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Effect of propolis supplementation on haematological and biochemical parameters in lambs

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Abstract

This study evaluated the effects of oral propolis supplementation on haematological and metabolic indicators and coagulation parameters in neonatal lambs. Forty healthy newborn lambs were randomly assigned to either a control group or a treatment group receiving 1.5 mL of propolis extract daily for 15 days. Blood samples were collected on days 0, 7, 21, and 45 to assess for changes over time. The results showed that propolis did not cause any harmful effects on liver or kidney function, and all blood parameters remained within normal physiological ranges. Although most changes in biochemical markers were time-related, lambs supplemented with propolis showed slight improvements in lipid metabolism, including numerically lower cholesterol and triglyceride levels at specific time points. Moreover, lambs in the propolis group tended to gain more weight over time, suggesting possible benefits for early growth. Minor differences were observed in some clotting parameters, but no adverse effects on blood coagulation were detected. These preliminary findings suggest that propolis may be considered a complementary strategy in lamb production, although further studies are needed to confirm its long-term benefits. Propolis-use may help facilitate a reduction in the use of synthetic additives in small ruminant production systems.

Keywords: antioxidant, alternative therapy, blood parameters, propolis

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Introduction

In recent years, there has been a notable increase in the pursuit of natural substitutes for synthetic growth enhancers and antibiotics in animal nutrition, driven by rising concerns about antimicrobial resistance and food safety. Of these natural alternatives, propolis – a resinous substance synthesised by bees from various plant materials – has garnered significant interest because of its wide-ranging biological properties, which include antimicrobial, antioxidant, anti-inflammatory, antithrombotic, and immunomodulatory effects (Ghisalberti, 1979; Castaldo & Capasso, 2002). Propolis is recognised as containing over 300 active compounds, such as flavonoids, phenolic acids, terpenoids, and essential oils, all of which contribute to its biological efficacy (Buratti *et al*., 2007).

The capacity of propolis to influence rumen microbial ecology, diminish harmful bacterial populations, and potentially improve nutrient utilisation has been extensively investigated, especially in

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small ruminants (Silva *et al*., 2015; Badawy, 2021). Evidence from both *in vivo* and *in vitro* research suggests that incorporating propolis into ruminant diets can result in favourable alterations in haematological and biochemical metrics. For example, da Silva *et al.* (2015) showed that ethanol extracts of propolis did not negatively impact dry matter digestibility or serum parameters, and assisted in maintaining the ruminal pH within physiological ranges. Furthermore, the addition of propolis resulted in stable blood glucose, urea, and total protein (TP) concentrations – factors closely linked to metabolic and liver health. In a similar vein, Badawy (2021) reported that propolis supplementation in pregnant ewes significantly enhanced nutrient digestibility, lowered ruminal ammonia-nitrogen and total bacterial counts, and improved the performance of both the ewes and their progeny without adversely affecting blood biochemical parameters. These results support the hypothesis that propolis can beneficially affect animal physiology and metabolism via its bioactive constituents.

In addition to its effects on digestion and metabolism, propolis has been demonstrated to modulate inflammatory and oxidative stress markers. A recent meta-analysis encompassing 14 randomised clinical trials indicated that propolis supplementation led to a significant decrease in insulin resistance, as well as in levels of fasting plasma glucose, liver enzymes, and proinflammatory cytokines such as tumour necrosis factor-α and interleukin-6 (Koya-Miyata *et al*., 2009; Hallajzadeh *et al*., 2021). Experimental studies in laboratory animals, especially rodents, have also demonstrated that propolis constituents, such as caffeic acid phenethyl ester and chrysin, inhibit platelet aggregation, influence coagulation pathways, and promote fibrinolytic activity (Kolayli *et al*., 2022). While most of these studies were performed on laboratory animals and humans, the mechanistic insights gained – particularly regarding the antioxidant and hepatoprotective properties of propolis – necessitate further exploration in veterinary settings. Despite the accumulation of evidence, studies assessing the haematological and biochemical impacts of propolis, specifically in lambs within practical husbandry environments, are scarce.

Because research on propolis supplementation to lambs is relatively new, and because the location, time, and method of propolis extraction influences its composition, human and animal studies yield varying results. The consensus is that propolis may be beneficial for metabolism. However, in human medicine, some studies have demonstrated that honey, propolis, or similar products can be dangerous for infants, causing illnesses such as infant botulism, and caution should thus be exercised when these products are used (Grant *et al*., 2013).

This study was conducted to investigate the effects of propolis on haematological, biochemical and coagulation parameters in neonatal lambs and to determine any possible side effects. Notably, it represents the first known investigation into the impact of propolis on lambs' coagulation factors. The findings are expected to improve health management practices in sheep production systems by elucidating the systemic biological responses to propolis administration. The enhanced haematological and biochemical stability associated with propolis use may support better growth performance, disease resilience, and overall productivity. This offers a natural and sustainable alternative to conventional additives in modern small ruminant husbandry.

Materials and methods

Animal material

This study was conducted with the approval of the Afyon Kocatepe University Animal Experiments Local Ethics Committee (approval number: 49533702/42).

A total of 40 clinically healthy singleton newborn lambs (n = 40) were selected from a private commercial sheep farm in Afyonkarahisar, Türkiye. All lambs were born to Merino ewes of a similar age (2–3 years). Only singleton lambs were included in the study to eliminate potential confounding effects related to birth weight and metabolic differences. The lambs remained with their dams throughout the 15-day experimental period. The animals were managed in an intensive production system in a naturally ventilated barn with group housing on straw bedding. Water was provided *ad libitum* to both ewes and lambs.

All ewes were given the same basal diet formulated to meet their nutritional requirements, while lambs were fed only their mothers' milk and had access to water. The ewe ration consisted of maize silage, wheat straw, alfalfa hay, barley meal, maize meal, cottonseed meal, soya bean meal, wheat bran, razmol, and a commercial vitamin-mineral premix. The approximate nutritional composition of the basal diet was as follows: 16% crude protein, 4.2% crude fat, 10.5% crude fibre, and 11.5 MJ

metabolisable energy per kilogram of dry matter. The feed was offered *ad libitum*, and no growth promoters or feed additives were used during the study period.

Lambs were randomly assigned to two groups (n = 20 per group). The experimental group received 1.5 mL of propolis extract (Bee & You®, 30% ethanol-based drops, SBS Bilimsel Bio Solutions AŞ) orally once daily for 15 consecutive days, while the control group received no supplementation. The dosage and duration of administration were based on the manufacturer's recommendations and previous literature (Cécere *et al.*, 2021; Linécio *et al.*, 2022).

Haematological and biochemical sampling

Clinical examinations performed included body temperature, pulse, and respiration measurements. Blood samples were collected from the jugular vein into ethylenediaminetetraacetic acid tubes for haematological analysis and serum tubes for biochemical and coagulation tests on days 0, 7, 21, and 45.

Haematological parameters such as the white blood cell count (WBC), lymphocyte percentage (Lymp%), mid-size cell percentage (MID%, including monocytes, eosinophils, and basophils), granulocyte percentage (Gran%), lymphocyte count (Lymp), mid-size cell count (MID), granulocyte count (Gran), haemoglobin, mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red blood cell count (RBC), mean corpuscular volume (MCV), red cell distribution width (RDW), haematocrit, platelet count (PLT), plateletcrit, and mean platelet volume (MPV) were comprehensively measured using the HumaCount® 80TS (Germany).

Serum samples were centrifuged and stored at −20 °C. Biochemical parameters, including glucose, TP, albumin, globulin, cholesterol, urea, triglyceride, low-density lipoprotein (LDL), high-density lipoprotein (HDL), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT) were analysed using a Randox® (Monaco) spectrophotometer.

Coagulation parameters, including prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen, and thrombin time (TT), were measured using the Coag 4D® (Hungary).

Statistical analyses

All statistical analyses were performed using the Statistical Package for the Social Sciences (version 16.0 for Windows; SPSS Inc., Chicago, IL, USA).

Parametric tests were used to analyse the data obtained in this study. The normality assumption for all variables was first tested by evaluating the kurtosis and skewness coefficients. The kurtosis and skewness values obtained were between −2 and +2, indicating that the relevant data sets were normally distributed and that parametric tests were appropriate (George & Mallery, 2010). A two-way repeated measures analysis of variance (ANOVA) was applied to assess the effects of group, time, and their interaction, including pairwise comparisons. The Tukey test was used to determine the significance of the differences.

The reported *P1* values indicate whether there was a significant difference between the propolis and control groups at each time point. In contrast, the *P2* values indicate the results of the two-way repeated measures ANOVA analysing the group differences, and examine how these differences changed over time. Thus, the *P2* value reveals not only the difference between the group means but also whether this difference varies depending on the trend over time. By accounting for repeated measurements across time, the within-subject variation of each individual is also determined.

The significance level was accepted as $P < 0.05$ for all statistical evaluations and all results are expressed as the mean ± the standard deviation.

Results

Serum biochemistry results

Evaluation of the biochemical parameter results revealed significant differences in the ALP (*P1* = 0.008), ALT (*P1* = 0.001), AST (*P1* = 0.001), GGT (*P1* = 0.001), and triglyceride levels (*P1* = 0.001) between the treatment groups at the different sampling times. In addition, the group × time interaction was statistically significant for TP, albumin, globulin, cholesterol, HDL, and LDL (*P2* <0.05). Although the results indicated statistically significant differences, an examination of Table 1 reveals that these differences predominantly occurred over time, rather than between the treatment groups.

Alanine aminotransferase and AST were present at similar levels in the propolis and control groups at the different sampling times, with some numerical differences but no statistically significant differences. Aspartate aminotransferase was numerically higher in the propolis group than in the control group on days 0, 7, and 45. Treatment group differences were also found for the TP (*P1* = 0.001), albumin (*P1* = 0.001), and globulin (*P1* = 0.001) levels and, in weekly comparisons, differences were observed in the cholesterol, HDL, LDL, and glucose levels (*P1* = 0.001). Moreover, while there were statistically significant differences in the glucose levels between day 0 and days 21 and 45 in the propolis-supplemented group, the control group's results were similar between days 0 and 45. There was a difference in the triglyceride levels in the propolis group between days 0 and 45, and in the control group between days 0 and 21. However, there were no significant differences between the control and propolis groups at any of the time points for this parameter. The cholesterol concentrations decreased significantly in the propolis group between days 7 and 45, and when days 21 and 45 were compared, the propolis group had numerically (but not statistically) lower values than the control group, reversing the trend seen on days 0 and 7. A significant decrease in the HDL concentration was found between days 7 and 45 in the propolis group, but a significant difference between the treatment groups was only seen on day 0. The LDL concentration was significantly lower in the propolis group on days 21 and 45 than on day 7, whereas the concentration in the control group did not significantly vary over the trial period. These findings suggest that propolis causes differential biochemical changes in these parameters over time. However, no significant differences were observed between the groups or in the group × time interactions for renal function indicators such as urea and blood urea nitrogen (BUN) (*P1* and *P2* >0.05).

Live weight during the trial period differed between the treatment groups at the different time points (*P1* <0.050). As expected, a statistical difference was found between days 7 and 45 in both groups, but, numerically, lambs in the propolis group weighed more on days 7, 21, and 45 (Table 1).

Haematological results

Differences in the WBC (*P1* = 0.001), Lymp% (*P1* = 0.001), MID% (*P1* = 0.003), Gran% (*P1* = 0.001), Lymp (*P1* = 0.001), and Gran (*P1* = 0.045) values were observed between the treatment groups at the different sampling times. No differences were observed in the haemoglobin (*P1* = 0.052), MCHC (*P1* = 0.061), RBC (*P1* = 0.631), haematocrit (*P1* = 0.061), plateletcrit (*P1* = 0.070), or platelet distribution width – standard deviation (PDWs) (*P1* = 0.144) values. However, differences were observed in the MCV (*P1* = 0.001), RDWs (*P1* = 0.001), RDWc (*P1* = 0.017), PLT (*P1* = 0.001), platelet distribution width – coefficient of variation (PDWc) (*P1* = 0.001), and MPV (*P1* = 0.003) values (Table 2). The Lymp%, Gran%, and Lymp were also found have statistically significant group × time interactions (*P2* <0.05). These results suggest that propolis affected how these parameters changed over time. However, for some parameters (e.g. the haemoglobin, MCHC, RBC, haematocrit, plateletcrit, and PDWs), no significant differences were found either between the groups or in the group × time interaction (*P1* and/or *P2* >0.05).

Coagulation factor results

According to the analysis of the coagulation parameters presented in Table 3, significant differences in the PT and TT values (*P1* <0.05) were found between the groups. The group × time interaction for the PT also tended towards significance (*P2* = 0.055), suggesting that the time-dependent effect of propolis administration may be substantial. No significant differences were observed in the APTT and fibrinogen values, either between the groups or in the group × time interaction (*P1* and *P2* >0.05).

Table 1 Serum biochemistry and live weight values (mean ± standard deviation) for propolis-supplemented and control lambs over a 45-day trial period

	Propolis-0	Propolis-7	Propolis-21	Propolis-45	Control-0	Control-7	Control-21	Control-45	*P1*	*P2*
Urea (mg/dL)	25.5 ± 11.7	25.8 ± 11.9	24.4 ± 12.6	29.4 ± 10.2	25.1 ± 5.1	28.0 ± 14.5	24.2 ± 8.0	38.1 ± 9.3	0.206	0.444
ALP (U/L)	1611.0^{ab} ± 676.0	1540.2^{ab} ± 495.2	1260.4^{ab} ± 475.0	1188.0^{ab} ± 344.2	1902.3^{a} ± 450.9	890.0^{b} ± 145.2	1443.7^{ab} ± 440.6	1087.2^{b} ± 395.9	0.008	0.068
ALT (U/L)	8.7^{a} ± 3.1	6.6^{abc} ± 2.4	5.7^{bc} ± 1.7	8.2^{ab} ± 1.9	6.5^{abc} ± 1.1	5.2^{c} ± 1.2	6.0^{abc} ± 1.4	6.5^{abc} ± 2.0	0.001	0.301
AST (U/L)	82.0^{a} ± 26.5	56.8^{bcd} ± 11.7	47.7^{cd} ± 10.4	81.4^{a} ± 20.1	68.3^{abc} ± 15.6	45.6^{d} ± 8.0	49.9^{cd} ± 4.2	73.2^{ab} ± 16.8	0.001	0.154
GGT (U/L)	316.1^{a} ± 279.4	126.6^{ab} ± 128.8	58.5^{b} ± 25.5	56.9^{b} ± 12.6	136.1^{ab} ± 85.8	90.5^{b} ± 34.7	58.7^{b} ± 16.2	60.1^{b} ± 16.7	0.001	0.183
TRIG (mg/dL)	81.6^{a} ± 39.5	54.4^{ab} ± 25.2	44.6^{ab} ± 20.4	39.8^{b} ± 15.8	81.1^{a} ± 46.9	49.1^{ab} ± 19.2	40.0^{b} ± 15.3	50.8^{ab} ± 29.2	0.001	0.704
TP (g/dL)	5.8^{a} ± 0.7	5.2^{ab} ± 0.5	4.7^{b} ± 0.4	5.1^{ab} ± 0.3	5.0^{b} ± 0.4	5.0^{b} ± 0.5	5.1^{ab} ± 0.3	4.9^{b} ± 0.5	0.001	0.000
ALB (g/dL)	2.6^{d} ± 0.2	2.8^{bcd} ± 0.1	3.17^{ab} ± 0.1	3.1^{bc} ± 0.3	2.6^{d} ± 0.3	2.8^{cd} ± 0.1	3.4^{a} ± 0.1	3.1^{bc} ± 0.2	0.001	0.046
Glo (g/dL)	3.2^{a} ± 0.7	2.3^{b} ± 0.5	1.5^{c} ± 0.2	2.0^{bc} ± 0.3	2.3^{b} ± 0.6	2.2^{bc} ± 0.5	1.6^{c} ± 0.2	1.8^{bc} ± 0.3	0.001	0.001
ALB/Glo	0.8^{d} ± 0.2	1.2^{cd} ± 0.3	2.1^{a} ± 0.3	1.5^{bc} ± 0.3	1.2^{cd} ± 0.3	1.3^{c} ± 0.3	2.0^{a} ± 0.4	1.7^{ab} ± 0.3	0.001	0.218
CHOL (mg/dL)	90.9^{a} ± 27.1	113.9^{a} ± 28.2	69.1^{bcd} ± 24.0	47.3^{d} ± 13.2	66.2^{bcd} ± 23.6	82.5^{abc} ± 38.2	91.7^{ab} ± 19.0	50.9^{cd} ± 12.4	0.001	0.002
HDL (mg/dL)	31.1^{a} ± 11.6	41.1^{a} ± 7.3	29.5^{ab} ± 10.2	12.7^{c} ± 5.2	21.1^{bc} ± 13.3	31.4^{ab} ± 11.2	33.6^{ab} ± 9.9	13.9^{c} ± 4.2	0.001	0.024
LDL (mg/dL)	43.4^{ab} ± 18.6	59.7^{a} ± 23.3	30.6^{b} ± 16.3	26.6^{b} ± 6.9	28.8^{b} ± 15.7	41.2^{ab} ± 27.7	50.0^{ab} ± 8.8	26.8^{b} ± 8.8	0.001	0.002
BUN (mg/dL)	11.9 ± 5.4	12.1 ± 5.5	11.4 ± 5.8	13.7 ± 4.8	11.7 ± 2.4	13.0 ± 6.7	11.3 ± 3.7	17.8 ± 4.3	0.205	0.444
GLU (mg/dL)	114.5^{a} ± 19.9	102.0^{ab} ± 12.3	77.1^{c} ± 13.3	85.5^{bc} ± 19.7	94.6^{abc} ± 20.0	98.1^{abc} ± 12.8	82.6^{bc} ± 8.0	77.0^{c} ± 10.0	0.001	0.066
Weight (kg)	7.0^{c} ± 2.2	8.9^{bc} ± 2.3	12.1^{ab} ± 3.3	16.6^{a} ± 4.6	6.4^{c} ± 2.9	7.9^{bc} ± 2.7	10.8^{bc} ± 3.3	15.8^{a} ± 4.9	0.001	-

^{a,b,c,d} Means in the same row with different superscript letters differ significantly according to Tukey's test (P <0.05). ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma-glutamyl transferase; TRIG: triglycerides; TP: total protein; ALB: albumin; Glo: globulin; CHOL: cholesterol; HDL: high-density lipoprotein; LDL: low-density lipoprotein; BUN: blood urea nitrogen; GLU: glucose. *P1*: significance level for one-way ANOVA; *P2*: significance level for repeated measures two-way ANOVA.

Table 2 Haematology findings (mean ± standard deviation) for propolis-supplemented and control lambs over a 45-day trial period

	Propolis-0	Propolis-7	Propolis-21	Propolis-45	Control-0	Control-7	Control-21	Control-45	*P1*	*P2*
WBC (10^9/L)	$7.2^{b} \pm 4.0$	$7.1^{b} \pm 3.2$	$7.7^{ab} \pm 2.0$	$11.3^{a} \pm 1.0$	$7.0^{b} \pm 2.2$	$9.3^{ab} \pm 3.5$	$5.9^{b} \pm 1.7$	$11.4^{a} \pm 0.7$	0.001	0.204
Lymp%	$59.1^{bc} \pm 18.9$	$78.6^{a} \pm 8.6$	$80.1^{a} \pm 6.6$	$73.7^{ab} \pm 12.7$	$72.3^{ab} \pm 6.9$	$53.9^{c} \pm 22.6$	$79.4^{a} \pm 4.9$	$76.8^{a} \pm 4.3$	0.001	0.000
MID%	$15.2^{a} \pm 6.6$	$12.6^{ab} \pm 7.0$	$10.6^{ab} \pm 4.0$	$7.5^{b} \pm 3.5$	$11.0^{ab} \pm 4.6$	$14.1^{ab} \pm 6.8$	$13.0^{ab} \pm 3.9$	$8.0^{ab} \pm 1.5$	0.003	0.339
Gran%	$25.0^{ab} \pm 19.8$	$8.7^{bc} \pm 4.3$	$9.2^{bc} \pm 5.8$	$18.6^{abc} \pm 13.8$	$16.5^{abc} \pm 8.4$	$31.8^{a} \pm 22.4$	$7.5^{c} \pm 1.8$	$15.1^{abc} \pm 3.1$	0.001	0.000
Lymp (10^9/L)	$12.8^{abc} \pm 8.2$	$17.4^{ab} \pm 9.9$	$23.2^{a} \pm 8.8$	$9.0^{bc} \pm 6.2$	$17.4^{ab} \pm 7.8$	$11.1^{bc} \pm 8.4$	$22.4^{a} \pm 4.6$	$6.2^{c} \pm 1.9$	0.001	0.013
MID (10^9/L)	$2.8^{abc} \pm 2.1$	$2.9^{abc} \pm 2.2$	$3.1^{ab} \pm 1.8$	$0.8^{bc} \pm 0.5$	$2.3^{abc} \pm 1.0$	$2.3^{abc} \pm 1.6$	$3.3^{a} \pm 1.3$	$0.6^{bc} \pm 0.3$	0.001	0.615
Gran (10^9/L)	$3.5^{ab} \pm 2.9$	$1.8^{ab} \pm 1.5$	$2.3^{ab} \pm 2.3$	$2.4^{ab} \pm 3.1$	$3.7^{ab} \pm 1.7$	$4.8^{a} \pm 3.1$	$2.0^{ab} \pm 0.3$	$1.2^{b} \pm 0.7$	0.045	0.041
Hb (g/dL)	12.1 ± 1.8	11.1 ± 1.2	11.2 ± 0.9	11.8 ± 0.8	12.9 ± 1.9	11.4 ± 1.7	11.2 ± 1.1	12.0 ± 0.8	0.052	0.519
MCH (pg)	$13.1^{abc} \pm 2.2$	$12.1^{abc} \pm 2.8$	$14.8^{ab} \pm 5.8$	$9.8^{bc} \pm 0.6$	$14.5^{abc} \pm 3.4$	$14.0^{abc} \pm 4.5$	$15.3^{a} \pm 6.2$	$9.6^{c} \pm 0.6$	0.001	0.648
MCHC (g/dL)	38.4 ± 4.1	35.8 ± 8.1	42.1 ± 10.5	34.8 ± 1.6	39.8 ± 6.9	40.8 ± 9.0	42.4 ± 11.3	35.8 ± 2.3	0.061	0.695
RBC (10^{12}/L)	7.1 ± 3.2	8.3 ± 2.5	7.7 ± 1.8	8.4 ± 2.2	8.4 ± 2.1	8.0 ± 2.2	6.6 ± 1.7	7.8 ± 2.5	0.631	0.781
MCV (fL)	$34.1^{a} \pm 3.2$	$32.2^{ab} \pm 4.0$	$34.3^{a} \pm 5.6$	$28.2^{b} \pm 1.7$	$36.3^{a} \pm 3.3$	$33.8^{a} \pm 2.9$	$35.0^{a} \pm 5.6$	$27.0^{b} \pm 2.7$	0.001	0.382
RDWs (fL)	$17.4^{a} \pm 4.7$	$17.7^{a} \pm 4.1$	$12.4^{abc} \pm 3.8$	$10.3^{bc} \pm 3.8$	$16.2^{ab} \pm 3.3$	$16.5^{ab} \pm 3.0$	$9.2^{c} \pm 7.1$	$9.0^{c} \pm 2.4$	0.001	0.542
RDWc (%)	$27.0^{ab} \pm 3.9$	$29.0^{a} \pm 3.7$	$27.0^{ab} \pm 4.2$	$27.2^{ab} \pm 2.8$	$24.0^{ab} \pm 2.7$	$26.6^{ab} \pm 1.8$	$21.6^{b} \pm 8.0$	$28.0^{a} \pm 4.5$	0.017	0.205
HCT (%)	31.7 ± 5.0	29.6 ± 5.2	28.1 ± 6.6	33.5 ± 4.5	31.7 ± 5.1	28.9 ± 6.5	27.7 ± 7.9	33.7 ± 2.8	0.061	0.858
PLT (10^9/L)	$393.2^{bc} \pm 159.4$	$477.8^{abc} \pm 139.5$	$543.4^{ab} \pm 145.7$	$636.6^{a} \pm 138.4$	$322.7^{c} \pm 149.9$	$495.8^{abc} \pm 159.5$	$546.1^{ab} \pm 164.1$	$617.0^{a} \pm 131.6$	0.001	0.335
PCT (%)	0.2 ± 0.1	0.3 ± 0.4	0.6 ± 0.5	0.3 ± 0.8	0.3 ± 0.4	0.4 ± 0.5	0.7 ± 0.6	0.3 ± 0.0	0.070	0.972
PDWs (fL)	5.6 ± 1.1	4.6 ± 0.7	5.3 ± 2.7	5.7 ± 0.4	5.5 ± 0.9	4.6 ± 1.3	4.1 ± 1.5	5.3 ± 0.9	0.144	0.260
PDWc (%)	$32.8^{a} \pm 3.3$	$29.7^{ab} \pm 3.4$	$26.2^{b} \pm 5.1$	$33.0^{a} \pm 3.1$	$31.8^{a} \pm 4.4$	$30.9^{ab} \pm 2.5$	$29.5^{ab} \pm 3.9$	$32.3^{a} \pm 2.4$	0.001	0.595
MPV (fL)	$6.3^{ab} \pm 3.8$	$6.9^{ab} \pm 5.2$	$12.9^{a} \pm 8.4$	$5.4^{b} \pm 0.2$	$8.1^{ab} \pm 6.2$	$7.6^{ab} \pm 6.0$	$9.5^{ab} \pm 6.0$	$5.2^{b} \pm 0.2$	0.003	0.192

[a,b,c] Means in the same row with different superscript letters differ significantly according to Tukey's test (P <0.05). WBC: white blood cell count; Lymp%: lymphocyte percentage; MID%: mid-size cell percentage (including monocytes, eosinophils, and basophils); Gran%: granulocyte percentage; Lymp: lymphocyte count; MID: mid-size cell count; Gran: granulocyte count; Hb: haemoglobin; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration; RBC: red blood cell count; MCV: mean corpuscular volume; RDWs: red cell distribution width – standard deviation; RDWc: red cell distribution width – coefficient of variation; HCT: haematocrit; PLT: platelet count; PCT: plateletcrit; PDWs: platelet distribution width – standard deviation; PDWc: platelet distribution width – coefficient of variation; MPV: mean platelet volume. *P1*: significance level for one-way ANOVA; *P2*: significance level for repeated measures two-way ANOVA.

Table 3 Coagulation factor findings (mean ± standard deviation) for propolis-supplemented and control lambs over a 45-day trial period

	Propolis-0	Propolis-7	Propolis-21	Propolis-45	Control-0	Control-7	Control-21	Control-45	*P1*	*P2*
PT (sec)	$28.1^{b} \pm 6.1$	$42.5^{ab} \pm 5.2$	$37.2^{ab} \pm 14.7$	$46.1^{ab} \pm 6.1$	$37.0^{ab} \pm 3.2$	$26.0^{b} \pm 4.1$	$46.0^{ab} \pm 3.6$	$53.6^{a} \pm 8.5$	0.007	0.055
APTT (sec)	39.9 ± 14.1	40.6 ± 9.2	37.5 ± 4.7	33.6 ± 5.5	41.9 ± 9.6	41.0 ± 7.3	38.0 ± 3.6	35.1 ± 2.6	0.894	0.182
FIB (sec)	14.2 ± 2.5	21.2 ± 6.6	19.5 ± 9.2	16.1 ± 2.0	11.3 ± 1.4	12.2 ± 4.5	11.6 ± 1.2	16.9 ± 1.2	0.103	0.292
TT (sec)	$29.6^{a} \pm 4.3$	$19.6^{b} \pm 2.8$	$20.6^{ab} \pm 3.8$	$27.8^{ab} \pm 0.4$	$23.0^{ab} \pm 6.1$	$20.6^{ab} \pm 5.1$	$23.8^{ab} \pm 1.9$	$24.0^{ab} \pm 1.4$	0.007	0.170

[a,b] Means in the same row with different superscript letters differ significantly according to Tukey's test ($P < 0.05$). PT: prothrombin time; APTT: activated partial thromboplastin time; FIB: fibrinogen; TT: thrombin time. *P1*: significance level for one-way ANOVA; *P2*: significance level for repeated measures two-way ANOVA.

Discussion

The current research examined the impact of dietary propolis supplementation on haematological, biochemical, and coagulation metrics in lambs. The results indicate that although specific parameters, including BUN and urea levels, did not change, several critical biomarkers, including liver enzymes, serum proteins, lipid profiles, and blood glucose levels, demonstrated enhancements in the group treated with propolis.

In previous studies on propolis extract, it has been found that propolis has a hepatoprotective effect on normal cells (Zhao *et al.,* 2009; Bhadauria, 2012; Silva *et al.,* 2019d). In addition, de Melo Garcia *et al.* (2022) reported that no histopathological changes were observed in the liver tissue of sheep treated with propolis, supporting the hepatoprotective effect of propolis at the tissue level. Hallajzadeh *et al.* (2021) reported that propolis supplementation decreased the concentration, on average, of ALT by 2.58 U/L, of AST by 1.84 U/L, and of ALP by 24.9 U/L. The results obtained in this study do not directly support the existence of a hepatoprotective effect of propolis. While there were some statistically significant differences, an examination of Table 1 reveals that most of the differences found occurred over time, rather than being differences between the treatment groups. Previous studies have reported that ALP and GGT levels in newborn lambs receiving colostrum may be affected by colostrum intake (Maden *et al*., 2004). We therefore conclude that the changes in ALP and GGT levels over time found in both the propolis and control groups were shaped by the lambs' colostrum intake.

Comparing the levels of liver parameters from previous studies to those found in the current study, we cannot conclude that propolis supplementation had a direct hepatoprotective effect on the newborn lambs. The hepatoprotective effect found in previous studies may have been due to the variety of propolis used, the amount of propolis administered, and the age of the animals used. Although no liver-protective effect was observed, liver parameters remained within reference ranges in both the propolis-supplemented and control lambs. Therefore, propolis supplementation did not damage the livers of the newborn lambs.

Castaldo & Capasso (2002) reported that the flavonoids present in propolis act as immunostimulants, exerting regulatory effects on the immune system. In ruminants, serum immunoglobulin-G levels are utilised to identify passive transfer deficiencies. Furthermore, it has been reported that estimated serum immunoglobulin-G levels can serve as indicators of immunoglobulin concentrations, as the absorption of immunoglobulins from colostrum increases the TP levels in the blood (Hogan *et al.,* 2015; Aydoğdu *et al.,* 2019). Although immunoglobulin-G was not measured in this study, propolis supplementation did not change TP levels, and we therefore cannot say that propolis supplementation affected immunoglobulin levels.

It has been reported that, depending on the dosage and duration, propolis supplementation can affect lipid metabolism (Samadi *et al*., 2017; Al-Tamimi, 2020; Salehi-Sahlabadi *et al*., 2020). Previous studies have reported that triglyceride, HDL, LDL, and cholesterol concentrations were lower in propolis-treated groups than in the control group (Tsamesidis *et al*., 2022). Moreover, de Melo Garcia *et al*. (2022) showed that propolis supplementation improved intestinal morphology, increasing nutrient absorption efficiency and possibly indirectly contributing to lipid profile improvements. In another study, it was reported that propolis has hypolipidemic effects and improves liver lipid clearance by inhibiting lipid peroxidation (Hallajzadeh *et al*., 2021). Since propolis was administered orally for 15 days in our study, the results would be most relevant on day 21, and cholesterol, LDL, and HDL concentrations were found to be numerically (but not significantly) lower in the lambs treated with propolis at this time. However, the differences between the propolis and control groups remained time-dependent, and no statistically significant differences were observed. While previous studies have suggested positive effects of propolis supplementation on lipid metabolism, our study did not yield results directly consistent with this information. These conflicting results may be explained by the dosage and duration of propolis application used in this study. Nonetheless, propolis supplementation to newborn lambs did not have a detrimental effect on the lipid profile, and may have been somewhat beneficial at specific times.

The levels of BUN and urea did not show statistically significant differences between the control and propolis groups; this indicates that the propolis did not impose a harmful burden on the renal system and is thus a safe supplement. Similarly, de Melo Garcia *et al*. (2022) reported that they did not observe any histopathological changes in the kidney tissues of animals supplemented with propolis. Propolis supplementation did not harm kidney function in the newborn lambs, aligning with the findings of previous studies.

A performance study by Abdlazez & Saleh (2016) showed that the daily administration of propolis extract to lambs resulted in a permanent and significant increase in their live weights. Similarly, de Melo Garcia *et al.* (2022) reported that the ruminal papilla flaps and exit passages of sheep fed with propolis increased, leading to 20.24% more weight gain than the control group. However, Silva *et al.* (2019a) reported no changes in lambs supplemented with 15 mL of brown propolis extract per kilogram of dry matter. Although the highest live weights were measured in the lambs supplemented with propolis, the results of this study are consistent with those of Silva *et al*. (2019a), as propolis supplementation did not cause a significant increase in the live weight gain of the lambs. However, the live weight gain observed in previous studies was likely related to the age and breed of the animals used, as well as the type and amount of propolis used.

It has been reported that exogenous agents can alter haematological parameters such as the RBC, haemoglobin, and WBC counts (Talas & Gulhan, 2009). No statistically significant differences were found between the treatment groups in the RBC, haemoglobin, plateletcrit, PDW, and MCHC values in this study. Statistically significant differences were found within the groups for other haematological parameters (Table 2). However, no adverse effects of propolis administration on the monitored blood parameters were detected, and the values measured in both groups were found to be within the reference ranges for these parameters. In conclusion, while some changes were observed in the haematological parameters of the lambs receiving propolis supplementation, these parameters remained within reference limits, suggesting that propolis supplementation had no adverse effects on the newborn lambs.

The impact of propolis supplementation on coagulation factors in lambs was examined, revealing statistically significant differences between the groups at the different sampling times in the PT and TT parameters. No previous studies have investigated the effects of propolis application on the coagulation factors of lambs. Tsamesidis *et al.* (2022) reported higher ferritin levels in donors supplemented with propolis, and the numerically (but not significantly) lower PT and TT values observed in the lambs treated with propolis after 21 days in this study are believed to stem from the positive effects of propolis on ferritin levels. Furthermore, previous research has indicated that caffeic acid phenethyl ester regulates platelet aggregation and coagulation cascades (Zhang *et al*., 2017; Okhura *et al*., 2020; Pérez *et al*., 2023). The lower PT and TT values may thus indicate a mild anticoagulant or fibrinolytic effect of propolis components. However, further detailed studies are necessary to clarify this issue, and our study concludes that propolis administration does not negatively affect clotting factors in lambs.

Conclusions

Propolis supplementation in neonatal lambs did not cause any adverse effects on liver, kidney, haematological, or coagulation parameters. While most changes were time-related, propolis demonstrated potential benefits for lipid metabolism and live weight gain during specific periods. These findings suggest that propolis can be safely used as a natural supplement in lamb production systems to support metabolic health and overall performance. Further long-term studies may clarify its role in improving the growth efficiency and disease resilience of lambs.

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Authors' contributions

A.C.T., E.K., and F.M.B.: Conception and design of the study, data acquisition, and manuscript drafting. A.C.T. and F.M.B.: Analysis and/or interpretation of the data and critical review/revision. All authors reviewed the manuscript and gave it their final approval.

Conflict of interest statement

The authors declare that they have no conflicts of interest.

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