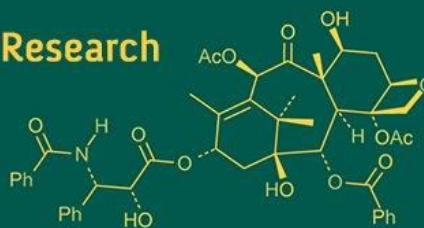


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SS Gaikwad
Department of Poultry Science;
Mumbai Veterinary College,
Maharashtra Animal and Fishery
Sciences University, Nagpur,
Mumbai, Maharashtra, India

DN Desai
Sectional Head and Professor,
Department of Poultry Science;
Mumbai Veterinary College,
Maharashtra Animal and Fishery
Sciences University, Nagpur,
Mumbai, Maharashtra, India

AS Ranade
Retd. Professor, Department of
Poultry Science, Mumbai
Veterinary College, Maharashtra
Animal and Fishery Sciences
University, Nagpur, Mumbai,
Maharashtra, India

MA Gole
Professor, Department of Poultry
Science; Mumbai Veterinary
College, Maharashtra Animal and
Fishery Sciences University,
Nagpur, Mumbai, Maharashtra,
India

RJ Zende
Professor, Department of
Veterinary Public Health, Mumbai
Veterinary College, Maharashtra
Animal and Fishery Sciences
University, Nagpur, Mumbai,
Maharashtra, India

MP Sawane
Professor, Department of Animal
Genetics and Breeding, Mumbai
Veterinary College, Maharashtra
Animal and Fishery Sciences
University, Nagpur, Mumbai,
Maharashtra, India

SD Jagadale
Assistant Professor, Department of
Animal Nutrition; Mumbai
Veterinary College, Maharashtra
Animal and Fishery Sciences
University, Nagpur, Mumbai,
Maharashtra, India

Corresponding Author:
SS Gaikwad
Department of Poultry Science;
Mumbai Veterinary College,
Maharashtra Animal and Fishery
Sciences University, Nagpur,
Mumbai, Maharashtra, India

Investigating the impact of milk thistle (*Silybum marianum*) on liver health, gut health and immune status of broilers

SS Gaikwad, DN Desai, AS Ranade, MA Gole, RJ Zende, MP Sawane and SD Jagadale

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Abstract

Milk thistle (*Silybum marianum*) is commonly utilized in poultry farming due to its content of silymarin which has a positive impact on liver function, immune response, and overall growth performance. This study aimed to assess the effects of milk thistle on the production performance of broiler chickens. A total of 300 Vencobb 430 Y broilers were raised over a five-week period. The chicks were randomly assigned to five groups (A, B, C, D, and E), with each group consisting of 60 birds, further divided into six replicates of 10 birds each. The treatment plan was as follows: Group A-control, receiving diet containing herbal choline at 250 g/T without milk thistle. Groups B, C, D, and E received diets supplemented with herbal choline at 250 g/T, but with milk thistle incorporated at 5 g/T, 10 g/T, 15 g/T, and 20 g/T, respectively. The parameters like liver function tests-SGPT, SGOT, Alkaline phosphatase, Total protein, Albumin, Globulin and GGT; Gut health parameters-Total Viable count and total coliform count and immunity parameters like HI titers for ND and IBD were carried out. The results reported no significant differences ($p \geq 0.05$) in any of the liver function values, total viable count, total coliform count and HI titers for ND and IBD for the birds from different treatment groups. However, the values of liver function tests and Total viable and total coliform counts were numerically lower in the treatment groups as compared to the control. Thus, it can be concluded that milk thistle is beneficial in improving the liver and gut health in broilers without any negative impact on the health and performance of the birds.

Keywords: Milk thistle, herbal choline, Silymarin, broilers

Introduction

The poultry sector is one of the most rapidly expanding industries globally. In recent years, broiler chickens have been intensively bred to promote faster growth and greater weight gain. Although this selective breeding has improved growth rates and feed conversion efficiency, it has also led to the accumulation of excessive and unwanted fat in modern broilers. This surplus fat is often regarded as waste by consumers and raises concerns regarding the nutritional value and health implications of poultry products. The use of high energy diets aims at shortening the rearing period may increase metabolic disorders such as fatty liver syndrome (FLS) in broiler chickens ^[1]. Increased abdominal fat pad ^[2], incidence of leg problems ^[3] and hypertension ^[4] are some other detrimental responses associated with high energy diets. Fatty liver syndrome is a condition that generally affects fast growing broilers fed high energy diets and caged layers with inadequate chance to move and exercise freely ^[5].

To mitigate these issues, poultry nutritionists are continuously investigating dietary strategies to achieve an optimal balance between growth performance and health of the animals. They are also considering herbal alternatives to the traditional chemical drugs as they are considered cheaper and safe. The natural additives are rich in bioactive compounds, including essential oils, tannins, glycosides, flavonoids, terpenes, and organic acids ^[6]. The active ingredients in these herbal products offer a range of benefits, such as anti-stress, antibacterial, antiviral, and antifungal properties ^[7]. Furthermore, they promote the secretion of digestive enzymes, enhancing metabolism and stimulating appetite in poultry. Additionally, herbal supplements are known to strengthen the immune system by enhancing

the activity of immune cells like lymphocytes and macrophages, thereby improving overall immunity [8]. Currently, milk thistle (*Silybum marianum*) is increasingly popular in animal nutrition [9]. Silymarin exhibits activity against chemical, viral, bacterial and fungal toxins, inhibits lipid peroxidation, and stabilizes the cell membranes of the liver parenchyma [10]. Silymarin presents a pharmacologically effective substance containing four main constituents: silybin, isosilybin, silychristin and silydianin [11]. Furthermore, milk thistle seeds contain betaine, trimethyl glycine, and essential fatty acids which are involved in the hepatoprotective and anti-inflammatory actions of the silymarin complex [12]. The different beneficial effects of silymarin have been previously reported in poultry production. It is considered as a potential feed additive to broilers in terms of enhancement of the growth performance, prevention of oxidative stress, improvement of the meat quality, increasing the production of polyunsaturated fatty acids, and stimulation of immune status [13, 14, 15].

Based on the above-mentioned findings, this article aims to show the different potential uses of milk thistle, in poultry production system regarding its effect on liver health, intestinal health and immune response.

Materials and Methods

Experimental design and data

The present study was conducted for a period of five weeks on 300-day-old broiler chicks. The birds were divided into five equal groups. Each group was further sub-divided into six replicates of ten birds each. The groups were subjected to the treatments

Group A: Control group-Herbal choline without Milk Thistle and used @ 250 g/T. *

Group B: Diet supplemented with Herbal Choline with Milk thistle @ 5g/T used @ 250 g/T.**

Group C: Diet supplemented with Herbal Choline with Milk thistle @ 10 g/T used @ 250 g/T.**

Group D: Diet supplemented with Herbal Choline with Milk thistle @ 15 g/T used @ 250 g/T.**

Group E: Diet supplemented with Herbal Choline with Milk thistle @ 20 g/T used @ 250 g/T.**

*The Products of herbal Choline and herbal choline with milk thistle were provided by-Nurture Aqua Technology Pvt. Ltd.

Feed

Diets were formulated according to the standards without the inclusion of choline chloride, liver tonic and betaine in the feed. The details of the feed formulation used in the trial are presented in Table 1. The calculated values of the nutrients of pre-starter, starter and finisher feed are presented in Table 2.

Table 1: Percent composition of pre-starter, starter and finisher feed

Ingredient	Pre-Starter	Starter	Finisher
Maize	57.30	61.00	64.20
MBM	4.50	4.00	3.50
Oil	0.50	1.00	2.00
DCP	0.10	0.10	0.10
LSP	0.60	0.50	0.50
Salt	0.23	0.22	0.26
Soda	0.35	0.35	0.40
Base Premix	0.30	0.30	0.30
DL-Methionine	0.27	0.23	0.23
Lysine Sulphate	0.42	0.38	0.47
L-Threonine	0.14	0.13	0.14
Soya Doc-42.5%	35.30	31.80	27.90
Total	100.00	100.00	100.00

*Base premix contains broiler vit. Premix, trace mineral mixture, BMD, enzyme, toxin binder, salinomycin, Phytase.

Table 2: Nutrient levels of pre-starter, starter and finisher feed

Nutrient	Pre-Starter	Starter	Finisher
Metabolizable energy (Kcal /Kg)	2925.00	2990.95	3084
Crude Protein (%)	22.50	21.04	19.51
Crude Fibre (%)	3.24	3.11	2.94
Methionine (%)	0.61	0.55	0.52
Lysine (%)	1.31	1.2	1.15
Calcium (%)	0.84	0.74	0.68
Total Phosphorus (%)	0.60	0.56	0.52

Liver Function Tests

The clinical chemical analyses of serum samples (SGPT, SGOT, Alkaline phosphatase, Total protein, Albumin, Globulin and GGT) were performed by Vetcore laboratories Ltd. using a semi-automatic biochemical analyzer (Ebra-Chem. 5X, USA) using commercial reagent kits, and procedure was performed by following standard methodology.

Total viable count and total coliform count of the intestine

At the end of the study, six birds from each group were randomly selected for sacrifice. Approximately 2 cm in length from the ileum of each bird was obtained through dissection. These ileum samples were placed in 50 ml containers filled with phosphate-buffered saline (PBS) at a pH of 6.8 and stored at 4°C until further analysis. 0.1 ml of

inoculum was taken from three different dilutions of the intestinal content and cultured on Plate Count Agar and MacConkey agar using the spread plate method. The agar plates were incubated at 37 °C for 24 hours and then bacterial colonies were counted. The total viable count and coliform count were determined by calculating the number of bacteria (CFU) per gram of sample, dividing the number of colonies by the dilution factor [16].

$$\text{CFU/g} = \frac{\text{No. of colonies} \times \text{Total dilution factor}}{\text{Volume of culture plate}}$$

HI titer for ND and IBD

At the end of the third and fifth week, 2 ml of blood was collected from six birds per group and transferred to a clot activator tube. After centrifugation, the clot was removed, leaving the serum, which was carefully extracted.

In a 96-well plastic v-bottom plate, 25 µl of phosphate-buffered saline (PBS) was dispensed into 12 wells of one row. A two-fold serial dilution of 25 µl of field serum was prepared up to the 11th well. An equal volume of 4 HA units of ND and virus was added to each well up to the 12th well, and the mixture was left at room temperature for 30 minutes. Subsequently, 25 µl of 1% (v/v) chicken RBC suspension was added to each well, and the RBCs were allowed to settle for 40 minutes. Agglutination was evaluated by tilting the plates, and samples displaying a distinct central button-like settling of RBCs were recorded as positive. The highest dilution showing complete inhibition was noted as the HI antibody titer [17].

Statistical Analysis

The data obtained in this study were analyzed statistically

by one-way ANOVA using the SPSS version 27.0@ software [17].

Results

The results for the liver function tests, total viable counts and total coliform counts were carried out at the end of the trial (35 days) and the results for HI titer for ND and IBD were carried out on the 21st day and 35th day.

Liver Function Tests

No significant difference ($p \geq 0.05$) was observed in any of the liver function values for the birds from different treatment groups (table 3). Further, the values of all the liver functional tests for the birds from all the treatment groups receiving milk thistle were numerically lower than that of the control group.

Table 3: Average liver function values

Groups	SGPT (ALT) IU/L	SGOT (AST) IU/L	(ALP) U/L	Total Protein gm/dl	Albumin gm/dl	Globulin gm/dl	GGT IU/L
A	5.33±0.06	261.25±41.84	2556.08±602.69	2.86±0.40	1.06±0.40	1.80±0.65	9.21±1.52
B	3.86±1.02	191.77±36.70	2313.60±763.68	2.30±0.43	0.86±0.41	1.44±0.68	8.17±2.63
C	5.14±1.99	199.00±55.58	2146.68±655.98	2.80±0.72	0.90±0.46	1.90±1.34	6.67±1.64
D	4.25±1.05	141.28±12.55	1986.75±493.94	1.72±0.21	0.64±0.21	1.08±0.33	5.37±0.79
E	4.06±0.37	244.17±50.22	1905.82±300.72	2.67±0.50	0.94±0.37	1.73±0.84	5.10±0.65
SEM	4.53±0.49	207.49±19.17	2181.79±246.73	2.47±0.21	0.88±0.38	1.59±0.83	6.90±0.73
P-value	0.853	0.312	0.936	0.449	0.429	0.457	0.332

SGPT (ALT)-Alanine amino transferase, SGOT (AST)-Aspartate aminotransferase, ALP-Alkaline Phosphatase, GGT-Gamma-glutamyl transferase. Note: ^{abc} Mean values with different superscripts within a row differ significantly.

Total viable count and Total Coliform count

The data pertaining to average total viable counts and average total coliform counts of the intestinal contents of birds from different groups were subjected to statistical analysis and the results indicated that the differences average total viable count of the intestinal contents of birds from different treatment groups were statistically non-significant ($p \geq 0.05$) (table 4) (figure1). From the data, it was noticed that at the end of the five weeks, the birds from

treatment groups C receiving herbal choline with milk thistle at the dose of 10 g and used at 250 g/T recorded numerically lowest total viable count followed by the birds from groups E, D, B and A, respectively, in their intestinal contents. Similarly, the birds from treatment groups B receiving herbal choline with milk thistle at the dose of 5 g and used at 250 g/T recorded numerically lowest total coliform count followed by the birds from groups E, C, D and A, respectively.

Table 4: Average total viable count and Total Coliform counts of intestinal contents

Groups	TVC	TCC
A	7.63±0.05	3.45±0.03
B	7.54±0.06	3.13±0.04
C	7.44±0.11	3.36±0.13
D	7.52±0.11	3.41±0.05
E	7.51±0.10	3.32±0.16
SEM	7.53±0.04	3.34±0.05
P-value	0.706	0.197

TVC-Total Viable Counts (Log₁₀ CFU/g), TCC-Total coliform counts (Log₁₀ CFU/g). Note: ^{abc} Mean values with different superscripts within a row differ significantly.

HI antibody titer (@ 8 HAU) for Newcastle Disease and IBD (ELISA) antibody titer

The data indicates that birds in group B, which received herbal choline with milk thistle at the dose of 5 g and used at 250 g/T, showed a higher average HI antibody titer (@ 8 HAU) for Newcastle Disease after three weeks and by the end of three weeks and the birds from group E receiving herbal choline with milk thistle at the dose of 20 g and used at 250 g/T recorded numerically higher average HI antibody titer (8 HAU) for Newcastle Disease at the end of five weeks (table 5). Further, it is revealed that at the end of three weeks, the birds from group C receiving herbal choline

with milk thistle at the dose of 10 g and used at 250 g/T recorded numerically the highest average HI antibody titer for IBD (ELISA) as compared to all the other treatment groups and the birds from group B receiving herbal choline with milk thistle at the dose of 5 g and used at 250 g/T reported numerically the highest average HI antibody titer as compared to the birds from all the other treatment groups. However, the statistical analysis revealed that the differences in the average HI antibody titer (@ 8 HAU) for Newcastle Disease and IBD (ELISA) of the birds from different groups for week three and week five was statistically non-significant ($p \geq 0.05$).

Table 5: Average values of HI antibody titer (@ 8 HAU) for Newcastle Disease and IBD (ELISA) antibody titer

Groups	ND titer		IBD titer	
	Week 3	Week 5	Week 3	Week 5
A	8.67±1.61	10.00±2.00	1387.83±152.05	1443.17±274.61
B	11.33±4.50	10.00±4.90	1057.83±198.37	1509.17±224.89
C	9.67±2.85	6.33±2.28	1441.50±283.36	1239.67±153.01
D	8.67±4.78	10.67±4.78	1152.17±167.07	1213.50±158.60
E	5.67±1.50	11.00±4.31	1297.17±120.24	1323.67±143.77
SEM	8.80±1.44	9.60±1.63	1267.30±84.22	1345.83±84.75
P-value	0.823	0.915	0.603	0.793

Note: ^{abc} Mean values with different superscripts within a row differ significantly.

Discussion

This study aimed to investigate the impact of herbal choline supplemented with milk thistle at different doses on the liver health, gut health and immune status of commercial broiler birds. The results demonstrate that there were no significant differences ($p \geq 0.05$) in the values of liver function tests, total viable counts, total coliform counts and HI antibody titer (@ 8 HAU) for Newcastle Disease and IBD (ELISA) antibody titer values. However, the values of liver function tests, total viable counts and total coliform counts for the birds from all the treatment groups receiving milk thistle were numerically lower than that of the control group, suggesting that supplementation of herbal choline with milk thistle had marginal effect on liver and gut health at such low dose rates, also suggesting that the effect could be higher at supplementation of herbal choline with milk thistle at higher doses.

The mechanisms responsible for the hepatoprotective effects of silymarin remain incompletely understood. The liver plays a crucial role in regulating various physiological functions in animals. In birds, nutritional status is influenced not only by dietary intake but also by liver function and metabolic processes [18]. The liver exhibits complex biochemical activity and is essential for poultry physiology. Toxins absorbed from the digestive tract first pass through the liver, where they may accumulate and contribute to disease development [19]. Liver damage can manifest in various forms, including acute hepatitis and hepatocellular carcinoma, driven by factors such as immune responses, inflammation, necrosis, fibrosis, apoptosis, altered gene expression, regeneration, and ischemia [20]. Silymarin has a relatively short half-life, undergoes rapid conjugation in the liver, and is primarily eliminated via bile. To effectively manage hepatic inflammation *in vivo*, it must be administered in high doses or at frequent intervals [21]. Research conducted in Italy indicated that a 240 mg/day dose of silymarin (silybin) led to a significant reduction in serum liver enzyme levels (ALT, AST, GGT, ALP), which serve as biomarkers of liver damage and stress, as well as total bilirubin in patients with chronic active hepatitis [22]. Silymarin in milk thistle plays a crucial role in liver protection through several mechanisms. It acts as a stabilizer and regulator of cell membrane permeability, preventing hepatotoxic substances from entering hepatocytes [23].

These results were in accordance with the findings of Abdalla *et al.* (2018) [24] who reported that the birds receiving fine ground ariel parts of milk thistle plant at 12.5 and 25 g/kg in diet significantly ($p \leq 0.05$) improved the levels of total protein AST, ALT, creatinine. Diets supplied with 0.5 and 1 g/kg levels of commercial silymarin significantly improved the liver function and lipid profile, since the activity of AST, ALT, ALP. Allasi and Allaw

(2020) [25] found significantly lower ($p \leq 0.05$) values of total protein, albumin, globulin, ALT and AST in the treatment groups supplemented with 0.5 % and 1% of milk thistle seed powder as compared to the control group receiving commercial feed without milk thistle. Further, Elnaggar *et al.* (2021) [26] also reported significantly improved values of AST, ALT, ALP in ducks supplemented with silymarin extract at levels of 0.6, 0.9 or 1.4 g/kg diet levels, respectively. However, Schiavone *et al.* (2007) [27] reported a slight, but not significant ($p \geq 0.05$) changes in the total protein values at 60 days and AST values on 40 days when the birds were supplemented with 40 and 80 ppm of silymarin in the diet. Further, Sakamoto *et al.* (2018) [28] reported no interactions in the ALT, AST and GGT values of the birds supplemented with 1500 µg/kg silymarin and contaminated or not with aflatoxins.

Multiple studies have shown that the bioactive compounds found in medicinal plants exhibit antibacterial properties through their hydroxyl groups. These hydroxyl groups can interact with bacterial membrane proteins, causing leakage of essential cellular components [29, 30]. Supporting these findings, Lahlah *et al.* (2012) [31] reported that both gram-positive and gram-negative bacteria are vulnerable to silymarin and silibinin. Likewise, research by Abed *et al.* (2015) [32] and Evren and Yurtcu (2015) [33] indicated that *Silybum marianum* demonstrated antibacterial effects against *Staphylococcus saprophyticus*, *Escherichia coli*, and *Klebsiella pneumoniae* under *in-vitro* conditions.

These findings align with the study by Shahsavan *et al.* (2021) [34], which found no significant differences ($p \geq 0.05$) in the cecal microbial population of birds fed diets containing 0%, 3%, 6%, 9%, and 12% of *Silybum marianum* oil extraction byproduct (SMOEB). In contrast, Elnaggar *et al.* (2021) [26] observed that supplementing duck diets with silymarin extract at levels of 0.6, 0.9, or 1.4 g/kg significantly reduced bacterial growth ($p \leq 0.05$) compared to the control group. Additionally, Hashemi Jabali *et al.* (2017) [35] reported that incorporating milk thistle meal at 15%, 30%, and 60% in the diets of Leghorn laying hens (Hy-Line W-36) led to a significant reduction in ileal microbial counts of *Salmonella* and *E. coli* compared to the control group, which received no milk thistle meal.

Studies carried out by Chand *et al.* (2011a) [36] reported that *Silybum marianum* extract had effect on immune cells and support immune system through the strong antioxidants and collecting free radicals. Additionally, Wilasrusmee *et al.* (2002) [37] and Thyagarajan *et al.* (2002) [38] highlighted the immune-boosting properties of *Silybum marianum*. Several studies have shown that administering water-soluble extracts from medicinal plants to broiler chickens enhances blood antibody titers against ND and IBD [39]. Furthermore, Chand *et al.* (2011b) [40] reported that incorporating 10 g of

Silybum marianum per kg of broiler chicken feed significantly improved immune response against ND and IBD.

These results are in accordance with Amiri Dumari *et al.* (2014) [41] who reported that the supplementation of milk thistle seeds alone at the rate of 0.5 % and 1% had no effect on the ND and IBD titers of the birds and the statistical results were non-significant ($p \geq 0.05$). Similarly, Zarei *et al.* (2016) [42] reported that In-ovo injection of the extract at the dose of 100 mg/L and 200 mg/L and inclusion of milk

thistle extract in the diet at the dose of 100 mg/Lg had no effect on the antibody titers of ND and IBD. However, Ahmad *et al.* (2020) [43] reported that supplementation of dried seeds of milk thistle at the rate of 10 g/kg and 15 g/kg in the diet significantly ($p \leq 0.05$) improved the antibody titer against New castle disease. Similarly, Zarei *et al.* (2016) [42] reported that inclusion of the milk thistle extract in the diet of broilers significantly ($p \leq 0.05$) improved the antibody titer against IBD.

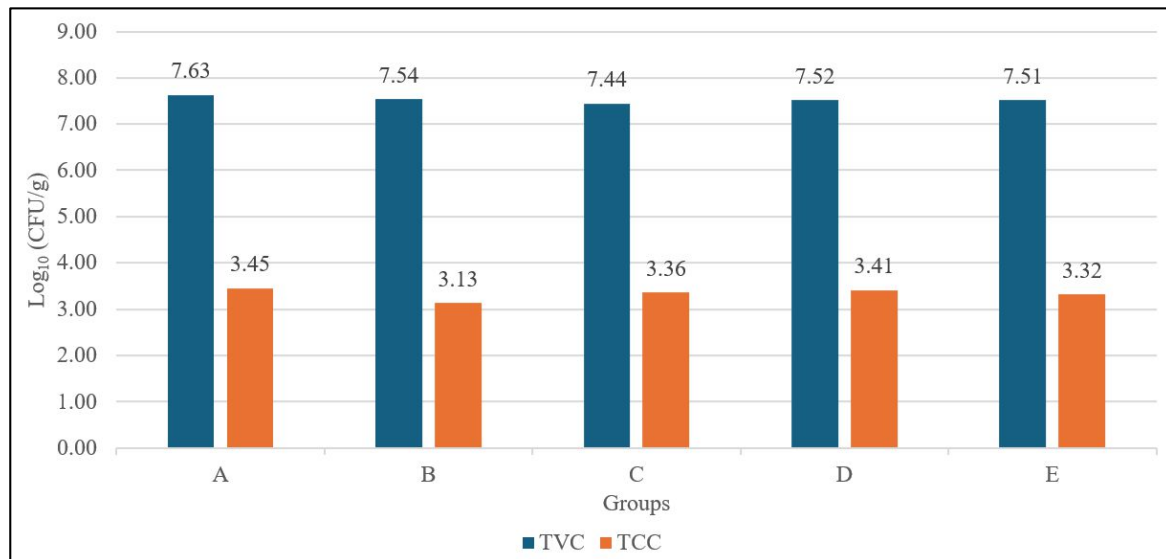


Fig 1: Average Total viable count (TVC) and Total coliform counts (TCC) of intestinal contents

Conclusion

In conclusion, the results of this study suggest that the supplementation of milk thistle (*Silybum marianum*) with herbal choline in the diets of broiler birds reported no significant differences in any of the liver function values, total viable count, total coliform count and HI titers for ND and IBD for the birds from different treatment groups. However, the values of liver function tests and Total viable and total coliform counts were numerically lower in the treatment groups as compared to the control. Thus, it can be concluded that milk thistle is beneficial in improving the liver and gut health in broilers without any negative impact on the health and performance of the birds.

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