

## SILYMARIN AS A DIETARY ADDITIVE: INFLUENCE ON IMMUNE FUNCTION AND OXIDATIVE STRESS IN BROILER TURKEYS

Lilyan Sotirov<sup>1✉</sup>, Veselin Ivanov<sup>2</sup>, Magdalena Oblakova<sup>3</sup>,  
Tsvetoslav Koynarski<sup>1</sup>, Pavlina Hristakieva<sup>3</sup>, Ivelina Ivanova<sup>3</sup>,  
Nadya Mincheva<sup>3</sup>, Nadya Bozakova<sup>4</sup>

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### Abstract

The present study aimed to evaluate the effects of silymarin on immune response, biochemical markers, and some traits of oxidative stress in broiler turkeys. The turkeys were fed ad libitum with a commercially prepared feed from the Agricultural Institute in Stara Zagora, supplemented with 2% silymarin over 126 days. Sixty-one-day-old female broiler turkeys were divided into two groups of 30. The control group received a standard age-matched feed without silymarin, while the experimental group received a feed supplemented with 2% silymarin. Serum lysozyme concentration, alternative complement activation pathway, and beta-lysin activity were not significantly affected by silymarin. Most biochemical parameters remained unaffected, except for uric acid levels, which were substantially lower in the silymarin-treated birds ( $P < 0.05$ ). Indicators of oxidative stress were notably higher in the silymarin-treated group ( $P < 0.01$ ), except for superoxide dismutase (SOD), where activity was significantly higher in the control group ( $P < 0.01$ ). This experiment was carried out during the warm summer season. More studies are needed during different seasons of the year. Based on the results obtained, it can be concluded that

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silymarin has no significant immunomodulatory potential in turkeys but had a noticeable effect on oxidative stress indicators in the experimental group.

**Key words:** silymarin, turkeys, immunity, biochemical indicators, stress factors

**Introduction.** In recent decades, the ban on antibiotic use in animal husbandry has spurred interest in biotechnological and natural products to enhance poultry productivity, health, quality, and safety. Immunomodulators and immunoprotecting substances are particularly notable for their ability to stimulate growth while simultaneously improving immune function. Research has largely focused on the impact of these substances on mortality rates, stress hormone levels, blood and muscle metabolism, and overall immune system function in domestic animals.

The alternative pathway of complement activation (APCA) is a well-recognized component of innate immunity, active against gram-negative bacteria, viruses, virus-infected cells, neoplastic cells, agarose, and lipopolysaccharides [1]. DE BOER et al. [2] reported that the cytotoxic effect of neutrophils on herpes virus-infected cells relies not only on the classical complement activation pathway but also on the alternative pathway of complement activation (APCA). Lysozyme, a hydrolytic enzyme in mucous secretions, is produced by macrophages and mucosal epithelial cells (Paneth cells) and exhibits bactericidal activity. When in contact with complement, lysozyme can initiate the lysis of antibiotic-resistant bacteria by hydrolyzing the bonds between N-acetylmuramic acid and N-acetylglucosamine in peptidoglycans. This process releases muramyl dipeptide, a potent natural immune stimulator. On mucosal surfaces, such as in the intestines, lysozyme and sIgA are key factors in resistance [3, 4]. KIM et al. [5] demonstrated that six months of silymarin supplementation in castrated male Hanwoo cattle led to decreased alanine aminotransferase activity and increased growth rates compared to a control group. The antioxidant effects of silymarin are linked to reduced lipid peroxidation and lower levels of malondialdehyde (MDA) in blood and tissues, thus mitigating the high levels of free radicals and protein carbonylation [6]. DE OLIVEIRA et al. [7] confirmed silymarin's antioxidant activity in vitro, noting its ability to prevent lipid peroxidation in brain tissue and maintain the antioxidant defense system. They also discovered that silymarin alters the activity of Na<sup>+</sup>/K<sup>+</sup>-ATPase and monoamine oxidase, indicating that its neuroprotective effects extend beyond antioxidant activity alone. KHAMISABADI [8] let us know that silymarin reduces HSP 70 gene expression in sheep, lowering HSP 70 mRNA levels. This suggests that silymarin supplementation can reduce oxidative stress and heat shock, thereby improving animal welfare under severe and prolonged stress conditions. The current experiment evaluated the effects of 2% silymarin supplementation on serum lysozyme concentrations, alternative pathway of complement activity (APCA), beta-lysine activity, and various biochemical parameters in broiler turkeys.

**Materials and methods. Animals and experimental groups.** This study was conducted at the Agricultural Institute in Stara Zagora, where sixty-one-day-old female broiler turkeys were randomly divided into two groups of 30 birds each. The chicks were reared in floor pens on sawdust bedding from day 1 to day 126. During the first week, temperature was maintained using circular brooders, and corrugated cardboard was used to prevent sawdust pecking. The birds had ad libitum access to feed and water, and continuous lighting was provided throughout the study. The turkeys were divided into two groups – a control and an experimental group. The control group was fed an age-appropriate basal diet without silymarin supplementation, while the experimental group received the same basal diet supplemented with 2% silymarin.

**Blood samples.** Blood for analysis was sampled from *v. subcutaneous ulnaris* in Vacutests KIMA – 11020 for a serum with clot activator – 6 ml, and Vacutests KIMA 135400 – for hematology with K2 EDTA 6 ml (Distributor CHIM-TEX LTD). The blood was allowed to clot for one hour at room temperature (25 °C), the samples were centrifuged at 2000 g for 10 min, and the extracted blood sera were stored at 4 °C. The sera were analyzed not later than 1 hour after the samples were taken.

**Determination of serum lysozyme concentrations.** Serum lysozyme concentrations were measured using the method described by LIE et al. [9].

**Determination of the alternative pathway of complement activation (APCA).** The activity of the APCA was performed by the method of SOTIROV [10].

**Determination of beta-lysin activity.** Beta-lysin activity in blood serum was determined using a spectrophotometric method as described by BUCARIN et al. [11], modified by KARAKOLEV and NIKOLOV [12].

**Investigation of electron paramagnetic resonance (EPR).** A standard resonator (Bruker BioSpin GmbH, Ettlingen, Germany) was used to perform all EPR measurements. All EPR experiments were repeated three times in triplicate.

**Evaluation of the reactive oxygen species (ROS) product levels.** The activities of ROS were assessed by the method of SHI et al. [13] with some modifications.

**Evaluation of the nitric oxide (NO) radical levels.** Based on the methods published by YOSHIOKA et al. [14] and YOKOYAMA et al. [15], the EPR method was developed and adapted for estimating NO radical levels.

**Evaluation of the ascorbate radicals (Asc) level.** The levels of Asc were studied according to BAILEY et al. [16], with some modifications.

**Evaluation of the biochemical traits.** The biochemical parameters of the blood were determined in the biochemical laboratory of the Faculty of Veterinary Medicine, Trakia University, Stara Zagora. The following biochemical parameters were studied: Blood sugar (mmol/l), Total protein (g/l), Creatinine (mmol/l), Uric acid (mmol/l), Cholesterol (mmol/l), Triglycerides (TG,  $\mu$ mol/l), Aspartate

transferase (AST, U/l), Alanine aminotransferase (ALAT, U/l), Gamma-glutamyl transferase (GGT, U/l). Blood samples for the determination of biochemical indicators were centrifuged for 10 min at 1500 g. Plasmas were immediately separated and stored at  $-20^{\circ}\text{C}$  until analysis. For the determination, an automatic biochemical analyzer BS-120 (Mindray, China) was used, using reagents from the company “Biomed”.

**Enzyme-linked immunosorbent assay.** The oxidative stress markers were measured with ELISA kits (Abcam, UK) following the manufacturer’s instructions.

**Statistical analysis.** Descriptive statistics for each variable were calculated and presented as the mean  $\pm$  standard error. A two-way ANOVA (Analysis of Variance) was performed using the General Linear Model (GLM) procedure to assess the effects of the independent variables. A  $P$ -value of less than 0.05 was considered statistically significant unless otherwise specified.

**Results.** The results of this study provide a comprehensive view of the impact of silymarin on various physiological parameters in broiler turkeys. Table 1 highlights that silymarin supplementation did not produce significant changes in three humoral factors of innate immunity: serum lysozyme concentration, alternative complement activation pathway activity, and beta-lysin levels, indicating that these aspects of immune function remained unaffected by the treatment.

T a b l e 1  
Effect of silymarin on lysozyme concentrations, APCA,  
and beta-lysin

| Groups    | Indicators       |                     |                  |
|-----------|------------------|---------------------|------------------|
|           | Lysozyme         | APCA                | Beta-lysin       |
| Control   | $4.29 \pm 0.46$  | $716.05 \pm 8.84$   | $8.94 \pm 1.20$  |
| Silymarin | $4.29 \pm 0.734$ | $715.99 \pm 15.584$ | $6.97 \pm 1.344$ |

In contrast, Table 2 shows that while most blood biochemical indicators were similarly unaffected, uric acid levels were significantly lower in the silymarin-treated group compared to the control group ( $P < 0.05$ ), suggesting a specific biochemical response to the supplement.

The data in Table 3 reveal that oxidative stress markers were significantly elevated in the silymarin-treated group ( $P < 0.01$ ), except superoxide dismutase (SOD) activity, which was notably higher in the control group ( $P < 0.01$ ), indicating a complex interaction between silymarin and oxidative stress mechanisms. These findings collectively suggest that while silymarin did not substantially alter immune parameters, it had a notable impact on certain biochemical indicators.

**Discussion.** The results of this study indicate that silymarin did not significantly impact serum lysozyme concentration, alternative complement activation pathway activity, or beta-lysine levels in the silymarin-treated groups. These

T a b l e 2

Effect of silymarin on some biochemical traits

| Indicators           | Groups          |                |
|----------------------|-----------------|----------------|
|                      | Control         | Silymarin      |
| Glucose (mmol/l)     | 16.75 ± 0.26    | 16.45 ± 0.94   |
| Total protein (g/l)  | 43.33 ± 1.14    | 39.27 ± 2.10   |
| Creatinine (mmol/l)  | 37.33 ± 0.49    | 37.67 ± 0.71   |
| Uric acid (mmol/l)   | 320.00 ± 33.41* | 225.50 ± 24.02 |
| Cholesterol (mmol/l) | 2.97 ± 0.14     | 3.26 ± 0.45    |
| TG (μmol/l)          | 1.25 ± 0.06     | 1.20 ± 0.10    |
| ASAT (U/l)           | 532.33 ± 26.08  | 496.83 ± 36.49 |
| ALAT (U/l)           | 12.33 ± 0.76    | 13.33 ± 1.26   |
| GGT (U/l)            | 4.17 ± 0.54     | 3.33 ± 0.95    |

\*  $P < 0.05$ 

T a b l e 3

Effect of silymarin on some traits of oxidative stress

| Traits                   | Groups          |                 |
|--------------------------|-----------------|-----------------|
|                          | Control         | Silymarin       |
| SOD (U/mL)               | 13.28 ± 0.14*** | 11.08 ± 0.56    |
| GSH (U)                  | 35.80 ± 1.26    | 63.52 ± 0.83*** |
| MDA (Mmol/L)             | 4.08 ± 0.16     | 12.36 ± 0.71*** |
| Asc radicals (Arb Units) | 1.30 ± 0.08     | 3.42 ± 0.09***  |
| NO radicals (Arb Units)  | 5.11 ± 0.38     | 11.88 ± 0.67*** |
| ROS products (Arb Units) | 2.97 ± 0.09     | 6.60 ± 0.21***  |

\*\*\*  $P < 0.001$ 

humoral factors play a critical role in the innate immune response, protecting against infectious diseases in animals and humans. According to BOZAKOVA et al. [17], lysozymes are enzymes that degrade bacterial cell walls by cleaving the bond between N-acetylglucosamine and N-acetylmuramyl pentapeptide carbohydrates, thereby disrupting bacterial life cycles. DENEV et al. [18] reported that ochratoxin A exerts an immunosuppressive effect on chickens and noted the positive antitoxic effect of silymarin on serum beta-lysine levels. BOZAKOVA et al. [19] reported that silymarin supplementation at a dose of 2% significantly increased serum lysozyme concentration, alternative complement pathway activity, and beta-lysine levels in lambs. Additionally, ZHOU et al. [20] observed that silymarin treatment in chicken broilers led to increased total antioxidant capac-

ity, superoxide dismutase (SOD) activity, and elevated mRNA expression levels of interferon- $\gamma$  (IFN- $\gamma$ ), interleukin-6 (IL-6), and IgM in the mucosa of the jejunum.

**Conclusions.** Based on the results obtained, it can be concluded that silymarin has no significant immunomodulatory potential in turkeys but had a noticeable effect on oxidative stress indicators in the experimental group.

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<sup>1</sup>*Department of Animal Genetics, Faculty of Veterinary Medicine, Trakia University, Students' campus, 6000 Stara Zagora, Bulgaria*  
e-mails: [sotirov54@gmail.com](mailto:sotirov54@gmail.com), [tkoynarski@gmail.com](mailto:tkoynarski@gmail.com)

<sup>2</sup>*Department of Social Medicine, Health Management and Disaster Medicine, Faculty of Medicine, Trakia University, Students' campus, 6000 Stara Zagora, Bulgaria*  
e-mail: [veskoasenov@abv.bg](mailto:veskoasenov@abv.bg)

<sup>3</sup>*Agricultural Academy, Agricultural Institute, Stara Zagora, Bulgaria*  
e-mails: [moblakova@abv.bg](mailto:moblakova@abv.bg), [poly\\_31@abv.bg](mailto:poly_31@abv.bg), [ivanova24@abv.bg](mailto:ivanova24@abv.bg), [minchevan@yahoo.bg](mailto:minchevan@yahoo.bg)

<sup>4</sup>*Department of Animal Hygiene, Ethology and Animal Protection, Faculty of Veterinary Medicine, Trakia University, Students' campus, 6000 Stara Zagora, Bulgaria*  
e-mail: [nadiab@abv.bg](mailto:nadiab@abv.bg)