

Nutraceuticals vs. antibiotic growth promoters: differential impacts on performance, meat quality, blood lipids, cecal microbiota, and organ histomorphology of broiler chicken

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ABSTRACT The main goal of this study was to evaluate the effect of nutraceuticals vs. in-feed antibiotics on performance, blood lipids, antioxidant capacity, cecal microbiota, and organ histomorphology of broiler chickens. A total of 320 one-day-old male broiler chickens were distributed into 5 treatment groups with 8 replicates each. The control group was fed on a basal diet without any additives (**NC**); the antibiotic group was fed on a basal diet supplemented with 100 mg kg⁻¹ avilamycin (**PC**); the algal group was fed on a basal diet supplemented with a mixture of *Spirulina platensis* and *Chlorella vulgaris* (1.5 g + 1.5 g/kg feed) (**SP+CV**); the essential oil group was fed with a basal diet containing 300 mg/kg feed rosemary oil (**REO**); and the probiotics group (a mixture of 1×10^{11} CFU/g *Bacillus licheniformis*, 1×10^{11} CFU/g *Enterococcus faecium*, 1×10^{10} CFU/g *Lactobacillus acidophilus*, and 2×10^8 CFU /g *Saccharomyces cerevisiae*) was fed with a basal diet supplemented with 0.05% probiotics (**PRO**). The experiment lasted for 35 d. A beneficial effect of SP+CV and PRO ($P < 0.01$) was noticed on final body weight, body weight gain, feed conversion ratio, and breast yield. The dietary

supplementation with SP+CV, REO, and PRO increased ($P < 0.001$) broilers' cecal lactic acid bacteria count compared to the control. Lower cecal *Clostridium perfringens* and *Coliform* counts ($P < 0.001$) were noticed in chickens fed the PC and supplemental diets. Malondialdehyde (MDA) concentration was decreased, while glutathione peroxidase (GPx), superoxide dismutase, and catalase enzymes were increased in the breast and thigh meat ($P < 0.001$) of broiler chickens fed SP+CV, REO, and PRO diets. Dietary SP+CV, REO, and PRO supplementation decreased ($P < 0.001$) serum total lipids, cholesterol, triglycerides, low-density lipoprotein, and MDA, but increased serum high-density lipoprotein and GPx compared to PC and NC. No pathological lesions were noticed in the liver, kidney, or breast muscle among broilers. The SP+CV, REO, and PRO groups had greater ($P < 0.001$) intestinal villi height and crypt depth while lower goblet cell densities ($P < 0.01$) than the control. The present findings suggest that PRO and SP+CV, followed by REO could be suitable alternatives to in-feed antibiotics for enhancing the performance, health, and meat quality of broiler chickens.

Key words: nutraceutical, antibiotic alternative, performance, meat quality, broiler chicken

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INTRODUCTION

The extraordinary development in poultry manufacturing can be attributed to advanced technologies in poultry breeding, nutrition, health, and welfare.

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Antibiotics as growth promoters (AGP) in animal feeds have influenced the intestinal microbiota and immunity, resulting in a significant capacity to control diseases. However, careless application of antibiotics can result in antibiotic-resistant microbes and increased antibiotic residues in poultry products, threatening the health of consumers and animals. Antibiotics in chicken feed were consequently banned by the European Union on January 1, 2006 ([Kulshreshtha et al., 2014](#); [Abd El-Hack et al., 2022](#)). Because of this, more research is being done to identify safer alternatives to AGP that can affect poultry and livestock productivity in a comparable or

better way. Green and healthful nutraceuticals can fill this gap and be used in poultry production instead of antibiotics. In poultry, nutraceuticals can be more beneficial in promoting immunological responses, enhancing growth performance, and improving the quality of the final product (Abd El-Hack et al., 2022; Yosi and Metzler-Zebeli, 2023).

Probiotics, live microbial products, are feed additives that play a beneficial role in maintaining the balance of gastrointestinal tract (GIT) microbiota (Yosi and Metzler-Zebeli, 2023). This balance will improve digestion and absorption of nutrients, boost immune response, and enhance growth performance and antioxidant capacity, eventually resulting in better profitability (Yosi and Metzler-Zebeli, 2023). Probiotics perform their functions through various mechanisms, involving nutritional competition with harmful microbes, adhesion to the GIT surface, and production of anti-pathogenic substances (Latif et al., 2023; Jha et al., 2020). So far, many microbial species have been identified for use as probiotics and feed supplements in poultry, such as *Lactobacillus*, *Bacillus*, *Enterococcus*, *Bifidobacterium*, and *Saccharomyces* (Jha et al., 2020). *Bacillus licheniformis* was reported to produce biologically active substances, including digestive enzymes, lysozyme, and antibacterial compounds, which augment nutrient digestibility, enhance the immune response, improve intestinal mucosal barrier function, prevent the colonization of pathogenic microbes, promote the multiplying of beneficial bacteria, and adjust the equilibrium of GIT microbiota (Sun et al., 2023). *Enterococcus faecium* has been indicated to enhance intestinal immunity and jejunal mucus secretion in broilers (Wu et al., 2019) and improve hosts' resistance to pathogenic microbes (Pogačar et al., 2020). Furthermore, *Saccharomyces cerevisiae* was shown to improve the growth and serum biochemistry, enhance GIT health and morphology, favorable bacterial proliferation, boost immunity, and augment the antioxidant capacity of birds (Hussein and Selim, 2018; Selim et al., 2022).

Microalgae are nutraceuticals with eminent nutritional benefits and are effectively applied in poultry nutrition with numerous aspects purposes such as to improve the pigmentation and nutritional value of meat and eggs and enhance health and welfare (Bonos et al., 2016; Selim et al., 2018). A blue-green filamentous algae, *Spirulina platensis* (SP), is widely recognized as a dietary supplement in poultry diets as a valuable source of protein (50–70%) and for their valuable contents of essential nutrients, including, vitamins, minerals, amino acids, fatty acids, and phytopigments (Bondar et al., 2023). SP is a potent candidate as an alternative to antibiotics in poultry feeds because their beneficial components exhibit antimicrobial, antioxidant, anti-inflammatory, antiviral, immune-stimulating, and hypocholesterolemic effects (El-Shall et al., 2023). Recent studies determined its application in poultry as a growth promoter, a maintainer of gut integrity, a booster of immunity, and an enhancer of meat yield and quality (Bonos et al., 2016; Sugiharto et al., 2018). These

investigations have shown several outcomes emphasizing the potential of SP in these different purposes of poultry production. Another important microalgae that garnered attention as a feed source is *Chlorella vulgaris* (CV), green microalgae, owing to its valuable contents of nutrients, including good-quality protein, vitamins, phytopigments, minerals, and polyunsaturated fatty acids (Chaves et al., 2021). These nutritional benefits of CV have led to its application as a feed supplement, with research indicating positive effects on growth performance, immune function, and meat quality in animals (Kotrbáček et al., 2015). Therefore, a combination of SP and CV can provide more potential benefits as a growth promoter and as an alternative to AGP in the diet of poultry.

Essential oils (EO) were recorded to protect GIT from pathogenic microorganisms, increase the potential of digestive secretion, boost the immune system, improve feed palatability, and thus augment production performance (Aberbour et al., 2023; Gumus and Gelen, 2023). Instead of antibiotics, essential oils can be added to feed to improve digestibility and nutrient absorption (Aberbour et al., 2023; Gumus and Gelen, 2023). *Rosmarinus officinalis* EO is one of the most noteworthy and effective supplements in poultry diets; it has been proven to postulate antioxidative properties, antimicrobial effects, and growth stimulator effects in poultry (Aberbour et al., 2023). It has also been implied that the phenolic substances of rosemary play a protective role against oxidative stress through free radical scavenging activity (Zhang et al., 2021). From this viewpoint, supplementing poultry diets with EO could be an effective approach to improve production and decrease the use of antibiotics (Zhang et al., 2021; Aberbour et al., 2023).

The goals of the current research were to examine the effects of different nutraceutical feed additives on growth performance, antioxidant capacity, meat quality, organ histomorphology, and cecal bacteria count in broiler diets as potential antibiotic alternatives. The hypothesis tested was that these nutraceuticals could induce beneficial impacts rather than antibiotics on production performance, meat quality, oxidation stability, cecal microbiota, and organ histomorphology of broiler chickens.

MATERIALS AND METHODS

Feed Additives

Avilamycin was purchased from Hangzhou Well Sunshine Biotech. Co. Ltd, Hangzhou, China. Probiotics were obtained from the Microbiological Resources Center (MIRCEN, Cairo, Egypt). The multi-strain probiotics (powder form) consisted of a mixture of *Bacillus licheniformis* (1×10^{11} CFU/g), *Enterococcus facieum* (1×10^{11} CFU/g), *Lactobacillus acidophilus* 1×10^{10} CFU/g, and *Saccharomyces cerevisiae* (2×10^8 CFU/g). SP and CV were supplied by the Algal Biotechnology Unit, National Research

Table 1. Nutrient composition of microalgae.

Item, %	<i>Spirulina platensis</i>	<i>Chlorella vulgaris</i>
Dry matter	94	93
Crude protein	55.5	49
Ether extract	8.01	10.98
Crude fiber	5.1	9.2
Ash	10.51	10.3

Centre (Giza, Egypt). The chemical composition of SP and CV is presented in **Table 1**. The chemical composition of SP and CV was performed according to AOAC (2005). Rosemary essential oil was purchased from the local market. The total flavonoid and phenolic contents of rosemary oil were determined according to the methods of Kim et al. (2003) and Al-Farsi et al. (2005), respectively. The total phenolic and flavonoid contents of rosemary oil were 63 mg GAE/g dry weight and 9.4 mg QE/g dry weight, respectively. The dosages of probiotics (Selim et al., 2022), algae (Abdelfatah et al., 2024), and essential oil (Gumus and Gelen, 2023) used in the current trial were established according to previous findings that proved plateau levels without an additional improvement in the growth performance of broiler chickens.

Ethical Statement

The trial was permitted by the Institutional Animal Care and Use Committee of the Faculty of Veterinary Medicine, University of Sadat City, Egypt (Ethical approval number is VUSC-001-1-24). All birds were handled along with the principles mentioned by the EC Directive 2010/63/EU.

Birds and Experimental Design

A total of 320 one-day-old male Arbor Acres plus broiler chickens with similar initial body weight (45.41 g \pm 0.16; mean \pm SEM) were procured from a commercial hatchery (Arab for Poultry Breeders Co., Ltd., Giza, Egypt). Chicks were randomly allotted into 5 treatment groups of eight replicates (8 chicks/replicate; $n = 64$ per group) in a completely randomized design for 35 days. The rearing period was allotted into 3 phases: 1 to 11 d, 12 to 24 d, and 25 to 35 d. The trial treatments were as follows: the Control group, a basal diet without any feed additives (**NC**); the antibiotic group, a basal diet supplemented with 100 mg avilamycin/kg diet (**PC**); the algae group, a basal diet supplemented with 1.5 g SP/kg diet + 1.5 g CV/kg diet (**SP+CV**); essential oil group, a basal diet supplemented with rosemary essential oil 300 mg/kg diet (**REO**); and the probiotic group, a basal diet supplemented with 0.5 g multi-strain probiotic/kg diet (**PRO**). Feed and water were kept ad libitum for 35 d of the experiment. As presented in **Table 2**, trial diets were provided in mash form and were formulated according to Arbor Acres plus nutritional guidelines (https://aviagen.com/assets/Tech_Center/AA_Broiler/AA-BroilerNutritionSpecifications2022-EN.pdf). The chemical composition of diets was performed according to AOAC (2005). The broilers were managed inconsistently with the breed's guidelines (Arbor Acres Broiler Commercial Management Guide). During the trial, birds were reared on a wire floor of equal dimensions in a well-established environment. The room temperature was 33°C for the first 3 d and then gradually dropped by 3°C per week to maintain a room temperature of 24°C and relative humidity of 40 to 70%. A lighting program was prepared for 24 h during the first 3 days and then continued for 23L:1D. The vaccination program was accomplished following the breeder standards.

Table 2. Experimental diets and their nutrient composition.

Ingredients, %	Starter, 1–11 d	Grower, 12–24 d	Finisher, 25–35 d
Yellow corn	48.00	52.60	55.24
SBM, 44 % CP	40.95	36.45	35.00
Corn gluten, 60% CP	1.36	1.27	0.00
Vegetable oil	4.80	5.10	5.60
Limestone	1.00	0.90	0.70
Dicalcium phosphate	1.97	1.80	1.80
L-lysine	0.90	0.80	0.70
DL-Methionine	0.42	0.39	0.36
Common salt	0.30	0.30	0.30
Premix ¹	0.30	0.30	0.30
Total	100	100	100
Nutrient composition			
ME, kcal/kg	3003.05	3078.90	3130.84
Crude protein, %	22.91	21.27	20.09
Calcium, %	1.17	1.06	0.94
Available phosphorus, %	0.48	0.42	0.40
Lysine, %	1.32	1.18	1.07
Methionine, %	0.54	0.51	0.48

¹Provided per kilogram of diet: vitamin A, 12,000 IU; vitamin D₃, 3,000 IU; vitamin E, 25 IU; vitamin K₃, 1.8 mg; vitamin B₁, 2.0 mg; vitamin B₂, 5.0 mg; vitamin B₆, 3.0 mg; vitamin B₁₂, 0.1 mg; vitamin B₃, 40 mg; vitamin B₅, 15 mg; vitamin B₉, 1.0 mg; vitamin B₇, 0.05 mg; Fe, 80 mg; Cu, 10 mg; Zn, 90 mg; Mn, 80 mg; I, 0.35 mg; Se, 0.20 mg.

Growth Performance, Carcass Traits, and Sampling

The birds were individually weighed on the day of their arrival and then, weekly until slaughtering. Feed intakes of birds were measured daily during the experiment. ADG, ADFI, and feed conversion ratio (**FCR**; g of feed/g of body gain) were calculated accordingly. At d 35 of age, 2 broiler chickens from each replicate (16 broilers/group) were selected, within the mean body weight of the group, for sampling. The birds were slaughtered by cervical dislocation following blood sampling. Birds were slaughtered after approximately 8 h of feed withdrawal and 4 h of water withdrawal. Thigh and breast muscles were collected from each broiler chicken for meat quality analysis. Internal organs were carefully excised for further analysis. The weights of the carcass, gizzard, liver, heart, abdominal fat, thigh, breast, and immune organs were recorded and represented as a percent of live body weight.

Blood Measurements

At d 35 of age, the blood of 2 chickens per replicate (16 broilers per treatment) was drawn using sterile syringes via the wing vein. The blood samples were centrifuged (at $3,000 \times \text{ rpm}$ for 5 min) to obtain serum and then stored at -20°C for additional analyses. The serum was used to assay total protein, albumin, glucose, total lipids, cholesterol, triglyceride (**TAG**), alanine transaminase (**ALT**), aspartate aminotransferase (**AST**), creatinine, glutathione peroxidase (**GPx**) and malondialdehyde (**MDA**) using commercial kits (Bio-diagnostic Co., Cairo, Egypt) according to the manufacturer's protocols using spectrophotometer UV4802 (Unico Co., Dayton).

Cecal Microbiota Count

On d 35 of age, the ceca of broiler chickens were dissected to determine the numeral of total aerobic, *Coliform*, *Clostridium perfringens*, and lactic acid bacteria (**LAB**). Immediately postmortem, the cecal contents were then transferred using an aseptic technique into sterile tubes. About 1 g of the homogenized cecal contents were diluted from 10^1 to 10^{-8} in sterile phosphate-buffered saline. Plate count agar, agar media, MacConkey agar, MRS, and Reinforced Clostridial agar were used to enumerate *Coliform*, total aerobic, LAB, and *Clostridium*, respectively. Each duplicate sample dilution was inoculated on agar plates and incubated at 37°C for 24 to 48 h before counting, whereas the *Clostridium* plates were incubated anaerobically at a similar temperature. Noticeable colonies were counted using a colony counter and presented as \log_{10} CFU/g of cecal digesta.

Meat Antioxidant Capacity and Oxidative Stability

On d 35 of age, after slaughtering, the breast and thigh meat of 16 broilers per group were excised and

stored in a refrigerator for 7 d at 4°C for lipid oxidation and antioxidant assays. The activities of superoxide dismutase (**SOD**), GPx, catalase (**CAT**), and the MDA contents in the meat were assessed using commercial kits (Spectrum Diagnostics, Cairo, Egypt) following the manufacturer's guidelines.

Organ Histomorphological Structure

Approximately 2 cm of the dissected duodenum, jejunum, ileum, and kidney were fixed in 10% buffered formalin as described in Selim et al. (2022) and Mousa et al. (2023). Liver samples were fixed in 10% neutral buffered formalin at room temperature for at least 48 h, then dehydrated in ascending grades of ethyl alcohols, cleared in methyl benzoate, and embedded in paraffin wax. Sections of 5 to 7 μm in thickness were obtained using a rotatory microtome and stained with the following stains: Harri's hematoxylin and eosin (**H&E**) for general histological studies, Masson's trichrome stain (**MT**) for demonstration of collagen fibers and muscle cells, Gomori's reticulin stain (**GR**) for demonstration of reticular fibers, and toluidine blue stained in semithin section of the liver. The stained sections were visualized using a Leica digital camera connected to a binocular microscope.

For scanning electron microscope of the small intestine, tissue blocks of the duodenum, ileum, and jejunum were removed, inserted into 2.5% glutaraldehyde in 0.1 M PBS pH7.4 at 4C for 2 h, post-fixed in 1% Osmic acid for 30min, washed 3 times with PBS (10 min. each), then dehydrated with ascending series of ethyl alcohol (30, 50, 70, 90% and absolute alcohol), and infiltrated with acetone, each concentration for 30 min. Samples were dried in SPI supplies, a critical point drying machine using liquid CO₂, mounted on aluminum stubs, coated with gold in an SPI- Module Vac/ Sputter, and then photographed using JEOL, JSM- 5200 LV scanning electron microscope (Japan) at the Electron Microscope Unit, Tanta University, Egypt.

Statistical Analysis

Data were subjected to One-way ANOVA using IBM SPSS software. Tukey's test was used to determine dissimilarities between the experimental groups and the probability value of <0.05 was statistically significant. The experimental unit was the replicate for performance parameters and the bird for other parameters. The results were reported as the mean and SEM.

RESULTS

Performance and Carcass Characteristics

Table 3. shows the growth performance of broilers fed the experimental diets. Significant variations in the growth performance were noticed during the entire trial period because of the main effects of PRO and SP+CV

Table 3. Growth performance of the experimental groups.

Item	Treatments ¹						SEM	P-value
	NC	PC	SP+CV	REO	PRO			
BW, g								
IBW	45.39	45.38	45.46	45.42	45.41	0.16	0.99	
7 d	190.99	192.16	194.35	193.63	196.24	2.13	0.17	
14 d	496.82 ^b	507.64 ^b	526.48 ^{ab}	532.34 ^{ab}	574.86 ^a	18.80	0.005	
21 d	1,030.25 ^b	1,137.23 ^{ab}	1,202.24 ^a	1,184.86 ^a	1,254.14 ^a	42.33	0.001	
28 d	1,770.86 ^c	1,810.98 ^{bc}	1,986.53 ^a	1,848.41 ^a	1,938.76 ^a	25.78	<0.001	
35 d	2,271.62 ^c	2,350.94 ^b	2,479.90 ^a	2,403.90 ^b	2,469.19 ^a	21.28	<0.001	
BWG, g								
1–7 d	145.60	146.78	148.89	148.21	150.83	2.12	0.17	
8–14 d	305.83 ^b	315.48 ^b	332.12 ^{ab}	338.71 ^{ab}	378.62 ^a	17.69	0.006	
15–21 d	533.42 ^b	629.59 ^{ab}	675.77 ^a	652.52 ^a	679.28 ^a	36.37	0.004	
22–28 d	740.61	673.75	784.28	663.55	684.62	49.10	0.11	
29–35 d	500.76	539.96	493.38	555.49	530.43	28.56	0.19	
1–35 d	2,226.23 ^c	2,305.55 ^b	2,434.44 ^a	2,358.48 ^b	2,423.78 ^a	21.21	<0.001	
Feed intake, g/d								
1–7 d	26.38	26.04	26.34	26.20	26.34	0.46	0.49	
8–14 d	64.88	64.99	65.49	64.87	65.45	2.65	0.87	
15–21 d	126.86	126.30	126.25	126.68	126.32	3.77	0.69	
22–28 d	157.33	155.14	153.64	155.35	151.68	6.75	0.94	
29–35 d	168.05	168.14	168.21	168.52	168.81	4.73	0.74	
1–35 d	108.70	108.12	107.99	108.32	107.72	1.56	0.85	
FCR, g feed/g gain								
1–7 d	1.27	1.24	1.24	1.24	1.22	0.03	0.74	
8–14 d	1.49 ^a	1.46 ^a	1.39 ^b	1.35 ^b	1.22 ^c	0.04	0.007	
15–21 d	1.76 ^a	1.40 ^b	1.31 ^b	1.36 ^b	1.30 ^b	0.09	0.04	
22–28 d	1.54 ^a	1.61 ^a	1.37 ^b	1.64 ^a	1.56 ^a	0.05	0.03	
29–35 d	2.36 ^a	2.21 ^b	2.39 ^a	2.13 ^c	2.24 ^b	0.04	0.02	
1–35 d	1.71 ^a	1.64 ^{ab}	1.55 ^c	1.61 ^{bc}	1.56 ^c	0.02	<0.001	

^{a,b,c}Means with no shared superscript in the same row at $P < 0.05$. SEM, standard error of the mean.

¹NC, negative control, basal diets; PC, positive control, basal diet supplemented with antibiotics; SP+CV, basal diets supplemented with a combination of *Spirulina platensis* and *Chlorella vulgaris*; REO, basal diets supplemented with rosemary essential oil; PRO, basal diets supplemented with probiotics.

dietary supplementation. At day 35 of age, the SP+CV and PRO broilers had greater final body weight and body weight gain (from 1 to 35 d of age) than the other treatment groups ($P < 0.001$). During the entire period (1–35 d), feed intake of birds did not vary among the treatment groups during the trial. The FCR of chickens in the SP+CV and PRO groups was better than those in the NC and PC groups ($P < 0.01$) during the whole experimental period (from 1 to 35 days). Overall, PRO followed by SP+CV outperformed the NC and PC groups during the entire period (1–35 d of age). Carcass traits and immune organ relative weights of the experimental broiler chickens are presented in Table 4. There were no significant differences in dressing percentage or

relative weights of the liver, heart, gizzard, abdominal fat, and thigh muscles among the treatment groups. Broiler chickens fed diets containing SP+CV and PRO had greater breast relative weights ($P < 0.01$) compared to those fed the NC and PC diets. The immune organ indexes, such as the thymus, spleen, and bursa of Fabricius did not differ among the treatment groups.

Blood Measurements

Blood biochemical constituents of the experimental broilers are shown in Table 5. Serum total protein concentrations of birds fed the SP+CV and PRO diets were

Table 4. Carcass traits and immune organs of the broiler chickens fed the experimental diets at 35 d of age.

Item, %	Treatments					SEM	P-value
	NC	PC	SP+CV	REO	PRO		
Carcass traits							
Dressing	77.37	77.31	78.06	77.93	79.50	1.85	0.09
Liver	1.95	1.96	1.83	1.86	1.84	0.07	0.39
Heart	0.52	0.51	0.49	0.50	0.51	0.02	0.49
Gizzard	1.71	1.79	1.67	1.65	1.71	0.05	0.09
Abdominal fat	2.14	2.04	2.02	2.03	2.03	0.07	0.50
Breast	21.80 ^b	22.08 ^b	25.15 ^a	23.72 ^{ab}	25.48 ^a	0.72	0.008
Thigh	18.53	19.36	19.12	18.68	20.09	0.98	0.55
Immune organs							
Spleen	0.101	0.105	0.102	0.095	0.106	0.007	0.60
Bursa of Fabricius	0.065	0.064	0.059	0.061	0.059	0.005	0.56
Thymus gland	0.090	0.090	0.082	0.086	0.086	0.006	0.63

^{a,b}Means with no shared superscript in the same row vary at $P < 0.05$. SEM, standard error of the mean. For abbreviations of treatments, see Table 3.

Table 5. Blood measurements of the broiler chickens fed the experimental diets at 35 d of age.

Items ¹	Treatments					SEM	P-value
	NC	PC	SP+CV	REO	PRO		
Total protein, g/dL	4.09 ^b	4.10 ^b	4.31 ^a	4.21 ^b	4.32 ^a	0.15	0.002
Albumin, g/dL	2.21	2.27	2.20	2.17	2.19	0.12	0.43
Globulin, g/dL	1.88 ^b	1.84 ^b	2.11 ^a	2.04 ^a	2.13 ^a	0.06	0.005
Glucose, mg/dL	272.63	261.67	262.77	260.77	263.71	4.64	0.15
Total lipids, mg/dL	425.54 ^a	418.53 ^a	341.61 ^c	361.34 ^b	329.01 ^d	6.63	<0.001
Cholesterol, mg/dL	171.40 ^a	167.49 ^a	142.95 ^b	137.63 ^b	133.22 ^b	3.25	<0.001
TAG, mg/dL	137.85 ^a	136.37 ^{ab}	130.63 ^b	130.85 ^b	118.25 ^c	1.90	<0.001
HDL, mg/dL	86.19 ^c	87.13 ^c	98.71 ^a	92.71 ^b	99.87 ^a	1.95	<0.001
LDL, mg/dL	61.76 ^a	60.52 ^a	44.97 ^b	40.19 ^e	39.23 ^c	1.73	<0.001
Creatinine	1.39	1.39	1.41	1.41	1.42	0.03	0.75
AST, U/L	38.92	39.02	41.00	40.45	40.46	1.73	0.34
ALT, U/L	18.14	17.51	17.88	18.14	17.82	0.75	0.15
GPx, mg/dL	3.85 ^b	3.48 ^b	4.94 ^a	4.79 ^a	4.87 ^a	0.12	<0.001
MDA, mg/dL	28.78 ^a	28.47 ^a	22.46 ^d	26.20 ^b	24.37 ^c	0.23	<0.001

^{a,b,c}Means with no shared superscript in the same row vary at $P < 0.05$. SEM, standard error of the mean. For abbreviations of treatments, see **Table 3**.

¹TAG, triglycerides; HDL, high-density lipoprotein; LDL, low-density lipoprotein; AST, aspartate aminotransferase; ALT, alanine transaminase; GPx, glutathione peroxidase; MDA, malondialdehyde.

higher ($P < 0.01$) than those fed the other diets. Supplementation of broilers' diets with SP+CV, REO, and PRO increased ($P < 0.01$) serum globulin contents of broilers compared to the control and antibiotic-supplemented diets. The dietary supplementation with SP+CV, REO, and PRO decreased ($P < 0.001$) serum concentrations of total lipids, cholesterol, triglycerides, and LDL compared to the control and antibiotic groups. On the other hand, serum HDL levels were increased ($P < 0.001$) in the SP+CV, REO, and PRO broilers compared to the control and antibiotic broilers. Serum glucose, AST, ALT, and creatinine levels did not vary among the treatment groups. Supplementation of broilers' diets with SP+CV, REO, and PRO increased serum GPx contents ($P < 0.001$) of broiler chickens, while reducing serum MDA levels ($P < 0.001$) compared to those fed the PC and NC diets.

Ceca Microbial Population

The ceca microbiota counts showed significant differences at the end of the experiment (35 d of age) owing to the main impacts of nutraceuticals. The dietary supplementation with SP+CV, REO, and RPO increased ($P < 0.001$) LAB counts in the ceca of broiler chickens compared to the NC and PC groups (**Table 6**). Lower *Clostridium perfringens* and Coliform counts ($P < 0.001$) were noticed in the ceca of broilers fed the PC and supplemented diets compared to the NC group

(**Table 6**). Total aerobic counts in the ceca of the SP+CV and PRO birds were lower ($P < 0.01$) than those in the ceca of NC, PC, and REO groups (**Table 6**).

Meat Antioxidant Capacity and Oxidative Stability

The impact of dietary nutraceuticals on the activities of meat antioxidant enzymes and MDA of broiler chickens is shown in **Table 7**. The MDA concentration was decreased in the breast and thigh meat ($P < 0.001$) of broiler chickens fed diets containing SP+CV, REO, and PRO compared to NC and PC ones. On the other hand, the activities of antioxidant enzymes, such as GPx, SOD, and CAT, were increased ($P < 0.001$) in the breast and thigh meat of broiler chickens fed the SP+CV, REO, and PRO-supplemented diets compared to those fed the NC and PC diets. The highest levels of antioxidant enzymes in both breast and thigh muscles were noticed in the PRO group, followed by the SP+CV group.

Organs Histomorphology

Small Intestine. Intestinal histomorphology of broiler chickens fed the experimental diets are shown in **Table 8** and **Figures 1 and 2**. Overall, we did not detect any pathological lesions or inflammatory changes in the small intestine of the studied broiler chickens (**Figure 1**).

Table 6. Ceca microbial population of broiler chickens fed the experimental diets at d 35 of age.

Bacteria, Log ₁₀ CFU/g	Treatments					SEM	P-value
	NC	PC	SP+CV	REO	PRO		
LAB ¹	8.485 ^b	8.630 ^b	9.965 ^a	9.592 ^a	9.965 ^a	0.12	<0.001
<i>Clostridium perfringens</i>	5.83 ^a	4.60 ^b	3.70 ^c	3.44 ^c	3.10 ^c	0.19	<0.001
Coliforms	8.84 ^a	6.45 ^b	5.29 ^c	6.13 ^b	5.18 ^c	0.16	<0.001
Total aerobic	8.94 ^a	8.75 ^a	8.09 ^{bc}	8.52 ^{ab}	8.08 ^c	0.13	0.001

^{a,b,c}Means with no shared superscript in the same row vary at $P < 0.05$. SEM, standard error of the mean. For abbreviations of treatments, see **Table 3**. LAB, lactic acid bacteria.

Table 7. Meat antioxidant capacity and oxidative stability of broiler chickens.

Meat type ¹	Treatments					SEM	P-value
	NC	PC	SP+CV	REO	PRO		
Breast							
MDA, nmol/g	42.07 ^a	43.15 ^a	23.64 ^c	20.06 ^c	23.08 ^c	1.41	<0.001
GPx, mg/g	2.54 ^d	2.43 ^d	3.86 ^b	3.02 ^c	5.30 ^a	0.15	<0.001
SOD, U/g	356.24 ^c	352.52 ^c	437.79 ^b	498.23 ^a	504.80 ^a	20.21	<0.001
CAT, U/g	7.83 ^d	8.56 ^d	14.62 ^b	11.79 ^c	19.26 ^a	0.73	<0.001
Thigh							
MDA, nmol/g	40.29 ^a	39.72 ^a	20.13 ^c	27.53 ^b	23.26 ^c	1.02	<0.001
GPx, mg/g	2.41 ^d	2.26 ^d	5.29 ^b	3.62 ^c	6.01 ^a	0.20	<0.001
SOD, U/g	389.19 ^e	405.63 ^e	474.83 ^b	540.64 ^a	556.03 ^a	15.98	<0.001
CAT, U/g	9.26 ^c	9.39 ^c	19.57 ^a	11.59 ^b	19.98 ^a	0.53	<0.001

^{a,b,c}Means with no shared superscript in the same row vary at $P < 0.05$. SEM, standard error of the mean. For abbreviations of treatments, see [Table 3](#).

¹MDA: malondialdehyde; GPx: glutathione peroxidase; SOD: superoxide dismutase; CAT: catalase.

The dietary addition of SP+CV, REO, and PRO increased ($P < 0.001$) the duodenal villi height (**VH**) and crypt depth (**CD**) of broiler chickens when compared with the NC group. The PC, SP+CV, REO, and PRO birds had lower duodenal goblet cell densities ($P < 0.01$) than the NC ones. In the jejunum, The REO and PRO groups had greater VH and CD ($P < 0.001$), but lower goblet cell density ($P < 0.01$) compared to other treatment groups. Ileal VH and CD were improved in all supplemented groups compared to the NC and PC groups ($P < 0.001$). However, ileal goblet cell density was reduced ($P < 0.01$) in the SP+CV, REO, and PRO birds.

Liver. The hepatic histomorphology of broiler chickens fed the experimental diets is shown in [Figure 3](#). Generally, the hepatic histomorphological structures of all the experimental groups were normal. No pathological lesions or inflammatory changes were observed in the liver of the examined broiler chickens. The hepatic lobules were well-distinct, and the parenchyma was formed from hepatic plates, hepatic sinusoid, the central veins with wide lumen, and portal areas. The hepatocytes appeared pyramidal with acidophilic cytoplasm and dense basophilic spherical nuclei situated toward

the hepatic sinusoids. The hepatocytes were arranged in 2 cell thick hepatic plates. Most of them appeared as tubules formed from 4 to 6 cells. The center of the tubular structure contained bile canaliculi. Hepatic sinusoids were lined by flat endothelial cells and Von Kupffer cells-aggregations of lymphatic cells of varying number, size, and irregular distribution throughout the liver parenchyma. The reticular fibers appeared as fine networks in the connective tissue (**CT**) capsule and the intralobular CT between hepatic plates and the CT of the portal area. The collagen fibers appeared as collagen bundles in the CT capsule and in the wall of blood vessels, CT of the portal area around the blood vessels and duct.

Kidney. The kidney histomorphology of broiler chickens fed the experimental diets is shown in [Figure 4](#). In general, no pathological lesions or inflammatory changes were observed in the kidneys of the examined broiler chickens. The kidneys of birds have many renal tubules divided into cortical and medullary parts. The tubules are convoluted in the cortex and straight in the medulla. The kidney of birds contains 2 types of nephrons: cortical (reptilian) type is more numerous, lacks a loop of Henle, and is located within the cortex. The medullary

Table 8. Intestinal histomorphology of broiler chickens fed the experimental diets at the end of the experiment.

	Treatments					SEM	P-value
	NC	PC	SP+CV	REO	PRO		
Duodenum							
Villus height (VH), μm	1,234.22 ^c	1,237.27 ^c	1,350.50 ^b	1,313.63 ^b	1,398.73 ^a	33.51	<0.001
Crypt depth (CD), μm	216.03 ^d	223.07 ^d	245.27 ^c	263.17 ^b	287.10 ^a	6.49	<0.001
VH/CD	5.71	5.55	5.50	4.99	4.87	0.12	0.39
Goblet cell density, No/ 100 μm	10.43 ^a	9.53 ^b	9.53 ^b	9.03 ^b	8.30 ^c	0.22	0.006
Jejunum							
Villus height, μm	1,098.72 ^b	1,093.77 ^b	1,108.37 ^b	1,226.93 ^a	1,255.10 ^a	24.63	<0.001
Crypt depth, μm	216.27 ^c	217.13 ^c	225.60 ^c	241.03 ^b	265.57 ^a	5.91	<0.001
VH/CD	5.08	5.04	4.91	5.09	4.73	0.08	0.45
Goblet cell density, No/ 100 μm	12.60 ^a	12.67 ^a	11.47 ^b	10.17 ^c	9.10 ^d	0.25	0.002
Ileum							
Villus height, μm	888.58 ^d	883.30 ^d	931.57 ^c	989.25 ^b	1068.73 ^a	18.03	<0.001
Crypt depth, μm	114.33 ^d	114.84 ^d	155.42 ^c	185.43 ^b	208.20 ^a	5.25	<0.001
VH/CD	7.77 ^a	7.69 ^a	6.00 ^b	5.34 ^c	5.13 ^c	0.10	<0.001
Goblet cell density, No/ 100 μm	12.54 ^a	12.72 ^a	11.52 ^b	10.93 ^b	9.92 ^c	0.23	0.006

^{a,b,c,d}Means with no shared superscript in the same row vary at $P < 0.05$. SEM, standard error of the mean. For abbreviations of treatments, see [Table 3](#).

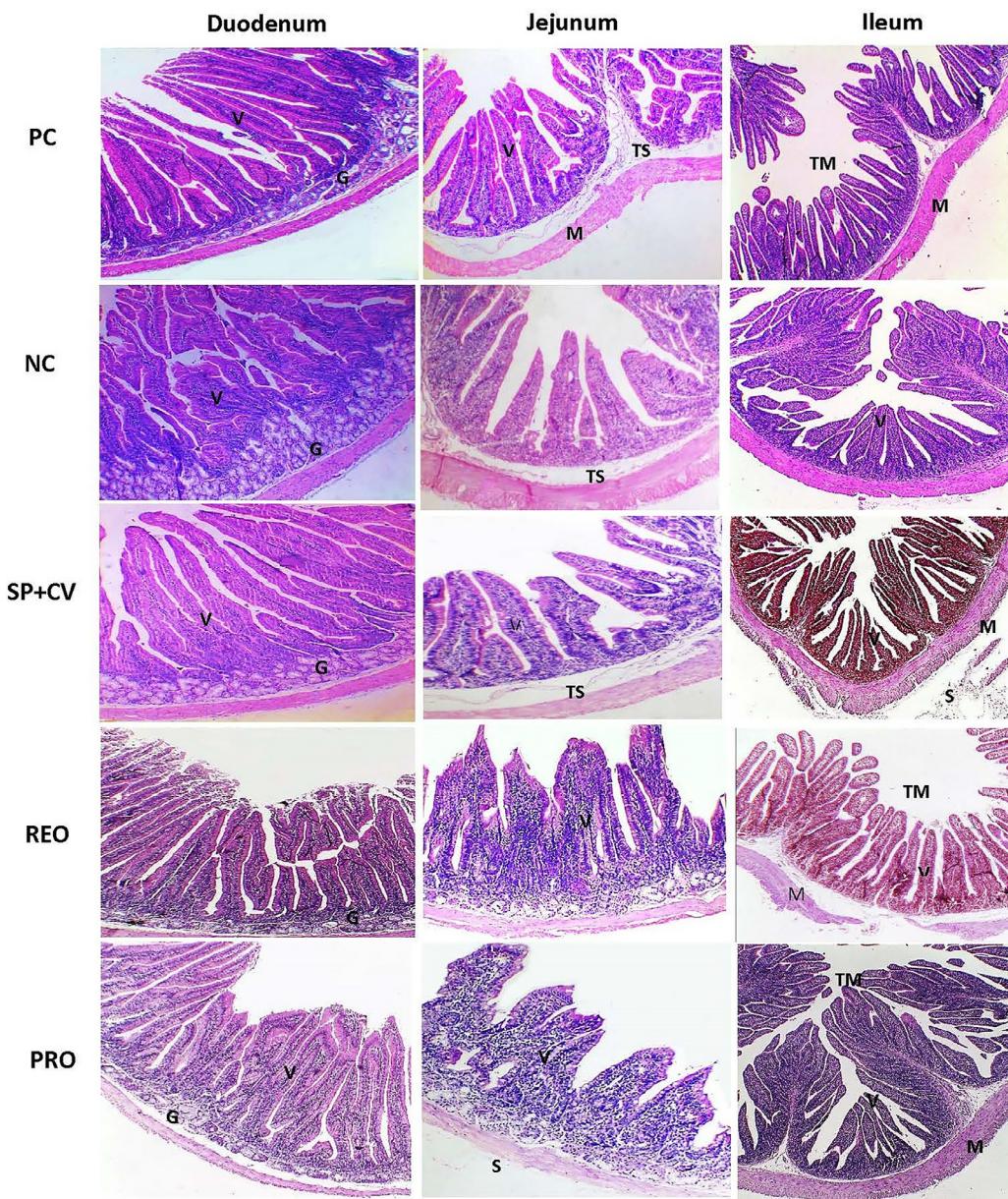


Figure 1. Histological examination of the small intestine (duodenum, jejunum, and ileum) of broiler chickens showed normal features without any pathological lesions. TM, tunica mucosa; V, intestinal villi; TS, tunica submucosa; G, goblet cells; M, tunica muscularis; S, tunica serosa. Small intestine stained by Hematoxylin and Eosin ($\times 40$ & $\times 100$). NC, negative control, basal diets; PC, positive control, basal diet supplemented with antibiotics; SP+CV, basal diets supplemented with a combination of *Spirulina platensis* and *Chlorella vulgaris*; REO, basal diets supplemented with rosemary essential oil; PRO, basal diets supplemented with probiotics.

(mammalian) type is less numerous and has a loop of Henle called the medullary loop, which extends into the medulla.

Breast Muscle. The breast histomorphology of broiler chickens fed the experimental diets is shown in Table 9 and Figure 5. There were no significant differences in myofibrillar fragmentation index (MFI), soluble and insoluble collagen, or sarcomere length of breast muscle between the treatment groups. Breast muscle in chicken is a type of skeletal muscle fiber. Breast muscle is formed from skeletal muscle fibers and CT. The whole muscle is coated by a sheath of dense CT called epimysium. Thin CT septa extend from the epimysium to surround and support the muscle bundles or fascicles called perimysium. Thin delicate reticular networks surround the

individual muscle cells containing blood capillaries, nerves, fibroblasts, and fixed macrophages. This layer is continuous with the perimysium called endomyxium. They have large diameters, less content of mitochondria, cytochrome, and few myoglobin. The cytoplasm is rich in glycogen.

DISCUSSION

Broiler production is presently in great demand worldwide. Growth performance parameters of broilers are adjusted for improved feed efficiency and ameliorated health status. The problem of antibiotic resistance and its residues has received substantial critical awareness in

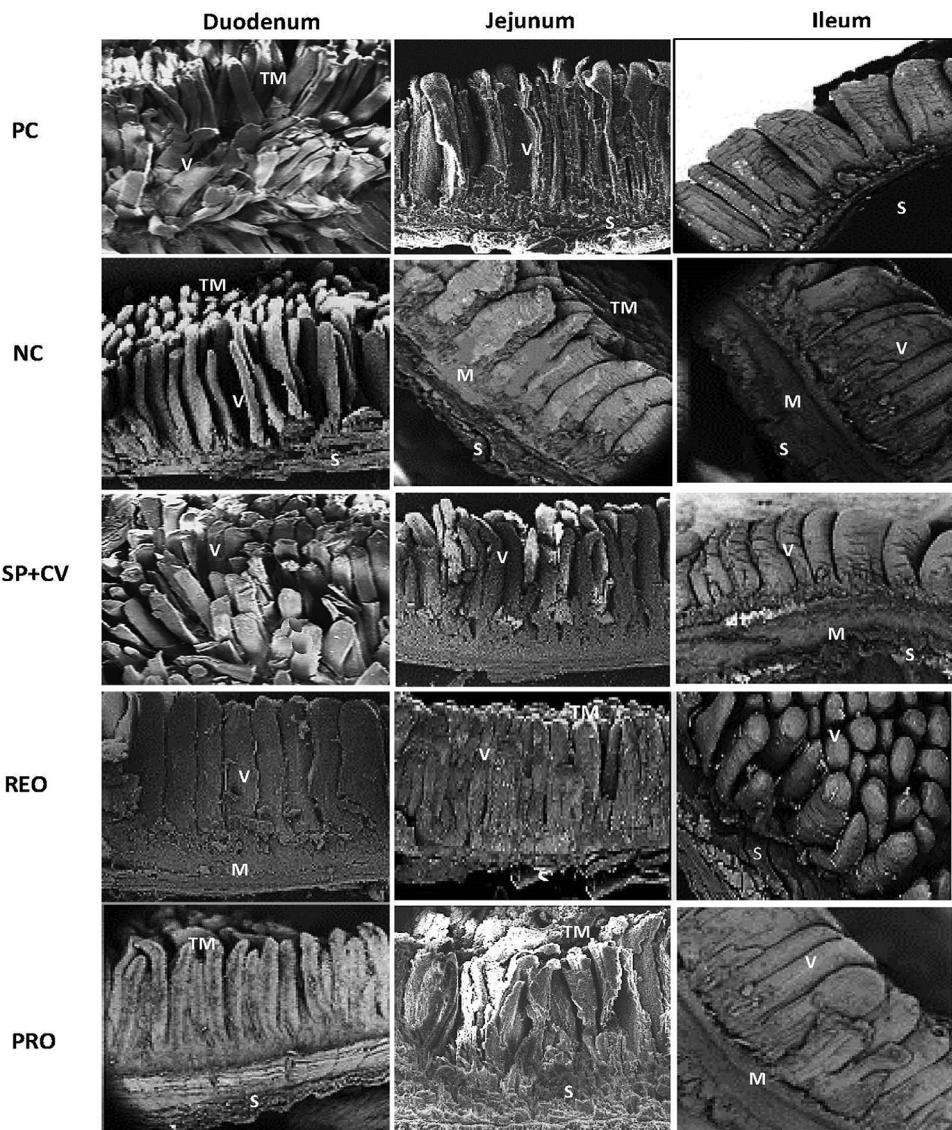


Figure 2. Scanning electron microscope of the small intestine (duodenum, jejunum, and ileum) of broiler chickens showed normal features. TM, tunica mucosa; V, intestinal villi; TS, tunica submucosa; M, tunica muscularis; S, tunica serosa. NC, negative control, basal diets; PC, positive control, basal diet supplemented with antibiotics; SP+CV, basal diets supplemented with a combination of *Spirulina platensis* and *Chlorella vulgaris*; REO, basal diets supplemented with rosemary essential oil; PRO, basal diets supplemented with probiotics.

recent years. The demand for antibiotic-free additives is now being utilized, thus the search for natural additives as an alternative to AGP has augmented research on nutraceuticals. Among these alternatives, SP, CV, EO, and PRO have enormous potential benefits because they are natural products and are free of harmful constituents and chemicals (El-Shall et al., 2023; Yosi and Metzler-Zebeli, 2023). Therefore, the main goal of the current trial was to determine the effect of SP+CV, REO, and PRO supplementation as alternatives to AGP on growth performance, blood biochemical constitutes, meat quality, cecal microbiota, and organ histomorphology of broilers.

The obtained results demonstrated a beneficial effect of SP+CV and PRO supplementation on final body weight, body weight gain, and FCR, without affecting the feed intakes of broilers. Previous research proved that microalgae such as SP and CV can be applied in

poultry nutrition to accelerate growth and promote intestinal microbiota (El-Bahr et al., 2020; Alghamdi et al., 2024). This could be because SP and CV can help birds sustain gut microbiota and lessen harmful microorganisms (Rubel et al., 2019), which supports them in digesting feed and achieving better metabolism via absorbing vitamins and minerals (Rubel et al., 2019; Alghamdi et al., 2024). Furthermore, Alghamdi et al. (2024) suggested that *microalgae* (a mixture of *Dunaliella salina* and *Spirulina*) at a level of 1 to 2 g/kg of diet might be applied in place of AGP as a growth stimulant in broiler chickens. Moreover, the improved body weight and FCR of broilers fed a combination of SP and CV can be due to their nutritional contents, including protein, carbohydrates, lipids, vitamins, and minerals. SP and CV supplementation can modulate beneficial intestinal microbiota which in turn improves the growth performance and immune status of broilers (Roques et

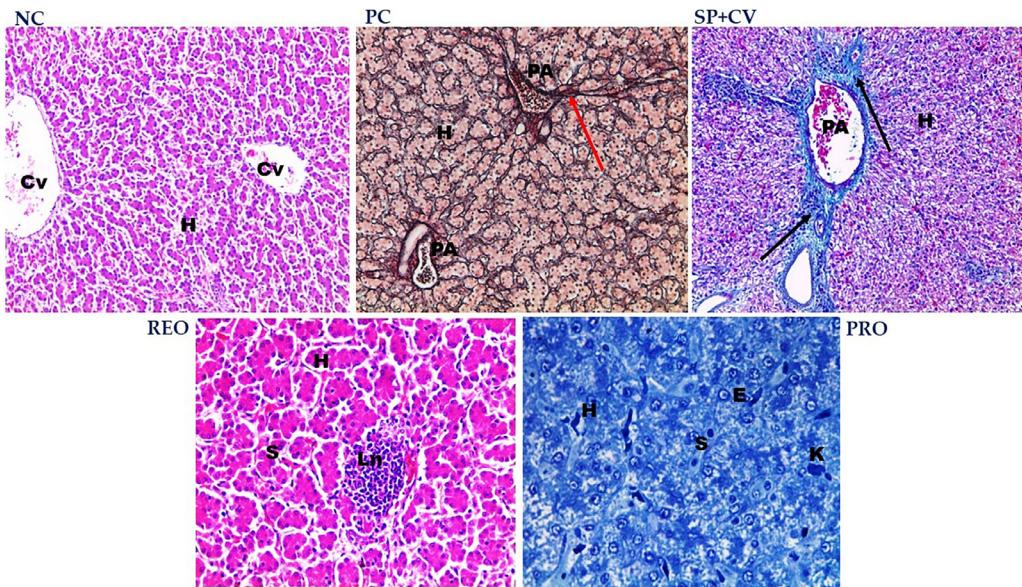


Figure 3. Histological structure of the liver of the treatment groups. The livers of broiler chickens showed normal features, normal arrangement of hepatic plates and hepatocytes (H), normal central vein (CV), portal area (PA), and normal sinusoids (S). A fine network of reticular fibers appeared in black color (red arrow) and collagen fiber was stained in green color (black arrow). NC, negative control, basal diets; PC, positive control, basal diet supplemented with antibiotics; SP+CV, basal diets supplemented with a combination of *Spirulina platensis* and *Chlorella vulgaris*; REO, basal diets supplemented with rosemary essential oil; PRO, basal diets supplemented with probiotics.

al., 2022; Alghamdi et al., 2024). On the other hand, the accelerated body gain and enhanced FCR of broilers fed a mixture of *Bacillus licheniformis*, *Enterococcus facieum*, *Lactobacillus acidophilus*, and *Saccharomyces cerevisiae* may be due to the accumulation of these strains in the gut of broilers and thus may be associated with improved intestinal microecology and histomorphology (Zeng et al., 2021; Zou et al., 2022). Our results were in line with earlier research using multi-strain

probiotics in broiler chickens (Hussein and Selim, 2018; Selim et al., 2022).

Dietary supplementation with REO improved body weight gain and FCR comparable to the antibiotic group, while there was no significant difference between REO and antibiotic treatment groups. Gumus and Gelen (2023) showed that dietary addition of REO with a level of 100 and 200 mg/kg of the diet did not affect body weight, body weight gain, feed intake, or FCR in

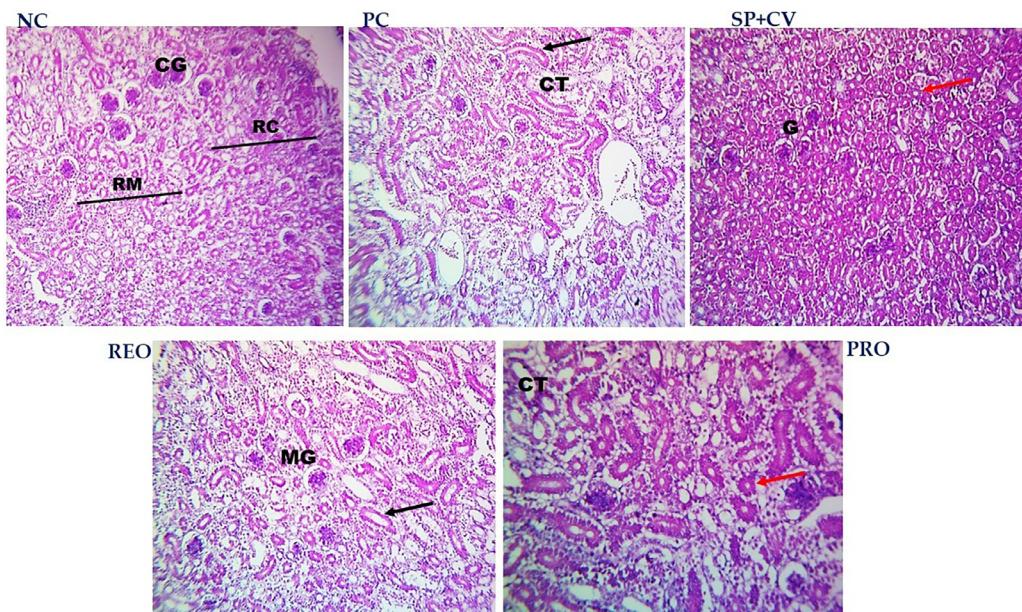


Figure 4. Histological structure of kidney of the treatment groups. Kidneys showed normal structure. RC, renal cortex; RM, renal medulla; CG, cortical glomeruli; MG, medullary glomeruli; CT, renal collecting tubules; proximal convoluted tubules (red arrow); and distal convoluted tubules (black arrow). (H&E stain). NC, negative control, basal diets; PC, positive control, basal diet supplemented with antibiotics; SP+CV, basal diets supplemented with a combination of *Spirulina platensis* and *Chlorella vulgaris*; REO, basal diets supplemented with rosemary essential oil; PRO, basal diets supplemented with probiotics.

Table 9. Breast myofibrillar fragmentation index (MFI), collagen, and sarcomere length of breast meat of broiler chickens.

	Treatments					SEM	P-value
	NC	PC	SP+CV	REO	PRO		
MFI	80.60	81.03	81.40	81.30	81.72	0.39	0.13
Soluble collagen, %	0.13	0.13	0.14	0.14	0.15	0.008	0.51
Insoluble collagen, %	0.15	0.14	0.15	0.15	0.15	0.007	0.22
Total collagen, %	0.28	0.27	0.29	0.29	0.30	0.009	0.13
Sarcomere, μm	1.58	1.58	1.63	1.65	1.69	0.06	0.20

For abbreviations of treatments, see Table 3.

broiler chickens. Al-Kassie (2008) reported that the dietary addition of 1% REO improves body weight gain and feed intake and enhances FCR. Furthermore, a literature report by Abd El-Latif et al. (2013) suggested that the supplementation of broiler rations with 100 and 200 mg kg⁻¹ REO significantly improved feed intake. Earlier studies have documented the beneficial impacts of essential oils on the secretion of digestive enzymes and intestinal mucosa (Gumus and Gelen, 2023). An in-vitro study by Mathlouthi et al. (2012) revealed that REO had numerous antimicrobial effects against pathogenic microorganisms while having the same effect on avilamycin as a growth promoter when included in broiler rations. The improved FCR in the supplemented groups may be because the studied nutraceuticals contain certain compounds with valuable impacts in stimulating the absorption of nutrients in GIT. These bioactive components have antimicrobial activities and antioxidants that can be responsible for the current enhancement.

To investigate whether these feed supplements have functional impacts, the intestinal morphology and cecal microbiota counts were assessed to gain insight into the mechanisms involved beyond the improved growth

performance and feed efficiency. The present study illustrated that dietary supplementation with SP+CV, REO, and RPO increased LAB counts while decreasing *Clostridium perfringens*, *Coliform*, and total aerobic counts in the ceca of broiler chickens compared to the NC and PC groups. The antimicrobial activities of microalgae can be attributed to its bioactive antimicrobial and antioxidant peptides such as lipopolysaccharides, alkaloids, eicosapentaenoic acids, and cyclic peptides (El-Bahr et al., 2020; Alghamdi et al., 2024). *Spirulina* addition at a level of 0.1–0.3% was reported to increase the cecal *Lactobacillus* count in broilers, according to Abdelfatah et al. (2024), but did not affect the cecal *Coliform* number. Moreover, Feshangchi et al. (2022) found that dietary *Spirulina* at a level of 1% decreased cecal *E. coli* counts in broiler chickens given aflatoxin-contaminated diets. Moreover, earlier literature recorded that rosemary extract had the potential to reconstruct the gut microbiota in mice (He et al., 2022), and rosemary leaf meal can increase *Lactobacillus* count but decrease *E. coli* and *Salmonella* populations in broilers (Ogwuegbu et al., 2021). Mathlouthi et al. (2012) reported that REO had numerous antimicrobial

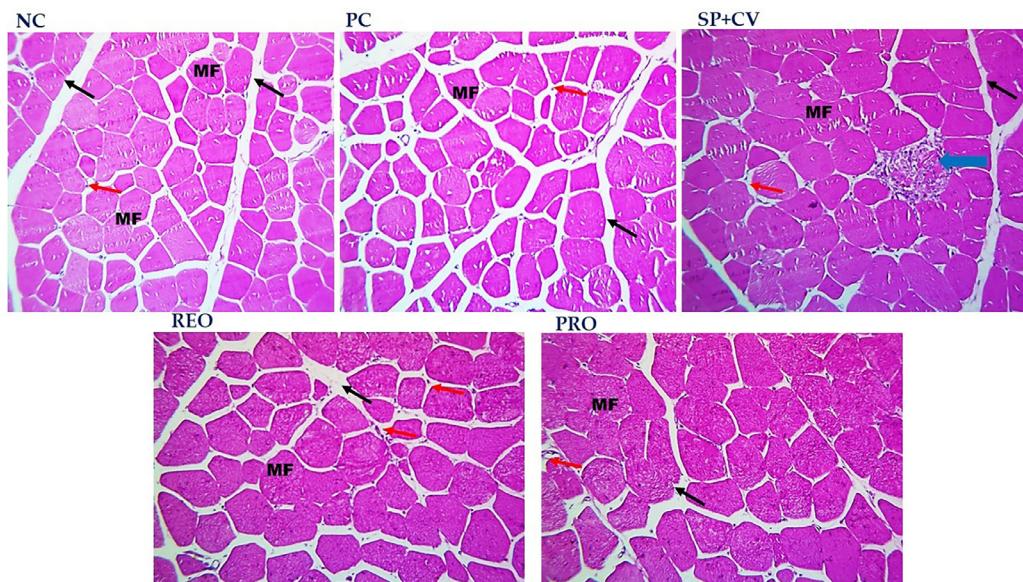


Figure 5. Cross section of breast muscle of the experimental broilers. Breast muscles show normal architecture without any pathological lesions. The muscle fibers (MF) appear rounded or polygonal in shape with their nuclei peripherally situated. Thin connective tissue septa surround and support the muscle bundles or fascicles, called perimysium (black arrow). A thin delicate reticular network surrounds the individual muscle cell containing blood capillaries, nerve (blue arrow), and fibroblasts called endomysium (red arrow). NC, negative control, basal diets; PC, positive control, basal diet supplemented with antibiotics; SP+CV, basal diets supplemented with a combination of *Spirulina platensis* and *Chlorella vulgaris*; REO, basal diets supplemented with rosemary essential oil; PRO, basal diets supplemented with probiotics.

effects on harmful pathogens and even had the same effect as avilamycin when included in broiler rations. The positive impact of REO on ceca microbiota counts may be due to the active constituents of REO, which have been recorded to play vital roles in regulating the intestinal barrier and microbiota (Liu et al., 2022; Zhang et al., 2023).

The PRO effect on the cecal microbial count can be owing to the favorable actions of the selected bacterial strains. Various mechanisms have been proposed, such as competitive prohibition, reducing the intestinal pH via acid fermentation, bacteriocins production, boosting the gut immune system, enhancing short-chain fatty acids production, and improving the integrity of mucosa (Latif et al., 2023; Sun et al., 2023). As a result of these actions, the intestinal condition favors the growth of beneficial microbes and hinders the multiplication of pathogenic microorganisms (Wu et al., 2019; Pogačar et al., 2020). Zou et al. (2022) found that probiotics can ultimately decrease the environmental contaminants in poultry feces by enriching intestinal microbiota. In this pilot study, it was shown that feeding AA+ male broilers a probiotic cocktail (0.05%) containing *Bacillus subtilis*, *Clostridium butyricum*, and *Enterococcus faecalis* for 42 d was an effective way to reduce the amount of *Salmonella* and *E. coli* found in their cecum while raising the amount of *Lactobacillus*. It can be concluded that the supplementation of multi-strain probiotics could sustain gut microbiota balance by boosting the number of favorable microbes and diminishing the number of harmful bacteria, resulting in superior growth performance.

The small intestine, the duodenum, the jejunum, and the ileum, perform a fundamental role in the digestion and absorption of nutrients in poultry. The development of the small intestine can be assessed using histomorphological measurements, such as VH, CD, and goblet cell intensity. These parameters are used as indicators of the health and digestive capacity of the small intestine, where both VH and CD are straightforwardly linked to intestinal absorption ability. Overall, we did not detect any pathological lesions or inflammatory changes in the small intestine of the studied broiler chickens. It is noteworthy that goblet cell densities were reduced but duodenal, jejunal, and ileal VH and CD were improved with PRO and SP+CV supplementation followed by REO. The current findings are consistent with earlier research which reported that SP or CV supplementation beneficially impacts intestinal VH, CD, and goblet cell numbers, ultimately augmenting nutrient absorption, FCR, and body weight gain in broiler chickens (Khan et al., 2020; Mirzaie et al., 2020).

Similar to the current trial, recent studies have also observed that the dietary addition of EO enhances intestinal VH and CD but decreases the goblet cell density in broilers (Du and Guo, 2021; Hosseinzadeh et al., 2023). Researchers have proposed that EO can boost the intestine epithelium and improve the intestinal absorptive area through antioxidant properties and enhancement of gut microbiota (Du and Guo, 2021; Hosseinzadeh

et al., 2023). It has been suggested that the decrease in small intestine goblet cell density may be directly or indirectly because of the EO effect on the gut microbial population (Du and Guo, 2021; Hosseinzadeh et al., 2023). Moreover, the current results are consistent with recent studies by Danladi et al. (2022) and Yosi and Metzler-Zebeli (2023) concerning the effect of PRO on the gut microstructure and improved intestinal histomorphology. It is well known that dietary PRO potentially modulates gut microbiota, regularly benefiting the bird and that GIT can adjust and modify its microstructure in response to changing situations such as diet (Danladi et al., 2022; Yosi and Metzler-Zebeli, 2023).

Based on our findings we suggest that nutraceuticals can have the potential to enhance the population of beneficial bacteria while hindering the growth of pathogenic bacteria owing to their bioactive components (Kotrbáček et al., 2015; Yosi and Metzler-Zebeli, 2023). This adjustment of the gut microbiota was in line with the improvement of gut microstructure which in turn can induce valuable outcomes for the health and well-being of broiler chickens, such as body weight gain and FCR. It has been documented that modification in the gut microbiota can impact gut mucosal morphology, prompt immune responses, and eventually affect energy expenditure and chicken growth (Hussein et al., 2020; Bondar et al., 2023).

Antioxidant enzymes' activities and oxidative product levels in blood and meat are critical for the evaluation of the oxidant status of poultry. SOD is an antioxidant enzyme that converts the superoxide radical into hydrogen peroxide and molecular oxygen, and this is represented as the first line of defense against reactive oxygen species (Sen et al., 2010). Consequently, hydrogen peroxide is scavenged by CAT or GPx (Young and Woodside, 2001). MDA was recognized as a marker of lipid peroxidation (Dröge, 2002). In the current study, dietary supplementation with PRO, SP+CV, and REO increased serum GPx concentrations as well as breast and thigh concentrations of GPx, CAT, and SOD compared to control and probiotic groups. Moreover, the MDA levels in serum and muscles were significantly decreased in broilers fed the PRO, SP+CV, and REO diets. Similarly, a recent study by Gümuş et al. (2023) reported an increase in breast muscle SOD activity with dietary thymol essential oil ($100\text{-}300 \text{ mg kg}^{-1}$) and REO ($100\text{-}200 \text{ mg kg}^{-1}$), and they suggested that these feed additives had positive effects on antioxidant metabolism. Moreover, the beneficial impacts of rosemary powder have been reported on serum antioxidant activity (Soltani et al., 2016). The active components in REO are mainly attributed to its oxidative damage alleviation and antioxidant enzyme augmentation, such as phenolic acids, flavonoids, and many others (Gümuş et al., 2023).

The Current finding of SP+CV agreed with previous research elucidating the high antioxidant activities of SP and CV in broiler chickens (El-Bahr et al., 2020; Mirzaie et al., 2020). This could be attributed to their elevated concentrations of beta-carotene, phycocyanin, zeaxanthin, and allophycocyanin (Selim et al., 2018;

Mirzaie et al., 2020). On the other hand, the observed reduction in MDA concentrations and elevated levels of antioxidant enzymes in the serum and muscles of broiler chickens fed the PRO diet may indicate that this supplement could improve the oxidative stability and antioxidant capacity in broiler chickens. This improvement could be related to radical-scavenging factors, or some antioxidant components found in the multi-strain PRO, such as those reported in *S. cerevisiae* and *Bacillus* sp. (Hossain et al., 2012; Hussein and Selim, 2018). Furthermore, it has been documented that PRO produces butyric acid and hydrogen, which may promote antioxidant secretion and free radical scavenging (Zheng et al., 2019). To sum up, supplementation of PRO and SP +CV, followed by REO into the diets of broiler chickens would offer advantages in terms of antioxidant status and hindering oxidative damage in meat.

Blood biochemical measurements are frequently used to determine poultry's physiological, nutritional, and pathological status. Modifications in metabolic and physiological conditions may influence avian health (Emam et al., 2023). Dietary SP+CV, REO, and PRO supplementation did not affect the serum biochemical indicators related to broiler chicks' liver (ALT and AST) and kidney (creatinine) functions. The current findings suggested that these feed supplements did not adversely affect hepatic and renal function and thus can be used safely in the diets of broiler chickens. Furthermore, these findings were confirmed with the histomorphological structures of the kidney and liver of the experimental groups, where there are no pathological lesions in these organs. On the other hand, the addition of SP+CV, REO, and PRO resulted in a noteworthy decrease in the serum concentration of total lipid, total cholesterol, LDL, and triglyceride but an increase in HDL compared to PC and NC broilers. Our outcomes are in harmony with previous studies (Gümüş et al., 2023; Yao et al., 2023; Alghamdi et al., 2024). Our findings revealed the hypocholesterolemic effect of microalgae and REO which can be due to a reduction in the gut synthesis and absorption of cholesterol in broiler chickens (Yao et al., 2023; Alghamdi et al., 2024). Furthermore, the antioxidant activities of SP+CV and REO bioactive components may decrease the concentration of blood lipids in microalgae-supplemented broilers because of lower pancreatic lipase activity, thereby reducing hepatic fatty acid synthesis (Deng and Chow, 2010), as well as a reduction in intestinal cholesterol absorption or/and the synthesis (Yao et al., 2023; Alghamdi et al., 2024). The current findings of PRO on blood lipids concur with previous studies (Iqramu et al., 2017; Hussein and Selim, 2018), which recorded a decrease in serum total cholesterol by feeding probiotics in poultry. Numerous mechanisms may play a role in the hypocholesterolemic effect of probiotics. PRO can decrease blood cholesterol by adjusting endogenic- or exogenic-originated cholesterol in the gut, diminishing, or preventing the expression of Niemann-Pick C1-like 1 a protein, on the enterocytes, which in turn lessens cholesterol absorption (Huang and Zheng, 2010).

Furthermore, probiotics produce bile salt hydrolase, an enzyme responsible for bile acids deconjugation (Kalavathy et al., 2003).

CONCLUSIONS

The present findings revealed that supplementing the broilers' diets with multi-strain probiotics and a mixture of *Spirulina platensis* and *Chlorella vulgaris*, followed by rosemary essential oil improved the growth performance and feed conversion efficiency of broiler chickens compared to control and antibiotic groups. The dietary supplementation of these supplements also improved antioxidant capacity, lessened the oxidation stability in the meat of broiler chickens, and resulted in a positive modulation of blood lipid indices. The small intestine histomorphology was improved and the cecal microbiota counts of broilers were positively modified after the dietary supplementation of the studied nutraceutical feed additives. No pathological lesions were noted in the liver and kidneys, as well as no alteration in hepatic and renal activities of broiler chickens fed the diets supplemented with multi-strain probiotics, algae, or essential oil. The present findings suggest that multi-probiotics and a mixture of *Spirulina platensis* and *Chlorella vulgaris*, followed by rosemary essential oil could be suitable alternatives to in-feed antibiotics for enhancing the performance, health, and meat quality of broiler chickens.

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DISCLOSURES

The authors declare no conflicts of interest.

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