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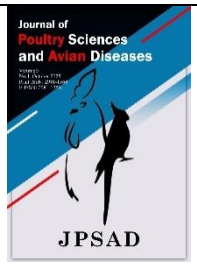


# Intestinal Microflora, Morphology, and Immune Response in Broiler Chickens Fed Various Organic Selenium and Probiotic Sources

Asma Shokrinejad Gerdin[1], Mohsen Afsharmanesh[1*], Mohammad Khajeh Bami[1*]

[1] Department of Animal Science, Faculty of Agriculture, Shahid Bahonar University of Kerman, Kerman, Iran

* Corresponding **authors' email addresses:** mafshar@uk.ac.ir; mkhaje@agr.uk.ac.ir

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A B S T R A C T

The purpose of this study was to investigate the effects of selenium-enriched yeast (SeY), selenium-chitosan (SeCh), and selenized glucose (SeGlu) as organic selenium sources, probiotics, and the interactions between selenium sources and probiotics on the intestinal microflora, intestinal morphology, and immune response in broilers. In a 3×2 factorial treatment design, 300 one-day-old Ross 308 broiler chickens were randomly assigned to six experimental groups. Selenium sources (0.3 mg/kg SeY, SeCh, and SeGlu) and probiotic levels (0 and 100 mg/kg) were among the factors investigated. Five-floor pens with 10 birds each have been used to replicate the treatments. Compared to SeY, broiler chickens fed SeCh or SeGlu had lower coliform bacteria counts, higher lactic acid bacteria counts, and lactic acid bacteria/coliform ratios in the ileum ($p$<0.05). Interaction results showed that birds fed diets supplemented with SeCh and SeGlu plus probiotics had higher villus height per crypt depth, villus surface area, and goblet cell density, as well as lower epithelial cell layer thickness in the ileum ($p$<0.05). At 28 and 42 days, birds fed diets supplemented with SeCh and SeGlu had the highest total antibody response to sheep red blood cells, IgG, and IgM titers ($p$<0.05). Birds fed diets supplemented with SeCh and SeGlu plus Probiotic had higher IgG levels than SeY without Probiotic ($p$<0.05). As a result, it is possible to conclude that SeCh and SeGlu, as novel and simple Se sources plus Probiotic, can improve intestinal microflora, morphology, and immune response in broiler chickens when compared to SeY alone.

***Keywords:*** *Broiler, Gut microflora, Immune response, Intestine, Selenium-chitosan, Selenized glucose, Probiotic.*

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## 1 Introduction

Selenium (Se) is an essential trace micronutrient that plays a crucial biological role in maintaining animal health (1, 2). Selenium has an effect on physiological functions via selenoproteins (3). It protects cell membranes from oxidative stress (4, 5). Selenium is an essential component of the enzyme glutathione peroxidase, which plays a crucial biological role in many of the body's systems (6-8). It has a positive effect on broiler immunity, intestinal morphology, microflora, and antioxidation (9-11). The bioactivity, metabolic pathways, bioavailability, physiological functions, and toxicity of selenium (Se) are known to be highly related to its chemical forms (3). In the diet, Se is found in both inorganic (sodium selenite) and organic (selenomethionine and selenium-enriched yeast) forms. Previous research has demonstrated that organic selenium (Se) has a greater impact on immune response, intestinal microflora, and intestinal morphology than inorganic selenium (11). A new type of Se has emerged in recent years. Selenium yeast (SeY) exhibits better physiological functions, higher bioavailability, and a greater influence, as well as lower toxicity, compared to inorganic selenium (12, 13). However, high production costs prevent the synthesis of organic Se types on a large scale (14). Furthermore, the production of organic Se, such as SeY, is typically time-consuming and yields only trace amounts of Se, which may hinder its widespread use. As a result, recent research has employed various methods to produce organic selenium.

Selenium-chitosan (SeCh) and selenized glucose (SeGlu) are novel synthetic organic Se sources that have a wide range of physiological processes (14-17). Selenium-chitosan is a chemically synthesized compound made from sodium selenite and chitosan . Recent research has shown that using SeCh improves broiler intestinal microflora, intestinal morphology, and immune response (11). Another source of synthetic organic Se is SeGlu, which is produced at a low cost by the selenide reaction of glucose with sodium hydrogen selenide (18). A study found that feeding SeGlu supplementation to laying hens increased antioxidant activity (14). There is limited research on SeGlu supplementation in broilers. On the other hand, it is well known that Se and probiotics can act synergistically and influence biological processes, as both are immune stimulants and improve microbial populations (19).

As a result, the purpose of this study was to evaluate the effect of dietary supplementation with SeCh, SeGlu, and SeY as organic forms of selenium, as well as the interaction of these compounds with probiotics, on the intestinal microflora, intestinal morphology, and immune response of broilers.

## 2 Materials and methods

### *2.1 Management, Birds, and Experimental Design*

A total of 300 one-day-old Ross 308 broiler chickens were randomly assigned to six experimental groups in a 3×2 factorial treatment arrangement. The experimental treatments were as follows: 1) basal diet + SeY, 2) basal diet + SeCh, 3) basal diet + SeGlu, 4) basal diet + SeY + probiotics, 5) basal diet + SeCh + probiotics, 6) basal diet + SeGlu + probiotics. Factors tested included organic Se sources (SeY, SeCh, and SeGlu at a level of 0.3 mg/kg) and probiotic levels (0 and 100 mg/kg in the diet). The treatments were replicated in five-floor pens with 10 chicks per pen. Feed and water were provided *ad libitum* to the chickens during the experimental period. Birds were raised for 42 d in cemented floor pens of identical measure (length 120 cm × width 120 cm × height 80 cm) and covered with wood chips. All animal experiments were performed according to the guidelines for the care and use of laboratory animals and were approved by the Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman (approval number: IR.UK.VETMED.REC.2019-03-05).

### *2.2 Diets and Supplementation*

The basal diets were freshly prepared each day and formulated according to the requirements suggested by Ross 308 guideline (20). Diets were formulated into starter (1 to 14 days), grower (15 to 21 days), and finisher (22 to 42 days) periods in mash form (Table 1). However, the mineral supplement was free of Se. At first, a single batch of diet (without Se supplement) was produced. Selenium (SeY, SeCh, or SeGlu) and probiotic supplements were then added to the main diet at defined doses. The SeY was purchased from the Radin Dam Fartak Company. The SeCh was prepared by mixing sodium selenite (Sigma-Aldrich Chemical Co., St. Louis, MO, USA) and chitosan (Sigma-Aldrich Chemical Co., St. Louis, MO, USA) according to the method described by Victor et al. (2019) (15). The chitosan solution was created by mixing 100 mL of 1% acetic acid with 1.0 g of chitosan. The chitosan solution was then treated with 0.4 g of $Na_2SeO_3$ for two hours to facilitate a reaction. After the mixture was filtered to remove any

insoluble materials, 70% ethyl alcohol was added. The alcohol mixture was allowed to precipitate for 12 hours before the solution was filtered. The filtrate was washed, then ground, and finally dried at low temperatures. The SeGlu was prepared using the method described by Zhou et al.2020. (18). A portion of sodium borohydride (NaBH4) was gently added after the Se powder suspension in EtOH solution was cooled to -25°C. The remaining NaBH4 was added at 15–17°C when the reaction started to drift toward equilibrium. Following the addition of glucose, the prepared NaHSe solution was agitated for 12 hours. The EtOH medium was then recovered through distillation, and the resulting powder was dried for 80 hours at 60-75°C and 0.1 atm of pressure. When finally obtained, the selenized glucose product was a white, powdery substance. The multi-strains probiotic that contains *Bacillus coagulans*, *Lactobacillus faecium*, *Bacillus Subtilis*, *Bacillus lichen formis*, *Lactobacillus rhamnosus*, and *Lactobacillus plantarum* (lyophilized probiotic powder, $2.3\times10^{11}$ CFU/g) was purchased from Pardis Roshd Mehregan Co., BioExir®, Iran. The light and room temperature were preserved as suggested, according to the management guide for Ross 308 broilers.

**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 | 58.25 | 62.32 |
| Soybean meal | 38 | 36.1 | 31.7 |
| Soybean oil | 1.6 | 1.8 | 2.2 |
| Dicalcium phosphate | 1.75 | 1.75 | 1.6 |
| Calcium carbonate | 1 | 1 | 1.1 |
| DL-methionine | 0.25 | 0.2 | 0.15 |
| L- Lysine | 0.4 | 0.1 | 0.13 |
| Threonine | 0.2 | 0 | 0 |
| Vitamin premix[1] | 0.25 | 0.25 | 0.25 |
| Mineral premix[2] | 0.25 | 0.25 | 0.25 |
| Salt | 0.3 | 0.3 | 0.3 |
| Calculated chemical composition | | | |
| Metabolizable energy (Kcal/kg) | 2995 | 2990 | 3047 |
| Crude protein (%) | 22.5 | 21.9 | 20 |
| Calcium (%) | 1 | 1 | 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 $D_3$ (Cholecalciferol), 5000 IU; vitamin $K_3$, 2.55 mg; thiamin, 3 mg; riboflavin, 7.5 mg; vitamin $B_6$ (pyridoxine), 4.5 mg; vitamin $B_{12}$ (cyanocobalamin), 0.02 mg; niacin, 51 mg; folic acid, 1.5 mg; biotin, 0.2 mg; pantothenic acid, 13.5 mg; choline chloride, 250 mg.

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

### *2.3 Intestinal Microflora*

At 42 d of age, one bird from each cage was randomly chosen and euthanized by cervical dislocation. Subsequently, the ileal digesta was collected and stored in sterile plastic bags at −80°C until microbial analysis. The ileal digesta were diluted in phosphate-buffered saline for coliforms (COL) and lactic acid bacteria (LAB) counts. The COL and LAB on MacConkey agar at 37°C for 24 h and on MRS agar at 37°C for 72 h were cultivated, respectively. Dilutions from 10^(-2) to 10^(-5) for coliforms and from 10^(-3) to 10^(-6) for lactic acid bacteria counts were used (21).

### *2.4 Intestinal Morphology*

To evaluate the structure of intestinal tissue, a 1 cm segment of the ileum was separated and fixed in 10% formaldehyde buffer, following which it was washed to measure the ileum structure. Each sample was then embedded in paraffin wax. Hematoxylin and eosin were used to stain the samples. To assay the morphological parameters of the intestine, 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 um) were measured. The slides were examined using an optical microscope (Micromaster, Fisher Scientific, Cat. No. 12-562-27, Fisher Scientific, Waltham, MA) with

the Image Pro Plus v4.5 software package (Media Cybernetics, Silver Spring, MD, USA;(11).

### 2.5 *Immune Response*

On d 21 and 35 of the experiment, two birds from each cage (10 birds/treatment) were injected with 1 ml of 0.5% sheep red blood cells (SRBC) suspension in the breast muscle to assay the humoral immune response. Seven days after each injection, blood samples were collected, and sera were frozen to measure antibody titers. The total and IgG anti-SRBC antibodies (mercaptoethanol-resistant antibodies) were determined according to the method described by Khajeh Bami et al. (2022b) and Wegmann and Smithies (1966) (21, 22). The difference between total and IgG titer measured the amount of IgM titer.

### 2.6 *Statistical Analysis*

Data were analyzed using a completely randomized design with treatments arranged in a 3×2 (three Se sources×two probiotic levels) factorial to evaluate three organic sources of Se (SeY, SeCh, and SeGlu), two levels of probiotic (0 and 100 mg/kg), and the interactions among these factors by the General Linear Model (GLM) procedure of SAS (2003; SAS Institute, Cary, NC). Means were compared using Tukey's test, and differences were considered significant at $p < 0.05$. The statistical model used was: $Y_{ijk} = \mu + S_i + P_j + SP_{ij} + e_{ijk}$, where $Y_{ijk}$ is the individual observation, $\mu$ is the experimental mean, $S_i$ is the Se source effect, $P_j$ is the probiotic level effect, $SP_{ij}$ is the Se source by probiotic level interaction, and $e_{ijk}$ is the error term.

## 3 Results

### 3.1 *Intestinal microflora*

Table 2 shows the effects of different Se and probiotic sources on the intestinal microflora of broilers at 42 days. In the ileum, broilers fed SeGlu had higher LAB counts and LAB/COL ratios than broilers fed SeY ($p$<0.05). Furthermore, dietary treatment with SeCh and SeGlu significantly reduced COL counts in the ileum more than SeY ($p$<0.05). There was no interaction between the Se source and probiotic level for the population of intestinal microflora.

**Table 2.** Effects of selenium-yeast (SeY), selenium-chitosan (SeCh), selenized glucose (SeGlu), probiotic and their various combinations on the ileal microflora (log cfu / g) of broilers at 42 d

| Items | Lactic acid bacteria | Coliforms | Lactic acid bacteria /Coliform ratios |
|---|---|---|---|
| Selenium Source (SeS) | | | |
| SeY | 5.077$^{b}$ | 2.697$^{a}$ | 1.895$^{b}$ |
| SeCh | 5.590$^{ab}$ | 2.334$^{b}$ | 2.409$^{a}$ |
| SeGlu | 6.072$^{a}$ | 2.312$^{b}$ | 2.650$^{a}$ |
| SEM | 0.20 | 0.08 | 0.09 |
| Probiotic (Pro) | | | |
| 0 mg/kg | 5.546 | 2.522 | 2.230 |
| 100 mg/kg | 5.613 | 2.373 | 2.406 |
| SEM | 0.16 | 0.07 | 0.07 |
| Interaction | | | |
| SeY- 0 mg/kg Pro | 5.076 | 2.760 | 1.835 |
| SeY- 100 mg/kg Pro | 5.077 | 2.634 | 1.955 |
| SeCh- 0 mg/kg Pro | 5.573 | 2.366 | 2.384 |
| SeCh- 100 mg/kg Pro | 5.607 | 2.303 | 2.434 |
| SeGlu- 0 mg/kg Pro | 5.955 | 2.439 | 2.470 |
| SeGlu- 100 mg/kg Pro | 6.190 | 2.184 | 2.830 |
| SEM | 0.28 | 0.13 | 0.07 |
| *p*-Values | | | |
| SeS | 0.007 | 0.008 | <0.001 |
| Pro | 0.772 | 0.161 | 0.108 |
| SeS × Pro | 0.875 | 0.741 | 0.468 |

[a-b] The heterogenous letters in the same column indicate significant differences ($p$≤0.05), and the homogenous letters indicate no significant difference ($p$>0.05).

### 3.2 *Intestinal morphology*

Table 3 and Figure 1 show the effects of different Se and probiotic sources on ileal morphology at 42 days. When SeCh and SeGlu were added to the diet instead of SeY, there was a significant increase in villus height, VH/CD, and goblet cell density, as well as a decrease in epithelial cell layer thickness and crypt depth ($p$<0.05). Birds fed SeCh-

supplemented diets had greater villus width than SeGlu and SeY and greater villus surface area than SeY-supplemented diets ($p$<0.05). The main effect of the Probiotic was that broilers fed the Probiotic had higher villus height, width, villus surface area, and goblet cell density, as well as a lower epithelial cell layer thickness ($p$ < 0.05). Broilers fed SeCh and SeGlu plus Probiotic had a higher villus surface area, VH/CD ratio, and goblet cell density, as well as a lower epithelial cell layer thickness, compared to broilers fed SeY alone ($p$ < 0.05).

**Table 3.** Effects of selenium-yeast (SeY), selenium-chitosan (SeCh), selenized glucose (SeGlu), probiotic and their various combinations on ileal morphology of broilers at 42 d

| 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) | | | | | | | |
| SeY | 1191.8$^{b}$ | 166.5$^{b}$ | 150.0$^{a}$ | 8.01$^{b}$ | 0.63$^{b}$ | 48.47$^{a}$ | 10.4$^{b}$ |
| SeCh | 1375.2$^{a}$ | 184.7$^{a}$ | 124.2$^{b}$ | 11.32$^{a}$ | 0.79$^{a}$ | 38.67$^{b}$ | 13.6$^{a}$ |
| SeGlu | 1366.6$^{a}$ | 167.1$^{b}$ | 128.4$^{b}$ | 10.73$^{a}$ | 0.72$^{ab}$ | 31.86$^{b}$ | 14.3$^{a}$ |
| SEM | 39.03 | 5.17 | 4.68 | 0.42 | 0.03 | 2.29 | 0.40 |
| Probiotic (Pro) | | | | | | | |
| 0 mg/kg | 1254.2$^{b}$ | 152.7$^{a}$ | 139.4 | 9.603 | 0.66$^{b}$ | 44.03$^{a}$ | 11.9$^{b}$ |
| 100 mg/kg | 1369.7$^{a}$ | 192.8$^{a}$ | 129.1 | 9.861 | 0.78$^{a}$ | 35.30$^{b}$ | 13.6$^{a}$ |
| SEM | 31.87 | 4.23 | 3.83 | 0.34 | 0.03 | 1.88 | 0.33 |
| Interaction | | | | | | | |
| SeY- 0 mg/kg Pro | 1052.4 | 162.1$^{bc}$ | 151.1 | 6.34$^{c}$ | 0.53$^{c}$ | 55.74$^{a}$ | 7.97$^{b}$ |
| SeY- 100 mg/kg Pro | 1331.2 | 170.9$^{bc}$ | 148.9 | 8.15$^{bc}$ | 0.72$^{bc}$ | 39.20$^{b}$ | 12.9$^{a}$ |
| SeCh- 0 mg/kg Pro | 1347.3 | 147.0$^{c}$ | 130.7 | 10.90$^{a}$ | 0.64$^{bc}$ | 41.04$^{ab}$ | 12.7$^{a}$ |
| SeCh- 100 mg/kg Pro | 1403.5 | 222.3$^{a}$ | 117.7 | 11.74$^{a}$ | 0.94$^{a}$ | 36.30$^{b}$ | 14.5$^{a}$ |
| SeGlu- 0 mg/kg Pro | 1360.9 | 171.1$^{bc}$ | 136.2 | 9.76$^{ab}$ | 0.60$^{c}$ | 33.33$^{b}$ | 13.4$^{a}$ |
| SeGlu- 100 mg/kg Pro | 1372.3 | 193.9$^{ab}$ | 120.7 | 11.50$^{a}$ | 0.83$^{ab}$ | 30.40$^{b}$ | 15.1$^{a}$ |
| SEM | 55.19 | 7.32 | 6.63 | 0.59 | 0.04 | 3.24 | 0.50 |
| $p$-Values | | | | | | | |
| SeS | 0.004 | 0.032 | 0.001 | <0.001 | 0.006 | 0.001 | <0.001 |
| Probiotic | 0.017 | <0.001 | 0.069 | 0.598 | 0.007 | 0.003 | <0.001 |
| SeS × Probiotic | 0.051 | <0.001 | 0.572 | 0.017 | <0.001 | 0.047 | <0.001 |

[a-d] The heterogenous letters in the same column indicate significant differences ($p$≤0.05), and the homogenous letters indicate no significant difference ($p$>0.05)

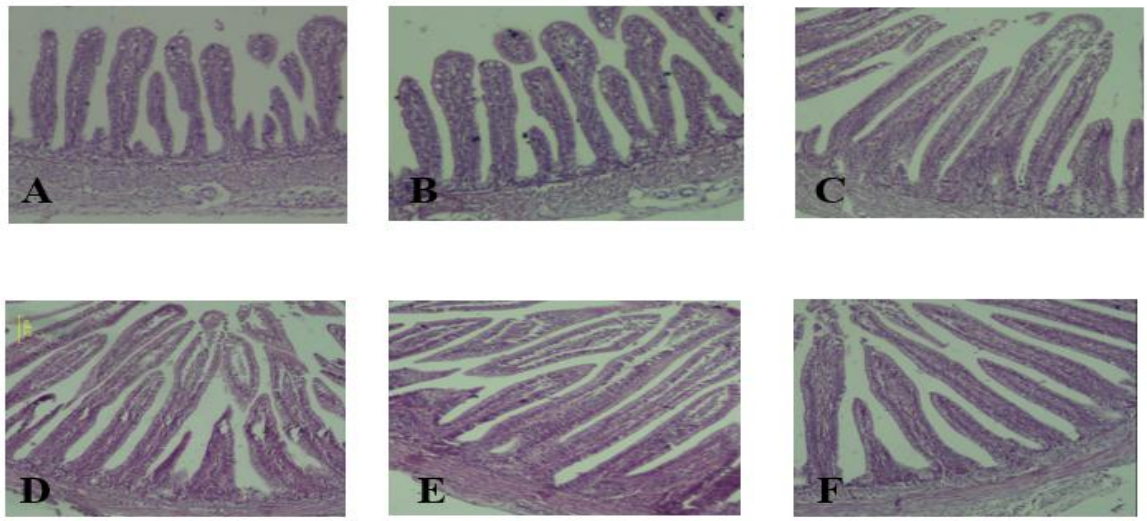


**Figure 1.** (A) selenium-yeast, (B) selenium-chitosan, (C) selenized glucose, (D) selenium-yeast + probiotic, (E) selenium-chitosan + probiotic, (F) selenized glucose + Probiotic

### 3.3 *Immune response*

Table 4 shows the effects of different Se and probiotic sources on the humoral immune response of broilers. The effect of Se source was observed at 28 and 42 days, with birds fed diets supplemented with SeCh and SeGlu exhibiting a higher total antibody response to SRBC, as well as higher IgG and IgM titers ($p < 0.05$). At 42 days, broilers fed probiotics had higher IgM and total antibody titers against SRBC than those fed unsupplemented probiotics ($p<0.05$). At 42 days, an interaction was observed between the Se source and probiotic levels for IgG ($p < 0.05$). Birds fed diets supplemented with SeCh and SeGlu, plus Probiotic, had higher IgG levels than those fed SeY without Probiotic.

**Table 4.** Effects of selenium-yeast (SeY), selenium-chitosan (SeCh), selenized glucose (SeGlu), probiotic and their various combinations on the antibody response to sheep red blood cells (log2) of broilers at 28 and 42 d

| Items | Total antibody | | IgG | | IgM | |
|---|---|---|---|---|---|---|
| | d 28 | d 42 | d 28 | d 42 | d 28 | d 42 |
| Selenium Source (SeS) | | | | | | |
| SeY | 3.3[b] | 4.5[b] | 1.4[b] | 2.1[b] | 2.0[b] | 2.5[b] |
| SeCh | 5.9[a] | 7.1[a] | 2.2[a] | 2.7[a] | 3.7[a] | 4.5[a] |
| SeGlu | 5.4[a] | 7.2[a] | 2.0[a] | 2.8[a] | 3.4[a] | 4.4[a] |
| SEM | 0.28 | 0.15 | 0.10 | 0.13 | 0.25 | 0.17 |
| Probiotic (Pro) | | | | | | |
| 0 mg/kg | 4.8 | 5.9[b] | 1.8 | 2.4 | 3.0 | 3.4[b] |
| 100 mg/kg | 4.9 | 6.3[a] | 1.8 | 2.5 | 3.0 | 4.1[a] |
| SEM | 0.23 | 0.13 | 0.08 | 0.74 | 0.20 | 0.14 |
| Interaction | | | | | | |
| SeY- 0 mg/kg Pro | 3.3 | 4.3 | 1.2 | 1.8[a] | 1.8 | 2.0 |
| SeY- 100 mg/kg Pro | 3.3 | 4.7 | 1.5 | 2.3[ab] | 2.1 | 2.9 |
| SeCh- 0 mg/kg Pro | 5.8 | 6.7 | 2.1 | 2.5[ab] | 3.6 | 4.2 |
| SeCh- 100 mg/kg Pro | 6.0 | 7.5 | 2.2 | 2.8[a] | 3.8 | 4.7 |
| 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 | 0.39 | 0.22 | 0.15 | 0.18 | 0.35 | 0.24 |
| *p*-Values | | | | | | |
| SeS | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Pro | 0.755 | <0.001 | 0.782 | 0.819 | 0.907 | <0.001 |
| SeS × Pro | 0.968 | 0.277 | 0.294 | 0.042 | 0.709 | 0.681 |

[a-b] The heterogenous letters in the same column indicate significant differences ($p\leq0.05$), and the homogenous letters indicate no significant difference ($p>0.05$).

IgG: immunoglobulin G, IgM: immunoglobulin M.

## 4 Discussion

In the current study, broilers fed diets supplemented with SeCh and SeGlu had lower COL counts and higher LAB/COL ratios in the ileum than broilers fed SeY. Furthermore, food supplementation with SeGlu significantly increased LAB numbers in the ileum as compared to SeY. Several studies have investigated the antibacterial effects of organic selenium sources. Khajeh Bami et al. (2022a) demonstrated that broiler chickens fed SeCh had higher LAB/COL ratios and lower COL counts in the ileum than those fed inorganic Se (11). According to one study, feeding Se nanoparticles to broilers decreased the number of COL in the cecum while increasing the number of LAB and the LAB/COL ratios in the ileum (21). According to Zhai et al.(2018), adequate selenium (Se) intake improved microbial balance in the intestines of mice (23). Trace elements in the diet can influence the diversity of intestinal microflora (24).

Furthermore, increasing the population of beneficial microbiota helps maintain the bird's health and reduces the presence of harmful bacteria (25, 26). In a recent study, feeding organic Se (bacterial organic Se or Se-yeast) versus inorganic Se reduced the number of COL and increased the counts of LAB in the cecum. Furthermore, this study shows that feeding organic Se reduces the ileum COL population (27). According to Lv et al.(2015), feeding Se-enriched probiotics versus inorganic Se increased LAB and decreased COL in piglets' intestines (28). Gangadoo et al. (2019) demonstrated that feeding Se nanoparticles to broilers

reduced the number of harmful bacteria (29). Diets containing Se supplementation with antioxidant function can modulate the diversity of the intestinal microbial population by suppressing oxidative stress, providing a more conducive environment for the growth and proliferation of beneficial bacteria (27). Selenium-chitosan and SeGlu can modulate intestinal microflora, which may improve broiler intestinal health (modulate gut barrier integrity) and immune response due to improved intestinal morphology and immune response.

In the current study, diets supplemented with SeCh and SeGlu increased villus height, VH/CD, and goblet cell density while decreasing epithelial cell layer thickness and crypt depth values in the ileum compared to the SeY diet. Furthermore, broilers given probiotic supplementation had higher villus height, villus width, villus surface area, and goblet cell density, as well as lower epithelial cell layer thickness in the ileum than broilers given unsupplemented Probiotic. Furthermore, interaction results revealed that birds fed diets supplemented with SeCh and SeGlu plus Probiotic had higher VH/CD, villus surface area, and goblet cell density, and lower epithelial cell layer thickness in the ileum compared to those fed SeY without Probiotic. According to these findings, a combination of synthetic organic Se supplementation and Probiotics appears to have a synergistic effect on improving broiler intestinal structure. The favorable response observed with SeCh and SeGlu could be attributed to improved absorption, enhanced compound stability, or anti-inflammatory activity. Khajeh Bami et al. (2022a) found that broilers fed diets supplemented with SeCh had higher VH/CD, villus surface area, and goblet cell density, as well as lower epithelial cell layer thickness, in the ileum and jejunum compared to sodium selenite (11). Muhammad et al.(2021) also demonstrated that feeding bacterial organic Se increased the villus height of the small intestin (27).

Furthermore, bacterial organic selenoprotein supplementation can affect intestinal morphology, as evidenced by increased villi height in the duodenum and ileum of broilers (8). Increasing villi height while decreasing crypt depth increases nutrient uptake and improves growth performance. On the other hand, the effect of Se supplement feeding on maintaining intestinal health is related to the regulation of microbial populations in the intestine (23). Furthermore, increasing the microbial population of the intestine increases nutrient absorption and stimulates the intestinal villi (30). The mechanism of action of SeCh and SeGlu in improving intestinal morphology is most likely due to a reduction in the growth of some harmful bacteria in the intestine. As a result, the findings of this study suggest that combining SeCh and SeGlu with probiotics in broiler diets may improve intestinal morphology by increasing the beneficial microbial population, as demonstrated in Table 3.

At 28 and 42 days, dietary supplementation with SeCh and SeGlu significantly enhanced total antibody response to SRBC, as well as IgG and IgM, compared to SeY. Furthermore, at 42 days, an interaction was observed between the Se source and Probiotic on serum IgG levels. Birds fed diets supplemented with SeCh and SeGlu, plus probiotics, had higher IgG levels than those fed SeY without probiotics. The improvement in immune response in this experiment may be associated with improvements in intestinal microbiota and morphology (30, 31). As a result, improving immune status could be attributed to improvements in intestinal morphology and microbial population. According to the current findings, nano-Se increased serum IgM and IgG levels compared to sodium selenite (29). According to Mohammadi et al.(2020) and Khajeh Bami et al. (2022b), feeding nano-Se versus inorganic Se improved the IgG, IgM, and total antibody response to SRBC (21, 32, 33). Selenium affects immune system regulation by reducing stress and increasing the activity of antioxidant enzymes (34). Studies have shown that using organic Se supplementation instead of sodium selenite in broilers resulted in an increase in serum total anti-SRBC and IgG titers (35, 36).

## 5 Conclusions

According to the findings of this study, SeCh and SeGlu, as new sources of organic selenium, are more effective than the common organic form of selenium (SeY). As a result, SeCh and SeGlu can be used as selenium additives in broiler diets. The addition of synthetic organic Se with Probiotics could improve broiler intestinal microflora, morphology, and immune response.

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## Conflict of Interest

The authors declare no competing interests.

## Author Contributions

Asma Shokrinejad Gerdin: Methodology, Investigation, Writing-original draft. Mohsen Afsharmanesh: Conceptualization, Methodology, Formal analysis, Writing – review and editing. Mohammad Khajeh Bami: Methodology, Investigation, Writing – review and editing, Formal analysis.

## Data Availability Statement

All data analyzed during this study are included in this article. Any other data are available from the corresponding author upon reasonable request.

## Ethical Considerations

All animal experiments were performed according to the guidelines for the care and use of laboratory animals and were approved by the Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman (approval number: IR.UK.VETMED.REC.2019-03-05).

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