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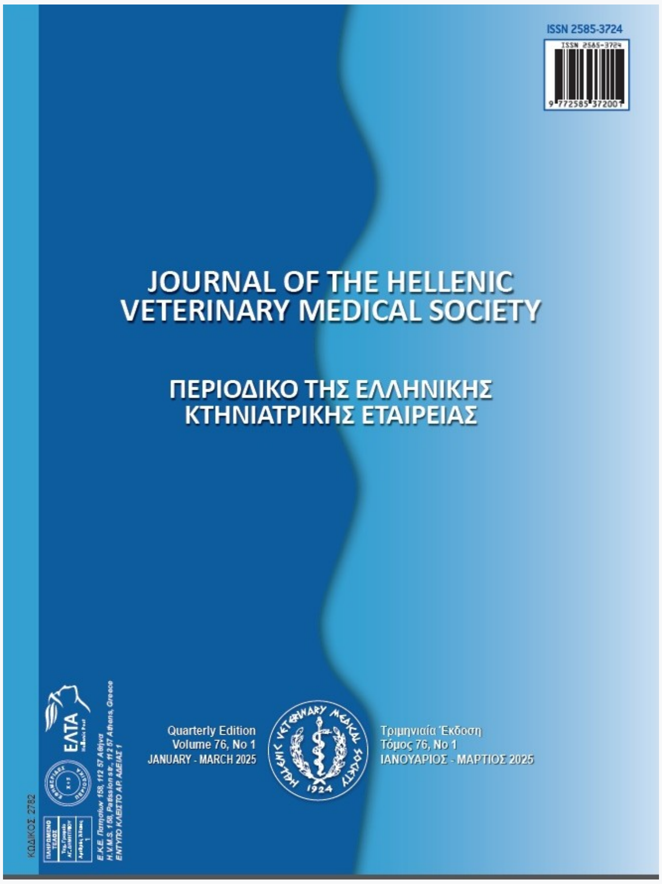

Effects of Dietary Prebiotic, Probiotic, and Synbiotic Supplementation on Growth Performance, Carcass Traits, Digestive Enzymes and Some Biochemical Parameters in Broiler Chickens

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Effects of Dietary Prebiotic, Probiotic, and Synbiotic Supplementation on Growth Performance, Carcass Traits, Digestive Enzymes and Some Biochemical Parameters in Broiler Chickens

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ABSTRACT : This study was conducted to determine and compare the effects of chitosan-oligosaccharide (COS), probiotics (Pro), and their combination on the growth performance, carcass parameters, digestive enzymes, and serum biochemical parameters of broiler chickens. A total of 300 one-day-old mixed-sex Ross 308 broiler chicks (36.29±0.93) were randomly divided into four treatment groups with five replicates each (15 chicks per replicate). No supplements were added to the basal diet in the control group; however, 1 g/kg COS, 1 g/kg Pro, and 1 g/kg COS + 1 g/kg Pro (Syn) were added to the basal diet of the respective treatment groups. At the end of the study, the results showed that the dietary supplementation of COS, Pro, and Synbiotic significantly ($P < 0.001$) increased the average daily gain, with values of 80.78±0.85 g/d, 80.21±0.36 g/d, and 79.94±0.7 g/d, respectively, compared to 73.28±0.49 g/d in the control group. Average feed intake was significantly higher in the supplemented groups ($P < 0.001$), with Pro showing the highest average feed intake of 149.00±1.17 g/d. COS supplemented group showed the lowest feed conversion ratio (1.82±0.00, $P = 0.004$) among all the study groups. There was no significant difference between the groups for carcass parameters and internal organ weights ($P > 0.05$), except for live weight at slaughter, which was found to be higher in all dietary-supplemented groups, 3006.22±29.52 g, 2984.51±13.75 g, and 2972.60±24.33 g respectively, than in the control group (2740.47±17.13 g). There was no significant effect ($P > 0.05$) of the different diets on digestive enzymes and serum biochemical parameters among the treatment groups ($P > 0.05$). In conclusion, dietary supplementation of COS and Pro individually and in combination with broiler diets improves performance parameters without adverse effects on serum metabolites and digestive enzymes.

Keywords: Broiler; Prebiotic; Probiotic; Synbiotic; Performance parameters.

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INTRODUCTION

The eradication of global hunger and the provision of feed to the steadily increasing population of the future are two of the most critical issues currently confronting the world (Van Dijk et al., 2021). Poultry served as a promising source for the global demand for animal protein. Chicken meat is a highly utilized and affordable source of protein with low cholesterol levels (Sarangi et al., 2016). However, the fast-growing poultry industry faces various challenges due to extensive production conditions, such as diseases and microbial stress. Antibiotics have long been used as antimicrobial and growth-promoting additives (Suresh et al., 2018). Increasing concerns regarding antibiotic resistance have led researchers to find alternative growth promoters in the poultry industry for antibiotic-free chicken meat and to stimulate growth, immunity, and health in broiler chickens (Hussain et al., 2023, Iqbal et al., 2021, Maqbool et al., 2023, Ningsih et al., 2023, Tayeri et al., 2018). Photobiotics, organic acids, antimicrobial peptides, bacteriophages, prebiotics, probiotics, and synbiotics are commonly used as growth promoter alternatives to antibiotics in the poultry industry (Gadde et al., 2017).

Probiotics are direct microbial feed supplements that can provide health benefits to the host when provided in an appropriate amount (FAO, 2016). In poultry nutrition, microbes used as probiotics mainly belong to *Lactobacillus, Bacillus, Streptococcus, Enterococcus, Bifidobacterium, Aspergillus,* and *Saccharomyces* (Park et al., 2016). *Bacillus subtilis* strains have widely been used as a probiotic in poultry production, and their isolation has a significant potential to be used as direct-fed microbes in the poultry sector (Shivaramaiah et al., 2011). *Bacillus*-based probiotics are reported to improve the growth performance parameters, carcass characteristics, intestinal health and morphology, immune response, and antioxidant status (Manafi et al., 2018, Rashid et al., 2023, Xu et al., 2021). In addition*, Bacillus* spp. particularly *Bacillus subtilis* and *Bacillus licheniformis*, contribute to enhanced nutrient digestion through the production of digestive enzymes, including protease, amylase, and lipase (Danilova and Sharipova, 2020, Latorre et al., 2016). These probiotic organisms also modulate the gut microbiota, promoting the proliferation of beneficial bacteria while reducing pathogenic microbes. Probiotics reduce pathogenic bacterial load through multiple mechanisms: competition for adhesion sites and nutrients, production of antimicrobial compounds, and modulation of the gut microbiota to favor beneficial bacteria. Furthermore, probiotics enhance immune responses, which subsequently contributes to the control of pathogenic populations and promotes the proliferation of beneficial microbes; thereby ultimately enhancing growth performance and immune response (Musa et al., 2019, Teo and Tan, 2006).

Prebiotics are dietary components that the host birds cannot digest; however, they promote the growth of beneficial bacteria in the gastrointestinal tract of animals (Tayeri et al., 2018). Prebiotics promote health-beneficial bacterial growth and reduce the pathogenic bacteria by competing for nutrients and attachment sites in the intestine. Prebiotics mainly include fructose-oligosaccharides, galacto-oligosaccharides, mannan-oligosaccharides, chitosan-oligosaccharides, raffinose, lactulose, inulin, stachyose and other oligosaccharides (Davani-Davari et al., 2019, Tufarelli et al., 2023), that showed improvement in growth performance, blood profile and gut health of broiler chickens (Ahmad et al., 2023). Chitosan-oligosaccharide (COS) is a chemical or enzymatically derived product from the second most abundant carbohydrate, chitosan. It is characterized by its low molecular weight, good solubility, and low viscosity, which make it an ideal prebiotic (Naveed et al., 2019). Dietary supplementation of COS improved growth performance, gut health, intestinal microbiota, nutrient digestibility, mineral retention, serum metabolic profile, meat quality, and antioxidant and immune status in broiler chickens (Chang et al., 2020, Egorov et al., 2022, Li et al., 2017, Li et al., 2007, Swiatkiewicz et al., 2014).

Synbiotics are the combination of prebiotics and probiotics, showing ~~possible~~ synergetic effects on animal health and production performance (Li et al., 2019). Synergetic effects can be attributed to the prebiotics, which promotes the survival of health-beneficial microbes because of substrate availability in the intestine. Some studies reported positive impacts of the synbiotics on the performance parameters (Tayeri et al., 2018), intestinal morphology (Awad et al., 2009), nutrient digestibility and meat quality (Cheng et al., 2017, Li et al., 2019), blood biochemical profile (Ghasemi et al., 2014), fat metabolism (Ghasemi and Taherpour, 2013), immunity (Żbikowski et al., 2020), antioxidant status (Li et al., 2019), meat composition (Ghasemi et al., 2016), and meat mineral contents (Cheng et al., 2017) of the birds. However, some studies demonstrated no differences in performance parameters, organ weights, carcass traits, blood metabolites, and mineral digestibility (Abd El Latif and Omar, 2023, Nisar et al., 2021, Sahin et al., 2008, Sarangi et al., 2016). This inconsistency in the results is attributed to the microbial strain, dose, birds' age, and nutrient composition, growing condition, broiler cross, and many other facotrs (Bilal et al., 2021).

B.subtilis with xylo-oligosaccharide and mannan-oligosaccharide was found to have a positive effect on growth performance, intestinal morphology, immune status, and antioxidant status of broiler chickens (Min et al., 2016). Encapsulated *L. lactis* and *B. bifidum* with chitosan improved the serum biochemical profile compared to those with only probiotic or encapsulated bacteria (Besharati et al., 2022, Yazhini et al., 2018). Li et al. (2016) reported that adding COS alone or with *L. casei* increased growth performance, meat quality, and antioxidant status of broiler chickens. However, information on the effects of chitosan-oligosaccharide with *Bacillus subtilis* and *Bacillus licheniformis* on growth performance, carcass characteristics, serum biochemical parameters, and digestive enzymes in broiler chickens is scarce. Therefore, we hypothesized that dietary inclusion of COS, probiotic and their combination may benefit the broiler chickens. Therefore, the present study aimed to whether the synergistic effects of these microorganisms and prebiotics can effect on the growth performance, carcass traits, serum biochemical parameters, and enzyme activity in broiler chickens.

MATERIALS AND METHODS

Animals, diets, and experimental design

The present study was conducted at Kafkas University, Kars, Turkey. In the study, 300 one-day-old mixed-sex Ross 308 broiler chicks were randomly allocated to four treatments, each treatment comprising 75 birds. Each experimental group was comprised of five replicates, with each replicate consisting of 15 birds. The control group was fed a basal diet without additional supplementation. The other groups received 1 g/kg COS, 1 g/kg probiotic (Pro), 1 g/kg COS + 1 g/kg Pro (Syn). The composition of the probiotic and the corresponding concentrations of the microbes are presented in Table 1. The experiment spanned a total of 42 days, comprising a preliminary adaptation phase of seven days, where all groups received a basal diet, followed by a subsequent 35-day experimental period. All experimental diets, in pelleted form, were formulated as isocaloric and isonitrogenous, according to NRC recommendations (NRC, 1994) (Table 2). Birds were housed within pens with 8-10 cm of wood shavings spread as litter material. The ambient temperature was maintained at 32°C during the first week of the study. Subsequently, adjustments were made, whereby the temperature was progressively lowered by 0.5°C each day until it reached a stable range of 24-26°C. This temperature range was maintained until the end of the experiment. A 24-hour light schedule was implemented for the initial three days, and it was then condensed to 18 h of light and 6 h of darkness during the trial. During the study period, ad libitum feed and fresh water were provided to the birds.

Growth performance

The birds' body weight, average daily gain, and average feed intake were recorded on d 7, 14, 21, 28, 35, and 42. The average daily gain (ADG), average feed intake (AFI), and feed conversion ratio (FCR) were calculated using the following formula:

Table 1 The probiotic formulation and its corresponding concentrations

Composition	Concentration
Lactobacillus farciminis	2×10^{11} cfu/kg
Saccharomyces cerevisiae	3.75×10^{11} cfu/kg
Bacillus subtilis	8×10^{9} cfu/kg
Bacillus licheniformis	8×10^{9} cfu/kg
Lactobacillus acidophilus	1×10^{9} cfu/kg
Enterecoccus faecium	2×10^{8} cfu/kg
Pediococcus acidilactici	1×10^{9} cfu/kg
Endo-1,4 beta-pentosanase	3300.00 epu/g
Alpha-amylase	1.10 skbu/g
1,4 Beta Glucanase	55.00 cfu/g
Protease	55.00 cfu/g
Galactomannanase	15.00 cfu/kg

cfu: colony-forming unit; skbu: sandstedt kneen blish unit; epu/g: endo-pentosanase units

Table 2 Ingredients and chemical composition of diets

Ingredients (%)	D 0 to 21	D 22 to 42
Corn	51.10	60.00
Soybean meal, 48% CP	34.00	30.00
Full-fat soybean	9.50	4.00
Vegetable oil	1.50	3.00
Di-calcium phosphate	1.90	1.44
Marble dust	1.00	0.80
DL-methionine	0.30	0.15
L-lysine sulfate	0.10	0.01
Salt	0.35	0.35
Vitamin-mineral premix*	0.25	0.25
Calculated chemical composition (%)		
Crude protein	24.10	20.90
Metabolic energy (kcal/kg)	3009	3107

* Vitamin-mineral premix: Vit A: 12000 IU; Vit D3: 3500 IU; Vit E: 50 IU; Vit B1: 3 mg; Vit B2: 6 mg; Vit B3: 20 mg; Vitamin B12: 5 mg; Folic Acid: 2.15 mg; Biotin: 0.75 mg; Vit C: 0.045 mg; Choline Chloride: 50 mg; Manganese: 125 mg; Iron: 80 mg; Zinc: 60 mg; Copper: 60 mg; Cobalt: 5 mg; Iodine: 0.2 mg; Selenium: 1 mg (per 1 kg).

$$ADG = \frac{FinalBodyWeight(g) - InitialBodyweight(g)}{NumberofDays}$$

$$AFI = \frac{TotalFeedConsumed(g)}{NumberofBirdsxNumberofDays}$$

$$FCR = \frac{TotalFeedIntake(g)}{TotalBodyWeightGain(g)}$$

In the case of mortality, adjustments were made to the growth performance metrics to ensure data accuracy.

Carcass characteristics and organ weights

On the 42nd day, birds were subjected to feed restriction for 6 hours before slaughtering, and a total of 40 broiler chickens, ten birds from each group, close to the average body weight of their respective pens were randomly selected and humanely slaughtered via decapitation. Carcass characteristics, including hot carcass weight and visceral organs (liver, heart, and gizzard), were measured using a precise weighing scale. Hot carcass weight was determined immediately after slaughter, and cold carcass weight was calculated after storage at +4 ºC for 24 h (Ahmad et al., 2023). Hot carcass weight, cold carcass weight, and organ weight percentages were determined relative to the live weights of the birds.

Serum biochemical parameters

On the 42nd day, blood samples were collected from two birds in each replicate (10 samples/group) from the brachial vein. The blood samples were then clotted, followed by centrifugation at 4500 rpm for 15 min using an NF 200 Bench-Top Centrifuge (Nüve, Ankara, Turkey) to separate the serum. Serum aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), glucose, total protein, albumin, uric acid, calcium (Ca), and phosphorus (P) levels were determined with a spectrophotometer (Spectra max plus, Moleculer Device, Sunnyvale, CA, USA) using commercial kits (Erba Lachema, Brno, Cz) (Aslam et al., 2021, Ölmez and Yörük, 2021).

Digestive enzymes

Amylase and lipase concentrations in the serum, derived from the blood samples collected at slaughtering, were quantified using commercially available assay kits as per mentioned protocol by the producer (Erba Lachema, Brno, Cz).

Statistical analysis

The data analysis was carried out using one-way ANOVA in the statistical software package SPSS (version 26.0, IBM Corp., Armonk, NY, US). Each replicate was considered an independent experimental unit and the confidence level was set at 95%. Duncan's multiple range test was used to discern any significantly different mean values between groups. The results are presented as mean ± standard error of the mean (SEM). Results were considered statistically significant at $P < 0.05$.

RESULTS

Growth performance

Table 3 shows the effects of COS, Pro, and Syn dietary supplements on the average daily gain of the broiler chickens. In the first week of the experiment, there was no significant difference (P > 0.05) in ADG between the study groups. The highest ADG was observed in the COS group (P < 0.05) between 21-28 days of the study, and the highest ADG in the Pro group (P < 0.001) was observed between 28-35 days. During 35-42, the highest ADG was recorded in the synbiotic group compared to the other study groups. Overall (d 7-42), it was determined that all groups fed diets supplemented with COS, Pro, and Syn had higher values for ADG than the control group (P < 0.001).

Average feed intake data is presented in Table 4. Analysis revealed that the group supplemented with Pro exhibited the highest AFI between d 14-21 of the experiment. However, the COS-supplemented group showed the highest AFI on d 21-28. For both the d 28-35 and the overall study duration, it was observed that all groups receiving diets supplemented with COS, Pro, or Syn demonstrated the highest AFI levels compared to the control group.

Table 5 shows the results of COS, Pro, and Syn supplementation in diets of broiler chickens on FCR. During the initial three-week period of the experiment, no statistically significant differences in FCR were observed among the groups. However, during d 28-35, both COS- and Pro-supplemented groups demonstrated the lowest FCR values (P = 0.017).

Table 3 Effects of dietary prebiotic, probiotic and symbiotic on body weight gain of broiler chickens

Average Daily Gain (g/d/bird)	Dietary Treatments				P-value
	Control	COS	Pro	Syn	
D 7-14	35.23±0.99	35.87±0.97	37.33±0.42	36.73±0.53	0.282
D 15-21	56.53±2.50	56.61±1.54	60.92±1.95	60.45±2.48	0.344
D 21-28	69.13±2.63^{b}	82.13±1.56^{a}	74.85±2.40^{b}	72.74±2.55^{b}	0.013
D 29-35	97.05±1.97^{c}	114.29±1.70^{ab}	118.81±1.62^{a}	111.76±2.08^{b}	<0.001
D 36-42	108.45±1.36^{b}	114.98±2.81^{ab}	109.15±2.59^{b}	109.15±1.05^{a}	0.019
D 7-42	73.28±0.49^{b}	80.78±0.85^{a}	80.21±0.36^{a}	79.94±0.71^{a}	<0.001

COS = chitosan-oligosaccharide 1g/kg of diet; Pro = Probiotic 1g/kg of diet; Syn= 1g COS + 1g probiotic/kg of diet
Means with distinct superscripts within the same row are statistically significant (P < 0.05).

Table 4 Impact of dietary prebiotic, probiotic and symbiotic on feed intake of broiler chickens

Average Feed Intake (g/d/bird)	Dietary Treatments				P-value
	Control	COS	Pro	Syn	
D 7-14	68.47±2.06	68.47±1.30	70.91±0.67	69.51±0.93	0.626
D 15-21	106.96±7.48^{b}	108.11±5.72^{b}	124.41±2.41^{a}	122.58±2.32^{ab}	0.052
D 22-28	128.01±4.32^{c}	152.54±6.44^{a}	144.37±3.40^{ab}	133.23±4.87^{bc}	0.017
D 29-35	174.37±2.25^{b}	198.08±3.38^{a}	204.91±2.43^{a}	207.30±3.93^{a}	<0.001
D 36-42	200.99±5.26	206.55±4.72	200.39±5.39	215.45±2.64	0.133
D 7-42	135.76±1.31^{b}	146.86±1.27^{a}	149.00±1.17^{a}	149.62±1.70^{a}	<0.001

COS = chitosan-oligosaccharide 1g/kg of diet; Pro = Probiotic 1g/kg of diet; Syn= 1g COS + 1g probiotic/kg of diet
Means with distinct superscripts within the same row are statistically significant (P < 0.05).

Table 5 Impact of dietary prebiotic, probiotic and symbiotic on feed conversion ratio of broiler chickens

Feed Conversion Ratio (g:g)	**Dietary Treatments[1]**				P-value
	Control	COS	Pro	Syn	
D 7-14	1.95±0.02	1.93±0.03	1.90±0.04	1.89±0.04	0.625
D 15-21	1.89±0.07	1.91±0.08	2.05±0.06	2.03±0.06	0.289
D 26-28	1.85±0.05	1.86±0.05	1.93±0.04	1.83±0.05	0.497
D 29-35	1.80±0.05^{ab}	1.73±0.01^{b}	1.73±0.01^{b}	1.86±0.02^{a}	0.017
D 36-42	1.85±0.04	1.80±0.03	1.84±0.02	1.83±0.02	0.622
D 7-42	1.85±0.01^{ab}	1.82±0.00^{b}	1.86±0.02^{ab}	1.87±0.02^{a}	0.004

COS = chitosan-oligosaccharide1g/kg of diet; Pro = Probiotic 1g/kg of diet; Syn= 1g COS + 1g probiotic/kg of diet
Means with distinct superscripts within the same row are statistically significant (P < 0.05).

Table 6 Impact of dietary prebiotic, probiotic and symbiotic on some carcass characteristics and organ weights of broiler chickens

Item (%)	Dietary Treatments				P-value
	Control	COS	Pro	Syn	
Slaughter Weight (g)	2740.47±17.13[b]	3006.22±29.52[a]	2984.51±13.75[a]	2972.60±24.33[a]	<0.001
Carcass Yield	72.13±0.23	72.41±0.13	72.34±0.09	72.17±0.20	0.36
Liver	0.55±0.01	0.52±0.01	0.53±0.01	0.51±0.01	0.919
Gizzard	1.26±0.01	1.27±0.04	1.25±0.02	1.25±0.03	0.986
Heart	0.55±0.01[a]	0.52±0.01[ab]	0.53±0.01[ab]	0.51±0.01[b]	0.041

COS = chitosan-oligosaccharide 1g/kg of diet; Pro = Probiotic 1g/kg of diet; Syn= 1g COS + 1g probiotic/kg of diet
Means with distinct superscripts within the same row are statistically significant (P < 0.05).

During the entire study period, the COS-supplemented group exhibited the lowest FCR (P = 0.004) among all the study groups.

Carcass characteristics and organ weights

The carcass characteristics of broiler chickens fed diets containing COS, Pro, and Syn are presented in Table 6. At the end of the experiment (42[nd] day), there was a significant difference (P < 0.001) in the live slaughter weights of the study groups. The highest live slaughter weight was found in the additive-fed study groups compared to the control group. However, there was no significant difference (P > 0.05) between the groups for carcass characteristics (hot carcass weight, and cold carcass weight) and organ weights (liver, and gizzard) except heat weight which was lowest in Syn group compared to the control group (P < 0.05).

Digestive enzymes

The digestive enzyme results of the study are given in Table 7. A statistically non-significant difference (P > 0.05) was observed between the amylase and lipase levels between the control and experimental groups.

Serum biochemical parameters

Serum biochemical levels of all the study groups are summarized in Table 8. It was determined that the COS, Pro, and Syn supplementation in broiler diets had no statistically significant (P > 0.05) effect on serum total protein, albumin, glucose, AST, ALP, GGT, uric acid, Ca, and P levels.

Table 7 Impact of dietary prebiotic, probiotic and symbiotic on digestive enzymes of broiler chickens

Item (IU/L)	Dietary Treatments				P-value
	Control	COS	Pro	Syn	
Amylase	349.69±38.90	356.74±48.69	482.29±104.55	285.38±36.91	0.195
Lipase	19.19±1.56	19.25±1.39	19.50±1.67	19.98±1.23	0.981

COS = chitosan-oligosaccharide 1g/kg of diet; Pro = Probiotic 1g/kg of diet; Syn= 1g COS + 1g probiotic/kg of diet
Means with distinct superscripts within the same row are statistically significant (P < 0.05).

Table 8 Impact of dietary prebiotic, probiotic and symbiotic on some serum biochemical parameters of broiler chickens

Item	Dietary Treatments				P-value
	Control	COS	Pro	Syn	
Total Protein (g/L)	4.45±0.07	4.40±0.10	4.27±0.08	4.46±0.09	0.373
Albumin (g/L)	1.65±0.04	1.65±0.06	1.60±0.08	1.71±0.03	0.569
Glucose (mg/dL)	214.32±8.84	208.33±7.17	218.50±5.04	229.44±13.38	0.430
AST (IU/dL)	198.34±13.11	188.06±6.49	170.88±9.36	177.50±7.04	0.199
ALP (IU/dL)	1931.96±20.66	1894.68±13.67	1922.29±21.45	1909.55±19.33	0.548
GGT (IU/dL)	16.89±0.79	16.98±0.60	16.56±0.85	15.66±0.91	0.643
Uric acid (mg/dL)	4.65±0.23	4.61±0.22	5.14±0.36	5.16±0.21	0.279
Ca (mg/dL)	9.82±0.39	10.46±0.41	10.72±0.47	10.09±0.30	0.404
P (mg/dL)	6.92±0.26	7.00±0.25	7.02±0.13	7.23±0.26	0.815

AST: aspartate aminotransferase; ALP alkaline phosphatase; GGT: gamma-glutamyl transferase; Ca: calcium, P: phosphorus
COS = chitosan-oligosaccharide 1g/kg of diet; Pro = Probiotic 1g/kg of diet; Syn= 1g COS + 1g probiotic/kg of diet
Means with distinct superscripts within the same row are statistically significant (P < 0.05).

DISCUSSION

Growth parameters are the key indicators for the performance of the birds, profitability, and sustainability of the farm. Prebiotic, probiotic and synbiotic in the diets increased the ADG and AFI of broiler chickens at 42nd day of the study. These results are similar to the findings of previous studies that showed an increase in ADG and AFI with supplementation of COS (Chang et al., 2020, Li et al., 2019) probiotic (Wang et al., 2022, Zhang et al., 2021), and synbiotic (Awad et al., 2009, Ghasemi and Taherpour, 2013, Mohammed et al., 2018, Salah et al., 2018). Some studies reported no effect of probiotics, prebiotics, and synbiotics on the ADG and AFI of the broiler birds (Al-Khalaifa et al., 2019, Li et al., 2019, 2019, Mookiah et al., 2014, Sahin et al., 2011, Sahin et al., 2008, Salehimanesh et al., 2016, Sarangi et al., 2016, Yalçın et al., 2003). However, improvement in the ADG of the broiler birds could be the result of the underlying growth-promoting mechanism of prebiotic, probiotic, and `synbiotic. Although, the precise mechanism on the effect of prebiotic, probiotic and synbiotic is unclear, however, many studies reported that probiotic supplementation modulates the intestinal microbiota, enhance the population of beneficiary bacteria (Jabeen et al., 2023, Mookiah et al., 2014), reduce pathological bacterial load (Tarabees et al., 2019, Tayeri et al., 2018), and improve the intestinal environment and health (Ghasemi and Taherpour, 2013, Tayeri et al., 2018), antioxidant status (Li et al., 2019), immune response (Ghasemi et al., 2014), nutrients digestibility (Abd El Latif and Omar, 2023, Nisar et al., 2021), which ultimately leads to improved birds performance. Prebiotic helps in the growth of selective beneficiary bacteria, which improves the intestinal environment and health and ultimately results in improved nutrient digestion, absorption, and, finally, improved growth performance (Li et al., 2019).

The results on the FCR showed that the birds fed diets with Syn have the highest FCR compared to the other treatment groups. The COS-supplemented group exhibited the lowest FCR among all treatment groups and no differences were found between probiotic and control group FCR. This result is contrary to the result of many studies that reported an improvement in FCR with the dietary supplementation prebiotic, probiotic and synbiotic (Ghasemi et al., 2014, Tayeri et al., 2018). However, some studies reported no effect of the dietary prebiotic, probiotic, and synbiotic on the FCR of the broiler chickens. These differences can be the result of higher AFI in this study, parallel to the higher ADG. The earlier studies reporting higher bird performance showed no change in AFI, which resulted in improved FCR (Ghasemi et al., 2014, Tayeri et al., 2018). In addition, the COS and Pro treatment groups showed statistically similar results compared to the Syn group, and no combination effect was observed in the Syn groups. These results conclude that combining COS and Bacillus probiotic has no synergetic impact. This study's results are similar to those of Mookiah et al. (2014) who reported that synbiotics showed no improvements in performance compared to the results of prebiotic and probiotic-fed treatment groups. However, several studies reported improved ADG and AFI when fed synbiotics compared to the prebiotic and probiotic-fed groups (Awad et al., 2009) The inconsistencies in the growth performance results could be attributed to the variations in the probiotic strains, bacterial count in the composition, prebiotics, and environmental factors for the broiler chickens.

The carcass yield and organ weights remained similar among all the treatments, and these results contradict some earlier research (Awad et al., 2009) that prebiotic, probiotic, and synbiotic improve the carcass yield and organs weights. This study's results support the finding of some other researchers who found that prebiotic, probiotic, and synbiotic do not affect the carcass yield and organ weights of broiler chickens (Cheng et al., 2017, Erdoğan et al., 2010, Ghasemi et al., 2014, Li et al., 2019, Ölmez et al., 2022, Sarangi et al., 2016). Salah et al. (2018) reported that dietary supplementation with synbiotics improves the carcass yield, however, it reduces the relative weights of the liver and gizzard. Some studies reported an increased weight of the liver with synbiotic supplementation (Abd El Latif and Omar, 2023, Awad et al., 2009) However, no probiotic, prebiotic, and synbiotic effect was observed on the gizzard and heart weight. Nisar et al. (2021) also reported an increase in liver weight with the dietary supplementation of synbiotics in broiler chicken. However, other organs' weights remained similar among the studied groups.

Digestive enzymes play a critical role in nutrient digestion, resulting in increased animal performance. The results for digestive enzymes showed a non-significant change in the serum values of digestive enzymes. Similar results were observed by Lan et al. (2024) who reported no alteration in serum values of digestive enzymes with supplementation of COS. Wang and Gu (2010) also reported no effect of probiotic supplementation on lipase activity, although amylase activity increased in the probiotic-supplemented groups compared with the control group. However, Mathivanan et al. (2006) reported an increase in lipase activity in broiler chickens fed a soybean-meal fermented diet with *Aspergillus niger*. These results are also not in agreement with those of some studies that reported that digestive enzymes increased with the supplementation of probiotics, prebiotics, and synbiotic (Abd El Latif and Omar, 2023, Gong et al., 2018, Kolodziejski et al., 2018, Sun et al., 2022). Abd El Latif and Omar (2023) found the highest enzyme values in the probiotic group compared to prebiotic and synbiotic, which suggests that the probiotic is mainly involved in altering enzyme activities. In this study, no change in the digestive enzyme values among all the treatment groups could be the result of probiotic strains, concentration, and prebiotic type because different probiotic strains, their combinations, and synbiotics have various capacities to modulate the production of digestive enzymes in broiler chickens (Wang and Gu, 2010).

The serum biochemical profile results of this study showed that the dietary prebiotic, probiotic, and synbiotic have no effect on the serum total protein, albumin, glucose, triglyceride, AST, ALP, GGT, Uric Acid, Ca, and P levels. These results are in agreement with the results of Żbikowski et al. (2020) who reported no effect of two different prebiotics and three different multistrain probiotics, and synbiotic on the total protein, albumin, glucose, triglyceride, ALP, GGT, Uric Acid, Ca, and P concentration at d 42 of the study. Only AST increased in the control group compared to the other treatment groups. It is well known that the AST is involved in protein transformation. Researchers found no correlation between the AST and total protein, albumin, and globulin (Żbikowski et al., 2020), which supports the results of this study. Similarly, Abd El Latif and Omar (2023) also reported an increased serum total protein, globulin, glucose, and triglyceride with the probiotic and synbiotic supplementation; however, albumin, cholesterol, ALT, and AST remained similar among all the treatment groups. No such results have been found in this study with the supplementation of probiotics combination, COS, and synbiotic.

In conclusion, dietary supplementation with 1 g/kg COS, 1 g/kg Pro, and 1 g/kg COS + 1 g/kg Pro improved growth performance and feed intake in broiler chickens. Relative to the other treatment groups, the specific benefits of COS supplementation were observed in improving FCR. No effects of COS, Pro, and Syn dietary supplementation were observed on carcass parameters, serum biochemical profiles, and digestive enzymes in broiler chickens. No synergetic effects were observed in this study with the combined supplementation of COS and Pro. Although the economic impact of these additives appears promising due to enhanced feed efficiency, further investigation is necessary to optimize dosages, combinations, and strain selection to maximize their efficacy. Therefore, the findings of this study require additional scientific research to confirm their results for practical industrial applications. In addition, further research on the effects of COS with multi-strain probiotics is needed to investigate the effects of synbiotics on gut health, gut microbiota, immune response, nutrient utilization, and meat composition.

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CONFLICT OF INTEREST

The authors declare no conflict of interest for this study.

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