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Supplementation of silymarin and chromium picolinate as feed additives in broilers enhances antioxidant status, liver, and kidney function

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ABSTRACT

The present investigation aimed to explore the impact of supplementation of silymarin (SM) and chromium picolinate (CP) as feed additives on the antioxidant status, along with liver, and kidney function of broilers. One hundred eighty day-old (mixed-sex) broiler chicks (Cobb 400) were used for the current study. The chicks were allocated in a completely randomized design in 4 treatments groups with 3 replicates per treatment, and 15 chicks in each replicate. The basal diet of control (CON) was supplemented with silymarin (500 mg/kg feed) as milk thistle powder (SM_{500}), chromium (400 µg/kg feed) (Cr_{400}), and a combination of SM (500 mg/kg feed) and chromium (400 µg/kg feed) ($SM_{500}+Cr_{400}$), from day old to 42 days of the experiment. Activities of superoxide dismutase (SOD), catalase (CAT), and total antioxidant capacity (TAC) were observed to be higher in the treatment groups supplemented either with SM (500 mg/kg), CP (400 µg/kg) alone, or in combination as compared to the control group. Thiobarbituric acid reactive substance (TBARS) level declined ($P<0.05$) in the treatment groups, but the decline was more pronounced in the $SM_{500}+CP4_{00}$ group compared to all other groups. Blood urea nitrogen, creatinine, alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase were declined, however, total protein, albumin, and globulin levels were increased in the broilers that fed SM (500 mg/kg) and CP (400 µg/kg). The present study concluded that the addition of silymarin and chromium in the diet of broilers improved antioxidant status and kidney and liver function, which might have increased the productive performance of broilers.

Keywords: Antioxidant status, Broilers, Kidney function, Liver function

Enhancing productive efficiency through cost-effective methods is a constant challenge in the poultry sector, which produces affordable, nutritious foods for human consumption. For several decades, various feed additives comprising antibiotics as growth promoters have been frequently utilized in the diet of livestock. However, the use of antibiotics in animal feed is prohibited due to concerns about antibiotic residues and disease resistance. As a result, the restrictions and bans on the use of antibiotics as growth promoters have prompted scientists to look for substitutes that may boost animal health performance. Several studies have shown that plant extracts, including those from ginger (*Zingiber officinale*), turmeric (*Curcuma longa*), and milk thistle (*Silybum marianum*), could be excellent in health performance (Saeed *et al*. 2017).

Milk thistle is a native herb with reddish-purple flowers and large, thorny leaves. Previously, its seeds were used as a natural drug to cure liver ailments. The seeds of milk thistle have approximately 60% silymarin (SM). SM has anti-inflammatory, hepatoprotective, cytoprotective, and anticarcinogenic effects (Madrigal-Santillán *et al.* 2014). The SM has powerful antioxidant potential; therefore, it protects the cell membrane from lipid peroxidation (Madrigal-Santillán *et al.* 2014). The addition of silymarin in the diet of broilers has beneficial effects on consumption and feed utilization (Tedesco *et al*. 2004) and also improves broiler productive performances (Shanmugam *et al*. 2022).

Chromium picolinate (CP) is one of the most common forms of organic chromium used as supplements in the diets of poultry and animals (Zhang *et al*. 2008). Chromium has a significant task in nutrient metabolism, such as carbohydrates, proteins, fats, amino acids, and nucleic acids; hence, it is used as a supplement in the diet of animals (Khan *et al*. 2014). The organic form of chromium

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supplementation has 15 to 30% bioavailability compared to inorganic forms that have only 1 to 3% bioavailability (Forbes and Erdman, 1983). CP boosts health status when it is incorporated into the diets of broilers (Huang *et al*. 2016). TP and GLOB levels were increased in broilers supplemented with chromium (Sarai *et al*. 2022).

Numerous studies have reported the positive effects of SM or CP in animal and human models, according to our knowledge, studies have reported the comparison of SM, CP individually or in combination. Therefore, the current study was designed to compare the effect of SM and CP supplementation individually or in combination on antioxidant status and kidney and liver function.

MATERIALS AND METHODS

Ethics approval: The experiment was conducted following approval by the Institute Animal Ethics Committee (Registration no. 2058/GO/Re/SL/19/CPCSEA) with approval no. IAEC/SVPUAT/2023/120.

Experiment design, animals, and diet: The experiment took place at the Poultry Research and Training Centre, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, UP, India. For this study, 180 day-old broiler chicks (Cobb 400) of mixed sex, procured from Venkey's Hatcheries Pvt. Ltd., Dehradun, UK, India, were used. Just after the arrival of chicks at the experimental house, they were weighed and allocated in a completely randomized design within 4 treatments, 3 replicates per treatment, and 15 chicks in each replication. The chicks of each replicate were reared over 42 days in a deep litter system in well-ventilated pens under standard climatic conditions with *ad libitum* access to water and feed. All over the study period, the chicks were exposed to 24h electric lighting. In the first experimental week, the ambient temperature was 32°C and then declined by 2°C every week until it reached 24°C in the last week of the experiment. The chicks were offered starter (1-3 weeks) followed by finisher (4-6 weeks) basal diet and formulated to meet the nutritional specifications of broilers as per guidelines of NRC standards (NRC, 1994) (Table 1). The basal diet was offered without any supplementation (CON) or supplemented with SM (500 mg/kg feed) as milk thistle powder (purity 80%, World of Nature, Pune, Maharashtra. India; SM_{500}), chromium (400 µg/kg feed) as CP (purity 96%, HiMedia Laboratories Pvt. Ltd, Mumbai, India; Cr_{400}), and a combination of SM (500 mg/kg feed) and chromium (400 µg/kg feed) (SM_{500}+Cr_{400}), respectively, from day old to 42 days of the experiment.

Sample collection: Nine blood samples per treatment (three birds/replication) were collected from the wing vein puncture in the EDTA-containing test tube on days 21 and 42 of life. The blood samples were transported to the laboratory in the icebox. Thereafter, blood samples were centrifuged in the refrigerated centrifuge at 3000 rpm for 30 min, and plasma was harvested, kept in the Eppendorf tube and stored at – 20 °C till further analysis of total protein (TP), albumin (ALB), globulin (GLOB),

Table 1. Ingredient composition and chemical analysis of starter and finisher diets

Attributes	Ration	
	Starter (0-21 d)	Finisher (22-42 d)
Ingredient composition (g per kg)		
Soybean meal	275.00	245.00
Yellow maize	580.00	615.00
Wheat bran	35.00	58.00
Fish meal	80.00	55.00
Limestone	18.00	16.50
L-lysine	1.50	1.00
DL-methonine	2.50	1.50
Common salt	3.50	3.50
Mineral and vitamin premix[a]	4.50	4.50
Chemical analysis of diet (%, as DM)		
ME (Kcal/kg)	2900.00	3000.00
Moisture	10.00	9.50
Dry matter	90.00	90.50
Organic matter	92.00	91.00
Crude protein	23.50	19.50
Ash	8.00	9.00
Ether extract	5.50	5.00

Premix composition per kg: Vitamin A 4000000 IU, Vitamin D3 800000 IU, Vitamin B2 800 mg, Vitamin E 300 IU, Vitamin K 400 mg, Calcium pantothenate 1000 mg, Niacinamide 4000 mg, Vitamin B_{12} 3.2 mg, Choline chloride 80000 mg, Zn 6000 mg, Iodine 400 mg, Mn 11000 mg, Cu 800 mg, Fe 3000 mg, Co 180 mg, Ca 300 g, Se 800 mg.

creatinine (CREAT), blood urea nitrogen (BUN), alanine aminotransferase (ALT), aspartate aminotransferase (AST), Alkaline Phosphatase (ALP), thiobarbituric acid reactive substances (TBARS), Total antioxidant capacity (TAC). The hemolysate was prepared from hematocrit volume and used in the estimation of SOD and CAT.

Plasma biochemistry: Using commercial kits (ERBA Diagnostics Mannheim GmbH Germany), TP, ALB, CREAT, and BUN concentrations, as well as ALT, AST, and ALP activities, were measured. The plasma concentration of GLOB was calculated by subtraction of ALB from TP. The activities of SOD and CAT were measured spectrophotometrically in hemolysate using the procedures described by Marklund and Marklund (1974) and Aebi (1983), respectively. The TBARS is an indicator of lipid peroxidation and was measured using the procedure of Asakawa and Matsushita (1979). Plasma circulating concentration of TAC was measured by the ferric-reducing antioxidant power assay method (Benzie and Strain, 1999).

Statistical analysis: The results of different variables were statistically analyzed by using the GLM procedure of SPSS (V19.0; SPSS Inc., Chicago, IL, USA), according to the following mathematical model:

Table 2. The effect of silymarin and chromium picolinate on the antioxidant status of broiler chicks

Variable	Day	Group				SEM
		CON	SM_{500}	CP_{400}	SM_{500}+CP_{400}	
SOD (Unit/g Hb)	21	899.67^{a}	914.67^{b}	916.33^{bc}	920.67^{c}	1.90
	42	940.33^{a}	957.33^{b}	958.67^{b}	964.67^{c}	1.20
	Mean	920.00^{a}	936.00^{b}	937.50^{b}	942.67^{c}	1.55
CAT, µmol of H_2O_2 consumed/ min/mg Hb	21	60.12^{a}	70.15^{b}	71.20^{bc}	74.19^{c}	1.20
	42	68.10^{a}	79.18^{b}	81.21^{b}	86.05^{c}	1.20
	Mean	64.09^{a}	74.23^{b}	76.25^{b}	80.33^{c}	1.20
TAC (µmole/ml)	21	1.60^{a}	1.80^{b}	1.83^{b}	1.90^{c}	0.01
	42	1.90^{a}	2.20^{b}	2.24^{c}	2.35^{d}	0.01
	Mean	1.75^{a}	2.00^{b}	2.04^{c}	2.12^{d}	0.01
LPO (nmol/ml)	21	3.30^{c}	3.15^{b}	3.13^{b}	3.09^{a}	0.01
	42	3.20^{c}	3.03^{b}	3.01^{b}	2.96^{a}	0.01
	Mean	3.25^{c}	3.09^{b}	3.07^{b}	3.02^{a}	0.01

CON, control group without supplementation of silymarin and chromium picolinate; SM_{500}, silymarin supplemented group (500 mg/kg feed); CP_{400}, chromium picolinate supplemented group (400 µg/kg feed); SM_{800}+CP_{400}, both silymarin (500 mg/kg) + chromium picolinate (400 µg/kg) supplemented group; SEM, standard error mean.

Mean bearing different superscripts (a, b, c, and d) in a row differ significantly at P<0.05.

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where Y_{ij} is the dependent variable, µ is the total mean, T_i is the effect of treatment (i = 1, 2, 3, and 4), and e_{ik} is the error. The significance of differences between mean values was verified using the Tukey's Honestly Significant Difference, a post hoc test at the significance level $\alpha \leq 0.05$.

RESULTS AND DISCUSSION

Antioxidant status: The effect of SM and CP supplementation on the antioxidant status of broilers has been presented in Table 2. Activities of mean SOD and CAT were higher (P<0.05) in broilers fed combination followed by CP_{400}, SM_{500}, and CON groups. Similarly, on 21 and 42 days of study, activities of SOD and CAT were significantly (P<0.05) higher in the treatment groups as compared to the CON groups. Moreover, TAC concentration in the blood plasma varied statistically (P<0.05) among the groups and was reported to be higher (P<0.05) in the SM_{500}+CP_{400} groups as compared to all other groups. The TAC concentration on 21 and 42 days of the study was observed to be higher in treatment groups either supplemented with SM (500 mg/kg), CP (400 µg/kg), or their combination compared to the CON group. The mean TBARS and TBARS levels on 21 and 42 days of study decreased significantly (P<0.05) in treatment groups as compared to the CON group. However, insignificant difference was observed in the LPO levels of the SM_{500} and CP_{400} groups.

The SOD, CAT, and TAC are biomarkers of antioxidant status; however, TBARS is the product of oxidation of cellular lipids and represents the extent of lipid peroxidation. In the present study, both SM and CP supplementation individually or in combination had a positive impact on SOD, CAT, and TAC with a negative impact on TBARS, but the effect was observed more pronounced in broilers who received combination. Similarly, SM statistically augmented hepatic mRNA expression of CAT and Mn-SOD in broilers (Baradaran *et al*. 2019), reflecting antioxidant potential. Moradi *et al.* (2017) observed that SM supplementation in quails reduced malondialdehyde but increased the activities of SOD, glutathione peroxidase, and TAC. Moreover, SM remarkably boosted TAA and GSH and declined MDA in the laying hen (Abdalla *et al*. 2018). Higher antioxidant status and lower lipid peroxidation in SM-fed broilers might be due to scavenging of free radicals and boosting of the activity of the antioxidant enzymes (Tedesco *et al.* 2004). In contrast with the present findings, Armanini *et al*. (2021) reported that SM supplementation (100 mg/kg) did not affect the TBARS and glutathione s-transferase in broilers. This discrepancy with our findings might be due to differences in doses of SM and the time of study. In corroboration with the present findings, Rama Rao *et al*. (2012) showed an increase in antioxidant status in broilers supplemented with different doses 100, 200, 300, and 400 µg/kg diet) of organic chromium. Moreover, Ognik *et al.* (2020) reported that the addition of Cr with doses of 3 and 6 mg/kg diet in broilers stimulated the antioxidant defense system.

Liver and kidney function: The variables for liver and kidney function are reported in Table 3. Activities of ALT, AST, and ALP were lowered significantly (P<0.05) in the group that received both SM (500 mg/kg) and CP (400 µg/kg) as compared to SM_{500}, CP_{400}, and control groups over a period of 42 days of study. BUN and CREAT levels were lowered significantly (P<0.05) in broilers fed on a diet supplemented either with SM (500 mg/kg), CP (400 µg/kg) alone or in combination, as compared to the control, while levels reported in groups SM_{500} and CP_{400} did not show a significant difference. TP and ALB levels were increased significantly (P<0.05) in broilers that received SM (500 mg/kg) and CP (400 µg/ml) either alone or in combination in comparison to the control group. GLOB level was observed

Table 3. The effect of silymarin and chromium picolinate on blood metabolites of broiler chicks

Variable	Day	Group				SEM
		CON	SM_{500}	CP_{400}	SM_{500}+CP_{400}	
BUN, mg/100 ml	21	3.00^{c}	2.95^{b}	2.94^{b}	2.90^{a}	0.01
	42	2.85^{c}	2.75^{b}	2.74^{b}	2.65^{a}	0.01
	Mean	2.92^{c}	2.85^{b}	2.84^{b}	2.77^{a}	0.01
CREAT, mg/100 ml	21	0.65^{c}	0.60^{b}	0.59^{b}	0.55^{a}	0.01
	42	0.55^{c}	0.47^{b}	0.46^{b}	0.41^{a}	0.01
	Mean	0.60^{c}	0.53^{b}	0.53^{b}	0.48^{a}	0.01
ALT, IU/L	21	38.01^{a}	36.90^{a}	36.30^{a}	35.60^{a}	1.18
	42	34.03^{c}	31.05^{bc}	31.15^{bc}	30.10^{a}	1.20
	Mean	36.02^{b}	33.98^{ab}	33.73^{ab}	32.85^{a}	1.19
AST, IU/L	21	219.70^{c}	215.50^{bc}	213.33^{ab}	209.30^{a}	1.53
	42	210.03^{c}	192.08^{b}	191.11^{b}	184.60^{a}	1.27
	Mean	214.87^{d}	203.79^{c}	202.22^{b}	196.95^{a}	1.40
ALP, IU/L	21	39.35^{c}	34.33^{b}	33.16^{b}	29.37^{a}	1.10
	42	31.12^{c}	23.30^{b}	21.63^{ab}	19.11^{a}	1.42
	Mean	35.24^{c}	28.82^{b}	27.40^{b}	24.24^{a}	1.26
TP, g/100 ml	21	3.21^{a}	3.31^{b}	3.38^{c}	3.48^{d}	0.02
	42	3.34^{a}	3.51^{b}	3.60^{c}	3.77^{d}	0.01
	Mean	3.27^{a}	3.41^{b}	3.49^{c}	3.63^{d}	0.02
ALB, g/100 ml	21	1.79^{a}	1.87^{b}	1.91^{b}	2.00^{c}	0.02
	42	1.90^{a}	2.04^{b}	2.10^{c}	2.24^{d}	0.01
	Mean	1.84^{a}	1.95^{b}	2.00^{c}	2.12^{d}	0.01
GLOB, g/100 ml	21	1.42^{a}	1.44^{ab}	1.47^{b}	1.49^{b}	0.02
	42	1.44^{a}	1.47^{ab}	1.50^{bc}	1.53^{c}	0.01
	Mean	1.43^{a}	1.45^{a}	1.49^{b}	1.51^{b}	0.02

CON, control group without supplementation of silymarin and chromium picolinate; SM_{500}, silymarin supplemented group (500 mg/kg feed); CP_{400}, chromium picolinate supplemented group (400 µg/kg feed); SM_{800}+CP_{400}, both silymarin (500 mg/kg) + chromium picolinate (400 µg/kg) supplemented group; SEM, standard error mean.

Mean bearing different superscripts (a, b, c, and d) in a row differ significantly at P<0.05.

to be greater (P<0.05) in broiler chicks that received either CP (400 µg/kg) or both SM (500 mg/kg) and CP (200 µg/kg); however, no statistical difference was noticed in the levels of GLOB of control and SM_{500} groups.

In the present experiment, SM and CP supplementation either alone or in combination improved the function of kidney and liver by lowering biomarkers of kidney function, including BUN and CREAT, and the biomarkers of liver function, comprised ALT, AST, and ALP. The reduction in the biomarkers of kidney and liver function was observed more prominent in broilers who were fed both supplements. Similarly, SM reduced the production of hepatic enzymes, ALT, AST, and ALP as a consequence of hepatic injuries from free radicals (Amiridumari *et al*. 2013) and also lowered lipid and protein oxidation, indicating the hepato-protecting effect of SM. Moreover, the hepatoprotective effect of SM (800 mg/kg) by lowering ALT activity was reported by Jamshidi *et al*. (2007) in broilers. The addition of 1 g/kg milk thistle seed powder to the diet of quail lowered ALT and AST activities (Alassi and Allaw, 2020). Gharahveysi (2019) supplemented milk thistle at a dose of 3% and significantly reduced blood CREAT and had protective effect on the liver and kidneys of broilers. ALP activity significantly decreased with CP supplementation in the form of chromium-methionine at doses of 200, 400, and 800 ppb (Ebrahimzadeh *et al*. 2013).

The SM and CP both improved the levels of TP, ALB, and GLOB in blood, but the improvement was better in broilers who received both supplements. In agreement with the present findings, Sahad *et al*. (2020) observed that SM supplementation in the form of milk thistle seed powder @1% of feed in quail increases TP and GLOB levels. Increased TP, ALB, and GLOB in the blood agreed with the results reported by Dubey *et al*. (2022) in Sahiwal calves and Singh *et al*. (2023) in buffalo calves; they reported higher TP, ALB, and GLOB in SM-supplemented groups. Likewise, Sarai *et al*. (2022) reported a significant concentration of TP and ALB in a broiler-fed diet supplemented with chromium.

The results of the present study concluded that supplementation of SM and CP either alone or in combination improved the antioxidant status and health of the liver and kidney in broilers.

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