Ankara Üniversitesi Veteriner Fakültesi Dergisi/Ankara Univ Vet Fak Derg; 2026:73(1): 1-6
DOI: 10.33988/auvfd.1700688
Research Article

# Effects of bee pollen supplementation in lactating ewes on maternal blood parameters and lamb growth performance

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## ABSTRACT

The aim of the study was to determine the effect of pollen supplementation on lactating ewes and their lambs. An experiment was carried out for a period of 4 weeks with 22 lactating ewes and their offspring of the breed Ile de France (28 lambs), matched for lambing date and live weight of the lambs. The sheep were divided into two groups: control and experimental. Feeding was the same for both groups. The experimental group was supplemented with bee pollen at a dose of 6 g/twice per week at regular intervals of 3 days. The live weight of the lambs was determined at the beginning and at the end of the experiment, and the average daily gain was calculated. Blood samples were taken from the ewes, and some biochemical parameters were examined. The live weights and growth of lambs at the beginning and end of the experiment showed a slight trend towards higher values in the experimental group, which were not significant. In the biochemical profile, the values of the parameters studied during both periods were within the reference range for the animals, with the exception of glucose, which was lower. Significant differences ($P<0.001$; $P<0.01$) in glucose, triglyceride, and AST levels were found within groups between the initial and final measurements. Bee pollen supplementation demonstrated no adverse effects on the health of ewes or their offspring. Furthermore, it may exert beneficial effects on productivity parameters. Further research is warranted to confirm these findings and elucidate optimal application strategies.

**Keywords**: Bee pollen, Biochemical profiles, Lactating ewes, Lambs

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**Received:** 16.05.2025, **Accepted:** 31.08.2025, **Publication Date:** 01.01.2026

**How to cite this article:** Todorova M, Ivanova T, Balkanska R (2026): Effects of bee pollen supplementation in lactating ewes on maternal blood parameters and lamb growth performance. Ankara Univ Vet Fak Derg, 73 (1), 1-6. DOI: 10.33988/auvfd. 1700688.

## Introduction

The nutrition of farm animals is a topic of utmost importance, as it also determines the quality of their production. The link between obtaining high-quality animal production and human health is gaining increasing popularity. The utilization of additives in animal nutrition has focused on the use of natural products that pose no real risk to human health (5). In this respect, plant extracts (containing phenols and flavonoids) and bee products have gained considerable popularity as natural additives. Bee products, such as honey, propolis, royal jelly, and bee pollen, are natural sources that are rich in carbohydrates, proteins, lipids, vitamins, and mineral substances. These products have seen increased use as food supplements or for therapeutic purposes against various diseases in humans and animals (5, 21, 29). Nevertheless, the extant scientific literature on the effects of these products on the nutrition of farm animals remains scant.

Pollen is defined as the male reproductive cells of plants. Bees collect pollen from plants and form it into small balls, which they can mix with nectar and secretions from their hypopharyngeal glands (2, 19). According to Thakur and Nanda (30), bee pollen contains carbohydrates, proteins, lipids, minerals, and phenolic compounds (average 30.59 mg/g gallic acid) with anti-inflammatory, antioxidant, and hepatoprotective effects. A notable aspect of bee pollen's chemical composition is the prevalence of derivatives of phenolic acids and polyphenolic compounds, predominantly flavonoid glycosides. Flavonoids have been demonstrated to possess significant physiological and pharmacological activities. These compounds exhibit a wide range of biological

properties, including antioxidant, antineoplastic, anti-inflammatory, anti-atherosclerotic, and cardioprotective effects. Flavonoids may also exert indirect effects by activating endogenous defense systems and by modulating various physiological processes in the body (15).

Fodail et al. (13) investigated the effect of oral administration of different doses of bee pollen (4.5 g and 9 g) before and after parturition on the performance of ewes as well as on the growth of lambs. The authors found that 9 g of pollen given before and after parturition had a positive effect on milk composition and quantity, colostrum composition, blood parameters of ewes, and growth performance of lambs. In a recent study, Hou et al. (16) examined the impact of providing sheep with unbroken pollen and showed that feeding unbroken bee pollen to sheep can enhance their growth, serum immune function, and economic benefits. The authors thus recommend incorporating 2 g per day of unbroken bee pollen into the sheep's diet.

In Bulgaria, research on the use of different bee products in animal nutrition is limited or non-existent. Additionally, there is a lack of recent information on the use of bee pollen in lactating ewes. The aim of this study is to evaluate the effect of supplementing the diet of lactating ewes with bee pollen during lactation on some of their blood biochemical parameters and the growth performance of their offspring.

## Materials and Methods

The experiment was carried out for a period of 4 weeks, in the Experimental Base of IAS – Kostinbrod, with 22 lactating ewes and their offspring of the Ile de France breed (28 lambs), matched for lambing date (30±5 days), live weight of the lambs, type of birth, and sex equality. During the experimental period (30 days), ewes and lambs were reared barn raised. The sheep were divided into two groups, control and experimental. Feeding was the same for both groups. The experimental group was supplemented with bee pollen at a dose of 6 g/twice per week at regular intervals of 3 days. The bee pollen was consumed by each ewe.

Chemical analysis of dry matter, crude protein, ether extract, crude fibre, crude ash (by Weende analysis), Ca and P contents (9) was carried out on the feeds used and the bee pollen. The chemical composition of the feed used is shown in detail in Table 1. Sheep received a concentrate mixture of 1 kg/day containing wheat 77%, sunflower meal 20% and vitamin mineral premix 3%. Lambs of both groups were fed *ad libitum* with a supplementary compound feed containing wheat 70.23%, sunflower meal 20%, soybean meal 7% and vitamin mineral premix 2.77%. Animals received meadow hay and alfalfa hay *ad libitum*. They were provided with constant access to water and mineral licking block. The live weight of the lambs was determined at the beginning and end of the experiment, and the average daily gain was calculated.

Blood samples were taken from the ewes at the beginning and end of the experiment. The following biochemical parameters were examined: glucose, total cholesterol, triglycerides, ALT (alanine aminotransferase), and AST (aspartate aminotransferase) (24). Blood samples were collected from *v. jugularis* in vaccine containers with anticoagulant EDTA. Plasma was separated by centrifugation for 10 min at 3000 rpm and was measured by commercial kits (Giesse Diagnostics) analyzed with a BioSystems BTS-350 semi-automated biochemical analyzer (S. A. Costa Brava Spain).

***Statistical Analysis:*** The obtained data were statistically processed by usual variation statistics methods MS Excel 2010. The difference between groups was analysed by the t-test of Student. The results are presented as mean ± standard error (X±SE) at the beginning and at the end of each period for each parameter. A value of $P<0.05$ was considered statistically significant.

## Results

Data about chemical composition of polyfloral bee pollen is presented in Table 2. The values obtained in chemical analysis of bee pollen were dry matter (87.34%), crude protein (21.64%) and ether extract (6.77%). Live weight data of the lambs at the beginning and end of the experiment (which lasted for 30 days) are presented in Table 3. During the experiment, one lamb (control group) sustained trauma and was excluded from all subsequent data collection. The live weights of lambs of the two groups measured at the beginning and at the end of the experiment were similar with a slight trend towards higher values in the experimental group, with no statistically significant differences. There were also no significant differences in average daily gain, which was 2.2% higher in lambs whose mothers received bee pollen.

**Table 1.** Chemical composition of the feeds

| Parameters | Dry matter, % | Crude protein, % | Ether extract, % | Crude fibre, % | Crude ash, % | Ca, % | P, % |
|---|---|---|---|---|---|---|---|
| Compound feed for sheep | 88.49 | 16.20 | 1.82 | 5.47 | 2.20 | 1.02 | 0.51 |
| Compound feed for lambs | 88.63 | 18.08 | 1.79 | 5.74 | 2.32 | 1.22 | 0.52 |
| Meadow hay | 86.72 | 5.91 | 1.79 | 34.85 | 4.95 | 0.52 | 0.12 |
| Alfalfa hay | 88.65 | 19.02 | 1.14 | 28.35 | 8.66 | 1.37 | 0.25 |

**Table 2.** Chemical composition of polyfloral bee pollen

| Parameter | Bee pollen |
|---|---|
| Dry matter, % | 87.34 |
| Crude protein, % | 21.64 |
| Ether extract, % | 6.77 |
| Crude ash, % | 2.20 |

**Table 3.** Live weight and average daily gain of lambs (Mean±SE)

| Parameter | n | Control group | n | Experimental group | P |
|---|---|---|---|---|---|
| Live weight at the beginning of the experiment (day 1st), kg | 14 | 9.35±0.60 | 14 | 10.83±0.82 | 0.162 |
| Live weight at the end of the experiment (day 30th), kg | 13 | 15.45±1.02 | 14 | 17.27±1.12 | 0.243 |
| Average daily gain (1-30 days), g/day | 13 | 203.31±20.58 | 14 | 207.79±24.15 | 0.889 |

**Table 4.** Biochemical analysis of sheep blood (Mean±SE)

| Parameter | | No | Control group (n=11) | Experimental group (n=11) | P |
|---|---|---|---|---|---|
| Glucose, mg/dL | At the beginning | 1 | 25.33±1.55 | 24.24±0.77 | 0.571 |
| | At the end | 2 | 32.10±1.40 | 33.59±1.64 | 0.576 |
| Total cholesterol, mg/dL | At the beginning | 3 | 63.38±4.56 | 65.64±2.75 | 0.524 |
| | At the end | 4 | 59.51±4.74 | 60.75±6.27 | 0.869 |
| ALT, IU/L | At the beginning | 5 | 37.18±2.72 | 40.27±4.06 | 0.616 |
| | At the end | 6 | 32.18±3.36 | 32.82±4.94 | 0.900 |
| AST, IU/L | At the beginning | 7 | 220.18±13.26 | 213.36±10.75 | 0.675 |
| | At the end | 8 | 174.91±7.66 | 164.45±8.40 | 0.278 |
| Triglycerides, mg/dL | At the beginning | 9 | 16.51±0.88 | 17.39±1.49 | 0.572 |
| | At the end | 10 | 24.16±2.35 | 28.50±3.40 | 0.269 |
| P | | | 2>1***; 7>8***; 10>9** | 2>1***; 7>8***; 10>9** | |

***P<0.001; ** P<0.01.

Table 4 presents the results of the study of the biochemical parameters of sheep blood at the beginning and at the end of the experiment. In our study, no statistically significant differences were found between the two groups regarding the biochemical profile of sheep at the beginning of the experiment. Even at the end of the experiment, despite the consumption of bee pollen by the animals in the experimental group, no statistically significant differences were found.

According to the results of the present study, statistically significant differences were between the initial and final examinations within the groups regarding the following parameters: glucose (P<0.001), triglycerides (P<0.01), and AST (P<0.001) (Table 4). In both groups of animals, the parameters exhibited a similar trend, likely reflecting physiological changes associated with different stages of lactation. In the second measurement glucose values were significantly higher (P<0.001). Moreover, a significant difference was observed between the first and the second measurements, with significantly higher levels (P<0.01) recorded in the second survey (Table 4).

The values for AST in both groups of sheep were higher when measured at the end of the experiment (P<0.001) (Table 4).

## Discussion and Conclusion

The values obtained in our study for moisture, protein, and fat content in bee pollen are close to those established by Barajas et al. (10) when examining various samples of polyfloral bee pollen. According to a review article by Rodríguez-Pólit et al. (27), summarizing data from various authors, the protein content of pollen ranges from 2% to

60%, and the lipid content from 1% to 20%. Thakur and Nanda (30) also presented large ranges for the parameters' protein and lipid content. The reason for the large ranges of these parameters may be due to multifloral composition of the bee pollen. Pollen composition and production depend on which plants the worker bees collect pollen from, as well as climatic and soil factors (10).

In our study, the live weight and average daily gain of lambs from both groups at the beginning and end of the experiment showed no significant differences. A slight trend towards higher live weights in the experimental lambs can be explained by bee pollen's properties, acting as a growth promoter and energy enhancer. Its nutritional and therapeutic values, including antioxidant properties, benefits for enhancing immune function and growth in livestock, are well-known (1, 6). The weight development of lambs was normal compared to previous studies conducted in the same flock (17, 26) and is typical for the breed and in particular for flocks reared in Bulgaria (3, 4, 20).

In a similar study, Fodail et al. (13) also found no positive effect on lamb growth of feeding pollen to ewes before and after parturition, neither at the low dose of 4.5 g/ewe/day nor at the high dose of 9 g/ewe/day used by them. When lambs were fed with bee pollen, Gaafar et al. (14) found a significant positive effect on their productivity. Bee pollen supplementation can modulate key hematological parameters in ewes, improving antioxidant status through its bioactive compounds (13).

In our study, no statistically significant differences were found between the two groups in the biochemical profile of the sheep at the beginning and end of the experiment. Apart from glucose, which was lower in both measurements, the values of the tested parameters in both periods were within the reference range for these animals (18, 23). This finding indicates that the animals exhibited good overall health. It is known that biochemical parameters serve to assess the health status and metabolic activity of animals. Many physiological factors can affect the physiological levels of blood parameters (12).

Throughout the experiment, plasma glucose levels in both groups remained below the specie's reference values at both measurement points. According to various studies, a negative energy balance is often observed at the beginning of lactation (7, 12). Glucose is essential for protein production and lipid metabolism. The blood glucose level decreases due to continuous energy loss with milk in lactating ewes. Increased glucose utilization for lactose synthesis in milk and insufficient dietary intake disrupt glucose homeostasis (12). With a pronounced negative energy balance, intense lipomobilization most often results in a reduced rate of gluconeogenesis followed by hypoglycemia in early lactation (7). The two hormones that directly regulate blood glucose levels are glucagon and insulin, as well as adrenocorticotropic hormone (ACTH), glucocorticoids, adrenaline, and thyroxine. All of these raise blood glucose levels, whereas insulin alone lowers them (32).

Blood triglycerides are an important component in milk fat synthesis (12). In our study, triglyceride levels in animals of both groups were within the results in some studies (18, 28, 31) in both study periods. Triglyceride concentration decreases in early lactation compared to the gestation period due to increased energy expenditure for milk production and milk fat synthesis in addition to a lower supply of available free fatty acids (28). According to Antunović et al. (8), negative energy balance during lactation reduces triglycerides and cholesterol, whereas no such effect was observed in this study.

The increase in the activity of AST and ALT in the blood of lactating ewes indicates an increase in liver metabolism (8). According to our results, statistically significant differences were observed between the initial and final studies in the groups regarding triglycerides and AST. Significantly higher ALT activity as well as insignificant AST activity in the blood of lactating ewes may be related to diet quality (7). Castillo et al. (11) and Mašek et al. (22) observed decreased AST activity in the blood of ewes with advancing lactation. Similarly, Patkowski et al. (25) received lower ALT activity in the blood of sheep on day 40 of lactation. The more intense liver function of lactating ewes is attributed to the higher energy and protein requirements necessary for maintenance and milk production (7). Monitoring biochemical parameters in lactating ewes after feeding with bee pollen could be used to ensure optimal physiological responses and validate the efficacy of supplementation.

As a conclusion, bee pollen supplementation demonstrated no adverse effects on the health of ewes or their offspring. Furthermore, it may exert beneficial effects on productivity parameters. Further research is warranted to confirm these findings and elucidate optimal application strategies.

## Financial Support

This research received no grant from any funding agency.

## Ethical Statement

This study was approved under European and Bulgarian legislation concerning the protection of animals used for experimental and other scientific purposes. The protocol was based on permit no. 227 for the use of animals in experiments from the Bulgarian Food Safety Agency (statement No. 193 from the Bulgarian Animal Ethics Committee, protocol no. 18, July 2, 2020).

## Conflict of Interest

The authors declared that there is no conflict of interest.

## Author Contributions

MT, TI and RB conceived the idea and planned the manuscript. MT, and TI contributed to sample preparation. MT and TI have made significant scientific support and also contributed to the interpretation of the results. All authors provided significant contributions by giving feedback and help shape the manuscript.

## Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

## Animal Welfare

The authors confirm that they have adhered to ARRIVE Guidelines to protect animals used for scientific purposes.

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