



## RESEARCH ARTICLE

### Effects of Postbiotic and Egg Yolk Powder Supplementation on the Growth Performance, Serum Biochemical Profile, Antioxidant Capacity and Gut Histomorphology of Broilers

Waqas M<sup>1,2,\*</sup>, Salman M<sup>1</sup> and Nastoh Ahmad A<sup>1</sup>

<sup>1</sup>Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine, Ondokuz Mayıs University, Samsun, Türkiye; <sup>2</sup>Department of Poultry Production, University of Veterinary and Animal Sciences, Lahore, Pakistan; \*Corresponding author: [m.waqas@uvas.edu.pk](mailto:m.waqas@uvas.edu.pk)

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#### ABSTRACT

This study explored the advantageous effects of *Bifidobacterium bifidum*-based postbiotic and egg yolk powder on performance parameters, serum biochemical profile, antioxidant/oxidant capacity, and gut histomorphology of broiler chickens. A total of 320 day-old Ross-308 unsexed broiler chicks were randomly allotted to four experimental groups, and each group was replicated four times with 20 birds per replicate. The control group was fed a basal diet. The postbiotic (PB), egg yolk powder (EYP), and PB + EYP groups received the following supplements in addition to their basal diet: 0.3% PB, 3.5% EYP, and 0.3% PB + 3.5% EYP, respectively. Feed intake, except in weeks three and four, was non-significant among the experimental groups. Significantly higher body weight of the birds throughout the experiment, and higher body weight gain for the first three weeks, were recorded in the treatment groups compared to the control group. Treatment groups had a significant effect on European production efficiency factor (EPEF) performance index (PI), and feed conversion ratio (FCR). Treatment groups had a lowering ( $P < 0.05$ ) effect on triglycerides. The total antioxidant capacity was significantly higher in the treatment groups. With respect to gut histomorphology, significantly better villus height and crypt depth of small intestine segment, with a notably higher VH: CD ratio in the ileum, were observed in the PB+EYP group. The results of the present study demonstrate that supplementing broiler chickens with postbiotic and egg yolk powder alone or in combination can improve the performance, triglycerides, total antioxidant capacity, and intestinal histomorphology.

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#### INTRODUCTION

Poultry meat provides high-quality proteins, essential minerals, vitamins, and healthy fats, thereby making it an important part of our daily diet (Shaukat *et al.*, 2025). Nonetheless, to fulfill the meat requirements of a huge human population, poultry is being produced in confinement to produce more meat per square meter area, due to which poultry industry is facing several significant challenges, like overstocking density, heat stress, disease load, and gut health issues, with disruption to gut health being a major economic concern (Khalique *et al.*, 2020). To address these gut issues in poultry, farmers have been using antibiotics, as well as using these antibiotics as growth promoters, commonly known as antibiotic growth promoters (AGP) (Mehdi *et al.*, 2018). Essential oils, enzymes, probiotics, prebiotics, synbiotics, antimicrobial

peptides, postbiotics, bacteriophage, organic acids, metals, clay, and egg yolk immunoglobulin Y (IgY) are being investigated as growth promoters to improve livestock production efficiency (Loh *et al.*, 2014). Probiotics have profound positive effects on the host animals (Chandrasekaran *et al.*, 2024); however, despite these advantages, probiotics face limitations, including specific storage requirements, host-specific strain selection, and potential risk of horizontal transfer of virulence genes from pathogenic microorganisms (Nayak, 2010; Merenstein *et al.*, 2023). These concerns related to probiotics have sparked growing interest in postbiotics, the metabolic products of probiotics, which are now considered as favorable alternatives to live probiotics. This transition is mainly attributed to their better safety profile, higher stability, ease of storage, and lower likelihood of contributing to antimicrobial resistance (Ma

*et al.*, 2023). According to Salminen *et al.* (2021), postbiotics are described as non-living microbial components or bioactive substances produced by beneficial microbes, which may include peptides, enzymes, muropeptides derived from peptidoglycan, surface proteins, polysaccharides, organic acids, and teichoic acids. Beneficial effects of postbiotics, such as immune modulation, growth enhancement, reduction of hypersensitivity, and antimicrobial, antiviral, antioxidant, anti-obesity, anti-diabetic, antihypertensive, antiproliferative, antimutagenic, and anticancer properties, have been studied both in *in-vivo* and *in-vitro* experiments (Martin-Gallausiaux *et al.*, 2021). Previous studies have demonstrated the beneficial effects of postbiotics on growth performance, gut histomorphology, gut health, blood biochemistry, antioxidant status and gene expression of broiler chickens (Izuddin *et al.*, 2020; Humam *et al.*, 2020; Monika *et al.*, 2024).

On the other hand, nutraceuticals, such as encapsulated immunoglobulins, phosvitin, carotenoids, vitamins, conjugated linoleic acids (CLAs), choline, chitin, chitosan and glucosamine are also being studied in poultry to investigate their advantages in poultry (Playford and Weiser, 2021; Zhang *et al.*, 2021; Yang *et al.*, 2025; Wang *et al.*, 2025). However, there is still a scarcity of research on the use of EYP in broiler production. Egg yolk is a nutritious and important part of the egg that includes useful proteins, pigments, vitamins, omega-3 fatty acids, and minerals (Nys and Guyot, 2011; Mahdavi *et al.*, 2021). The protein of the egg yolk is predominantly made up of livetins and lipovitellins, with g-livetin being commonly referred to as immunoglobulin Y or IgY, and phosvitin proteins being of particular interest because of their functional and bioactive nature (Kovacs-Nolan and Mine, 2004; Chalamaiah *et al.*, 2018). These nutrients act as antioxidants and immunoregulators, thus may help to improve the overall health of chicks and aid in their proper growth and development (Sunwoo and Gujral, 2015). Immunoglobulin Y (IgY), found in chicken serum, serves as the main antibody involved in treating animal and human diseases (Hatta *et al.*, 1993; Cook and Trott, 2010), and also plays an important role in recognizing and controlling pathogenic microorganisms in food products and in immunodiagnoses (Li *et al.*, 2016). Egg yolk-phosvitin is known for its metal-binding, antioxidant, antimicrobial, emulsifying, and cytotoxic properties (Lee *et al.*, 2017a). Moreover, the omega-3 fatty acids present in egg yolk are vital for supporting both brain and ocular health and functions (Maki *et al.*, 2003). The studies by Gadde *et al.*, (2015), Esmailzadeh *et al.* 2016), Abbas *et al.* (2019), Hussein *et al.* (2020), and Rehan *et al.* (2022) reported promising effects of supplementation with IgY and egg-by-products in the poultry diets on their growth performance and meat quality. According to our search and data review, previous research has explored the beneficial effects of postbiotics, egg by-products, and immunoglobulin Y individually, but not in combination, in poultry. Moreover, *Bifidobacterium bifidum*-based postbiotic and egg yolk powder have also not been studied either alone or in combination in broiler chickens. To fulfill this literature gap, the present study aimed to evaluate the independent and synergistic effects of *Bifidobacterium bifidum*-derived postbiotic and egg yolk powder on growth performance,

serum biochemistry, oxidative status, and intestinal histomorphology in broiler chickens. The study hypothesized that broiler chickens fed on diets containing *B.bifidum*-based postbiotic and egg yolk powder would exhibit improvements in growth performance, serum biochemical profile, antioxidant status, and gut histomorphology.

## MATERIALS AND METHODS

**Experimental ethics, design, and diets:** Local Ethics Committee approval was obtained from Ondokuz Mayıs University, Türkiye (decision number E-68489742-604.01-2400073282 dated 16/04/2024). To conduct this trial, day-old broiler chicks (Ross-308) were procured from the Ay-Pi Tavukculuk (Samsun, Türkiye). Chicks were weighed individually, and subgroups were formed based on their weight. In this study, 320 day-old chicks were randomly divided into four experimental groups, each containing 80 chicks, and each group was replicated four times, with 20 birds per replicate. Before the placement of chicks, the house temperature was set 35°C±1 and was reduced to 24°C with a decrease of 2-3°C per week with 60-70% humidity. The trial was executed for forty days. Groups were formed as follows: (i) Control group; fed solely the basal diet, (ii) Postbiotic group (PB); 0.3% postbiotic with basal diet, (iii) Egg Yolk Powder group (EYP); 3.5% egg yolk powder with basal diet and (iv) Postbiotic and Egg Yolk Powder group (PB+EYP); 0.3% postbiotic and 3.5% EYP with basal diet. Postbiotic supplemented in this trial, namely ATA-BSPI7O9P (ATABIO-Postbiotic) derived from *Bifidobacterium bifidum* bacteria with Lot:ATB24014), was funded by ATA-BIO Technology, Istanbul, Türkiye. The metabolic products of *Bifidobacterium bifidum*-based postbiotic were analyzed by GC-MS, and the following components, such as, lactic acid, glycolic acid, stearic acid, heptadecanoic acid, 3-hydroxybutyric acid, succinic acid, 3-hydroxypropionic acid, and exopolysaccharides, were identified while egg yolk powder (Lot:PNO:3201-180) was funded by Kor Agro Organic Food Inc.(Izmir). Proximate and microbial analysis of postbiotic and EYP are presented in Table 1. A live virus vaccine was applied by spray method on days 13 and 32 to protect birds from Newcastle (NDV-CL/79) and infectious bronchitis (MassH120) diseases. The nutrient requirements of birds are presented in Table 2. The diets were prepared following the guidelines of Ross 308 (Aviagen, 2007).

**Table 1:** Chemical and microbial analysis of postbiotic and egg yolk powder

| Contents                               | <i>Bifidobacterium bifidum</i> based Postbiotic | Egg yolk powder |
|--|---|-----------------|
| Moisture %                             | 3.6   | 1.87            |
| Crude protein %                        | 27.3  | 35.25           |
| Saturated fatty acids %                |   | 19.50           |
| Monounsaturated fatty acids %          |   | 29.29           |
| Polyunsaturated fatty acids            |   | 10.29           |
| Total fat                              |   | 59.08           |
| Ash %                                  |   | 3.72            |
| <i>Bifidobacterium bifidum</i> , cfu/g | 2.00E+10  |                 |
| <i>E.Coli</i> , cfu/g                  | 0   |                 |
| Salmonella, cfu/g                      | 0   | 0               |
| Total viable bacteria, cfu/g           | <10   |                 |
| Immunoglobulin Y (µg/ml)               |   | 6100.34         |

CFU: colony-forming unit.

**Growth performance:** Live weight and feed consumption were recorded weekly. Feed conversion ratio was measured on a weekly basis using feed consumed per bird per week to weekly body weight gain, while cumulative FCR was calculated by using the cumulative feed consumed per bird to the final body weight of the bird.

Times of gain were calculated by following the formula: current week weight (g)/previous week weight (g)

**Production efficiency indices:** At the end of the trial, production efficiency indices such as the European production efficiency factor (EPEF) and the performance index (PI) were measured according to Waqas *et al.* (2018).

$$\text{EPEF} = \frac{\text{final BW (kg)} \times \text{livability\%}}{\text{FCR} \times \text{market age in days}} \times 100$$

The performance index was calculated by following the formula:

$$\text{Performance index} = \frac{\text{live weight (kg)}}{\text{FCR}} \times 100$$

Livability percentage was measured by following the given formula:

$$\text{Livability (\%)} = (A / B) \times 100$$

A: total live birds at the end of the trial, B: total number of birds at the end of the trial.

**Determination of serum biochemical profile:** At the end of the trial, blood was randomly collected from three birds (n=48) from each subgroup to determine the serum biochemical parameters, such as glucose (Biosystem Spain, lot: 11803), total protein (Biosystem Spain, lot:11800), cholesterol (Biosystem Spain, lot: 11805), and triglycerides (Biosystem Spain, lot: 11828), following the spectrophotometric method using specific kits on an automatic analyzer (Biosystem, Spain).

**Determination of antioxidant and oxidant capacities:** Total antioxidant capacity (TAC) and total oxidant capacity (TOC) were measured using commercial kits with a spectrophotometric method (TAC: RL0017 and TOC:RL0024; RelAssay, Diagnosticskit, Gaziantep, Türkiye). TAC was expressed in mmol Trolox equivalents per litre, whereas TOC was reported in  $\mu\text{mol}$  hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) equivalents per litre (Erel, 2005).

**Histological analysis of the intestine:** Villus height (VH), crypt depth (CD) and VH/CD ratio were determined in the small intestines of birds. Histological analysis of each section of the small intestine was performed according to Choe *et al.* (2013). Intestinal segments were identified using ImageJ software (National Institute of Health, USA).

**Statistical analysis of data:** Data collected were analyzed using the SPSS (V21.0; IBM) software (OMU, Samsun, Türkiye). To determine the differences in the mortality rates among treatment groups, the Chi-square test was applied. Differences between experimental groups were determined using ANOVA, and group differences were evaluated with Duncan's Multiple Range Test. Livability differences among the experimental

groups were analyzed using the Chi-square test. Descriptive data (Mean $\pm$ SEM) are presented in the tables. GraphPad Prism (v.10.4.1) was used to generate the graphs.

**Table 2:** Physical and chemical composition of experimental diets for broiler chicks

| Feed ingredients         | Starter diet (1-21 days) |            |            |            | Grower diet (22-40 days) |            |            |            |
|--------------------------|--------------------------|------------|------------|------------|--------------------------|------------|------------|------------|
|                          | Contr                    | PB         | EYP        | PB+EY      | Contr                    | PB         | EYP        | PB+EY      |
| Physical composition (%) |                          |            |            |            |                          |            |            |            |
| Corn                     | 48.7                     | 48.4       | 51.4       | 51         | 53.45                    | 53.08      | 55.70      | 55.50      |
| Soybean meal             | 30                       | 30         | 29.13      | 29.23      | 24.00                    | 24.50      | 21.00      | 24.00      |
| Full-fat soybean         | 14                       | 14         | 11.1       | 11.1       | 14.80                    | 14.30      | 14.80      | 11.10      |
| Limestone                | 1.11                     | 1.11       | 1.2        | 1.2        | 1.20                     | 1.15       | 1.33       | 1.30       |
| Dicalcium phosphate      | 1.9                      | 1.9        | 1.75       | 1.75       | 1.40                     | 1.40       | 1.20       | 1.20       |
| DL-Methionine            | 0.19                     | 0.19       | 0.14       | 0.14       | 0.08                     | 0.10       | 0.02       | 0.05       |
| Sodium bicarbonate       | 0.2                      | 0.2        | 0.18       | 0.18       | 0.14                     | 0.14       | 0.14       | 0.14       |
| Salt                     | 0.25                     | 0.25       | 0.25       | 0.25       | 0.18                     | 0.18       | 0.16       | 0.16       |
| Phytase                  | 0.15                     | 0.15       | 0.15       | 0.15       | 0.15                     | 0.15       | 0.15       | 0.15       |
| Coccidiostat             | 0.05                     | 0.05       | 0.05       | 0.05       | 0                        | 0          | 0          | 0          |
| Vitamin-Mineral premix*  | 0.25                     | 0.25       | 0.25       | 0.25       | 0.25                     | 0.25       | 0.25       | 0.25       |
| Vegetable oil            | 3                        | 3          | 0.7        | 0.7        | 4.20                     | 4.30       | 1.60       | 2.20       |
| Salkit**                 | 0.15                     | 0.15       | 0.15       | 0.15       | 0.15                     | 0.15       | 0.15       | 0.15       |
| Toxin binder-Notox       | 0.05                     | 0.05       | 0.05       | 0.05       | 0                        | 0          | 0          | 0          |
| Egg yolk powder (EYP)    | 0                        | 0          | 3.5        | 3.5        | 0                        | 0          | 3.50       | 3.50       |
| Postbiotic (PB)          | 0                        | 0.3        | 0          | 0.3        | 0                        | 0.30       | 0          | 0.30       |
| <b>Total</b>             | <b>100</b>               | <b>100</b> | <b>100</b> | <b>100</b> | <b>100</b>               | <b>100</b> | <b>100</b> | <b>100</b> |
| Chemical composition     |                          |            |            |            |                          |            |            |            |
| CP,%                     | 23.07                    | 23.05      | 23.01      | 23.03      | 21.01                    | 21.05      | 21.05      | 21.00      |
| ME,kcal/kg               | 3047.0                   | 3037.0     | 3043.0     | 3032.0     | 3202.3                   | 3203.3     | 3203.3     | 3197.9     |
| Ca,%                     | 1.00                     | 1.00       | 1.00       | 1.00       | 0.90                     | 0.90       | 0.90       | 0.89       |
| Available P,%            | 0.44                     | 0.44       | 0.45       | 0.45       | 0.35                     | 0.35       | 0.35       | 0.35       |
| Met+Cyst,%               | 0.90                     | 0.90       | 0.90       | 0.90       | 0.74                     | 0.73       | 0.73       | 0.76       |
| Lysine,%                 | 1.27                     | 1.27       | 1.28       | 1.28       | 1.14                     | 1.16       | 1.16       | 1.15       |
| Threonine,%              | 0.87                     | 0.87       | 0.87       | 0.87       | 0.79                     | 0.80       | 0.80       | 0.80       |
| Tryptophan,%             | 0.32                     | 0.32       | 0.32       | 0.32       | 0.28                     | 0.28       | 0.28       | 0.28       |
| Linoleic acid,%          | 2.19                     | 2.19       | 1.99       | 1.99       | 2.32                     | 2.35       | 2.35       | 2.04       |

CP: crude protein, ME: metabolizable energy, P: phosphorus, Met: methionine, Cyst: cysteine, Ca: calcium \*Vitamin/mineral composition per kg of ration: Retinyl acetate:4.000.000IU, Cholecalciferol:1.200.000IU, All-rac-alpha-tocopherol acetate:40.000mg, Menadiolone-sodium bisulfite:1.600mg, Thiamine mononitrate:1.200mg, Riboflavin:3.200mg, Pyridoxine hydrochloride:1.600mg, Cobalamin:12mg, Niacin-nicotinic acid:16.000mg, Calcium D-pantothenate:6.000mg,Folic acid:400mg, Biotin:40mg, Choline chloride:160.000mg,Manganese sulfate monohydrate:40,000mg,Iron (II)sulfate monohydrate:24.000mg,Zinc oxide:32.000mg,Copper(II)sulfate pentahydrate:4.000mg,Anhydrous calcium iodate:600mg,Sodium selenite:80mg,Mg<sub>4</sub>Si<sub>6</sub>O<sub>15</sub>(OH)<sub>2</sub> 6H<sub>2</sub>O: 100.000mg. Furthermore, it contains Salicylic acid at 7.500mg, \*\*Salkil (ammonium salts of formic and propionic acids impregnated in silica) in the range of 2.000–4.000mg.

## RESULTS

### Growth performance parameters

**Feed intake:** Table 3 presents the individual and synergistic effects of feed additives on the weekly feed intake of broilers. During the first, second, fifth, and sixth weeks of trial, postbiotic and egg yolk powder either alone or in combination had no effect on feed intake; however, significantly higher feed intake was ascertained in the control during the third and fourth weeks as compared to PB, EYP, and PB+EYP groups. The cumulative feed intake, from week one through week six, was statistically non-significant among the experimental groups.

**Table 3:** Effect of dietary postbiotic, egg yolk powder, and their combination powder on weekly feed intake (g) of broiler chickens (Mean±SEM)

| Week | Experimental Groups       |                           |                          |                       | P-Value |
|------|---------------------------|---------------------------|--------------------------|-----------------------|---------|
|      | Control                   | PB                        | EYP                      | PB+EYP                |         |
| 1    | 200.33±9.01               | 188.07±6.17               | 198.32±5.11              | 196.23±0              | 0.526   |
| 2    | 319.05±6.78               | 296.80±5.63               | 315.05±10.10             | 314.48±0              | 0.190   |
| 3    | 596.14±1.62 <sup>a</sup>  | 583.18±3.97 <sup>b</sup>  | 583.33±2.40 <sup>b</sup> | 585.81±0 <sup>b</sup> | 0.042   |
| 4    | 944.00±14.09 <sup>a</sup> | 858.19±10.13 <sup>b</sup> | 877.01±6.57 <sup>b</sup> | 874.47±0 <sup>b</sup> | 0.002   |
| 5    | 1188.50±32.74             | 1234.17±42.20             | 1237.38±25.30            | 1169.24±0             | 0.537   |
| 6    | 950.97±6.33               | 952.96±30.23              | 951.38±6.68              | 943.90±0              | 0.978   |
| 0-6  | 4198.98±43.99             | 4113.37±28.95             | 4162.47±39.71            | 4084.12±0             | 0.296   |

<sup>a,b</sup> Means in the same row that carry different letters indicate significant differences (P<0.05)

**Body weight:** There was no significant difference among the experimental groups in terms of body weight on day 0 (Table 4). The treatment groups of PB+EYP and EYP expressed significantly higher BW as compared to PB and control groups during week one. From week second through six, treatment groups of PB, EYP, and PB+EYP exhibited higher (P<0.05) body weight than the control group.

**Body weight gain:** Body weight gain (BWG) of the birds is presented in Table 5. During the first week, PB+EYP showed significantly (P<0.05) higher BWG, followed by EYP, PB, and the control groups. During the second and third weeks, treatment groups increased their body weight (P<0.05) as compared to the control group, while during weeks five and six, no effect on the BWG of the control and experimental groups was noticed. However, higher BWG was noted in the PB group during week five and in the PB+EYP group during week six. Livability percentage (%) remained unaffected (P>0.05) in all the experimental groups.

**Table 4:** Effect of dietary postbiotic, egg yolk powder, and their combination on the weekly cumulative body weight (g) of broiler chickens (Mean±SEM)

| Week         | Experimental Groups        |                            |                            |                            | P-Value |
|--------------|----------------------------|----------------------------|----------------------------|----------------------------|---------|
|              | Control                    | PB                         | EYP                        | PB+EYP                     |         |
| 0            | 41.1±0.32                  | 41.25±0.36                 | 41.22±0.32                 | 41.34±0.18                 | 0.954   |
| 1            | 156.91±1.80 <sup>c</sup>   | 180.09±4.33 <sup>b</sup>   | 185.99±4.49 <sup>ab</sup>  | 193.78±3.93 <sup>a</sup>   | <0.001  |
| 2            | 356.70±6.60 <sup>b</sup>   | 429.36±9.60 <sup>a</sup>   | 438.19±11.08 <sup>a</sup>  | 447.57±10.42 <sup>a</sup>  | <0.001  |
| 3            | 736.70±14.29 <sup>b</sup>  | 895.83±10.15 <sup>a</sup>  | 904.98±12.96 <sup>a</sup>  | 922.33±11.33 <sup>a</sup>  | <0.001  |
| 4            | 1261.55±17.86 <sup>b</sup> | 1443.04±11.99 <sup>a</sup> | 1466.82±26.28 <sup>a</sup> | 1491.47±11.23 <sup>a</sup> | <0.001  |
| 5            | 1821.11±19.87 <sup>b</sup> | 2009.06±37.56 <sup>a</sup> | 2020.51±48.45 <sup>a</sup> | 2009.11±52.41 <sup>a</sup> | 0.003   |
| 6            | 2220.80±27.21 <sup>b</sup> | 2393.22±47.85 <sup>a</sup> | 2437.21±58.08 <sup>a</sup> | 2384.95±75.69 <sup>a</sup> | 0.026   |
| Livability % | 96.25                      | 96.25                      | 98.75                      | 97.50                      | 0.77    |

<sup>a,b,c</sup> Means in the same row that carry different letters indicate significant differences (P<0.05)

**Table 5:** Effect of dietary postbiotic, egg yolk powder, and their combination on the weekly body weight gain (g) of broiler chickens (Mean±SEM)

| Week | Experimental Groups       |                          |                           |                           | P-Value |
|------|---------------------------|--------------------------|---------------------------|---------------------------|---------|
|      | Control                   | PB                       | EYP                       | PB+EYP                    |         |
| 1    | 116.47±2.50 <sup>c</sup>  | 138.84±4.32 <sup>b</sup> | 144.77±4.76 <sup>ab</sup> | 152.44±3.87 <sup>a</sup>  | <0.001  |
| 2    | 199.59±7.58 <sup>b</sup>  | 249.28±5.90 <sup>a</sup> | 252.19±6.81 <sup>a</sup>  | 253.79±8.19 <sup>a</sup>  | <0.001  |
| 3    | 383.56±12.65 <sup>b</sup> | 466.46±4.42 <sup>a</sup> | 466.79±5.44 <sup>a</sup>  | 474.76±10.03 <sup>a</sup> | <0.001  |
| 4    | 523.55±7.28               | 547.21±7.44              | 561.84±14.92              | 569.14±16.02              | 0.089   |
| 5    | 560.99±14.33              | 566.02±28.63             | 553.68±23.87              | 517.64±44.56              | 0.667   |
| 6    | 397.05±25.73              | 384.16±11.19             | 416.7±16.49               | 451.09±13.17              | 0.088   |

<sup>a,b,c</sup> Means in the same row that carry different letters indicate significant differences (P<0.05)

**Feed conversion ratio (FCR):** FCR is rendered in Table 6. During the first four weeks, treatment groups PB, EYP, and PB+EYP showed significantly (P<0.05) better FCR

than the control group. During weeks five and six, FCR remained unaffected (P>0.05), while the cumulative FCR was significantly better in the treatment groups than the control group.

**Table 6:** Effect of dietary postbiotic, egg yolk powder, and their combination on the weekly feed conversion ratio of broiler chickens (Mean±SEM)

| Week | Experimental Groups    |                        |                        |                        | P-Value |
|------|------------------------|------------------------|------------------------|------------------------|---------|
|      | Control                | PB                     | EYP                    | PB+EYP                 |         |
| 1.   | 1.72±0.07 <sup>a</sup> | 1.36±0.02 <sup>b</sup> | 1.37±0.04 <sup>b</sup> | 1.29±0.03 <sup>b</sup> | <0.001  |
| 2.   | 1.6±0.027 <sup>a</sup> | 1.19±0.01 <sup>b</sup> | 1.25±0.02 <sup>b</sup> | 1.24±0.03 <sup>b</sup> | <0.001  |
| 3.   | 1.56±0.05 <sup>a</sup> | 1.25±0.01 <sup>b</sup> | 1.25±0.02 <sup>b</sup> | 1.24±0.03 <sup>b</sup> | <0.001  |
| 4.   | 1.8±0.02 <sup>a</sup>  | 1.57±0.01 <sup>b</sup> | 1.56±0.03 <sup>b</sup> | 1.54±0.02 <sup>b</sup> | <0.001  |
| 5.   | 2.12±0.08              | 2.19±0.05              | 2.24±0.07              | 2.26±0.13              | 0.556   |
| 6.   | 2.43±0.16              | 2.49±0.15              | 2.29±0.08              | 2.1±0.05               | 0.135   |
| 0-6  | 1.89±0.02 <sup>a</sup> | 1.72±0.03 <sup>b</sup> | 1.71±0.03 <sup>b</sup> | 1.71±0.02 <sup>b</sup> | <0.001  |

<sup>a,b</sup> Means in the same row that carry different letters indicate significant differences (P<0.05)

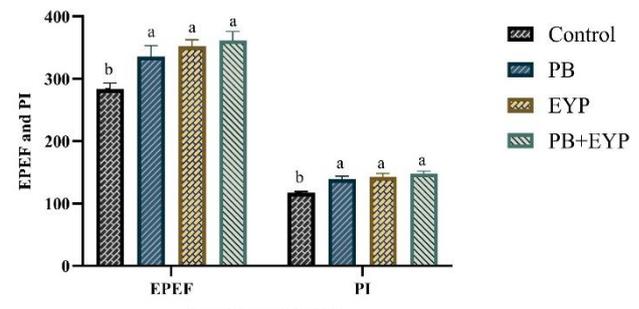
**Times of gain:** Table 7 demonstrates that times of gain (TOG) of broiler chicks were higher (P<0.05) in PB+EYP, followed by PB, EYP, and the control group during week one. In the second and third weeks, TOG remained unaffected (P>0.05). During weeks four and five, TOG was higher (P<0.05) in the control group compared to the treatment groups, while in week six, TOG remained unaffected.

**Table 7:** Effect of dietary postbiotic, egg yolk powder, and their combination on the weekly times of weight gain of broiler chickens (Mean±SEM)

| Week | Experimental Groups    |                         |                        |                        | P-Value |
|------|------------------------|-------------------------|------------------------|------------------------|---------|
|      | Control                | PB                      | EYP                    | PB+EYP                 |         |
| 1    | 3.85±0.07 <sup>b</sup> | 4.37±0.11 <sup>a</sup>  | 4.52±0.14 <sup>a</sup> | 4.69±0.09 <sup>a</sup> | <0.001  |
| 2    | 2.27±0.03              | 2.38±0.02               | 2.36±0.02              | 2.31±0.04              | 0.056   |
| 3    | 2.07±0.02              | 2.09±0.03               | 2.07±0.03              | 2.06±0.04              | 0.934   |
| 4    | 1.71±0.02 <sup>a</sup> | 1.61±0.01 <sup>b</sup>  | 1.62±0.01 <sup>b</sup> | 1.62±0.02 <sup>b</sup> | 0.003   |
| 5    | 1.44±0.02 <sup>a</sup> | 1.39±0.02 <sup>ab</sup> | 1.38±0.01 <sup>b</sup> | 1.35±0.03 <sup>b</sup> | 0.028   |
| 6    | 1.22±0.02              | 1.19±0.00               | 1.21±0.01              | 1.23±0.01              | 0.192   |

<sup>a,b</sup> Means in the same row that carry different letters indicate significant differences (P<0.05)

**Performance indices:** Fig. 1 illustrates that performance indices such as EPEF and PI were significantly exceeded in the treatment groups than those of the control group. The PB+EYP group demonstrated significantly higher values for EPEF and PI as compared to the experimental group.

**Fig. 1:** The performance indices of broiler chickens. EPEF: European Production Efficiency Factor, PI: Performance Index

<sup>a,b</sup> Different letters on the bars indicate significance between means (P<0.05).

**Serum biochemical profile:** Findings about the serum biochemical profile of the present study are presented in Table 8. Serum glucose, cholesterol, and total protein levels remained unaffected in all the experimental groups while

serum triglyceride (TG) levels were found to be significantly lower in the EYP group compared to the other groups.

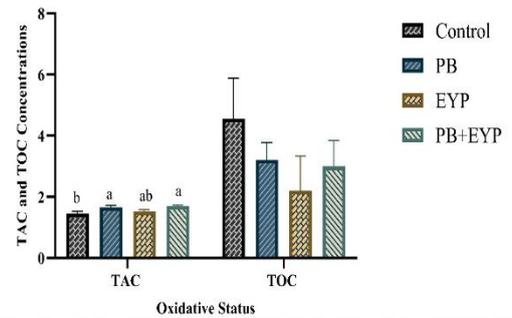
**Table 8:** Effect of dietary postbiotic, egg yolk powder, and their combination on the serum biochemical parameters of broiler chickens (Mean±SEM)

| Parameters            | Experimental Groups     |                         |                         |                          | P-Value |
|-----------------------|-------------------------|-------------------------|-------------------------|--------------------------|---------|
|                       | Control                 | PB                      | EYP                     | PB+EYP                   |         |
| Glucose (mg/dL)       | 198±4.58                | 187.75±2.63             | 194.58±3.74             | 184±6.28                 | 0.122   |
| Cholesterol (mg/dL)   | 135.42±6.00             | 127±4.80                | 126.33±3.65             | 129.64±5.37              | 0.561   |
| Triglycerides (mg/dL) | 28.42±2.48 <sup>a</sup> | 21.83±1.78 <sup>b</sup> | 18.67±1.96 <sup>b</sup> | 22.45±2.40 <sup>ab</sup> | 0.021   |
| Total protein (g/L)   | 39.2±1.29               | 37.33±1.14              | 4.97±1.34               | 36.65±0.93               | 0.104   |

<sup>a,b</sup> Different letters in the same row indicate differences between means (P<0.05)

**Antioxidant and oxidant capacities:** Fig.2. demonstrates that total antioxidant capacity (TAC) values were significantly higher in the treatment groups than in the control group, with significantly higher TAC values being observed in the PB+EYP group. Findings regarding TOC values revealed that neither the treatment groups nor the control group had an effect on the TOC values. However, the control group depicted higher values for TOC.

**Histological analysis of the intestine:** Each segment of the small intestine was improved in the PB+EYP group compared to the other groups (Table 9). With respect to crypt depth (CD), it was found that the CD of the duodenum was significantly (P<0.05) greater in the PB+EYP group compared to other groups. Jejunal crypt depth was significantly shallower (P<0.05) in the PB+EYP group, along with the EYP, PB, and control groups. In the ileum, the experimental groups showed significantly deeper crypts (P<0.05) compared to the control group. It was found that the ratio VH: CD of duodenum and jejunum was significantly (P<0.05) higher in the control group, followed by the PB+EYP and other treatment groups. while VH: CD of the ileum was recorded significantly (P<0.05) higher in PB+EYP, followed by control, PB, and EYP groups. Histomorphological images (H&E) of the segments of gut such as duodenum (A), jejunum (B), and ileum (C) have been illustrated in Fig.3.



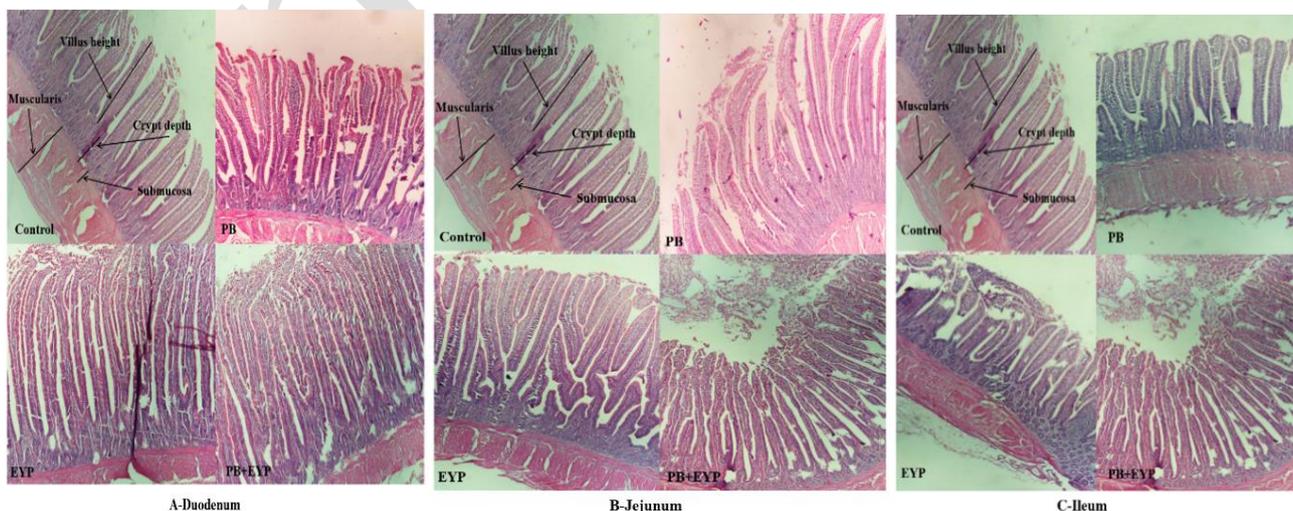
TAC: Total antioxidant Capacity (mmol Trolox Eq/L), TOC: Total Oxidant Capacity (µmol H2O2 Eq/L)

**Fig. 2:** The TAC and TOC parameters of broiler chickens. <sup>a,b</sup>: Different letters on the bars indicate significance between means (P<0.05).

## DISCUSSION

Growth performance is an important economic factor in broiler production since it is directly related to profitability (Kithama *et al.*, 2023). Feed intake, body weight, and FCR are some key indicators of growth performance. Presently, postbiotics have gained attraction for their supplementation in the diet of broilers due to their potential role in the improvement of production efficiency and health of birds.

In the current study, weekly and cumulative feed intake remained unaffected, in both the treatment and the control group, except in weeks three and four, in which significantly higher feed intake was seen in the control group. Compatible with these outcomes, Chang *et al.* (2022) revealed that it did not affect feed consumption in broilers supplemented with either *Lactiplantibacillus plantarum* postbiotics or antibiotics and a control diet throughout the study. On the same note, Humam *et al.* (2019) also indicated that the feed consumption of broiler birds was not affected by the augmentation of postbiotic in their diet. Kierenczyk *et al.* (2017) indicated that nisin, a bacterial metabolite, when added to the diet of broilers, led to improvement of the feed intake (FI), BWG, and FCR. Treatment groups had much higher BW, BWG, and better FCR, and times of gain in this experiment. Compatible with these outcomes, Humam *et al.* (2019), Jansseune *et al.* (2024), and Monika *et al.* (2024) demonstrated that broiler chicks fed on postbiotic supplement had improved final BW and FCR. The addition of inulin with postbiotics



**Fig. 3:** The histomorphology of each segment (A: Duodenum; B: Jejunum; C: Ileum) of the small intestine in broiler chickens (µm).

**Table 9:** Effect of dietary postbiotic, egg yolk powder, and their combination on gut histomorphological parameters of broiler chickens (Mean±SEM)

| Parameters                        | Experimental Groups         |                             |                             |                             | P-Value |
|-----------------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|---------|
|                                   | Control                     | PB                          | EYP                         | PB+EYP                      |         |
| <b>Villus height (µm)</b>         |                             |                             |                             |                             |         |
| Duodenum                          | 1347.39±24.328 <sup>d</sup> | 1490.37±10.521 <sup>b</sup> | 1400.43±18.335 <sup>c</sup> | 1685.85±18.634 <sup>a</sup> | <0.001  |
| Jejunum                           | 1030.3±21.387 <sup>d</sup>  | 1175.82±11.142 <sup>b</sup> | 1100.41±16.899 <sup>c</sup> | 1244.68±14.638 <sup>a</sup> | <0.001  |
| Ileum                             | 731.42±15.782 <sup>c</sup>  | 917.06±12.636 <sup>b</sup>  | 918.17±17.717 <sup>b</sup>  | 996.62±15.891 <sup>a</sup>  | <0.001  |
| <b>Crypt depth (µm)</b>           |                             |                             |                             |                             |         |
| Duodenum                          | 249.63±5.462 <sup>c</sup>   | 290.04±3.198 <sup>b</sup>   | 299.42±4.241 <sup>b</sup>   | 318.07±4.629 <sup>a</sup>   | <0.001  |
| Jejunum                           | 194.39±4.367 <sup>c</sup>   | 239.31±2.283 <sup>b</sup>   | 248.65±3.915 <sup>ab</sup>  | 249.82±3.03 <sup>a</sup>    | <0.001  |
| Ileum                             | 152.41±4 <sup>b</sup>       | 197.58±2.388 <sup>a</sup>   | 204.48±3.909 <sup>a</sup>   | 203.69±2.705 <sup>a</sup>   | <0.001  |
| <b>Villus height: crypt depth</b> |                             |                             |                             |                             |         |
| Duodenum                          | 5.58±0.111 <sup>a</sup>     | 5.19±0.053 <sup>b</sup>     | 4.78±0.084 <sup>c</sup>     | 5.43±0.098 <sup>ab</sup>    | <0.001  |
| Jejunum                           | 5.45±0.111 <sup>a</sup>     | 4.95±0.059 <sup>b</sup>     | 4.54±0.087 <sup>c</sup>     | 5.05±0.076 <sup>b</sup>     | <0.001  |
| Ileum                             | 4.94±0.095 <sup>ab</sup>    | 4.69±0.072 <sup>bc</sup>    | 4.64±0.1 <sup>c</sup>       | 4.97±0.094 <sup>a</sup>     | 0.016   |

<sup>a-c</sup> Different letters in the same row indicate differences between means (P<0.05).

ameliorated the growth status of broilers (Kareem *et al.*, 2016). The positive changes in the host animals can be attributed to the positive effects of the secondary metabolites or postbiotics of the *B. bifidum* (Vlasova *et al.*, 2016), such as the teichoic acid, bacteriocins, organic acids, peptidoglycan (PGN), exopolysaccharides, neurotransmitters, fimbriae and antioxidant compounds (Zolkiewicz *et al.*, 2020). These metabolic products serve to positively alter the intestinal microarchitecture, improve mucosal barrier activity, increase nutrient uptake, maintain microbial-host balance with more beneficial bacteria in gut, and, consequently, enhance animal performance (Zhao *et al.*, 2024).

In the current study EPEF and PI were noticed to be superior in the treatment groups. The findings of Bastamy *et al.* (2024) corroborated the outcomes of this study, and manifested that the European production efficiency factor of broilers fed with lysozyme extracted from *Acremonium alcalophilum* was significantly higher than that of the lysozyme-free treatment.

In the present study, significant effects of egg yolk powder on the body weight, FCR, and performance indices of EPEF and performance index were observed. These improvements can be linked to the bioactive components of eggs, which possess antioxidant, antibacterial, and antihypertensive properties (Lee *et al.*, 2017b; Zhang *et al.*, 2021), which may contribute to better gut health and consequently an enhanced growth performance. Our findings are in agreement with the findings of Chalghoumi *et al.* (2009) and Mahdavi *et al.* (2010), who demonstrated that dehydrated egg yolk contributed to the increase in growth rate. Esmailzadeh *et al.* (2016) also noticed enhancement (P≤0.05) in BW, FI, FCR, and EPEF when 40 g/kg egg powder was added to the starter diet. In line with these observations, Lei and Kim (2013) reported that supplementing broilers' diet with 3% whole egg powder enhanced growth performance up to day 35. Likewise, Rehan *et al.* (2020) also revealed that egg yolk-immunoglobulin Y addition in the diet of broilers resulted in an improvement in FCR.

Serum biochemical parameters were evaluated in this study because they are important physiological, nutritional, and pathological indicators of animals. Changes happening in these parameters can be attributed to the action of diet ingredients and additives supplementation in animals (Kang *et al.*, 2016). Treatment groups had no effect on cholesterol, glucose, and total protein values except TG. Humam *et al.* (2020) found that supplementation of R111 postbiotics in the diet of heat-stressed broilers gave rise to

a reduction in serum cholesterol level. Likewise, Reuben *et al.* (2021) reported that probiotic supplementation in broilers resulted in elevated serum protein levels and a reduction of total cholesterol, TG, LDL-cholesterol, and glucose.

These lower TG levels in the postbiotic group can be attributed to the probiotic properties of postbiotics, as postbiotics are the mirror image of probiotics in their function. Tomaro-Duchesneau *et al.* (2014), Sultan *et al.* (2024) and Marras *et al.* (2021) reported that multi-strain probiotics effectively lowered cholesterol and TG concentrations. This hypocholesterolemic effect of postbiotic may be credited to the inhibition of 3-hydroxy-3-methylglutaryl-CoA reductase by ferulic acid released by probiotics, as well as the enhanced hydrolysis of bile salts by the bile salt hydrolase activity of probiotics. The increased bile salt hydrolysis may reduce the absorption of lipids in the small intestine, hence contributing to decreased TG levels in birds supplemented with postbiotic (Alkhalif *et al.*, 2010; Jones *et al.*, 2013).

Egg yolk powder supplementation also exhibited tremendous positive effects. The current study showed that EYP reduced triglyceride concentration compared to the control. These findings are endorsed by Jeon *et al.* (2016), who demonstrated that dietary supplementation of IgY in the diet of ducks led to a decrease in the concentrations of LDL and total cholesterol in the meat, indicating the positive role of IgY in lipid metabolism. The cholesterol-lowering effects associated with the gut probiotics, as Zhang *et al.* (2021) stated that including phosvitin and immunoglobulin Y in broilers diet, supported the growth of health-promoting bacteria in the gut of broilers, hence these probiotic can bind cholesterol to their cell surfaces and incorporate it into their membranes as they grow, which reduces the amount of free cholesterol and triglycerides available for absorption in the intestine (Reis *et al.*, 2017).

In the present study, significantly higher TAC levels were observed in the treatment groups, with the highest TAC values observed in the PB+EYP group. These results are similar to those observed by Humam *et al.* (2020) for broilers under heat stress, which showed higher TAC levels when their diets were supplemented with postbiotics. Similarly, Atan Cirpici and Kirenkaya (2025) reported that broilers fed with encapsulated postbiotics disclosed an increase in the serum TAC levels and a reduction in TOC levels. The improvement in antioxidant status may be associated with the antimicrobial and antioxidant attributes of postbiotics, which provide a safer way to reduce oxidative stress; hence in this way they support animal health and overall well-being

(Li *et al.*, 2019; Gao *et al.*, 2019). Assessment of intestinal morphology is a valuable approach for evaluating gut health and nutrient absorption efficiency. Key indicators of improved intestinal histomorphology include greater villus height, reduced crypt depth, and an increased villus height to crypt depth ratio, all of which reflect enhanced absorptive capacity and overall intestinal function (Jha *et al.*, 2020). Villi are the main absorption sites found in the small intestine and thus, longer villi and a shallower crypt depth are linked to high nutrient absorption, low intestinal secretions, and better growth performance of poultry birds (Caspary *et al.*, 1992). In addition, Fan *et al.* (1997) postulated that the larger the ratio of VH to CD, the greater is the epithelial cell turnover, that is, the more active and functional the intestinal lining will be.

In the present study, a significant improvement was observed in villus height, crypt depth, and VH:CD in the PB+EYP group. In agreement with these findings, Danladi *et al.* (2022) reported that paraprobiotics and postbiotics enhanced intestinal histomorphology in broilers. Compatible with these outcomes of the current study, Humam *et al.* (2019), Incharoen *et al.* (2019), and Tukaram *et al.* (2022) admitted that the dietary postbiotic supplementation exerted immense effects on gut histomorphology in the form of enhanced villus height and VH: CD ratios in the intestinal segments. These results indicate that improvement in gut histomorphology is linked to probiotic properties of postbiotics, which help to improve gut histomorphology by increasing lactic acid bacteria (LAB) (Kareem *et al.*, 2016). The improvement of villus microarchitecture, particularly the enhancement of VH, is indicative of enhanced nutrient absorption and, ultimately, improved growth performance (Wang *et al.*, 2025). In the present study, EYP positively affected gut histomorphology, and the study of Han *et al.* (2021) endorsed these findings, who reported that piglets supplemented with IgY and phytomolecules exhibited significantly enhanced height of the villus in duodenum and ileum, along with a higher ratio of VH:CD of duodenum and jejunum. In this study, EYP alone or in combination with postbiotic (PB+EYP) treatment had a positive effect on the histomorphological parameters of the small intestine. IgY controls harmful bacteria and enterotoxins through opsonization (Borchers *et al.*, 2005), leading to intestinal healing.

**Conclusions:** The present study exhibits that augmenting the broilers' diet with the postbiotic and egg yolk powder, either alone or in synergy, has a positive influence on the overall performance and physiological parameters of broilers. These supplements significantly improved the performance parameters and performance indices of broilers. Birds supplemented with postbiotic and egg yolk powder exhibited reduced serum triglyceride levels, higher total antioxidant capacity, and improved intestinal histomorphology, particularly villus height and crypt depth. The combination of postbiotic (0.3%) and EYP (3.5%) showed strong potential as a natural alternative to AGP. Therefore, further research is needed to elucidate the biological mechanisms in this regard.

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**Contributions of authors:** Conceptualization; MW, MS, Methodology; MW, MS, Data curation; MW, MS, NAN, Formal analysis; MW, MS; Visualization; MW, MS, NAN, Supervision; MS, Drafting and original writing; MW, MS, and Review and editing; MW, MS, NAN.

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