts), and urea content ($p<0.05$). Moreover, ATC supplementation elevated serum levels of immunoglobulin A (IgA), urea, and superoxide dismutase (SOD) in pregnant and lactating ewes, while decreasing the interleukin-6 (IL-6) level

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($p < 0.05$). For lambs, the supplementation of ATC in ewes' diets significantly improved the average daily gain (ADG) during the 1–45-day nursing period ($p < 0.05$) and showed a trend toward increased weaning weight at 45 days of nursing ($p = 0.061$). Biochemically, lambs from the ATC - supplemented group exhibited significantly higher serum concentrations of urea, IgA, interleukin-4 (IL-4), catalase (CAT), SOD, and total antioxidant capacity (T-AOC), along with lower serum tumor necrosis factor-α (TNF-α) content ($p < 0.05$).

Conclusions Maternal dietary supplementation with ATC demonstrated dual zootechnical benefits, effectively augmenting ovine productive efficiency through enhanced milk synthesis capacity, improving the immune and antioxidant levels of the body, while concurrently stimulating neonatal development of pre-weaning growth velocity.

Keywords *Acremonium terricola* culture, Ewe, Milk ingredients, Lamb, Immune, Antioxidant

Background

Late pregnancy and lactation are critical periods that determine the growth and development of young animals. During these stages, ewes undergo profound physiological adaptations as they transition from pregnancy to parturition and lactation. Ewes in late pregnancy and lactation exhibit high metabolic activity, during this time, they experience dynamic alterations in hormonal profiles, metabolic processes, and immune status to meet the nutritional and developmental needs of fetuses. However, these physiological adjustments render them susceptible to oxidative stress and inflammation, primarily due to the elevated metabolic demand and physiological burden [1, 2]. The third trimester of pregnancy marks a phase of rapid embryonic development, during which approximately two - thirds of the fetal weight is accumulated. Concurrently, as the mammary gland rapidly develops in late pregnancy to prepare for postpartum lactation, the ewe's demand for nutrients escalates significantly. This phenomenon is particularly pronounced in polytomous animals [3]. Consequently, the nutritional status and health of ewes during this crucial stage directly influence the survival rate and production performance of their newborn offspring.

Previous studies have shown that when the body experiences physiological load caused by pregnancy, calving, and lactation during the perinatal period, reasonable feeding management can reduce the negative energy balance of the body and mitigate the risk of oxidative stress [4]. Recent studies have indicated that supplementary feeding of *Saccharomyces cerevisiae* to ewes' diets can enhance dry matter (DM) intake, boost nutrient digestibility, elevate milk yield, and increase lambs' average daily gain [5]. It has also been found that selenium supplementation in the diets of late-gestation ewes and lambs can enhance oxidative stability and improve lambs' metabolic and immune functions [6]. Therefore, enhancing the nutritional health of female animals during late gestation and lactation via nutritional regulation represents a viable and effective approach.

Cordyceps gunnii is a major entomogenous fungus, belonging to Ascomycota, Sclerotiniaceae, Xylariales, and Ophiocordycipitaceae. *Cordyceps gunnii* contains *cordycepin*, *cordycepic acid*, *cordyceps polysaccharide* and other bioactive substances [7]. In recent years, a novel feed additive—*Acremonium terricola* culture, produced via the solid-state fermentation of Cordyceps gunnii, has gradually garnered attention. ATC exhibits bioactive components and pharmacological functions analogous to those of cordyceps, including antioxidant, immune - regulating, and intestinal flora-modulating properties [8, 9]. Therefore, owing to its distinctive bioactive components, eco-friendly and cost - effective solid - state fermentation process, and natural origin, ATC has emerged as an economical, efficient, and sustainable option for enhancing animal health and performance.

Currently, the application of ATC in the field of animal nutrition has attracted widespread attention. In monogastric animal production, the utilization of ATC has reached a relatively advanced stage. For instance, in pig production, ATC can significantly augment the growth performance, antioxidant capacity, and immune function of pigs [10, 11]. Analogously, there are relevant application studies in poultry production [12, 13]. In the ruminant domain, research on ATC has achieved some progress; nevertheless, it predominantly centers on studies in dairy cows [14, 15] and fattening sheep [16, 17]. Notably, research on the effects of ATC on ewes and suckling lambs remains relatively scarce, particularly studies that adopt an integrated maternal - offspring perspective and investigate nutritional regulation during the gestation and lactation periods of ewes. Based on the potential role of ATC in nutritional regulation, we hypothesized that maternal dietary supplementation with ATC during gestation and lactation periods might improve both ewe and offspring productivity dual mechanisms of metabolic modulation and enhanced immunoglobulin transmission. To verify this hypothesis, the present study was designed to systematically evaluate the impacts of ATC supplementation on maternal production parameters, hematological profiles reflecting immune competence and oxidative homeostasis, and biochemical indicators of metabolic status in periparturient ewes. Furthermore, we sought to elucidate whether bioactive components in ATC could be transferred via mammary pathways to exert developmental effects on suckling lambs.

Methods

All experimental procedures involving animals were conducted under strict compliance with ethical standards at Lvrong Animal Husbandry Development Co., Ltd. (Anhui, China). The study protocol received formal authorization with the guidelines of the Experimental Ethics Committee of Anhui Science and Technology University (No. 48 / 05.08.2023) and the ARRIVE guidelines (https://arriveguidelines.org/).

Experimental design and treatments

The experiment was conducted at the sheep farm of Lvrong Animal Husbandry Development Co., Ltd., which is located in Linquan County, Anhui Province. Based on the estrus synchronization records, eighteen ewes with the following characteristics were selected: consistent parity, similar age and body weight (46.2 ± 3.9 kg), synchronized gestational progression (80 days post-conception), and confirmed by ultrasound to be carrying viable twin fetuses.

The experimental cohort was stratified through a computer-generated randomization algorithm (SPSS 23.0), employing a randomized block design with triplicate pens (n = 3 ewes/pen) per treatment. Dietary interventions consisted of: (1) Control group (CON, n = 9) receiving a standard total mixed ration (TMR) formulation; (2) ATC group (ATC, n = 9) administered identical basal diet supplemented with 9 g/d ATC premix through top-dressing method. The dietary supplementation dosage was determined in accordance with previously established protocols [18]. The total trial period lasted 115 days, including a 10-day pretrial period and a 105-day intervention period. The strain of *Acremonium terricola* culture used in this study was isolated from the sclerotium of *Cordyceps gunnii* and is deposited in the China Microbial Culture Collection (CGMCC) under the number 0346. The active ingredient was produced through artificial solid-state fermentation by Hefei Micro Biological Engineering Co., Ltd. (Hefei, China). The fermentation process involved inoculating *Acremonium terricola* onto a solid medium composed of 69.9% corn, 20% soybean meal, 10% wheat bran, 0.08% potassium hydrogen phosphate (KH_2PO_4), and 0.02% magnesium sulfate ($MgSO_4$). The culture was maintained at a relative humidity of 80–90% and a temperature of 25 °C for 76 h, then dried at 80 °C for 1 h and passed through a 0.15 mm sieve. The bioactive components of the dried *Acremonium terricola* culture included cordycepic acid (D-mannitol) at 84.50 g/kg dry matter (DM), Cordyceps polysaccharide (galactomannan) at 44.60 g/kg DM, cordycepin (3′-deoxyadenosine) at 0.432 g/kg DM, ergosterol at 0.597 g/kg DM, total amino acids at 218.10 g/kg DM, γ-aminobutyric acid (GABA) at 50 g/kg DM, and adenosine at 382.3 mg/kg DM. Additionally, the culture 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 total mixed diet (TMR) was prepared according to the basic diet nutrition level as per the feeding standard of the NY/T816-2004 *feeding standard for mutton sheep* (Ministry of Agriculture of the People's Republic of China, 2004). The diet composition and nutritional level are shown in Table 1.

Table 1 Effects of adding ATC on the feed intake of Ewes (kg/d)

Items[1]	Treatments		SEM	*P*–value
	CON	ATC		
Pregnancy				
90–110 d	1.63	1.72	0.03	0.078
111–130 d	1.84[b]	1.94[a]	0.02	0.005
131–145 d	2.03	2.10	0.09	0.713
90–145 d	1.83	1.92	0.04	0.265
Lactation				
1–15 d	1.82	1.95	0.05	0.162
16–30 d	1.84[b]	1.99[a]	0.03	0.001
31–45 d	1.92[b]	2.09[a]	0.04	0.015
1–45 d	1.86[b]	2.01[a]	0.02	0.001

[1] "90 d" refers to "90 days of pregnancy"; "110 d" to "110 days of pregnancy"; "130 d" to "130 days of pregnancy"; "145 d" to "145 days of pregnancy"; "1 d" refers to "1 day of lactation"; "15 d" to "15 days of lactation"; "30 d" to "30 days of lactation" and "45 d" to "45 days of lactation"

[2] CON = Control group; ATC = *Acremonium terricola* culture group. The data are expressed as the mean and SEM (n = 9). In the same row, values with different small letters are significantly different (P < 0.05). The same as Tables 2, 3, 4, 5 and 6

Feeding management

All equipment and surfaces within the experimental area were sanitized prior to the commencement of feeding trials. During the experiment, the conditions within the sheep house were kept consistent, and each sheep was clearly tagged. From the late pregnancy stage (approximately 80 days of gestation) until 45 days postpartum, all the 18 ewes were housed in 6 well-ventilated sheep pens (4 m×5 m) with controlled temperature and humidity. Experimental pens were configured to house three multiparous ewes each with their respective litters (2 lambs per ewe). All sheep had free access to water throughout the entire experiment, with scheduled feeding twice daily at 0700 a.m. and 1700 p.m. Every day, we measured the specific amount of ATC to be mixed into a portion of the morning total mixed ration (TMR). After offering this special feed, the regular TMR was provided as usual.

When the ewe gives birth, the farrowing time, the number of lambs, and other relevant information are accurately recorded, and the health status of the ewe is noted. After the lamb is born, the mucus in its mouth and nose is removed, the umbilical cord is disinfected after it is cut, the ewe is allowed to lick the amniotic fluid and mucus off the lamb's body to dry it, the newborn lamb is weighed to determine its birth weight, and an ear tag is attached to its ear. The lambs were nursed by their mothers; at 7 days of age, a lamb feeding pen was set up within the ewe pen. The feed for the lambs (purchased locally) mainly consisted of corn, soybean meal, extruded soybean, cottonseed meal, secondary flour, DDGS, molasses, wheat bran, etc. The nutritional content was as follows: crude protein ≥ 18% (% is added to make the unit clear), calcium 0.3–1.5%, crude fiber ≤ 14%, total phosphorus ≥ 0.3%, crude ash ≤ 10%, common salt 0.3–1.6%, lysine ≥ 0.65%, and moisture ≤ 14% (Henan Haida Jiuzhou Biotechnology Co., Ltd., China). All lambs were weaned at 45 days of age.

Sample and data collection

Growth performance

The feed intake record involved weighing the feeding amount and leftover amount to calculate the average daily dry matter feed intake of each column. Finally, the feed intake of each sheep was calculated by dividing the total feed intake of each column by the number of sheep in that During the experiment, the weight changes of the ewes and lambs were recorded, and the daily health status of the ewes was monitored. The body weight (BW) of the ewes was measured at 90 days (initial weight), 120 days, and 145 days of gestation, as well as at 1 day, 30 days, and 45 days after parturition. Lambs were weighed at birth, and at 30 days and 45 days of age, and the average daily gain (ADG) at each stage was calculated.

For the feed intake record, the amount of feed offered and the remaining amount were weighed to calculate the average daily dry matter feed intake of each pen. Finally, the feed intake of each sheep was calculated by dividing the total feed intake of each pen by the number of sheep in that pen. column.

Milk production and composition

To measure the milk production of the ewes, the method described in reference [19] was used. This method determines the milk production by calculating the weight difference of the lambs before and after nursing. The difference in the body weight of lambs before and after two consecutive nursing sessions represents the milk intake of each nursing event, and the sum of the daily milk intakes constitutes the total milk production of the ewe for that day. The specific operational steps were as follows: At 1, 7, 15, 30, and 45 days after the ewe gave birth, the ewe was separated from the lamb in advance (the separation took place at 2100 p.m. the previous day). Before the lambs reached 15 days of age, they received maternal nursing 5 times a day, starting at 0600 a.m., with a 4-hour interval between each feeding. After the lambs were 15 days old, they received maternal nursing 4 times a day, starting at 0700 a.m., with a 5-hour interval between each feeding. Each nursing session maintained approximately 10–15 min.

After parturition, milk samples were collected from ewes on the 1st, 7th, 30th, and 45th days of lactation through artificial milking. The milk samples of ewes were collected twice a day at 09:00 and 15:00, respectively. Subsequently, the collected milk samples were thoroughly mixed and temporarily stored at + 4℃ for the determination of milk components (such as milk fat, milk protein, lactose, total solids, and urea) and the levels of immunoglobulin A (IgA), immunoglobulin G (IgG), and immunoglobulin M (IgM). The composition and content of the milk were determined using the automatic milk analyzer FOSS MilkoScan™ FT+ (FOSS, Hillerød, Denmark). The concentrations of IgA, IgG, and IgM in the milk were detected using ELISA kits, and the tests were conducted by Jiangsu Nanjing Aoqing Biotechnology Co., Ltd.

Serum biochemical indices and immune and antioxidant indices

Blood samples were collected from ewes at 90, 120, and 145 days of pregnancy and at 1, 30, and 45 days of lactation. On the 1st, 30th, and 45th days of lactation, before the morning feeding, six lambs were randomly selected from both the control group and the ATC group for jugular vein blood sampling (CON = 6, ATC = 6). The collected blood samples were centrifuged at 3000 r/min for 15 min. After centrifugation, the serum was extracted and stored at -20℃.

The levels of serum alkaline phosphatase (ALP), globulin (GLB), total cholesterol (TCHO), triglyceride (TG), total protein (TP), and urea (UREA) were determined using an automatic biochemical analyzer. The analyses were conducted by Jiangsu Nanjing Aoqing Biotechnology Co., Ltd.

The contents of immunoglobulin A (IgA), immunoglobulin G (IgG), and immunoglobulin M (IgM) in the serum were determined by enzyme-linked immunosorbent assay (ELISA). The specific determination method was in accordance with the kit instructions provided by Jiangsu Nanjing Aoqing Biotechnology Co., Ltd. The levels of interleukin-1 beta (IL-1β), interleukin-4 (IL-4), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) in the serum were also determined by ELISA.

The specific operation was carried out according to the instructions of Jiangsu Nanjing Aoqing Biotechnology Co., Ltd. The total antioxidant capacity (T-AOC), glutathione peroxidase (GSH-Px), catalase (CAT), superoxide dismutase (SOD) activity, and malondialdehyde (MDA) content in the serum were determined by colorimetric methods, and the specific operation was performed following the manual from Jiangsu Nanjing Aoqing Biotechnology Co., Ltd.

The ELISA kits were purchased from Jiangsu Nanjing Aoqing Biotechnology Co., Ltd. Each sample was measured repeatedly to ensure accuracy during the detection process.

Immune and inflammatory factors were measured using the double-antibody sandwich method. Antioxidant indicators and other biochemical markers were measured using the micro method. CAT was measured using the ammonium molybdate colorimetric method. Repeated measurements were performed on each sample during the detection process. The specific product codes of the kits are as follows:

Statistical analysis

Before analysis, the normality and homogeneity of the data were tested. Subsequently, all the data were analyzed by one-way ANOVA with SPSS statistical software (version 23.0 for Windows; SPSS, Chicago, USA). The Duncan test was utilized to analyze the differences between the two treatments. The data are presented as the means and standard errors of the means (SEMs). A significance level was indicated at $p<0.05$, and $0.05 \le p<0.10$ represented a tendency.

Results

Production performance of Ewes

The effect of dietary supplementation with ATC on the feed intake of ewes is shown in Table 1. The results showed that the supplementation of ATC significantly increased the feed intake of ewes at 110–130 d of gestation ($P<0.05$) and tended to increase the feed intake at 90–110 d of gestation ($P=0.078$). During lactation, supplementation with ATC significantly increased the average feed intake at 15 to 30 d, 30 to 45 d, and 1 to 45 d postpartum ($P<0.05$).

The effect of ATC supplementation on the body weight of the ewes is shown in Table 2. ATC supplementation significantly increased the body weight of ewes at 145 d of gestation ($P<0.05$).

The effect of the supplementation of ATC on postpartum lactation in ewes is shown in Table 3. The results showed that ATC supplementation tended to increase the average daily lactation ($P=0.065$), and compared with those of the CON group, the lactation periods 1 d, 15 d, 30 d, and 45 d and the total lactation volume of the ATC group increased by 25.36%, 18.78%, 19.43%, 32.92%, 13.82% and 15.18%, respectively ($P>0.05$) (Tables 4, 5, 6 and 7).

The effects of ATC supplementation to the diet of pregnant ewes on milk composition and immunoglobulin levels in milk during lactation are shown in Fig. 1. The results indicated that the supplementation of ATC significantly increased the contents of total solids (Ts) and urea in 1-day milk, as well as fat and urea in both 7-day and 30-day milk ($P<0.05$). However, it had no significant effect on the contents of milk protein and lactose ($P>0.05$). Additionally, while the supplementation of ATC during the gestation and lactation periods of ewes did not significantly affect the concentration of immunoglobulins in ewes' milk, it did show a positive trend toward increased immunoglobulin levels. ($P>0.05$).

Table 2 Effects of adding ATC on the body weight of Ewes (kg)

Items[1]	Treatments		SEM	*P*–value
	CON	ATC		
Pregnancy				
Day 90	45.57	46.84	0.93	0.510
Day 120	50.93	52.71	0.78	0.263
Day 145	54.60[b]	57.66[a]	0.78	0.044
Total weight gain from days 90 to 145	9.03	10.83	0.70	0.210
Lactation				
Day 1	43.77	47.20	1.24	0.171
Day 30	41.91	45.39	1.39	0.220
Day 45	41.49	44.39	1.29	0.273
Weight loss				
Before and after delivery	10.83	10.46	0.75	0.815
Days l to 30 during lactation	1.86	1.81	0.45	0.963
Days 3 L to 45 during lactation	0.42	1.00	0.44	0.531
Days l to 45 during lactation	13.11	13.27	0.86	0.928

[1] "90 d" refers to "90 days of pregnancy"; "120 d" to "120 days of pregnancy"; "145 d" to "145 days of pregnancy"; "1 d" refers to "1 day of lactation"; "30 d" to "30 days of lactation"; and "45 d" to "45 days of lactation". The same as below

Table 3 Effect of the addition of ATC on postpartum lactation in Ewes (kg)

Items[1]	Treatments		SEM	*P*–value
	CON	ATC		
Milk yield				
Day 1	0.50	0.61	0.04	0.123
Day 7	0.83	0.78	0.04	0.857
Day 15	1.13	1.32	0.09	0.265
Day 30	0.53	0.61	0.05	0.301
Day 45	0.39	0.52	0.04	0.140
Total milk yield	30.36	34.55	1.88	0.277
Average daily milk yield	0.78	0.90	0.03	0.065

[1] "1 d" refers to "1 day of lactation"; "7 d" to "7 days of lactation"; "15 d" to "15 days of lactation"; "30 d" to "30 days of lactation"; and "45 d" to "45 days of lactation". The same as below

Serum immune and antioxidant function of Ewes

The effects of diets supplemented with ATC during pregnancy and lactation on blood biochemical indices, immunity, and antioxidation in ewes are shown in Fig. 2. The results indicated that, compared to that in the CON group, supplementation of ATC significantly increased the concentration of serum urea at 120 d of pregnancy

Table 4 Effects of dietary supplementation with ATC on the milk composition of pregnant and lactating Ewes (%)

Items	Time	Treatments		SEM	*p*-value
		CON	ATC		
Fat	1 d	12.21	13.72	0.46	0.102
	7 d	8.05[b]	8.97[a]	0.24	0.031
	30 d	5.63	7.22	0.50	0.111
	45 d	7.46	7.62	0.42	0.867
Protein	1 d	16.44	15.84	0.43	0.548
	7 d	5.44	5.37	0.28	0.912
	30 d	4.48	4.67	0.10	0.394
	45 d	5.40	5.53	0.18	0.759
Lactose	1 d	3.41	3.28	0.08	0.453
	7 d	5.12	5.10	0.10	0.944
	30 d	5.48	5.44	0.06	0.744
	45 d	4.94	5.38	0.24	0.421
Total solids	1 d	43.32[b]	44.58[a]	0.32	0.023
	7 d	25.20	25.47	0.67	0.867
	30 d	19.98	22.36	0.74	0.111
	45 d	23.14	24.04	0.47	0.397
Urea	1 d	45.70[b]	51.20[a]	1.46	0.036
	7 d	22.27	21.53	0.89	0.728
	30 d	13.70[b]	16.07[a]	0.53	0.001
	45 d	15.07	17.03	1.27	0.501

Table 5 Effects of dietary supplementation with ATC on Immunoglobulins in the milk of pregnant and lactating Ewes

Items[1]	Time	Treatments		SEM	*p*-value
		CON	ATC		
IgA(mg/mL)	1 d	17.87	17.31	0.92	0.796
	7 d	17.22	15.94	0.43	0.147
	30 d	12.23	11.30	0.74	0.592
	45 d	12.03	12.42	0.94	0.861
IgG(mg/mL)	1 d	38.35	34.09	1.63	0.222
	7 d	28.18	30.51	1.98	0.612
	30 d	23.95	28.82	3.72	0.573
	45 d	21.15	25.22	2.47	0.473
IgM(mg/mL)	1 d	7.55	7.41	0.71	0.939
	7 d	6.70	6.57	0.75	0.942
	30 d	6.36	6.60	0.20	0.618
	45 d	4.89	5.66	0.45	0.454

[1] IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M;

Table 6 Effects of dietary ATC supplementation during pregnancy and lactation on blood biochemical indices of Ewes

Items[1]	Time	Treatments		SEM	*p*-value
		CON	ATC		
ALP (U/L)	90 d	162.85	105.87	16.85	0.090
	120 d	179.73	180.57	23.92	0.987
	145 d	156.02	177.43	30.05	0.740
	1 d	121.25	118.00	13.60	0.919
	30 d	144.27	110.45	86.63	0.525
	45 d	141.95	100.08	63.03	0.269
GLB (g/L)	90 d	43.10	45.70	1.34	0.355
	120 d	40.23	41.17	0.97	0.651
	145 d	40.65	41.93	0.90	0.503
	1 d	42.30	43.07	0.70	0.610
	30 d	46.23	47.23	0.70	0.718
	45 d	47.30	51.15	1.31	0.148
TCHO (mmol/L)	90 d	2.03	1.80	0.09	0.198
	120 d	1.95	1.90	0.09	0.781
	145 d	2.00	1.97	0.12	0.918
	1 d	1.40	1.73	0.10	0.096
	30 d	1.71	1.57	0.09	0.487
	45 d	1.82	1.73	0.06	0.493
TG (mmol/L)	90 d	0.75	0.43	0.09	0.089
	120 d	0.87	0.62	0.08	0.108
	145 d	0.78	0.57	0.06	0.091
	1 d	0.28	0.27	0.02	0.721
	30 d	0.32	0.33	0.01	0.549
	45 d	0.35	0.33	0.03	0.787
TP (g/L)	90 d	71.98	74.42	1.63	0.481
	120 d	68.42	70.18	1.24	0.503
	145 d	69.95	70.57	1.02	0.779
	1 d	67.28	69.47	0.85	0.214
	30 d	70.78	71.38	0.75	0.710
	45 d	71.52	73.40	1.23	0.472
Urea (mmol/L)	90 d	4.21	4.68	0.28	0.430
	120 d	2.99[b]	3.89[a]	0.20	0.016
	145 d	2.92	3.27	0.17	0.332
	1 d	4.96	4.94	0.32	0.969
	30 d	1.31	1.53	0.10	0.276
	45 d	1.37	1.75	0.18	0.316

[1] ALP, alkaline phosphatase; GLB, globulin; TCHO, total cholesterol; TG, triglyceride; TP, total protein

[2]CON = Control group; ATC = *Acremonium terricola* culture group. The data are expressed as the mean and SEM ($n = 6$). In the same row, values with different small letters are significantly different ($P < 0.05$). The same as Tables 7, 8, 9, 11, 12 and 13

($p < 0.05$). and there was a significant decrease in the serum ALP, TG, and 145d serum TG on the levels at 90 d of pregnancy ($p = 0.090$, $p = 0.089$, $p = 0.091$). Conversely, the serum TCHO concentration on the first day of lactation tended to increase ($p = 0.096$).

The effect of ATC on the immune function of ewes during pregnancy and lactation is shown in Fig. 3. The results showed that the supplementation of ATC significantly increased the serum IgA level of ewes at 90 d, 145 d, 1 d, and 30 d of pregnancy and significantly decreased the serum IL-6 level at 120 d and 145 d of pregnancy ($p < 0.05$). Moreover, the serum IgA concentration tended to increase at 120 d of pregnancy, and the serum IgM and IL-1β concentrations tended to increase at 30 d of lactation ($p = 0.072$, $p = 0.083$, $p = 0.062$), but there was no significant effect on the serum indices, such as IL-4 and TNF-α. ($p > 0.05$). (Fig. 4)

Table 8 shows that the supplementation of ATC significantly increased the serum SOD level of ewes at 145 days of gestation ($p < 0.05$). There was also a trend toward an increase in serum CAT at 30 d of lactation ($p = 0.060$),

Table 7 Effects of dietary supplementation with ATC during pregnancy and lactation on the immune function of Ewes

Items[1]	Time	Treatments		SEM	*p*-value
		CON	ATC		
IgA(μg/mL)	90 d	27.66[b]	31.09[a]	0.90	0.050
	120 d	24.39	28.84	1.25	0.072
	145 d	21.34[b]	28.95[a]	1.48	0.003
	1 d	17.02[b]	22.00[a]	1.09	0.013
	30 d	23.25[b]	26.88[a]	0.81	0.017
	45 d	29.50	30.14	0.98	0.758
IgG(μg/mL)	90 d	193.55	218.29	12.86	0.353
	120 d	169.12	179.69	18.41	0.789
	145 d	155.23	188.56	17.41	0.363
	1 d	84.42	93.55	10.96	0.697
	30 d	114.96	140.49	8.31	0.130
	45 d	148.10	159.77	13.26	0.681
IgM(μg/mL)	90 d	26.68	25.27	1.45	0.649
	120 d	22.91	29.19	2.05	0.130
	145 d	23.08	25.31	1.46	0.473
	1 d	16.33	14.98	1.16	0.583
	30 d	19.14	22.41	0.95	0.083
	45 d	22.41	23.59	1.62	0.735
IL-1β(pg/mL)	90 d	245.69	233.46	18.39	0.756
	120 d	328.45	308.83	15.72	0.558
	145 d	373.31	360.55	27.77	0.831
	1 d	409.93	399.89	17.85	0.793
	30 d	343.58	414.88	19.45	0.062
	45 d	294.51	290.95	19.50	0.932
IL-4(pg/mL)	90 d	259.54	283.74	9.55	0.221
	120 d	227.11	247.72	10.95	0.372
	145 d	209.77	210.03	8.32	0.988
	1 d	176.71	190.84	13.25	0.618
	30 d	227.81	210.67	11.56	0.485
	45 d	254.42	271.21	12.04	0.512
IL-6(pg/mL)	90 d	45.01	43.88	2.20	0.811
	120 d	55.92[a]	46.03[b]	2.34	0.026
	145 d	58.04[a]	52.61[b]	1.29	0.027
	1 d	63.72	58.72	1.90	0.202
	30 d	55.80	54.96	2.20	0.860
	45 d	48.81	44.39	2.08	0.310
TNF-α(pg/mL)	90 d	28.73	26.54	0.84	0.203
	120 d	30.90	29.82	0.79	0.520
	145 d	34.15	31.87	0.98	0.265
	1 d	35.06	33.19	0.66	0.167
	30 d	30.10	30.95	0.68	0.555
	45 d	29.13	26.72	0.83	0.154

[1] IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; IL-1β, interleukin-1 beta; IL-4, interleukin-4; IL-6, interleukin-6; TNF-α, tumor necrosis factor-alpha

and there was no significant effect on serum indices such as CAT and GSH-Px ($p > 0.05$).

Lamb growth performance

According to Table 9, the supplementation of ATC can significantly increase the average daily gain (ADG) of lambs during the nursing period of 1–45 days ($p < 0.05$), and there is a trend toward increasing the weight at 45 days of nursing ($p = 0.061$) (Tables 10, 11 and 12 and 13).

Serum immune and antioxidant properties of lambs

The effects of ATC supplementation to ewes during pregnancy and lactation on the blood biochemical indices of lambs are shown in Fig. 5. The results showed that the supplementation of ATC significantly increased the serum urea level of lambs at 30 d of lactation ($p < 0.05$). Compared with those in the CON group, the supplementation of ATC tended to increase the serum GLB and TP concentrations at 30 d of lactation ($p = 0.057$, $p = 0.082$), and the serum TCHO concentration on the same day tended to decrease ($p = 0.074$). However, it had no significant effect on other serum indices, although there was some improvement ($p > 0.05$).

As Fig. 6 indicates, the supplementation of ATC to the diets of pregnant and lactating ewes significantly enhanced serum IgA levels on days 1, 30, and 45 of lactation, as well as IL-4 levels on day 45, while it significantly reduced the serum TNF-α content on day 45 of lactation ($p < 0.05$). The serum IgG level of lactating lambs on day 45 tended to increase ($p = 0.072$) but had no significant effect on other serum indices ($p > 0.05$).

The effect of ATC supplementation to ewes during pregnancy and lactation on the antioxidant performance of lambs is shown in Fig. 7. The results showed that the supplementation of ATC significantly increased the serum CAT concentration at 1 d, 30 d, and 45 d, the serum SOD concentration at 45 d, and the serum T-AOC at 1 d ($p > 0.05$). Additionally, ATC tended to reduce the serum MDA content at 45 days and increase the serum SOD content at 30 days of lactation ($p = 0.086$, $p = 0.074$). Although there were no significant effects on other serum indicators, their values improved ($p > 0.05$).

Discussion

Feed intake is crucial for the normal development of the fetus during late pregnancy and for milk secretion during lactation. At present, the research of ATC is mainly focused on fattening sheep, and there are limited reports on its application in ewes. Studies in poultry [12, 20] and calves [8, 21] have shown that the supplementation of ATC can increase feed intake, reduce feed-to-weight ratio, improve meat quality, and improve animal performance. The results of this study showed that dietary supplementation with ATC significantly increased the feed intake of ewes during 90–130 days of gestation and 16–45 days of lactation, which was consistent with the results of previous studies. This positive effect on feed intake may be attributed to the inherent sweetness of ATC, which could enhance the palatability and flavor of the feed, but the specific mechanism of increasing feed intake still

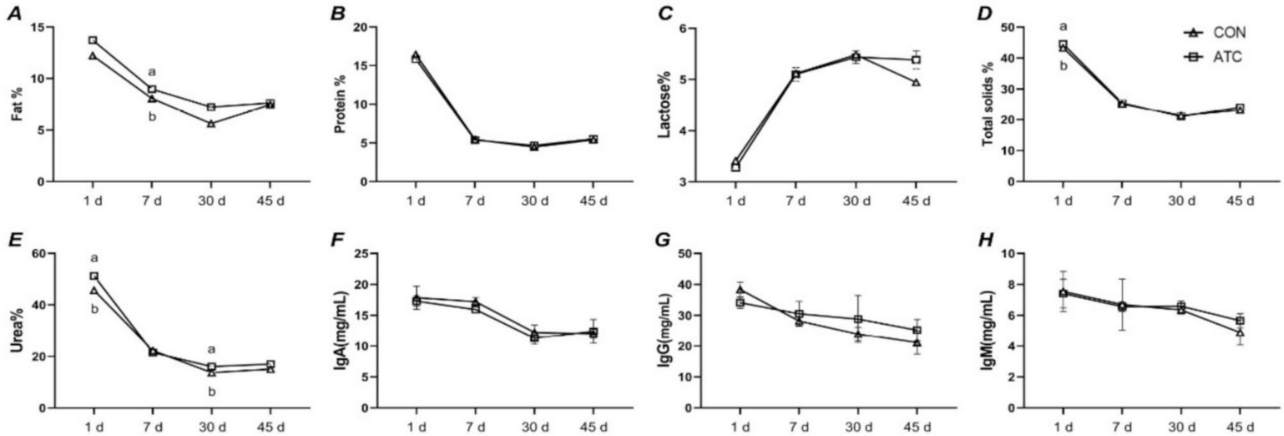

Fig. 1 Effects of ATC supplementation in diet of pregnant ewes on milk composition and immunoglobulin during lactation. IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M

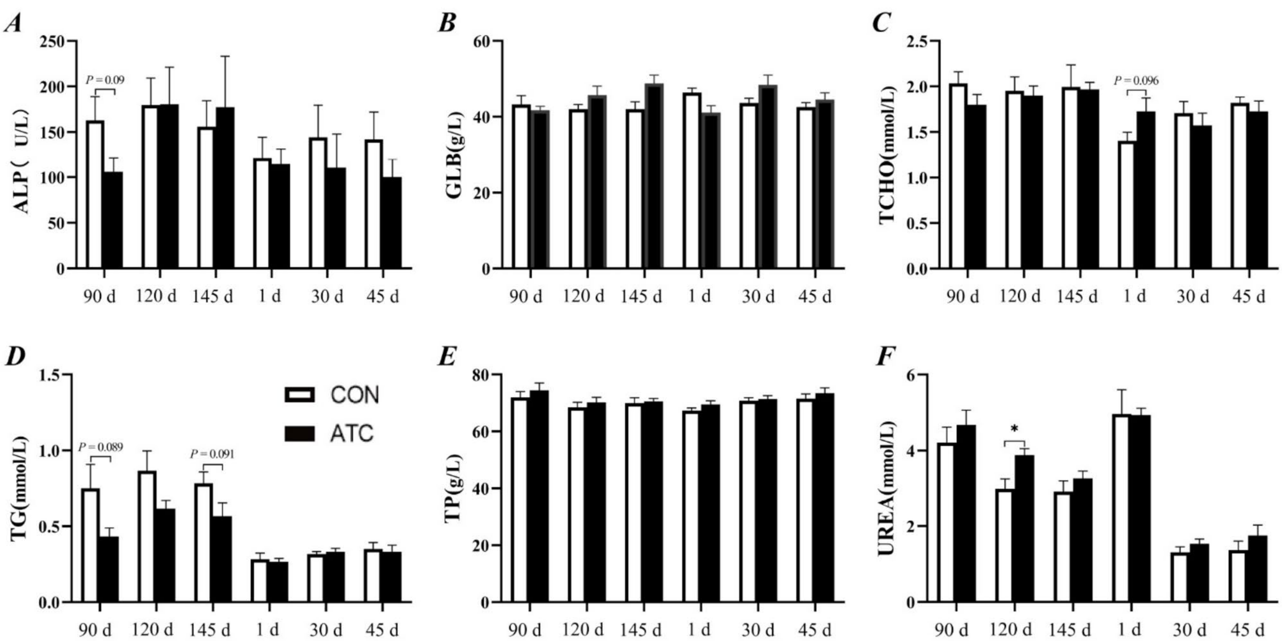

Fig. 2 Effects of dietary ATC supplementation during pregnancy and lactation on blood biochemical indices of ewes. [1] ALP, alkaline phosphatase; GLB, globulin; TCHO, total cholesterol; TG, triglyceride; TP, total protein. [2]CON = Control group; ATC = *Acremonium terricola* culture group. The data are expressed as the mean and SEM ($n = 6$). In the same row, values with different small letters are significantly different ($P < 0.05$). The same as Tables 7, 8, 9 and 10–12

needs further study. The change in maternal body weight is an important index reflecting the nutritional and metabolic balance of the body. Excessive postpartum weight loss can prolong the estrus interval and reduce production performance. The results of this study showed that the supplementation of ATC significantly increased the body weight of ewes at 145 d of gestation but had no significant effect on body weight at other time points. The weight gain of ewes may be related to the increase in propionic acid concentration in the rumen caused by ATC, which could enhance gluconeogenesis and blood glucose concentration and availability [9]. This, in turn, reduces the need for body fat mobilization. However, the sample size of this study is relatively small, which may limit the universality of the results. A larger sample size will provide greater statistical power and improve the robustness of the findings, making the results more universally applicable and persuasive. Future studies should consider a larger sample size to verify these findings and further explore the mechanism of the effect of ATC on feed intake and body weight of ewes.

The lactation performance of ewes plays a crucial role in the growth and development of lactating lambs. The results of this experiment showed that the changing trend in the milk yield of ewes in each group was similar, with a downward trend observed after reaching the

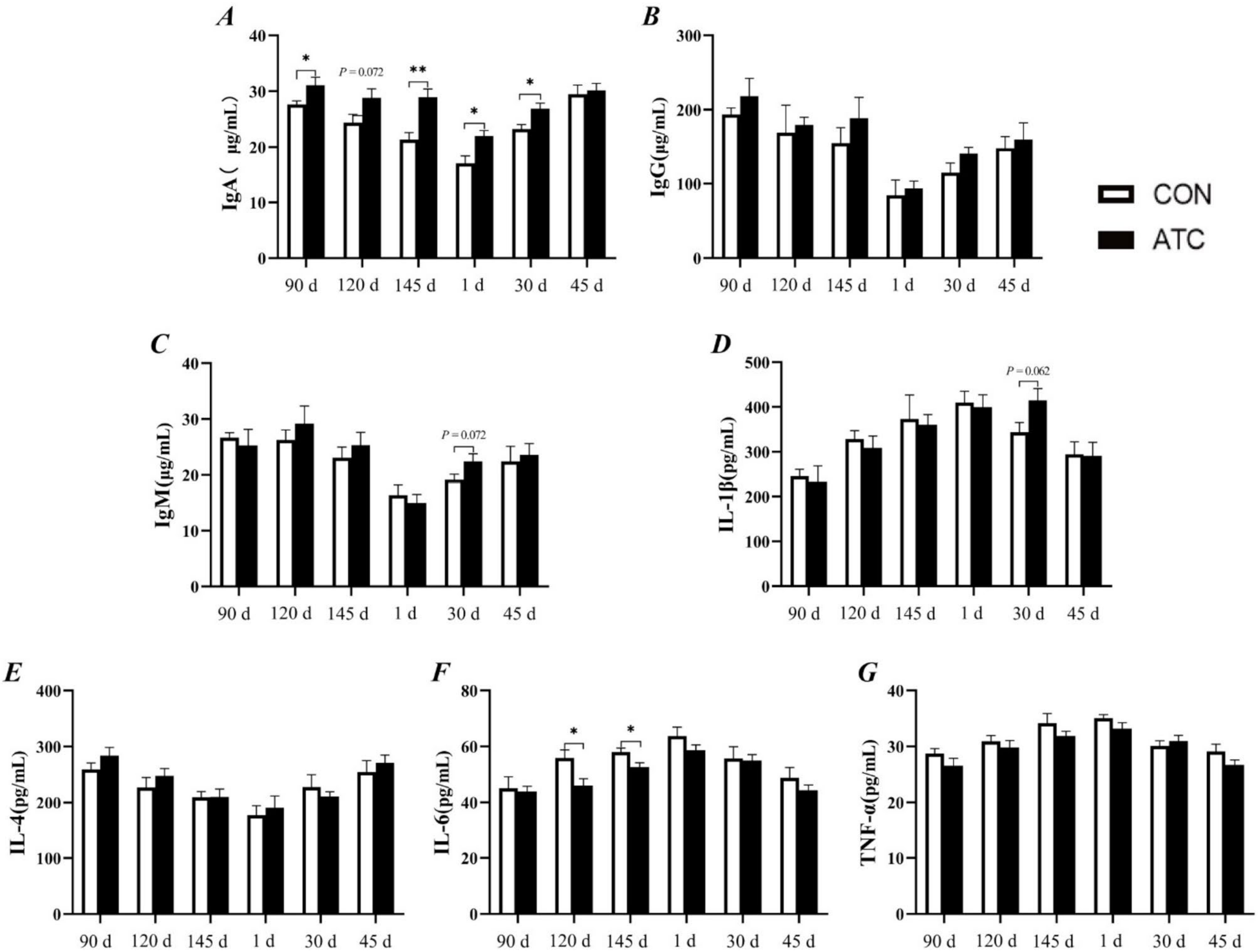

Fig. 3 Effects of dietary supplementation with ATC during pregnancy and lactation on the immune function of ewes. [1] IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; IL-1β, interleukin-1 beta; IL-4, interleukin-4; IL-6, interleukin-6; TNF-α, tumor necrosis factor-alpha

peak of lactation at 15 days postpartum. However, the milk yield of the ATC group remained greater than that of the CON group after 15 d postpartum, indicating that the supplementation of ATC could delay the decrease in milk yield. This finding aligns with the results of Wang et al. [9] Incorporating ATC into the diet of dairy cows can increase milk production, with outcomes consistently surpassing those of the control group throughout the trial period. This increase in milk yield may be attributed to the ability of ATC to improve the immune function and antioxidant capacity of dairy cows, reduce the number of somatic cells, and thereby enhance milk production [9].

Maternal colostrum/milk is the primary source of nutrition and antibodies for newborn animals, directly affecting their survival, growth, and development, and it also plays a certain role in their future productive performance. At present, the research on the effect of ATC on milk composition is mainly concentrated in dairy cattle production, and the research on goat milk composition has not been reported, and the research results are not the same due to differences in animal age and breed. For instance, it has been reported that supplementing the diet of lactating Holstein cows with ATC can significantly increase the fat and lactose content of milk without substantially affecting the protein content [14]. Conversely, other findings suggest that ATC supplementation does not significantly alter the levels of milk fat, protein, lactose, total solids, or milk urea nitrogen [8, 9]. The present study revealed that the supplementation of ATC significantly increased the Ts and urea contents in lactating milk at 1 day postpartum and the fat and urea contents at 7- and 30-days during lactation. The change in milk composition may be because ATC increased the concentration of total volatile fatty acids and ammonia nitrogen (NH_3-N) in the rumen and improved the milk composition by increasing the content of acetic acid and propionic acid, the precursors of synthetic milk fat, and improving the utilization efficiency of nitrogen [14].

The sensory structure, density, color, and chemical composition of colostrum are distinct from those of

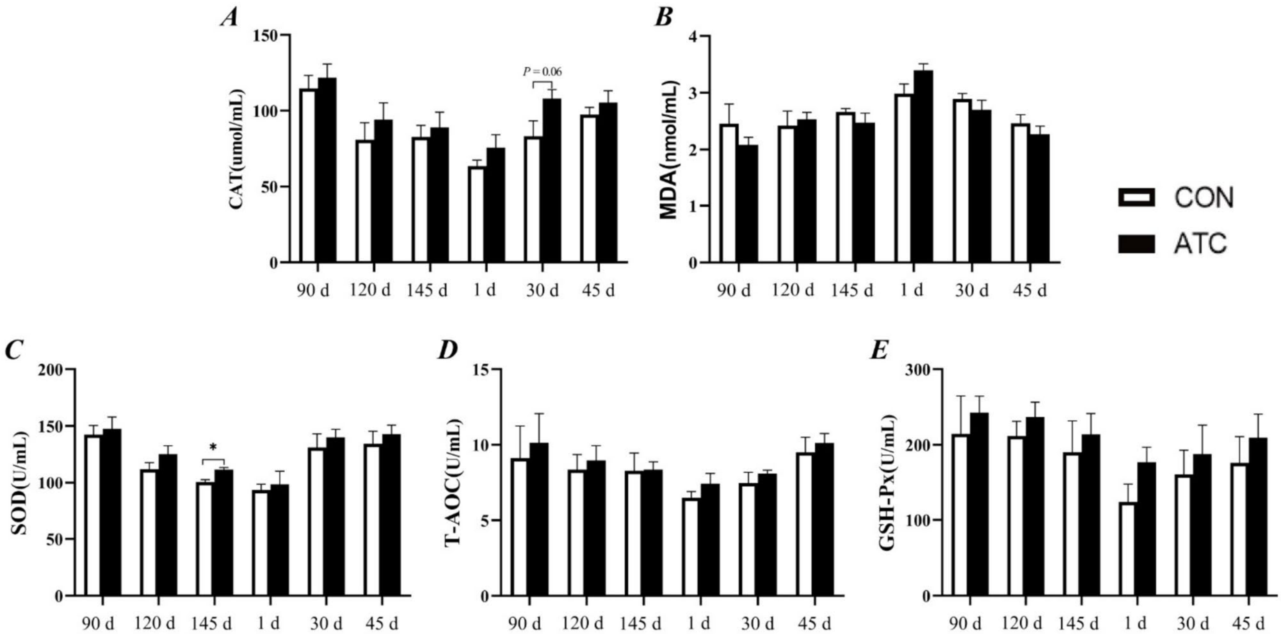

Fig. 4 Effects of dietary supplementation with ATC during pregnancy and lactation on the antioxidant performance of ewes. [1] T-AOC: total antioxidant capacity; GSH-Px: glutathione peroxidase; CAT: catalase; SOD: superoxide dismutase; MDA: malondialdehyde; The same as below

regular milk. It represents the first secretion from the mammary glands immediately following parturition. During colostrum formation, IgG is transferred from the bloodstream to the mammary glands through FcRn receptors [14]. In addition to supplying energy and nutrients, the paramount function of colostrum is to stimulate the immune system, conferring both specific and non-specific immune protection to neonates [23]. The level of IgG in colostrum varies between breeds and ranges from 6.2 to 65.4 mg/mL depending on the ovine breed [24], in line with the highest level of 38.35 mg/mL observed in the ewes monitored in our study. The placental structure of a ruminant does not allow any transfer of immunoglobulin (Ig) from a dam vascular system to the fetus, thus depriving the newborn ruminant of antibodies at birth. Furthermore, colostrum ingestion is of paramount importance for neonates because it promotes immunoglobulin Ig transfer from the dam to the newborn (known as immune passive transfer, IPT) and provides protection against infections [25]. Some studies have shown that serum IgG < 10 mg/mL in newborn lambs after they eat colostrum may be considered an indication of the failure of passive transfer of colostrum immunoglobulin [26]. In our study, the serum IgG level of the lambs was greater than the standard, indicating that the passive immunization of the lambs was successful. Our results showed a drastic decrease in colostral IgG concentration over time, an observation also observed by others [27, 28]. There have been a lot of studies on the effect of ATC on milk composition, but the effect of ATC on milk immunoglobulin has not been reported. In this experiment, the effect of ATC on immunoglobulin in milk was investigated by supplementing ATC to the diet of ewes. The results showed that supplementation of ATC had no significant effect on the content of immunoglobulins in ewes' milk, though a trend toward increased levels was observed.

Blood serves as the carrier for metabolic processes, and serum biochemical indices can be used to reflect metabolic activity and nutrient health status. The concentration of serum protein is positively correlated with the level of protein metabolism [29]. Previous research has shown that the supplementation of ATC did not significantly affect the serum TP, ALB, or GLB levels in weaning calves after 40 d [21]. In this study, there were no significant differences in serum TP, ALB, or GLB between the control group and the ATC group. This indicates that ATC supplementation did not have a negative impact on material metabolism in ewes, which is consistent with the findings of previous studies. The liver is the body's principal detoxification organ and, to some extent, reflects the efficiency of nutritional and metabolic conversion. ALP is an important indicator for assessing liver function [21]. In this study, the inclusion of ATC in the diet resulted in a decreased concentration of serum ALP in ewes during a 90-day pregnancy period. This finding suggested that ATC may have a protective effect on the liver and could enhance metabolic efficiency. In serum, urea is a waste product generated from protein metabolism. Studies have shown that high-fat and ketogenic dairy cows have lower serum urea concentrations in early lactation than healthy cows, which may be due to reduced dry matter intake related to liver metabolism

Table 8 Effects of dietary supplementation with ATC during pregnancy and lactation on the antioxidant performance of Ewes

Items[1]	Time	Treatments		SEM	*p*-value
		CON	ATC		
CAT(umol/mL)	90 d	114.68	121.78	6.02	0.580
	120 d	80.84	94.21	7.73	0.413
	145 d	82.69	89.05	6.09	0.625
	1 d	63.50	75.69	4.80	0.219
	30 d	83.17	108.08	6.74	0.060
	45 d	97.61	105.43	4.46	0.406
GSH-Px(U/mL)	90 d	214.69	242.59	26.31	0.620
	120 d	211.56	236.97	13.64	0.377
	145 d	190.35	213.88	23.92	0.646
	1 d	123.91	177.04	16.96	0.121
	30 d	160.76	187.99	24.10	0.597
	45 d	176.04	209.43	22.88	0.492
MDA(nmol/mL)	90 d	2.46	2.08	0.19	0.339
	120 d	2.42	2.53	0.14	0.698
	145 d	2.66	2.47	0.09	0.315
	1 d	3.65	3.39	0.39	0.756
	30 d	2.89	2.70	0.10	0.344
	45 d	2.46	2.27	0.10	0.377
SOD(U/mL)	90 d	142.35	147.51	6.34	0.703
	120 d	111.99	125.22	4.85	0.184
	145 d	100.75[b]	111.66[a]	2.08	0.002
	1 d	93.24	98.59	6.22	0.688
	30 d	131.15	140.12	6.70	0.529
	45 d	134.42	142.97	6.53	0.539
T-AOC(U/mL)	90 d	9.12	10.13	1.37	0.732
	120 d	8.36	8.97	0.67	0.672
	145 d	8.28	8.36	0.61	0.951
	1 d	6.50	7.43	0.40	0.259
	30 d	7.47	8.10	0.36	0.410
	45 d	9.51	10.12	0.56	0.607

[1] T-AOC: total antioxidant capacity; GSH-Px: glutathione peroxidase; CAT: catalase; SOD: superoxide dismutase; MDA: malondialdehyde; The same as below

Table 9 Effects of ATC supplementation in Ewe diet on growth performance of lactating lambs

Items[1]	Treatments		SEM	*p*-value
	CON	ATC		
Total number of lambing/head	18	18		
Birth weight(kg)	2.89	2.92	0.13	0.920
30 d BW(kg)	6.75	7.95	0.38	0.118
45 d BW(kg)	9.01	10.88	0.50	0.061
1–45 d ADG (g/d)	135.96	177.00	9.84	0.035

[1]"30 d" to "30 days of lactation" and "45 d" to "45 days of lactation". ADG, average daily gain;

[2]CON = Control group; ATC = *Acremonium terricola* culture group. The data are expressed as the mean and SEM (n = 18). In the same row, values with different small letters are significantly different (P < 0.05)

Table 10 Effects of ATC supplementation in the diet of Ewes on the immune function of lactating lambs

Items	Time	Treatments		SEM	*p*-value
		CON	ATC		
IgA(μg/mL)	1 d	26.61[b]	32.79[a]	1.01	0.001
	30 d	28.76[b]	34.61[a]	0.94	0.001
	45 d	30.14[b]	36.50[a]	0.99	0.001
IgG(μg/mL)	1 d	41.29	62.38	7.73	0.165
	30 d	79.14	87.24	5.89	0.518
	45 d	99.26	119.14	5.59	0.072
IgM(μg/mL)	1 d	12.22	14.66	0.79	0.127
	30 d	16.04	16.99	0.56	0.420
	45 d	18.12	19.74	0.50	0.109
IL-1β(pg/mL)	1 d	263.36	298.77	16.41	0.302
	30 d	233.53	224.90	21.48	0.852
	45 d	190.75	199.57	18.92	0.828
IL-4(pg/mL)	1 d	270.35	308.98	12.97	0.143
	30 d	315.77	346.98	11.76	0.197
	45 d	318.78[b]	369.04[a]	12.85	0.044
IL-6(pg/mL)	1 d	42.94	37.96	1.63	0.132
	30 d	37.68	34.27	1.59	0.304
	45 d	32.78	27.99	1.82	0.202
TNF-α(pg/mL)	1 d	26.89	25.98	0.71	0.550
	30 d	25.23	24.44	0.72	0.607
	45 d	27.96[a]	22.53[b]	1.06	0.003

Table 11 Effects of ATC supplementation in the diet of Ewes on the antioxidant function of lambs

Items	Time	Treatments		SEM	*p*-value
		CON	ATC		
CAT(μmol/mL)	1 d	42.78[b]	52.28[a]	1.98	0.008
	30 d	51.87[b]	68.28[a]	2.81	0.023
	45 d	64.97[b]	78.02[a]	3.98	0.017
GSH-Px(U/mL)	1 d	109.43	152.99	20.41	0.308
	30 d	147.45	173.74	31.71	0.699
	45 d	166.37	230.39	19.66	0.105
MDA(nmol/mL)	1 d	1.93	1.54	0.19	0.333
	30 d	1.31	1.00	0.19	0.442
	45 d	1.09	0.95	0.04	0.086
SOD(U/mL)	1 d	63.93	69.00	8.81	0.788
	30 d	69.00	79.54	2.98	0.074
	45 d	80.19[b]	93.89[a]	3.34	0.032
T-AOC(U/mL)	1 d	3.69[b]	6.08[a]	0.52	0.012
	30 d	6.51	7.00	0.70	0.740
	45 d	6.34	7.66	1.05	0.553

[31]. In this study, the inclusion of ATC in the diet significantly increased the serum urea concentration in ewes after 120 d. This increase may be related to the increase of feed intake of ewes at 120 days of pregnancy. After ewes ingest too much high-protein feed, the protein metabolism in the body is enhanced, and the ammonia produced by the decomposition of excess protein is converted into urea in the liver and enters the blood, resulting in an increase in serum urea concentration. Previous studies have shown that the supplementation of ATC to the diet of Holstein dairy cows during the dry period can significantly reduce the serum TG level, and there is a tendency to reduce the serum CHOL content [32]. The

Table 12 Basic diet composition and nutrition level (DM basis, %)

Ingredients	Content	Nutrientlevels[2]	Content
Corn straw silage	42.50	ME(MJ/kg)	8.31
Peanut vine	16.50	CP	12.16
Corn	25.00	EE	5.32
Soybean meal	9.00	NDF	48.57
Wheat bran	4.00	ADF	27.01
Premix[1]	1.00	Ca	0.75
$NaHCO_3$	0.75	P	0.34
NaCl	0.50		
$CaHPO_4$	0.75		
Total	100.00		

[1]The premix provided the following per kg of the diet: VA 300 KTU, VD3 60 KTU, VE 4000 mg, Cu (as copper sulfate) 250 mg, Fe (as ferrous sulfate) 1801 mg, Zn (as zinc sulfate) 1400 mg, Mn (as manganese sulfate) 1120 mg, Se (as sodium selenite) 10 mg, I (as potassium iodide) 15 mg, and Mg (as manganese sulfate) 8-200 mg

[2]ME was a calculated value, and the others were measured values

CP = crude protein; EE = ether extract; NDF = neutral detergent fiber; ADF = acid detergent fiber; Ca = calcium; P = phosphorus

Table 13 Effects of ATC supplementation in the diet of Ewes on blood biochemical indexes of lactating lambs

Items	Time[1]	Treatments		SEM	*p*-value
		CON	ATC		
ALP(U/L)	1 d	918.63	761.17	83.49	0.370
	30 d	546.45	411.47	58.26	0.266
	45 d	435.57	325.78	42.40	0.210
GLB(g/L)	1 d	39.87	42.15	3.45	0.758
	30 d	31.83	37.87	1.62	0.057
	45 d	33.53	34.00	1.21	0.857
TCHO(mmol/L)	1 d	2.38	1.83	0.17	0.105
	30 d	4.21	3.18	0.29	0.074
	45 d	2.18	2.72	0.21	0.204
TG(mmol/L)	1 d	1.18	1.45	0.22	0.570
	30 d	0.77	0.93	0.14	0.588
	45 d	1.05	1.05	0.19	1.000
TP(g/L)	1 d	62.47	64.38	3.33	0.789
	30 d	57.67	63.10	1.57	0.082
	45 d	58.53	59.52	1.16	0.693
Urea(mmol/L)	1 d	4.88	5.74	0.71	0.573
	30 d	2.98^b	4.94^a	0.41	0.007
	45 d	3.72	3.90	0.22	0.705

[1]"1 d" refers to "1 day of lactation", "30 d" to "30 days of lactation" and "45 d" to "45 days of lactation". The same as below

concentration of serum CHOL serves as an indicator of lipid metabolism in the body, while TG plays a direct role in the synthesis of CHOL and is a major component of blood lipids. In this study, the group receiving ATC exhibited a significant decrease in serum TG content and a tendency toward a reduction in serum CHOL content. These findings are consistent with those of previous research and may be related to the capacity of Cordyceps extract to prevent excess weight gain, fat accumulation, and liver enlargement, as well as to reduce triglyceride levels in rats [33]. However, this study only focused on the lipid metabolism and protein metabolism of ewes during pregnancy and lactation. The research period was relatively short, and the effects of ATC on the long-term health, production performance and fecundity of ewes were not fully evaluated, which was the deficiency of this study. In future studies, we will extend the research cycle and conduct long-term follow-up monitoring of ewes to assess the impact of ATC on their health and production performance throughout their life cycle. In addition, we will further explore the long-term mechanism of ATC on the fertility of ewes in combination with reproductive performance indicators, such as estrus cycle, conception rate and lambing rate, so as to provide a more scientific basis for the health management of ewes.

Immunoglobulins are essential proteins that bind to foreign substances such as bacteria and viruses, aiding in their elimination from the body, activating complement systems, and neutralizing toxins [34]. Current findings indicate that the inclusion of ATC in the diet can increase the concentrations of serum IgG and IgM in weaned calves and lactation cows [8, 14, 21], which is consistent with our results. It has been reported that maternal immune function is weakened during the perinatal period, mainly affecting cellular and humoral immune responses, and plasma IL-6 levels fluctuate significantly [35]. IL-6 is a proinflammatory factors that can accelerate inflammatory responses. In this study, the inclusion of ATC significantly decreased the serum IL-6 levels on days 120 and 145 of pregnancy, which is in agreement with the observations of Li et al. [14] in lactating dairy cows. The effect of ATC on reducing pro-inflammatory cytokines may be attributed to cordycepin (one of the active components of ATC) activating Nrf2 / Keap1 signaling pathway, up-regulating HO-1 expression, and enhancing the activity of autophagy-related genes (such as LC3, ATG5, ATG12, etc.) [36]. This finding suggests that ATC may play an immunoregulatory role by removing damaged mitochondria and oxides and protecting cells from oxidative damage.

The body naturally produces free radicals during metabolic and physiological processes. However, when the production of free radicals exceeds the body's capacity to counteract them, these radicals can cause tissue oxidative stress and potentially induce disease. The key indicators of oxidative stress in animals include SOD, GSH-Px, T-AOC, CAT, and MDA [37]. Under normal physiological conditions, SOD, GSH-Px, and CAT function to remove excess reactive oxygen species (ROS), maintaining a balance that reduces oxidative stress and prevents cellular damage [38]. Studies have found that oxidative stress can have a serious impact on maternal fertility. Oxidative stress can damage ovarian function (follicular development, maturation and ovulation), destroy

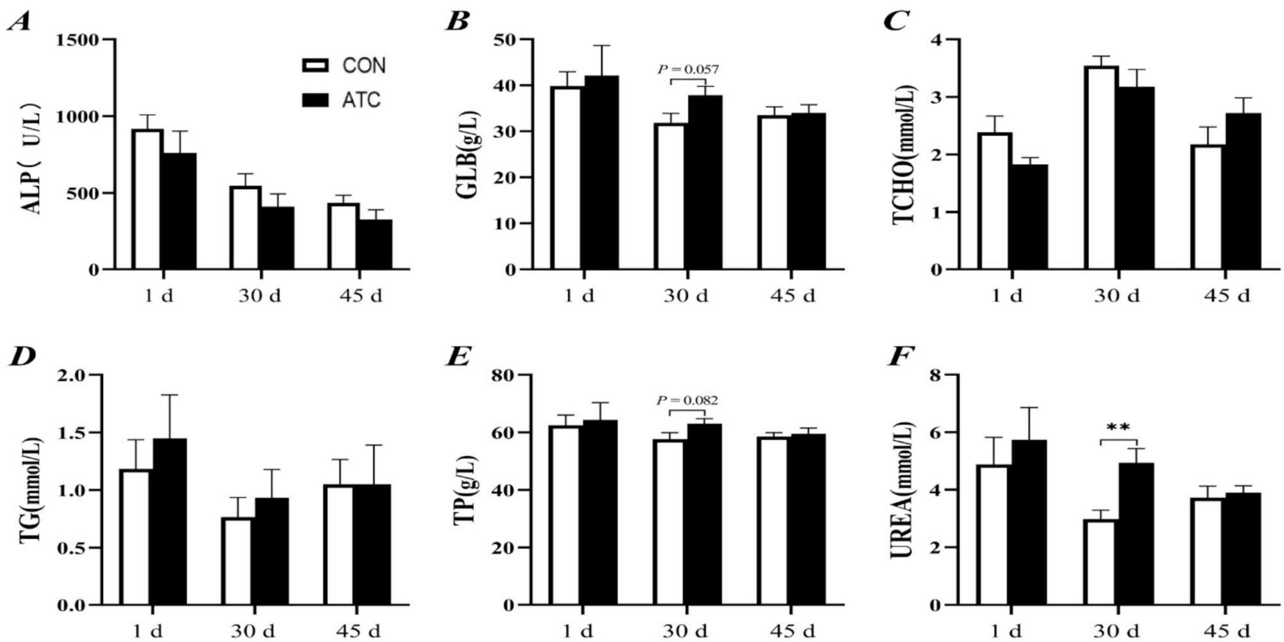

Fig. 5 Effects of ATC supplementation in the diet of ewes on blood biochemical indexes of lactating lambs. [1]"1 d" refers to "1 day of lactation", "30 d" to "30 days of lactation" and "45 d" to "45 days of lactation". The same as below

the morphological structure of uterine tissue, and cause diseases such as polycystic ovary syndrome, endometrial cancer and intrauterine growth restriction [39, 40]. At the same time, it leads to placental vascular dysfunction, reduced oxygen supply, abnormal differentiation of placental trophoblast, damage to mammary gland cells, aging and apoptosis, reduced placental blood flow, fetal tissue damage and mastitis [41, 42]. In this study, the supplementation of ATC was found to increase the activity of serum SOD and CAT, while the concentration of MDA in plasma was reduced. This is consistent with findings from other studies, where ATC supplementation increased SOD activity in weaned calves and serum, with a numerical decrease in serum MDA levels, without significantly affecting other markers [9, 21]. ATC has the potential to reduce serum lipid peroxidation and enhance antioxidant capacity, which may be attributed to its ability to inhibit mitogen-activated protein kinase signaling pathways, decrease the expression of proinflammatory factors, and increase the activity of antioxidant enzymes [42].

The nutritional and metabolic status of female animals during late pregnancy and lactation is closely related to their production performance and that of their offspring. As a brown powder-shaped solid fermentation feed additive of Ophiocordyceps sinensis, ATC can be directly mixed into the basic feed to achieve continuous and stable intake. It has extremely high application convenience and practicality, and possesses significant application value and broad promotion prospects in large-scale agricultural production. The results from studies on weaned calves and weaned piglets have shown that the direct supplementation of ATC to their diets can improve growth performance, antioxidant capacity, and immunity in these young animals [10, 21]. In this study, the supplementation of ATC to the diet of pregnant ewes had a tendency to increase the body weight and average daily gain of lambs at 45 days of age, which was consistent with the results of previous studies. This may be related to the significant increase in body weight of ewes in the ATC group at 145 days of pregnancy. At the same time, the ewes in the ATC group had greater dry matter intake and lactation, and had better milk sources, which may also be an important reason for the weight gain of lambs. Under the conditions of this experiment, supplementation of ATC in ewe diets exerted certain effects on the growth performance of lambs, suggesting that ATC may exert its effects through multiple mechanisms. However, the optimal supplementation strategy requires further investigation. In addition, the performance of lambs during lactation may also be affected by factors such as feed intake. In this study, due to the limited test conditions, the feed intake of lambs was not measured, and the test period was relatively short, so the feed efficiency and economic benefits could not be calculated. However, the effect of ATC on feed efficiency and economic benefits of lambs is worthy of attention, which will also be one of the key directions of our future research.

Research has shown that respiratory and digestive system diseases are the main causes of mortality in lambs before weaning [44, 45]. Therefore, providing proper maternal nutrition and antioxidants during the early stages of lactation is crucial. This practice could improve

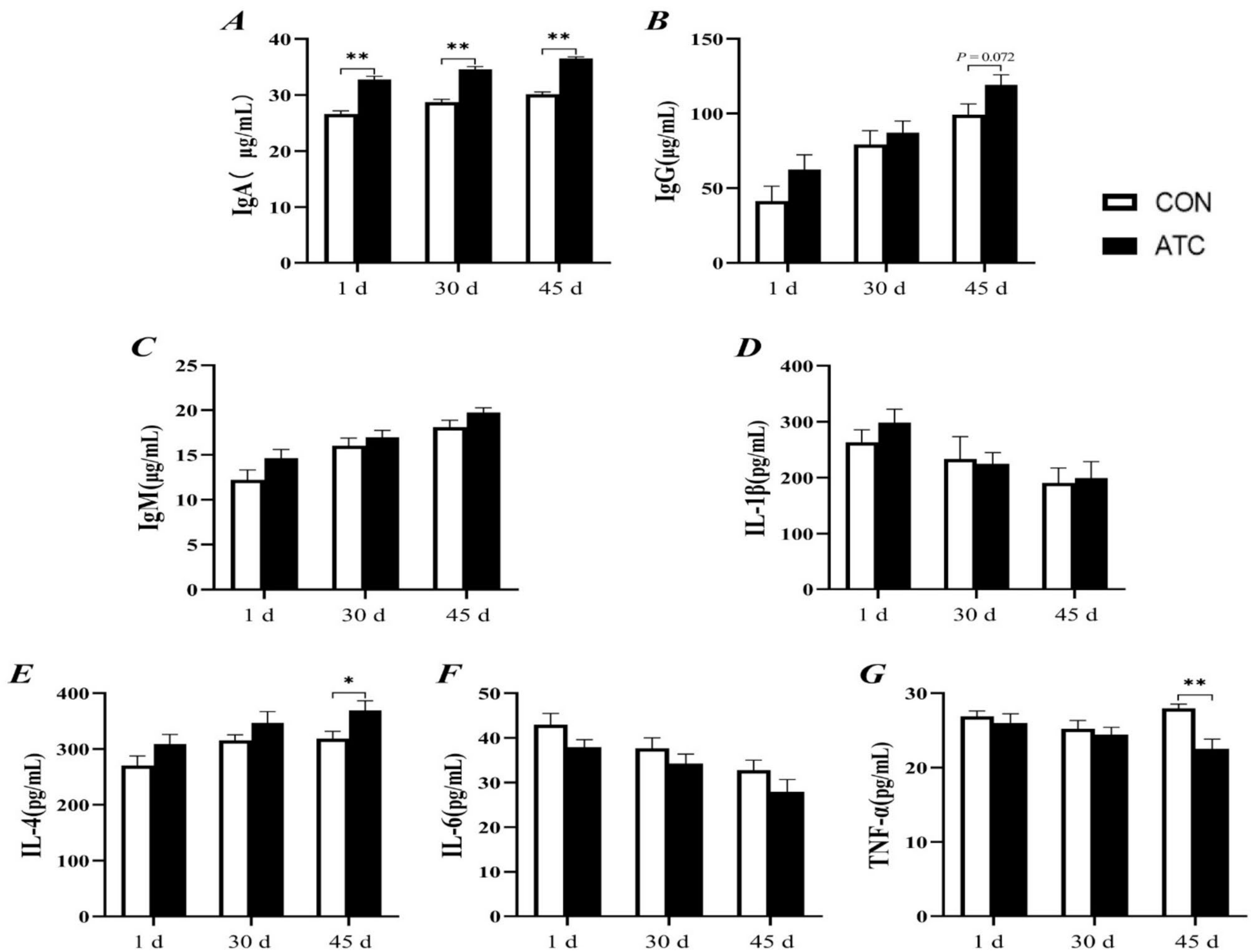

Fig. 6 Effects of ATC supplementation in the diet of ewes on the immune function of lactating lambs

the health status and milk production of ewes but also enhance the growth and health of their lambs [46–48]. Some of the antibodies and immunity acquired by the mother can be transferred to her offspring through specific pathways, thereby boosting the immunity of both the mother and her progeny. Numerous studies have explored the effects of maternal nutritional interventions during late pregnancy and lactation on offspring performance, yielding varied results due to the multitude of influencing factors. While some investigations have shown that improved maternal body condition can enhance the status of offspring [6, 49], others have observed no significant effect [50, 51]. In the present study, the dietary supplementation of ATC to pregnant and lactating ewes significantly increased the serum urea level in lambs at 30 days of lactation. Compared to the control group, ATC supplementation tended to increase the serum GLB and TG concentrations in lambs at 30 days of lactation. Since the serum urea level is positively correlated with nitrogen nutrient intake [52], this observation may be related to a greater intake of starter food in the ATC group. The increases in GLB and TG content suggest that ATC improved the body condition of ewes, potentially enhancing the milk protein supply from the mother to the lamb, thereby increasing the protein level of the lamb and promoting fat deposition.

Immunoglobulins play a crucial role in the immune system by activating the complement system, blocking enzymes to prevent pathogenic effects, initiating antimicrobial activity, neutralizing viruses, inhibiting microbial attachment, and suppressing bacterial metabolism [34]. IL-4 contributes to the immune response by binding to its receptor and mediating the phosphatidylinositol-3 kinase/protein kinase B/mammalian target rapamycin (PI3K/Akt/mTOR) signaling pathway, which helps to counteract oxidative stress [52]. TNF-α is mainly produced by activated macrophages, and binding to the corresponding receptor can activate the caspase cascade, which in turn induces oxidative stress and apoptosis [54]. The results of this study demonstrate that ATC supplementation can increase the serum levels of IgA and IgG in lactating lambs at 1, 30, and 45 days and increase the

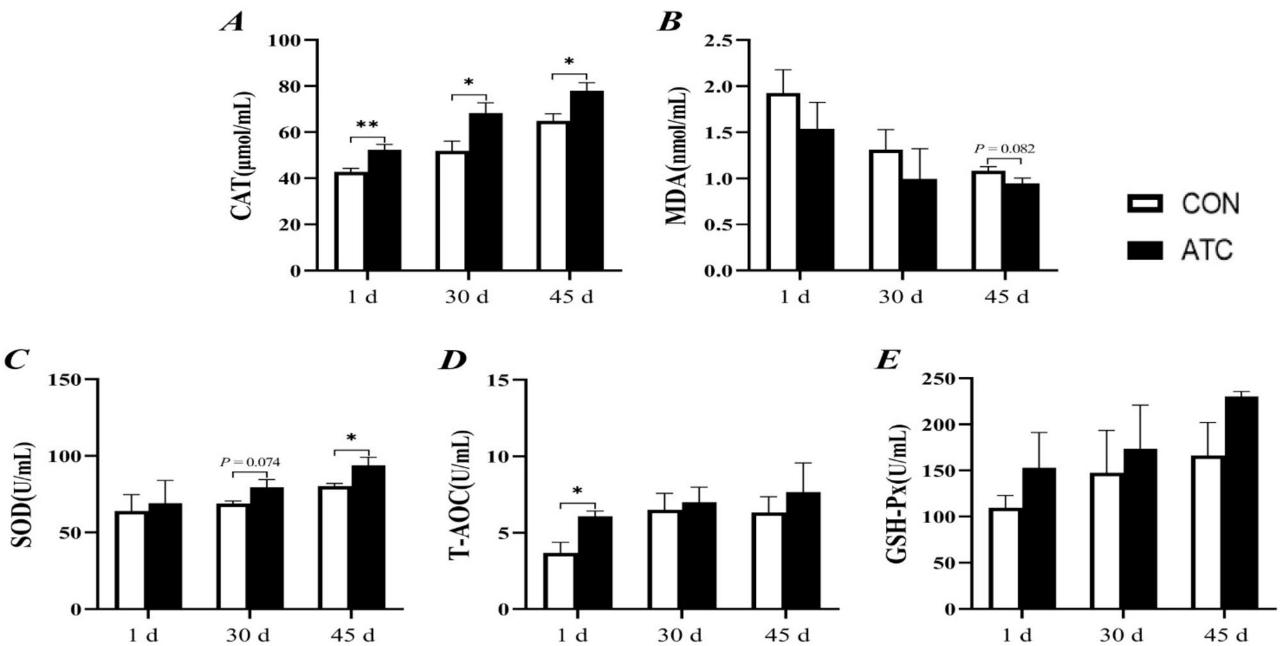

Fig. 7 Effects of ATC supplementation in the diet of ewes on the antioxidant function of lambs

serum IL-4 level at 45 days during lactation. Additionally, ATC significantly reduced the serum TNF-α concentration at 45 days. This finding is similar to the findings that ATC increases serum IgA and IgM levels and decreases serum IL-6 levels in ewes. This study shows that ATC can increase lamb immunoglobulin levels through ewe milk delivery and reduce oxidative stress by regulating the levels of immune system regulators. Furthermore, low birth weight and frailty in lambs often result in insufficient colostrum intake, leading to lower serum immunoglobulin levels. This combination of factors contributes to higher mortality rates in these lambs [55]. In this study, the serum immunoglobulin content of the lambs was high, indicating an adequate milk supply to meet their nutritional needs. Therefore, prenatal interventions can significantly affect both the passive and acquired immune functions of offspring [56].

As a comprehensive index of animal antioxidant function, the T-AOC can reflect the body's health. Antioxidant enzymes such as SOD and GHS-Px are effective at scavenging free radicals, and the MDA content can reflect the degree of cellular damage in animals [35]. An imbalance between oxidants and antioxidants can lead to apoptosis or necrosis, causing oxidative damage in animals [35]. Studies have shown that T-SOD, GSH-PX, and MDA in sow serum can be transmitted to offspring through milk [58]. The study demonstrated that the supplementation of ATC to the diet of ewes increased serum CAT levels in lambs at 1, 30, and 45 days of the suckling period, serum SOD levels at 45 days, and serum T-AOC levels at 1 day. which also verified the transfer of antioxidant capacity between maternal colostrum/milk and offspring serum, indicating that the active ingredient of ATC could be vertically transmitted to offspring through milk, thereby increasing the activity of antioxidant enzymes and reducing the oxidative damage induced by reactive oxygen species.

Conclusion

Dietary supplementation of ATC in ewes demonstrates multiple beneficial effects, including enhanced feed intake, significant body weight gain, and elevated milk production. Notably, the intervention improves milk nutritional quality, while concurrently enhancing immune parameters and antioxidant capacity. Maternal supplementation of ATC during gestation enhances lamb growth performance, significantly improves neonatal immunity and antioxidant capabilities. Which confirmed that the active ingredients of ATC can be transmitted from ewes to lambs through maternal nursing. This study provides new insights into the nutritional regulation of ruminants. It could support the application of ATC as a feed additive, thereby improving maternal health, promoting offspring growth, and enhancing overall productivity. Future research should focus on examining the content of ATC in the placenta, blood, and milk of ewes and lambs, exploring its long-term effects on the production performance, health, and reproductive capacity of both mothers and offspring, and investigating the potential mechanisms underlying these improvements facilitated by ATC.

Abbreviations

ATC	*Acremonium terricola* culture
BW	Body weight
ALP	Alkaline phosphatase
GLB	Globulin
TCHO	Total cholesterol
TG	Triglyceride
TP	Total protein
UREA	Urea
CAT	Catalase
Ts	Total solids
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
ELISA	Enzyme-linked immunosorbent assay
IL-1β	Interleukin-1 beta
IL-4	Interleukin-4
IL-6	Interleukin-6
TNF-α	Tumor necrosis factor-alpha
T-AOC	Total antioxidant capacity
GSH-Px	Glutathione peroxidase
CAT	Catalase
SOD	Superoxide dismutase
MDA	Malondialdehyde
DM	Dry matter

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Author contributions
Mengen Zhang: Data curation, Methodology, Writing – original draft. Rui Han, Anguo Zhang, and Chao Xu: Investigation, Software, Writing – review & editing. Guohong Zhao and Xunsheng Pang: Formal analysis, methodology, writing – review & editing. Xichun Jiang and Shiqin Wang: Funding acquisition, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing. All the authors have read and approved the final manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
All animals were treated and used by following ethical approval from the Institutional Animal Care and Use Committee (IACUC) guidelines under current approved protocols at Anhui Science and Technology University (No.48 / 05.08.2023). The experiments were also carried out in accordance with relevant guidelines and regulations and the study was carried out in compliance with the ARRIVE guidelines (https://arriveguidelines.org/). Informed consent was obtained from all participants involved in the study, and the participating farms were informed of the study and agreed to participate in the entire animal experiment.

Competing interests
The authors declare no competing interests.

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