

The Effect of Selenized Glucose with Probiotic on Broiler Growth Performance, Immune Response, Intestine Microflora and Morphology

Research Article

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ABSTRACT

A total of 300 one-day-old broilers were randomly assigned to six dietary treatments in a 3 by 2 factorial treatment arrangement to evaluate the effects of sodium selenite (SS), selenium yeast (SeY), and selenized glucose (SeGlu) supplements, as well as their combination with probiotic, on broiler growth performance, histomorphology, microbial population of the intestine, and immune response. Among the factors investigated were selenium sources (0.3 mg/kg) and probiotic levels (0 and 100 mg/kg of diet). The treatments were tested on five floor pens with ten birds each. Over the entire period, broilers fed SeGlu had a higher body weight gain and a lower feed conversion ratio than broilers fed SeY or SS ($P < 0.05$). Interaction results showed that broilers fed SeGlu plus probiotic had higher lactic acid bacteria counts and lactic acid bacteria/coliform ratios in the ileum than those fed SeY without probiotic ($P < 0.05$). Furthermore, when compared to SS alone, broilers fed SeGlu plus probiotic had greater villus height, villi height to crypt depth ratio, villus surface area, and goblet cell density ($P < 0.05$). Broilers fed diets containing supplemental SeGlu had higher total anti-sheep red blood cells (SRBC) titre, IgG, and IgM titers than SeY and SS ($P < 0.05$). Furthermore, broilers fed SeGlu plus probiotic dietary supplementation had higher IgG at 42 d. As a result, it could be argued that SeGlu, as a novel and simple Se source plus probiotic, is more effective than SS and SeY in improving broiler performance, microbial population, intestinal morphology, and immune response.

KEY WORDS gut health, immunity, microbial population, selenium-yeast, sodium selenite.

INTRODUCTION

Selenium (Se) is a trace micronutrient that is essential for both human and animal health (Zhang *et al.* 2018; Li *et al.* 2019). Selenium, through oxidative equilibrium, aids in the prevention of cell membrane damage (Mahmoud *et al.* 2016; Silva *et al.* 2019). It improves broiler immunity, intestinal morphology, microflora, and antioxidation (Bakhshalinejad *et al.* 2019; Khajeh Bami *et al.* 2022a). Selenium can be found in both inorganic (sodium selenite) and organic (selenomethionine and selenium-enriched yeast) forms in the diet. Previous research has shown that organic Se, such as selenium-yeast (SeY), has greater bio-

activity, metabolic pathways, bioavailability, physiological functions, and efficacy, as well as less toxicity, when compared to inorganic Se (Bakhshalinejad *et al.* 2018; Marković *et al.* 2018; Lu *et al.* 2019). However, high production costs prevent large-scale synthesis of organic Se types (Zhao *et al.* 2021a; Zhao *et al.* 2021b). Furthermore, the production of organic Se, such as SeY, is usually time consuming and contains only trace amounts of Se, which may inhibit widespread use. Selenized glucose (SeGlu) is a novel organic and synthetic Se source with numerous physiological activities such as antioxidant, free radical elimination, immunological response modulation, anti-virus, and blood glucose management. The selenide reac-

tion of glucose with sodium hydrogen selenide produces SeGlu at a low cost (Zhao *et al.* 2021a; Zhao *et al.* 2021b).

In laying hens, studies show that SeGlu supplementation increased antioxidant activity and decreased free radicals in the spleen, liver, and oviduct (Zhao *et al.* 2021a) and enhanced the Se deposition and antioxidant activity of eggs (Zhao *et al.* 2021b). Investigating the effect of SeGlu as a novel organic Se in broiler chickens is rare. On the other hand, it is well known that Se and probiotics can work together to influence biological processes because both are immune stimulants and improve microbial population (Yuan *et al.* 2012).

As a result, the purpose of this study was to determine how dietary supplementation of SS, SeY, and SeGlu as Se sources, as well as the interaction of these compounds with probiotic, affected broiler chicken growth performance, intestinal microflora, intestinal morphology, and immune response.

MATERIALS AND METHODS

Diets, birds, management, and experimental design

In a 3 × 2 factorial treatment arrangement with five replicate pens and ten birds per replicate, 300 one-day-old Ross 308 broilers were randomly assigned to six experimental groups. For 42 days, the broilers were raised in pens of equal size (100×100 cm floor area and 80 cm height). Birds were placed in identically sized cemented floor cages, and the floor was covered with wood chips. Se sources (SS, SeY, and SeGlu at 0.3 mg/kg) and probiotic levels (0 and 100 mg/kg of diet) were tested. The experimental treatments were as follows: 1) basal diet + SS, 2) basal diet + SeY, 3) basal diet + SeGlu, 4) basal diet + SS + probiotics, 5) basal diet + SeY + probiotics, 6) basal diet + SeGlu + probiotics. The environmental conditions, such as ventilation, light, and temperature, were maintained in accordance with broiler breeding standards. The daily basal diets were prepared fresh and formulated in accordance with the Ross 308 guideline (Aviagen, 2014). During the experiment, the chickens were given unlimited access to water and food. In mash form, diets were designed for starter (1 to 14 days), grower (15 to 21 days), and finisher (22 to 42 days) (Table 1). Mineral supplements, on the other hand, were free of Se. Initially, only one batch of diet (without Se supplement) was made. Then, at predetermined doses, Se and probiotic supplements were added to the main diet. Radin Dam Fartak Company purchased the SeY. Zhou *et al.* (2020) described a method for producing selenized glucose.

The multi-strain probiotic (lyophilized probiotic powder, 2.3×10^{11} CFU/g) obtained from Pardis Roshd Mehregan (Co., BioExir®, Iran) contained *Bacillus coagulans*,

Lactobacillus faecium, *Bacillus subtilis*, *Bacillus licheniformis*, *Lactobacillus rhamnosus*, and *Lactobacillus plantarum*.

Growth efficiency

Birds were weighed in groups on days 1 and 42 to determine body weight gain (BWG). Feed intake (FI) was calculated by subtracting feed supplied from feed refused. The feed conversion ratio (FCR) for mortality was corrected after checking mortality twice a day.

Intestinal microflora

At 42 days, one bird from each cage was chosen at random and euthanized via cervical dislocation. The ileal digesta was then collected and kept in sterile plastic bags at -80°C until microbial analysis. For coliforms (COL) and lactic acid bacteria (LAB) accounts, the ileal digesta were diluted in phosphate buffered saline. The COL and LAB were grown on Mac Conkey agar at 37°C for 24 hours and on MRS agar at 37°C for 72 hours, respectively. For COL, dilutions ranging from 10^{-2} to 10^{-5} were used, and for LAB counts, dilutions ranging from 10^{-3} to 10^{-6} were used (Khajeh Bami *et al.* 2022b).

Intestinal morphology

To assess the structure of intestinal tissue, a 1 cm segment of the ileum was separated and fixed in 10% formaldehyde buffer after washing to measure the ileum structure before being embedded in paraffin waxes. The samples were stained with hematoxylin and eosin. Villus height, villus width, crypt depth, villus height per crypt depth (VH/CD), villus surface area, epithelial cell layer thickness, and goblet cell density (per 100 μm) were measured to assess intestine morphological parameters. An optical microscope (Micromaster, Fisher Scientific, Cat. No. 12-562-27, Fisher Scientific, Waltham, MA) with the Image Pro Plus v 4.5 software packet was used to examine the slides (Media Cybernetics, Silver Spring, MD, USA; Khajeh Bami *et al.* 2022a).

Immune response

Two birds from each cage—a total of ten birds—were injected with 1 ml of a 0.5 percent suspension of sheep red blood cells (SRBC) in the breast muscle on days 21 and 35 of the experiment to measure the humoral immune response. Blood samples were taken seven days after each injection, and sera were frozen to calculate antibody titers. Using the procedure outlined by Khajeh Bami *et al.* (2022b), the total and IgG anti-SRBC antibodies (mercaptoethanol-resistant antibody) were measured. The difference between total and IgG titers was used to calculate the IgM titer.

Table 1 Ingredients and composition (as-fed basis) of the basal diets

| Item | Starter diet (d 1 to 14) | Grower diet (d 15 to 21) | Finisher diet (d 22 to 42) |
|--|-----------------------------|-----------------------------|-------------------------------|
| Ingredients (%) | | | |
| Corn | 56.00 | 58.25 | 62.32 |
| Soybean meal | 38.00 | 36.10 | 31.70 |
| Soybean oil | 1.60 | 1.80 | 2.20 |
| Dicalcium phosphate | 1.75 | 1.75 | 1.60 |
| Calcium carbonate | 1.00 | 1.00 | 1.10 |
| DL-methionine | 0.25 | 0.20 | 0.15 |
| L-lysine | 0.40 | 0.10 | 0.13 |
| Threonine | 0.20 | 0 | 0 |
| Vitamin premix ¹ | 0.25 | 0.25 | 0.25 |
| Mineral premix ² | 0.25 | 0.25 | 0.25 |
| Salt | 0.30 | 0.30 | 0.30 |
| Calculated chemical composition | | | |
| Metabolizable energy (Kcal/kg) | 2995 | 2990 | 3047 |
| Crude protein (%) | 22.50 | 21.90 | 20.00 |
| Calcium (%) | 1.00 | 1.00 | 0.99 |
| Available phosphorous (%) | 0.45 | 0.45 | 0.41 |
| Methionine + cysteine (%) | 0.55 | 0.51 | 0.44 |
| Lysine (%) | 1.52 | 1.26 | 1.15 |
| Arginine (%) | 1.37 | 1.35 | 1.21 |
| Threonine (%) | 0.98 | 0.78 | 0.72 |

¹ Supplied per kg of diet: vitamin A (retinol): 12000 IU; vitamin D₃ (cholecalciferol): 5000 IU; vitamin K₃: 2.55 mg; vitamin B₆ (pyridoxine): 4.5 mg; vitamin B₁₂ (cyanocobalamin): 0.02 mg; Thiamin: 3 mg; Riboflavin: 7.5 mg; Niacin: 51 mg; Folic acid: 1.5 mg; Biotin: 0.2 mg; Pantothenic acid: 13.5 mg and Choline chloride: 250 mg.

² Supplied per kg of diet: Mn: 120 mg; Cu: 16 mg; I: 1 mg; Fe: 40 mg and Zn: 100 mg.

Statistical analysis

To examine three organic sources of Se (SeY, SeCh, and SeGlu), two levels of probiotic (0 and 100 mg/kg), and the interactions between these factors, data were analyzed using a totally randomized design with treatments arranged in a 3 × 2 factorial (SAS, 2004). Tukey's test was used to compare the means, and differences were deemed significant at $P < 0.05$.

RESULTS AND DISCUSSION

Table 2 shows the effects of different forms of Se and probiotics on broiler growth performance (1 to 42 days). Dietary supplementation with Se source had an effect on broiler growth performance ($P < 0.05$). Broilers fed SeGlu performed better than SS and SeY in terms of BWG and FCR. Throughout the experiment, there was no interaction between Se source and probiotic on growth performance ($P > 0.05$). Furthermore, the effects of probiotics on broiler growth performance were not statistically significant ($P > 0.05$). Similar findings were obtained by Bakhshalinejad *et al.* (2018) and Selim *et al.* (2015), who demonstrated that dietary Se source affects the FCR and BWG of broilers. Because of active absorption, organic Se is more effective in intestinal absorption than inorganic Se. The efficiency of the bird improves as the beneficial intestinal microbial balance improves (Clavijo and Florez, 2018).

As a result of these findings, including SeGlu in broiler diets may improve growth performance by increasing the beneficial microbial population, as shown in Table 3. In contrast to our findings, Khajeh Bami *et al.* (2022b) concluded that dietary supplementation with nano-Se had no effect on broiler growth performance when compared to controls. Several other studies have found that Se sources and levels have no effect on broiler chicken growth performance (Bakhshalinejad *et al.* 2019; Mohammadi *et al.* 2020).

The main effect of probiotics on growth performance was insignificant. In another study, broiler chickens fed probiotic diets outperformed those fed a control diet in terms of BWG and FCR (Zhang and Kim, 2014). Furthermore, there was no interaction between the Se source and the probiotic on growth response. In contrast, other studies found that adding Se supplements in various forms had no effect on broiler growth performance (Habibian *et al.* 2014; Chadio *et al.* 2015). The effects of Se on broiler growth performance vary, which could be due to Se levels, experimental setups, or the chicken breeds used.

Table 3 shows the effects of different forms of Se and probiotics on the intestinal microflora of broilers at 42 days. Broiler chickens fed supplemental SeGlu or SeY diets had lower intestinal COL counts than SS. The number of intestinal LAB and LAB/COL ratios was higher in SeGlu-fed birds than in SeY and SS-fed birds ($P < 0.05$).

Table 2 Effects of selenite sodium (SS), selenium-yeast (SeY), selenized glucose (SeGlu), probiotic and their various combinations on the growth performance of broilers (1 to 42 d)

| Items | Body weight gain (g/b/d) | Feed intake (g/b/d) | Feed conversion ratio (g/g) |
|-----------------------|--------------------------|---------------------|-----------------------------|
| Selenium source (SeS) | | | |
| SS | 50.37 ^b | 100.7 | 2.006 ^a |
| SeY | 50.51 ^b | 103.2 | 2.046 ^a |
| SeGlu | 54.51 ^a | 101.2 | 1.866 ^b |
| SEM ¹ | 1.02 | 0.84 | 0.03 |
| Probiotic (Pro) | | | |
| 0 mg/kg | 51.75 | 102.2 | 1.984 |
| 100 mg/kg | 52.32 | 101.4 | 1.942 |
| SEM | 0.83 | 0.69 | 0.02 |
| Interaction | | | |
| SS-0 mg/kg Pro | 49.21 | 101.4 | 2.037 |
| SS-100 mg/kg Pro | 51.52 | 100.0 | 1.974 |
| SeY-0 mg/kg Pro | 50.29 | 104.7 | 2.064 |
| SeY-100 mg/kg Pro | 50.72 | 101.7 | 2.028 |
| SeGlu-0 mg/kg Pro | 53.72 | 101.8 | 1.882 |
| SeGlu-100 mg/kg Pro | 55.31 | 101.0 | 1.850 |
| SEM | 1.44 | 1.19 | 0.06 |
| P-values | | | |
| SeS | 0.020 | 0.115 | 0.003 |
| Pro | 0.634 | 0.422 | 0.246 |
| SeS × Pro | 0.402 | 0.220 | 0.329 |

The means within the same row with at least one common letter, do not have significant difference ($P>0.05$). SEM: standard error of the means.

Table 3 Effects of selenite sodium (SS), selenium-yeast (SeY), selenized glucose (SeGlu), probiotic and their various combinations on the intestinal microflora population of broilers at 42 d of age

| Items | Lactic acid bacteria | Coliforms | Lactic acid bacteria/coliform ratios |
|-----------------------|----------------------|--------------------|--------------------------------------|
| Selenium source (SeS) | | | |
| SS | 4.688 ^b | 4.489 ^a | 1.099 ^c |
| SeY | 5.077 ^b | 2.697 ^b | 1.895 ^b |
| SeGlu | 6.072 ^a | 2.312 ^b | 2.650 ^a |
| SEM | 0.20 | 0.19 | 0.09 |
| Probiotic (Pro) | | | |
| 0 mg/kg | 5.099 | 3.232 | 1.879 |
| 100 mg/kg | 5.459 | 3.099 | 1.883 |
| SEM | 0.16 | 0.15 | 0.07 |
| Interaction | | | |
| SS-0 mg/kg Pro | 4.030 ^b | 4.879 | 0.854 ^c |
| SS-100 mg/kg Pro | 5.346 ^a | 4.098 | 1.343 ^{de} |
| SeY-0 mg/kg Pro | 5.076 ^{ab} | 2.760 | 1.835 ^{cd} |
| SeY-100 mg/kg Pro | 5.077 ^{ab} | 2.634 | 1.955 ^{bc} |
| SeGlu-0 mg/kg Pro | 5.955 ^a | 2.439 | 2.470 ^{ab} |
| SeGlu-100 mg/kg Pro | 6.190 ^a | 2.184 | 2.830 ^a |
| SEM | 0.28 | 0.26 | 0.12 |
| P-values | | | |
| SeS | < 0.001 | < 0.001 | < 0.001 |
| Pro | 0.135 | 0.540 | 0.974 |
| SeS × Pro | 0.025 | 0.120 | 0.009 |

The means within the same row with at least one common letter, do not have significant difference ($P>0.05$). SEM: standard error of the means.

Furthermore, for LAB counts and LAB/COL ratios in the ileum, there was an interaction between Se source and probiotic, such that birds fed diets supplemented with SeGlu plus probiotic had higher LAB bacteria counts and LAB/COL ratios than those fed SeY without probiotic ($P<0.05$). The diversity of the intestinal microbial population is influenced by dietary essential trace elements

(Kasaikina *et al.* 2011). There have been few studies on the antibacterial effects of Se organic sources. Khajeh Bami *et al.* (2022b) found that feeding nano-Se increased LAB counts and LAB/COL ratios in the ileum while decreasing COL counts in the cecum of broiler chickens. Increasing the population of beneficial microbiota aids in the health of the bird (Khan *et al.* 2012).

Table 4 Effects of selenite sodium (SS), selenium-yeast (SeY), selenized glucose (SeGlu), probiotic and their various combinations on ileal morphology of broilers at 42 d of age

| Items | Villus height (µm) | Villus width (µm) | Crypt depth (µm) | Villus height/crypt depth (µm) | Villus surface area (mm ²) | Epithelial cell layer thickness (µm) | Goblet cell density |
|-----------------------|----------------------|---------------------|--------------------|--------------------------------|--|--------------------------------------|---------------------|
| Selenium source (SeS) | | | | | | | |
| SS | 1084.1 ^b | 143.9 ^b | 152.3 ^a | 7.132 ^b | 0.499 ^b | 49.86 ^a | 9.2 ^b |
| SeY | 1191.8 ^b | 166.5 ^a | 150.0 ^a | 8.008 ^b | 0.629 ^a | 48.47 ^a | 10.4 ^b |
| SeGlu | 1366.6 ^a | 167.1 ^a | 128.4 ^b | 10.73 ^a | 0.716 ^a | 31.86 ^b | 14.3 ^a |
| SEM | 37.62 | 4.69 | 3.66 | 0.38 | 0.03 | 2.80 | 0.35 |
| Probiotic (Pro) | | | | | | | |
| 0 mg/kg | 1127.7 ^b | 157.3 | 150.2 ^a | 8.445 | 0.572 ^b | 49.15 ^a | 10.05 ^b |
| 100 mg/kg | 1300.7 ^a | 160.9 | 136.9 ^b | 8.807 | 0.658 ^a | 37.64 ^b | 12.51 ^a |
| SEM | 30.72 | 3.83 | 2.99 | 0.31 | 0.02 | 2.29 | 0.29 |
| Interaction | | | | | | | |
| SS-0 mg/kg Pro | 969.7 ^b | 116.0 ^d | 163.3 | 6.870 ^c | 0.353 ^c | 57.74 | 7.12 ^c |
| SS-100 mg/kg Pro | 1198.5 ^{ab} | 140.2 ^{cd} | 148.9 | 7.394 ^c | 0.647 ^{ab} | 43.33 | 11.2 ^b |
| SeY-0 mg/kg Pro | 1052.4 ^b | 162.1 ^{bc} | 151.1 | 7.120 ^c | 0.533 ^{bc} | 56.40 | 7.97 ^c |
| SeY-100 mg/kg Pro | 1331.2 ^a | 170.9 ^{ab} | 141.3 | 8.896 ^{bc} | 0.725 ^{ab} | 39.20 | 12.9 ^{ab} |
| SeGlu-0 mg/kg Pro | 1360.9 ^a | 171.7 ^{ab} | 136.2 | 10.13 ^{ab} | 0.603 ^b | 33.33 | 13.4 ^a |
| SeGlu-100 mg/kg Pro | 1372.3 ^a | 193.9 ^a | 120.7 | 11.34 ^a | 0.829 ^a | 30.40 | 15.1 ^a |
| SEM | 53.20 | 6.64 | 5.18 | 0.54 | 0.04 | 3.96 | 0.50 |
| P-values | | | | | | | |
| SeS | < 0.001 | 0.002 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 |
| Probiotic | 0.001 | 0.512 | 0.005 | 0.418 | 0.026 | 0.002 | < 0.001 |
| SeS × Pro | 0.044 | < 0.001 | 0.174 | 0.034 | < 0.001 | 0.158 | < 0.001 |

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

SEM: standard error of the means.

Table 5 Effects of selenite sodium (SS), selenium-yeast (SeY), selenized glucose (SeGlu), probiotic and their various combinations on immune response (log₂) of broilers at 28 and 42 d of age

| Items | Total anti-SRBC titre | | IgG | | IgM | |
|-----------------------|-----------------------|------------------|------------------|-------------------|------------------|------------------|
| | d 28 | d 42 | d 28 | d 42 | d 28 | d 42 |
| Selenium source (SeS) | | | | | | |
| SS | 3.0 ^b | 3.9 ^c | 1.5 ^b | 1.7 ^b | 1.6 ^b | 2.3 ^b |
| SeY | 5.3 ^b | 4.5 ^b | 1.4 ^b | 2.1 ^b | 2.0 ^b | 2.5 ^b |
| SeGlu | 5.4 ^a | 7.2 ^a | 2.0 ^a | 2.8 ^a | 3.4 ^a | 4.4 ^a |
| SEM | 0.23 | 0.15 | 0.12 | 0.12 | 0.19 | 0.16 |
| Probiotic (Pro) | | | | | | |
| 0 mg/kg | 3.7 ^b | 4.7 ^b | 1.5 | 2.1 | 2.1 | 2.6 ^b |
| 100 mg/kg | 4.1 ^a | 5.6 ^a | 1.6 | 2.2 | 2.4 | 3.5 ^a |
| SEM | 0.19 | 0.12 | 0.09 | 0.09 | 0.16 | 0.13 |
| Interaction | | | | | | |
| SS-0 mg/kg Pro | 2.4 | 3.3 | 1.2 | 1.5 ^c | 1.2 | 1.8 |
| SS-100 mg/kg Pro | 3.6 | 4.6 | 1.7 | 1.8 ^{bc} | 1.9 | 2.8 |
| SeY-0 mg/kg Pro | 3.3 | 4.3 | 1.2 | 1.8 ^{bc} | 1.8 | 2.0 |
| SeY-100 mg/kg Pro | 3.3 | 4.7 | 1.5 | 2.3 ^{ab} | 2.1 | 2.9 |
| SeGlu-0 mg/kg Pro | 5.3 | 6.6 | 1.9 | 2.6 ^a | 3.3 | 4.0 |
| SeGlu-100 mg/kg Pro | 5.4 | 7.7 | 2.0 | 2.9 ^a | 3.5 | 4.8 |
| SEM | 0.32 | 0.21 | 0.16 | 0.17 | 0.27 | 0.23 |
| P-values | | | | | | |
| SeS | < 0.001 | < 0.001 | 0.001 | < 0.001 | < 0.001 | < 0.001 |
| Pro | 0.167 | < 0.001 | 0.461 | 0.808 | 0.244 | < 0.001 |
| SeS × Pro | 0.088 | 0.084 | 0.061 | 0.028 | 0.276 | 0.907 |

SRBC: sheep red blood cells; IgG: immunoglobulin G and IgM: immunoglobulin M.

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

SEM: standard error of the means.

In one study, feeding bacterial organic Se or Se-yeast rather than inorganic Se reduced the number of COL while increasing the number of LAB in the cecum. Furthermore, feeding organic Se reduces the ileum COL population, according to this study (Muhammad *et al.* 2021).

Furthermore, researchers discovered that broiler chickens fed Se-Chitosan had higher LAB/COL ratios and lower COL counts in the ileum than those fed inorganic Se (Khajeh Bami *et al.* 2022a).

Dalia *et al.* (2018) discovered that feeding Se-enriched bacteria to broilers reduces pathogenic bacteria while increasing useful bacteria and the balance of bacterial populations in the cecum. Diets containing Se supplementation with antioxidant function can modulate the diversity of the intestinal microbial population by suppressing oxidative stress, making the environment more conducive to the growth and proliferation of beneficial bacteria (Muhammad *et al.* 2021). Due to improved intestinal morphology, SeGlu has the ability to modulate intestinal microflora, which may improve broiler intestinal health and immune response.

Table 4 shows the effects of different forms of Se and probiotics on ileal morphology at 42 days. The main effect of probiotic demonstrated that broilers fed probiotic had higher villus height, villus surface area, and goblet cell density and lower crypt depth and epithelial cell layer thickness than those fed unsupplemented probiotic ($P < 0.05$). When compared to SS or SeY, broilers fed SeGlu diets had higher villus height, VH/CD, goblet cell density, and lower crypt depth and epithelial cell layer thickness ($P < 0.05$).

Furthermore, interaction results revealed that birds fed SeGlu plus probiotic diets had highest villus height, villus width, VH/CD, villus surface area, and goblet cell density ($P < 0.05$). According to these findings, the combination of SeGlu supplementation and probiotics appears to have a synergistic effect in improving the intestinal structure of broilers. Probiotic supplementation improved intestinal morphology by lowering pH and eliminating harmful bacteria in the gut (Beski and Al-Sardary, 2015).

According to Khajeh Bami *et al.* (2022a), broilers fed diets supplemented with Se-Chitosan as an organic Se compared to inorganic Se increased VH/CD, villus surface area, and goblet cell density and decreased epithelial cell layer thickness in the ileum and jejunum. Changes in intestinal morphology are influenced by gut functional development, the number of secretory goblet cells, nutrient absorption surface area, disease resistance, and intestinal immunity (Tang *et al.* 2020). Furthermore, feeding bacterial organic Se on the villus height of the small intestine was beneficial (Muhammad *et al.* 2021).

In a study Dalia *et al.* (2020) discovered that adding bacterial organic selenoprotein supplementation improved the morphology of broiler intestine, as evidenced by increased villi height in the duodenum and ileum.

Moghaddam *et al.* (2017) also showed that diet supplementation with organic Se substantially increases the jejunal villus height and villus surface area of broiler chickens compared to inorganic Se, which is in accordance with the results of the present research. Improving villi height and crypt depth improves nutrient uptake and growth performance. Furthermore, Se supplementation in the diet is effective in regulating intestinal health by regulating the intestinal microbial population (Zhai *et al.* 2018). The mechanism by which SeGlu with probiotic improves intestinal histomorphology is most likely due to growth suppression of some intestinal harmful bacteria. As a result, as shown in Tables 3 and 4, combining SeGlu with probiotics in broiler diets may improve intestinal morphology by increasing the beneficial microbial population.

Table 5 shows the effects of different forms of Se and probiotics on the humoral immune response of broilers at 28 and 42 days. At 28 and 42 days, dietary supplementation with SeGlu significantly increased total anti-SRBC titre, IgG, and IgM levels compared to SS and SeY ($P < 0.05$). At 42 day, the effect of probiotic was observed, with birds fed probiotic-supplemented diets having a higher total antibody response to SRBC and IgM titres than those fed unsupplemented probiotic ($P < 0.05$).

There was an interaction between Se source and probiotic levels for IgG at 42 days ($P < 0.05$). Birds fed SeGlu alone or in combination with probiotic had higher IgG levels than SeY alone or in combination with probiotic. Changes in intestinal microbiota and improvements in intestinal morphology influence immune response improvement, as observed in the current study (Tang *et al.* 2020).

As a result, improving immune status could be attributed to improvements in intestinal morphology and microbial population. When chemical nano Se was fed instead of SS, serum IgM and IgG levels increased (Boostani *et al.* 2015).

According to Mohammadi *et al.* (2020), feeding chemical nano Se improved total antibody response to SRBC, IgG, and IgM more than inorganic Se. In another study, broilers fed Se-Chitosan as organic Se had a higher total antibody response to SRBC and IgG than sodium selenite (Khajeh Bami *et al.* 2022a). Selenium regulates the immune system by reducing stress and increasing the activity of antioxidant enzymes (Rao *et al.* 2013). According to Mohammadi *et al.* (2020), using Se-enriched yeast as an organic Se source rather than inorganic Se increased broiler serum IgM.

CONCLUSION

In conclusion, the current study found that SeGlu, a new source of synthetic organic Se, is more efficient than SeY, the common organic form of Se, and inorganic Se. As a result, SeGlu can be thought of as a Se additive in birds. Furthermore, probiotic and SeGlu supplementation may be effective in improving broiler intestinal microflora, morphology, and immune response.

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