



RESEARCH ARTICLE

Effects of Selenium and Vitamin E Supplementation on Physiological, Biochemical Indicators, and Intestinal Microbiota of White Cashmere Goat

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ABSTRACT

The impact of Selenium (Se) and Vitamin E (VE) on physiological and biochemical parameters, secondary hair follicle development, and intestinal microbiota in White cashmere goat ewes and their offspring in Tibet is explored in the current paper. Twelve ewes and their lambs were randomly distributed into an experimental control group (EC) fed a basal diet and an experimentally supplemented group (ESE) supplemented with 0.4mg/kg Se and 200IU/kg VE. Their offspring were then divided into two groups: a lamb control group (LC) and an experimental lamb group (LSE). Lambs in the LSE group possessed a higher content of BUN in blood compared to those in the LC group ($P<0.05$). Ewes in the ESE group had significantly lower AST activities than ewes in the EC group ($P<0.05$). ALT activity was significantly higher in the LC group as opposed to that in the LSE group of lambs ($P<0.05$). Antioxidant capacity indicators, such as T-AOC, T-SOD, GSH-Px, and CAT, were significantly increased in both ewes and lambs after Se and VE supplementation ($P<0.05$). In the LSE lambs, the number of mature secondary hair follicles and the mature Sf:P ratio were increased ($P<0.05$), while Se and VE supplementation repressed these parameters in ewes ($P<0.05$). According to the analysis of microbiota, Se and VE supplementation showed no significant alteration in fecal microbiota composition in cashmere goats, including ewes and lambs. Conclusion: Dietary supplementation of Se and VE enhances antioxidant capacity, improves serum biochemical indices, and develops secondary hair follicles in lambs, further exerting a positive effect on cashmere production performance.

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INTRODUCTION

With cashmere goats produced throughout the country, China is the world's largest producer, creating the most value within this sector. Cashmere is considered the best animal fiber, commonly known as the "gem of fibers" or "soft gold," a raw material in the textile industry (Zhang *et al.*, 2024; Zhang *et al.*, 2025). Attributed to the increasing demand for high-quality cashmere products, the textile market sets higher requirements concerning fiber quality, notably fineness. International demand for fine cashmere is

steadily increasing, coupled with upward trends in market prices (Wu *et al.*, 2022; Pallotti *et al.*, 2023). According to the China Statistical Yearbook in 2020, there are about 3.3 million cashmere goats in the Northwest Xizang region. The dominant and representative breeds within the area are the white cashmere goats from Nagqu and Ngari Prefectures (Tian *et al.*, 2023; Li *et al.*, 2024).

Cashmere goats possess two skin follicle types: primary and secondary. Primary follicles generate the coarse guard hair, while the secondary follicles generate the fine cashmere fibers. The primary follicle development

takes place at the fetal stage of growth and is usually completed at birth; the formation and maturation are almost independent of postnatal nutrition or environmental conditions (Zhao *et al.*, 2024). The secondary hair follicles initiate morphogenesis in utero but attain full maturity at 3–6 months after birth (Gao *et al.*, 2016). The S:P (secondary to primary) follicle ratio established at birth is difficult to alter later in life, reflecting in utero secondary follicle formation. Gestational factors such as maternal undernutrition and physiological stressors-maternal heat stress or intrauterine hypoxia-have been shown to adversely affect the development and maturation of secondary hair follicles in pregnancy (Zhou *et al.*, 2018). Nutritional requirements for cashmere growth are relatively low; antioxidant supplementation may promote follicular development and increase cashmere yield accordingly (Yang *et al.*, 2019).

The overproduction of reactive oxygen species and the subsequent oxidative stress are major reasons that limit the morphological development and maturation of secondary hair follicles (Yang *et al.*, 2019). Melatonin and its metabolites are well-documented potent antioxidants that reduce oxidative damage and inhibit apoptosis (Ressmeyer *et al.*, 2003). Therefore, identifying antioxidant substances that would be suitable for supplementation in Northwest Xizang grazing systems is urgent for enhancing antioxidant activity in blood and skin and offering support for follicular health development and improving cashmere production. Selenium is an important trace mineral with critical biological functions, especially in animals. It acts mainly in the form of selenoproteins, among which glutathione peroxidase (GSH-Px) is included, to eliminate the intermediate product of lipid peroxidation, protect cellular membranes, and keep cells functioning normally. Selenium also takes part in the synthesis of coenzyme A and coenzyme Q, thus contributing to the tricarboxylic acid cycle and the electron transport chain (Flohe *et al.*, 2022; He *et al.*, 2024). Moreover, Se acts synergistically with VE: proper Se supplementation can enhance growth performance, improve disease resistance, and prevent nutritional muscular (white muscle disease) dystrophy (Martin Rodriguez *et al.*, 2018). Supplementation with Se improves milk production in Guanzhong dairy goats, as organic Se elevates blood Se levels more effectively compared to the inorganic form (Zhang *et al.*, 2018). VE is a fat-soluble micronutrient, well-documented for possessing antioxidant, anti-infertility, anticarcinogenic, and immunomodulatory properties. The antioxidant mechanism of VE is composed of the following: stopping oxidation and peroxidation of polyunsaturated fatty acids, neutralizing peroxides, and maintaining cell membrane integrity, which reduces oxidative stress and supports defense systems (Lewis *et al.*, 2019; Ghazali *et al.*, 2022; Renke *et al.*, 2023). Many studies have demonstrated that Se and VE in the blood of animals are related to their dietary intake, and these two nutrients exhibit a synergistic effect on antioxidant activity in animals (Chauhan *et al.*, 2015; Wang *et al.*, 2021).

Although the nutritional, reproductive, and immune functions of Se and VE have been extensively studied, their specific roles in cashmere development and hair follicle biology of pregnant ewes and their offspring remain incompletely understood. The present study was conducted to determine the effect of maternal Se and VE

supplementation during pregnancy on physiological traits, antioxidant capacity, hair follicle development, and production performance in White cashmere goat offspring in Northwest Tibet. Based on these results, it is expected to lay a scientific basis for improving cashmere yield and quality in practical farming.

MATERIALS AND METHODS

Ethics statement: All procedures involving animals were approved by the Animal Ethics Committee of the Tibet Academy of Agricultural and Animal Husbandry Sciences: TAAA202401125.

Design of experimentation and feeding management: Twelve healthy White cashmere goat ewes of similar body weight, with each ewe and her offspring, were enrolled and randomly allocated into two groups. Each group consisted of six replicates (one ewe/replicate). Ewes in the control group (EC) were fed a basal diet, whilst ewes in the experimental group (ESE) were fed the basal diet supplemented with 0.4 mg/kg Se and 200 IU/kg VE. Feeding was provided at 07:00 and 17:00 twice every day, with free access to water. The basal pelleted diet was formulated according to the nutrient specifications in the Nutritional Requirements for Cashmere Goats (NY/T 4048–2021) (Table 1). Lambs in the control group (LC group) grazed freely and suckled three times daily (07:00, 13:00, 19:00). Lambs in the experimental group (LSE group) were fed milk supplemented with Selenium and Vitamin E at the same schedule. The experiment lasted 60 days, including a 10-day adaptation period and a 50-day formal trial period. All animals were housed at the White Cashmere Goat Experimental Base, Ritu County, Ngari Prefecture, Xizang.

Table 1: Basic Diet Composition and Nutritional Levels (Air-dried basis)

Raw material composition	Conte nt %	Nutritional level ²⁾	Conte nt %
Oat grass granules	15.00	Crude protein	12.56
Rice husk powder	5.00	Metabolic energy ME/(MJ·kg ⁻¹ DM)	10.35
Corn	50.00	Coarse fiber	6.06
Wheat bran	10.00	Crude fat	3.38
Soybean meal	8.00	Neutral detergent fiber	16.25
Rapeseed meal	5.00	Acidic washing fiber	7.27
Spray corn husks	2.00		
Baking soda	1.00		
Calcium hydrogen phosphate	1.00		
Stone powder	1.00		
Sodium chloride	1.00		
Premix ¹⁾	1.00		
Total	100.00		

Note: 1. Premixes are provided per kilogram of diet: Vitamin A 15,000 IU, Vitamin D 5,000 IU, Vitamin E 50 mg, Antioxidant 100 mg, Fe 90 mg, Cu 12.5 mg, Mn 50 mg, Zn 100 mg, Se 0.3 mg, I 0.5 mg, Co 0.5 mg; 2. Nutritional levels are calculated values.

Sample collection: On day 50 of the trial, jugular venous blood was collected from each ewe and lamb before morning feeding. Serum was separated by centrifugation at 4°C and 3000 r/min for 10 min, then aliquoted into 2 mL tubes and stored at –20°C until analysis. Fresh fecal samples were obtained from both ewes and lambs, immediately flash-frozen in liquid nitrogen, and preserved at –80°C. Skin specimens (diameter approximately 1cm) were obtained from the upper third of the area located between the posterior scapular margin and the dorsal

midline, using a circular biopsy punch. After a short rinse with phosphate-buffered saline, the samples were transferred to pre-labeled containers and fixed in 4% paraformaldehyde.

Serum enzymes and antioxidant indicators detection:

Levels of serum biochemical indices related to protein and lipid metabolism, such as TP, ALB, BUN, CHOL, and TG, were estimated with the help of an automatic biochemical analyzer with commercial kits from Wako, Japan, in compliance with the manufacturer's instructions. The activities of serum enzymes, such as ALT, AST, AKP, and LDH, together with the levels of antioxidant-related indices, including T-AOC, T-SOD, GSH-Px, CAT, and MDA, were assayed with kits from Nanjing Jiancheng Bioengineering Institute, China, using the protocols provided by the manufacturers.

Skin hair follicle morphology analysis: Fixed skin samples in 4% paraformaldehyde were processed for routine paraffin embedding and sectioning. Sections were deparaffinized, then stained, dehydrated, cleared, and mounted. Slides were scanned using the panoramic digital imaging system. The numbers of primary follicles, secondary follicles, and mature secondary follicles were quantified using Image-Pro Plus 6.0 (IPP 6.0). The following indices were calculated:

S:P = Number of secondary hair follicles/Number of primary hair follicles

Sf:P = Number of mature secondary hair follicles/Number of primary hair follicles

16s rRNA sequencing of rectal contents: Total DNA from rectal contents was extracted using the Tiangen (Beijing) Stool DNA Extraction Kit following the manufacturer's protocol. DNA integrity was assessed by 1.0% agarose gel electrophoresis, and concentration and purity were determined using a UV spectrophotometer (NC 2000, Thermo Scientific). The V3-V4 region of the bacterial 16S rRNA gene was amplified using universal primers (Table 2). The PCR reaction components included Reaction buffer 12.5μL, Forward primer 2.5μL, Reverse

primer 2.5μL, DNA template 2μL, and ddH₂O 5μL. Amplicons were submitted to Shanghai Paisenuo Biotechnology Co., Ltd. for sequencing. Bioinformatic analyses included sequence assembly, OTU clustering, Venn diagram construction, rarefaction curve evaluation, alpha diversity, beta diversity, and microbial community composition analysis. Functional prediction of microbiota was performed using PICRUST2 to analyze differences in metabolic pathways.

Table 2: Primer Information in this study

Items	Gene sequence
Forward primer	5'-ACTCCTACGGGAGGCAGCA-3'
Reverse primer	5'-GGACTACHVGGGTWTCTAAT-3'

Statistical analysis: Data were prepared in Microsoft Excel 2021 and analyzed with independent-samples t-tests using SPSS software (version 25.0). Results are reported as mean ± standard error of the mean (SEM). Differences were evaluated for statistical significance with multiple t-tests, where a threshold of $P < 0.05$ was considered statistically significant and $P < 0.01$ as highly significant.

RESULTS

Effects of Se and VE on serum biochemical indicators, enzyme activities, and antioxidant indicators of cashmere goats, ewes, and lambs:

Analysis of serum biochemical indicators showed that supplementation with Se and VE did not significantly affect the biochemical parameters among the groups of cashmere goats and ewes, except for blood urea nitrogen (BUN). The BUN concentration in the LES group was significantly higher than that in the LC group ($P < 0.05$), while no significant differences were observed in other biochemical indices (Fig. 1a). Serum activities of hepatic enzymes (AST, ALT, ALP, and LDH) were assessed in both ewe and lamb groups. Among ewes, AST activity was significantly higher in the EC group compared to the ESE group ($P < 0.05$, Fig. 2a). In lambs, ALT activity was significantly elevated in the LC group relative to the LSE group ($P < 0.05$), while no significant differences were found for AST, ALP, or LDH activities (Fig. 2b).

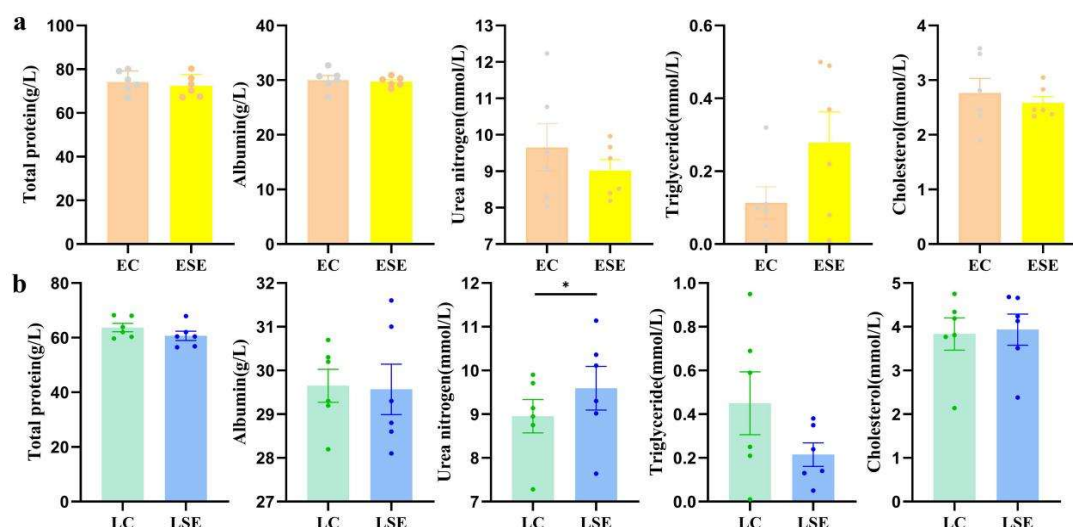


Fig. 1: The effects of Selenium and Vitamin E on serum biochemical indicators of cashmere goats, ewes, and lambs. (a): Ewes (b): Lamb.

Assessment of oxidative stress markers demonstrated that supplementation with Selenium and Vitamin E significantly improved the antioxidant capacity in both ewes and their lambs. Compared to the EC group, the ESE group exhibited significantly higher serum activities of T-AOC, SOD, GSH-Px, and CAT ($P < 0.05$; Fig. 3a). Correspondingly, in lambs, the LSE group displayed significantly elevated activities of these same antioxidant enzymes relative to the LC group ($P < 0.05$, Fig. 3b).

Effects of Se and VE on skin hair follicle development in cashmere goats, ewes, and lambs: In ewes, dietary supplementation with Selenium and Vitamin E significantly reduced the number of mature secondary hair follicles in the ESE group compared with the EC group ($P < 0.01$). The ratio of mature secondary to primary follicles (Sf:P) was also significantly lower ($P < 0.05$) (Fig.

4a). In lambs, no significant differences were observed between the LC and LSE groups in the number of primary follicles, secondary follicles, mature secondary follicles, or in follicle ratio indices (Fig. 4b).

Effects of Se and VE on the fecal microbiota composition of cashmere goats, ewes, and lambs: 16s rRNA gene sequencing experiments were conducted on 12 fecal samples of ewes and 12 lambs, respectively. A total of 19,741 OTUs were identified from ewes and 16,591 from lambs at a 97% similarity threshold. The number of unique OTUs in the EC group and the ESE group was 6,995 (35.43%) and 7,096 (35.95%), respectively. There were total of 2,825 OTUs in the two groups, accounting for 14.31% of the total. The number of unique OTUs in the LC group and the LSE group was 6,686 (40.30%) and 5,621 (33.88%), respectively. There were total of 2,142 OTUs in

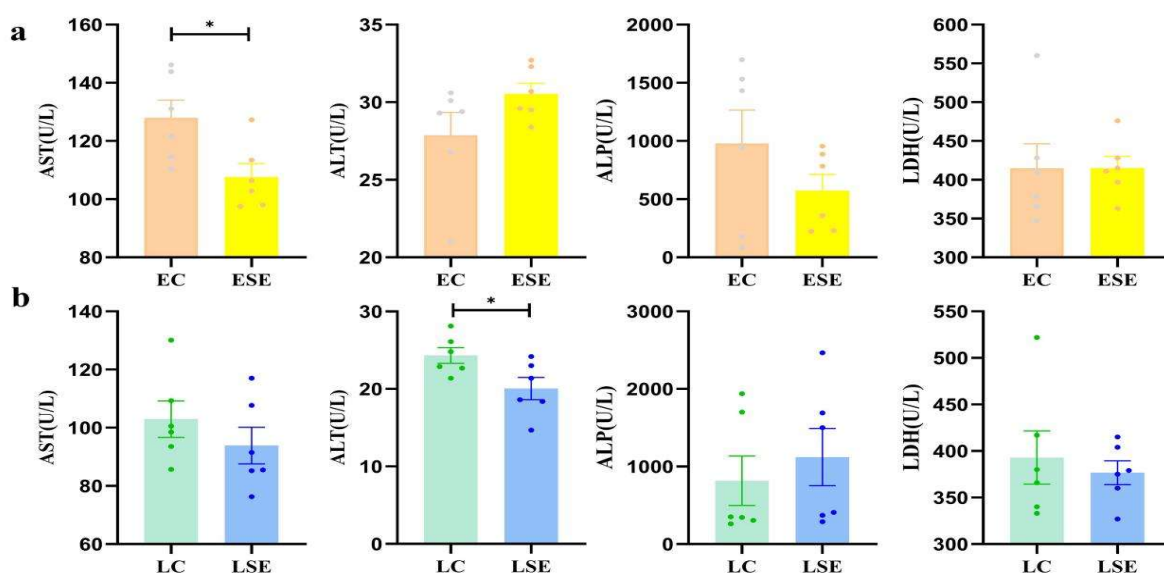


Fig. 2: The effects of Selenium and Vitamin E on serum enzyme activities in cashmere goats, ewes, and lambs. (a): Ewes (b): Lamb.

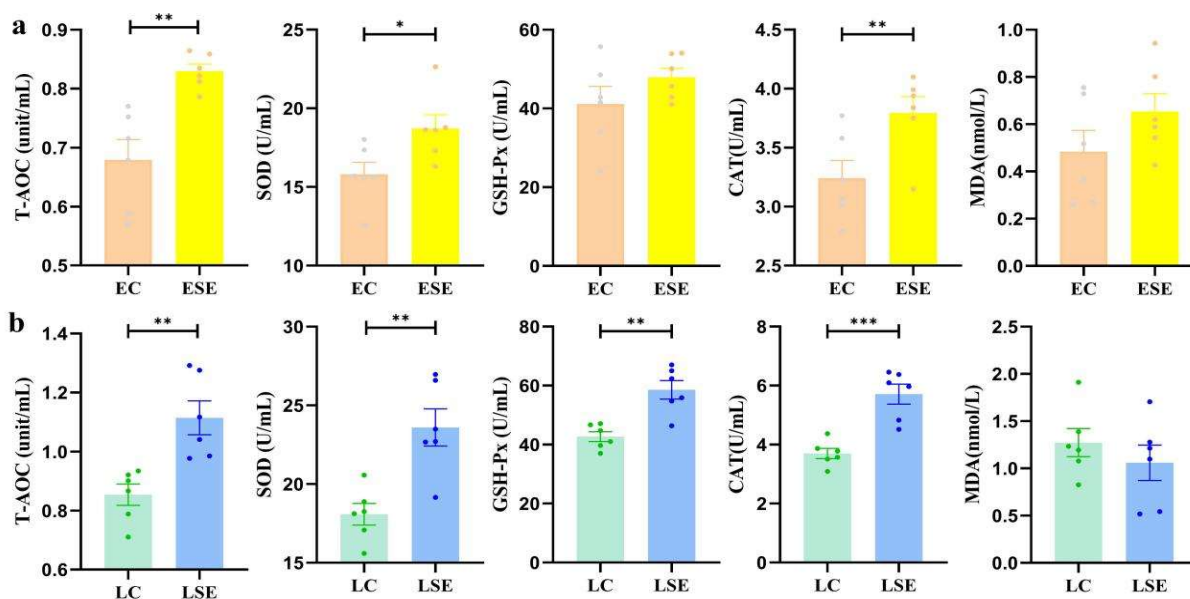


Fig. 3: The effects of Selenium and Vitamin E on the antioxidant indices of serum in cashmere goats, ewes and lambs. (a): Ewes (b): Lamb.

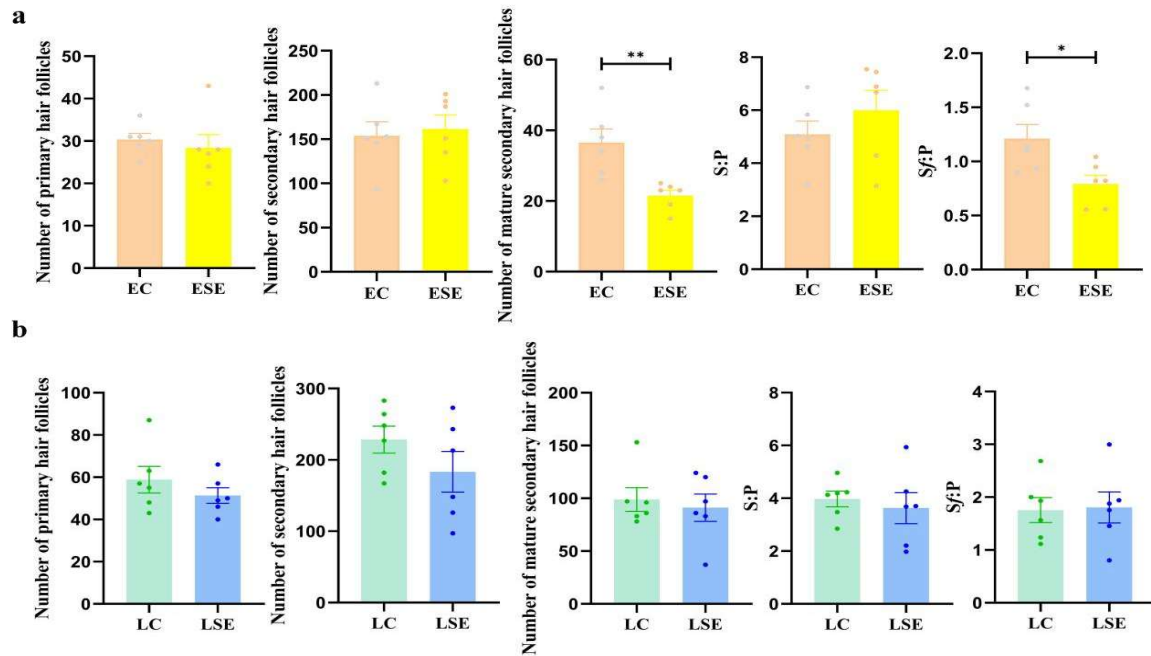


Fig. 4: The Effects of Selenium and Vitamin E on the hair follicle traits of cashmere goats, ewes, and lambs. (a): Ewes (b): Lamb.

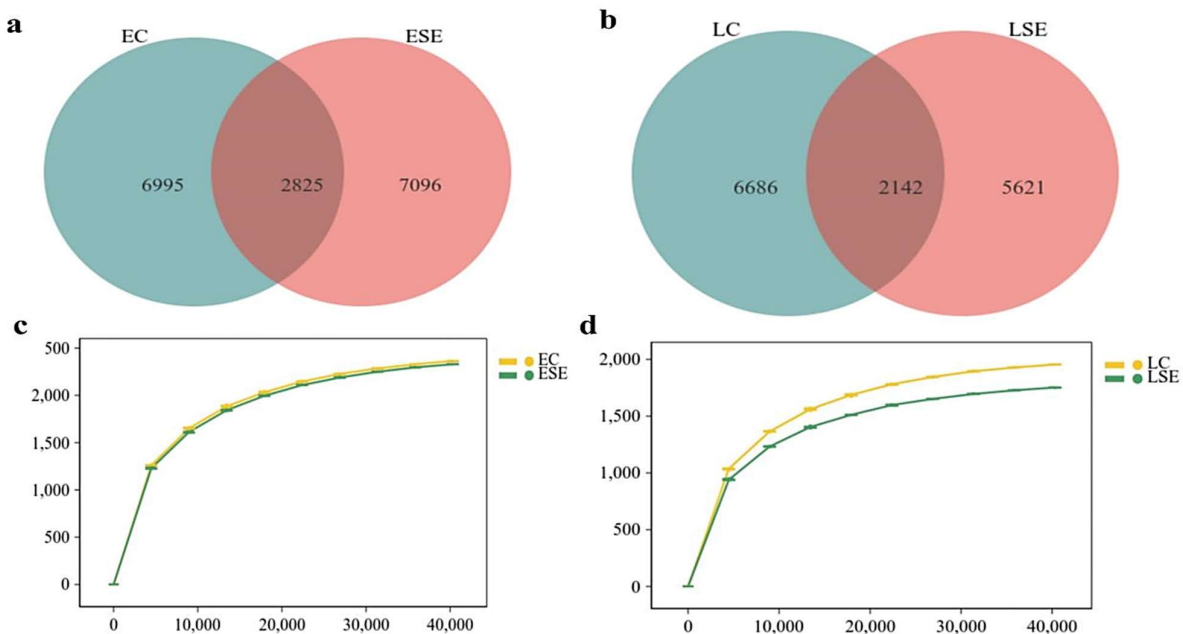


Fig. 5: Venn plot and species rarefaction curve. (a)(b): Venn plot. (c)(d): rarefaction curve. (a)(c): Ewes. (b)(d): Lamb.

the two groups, accounting for 12.90% of the total (Fig. 5a, b). The species rarefaction curve indicates that the sequencing depth achieved was adequate to capture the majority of microbial diversity present, confirming that sample quality met the necessary standards for subsequent sequencing and analytical procedures (Fig. 5c, d). Alpha diversity indices showed no significant differences between the EC and ESE groups in ewes, nor between the LC and LSE groups in lambs (Fig. 6a, b). By PCoA based on Bray-Curtis distance, some dispersions among groups were observed, reflecting mild differences in community structure, but these were not statistically significant (Fig.

6c, d). Se and VE supplementation did not influence the composition of fecal microbiota in cashmere goats, ewes, and lambs. Bacteroidetes (49.17%) and Firmicutes (33.27%) were dominant at the phylum level both in ewes and lambs, collectively accounting for over 90% of the fecal microbiota. No significant difference was observed between EC and ESE groups, or between LC and LSE groups (Fig. 7a, b). The dominant genera were 5-7N15, Ruminococcus, Oscillospira, and Dorea, each consisting of over 10% of the total bacterial community, with no significant differences among their relative abundances in each group (Fig. 7c, d).

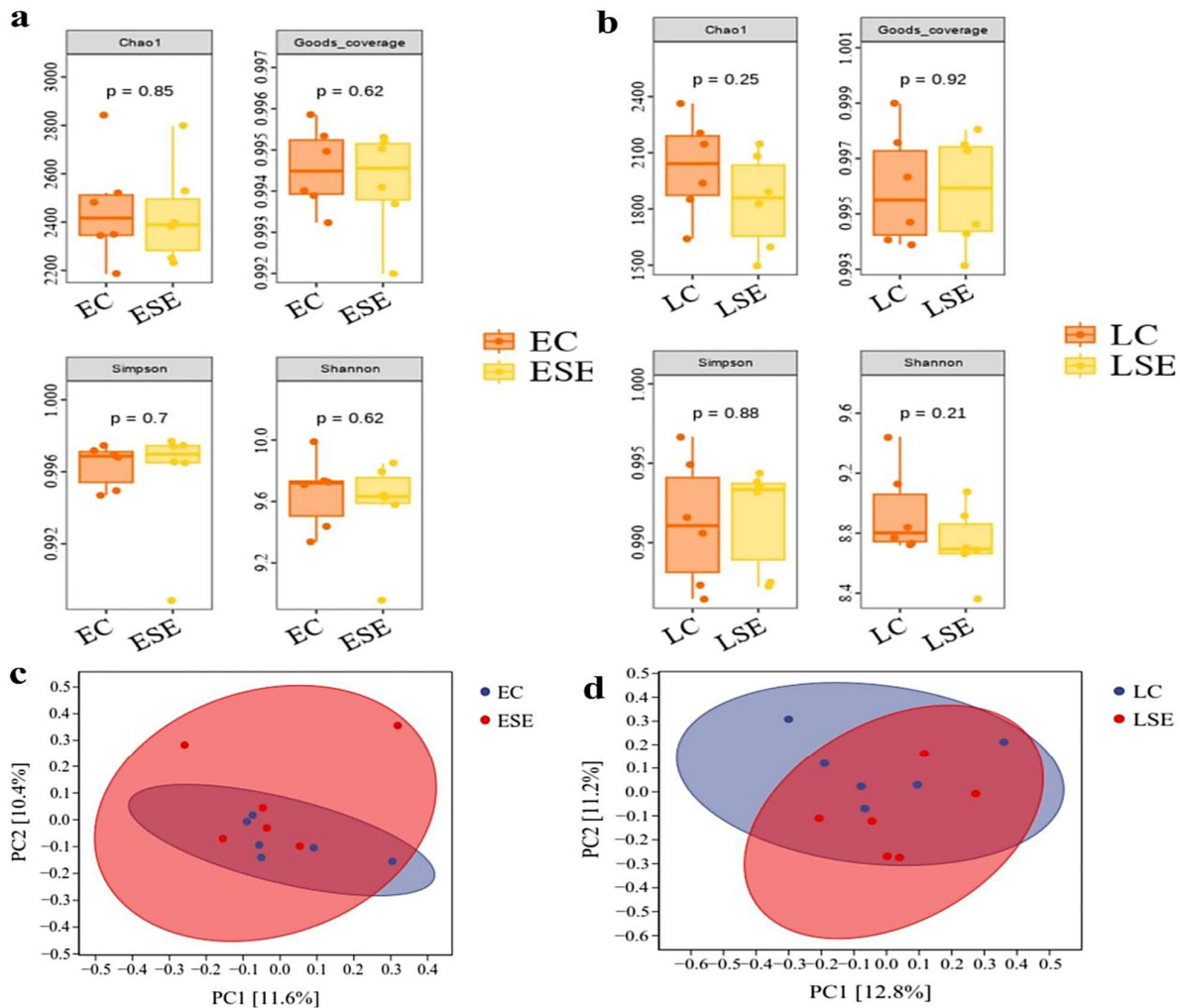


Fig. 6: The effects of Selenium and Vitamin E on the diversity of intestinal flora in cashmere goats, ewes, and lambs. (a)(b): Alpha Diversity. (c)(d): Beta Diversity. (a)(c): Ewes. (b)(d): Lamb.

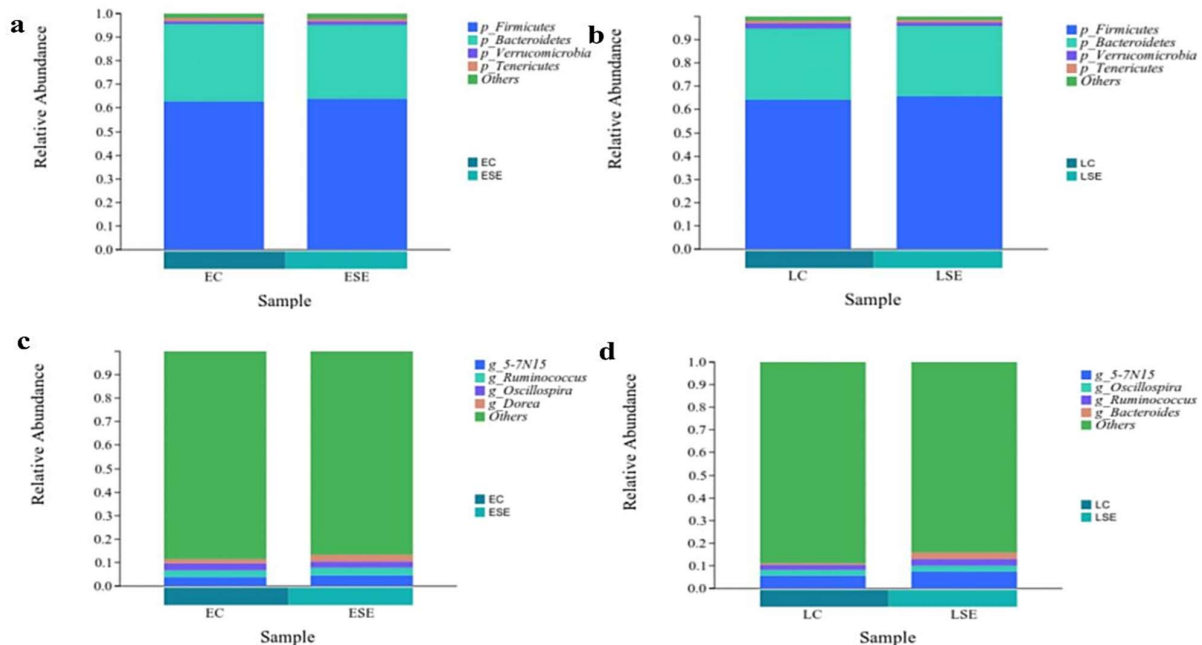


Fig. 7: The effects of Selenium and Vitamin E on the species composition of cashmere goats, ewes, and lambs at different taxonomic levels. (a)(b): Phylum. (c)(d): Genus. (a)(c): Ewes. (b)(d): Lamb.

DISCUSSION

Serum total protein, albumin, and urea nitrogen are important parameters of protein metabolism and reflect the effectiveness of protein digestion, absorption, and anabolic processes in animals (Fouillet *et al.*, 2008). In addition, one study reported that nano-Selenium supplementation to the diet had significantly affected serum albumin concentrations in laying hens (Zhou *et al.*, 2021). In this experiment, lambs supplemented with Se and VE had significantly increased serum BUN levels. The most possible explanation is that Se and VE supplementation improved the antioxidant capacity of immune cells to protect cellular structure and function and may cause increased protein catabolism or nitrogen metabolism, leading to high levels of BUN. AST is mainly present in the mitochondria of hepatocytes. Its activity in serum significantly increases with serious hepatic injury or necrosis. ALP has a wide distribution but shows particularly high activity in bone, liver, and kidney. Increased ALP usually indicates hepatobiliary dysfunction, whereas decreased ALP activity suggests chronic nephritis, anemia, and hypothyroidism (Riancho-Zarrabeitia *et al.*, 2016; Kwo *et al.*, 2017). In this study, serum AST and ALP activities were significantly decreased by dietary Se and VE supplementation in ewes, which contrasted with certain previous studies. The difference could be due to supplementation doses or different physiological stages. A moderate supplementation of Se and VE may reduce oxidative damage of hepatocytes and bone cells, thus improving the immune function of ewes. Well-coordinated interactions between these epidermal and dermal stem cell populations are very crucial for follicular morphogenesis, involving secondary hair follicle development and maturation. Excessive oxidative stress, along with ROS, can cause damage to proteins and DNA in those stem cells and consequently result in defective self-renewal and differentiation; as a result, the formation of secondary follicles will be impeded (Sengupta *et al.*, 2010). There is a large body of evidence showing that supplementation with antioxidants can enhance the overall antioxidant status and support the development of secondary hair follicles. For example, supplementation of coenzyme Q10 through the diet increases the levels of total antioxidant capacity and enhances follicle density in gonadectomized mice (Ozcan *et al.*, 2016), and supplementation of Selenium increases antioxidant enzyme activities in laying hens (Muhammad *et al.*, 2022). Total antioxidant capacity is the key marker reflecting the overall antioxidant capability of the body, while CAT and GSH-Px represent key enzymatic activities that scavenge free radicals (Grzeszczak *et al.*, 2023; Saleem *et al.*, 2023). Selenium has been demonstrated to strikingly upregulate the activity of GSH-Px in placental cells, protecting these cells against oxidative injury provoked by oxidative agents such as H₂O₂ (Khera *et al.*, 2017; Emilio *et al.*, 2019). Hussain *et al.* (2021) observed the dose-dependent increase in GPx activity of placental cell lines after Selenium supplementation, decreasing intracellular ROS and improving survival following an oxidative challenge. The improvement in T-AOC after Selenium might involve increased activities of key enzymatic antioxidants, such as CAT, GSH-Px, and SOD, along with higher levels of the non-enzymatic antioxidant glutathione (Ghneim and Al-Sheikh, 2011; Wu *et al.*, 2025). In this

experiment, dietary Se and Vitamin E improved serum T-AOC, the activities of CAT, T-SOD, and GSH-Px in ewes and lambs, and dietary Se and VE supplementation in ewes decreased serum MDA content in the current study, which agrees with the reports of Hussain *et al.* (2021). Primary hair follicles develop prenatally and are relatively insensitive to postnatal nutrition and environmental factors in cashmere goats (Diao *et al.*, 2023). The present study found no significant changes in the number of primary hair follicles in either ewes or lambs, which agrees with previous works. Secondary hair follicle morphogenesis appears to be governed instead by complex interactions between epidermal keratinocytes and dermal mesenchymal cells (Wang *et al.*, 2016). Cashmere fibers are products of secondary hair follicles, and improvements in cashmere production are highly related to the increase and maturity in these follicles. The processes that are important in governing secondary follicle morphogenesis and further cashmere growth include the proliferation and differentiation of hair bulb matrix cells (Lee *et al.*, 2024). *In-vitro*, Selenium has also been shown to enhance the migration of keratinocytes through the induction of matrix metalloproteinase expression in HaCaT cells, allowing interaction between keratinocytes and components of the dermal matrix (Kim *et al.*, 2020). The differentiation of keratinocytes and their molecular interactions at the cell-cell and cell-extracellular matrix levels within the basement membrane and epidermis, respectively, are essential for the maintenance of skin structural integrity and developmental support. Selenoproteins are implicated in keratinocyte function and overall skin morphogenesis as well (Sengupta *et al.*, 2010). Targeted disruption in mice of the gene encoding the tRNA for selenocysteine has been shown to lead to abnormal follicular morphology, an effect that is reversed by supplementation with Vitamin E, supporting an antioxidant protective pathway for Selenium (Jobeili *et al.*, 2017). Further supporting evidence suggests that Selenium can delay aging of keratinocytes (Rafferty *et al.*, 1998), protect DNA, and enhance the capacity for DNA repair (Wu *et al.*, 2011), thus maintaining skin homeostasis and promoting follicular development. In the present study, Selenium and Vitamin E significantly enhanced the counts of mature secondary follicles and the secondary-to-primary follicle (Sf:P) ratio in lambs, indicating a stimulatory effect on secondary follicle development. Overall, findings from these studies indicate that Selenium and Vitamin E in the diet effectively improve secondary follicle maturation, enhance cashmere quality, and increase cashmere yield. Microbial richness and diversity are considered important indicators of intestinal ecosystem stability. Higher richness supports community resilience through complementary interactions among species (Muhammad *et al.*, 2021). In the present study, Se and VE supplementation did not significantly alter microbial richness, diversity, or community structure in either ewes or lambs. Studies examining the effects of Selenium and Vitamin E on the fecal microbiota of cashmere goats remain limited. Based on the current findings, these supplements appear to have minimal impact on the gut microbiota of cashmere goats, ewes, and lambs.

Conclusions: Dietary supplementation with Selenium and Vitamin E increased serum BUN concentrations in lambs, reduced serum AST and ALP activities in ewes, and

significantly enhanced total antioxidant capacity and antioxidant enzyme activities in both ewes and lambs. Although the development of primary hair follicles was not altered, supplementation with Selenium and Vitamin E enhanced the maturation of secondary hair follicles and elevated the secondary-to-primary follicle ratio, thereby increasing the potential for cashmere production. In summary, Selenium and Vitamin E serve as effective nutritional interventions for boosting antioxidant capacity and promoting secondary hair follicle development in cashmere goats.

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Authors contribution: CA, JD, JB, JG, YW and LS performed conceptualization and methodology; CA and JD performed software analysis validation, formal analysis, investigation and data curation; CA, RAEM and EAI performed writing—original draft preparation, writing—review and editing; LS did visualization and supervision, project administration and funding acquisition; All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest: None.

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