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Determination of the effects of glucomannan supplementation into probiotic & enzyme combination on performance, some blood parameters and oxidative stress index in broilers

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ABSTRACT

Objectives: This trial investigated the effects of adding plant-derived glucomannan to diets containing a probiotic and enzyme complex (PEx) on the performance and certain blood and oxidative stress parameters in broilers.

Materials and Methods: A total of 150 one-day-old male broiler chicks were randomly allocated into three groups: as a control (C), probiotic-enzyme (PE), and probiotic-enzyme plus glucomannan (PEG) group. All birds received standard starter and grower diets. Throughout the 42-day experimental period, the control group fed basal diet, the PE group received 0.1% PEx, and the PEG group received 0.1% PEx plus additional glucomannan.

Results: Significant differences were observed among groups for body weight (BW), body weight gain (BWG), feed intake (FI), and carcass weight ($p<0.05$), with the PEG group was higher than the others. Feed conversion ratio (FCR), European Production Efficiency Factor (EPEF), carcass yield, and relative organ weights (heart, liver, pancreas, spleen, gizzard) were insignificant ($p>0.05$). However, the bursa of Fabricius was relatively larger in the PE and PEG groups. Among biochemical traits, globulin, albumin, and HDL-cholesterol levels differed significantly ($p<0.05$), with higher globulin and HDL-cholesterol and lower albumin levels observed in the PE- and PEG-supplemented groups compared with the control group. There were no significant differences in other biochemical or oxidative stress indicators (TAS, TOS, OSI) ($p>0.05$).

Conclusions: Dietary inclusion of 0.1% PEx combined with glucomannan positively influenced broiler performance and serum HDL levels, suggesting a potential synergistic effect. While the findings indicate benefits to growth and metabolic health, further research is needed to clarify the long-term and immunological impacts of these supplements.

Keywords: *Broiler, Enzyme, Glucomannan, Oxidative stress, Prebiotic, Probiotic*

INTRODUCTION

Digestive enzymes are biological catalysts naturally produced in the digestive systems of living organisms, enabling the breakdown of dietary nutrients into smaller and more absorbable components. In particular, during neonatal and adolescent stages, and occasionally later in life, insufficiencies or dysfunctions in the endogenous production of these enzymes may lead to digestive problems. Furthermore, when low-quality feed ingredients are included in diets, the complex compounds within plant cell walls can hinder the activity of digestive enzymes, thereby reducing nutrient digestion and bioavailability. The

combination of relatively short digestive tracts, rapid passage of digesta, and limited capacity for microbial fermentation leads to a less efficient digestive process in monogastric poultry species. As a countermeasure, exogenous supplementation of digestive enzymes such as phytase, β-glucanase, xylanase, and pectinase has been investigated. It has been demonstrated that such supplementation can enhance digestive efficiency and/or mitigate adverse effects (Adeola and Cowieson, 2011; Jha and Mishra, 2021; Yıldız et al., 2021). The oral administration of these enzymes, which are not produced endogenously in sufficient quantities by animals, is particularly important in poultry production, as it enhances nutrient digestibility and feed efficiency. In addition, this practice can substantially reduce feed costs, thereby delivering significant economic benefits to livestock enterprises (Kiarie et al., 2013; Azzaz et al., 2019).

Probiotics are defined as live microorganisms, that, when administered in adequate amounts, colonize specific organs and systems of the host and confer beneficial effects on overall health. They typically include certain bacteria, yeasts, fungi, algae, and bacteriophages. Generally, probiotic microorganisms are identical or closely related variants of microbes that constitute the natural microbiota of living organisms or are found in fermented foods (Song et al., 2012; Fijan, 2014). These agents provide significant benefits for both humans and animals, particularly by supporting gastrointestinal health, enhancing growth and developmental performance, strengthening the immune system to improve disease resistance, and controlling pathogenic microorganisms (Kiani et al., 2020; Oleskin and Boyang, 2022; Maftei et al., 2024). Furthermore, such microorganisms can also be cultured under laboratory conditions and made available as supplemental formulations. In broiler production, specific probiotic strains used for feed supplementation predominantly include species of *Bacillus*, *Lactobacillus*, *Bifidobacteria, Streptococcus, Enterococcus, Pediococcus,* as well as the yeast *Saccharomyces cerevisiae*, due to their documented effects on gastrointestinal health and production performance (Mountzouris et al., 2007; Naeem and Bourassa, 2025).

Prebiotics are defined as compounds that, when consumed, cannot be hydrolyzed by the host's endogenous digestive enzymes but can be degraded and utilized by beneficial microorganisms in the gut, thereby supporting their activity and growth while contributing to the maintenance of a balanced intestinal microflora. Essentially, prebiotic compounds work synergistically with probiotic microorganisms to produce potent effects that improve intestinal and overall bodily health (Swanson et al., 2025). Prebiotics are naturally present in various fruits, vegetables, whole grains, and some dairy products in the form of dietary fibers or complex carbohydrates. The most common prebiotics include inulin, fructo-, galacto-, and mannan-oligosaccharides.

Experimental studies have shown that prebiotic compounds, when administered alongside probiotic microorganisms, can create a synergistic interaction that produces results significantly more effective than when either is used alone. This dual combination has been termed "synbiotics" (Patterson and Burkholder, 2003; Jenkins and Mason, 2022; Ogwiji et al., 2024). Furthermore, it is aimed to include digestive enzymes in this interaction to maximize the nutritional benefits obtained from the consumed feed (Güçlü and Kara, 2009). In line with this objective, recent years have witnessed a growing interest in both human and animal nutrition research focusing on the application of such triple combinations (Webb et al., 2019; Xing et al., 2022; Ogwiji et al., 2024). It has been demonstrated that the combined use of probiotics, prebiotics, or enzymes can regulate intestinal microbiota, pH, and digesta viscosity; reduce mortality rates in animals; enhance feed intake and feed efficiency; and consequently lead to improvements and significant increases in performance parameters such as body weight gain, meat, milk, and egg production, as well as product quality (Gaggìa, 2010; Kırkpınar et al., 2018; Dong et al., 2024). In addition to their beneficial effects on the digestive system, evidence also indicates that such nutritional strategies may modulate the immune system and exert protective effects against certain inflammatory diseases (Hardy et al., 2013; Markowiak and Śliżewska, 2017; Tomczyk et al., 2024). Moreover, the incorporation of these combinations into diets is anticipated to reduce the use of antibiotics, thereby preventing the development of antimicrobial resistance and limiting the dissemination of antibiotic residues into the environment (Hu et al., 2022; Zhao et al., 2024). Previous studies in broiler chickens have shown that probiotics enhance intestinal barrier integrity, increase short-chain fatty acid production, and consequently improve feed conversion efficiency, while also modulating

immune responses and enhancing disease resistance. Prebiotics have been reported to improve intestinal morphology by increasing villus height and nutrient absorptive capacity; in particular, mannan-oligosaccharides reduce the adhesion of pathogenic bacteria to the intestinal mucosa, thereby supporting growth performance. Moreover, the combined use of probiotics and prebiotics as synbiotics has been associated with more pronounced improvements in body weight gain, faster stabilization of intestinal microflora, and increased resilience to environmental and nutritional stressors compared with their individual application (Abdel-Raheem et al., 2012; Khomayezi and Adewole, 2022; Atuahene et al., 2025).

While the individual or collective effects of probiotics, digestive enzymes, and prebiotics on broiler chickens have been extensively investigated, research concerning their synergistic application remains sparse. Specifically, the efficacy of enzyme-assisted probiotics when administered alongside natural prebiotic sources has not been fully elucidated. Moreover, information on the efficacy of plant-based glucomannan as a prebiotic component in broiler diets remains limited. Therefore, the present study aimed to address this knowledge gap by assessing the potential synergistic effects of combining a commercial probiotic and enzyme complex with a plant-derived glucomannan source on broiler performance and associated physiological parameters. Glucomannan, a polysaccharide of botanical and natural origin, is found in its highest concentration within the roots of the *Amorphophallus konjac* plant, where it constitutes between 40% and 60% of the dry matter content. In konjac gum, which is extracted from these roots, this proportion can reach up to 90%. (Chua et al., 2010; Jian et al., 2024). Glucomannan is a polysaccharide composed of glucose and mannose units, characterized by its high water-absorbing capacity, which underlies its widespread use in the food industry as a thickener, gelling agent, and carrier. Previous studies conducted in humans, mice, and chickens have demonstrated that glucomannan may regulate blood pressure as well as glucose, cholesterol, and triglyceride levels, in addition to exhibiting laxative, toxin-binding, anti-obesity, anti-inflammatory, and antitumor activities (Murthy et al., 2002; Behera and Ray, 2016; Kapoor et al., 2024).

In light of all these findings, it is evident that further research is essential to determine the optimal formulations of enzyme, probiotic, and prebiotic combinations in dietary applications for various animal species. This research should aim to clearly elucidate their beneficial effects, potential adverse effects, synergistic interactions, and overall contributions to production performance. This study aimed to provide valuable insights into the effects of specific dietary supplements on the performance and health outcomes of broiler chickens.

MATERIALS and METHODS

A total of 150 one-day-old male broiler chicks (Ross 308) were used in the trial. The chicks were allocated into three main groups of 50 birds each: control group (C) and experimental groups (designated as PE and PEG, respectively). Each main group was further subdivided into five subgroups, with 10 chicks per subgroup (a total of 15 subgroups). The chicks were fed a standard basal diet (+additives in experimental groups) and provided drinking water ad libitum for a period of 42 days, under a group-feeding system. On the first day of hatching, the chicks were vaccinated against Newcastle and infectious bronchitis.

Basically, all groups were provided with basal diets formulated according to the nutritional requirements of broilers for each rearing phase, with no additional supplements used apart from the feed ingredients listed in Table 1. The prepared basal diets were offered to the control group (C) without any additive supplementation. In the first experimental group (PE), the basal diet was supplemented with 0.1% of a commercial product (Prozyme©; Farmavet International, Manisa, Türkiye) containing a probiotic and enzyme complex (hereafter referred to as "PEx"). According to the product label, the composition of this commercial PEx preparation was as follows: *Bacillus subtilis & Bacillus licheniformis*, 7.5×10^{12} CFU/kg; α-amylase, 4×10^{5} U/kg; protease, 4×10^{6} U/kg; cellulase, 5×10^{6} U/kg; pectinase, 1×10^{5} U/kg; β-xylanase, 2.5×10^{6} U/kg. In the second experimental group (PEG), the basal diet was supplemented with 0.1% of the same commercial PEx product together with 0.1% konjac gum (E425; Smart Kimya, İzmir, Türkiye), derived from the Amorphophallus konjac plant. Based on the technical data provided by the manufacturer, the konjac gum contained at least 88% glucomannan. Based on this specification, the

actual glucomannan content in the diet of the PEG group was calculated to be a minimum of 0.088%.

Throughout the experimental period, birds were weighed weekly to determine the average body weight (BW) and body weight gain (BWG); feed intake (FI), and feed conversion ratio (FCR) were calculated based on these parameters. Additionally, indicators of poultry production efficiency, including the European Broiler Index (EBI) and the European Production Efficiency Factor (EPEF), were calculated according to the following equations: EBI = (daily weight gain × survival rate) / (10 × feed conversion ratio); EPEF = [(live weight × survival rate) / (feed conversion ratio × days of age)] × 100. At the end of the rearing period, four birds were randomly selected from each replicate, resulting in a total of 20 birds per main group. During the slaughtering phase, firstly, blood samples were taken from the jugular vein, then a cervical incision was made, and the slaughtering process was completed. Subsequently, the weights of the hot carcass weight (HCW), heart, liver, spleen, pancreas, gizzard, and bursa of Fabricius were recorded, and then carcass yields (RCW) as well as relative organ weights were calculated. Blood samples were analyzed for concentrations of glucose, total protein, albumin, triglycerides, total cholesterol, HDL-C, non-HDL-C, LDL-C, urea, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), creatine phosphokinase (CPK), and alkaline phosphatase (ALP). These analyses were performed using an autoanalyzer and commercial test kits based on colorimetric/spectrophotometric principles (Cobas® c 703 Analytical Unit, Roche Diagnostics, Basel, Switzerland). Total oxidant status (TOS) and total antioxidant status (TAS) were determined using a separate autoanalyzer operating on the same measurement principle (Mindray BS-800M, Shenzhen, China) with commercial kits (Rel Assay Diagnostic, Gaziantep, Türkiye). The oxidative stress index (OSI) was subsequently calculated based on these measurements.

Table 1. Botanical composition and chemical analysis of diets (DM%).

Item	Starter diet (0-21. day)	Grower diet (22-42. day)
Maize	50.50	57.51
Soybean meal	30.00	17.55
Full-fat soybean	14.92	20.26
Vegetable oil	1.00	1.00
Calcium carbonate	1.08	1.18
Vitamin & mineral premix*	2.50	2.50
Calculated nutrient levels		
Calcium	1.00	1.00
Phosphorus	0.48	0.48
Lysine	1.52	1.30
Methionine	0.72	0.68

Chemical compositions of experimental diets (DM%)						
	C		PE		PEG	
	(0-21.day)	(22-42.day)	(0-21.day)	(22-42.day)	(0-21.day)	(22-42.day)
Dry matter	94.69	93,75	94.28	93.31	93.92	92.32
Organic matter	86.94	90.79	86.90	90.74	86.89	90.78
Crude protein	26.90	22.99	26.52	23.01	26.66	22.78
Crude fiber	3.89	4.60	4.08	4.77	4.04	3.84
Ether extract	7.50	7.85	7.82	8.02	7.26	8.49
Ash	13.06	9.21	13.10	9.26	13.11	9.22
ME (Kcal/kg)**	3224	3299	3218	3289	3193	3317

*Vitamin-Mineral premix (in 1 kg): Vit. A, 13000.00 IU; Vit. D_3, 5000.00 IU; Vit. E, 70.00 mg; Vit. K_3, 3.00 mg; Vit. B_1, 3.00 mg; Vit. B_2, 8.00 mg; Vit. B_6, 4.50 mg; Vit. B_{12}, 0.20 mg; Niacin, 60.00 mg; Biotin, 0.25 mg; Pantothenic acid, 16.00 mg; Folic Acid, 2.00 mg; Copper, 16.00 mg; Iron, 20.00 mg; Manganese, 120.00 mg; Iodine, 1.25 mg; Zinc, 110.00 mg; Selenium, 0.30 mg.

**Determined by calculation (TSE, 1991)

The ingredient composition of the experimental diets was formulated in accordance with the guidelines set by the National Research Council (NRC, 1994). Nutrient content analyses were performed following the procedures outlined by the Association of Official Analytical Chemists (AOAC, 2005). The ingredient composition and chemical analysis data of the diets are presented in Table 1.

Statistical analysis

All data obtained from the experiment were evaluated for compliance with the assumptions of parametric analysis. Statistical analyses were performed using the SPSS software package (SPSS, 2006). Normality of data distribution was assessed using the Shapiro–Wilk test, and homogeneity of variances among groups was evaluated using Levene's test. Subsequently, the data were analyzed by one-way analysis of variance (ANOVA). When significant differences were detected, mean values were compared using Duncan's multiple range test. Differences were considered statistically significant at $P<0.05$.

Ethical approval

This study was approved by the Local Ethics Committee for Animal Experiments of Kırıkkale University (Decision no: 2025/01-01, dated 26/02/2025).

RESULTS

In the present study, the data obtained are presented as follows: the mean body weight values of the C, PE, and PEG groups are shown in Table 2; mean body weight gain, feed intake, feed conversion ratio and productivity indices are presented in Table 3; carcass parameters are shown in Table 4; serum biochemical parameters are presented in Table 5; and serum oxidative stress parameters are shown in Table 6.

According to the results, the mean body weights of the C, PE, and PEG groups were 2024.42 g, 2016.93 g, and 2194.30 g, respectively. These findings indicated that a statistically significant difference occurred among the groups ($p<0.05$); while the data of the control and PEx-supplemented groups were similar, a notable increase in BW was observed in the PEG group, which received PEx in combination with glucomannan (Table 2). When the changes in group BW were examined on a weekly basis, it was observed that the divergence in favor of the PEG group emerged as early as the first week and persisted throughout the rearing period.

Table 2. Mean and standard error mean of the body weight (g).

Age (Week)	**Trial Groups** (mean±SEM)			
	C	**PE**	**PEG**	***p* value**
1st day	36.91±0.31	37.09±0.30	37.09±0.43	0.903
1.	115.71^{b}±2.30	115.64^{b}±2.10	123.60^{a}±2.15	0.026
2.	233.46^{b}±5.17	227.06^{b}±3.83	274.54^{a}±5.99	<0.001
3.	535.97^{b}±11.21	528.06^{b}±10.94	623.84^{a}±14.28	<0.001
4.	977.13^{b}±16.31	965.06^{b}±17.83	1030.77^{a}±19.28	0.037
5.	1512.32^{b}±20.72	1494.97^{b}±27.82	1644.94^{a}±26.81	<0.001
6.	2024.42^{b}±25.29	2016.93^{b}±32.43	2194.30^{a}±31.51	<0.001

$^{a, b:}$ Values in the same row with different letters indicate statistically significant differences ($p<0.05$).

The evaluation of BWG and FI, as detailed in Table 3, revealed results parallel to the BW outcomes, with the PEG group significantly outperforming the other two groups ($p<0.05$). However, no significant difference was detected in the FCR either on a weekly basis or in total results ($p>0.05$). Additionally, two chick mortalities were recorded in the control group, while three mortalities were observed in each of the experimental groups, resulting in a total of eight deaths. Furthermore, efficiency and profitability indices were calculated to assess the overall production performance. The EBI values ranged from 245 to 266, whereas the EPEF scores fluctuated between 249 and 271. Although these indices followed a numerical ranking of PE < C < PEG, no statistically significant differences were observed among the experimental groups ($p>0.05$).

Significant differences were observed among groups in slaughter and carcass weights ($p<0.05$). Mean carcass weights were 1411.50 g, 1439.37 g, and 1526.58 g in the C, PE, and PEG groups, respectively. Relative liver weight was significantly lower in the PE and PEG groups compared with the

control group (p<0.05), whereas the relative weight of the bursa of Fabricius tended to be higher in the supplemented groups (p=0.057). No significant differences were found for other carcass parameters (p>0.05).

Serum biochemical analysis showed significant differences among groups in albumin, globulin, and HDL-cholesterol levels (p<0.05). Compared with the C group, albumin concentrations were lower, whereas globulin and HDL-cholesterol levels were higher in the PE and PEG groups (Table 5). No significant differences were observed among the groups for the remaining serum biochemical parameters (p>0.05). Likewise, an examination of Table 6 reveals that no statistically significant differences were identified among the groups with respect to TAS, TOS, or OSI values (p>0.05).

Table 3. Mean and standard error mean of the body weight gain, feed intake, feed conversion ratio, and productivity indices of broilers.

		Trial Groups (mean±SEM)			
	Age (Weeks)	**C**	**PE**	**PEG**	***p* value**
BWG (g)	1-3.	498.72^{b}±10.98	490.64^{b}±10.73	586.57^{a}±13.92	<0.001
	4-6.	1484.33^{b}±16.30	1485.16^{b}±23.63	1572.13^{a}±21.37	0.007
	1-6.	1986.97^{b}±25.06	1979.40^{b}±32.22	2156.77^{a}±31.13	<0.001
FI (g)	1-3.	704.58^{b}±28.83	710.35^{b}±21.03	814.06^{a}±28.81	0.023
	4-6.	2828.68^{b}±53.17	2839.17^{b}±52.76	3039.95^{a}±62.90	0.039
	1-6.	3533.26^{b}±78.96	3549.52^{b}±72.02	3854.01^{a}±85.84	0.026
FCR (FI/BWG)	1-3.	1.44±0.03	1.49±0.03	1.42±0.03	0.210
	4-6.	1.91±0.02	1.95±0.04	1.95±0.03	0.584
	1-6.	1.79±0.02	1.83±0.03	1.80±0.03	0.529
Mortality (Number of death)	1-6.	%4 (2)	%6 (3)	%6 (3)	-
EBI*	1-6.	252.36±5.61	244.74±7.25	266.06±7.20	0.101
EPEF*	1-6.	257.11±5.69	249.34±7.35	270.68±7.31	0.107

$^{a, b}$ Values in the same row with different letters indicate statistically significant differences (p<0.05).

Table 4. Mean and standard error mean of the carcass parameters .

Parameters	**Trial Groups** (mean±SEM)			
	C	**PE**	**PEG**	***p* value**
Live weight (g)	2043.64^{b}±27.77	2072.79^{b}±27.64	2204.74^{a}±28.71	0.001
Hot carcass weight (g)	1411.50^{b}±21.43	1439.37^{b}±22.85	1526.58^{a}±23.01	0.003
Carcass yield (BW/HCW)	69.03±0.22	69.15±0.25	69.21±0.27	0.870
Heart weight (g)	11.18±0.27	11.45±0.25	11.69±0.21	0.408
Heart relative weight (g/100g BW)	0.55±0.01	0.55±0.01	0.53±0.01	0.427
Liver weight (g)	49.83±1.10	47.60±0.68	50.10±1.54	0.195
Liver relative weight (g/100g BW)	2.44^{a}±0.05	2.30^{b}±0.03	2.27^{b}±0.06	0.027
Spleen weight (g)	4.52±0.24	4.45±0.28	4.86±0.37	0.607
Spleen relative weight (g/100g BW)	0.22±0.01	0.22±0.01	0.22±0.02	0.905
Pancreas weight (g)	4.94±0.19	4.62±0.16	4.88±0.26	0.454
Pancreas relative weight (g/100g BW)	0.24±0.01	0.22±0.01	0.22±0.01	0.212
Bursa of Fabricius weight (g)	4.35^{b}±0.27	5.21^{a}±0.25	4.88^{ab}±0.27	0.054
Bursa of Fabricius relative weight (g/100g BW)	0.21±0.01	0.25±0.01	0.22±0.01	0.057
Gizzard weight (g)	30.92±0.81	31.27±0.43	31.37±0.77	0.884
Gizzard relative weight (g/100g BW)	1.52±0.04	1.51±0.03	1.42±0.03	0.117

$^{a, b}$ Values in the same row with different letters indicate statistically significant differences (p<0.05).

Table 5. Mean and standard error mean of the serum biochemical parameters

Parameters	Trial Groups (mean±SEM)			
	C	PE	PEG	*p* value
Glucose (mg/dL)	205.46±2.52	211.00±2.12	202.29±2.89	0.064
Total protein (g/dL)	2.47±0.04	2.54±0.05	2.54±0.06	0.483
Albumin (g/dL)	1.42[a]±0.02	1.34[b]±0.02	1.33[b]±0.03	0.009
Globulin (g/dL)	1.05[b]±0.03	1.20[a]±0.03	1.21[a]±0.03	0.001
Albumin/Globulin	1.37[a]±0.03	1.14[b]±0.03	1.10[b]±0.02	<0.001
Trigliserit (mg/dL)	46.21±3.04	44.29±2.03	47.61±3.50	0.721
Total cholesterol (mg/dL)	113.19±3.43	123.25±3.98	119.17±4.34	0.159
HDL-C (mg/dL)	74.29[b]±2.42	86.18[a]±2.75	82.68[a]±3.45	0.008
non-HDL-C (mg/dL)	38.90±1.48	37.07±1.90	36.49±1.78	0.598
LDL-C (mg/dL)	29.66±1.36	28.21±1.66	26.97±1.66	0.507
CK	13645.71±1511.30	11577.32±987.35	13070.41±1094.32	0.479
ALP	1193.50±87.77	1254.50±63.77	1464.68±169.59	0.194
ALT	5.07±0.07	<5.00±0.00	<5.00±0.00	0.438
AST	341.75±13.73	350.43±18.85	352.95±14.32	0.879
AST/ALT	67.55±2.76	70.09±3.77	70.59±2.86	0.779
GGT	17.68±0.63	16.50±0.40	17.42±0.68	0.278
Urea	1.20±0.11	1.14±0.05	1.13±0.08	0.797

[a, b:] Values in the same row with different letters indicate statistically significant differences ($p<0.05$).

Table 6. Serum oxidative stress parameters.

Parameters	Trial Groups (mean±SEM)			
	C	PE	PEG	*p* value
TAS (mmol Trolox Eq/L)	1.71±0.04	1.62±0.05	1.74±0.04	0.108
TOS (μmol H_2O_2 Eq/L)	1.82±0.28	1.59±0.21	1.71±0.31	0.811
Oxidative stress index	0.11±0.02	0.10±0.01	0.10±0.02	0.915

DISCUSSION

This study aimed to evaluate the effects of dietary supplementation of PEx and glucomannan on growth performance in broilers. The primary findings demonstrate that the synergistic application of PEx and glucomannan (PEG group) significantly enhances BW and BWG compared to both the control and sole PEx supplementation. Notably, this growth-promoting effect emerged as early as the first week and was consistently maintained throughout the rearing period. Although the PEG group exhibited superior performance in terms of BW and FI, the lack of significant differences in FCR across all groups suggests that the observed weight gain is primarily driven by increased feed consumption rather than improved metabolic efficiency. Furthermore, the similarity in mortality rates among groups indicates that experimental diets did not exert any detrimental effects on bird viability.

These additive groups have been frequently investigated either individually or in binary combinations (such as prebiotic + probiotic or probiotic + enzyme complex) in literature. Several studies have reported that the combined use of these additives generally produces more effective outcomes compared to their individual applications, leading to significant improvements in live performance, carcass traits, blood biochemistry, and certain organ health parameters (e.g., gastrointestinal tract, immune system) of animals (Kırkpınar et al., 2018; Meijerink et al., 2021). However, there are also studies reporting no significant effects, contrary to expectations. For instance, the data presented by Rehman et al. (2020) indicated that the supplementation of a probiotic and prebiotic combination at the 0.1% level did not

result in any differences in BWG, FI, FCR, or in the weights of the heart, liver, and gizzard.

There is limited literature available on the simultaneous use of these three groups of additives. Examination of the limited number of available studies indicates that the use of a triple combination may exhibit synergistic effects on digestive system functions, thereby potentially providing stronger and more comprehensive benefits (Swanson et al., 2020; Zhao et al., 2024). Dong et al. (2024) reported that the combined use of probiotics, prebiotics, and enzymes could reduce mortality rates in animals, improve FI and FCR, and consequently lead to positive developments and significant increases in live weight, carcass yield, and other performance parameters. Zhao et al. (2024) demonstrated that broiler chickens fed with a 0.1% "synbiotic & enzyme complex" exhibited a marked improvement in average BWG and FCR compared to those in the "control," "antibiotic-only," and "prebiotic + probiotic" groups. Similarly, Ölmez et al. (2025) reported that diets supplemented with the triple combination at levels of 0.1-0.2% resulted in increased BWG and FI, while no changes were observed in FCR. Nevertheless, some studies report a lack of significant effects from such supplementation. Saiyed et al. (2015) found that groups fed diets containing 0.05% enzyme supplementation, along with synbiotics, did not show significant changes in live performance parameters. Similarly, Żbikowski et al. (2020) reported that the final BW, FI, and FCR values were lower in the experimental groups receiving "digestive enzyme+0.05% symbiotic" supplementation compared to the control group fed only with a digestive enzyme complex; they also reported that there was no statistically significant difference between the groups in terms of EPEF values.

In addition, although limited, studies specifically focusing on glucomannan can also be found in the literature. Kamalzadeh et al. (2009) reported that the use of a commercial combined product containing glucomannan (Mycosorb®) at levels of 0.05-0.15% significantly improved BW, FCR, and the productivity index in broiler chickens, while reducing mortality rates. However, Oliveira et al. (2015) observed no significant changes in BWG, FI, or FCR with the use of 0.1% esterified glucomannan. Additionally, Perdinan et al. (2019) reported that dietary supplementation with glucomannan alone at levels of 0.05-0.2% improved intestinal microflora balance and immune resistance but had no significant effects on the relative weights of the bursa of Fabricius and spleen, as well as on BWG, FI, and FCR values. Similarly, Larasati et al. (2021) reported comparable findings regarding the use of glucomannan at concentrations ranging from 0.05% to 0.15%.

When the live performance data is evaluated, it is clearly demonstrated that the significant increases observed in BW, BWG, and FI values of the PEG group compared to the C and PE groups indicate a noteworthy and positive effect of the triple additive combination used. When the groups' FCR values are examined, it is understood that, due to the proportional increases in FI and BWG across the groups, the FCR ratios between the groups are also similar. However, it is important to note that no differences were observed between the PEx-supplemented group and the control group. This observation supports the notion, as also indicated by some researchers, that certain additive combinations may not exert a pronounced effect (Sarangi et al., 2016; Al-Khalaifa et al., 2019; Luo et al., 2022).

In this study, EBI scores ranged from 245 to 266, while EPEF scores ranged from 249 to 271. When evaluated on a group basis, the indices were ranked as follows: PE<C<PEG. However, no significant differences were found between the groups. The minimum EPEF threshold for profitability in broiler farming has been established at 260, with the initial goal of exceeding this level (Mavromati et al., 2018). Nevertheless, producers in Europe generally aim to exceed 300 points to achieve high profitability (Curea et al., 2023). Variations in these indices may result from numerous factors related to farm management, animal-specific characteristics, and environmental conditions (Van Limbergen et al., 2020). According to the findings of the current study, while the PEG group was the only group to achieve the minimum profitability target, it is understood that the reason for the low indices observed in the PE group is that the dietary supplement given does not contribute sufficiently to the live performance efficiency of the birds.

It is established that glucomannan, when used alone, does not significantly affect broiler performance parameters, as reported by Oliveira et al. (2015), Perdinan et al. (2019), and Larasati et al. (2021). Therefore, based on the findings obtained in the present study, the performance improvement observed in the PEG group is likely attributable to a synergistic interaction between the PEx product

and glucomannan. Nevertheless, further studies are required to clarify this relationship more precisely.

When all these findings are collectively considered, it is reasonable to conclude that, despite the presence of inconsistent results in the literature, the positive outcomes observed in the present study may be explained by the combined effects of dosage, strain specificity, and synergistic interactions among the dietary additives used. The applied dose may have been sufficient to elicit a biological response without inducing adverse effects, while the selected probiotic strains supported by enzyme supplementation could have enhanced gastrointestinal functionality. Additionally, the inclusion of glucomannan may have further strengthened these effects by promoting beneficial microbial activity. Nevertheless, further studies are required to clarify the relative contribution of each of these factors.

Carcass characteristics are widely accepted as important indicators of growth performance and nutrient utilization in broiler production. In the present study, dietary supplementation resulted in significant differences in slaughter and carcass weights among the experimental groups ($p<0.05$). While the carcass weights of the C and PE groups were comparable, the higher carcass weight observed in the group given glucomannan combined with PEx suggests a potential advantage of the combined dietary strategy. Additionally, two parameters that demonstrated significant results were the relative weights of the liver and the bursa of Fabricius, both of which exhibited differences among the groups. This difference was particularly distinct in the supplemented groups compared to the control group. Relative liver weights were significantly lower in the PE and PEG groups ($p<0.05$), while bursa of Fabricius weights tended to be higher, with a value approaching significance ($p=0.054$). For the other carcass parameters examined, no significant differences were detected among the groups ($p>0.05$).

Upon reviewing the literature, Saiyed et al. (2015) reported that the supplementation of synbiotics in enzyme-enhanced diets led to an improvement in carcass yield and a reduction in abdominal fat content, while no significant changes were noted in other carcass parameters. Dong et al. (2024) stated that the combined use of probiotics, prebiotics, and enzymes could reduce mortality rates in animals and lead to improvements and significant increases in carcass performance parameters. Similarly, Zhao et al. (2024) stated that the group fed with the triple combination supplement exhibited a higher villus height/crypt depth ratio compared to the other groups, which enhanced nutrient absorption efficiency and consequently contributed both to growth performance and to the development of immune organs (spleen, thymus, and bursa of Fabricius). Furthermore, Ölmez et al. (2025) indicated that groups fed diets containing 0.1-0.2% of triple combination exhibited higher slaughter weights compared to the control group, while a reduction in heart weight was observed; however, carcass yield as well as liver and gizzard weights were found to be similar among the groups. Regarding studies specifically focusing on glucomannan, Perdinan et al. (2019) reported that supplementation with glucomannan at levels between 0.05% and 0.2% improved intestinal microflora balance and overall body resistance, although it had no significant effect on the relative weights of the bursa of Fabricius and spleen. Similarly, Meijerink et al. (2021) indicated that glucomannan could modulate the intestinal microflora (microbiota) in broilers and enhance intraepithelial immune function. As can be inferred from these reports, most studies in this field have primarily concentrated on the digestive system and microbiota, as well as their relationship with the immune system. In contrast, the presented study did not conduct such an investigation.

Based on the carcass weight data obtained in the study, it was determined that supplementation with PEx alone (in the PE group) did not have a distinct effect and produced results comparable to those of the control group. However, the combination of PEx with glucomannan, i.e., supporting the probiotic and enzyme complex with a prebiotic, resulted in a marked difference. This finding may indicate that the inclusion of a prebiotic component alongside probiotic and enzyme supplementation contributes to improved feed intake and enhanced growth performance.

In this context, while the absolute liver weights of the groups remained within the normal range, the observed differences in relative liver weights in the supplemented groups appear not to be attributable to hepatic atrophy. It is understood that the difference in relative liver weights observed in the PE and PEG groups is due to the fact that the slaughter weights of the animals selected for slaughter were higher than those in the control group and the liver sizes remained normal in all groups, rather than the formation of liver atrophy. Indeed, when the serum biochemical parameters

related to liver function were examined, no evidence was found to suggest the presence of acute or chronic inflammation or exposure to oxidative stress in this organ (Tables 5-6).

The numerical increases observed in the bursa of Fabricius parameters may be attributed to the beneficial effects of probiotic-prebiotic supplementation. This may have promoted functional enhancement in this lymphoepithelial organ and, in parallel, contributed to improvements in the immunological parameters in which it plays a role. Indeed, evaluation of blood parameters revealed that globulin levels were significantly higher in the PE and PEG groups compared to the control group. While no significant differences were detected among the groups with respect to total serum protein concentrations, the increase in the serum globulin fraction, particularly in the PE and PEG groups, suggests a potential immunostimulatory effect of these feed additives. However, to confirm this interpretation, it would be necessary to measure the levels of γ-globulins (immunoglobulins), which constitute a subfraction of total globulins. Moreover, it is thought that to understand whether these results are truly positive developments in terms of organ or body health, they will only gain meaning by establishing similar study designs and making more specific histopathological and biochemical evaluations on organs-systems. With respect to the remaining organs other than the liver and the bursa of Fabricius, no significant differences were observed among the groups, indicating that the effects of dietary supplementation were selective rather than systemic and were limited to specific physiological responses.

In this study, the evaluation of biochemical parameters revealed statistically significant differences between the groups in terms of serum albumin, globulin, and HDL-cholesterol levels ($p<0.05$). Compared to the control group, albumin concentrations were lower, whereas globulin and HDL-C levels were higher in the supplemented PE and PEG groups. For the other serum biochemical parameters examined, no significant differences were observed among the groups ($p>0.05$).

When the literature was reviewed, Żbikowski et al. (2020) reported that in their studies by adding 0.05% different synbiotic formulations to diets containing enzyme complex, almost all of the biochemical parameters measured at the end of the trial, including glucose, total protein, albumin, globulin, cholesterol, triglycerides, uric acid, ALT, ALP, Ca and P (except AST), gave similar results between the groups. Similarly, Ölmez et al. (2025) reported that in their study by adding a product containing 0.1-0.2% probiotic & enzyme complex and another product with prebiotic effect to broiler diets (as a synbiotic), no statistically significant difference was found between the groups in serum total protein, albumin, glucose, uric acid, AST, ALP, GGT, Ca, P values. Furthermore, studies specifically focusing on glucomannan have demonstrated that this additive may regulate blood pressure as well as blood glucose, cholesterol, and triglyceride levels, and may also possess laxative, anti-obesity, anti-inflammatory, and antitumoral activities (Behera and Ray, 2016; Kapoor et al., 2024).

According to the findings of the presented study, the absence of significant changes in serum glucose -a key indicator of carbohydrate metabolism in the body- as well as in AST, ALT, GGT, CK, ALP, and urea levels, which serve as markers of functional impairment or inflammatory responses in the liver, heart, muscle, kidney, and other tissues, is considered an important and favorable outcome, as it demonstrates that the additives used did not exert toxic or harmful effects on body tissues or the organism as a whole. Examination of the serum lipid profile revealed that HDL cholesterol levels were significantly higher in the groups fed supplemented diets compared to the control group ($p<0.05$). This finding suggests that the additive combination positively influences lipid metabolism without disrupting the serum lipid profile; that is, it does not induce any changes or adverse effects in serum triglycerides, total cholesterol, non-HDL cholesterol, or LDL cholesterol levels. Therefore, it may contribute positively to overall health.

Another important finding of the present study was that serum globulin levels were higher in the supplemented groups, whereas albumin concentrations were reduced compared to the control group. The most critical fraction of the serum globulin profile generally consists of γ-globulins, also known as immunoglobulins. The observed increase in globulin and the decrease in the albumin/globulin ratio in the PE and PEG groups may be attributed to the applied additive combinations stimulating the organism's defense mechanisms and eliciting a robust immune response. Furthermore, despite the significant differences observed among the groups in albumin and globulin levels, the absence of any change in total protein levels may appear contradictory. It

should first be known that the determination of the serum protein profile is based on the equation "albumin + globulin = total protein" (Eckersall, 2008). Considering this equation, the observation that total protein concentrations remained unchanged across the groups, while albumin and globulin levels varied significantly, may be explained by a compensatory mechanism in which the organism suppresses albumin synthesis in inverse proportion to the increase in globulin, thereby maintaining serum protein homeostasis. In conclusion, to clarify the exact cause of the changes observed in the globulin fraction, further studies are required, focusing on more specific parameters.

When the serum TAS, TOS, and OSI values of the groups were compared, no statistically significant differences were detected among them ($p>0.05$). These findings indicate that the feed additives used did not alter the balance between antioxidant defense mechanisms and oxidative load. Although no pronounced effect of the additives on oxidative balance was observed, the fact that they did not disrupt these parameters and helped maintain the existing equilibrium can still be considered a favorable outcome.

CONCLUSION

In conclusion, supplementing broiler diets with 0.1% PEx and glucomannan appears to have significant positive effects, especially on live performance parameters. Furthermore, tendencies toward improvement were observed in certain carcass traits and serum biochemical parameters. Among these, the increase in serum HDL levels stands out as an important finding of the study. However, the observed elevations in serum globulin concentrations are not considered to be associated with inflammatory processes. Nevertheless, further comprehensive studies are required to determine whether these elevations are indeed related to an enhancement of the immune system. Considering the positive effects on growth performance observed in the present study, the dietary combination of PEx and glucomannan used herein may be recommended, as the associated improvements in production efficiency are likely to have the potential to compensate for the additional feed cost.

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