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RESEARCH ARTICLE

Effects of *Moringa oleifera* Leaves Powder and *Saccharomyces cerevisiae* alone or in Combination on Blood Biochemical Profile, Total Antioxidant and Oxidant Capacity and Intestinal Morphology in Broiler Chickens

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A B S T R A C T

The present study aimed to investigate the synergistic effect of dietary supplementation of *Moringa oleifera* (MO) leaves powder and the yeast (*Saccharomyces cerevisiae)* (SC) based prebiotic on the blood biochemical profile, antioxidant capacity, and intestinal morphology of broiler chickens. A total of 320 (Ross 308) straight-run one-day-old broiler chicks were acquired and randomly allocated into four treatment groups: The control group was fed a basal diet (without MO and SC addition); MO group (basal diet+1.5% dried MO leaf powder; SC group (basal diet +1.5%SC); and the MO + SC (basal diet supplemented with 1.5% MO + 1.5% SC). Each experimental group was replicated 4 times with 20 chicks each. The experiment lasted for 40 days. The combined use of MO+SC significantly reduced serum cholesterol and total protein levels compared to their separate use and the control group. The experimental groups significantly affected total antioxidants and oxidant capacity compared to the control group. The use of MO alone or in combination with SC had a notable effect on villus height, while crypt depth was affected by MO alone in the small intestine, except for villus height of duodenum and crypt depths, the use of MO in combination with SC as MO+SC in broiler rations positively influenced the blood biochemical parameters, total antioxidant and oxidant capacity, and villus height of jejunum and ileum. Based on these findings, supplementing *Moringa oleifera* leaf powder and yeast-based prebiotics in combination at the dose level of 1.5% in broiler rations can significantly improve blood biochemistry, antioxidant capacity, and gut morphometry.

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INTRODUCTION

Over the past two decades, there has been increasing global interest in natural, safe, and cost-effective alternatives to synthetic antioxidants, antimicrobial growth promoters, and pharmaceuticals. To this end, probiotics, prebiotics, organic acids, herbs, medicinal plants, and their extracts have been studied and recognized as natural, residue-free, less toxic, and safer substitutes for conventional synthetic products (Gheisar and Kim, 2017; Aydogan *et al.*, 2020). *Moringa oleifera*, also known as the drumstick tree, is considered one of the most remarkable medicinal plants. It belongs to the family Moringaceae and is widely distributed across northern India, Pakistan, Afghanistan, and Nepal (Klimek-Szczykutowicz *et al.*, 2024). *Moringa oleifera* is considered a promising alternative to antibiotic growth promoter because it contains 'Pterygospermin' a compound that readily dissociates into benzyl isothiocyanate, presents potent antibiotic and antifungal properties, indicating its potential as a natural growth enhancer for poultry (Akib *et al.*, 2024). Remarkably, the Moringa plant leaves contain minerals, vitamins, glutathione, omega-3–fatty acids, and phytochemicals, including alkaloids, flavonoids, sterols, tannins, saponins, terpenoids, phenolics, and glycoside compounds (Choudhary *et al.*, 2013; Jung, 2014). Due to the presence of aromatic compounds, *Moringa oleifera* plant has antioxidant hypoglycemic, anti-inflammatory, hypolipidemic, cholesterol-lowering, and hepatoprotective capabilities (Rahman *et al.*, 2017; Bayraktar *et al.*, 2023). Cui *et al.* (2018) reported a quadratically increased total

antioxidant capacity (T-AOC), along with a decline of malondialdehyde (MDA) in the breast muscle of the broiler when the feed had been supplemented with *Moringa oleifera* leaves. Similarly, lipid oxidation of the broiler breast muscle decreased with the supplementation of 5% moringa leaf in the diet (Abu Hafsa *et al.,* 2020). Khan *et al.* (2017) reported an improvement in body weight and intestinal microarchitecture by the supplementation of *Moringa oleifera* leaf powder in broilers. Akib *et al*. (2024) reported that *Moringa oleifera* leaf powder supplementation in broilers exhibited hypocholesterolemia and hypolipidemic effects compared to non-supplemented groups.

Saccharomyces cerevisiae, a eukaryotic, unicellular microorganism, has drawn much interest as a probiotic and prebiotic substitute for antibiotics (Qui, 2023; Ali *et al.,* 2024). Yeast is rich in easily digested protein and amino acids and a good source of vitamins B1, B2, B3, B_5, B_7, magnesium, and zinc (Klis *et al.,* 2002). Prebiotics are essential for enhancing the survival and proliferation of probiotics in the gut. They serve as substrates that support the growth and activity of probiotics in the lower gut, functioning symbiotically (Naseer *et al.,* 2020). Studies reported a significant influence of mannan oligosaccharide-prebiotics on blood glucose and cholesterol levels and improvements in nutritional digestibility, intestinal morphology, and nutrient absorption in broilers (Hossain *et al.,* 2024). Zheng *et al.* (2018) demonstrated that adding dietary mannan-oligosaccharides (MOS) can function as a free radical scavenger, enhancing the body's antioxidant capacity by inhibiting lipid peroxidation and/or increasing antioxidant enzyme activities in laying hens, broilers, and sheep. Miscellaneous investigations have been done on poultry using *Moringa oleifera* and *Saccharomyces cerevisiae* as prebiotics separately to impose positive effects on the blood biochemistry, oxidation status, antioxidant capacity, and intestinal morphology. However, there is a scarcity of literature about the combined or synergistic effect of *Moringa oleifera* and *Saccharomyces cerevisiae* in broilers. *Moringa oleifera* is rich in bioactive compounds that can enhance gut health, immunity, and overall performance in poultry. Yeast-based prebiotics promote beneficial gut microbiota, binding pathogenic bacteria with them and expelling them out of the body, hence helping to improve nutrient absorption and support immune function. Given their individual benefits, combining MO and yeast-based prebiotics at a 1.5% inclusion level may have a complementary or synergistic effect, leading to enhanced blood biochemistry, antioxidant status, and intestinal morphology compared to their dual use. In the present study, it was hypothesized that a combination of these supplements would improve broiler chickens' blood biochemistry, antioxidant status, and intestinal morphology, ultimately leading to improved productivity and overall health of broilers.

MATERIALS AND METHODS

Ethical approval: The study was approved by the Local Ethics Committee for Animal Experiments of the Ondokuz Mayıs University with decision number 2023/99, dated 26/12/2023.

Experimental design: Before the arrival of chicks, the temperature in the poultry house was initially set to 35±1°C and gradually reduced to 24°C over a period of weeks. To execute this study, a total of 320 one-day-old Ross-308 broiler chicks were used, with 80 chicks in each treatment group, and each group had 4 replicates, with 20 chicks per replicate, and the chicks were weighed individually. A total of 4 treatment groups were established in this study in the following manner: The control group was fed a basal diet (without MO and SC addition); the MO group (basal diet+1.5% dried MO leaf powder; SC group (basal diet +1.5%SC); and the MO + SC (basal diet supplemented with 1.5% MO + 1.5% SC). Rice husk was used as a bedding material. Each replicate housed 15 chicks/m². On the 13th and 32nd days, the chicks were vaccinated with Newcastle disease virus CL/79 strain and Infectious bronchitis Mass H120 strain by spray method. The trial was carried out for 40 days. After one week of the trial, the lighting schedule was followed, with 23 hours of light and 1 hour of dark period using fluorescent lamps. Birds were offered feed and water *ad-libitum*, and the feeders were continuously cleaned and disinfected. Starter (0-20 days) and grower phase (21-40 days) rations were formulated according to Ross-308 guidelines, as shown in Table 1.

Blood biochemistry: To determine the blood biochemical parameters, on day 40, blood samples were randomly collected in vacutainers from three broilers in each replicate through the vena jugularis at the time of slaughtering, and birds were slaughtered by Halal method (Farouk *et al.,* 2014). Blood-containing vacutainers were kept at room temperature for 30 minutes undisturbed; after that, blood samples were centrifuged at 3000 rpm for 15 minutes at 4°C, and the serum was transferred aseptically into Eppendorf tubes and stored at -20°C until analysis. Glucose, total protein, cholesterol, and triglyceride levels in the serum samples were measured using an A25 Random Access Analyzer (Biosystems, Spain).

Total antioxidant (TAC) and oxidant capacity (TOC): The serum samples (48 samples) were used to determine the total antioxidant capacity and total oxidant capacity in broiler chickens. The total antioxidant capacity (TAC) was measured using a commercial kit (Relassay, Turkey) with a spectrophotometer and expressed as mmol Trolox equivalent/L. The total oxidant capacity (TOC) was measured using a commercial kit (Relassay, Turkey) with a spectrophotometer and expressed as µmol H_2O_2 equivalent/L (Erel, 2005).

Histological Analysis: Tissue samples were collected from the duodenum, jejunum, and ileum from each replicate at day 40 (n=3). The tissue was washed with normal saline, fixed in 10% neutral buffered formalin, embedded in paraffin, and sectioned (4µm). The sections were further stained with hematoxylin (H) and eosin (E), mounted, and examined under a light microscope as described by Choe *et al.* (2013).

Villus height (µm) was measured from the tip of the villus to the junction between the villus and crypt, and crypt depth was (µm) from the junction to the basal membrane of epithelial cells-base of the crypt.

Table 1: Composition and nutrient levels (%) of the rations during the starter period (0–21 days) and grower period for each treatment group

Feed ingredients	Treatment Groups (Starter period=0-21 days)				Treatment Groups (Grower period=22-40 days),			
	Control	MO	SC	MO+SC	Control	MO	SC	MO+SC
Maize	48.50	48.20	48.40	48.25	53.25	53.00	53.10	53.04
Soybean meal, (45%)	29.60	30.00	28.10	28.50	23.50	24.00	22.50	23.00
Full-fat soybean	14.60	13.00	14.70	13.00	15.50	13.71	15.00	13.20
Vegetable oil	3.00	3.00	3.00	3.00	4.20	4.30	4.30	4.30
Limestone	1.11	1.11	1.11	1.10	1.20	1.13	1.34	1.20
Dicalcium phosphate	1.90	1.90	1.90	1.85	1.40	1.40	1.30	1.30
DL-Methionine	0.19	0.19	0.19	0.20	0.08	0.10	0.10	0.10
Sodium Bicarbonate	0.20	0.20	0.20	0.20	0.14	0.13	0.13	0.13
Salt	0.25	0.25	0.25	0.25	0.18	0.18	0.18	0.18
Phytase	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Coccidiostat	0.05	0.05	0.05	0.05	-	-	-	-
Vitamin-Mineral mix*	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Salkil**	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Toxin binder	0.05	0.05	0.05	0.05	-	-	-	-
Saccharomyces cerevisiae (prebiotic)			1,50	1.50			1.50	1.50
Moringa oleifera		1.50		1.50		1.50		1.50
Nutrient composition								
CP,%	23.10	23.07	23.12	23.07	21.04	21.00	21.04	21.01
ME,kcal/kg	3051	3038	3047	3036	3210	3196	3201	3194
Ca,%	1.00	1.02	1.00	1.01	0.90	0.90	0.90	0.90
Available P,%	0.44	0.45	0.45	0.45	0.35	0.35	0.34	0.35
Met+Cys,%	0.90	0.89	0.90	0.90	0.74	0.75	0.76	0.75
Lysine,%	1.27	1.27	1.28	1.28	1.14	1.14	1.15	1.15
Threonine,%	0.87	0.87	0.88	0.88	0.79	0.79	0.80	0.80
Tryptophan,%	0.32	0.32	0.32	0.32	0.28	0.28	0.28	0.28

CP: crude protein; ME; metabolizable energy; P: phosphorous; Ca: calcium; Met+Cys: methionine and cysteine; *The basal ration contains a vitamin and mineral premix with the following composition per kilogram: Vitamin A (Retinyl acetate) 4,000,000IU, Vitamin D3 (Cholecalciferol) 1,200,000IU, Vitamin E (all-rac-alpha-tocopheryl acetate) 40,000mg, Vitamin K3 (Menadione sodium bisulfite) 1,600mg, Vitamin B1 (Thiamine mononitrate) 1,200mg, Vitamin B2 (Riboflavin) 3,200mg, Vitamin B6 (Pyridoxine hydrochloride) 1,600mg, Vitamin B12 (Cobalamin) 12mg, Vitamin B3 (Niacin-nicotinic acid) 16,000mg, Vitamin B5 (Calcium D-pantothenate) 6,000 mg, Vitamin B9 (Folic acid) 400mg, Vitamin H (Biotin) 40mg, and Choline chloride 160,000 mg. Additionally, it includes Manganese (Manganese sulfate monohydrate) 40,000mg, Iron (Iron (II) sulfate monohydrate) 24,000mg, Zinc (Zinc oxide) 32,000mg, Copper (Copper (II) sulfate pentahydrate) 4,000mg, Iodine (Anhydrous calcium iodate) 600mg, Selenium (Sodium selenite) 80mg, and Sepiolite (E562-mineral clay) magnesium silicate ($Mg_4Si_6O_{15}(OH)_2 \cdot 6H_2O$) 100,000mg. Furthermore, it contains Salicylic acid (precipitated and dried) at 7,500mg and **Salkil (ammonium salts of formic and propionic acids impregnated in silica) in the range of 2,000–4,000mg.

Villus height and crypt depth were measured using ImageJ software (National Institutes of Health, USA).

Statistical analysis: The data collected were analyzed by applying a One-way analysis of variance technique using SPSS software (IBM) version 23.0. The means of the treatment groups were compared through Duncan's multiple-range test. The probability value ($P<0.05$) indicated that the results were statistically significant, and data were expressed as Mean±SEM. Graphs were constructed using GraphPad Prism software (version 10.4.1).

RESULTS

Blood biochemistry: The effects of dietary (MO), (SC), and their combination (MO+SC) supplementation on the blood serum biochemistry of the broilers are presented in Table 2. Serum glucose and triglyceride levels did not differ significantly across groups. Significant variations were observed in cholesterol ($P<0.01$) and total protein levels ($P<0.05$). The MO+SC group exhibited significantly ($P<0.05$) the lowest cholesterol and total protein levels, respectively, compared to the MO, SC, and control groups, demonstrating a synergistic cholesterol-lowering effect for the MO+SC group. Conversely, the MO group exhibited the highest cholesterol and total protein concentrations, compared to other treatment groups.

Total antioxidant (TAC) and oxidant capacity (TOC): The bar graph in Fig. 1 illustrates the significant impact of dietary treatment groups of MO, SC, and MO+SC on broiler TAC and TOC values. Treatment groups are on the X-axis, and TAC (mmol Trolox Eq/L) and TOC (µmol H_2O_2 Eq/L) values are presented on the Y-axis, respectively. Results given in the bar chart indicate that significantly ($P<0.05$) lower TAC levels and significantly ($P<0.05$) higher TOC levels were recorded in the control group as compared to treatment groups. It can be observed that there was no significant difference among the dietary treatment groups regarding TAC and TOC levels compared to the control group.

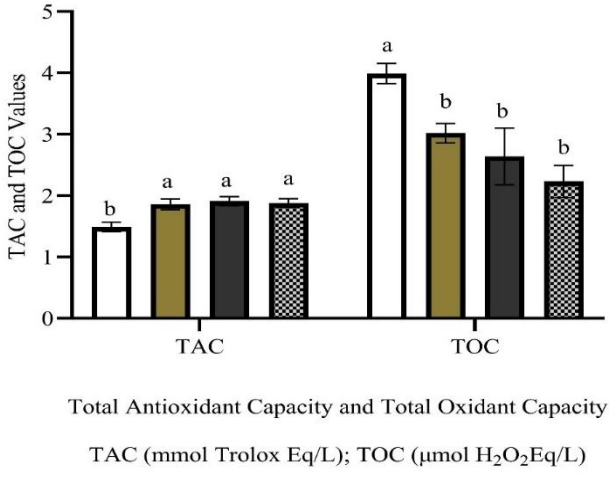

Fig. 1: Effect of *Moringa oleifera* leaf powder and *Saccharomyces cerevisiae* on total antioxidant and total oxidant capacity of broiler chickens with $P<0.05$. TAC: total antioxidant capacity; TOC: total oxidant capacity: MO: *Moringa oleifera*; SC: *Saccharomyces cerevisiae* MO+SC: *Moringa oleifera+Saccharomyces cerevisiae*

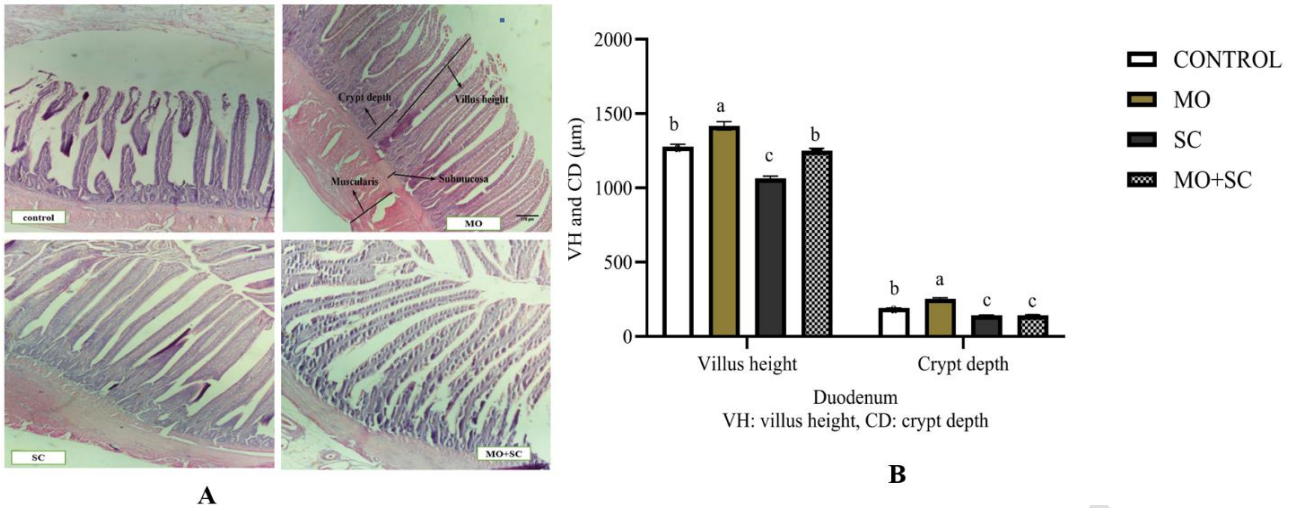

Fig. 2: Histological analysis of the effect of *Moringa oleifera* leaf powder and *Saccharomyces cerevisiae* on the histomorphology of duodenum in broilers. (A) H&E staining of duodenum showing different histological structures in duodenum. Crypt depth (µm), tunica muscularis (µm), villus height (B). Bar diagram showing a comparative difference in the mean values+SEM of Crypt depth (µm), and villus height in different groups in duodenum. Control: MO. Different letters (a, b, c) represent significantly different means (P<0.05).

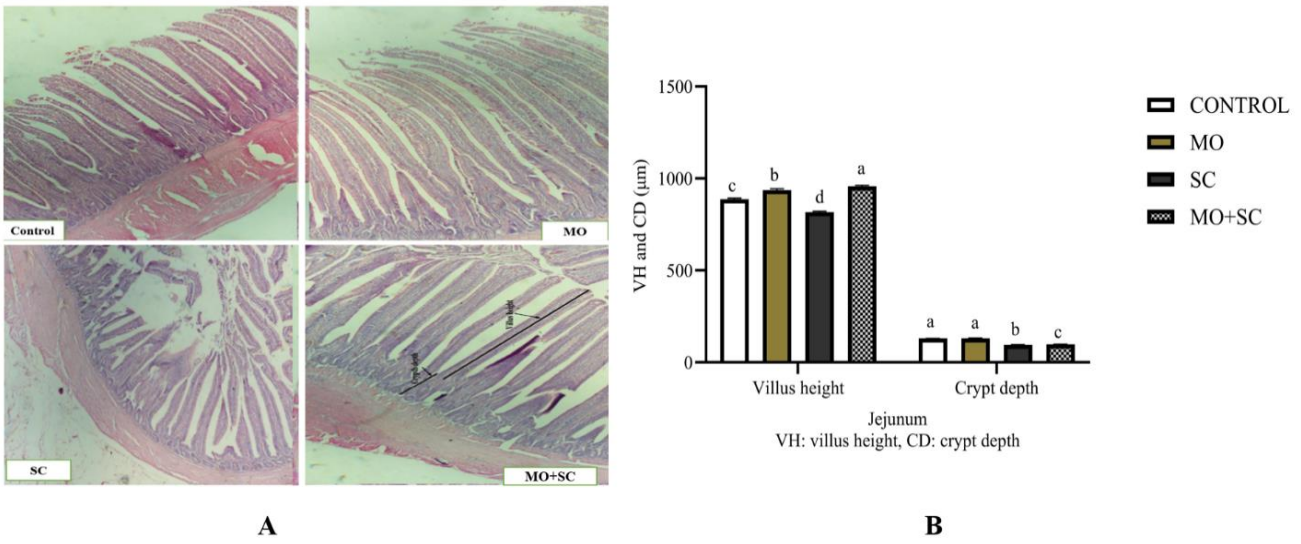

Fig. 3: Histological analysis of the effect of *Moringa oleifera* leaf powder and *Saccharomyces cerevisiae* on the histomorphology of jejunum in broilers. (A) H&E staining of jejunum showing different histological structures in jejunum. Crypt depth (µm), tunica muscularis (µm), villus height (B). Bar diagram showing a comparative difference in the mean values+SEM of Crypt depth (µm), and villus height in different groups in jejunum. Control: MO. Different letters (a, b, c) represent significantly different means (P<0.05).

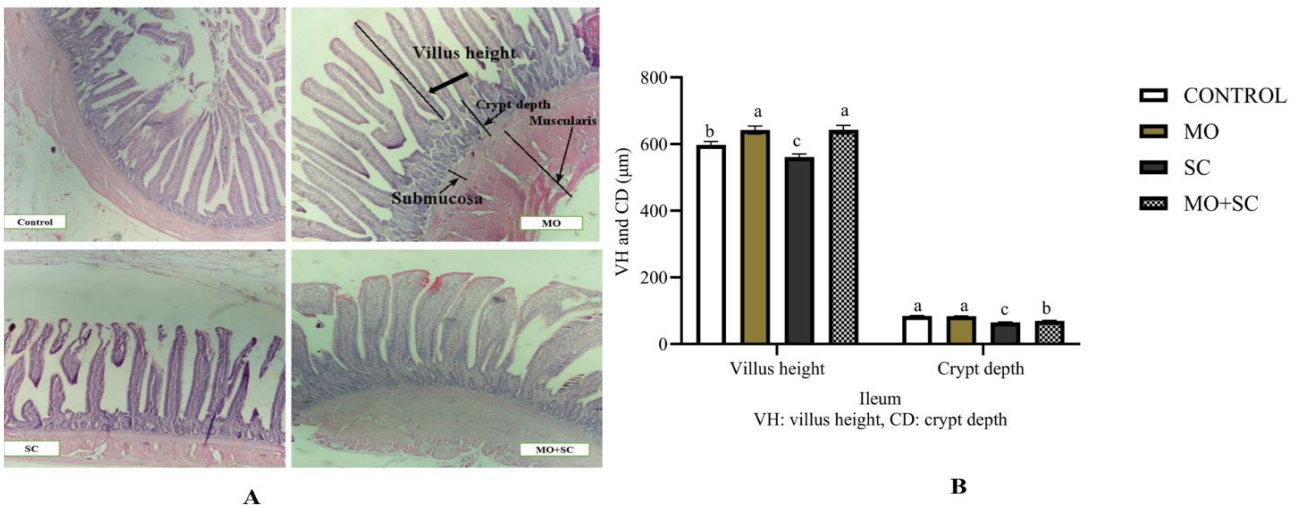

Fig. 4: Histological analysis of the effect of Moringa oleifera leaf powder and Saccharomyces cerevisiae on the histomorphology of ileum in broilers. (A) H&E staining of ileum showing different histological structures in ileum. Crypt depth (µm), tunica muscularis (µm), villus height (B). Bar diagram showing a comparative difference in the mean values+SEM of Crypt depth (µm), and villus height in different groups in ileum. Control: MO. Different letters (a, b, c) represent significantly different means (P<0.05)

Table 2: Effect of *Moringa Oleifera* leaves powder and *Saccharomyces cerevisiae* on blood Biochemical parameters of broiler chickens

Parameters	Treatment Groups				P-value
	Control	MO	SC	MO+SC	
Cholesterol, mg/dL	139.67±5.59^{ab}	161.09±5.29^{a}	146.18±4.87^{a}	121.42±13.10^{b}	0.013
Total protein, g/L	42.45±2.14^{ab}	47.44±2.13^{a}	46.33±2.23^{a}	35.49±4.55^{b}	0.032
Glucose, mg/dL	197.92±4.68	205.09±5.55	204.36±4.91	183.44±13.16	0.215
Triglycerides, mg/dL	30.25±2.14	30.91±2.43	29.45±2.06	27.56±3.10	0.792

Results are presented as mean+SEM and within the same row, means with different superscripts^{a,b} differ significantly (P<0.05). MO: *Moringa oleifera*; SC: *Saccharomyces cerevisiae* MO+SC: *Moringa oleifera+Saccharomyces cerevisiae.*

Histological Analysis: Hematoxylin and eosin-stained sections of duodenum, jejunum, and ileum have been shown in Fig. 2A, 3A, and 4A, respectively, as affected by different treatment groups. The effects of MO, SC, and their combination (MO+SC) supplementation on the intestinal morphology of the duodenum, jejunum, and ileum of broilers have been shown in Fig. 2B, 3B, and 4B, respectively. Duodenum villus height was significantly higher in the MO group (P<0.05), followed by MO+SC, control, and SC. The MO+SC group exhibited the greatest (P<0.05) Jejunum villus height, followed by the MO, control group, and SC groups. MO and MO+SC groups demonstrated comparable villus heights in the ileum; nevertheless, both were significantly higher (P<0.05) than the control and SC groups. The MO group recorded a greater (P<0.05) duodenum crypt depth than the control group and other treatment groups, and MO+SC showed a similar trend to that of SC. The MO and control groups displayed similar crypt depths in the jejunum and ileum; significantly higher crypt depth (P<0.001) was observed in the MO and control groups than in the SC and MO+SC groups.

DISCUSSION

The blood profile of healthy animals is affected by feed formulation and nutritional status (Iheukwumere and Herbert, 2003). The analysis of blood biomarkers in broiler chickens provides insight into their physiological status (Bagno *et al*., 2021). In the present study, the MO+SC treatment group showed significantly lower cholesterol compared to the MO, SC, and control groups. Similar to the findings of the present study, Alnidawi *et al*. (2016), SH *et al*. (2019), and Mousa *et al*. (2017) reported a reduction in cholesterol levels with increasing dietary inclusion of *Moringa oleifera* leaf meal (MOLM) in broilers. Olugbemi *et al*. (2010b) further demonstrated the hypocholesterolemia potential of MOLM, particularly in reducing egg cholesterol content. This cholesterol-lowering effect of *M. oleifera* leaf extracts is likely due to the presence of bioactive compounds that inhibit the intestinal absorption of dietary cholesterol (Maheshwari *et al*., 2014). Similarly, Kannan *et al*. (2005) observed that mannan-oligosaccharides (MOS) supplementation led to a decrease in certain biochemical parameters in broilers, including serum total cholesterol (TC) and total globulin. The reduction in cholesterol levels may also be attributed to the intake of prebiotics, which enhances mineral absorption and lowers serum cholesterol by inhibiting cholesterol absorption and deconjugating bile salts in the gastrointestinal tract (Moravej, 2009; Tang *et al*., 2017; Waqas *et al*., 2018). Therefore, the observed reduction in serum cholesterol in the MO+SC group suggests a synergistic effect between *Moringa oleifera* and the yeast-derived prebiotic, highlighting their combined potential to improve lipid metabolism in broilers.

The level of total protein in circulation primarily reflects the synthesis or breakdown of two major proteins: albumin and globulin (Bagno *et al*., 2021). In the current study, total protein levels were significantly higher in the MO and SC groups compared to the control and other treatment groups. This aligns with the findings of Onu and Aniebo (2011) and Hassan *et al*. (2016), who reported a significant increase in plasma total protein in broilers with increasing inclusion levels of *Moringa oleifera* leaf meal (MOLM). Similarly, Alqhtani *et al*. (2024) observed that dietary supplementation with yeast (*Saccharomyces cerevisiae*) cell wall (YCW) enhanced total protein (TP) and globulin (GLO) levels, suggesting that YCW may stimulate protein anabolism, thereby supporting improved growth efficiency, particularly in challenged birds. However, contrasting findings were reported by Divya *et al.* (2014), who noted a reduction in serum protein levels in broilers fed MOLM. In contrast, studies on other species, such as West African Dwarf goats, showed an increase in total serum protein when MOLM was included in the feed (Safa and Ibrahim, 2014; Yusuf *et al*., 2018). Moreover, other studies have reported no significant changes in serum total protein, triglyceride, or urea nitrogen levels in broilers receiving diets supplemented with *Saccharomyces cerevisiae* (Yalçın *et al*., 2013; He *et al*., 2021; Okasha *et al*., 2023). These discrepancies in findings may be attributed to variations in the inclusion levels and quality of *Moringa oleifera* and *S. cerevisiae* used in different studies (Modisaojang-Mojanaga *et al*., 2019).

Concerning results about glucose and triglyceride levels, there was no statistical difference among the treatment groups. Contrary to the present study, Zanu *et al.* (2012) and Castillo *et al. (*2018) found that triglycerides were reduced significantly with *Moringa Oleifera* leaf meal (MOLM) supplementation in birds; similar to the present study, glucose levels remained unchanged when Japanese quail fed 7 to 21 % Moringa supplementation (Castillo *et al.*, 2018). Several studies have also reported that serum concentrations of triglycerides and urea nitrogen were not significantly affected in broilers-fed diets supplemented with *Saccharomyces cerevisiae* (Okasha *et al*., 2023). Similarly, Yalçinkaya *et al*. (2008) and Konca *et al*. (2009) observed no significant differences in serum cholesterol and triglyceride levels in poultry birds fed either control, *Moringa oleifera* (MO), or *S. cerevisiae* (SC) supplemented diets. In contrast to the findings of the current study, Osita *et al*. (2020) reported a marked decrease in serum glucose and triglyceride levels, without any significant change in total protein, following dietary supplementation with *S. cerevisiae*.

Total antioxidant capacity is a comprehensive indicator of various factors that assess the serum's ability to counter oxidative stress (Silvestrini *et al.*, 2023). Total antioxidant capacity is a mechanism used to measure the extent of free radical scavenging in a test sample (Bibi Sadeer *et al.*, 2020), making it a valuable tool for evaluating the antioxidant capacity of biological samples

(Marques *et al.,* 2014). In the present study, all the treatment groups have a positive effect on broilers' total antioxidant and oxidant capacity. Treatment groups showed significantly higher TAC and lower TOC values than the control group. The results of MO are consistent with the observations of Cui *et al.* (2018), who reported that plasma total antioxidant capacity, total superoxide dismutase, and glutathione peroxidase activities increased quadratically ($P < 0.01$), while MDA decreased quadratically ($P < 0.001$) in broilers fed with MOLP. These findings are also supported by the results found by Lu *et al.* (2016), in which glutathione peroxidase linearly and quadratically increased, and plasma MDA level was lowered in MOL at the level of 10% and 15%. This trend was also fortified by 1% Moringa supplementation in the rabbit diet, in which catalase (CAT), glutathione peroxidase, and superoxide dismutase significantly increased, and MDA decreased (Helal *et al.,* 2017). Some other studies also reported that MDA was reduced in the groups supplemented with MOL up to 6% (Mohamed *et al.,* 2022). Findings about yeast-based prebiotics have been supported by Qiu *et al.* (2024), who reported that glutathione peroxidase (GSH-Px) and serum total antioxidant capacity (T-AOC) were both increased by yeast culture supplementation, while malondialdehyde (MDA) level decreased ($p < 0.05$) in laying hens. Identical outcomes were observed using spent ginger yeast culture in laying hens (Liu *et al.,* 2022). Contrary to the findings of the present study, supplementation of 400mg/kg of dietary yeast glycoprotein increased the total antioxidant capacity in broiler chickens (Wassie *et al.,* 2022). Alqhtani *et al.* (2024) in their study revealed that supplementing the diets with YCW resulted in heightened levels of T-AOC and T-SOD. Contrary to the findings of the present study, using dietary yeast probiotics up to 1.0 mL did not alter the levels of SOD and MDA in broiler chickens (Aluwong *et al.,* 2013). The reduction in total oxidant capacity and increase in antioxidant capacity of broilers chickens can be attributed to the antioxidant activity of *Moringa oleifera* leaf powder (Bayraktar *et al.,* 2023) and of yeast-based prebiotic (Zheng *et al.* (2018). Therefore, the combined supplementation of Moringa and SC-prebiotics demonstrates a synergistic effect in lowering oxidative stress in broilers.

The structural features of the chicken's intestine are essential for nutrient utilization and serve as a sign of healthy physiology (Mahfuz and Piao, 2019). The effects of MO, SC, and MO+SC on intestinal morphology in the duodenum, jejunum, and ileum have been presented in Fig. 2B, 3B, and 4B, respectively. According to the data obtained in this investigation, 1.5% MO significantly ($P < 0.001$) improved the villus height in the duodenum, jejunum, and ileum, but crypt depth did not change except for an improvement in the duodenum. Similar to the present study, (Khan *et al.,* 2017) reported that the inclusion of 1.2% MOLM in broilers' diet enlarged the villus height in the different parts of the small intestine, villus surface area of the duodenum and villus height: crypt depth of the ileum in contrast to the broilers control diet. These results were in tandem with the earlier reports of others that MOLM increases the length of the villi across the three anatomical parts of the small intestine in broilers (Tesfaye *et al.,* 2013; Nkukwana *et al.,* 2014). In the current study, 1.5% of dietary SC resulted in a significant decrease ($P < 0.001$) in villus height and crypt depth in all parts of the small intestine compared to the control. Contrary to the current findings, Adebiyi *et al.* (2012) observed greater villus height and crypt in the ileum by using 1.5 g/kg SC. However, the combination of MO+SC remarkably ($P < 0.001$) improved the villus height in the jejunum and ileum and reduced the crypt depth in all parts of the small intestine. Similar to these findings, Qiu *et al.* (2024) also found considerable enhancement in the duodenum by supplementing 1 to 2 g/kg yeast culture. Dietary yeast cell walls significantly improved villus height compared to the control, but no discrepancy was observed in crypt depth (Zhang *et al.,* 2005). Contrary to the findings of the present study, no dramatic differences were found in the villus height and crypt depth of the duodenum, jejunum, and ileum of the laying hens and broilers using yeast culture (Lin *et al.,* 2023). A significant improvement in the villus height of jejunum and ileum in the MO+SC group; can be attributed to the synergetic effects of both *Moringa oleifera* and SC-based prebiotic.

Conclusions: The results of the study indicate that the combination of MO+SC demonstrated significant reductions in serum cholesterol, while MO and SC groups have increased the total protein. The addition of MO, SC, and their combination reduced the oxidative stress markers (TOC) and has positively influenced antioxidant capacity (TAC). It is concluded that the use of these additives, whether individually or in combination, has the potential to improve the blood biochemical profile, intestinal histomorphology, total antioxidant capacity (TAC), and total oxidative capacity (TOC) in broiler chickens. Further investigations are recommended to explore their long-term effects, dose rate, interaction with other additives, and underlying mechanisms of these dietary interventions, as well as the utilization of their extracts and metabolites in the diet of poultry.

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