

**INFLUENCE OF SILYMARIN ON THE HUMORAL
IMMUNITY OF LAMBS LACAUNE BREED DURING
THE THERMONEUTRAL, HOT, AND COLD SEASONS**

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Abstract

Infectious diseases cause devastating economic losses in the sheep industry. The current study aimed to evaluate blood serum concentrations of lysozyme, gamma interferon, immunoglobulin G, immunoglobulin M, cortisol, serotonin, the activity of the alternative pathway of complement activation (APCA), and β -lysine activity in lambs, whose rations were supplemented with 2 g/kg Silymarin for 60 days – from the 15th day to the 75th day after birth in manure-pasture cultivation during a thermoneutral, hot summer and cold winter period. The lambs were divided into two groups – control and experimental. To the experimental animals, after 15 days of age, Silymarin was added to the standard concentrated feed, at a dose of 2 g/kg feed. It was found that Silymarin reliably increases the values of the investigated indicators in all three annual seasons. It could be concluded that the studied Silymarin possesses an important immunomodulating potential in lambs, which could improve their health.

Key words: lambs, silymarin, lysozyme, complement, β -lysine, IgG, IgM, cortisol, serotonin

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Introduction. For the last few decades, the ban on using antibiotics in livestock husbandry has increased the interest in biotechnological and natural products to improve birds' productivity, health, quality, and safety of produce [1]. Immunomodulators constitute a new class of growth promoters that recently have gained importance in food industry to produce functional foods. Research has been focused mainly on the effect of immunomodulators on mortality, stress hormones, blood, muscle metabolism, and even the immune system function of domestic animals. The alternative pathway of complement activation (APCA) is known to be an important factor in innate immunity. It is active against Gram-negative bacteria, viruses, virus-infected cells, neoplastic cells, agarose, lipopolysaccharides, contrast media used in radiology, etc. [2]. GREWAL and BABIUK [3] have studied the cytotoxic effect of neutrophils against herpes virus-infected cells and reported that it was dependent not only on the classical pathway of complement activation but on APCA as well. The antiviral activity of APCA was confirmed by the experiment of OHTA et al. [4]. They treated cell cultures of chicken embryos infected by the fowlpox virus with normal chicken serum and observed the lack of cytopathogenic effect, viral growth, and plaque formation [2]. Lysozyme is an enzyme that breaks down bacterial cell walls by attacking peptidoglycan. The increased serum concentration of lysozyme serves as an indirect marker of inflammation and provides information on the activity of granulocytes and the functionality of the monocyte-macrophage system. In addition, it is a potential indicator of the number of pathogens in the environment [5]. The stimulating effect of Silymarin on the live weight of lambs is due to its strong antioxidant and hepatoprotective action, as well as its antitoxic effect. Data on the stimulatory effect of Silymarin on ruminant productivity are presented by KIM et al. [6] who investigated the effect of herbal plant supplements including Silymarin on blood metabolites and carcass characteristics during the late feeding period in castrated male Hanu cattle. After 6 months of dietary supplementation, the authors found a decrease in the activity of alanine aminotransferase (an important liver enzyme) and an increase in calf growth in the Silymarin-supplemented group compared to the control group. Therefore, Silymarin can be used as an effective, harmless feed additive, as an alternative to nutritional antibiotics, to stimulate productivity during the late feeding stage of male castrated Hanu cattle. The strong antioxidant effect of Silymarin is associated with a reduction of lipid peroxidation and MDA levels in blood and tissues and thus with a reduction of high levels of free radicals, lipid peroxidation, and protein carbonylation, which lead to DNA strand damage and pathology in the body [7]. In addition, KHAMISABADI [8] found that Silymarin reduced HSP 70 gene expression in sheep blood serum (reducing HSP 70 mRNA levels). Silymarin supplementation may therefore reduce oxidative stress and heat shock protein activity and thus improve the welfare of sheep under severe and prolonged stress. KOSINA et al. [9] and GRELA et al. [10] present additional data on growth promotion in rabbits and pigs.

The current study aimed to evaluate blood serum concentrations of lysozyme, interferon gamma, immunoglobulin G, immunoglobulin M, the activity of the alternative pathway of complement activation (APCA), β -lysine activity, cortisol, and serotonin in lambs, whose rations were supplemented with 2g/kg Silymarin.

Materials and methods. Experimental design. The experiment was carried out in 2022, with 12 female lambs of the French Lacaune breed, during manure-pasture rearing on a private sheep farm. The area is located in the transitional Mediterranean climate region, characterized by warm and dry summers and mild winters. The lambs were divided into two groups – control and experimental. For the experimental animals, after 15 days of age, Silymarin was added in a dose of 2 g/kg feed to the standard concentrated feed (corn, wheat, barley, sunflower meal, soybean meal, bran, chalk, salt, monocalcium phosphate, vitamin-mineral premix; guaranteed analysis (in %): crude protein – 15.50, raw ash – 5.00, raw fibres – 6.20, calcium – 0.67, phosphorus – 0.57). In addition to the concentrated fodder, the lambs were also fed good quality meadow hay. The lambs were reared in group pens, with an area of 2 m² provided per animal, with a norm of 0.7–1 m² (Regulation No. 44/2006). Lambs had ad libitum access to feed and water and lighting was provided continuously. The experiments were conducted within standard ethical norms and no lambs were subjected to undue stress. The minimum requirements for the protection and welfare of experimental animals and the requirements for facilities for their use, keeping, and/or supply are set out in Ordinance No. 20 of 1.11.2012 on the minimum requirements for protection and welfare of experimental animals and the requirements for sites for use (8.1.2018), breeding and/or delivery, which transposes Directive 2010/63/EU.

Assay methods. Blood samples for immunology research and hormone research (serotonin and cortisol) were taken during a thermoneutral period (May 21, 2022), during a hot summer period (July 23, 2022) and during a cold winter period (January 3, 2023) from the jugular vein (v. jugularis) of six lambs from each group in sterile vacuum containers with anticoagulant (for the study of serotonin and cortisol concentrations) and without anticoagulant (for the immunological parameters). Serum lysozyme concentrations were determined by the method of LIE et al. [11], the alternative pathway of complement activation (APCA) was evaluated by the method of SOTIROV [12], and β -lysine activity was assessed by the method of BUHARIN et al. [13]. Our previous publication described these methods in detail [14]. Concentrations of interferon gamma (IFN- γ), immunoglobulin G (IgG), immunoglobulin M (IgM), cortisol, and serotonin in lamb's samples were determined by ELISA tests mentioned below:

1. Sheep IFN α /Interferon Alpha ELISA Kit – LS-F74396 (LSBio, USA)
2. Sheep IFN γ /Interferon Gamma ELISA Kit – LS-F50569 (LSBio, USA)

3. Sheep IgG (Sandwich ELISA) ELISA Kit – LS-F32254 (LSBio, USA)
4. Sheep IgM (Sandwich ELISA) ELISA Kit – LS-F44932 (LSBio, USA)

Silymarin is an extract from the seeds of the milk thistle plant *Silybum marianum* L. Gaertn. The product used in the experiment was manufactured by the Chinese company Wuqiao West Road Wuxi Jiangsu China.

Statistical analysis. Data were processed by one-way analysis of variance (ANOVA) with the fixed effect model using the Data analysis tool pack, Microsoft Excel 2016, Microsoft Corporation Ltd. at a level of significance $P < 0.05$.

Results. The results presented in Table 1 show that no significant differences were observed for lysozyme concentrations between tested seasons in the control groups. Lysozyme concentrations in the group treated with Silymarin are significantly higher than the control one ($P < 0.001$). Looking at the results for the alternative pathway for complement activation in the experimental group, a significant increase in the values of this indicator was observed consistently from the thermoneutral to the cold winter period ($P < 0.001$). The same was observed in the control group but with lower values ($P < 0.05$). For β -lysine, significant differences were found between the studied seasons in the control group for the thermoneutral period ($P < 0.001$), but the group treated with Silymarin showed significantly higher values compared to the control group ($P < 0.001$). IgG in the control group in winter showed a significant increase in blood serum concentration

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Influence of Silymarin on some humoral factors of immunity in lambs (S \pm Sx)

Seasons	Groups	<i>n</i>	Thermoneutral period	Hot summer period	Cold winter period
Lysozyme (mg/L)	Silymarin	6	0.18 \pm 0.02***	0.20 \pm 0.03***	0.18 \pm 0.03***
	Control	6	0.07 \pm 0.01	0.08 \pm 0.01	0.07 \pm 0.01
APCA (CH50)	Silymarin	6	221.14 \pm 1.87***	210.61 \pm 0.95***	230.80 \pm 1.61***
	Control	6	178.60 \pm 6.80	188.94 \pm 4.98	206.04 \pm 6.35*
β -lysine (%)	Silymarin	6	18.01 \pm 1.01**	13.61 \pm 0.94***	16.21 \pm 0.97
	Control	6	17.20 \pm 0.83***	7.01 \pm 0.34	13.37 \pm 1.11
IgG (mg/ml)	Silymarin	6	21.87 \pm 0.14	20.96 \pm 0.19	25.55 \pm 0.20***
	Control	6	20.94 \pm 0.70	19.52 \pm 0.76	24.44 \pm 0.72***
IgM (mg/ml)	Silymarin	6	59.38 \pm 0.65	69.71 \pm 0.91***	64.53 \pm 0.69
	Control	6	59.09 \pm 1.05	68.15 \pm 1.53***	62.94 \pm 1.63
IFN- γ (pg/ml)	Silymarin	6	10.97 \pm 2.72	31.78 \pm 2.11***	19.71 \pm 1.13
	Control	6	10.23 \pm 2.81	33.39 \pm 1.60***	20.65 \pm 1.01
Cortisol (ng/ml)	Silymarin	6	16.20 \pm 0.51	19.57 \pm 0.67**	17.95 \pm 0.28
	Control	6	26.75 \pm 0.42	32.97 \pm 0.56	39.37 \pm 0.31***
Serotonin (ng/ml)	Silymarin	6	109.84 \pm 1.50	104.32 \pm 1.50	110.97 \pm 1.19***
	Control	6	99.08 \pm 1.96***	71.73 \pm 2.03	62.44 \pm 2.27

*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$

($P < 0.001$). The experimental group observed the same but with significantly higher values ($P < 0.001$). The dynamics in the concentration of IgM was similar to that of IgG, but for this indicator, the highest values in both groups were observed during the hot summer season ($P < 0.001$). If comparing the control with the experimental groups, no differences were found in both indicators. Similar to IgM are the changes in IFN- γ dynamics. Again, the highest values were observed during the hot summer ($P < 0.001$). For Cortisol, the highest values were found in the cold winter season in the control group ($P < 0.001$). In the experimental group, the concentration of the indicator was highest in the hot summer season ($P < 0.001$). When comparing the control with the experimental group, the control group has significantly higher values ($P < 0.001$). Serotonin had the highest values in the cold winter in the Silymarin-treated group ($P < 0.001$). Contrariwise in the control group the highest values were observed in the thermoneutral period ($P < 0.001$).

Discussion. As can be seen from the results presented, the immunomodulator Immunobeta[®] reliably increases the values of the investigated indicators. It is known that these indicators play a decisive role in the protection of animals and humans from infectious diseases. PRIMO et al. [15], and BOZAKOVA et al. [16] let us know that lysozymes are enzymes that break down the bacterial cell wall and disrupt the bacterial life cycle by cleaving the linkage between the N-acetylglucosamine and N-acetyl muramyl pentapeptide carbohydrates. DENEV et al. [17] reported that ochratoxin A had an immunosuppressive effect on chickens treated at a dose of 3 mg/kg feed and had a positive antitoxic effect of Silymarin on serum β -lysine. GUERRINI and TEDESCO [18] also found an antitoxic effect of Silymarin in chickens treated with mycotoxins. Similar results were reported for carp by GRĂDINARIU et al. [19]. WANG et al. [20] showed that Silymarin is a potential nutritional product that can improve the growth and health status of turbot fed a high plant protein diet. The addition of 100 mg/kg Silymarin to the vegetable protein diet achieved optimal performance in turbot.

Conclusion. Based on the obtained results, it could be concluded that Silymarin possesses an important immunomodulating potential in lambs, which could improve their health and consequently, their productive performance.

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