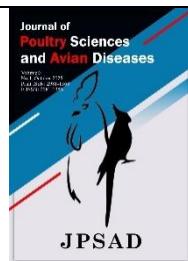


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Intestinal Microflora, Morphology, and Immune Response in Broiler Chickens Fed Various Organic Selenium and Probiotic Sources

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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 Na₂SeO₃ 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 saecum*, *Bacillus Subtilis*, *Bacillus lichen formis*, *Lactobacillus rhamnosus*, and *Lactobacillus plantarum* (lyophilized probiotic powder, 2.3×10^{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

¹Supplied per kg of diet: vitamin A (retinol), 12000 IU; vitamin D₃ (Cholecalciferol), 5000 IU; vitamin K₃, 2.55 mg; thiamin, 3 mg; riboflavin, 7.5 mg; vitamin B₆ (pyridoxine), 4.5 mg; vitamin B₁₂ (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 μ m) 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 \times 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, μ 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 \times Pro	0.875	0.741	0.468

^{a-b} The heterogenous letters in the same column indicate significant differences ($p \leq 0.05$), 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 ($p \leq 0.05$), and the homogenous letters indicate no significant difference ($p > 0.05$)

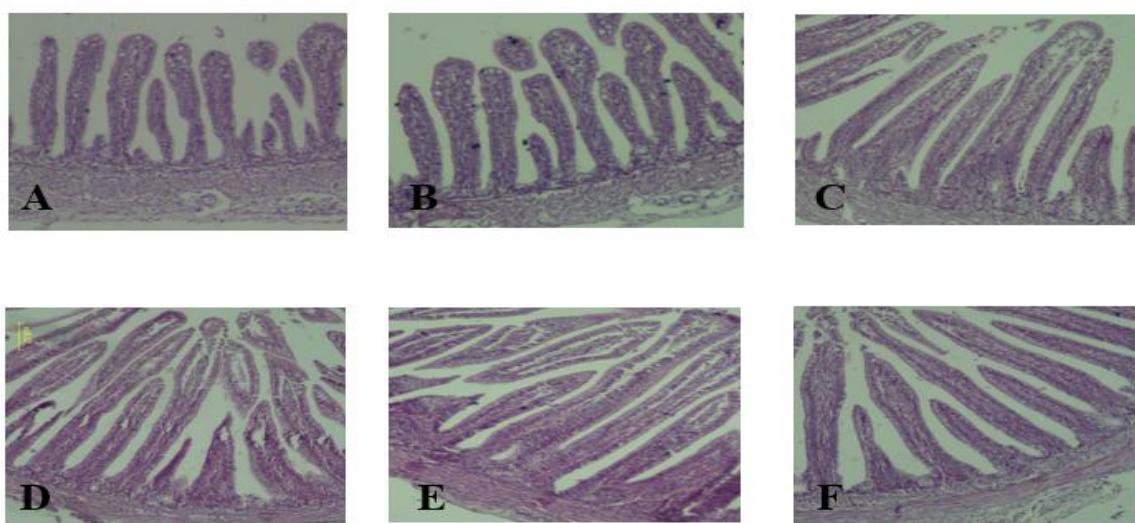


Figure 1. (A) selenium-yeast, (B) selenium-chitosan, (C) selenized glucose, (D) selenium-yeast + probiotic, (E) selenium-chitosan + probiotic, (F) selenized glucose + Probiotic

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 ($p \leq 0.05$), 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 intestine (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 Shokrinezad 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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References

1. Zhou X, Wang Y. Influence of dietary nano elemental selenium on growth performance, tissue selenium distribution, meat quality, and glutathione peroxidase activity in Guangxi Yellow chicken. *Poultry Science*. 2011;90(3):680-6. [PMID: 21325242] [DOI]
2. Li J, Shen B, Nie S, Duan Z, Chen K. A combination of selenium and polysaccharides: Promising therapeutic potential. *Carbohydrate Polymers*. 2019;206:163-73. [PMID: 30553309] [DOI]
3. Xia IF, Cheung JS, Wu M, Wong KS, Kong HK, Zheng XT. Dietary chitosan-selenium nanoparticles (CTS-SeNPs) enhance immunity and disease resistance in zebrafish. *Fish & Shellfish Immunology*. 2019;87:449-59. [PMID: 30703551] [DOI]
4. Mahmoud HED, Ijiri D, Ebeid TA, Ohtsuka A. Effects of dietary nano-selenium supplementation on growth performance, antioxidative status, and immunity in broiler chickens under thermoneutral and high ambient temperature conditions. *Journal of Poultry Science*. 2016;53(4):274-83. [PMID: 32908394] [PMCID: PMC477162] [DOI]
5. Silva VA, Clemente AHS, Nogueira BRF, De Carvalho AC, De Freitas LFVB, Ramos ADLS. Supplementation of selenomethionine at different ages and levels on meat quality, tissue deposition, and selenium retention in broiler chickens. *Poultry Science*. 2019;98(5):2150-9. [PMID: 30590669] [DOI]
6. Bakhshalinejad R, Akbari Moghaddam Kakhki R, Zoidis E. Effects of different dietary sources and levels of selenium supplements on growth performance, antioxidant status and immune parameters in Ross 308 broiler chickens. *British Poultry Science*. 2018;59(1):81-91. [PMID: 28906132] [DOI]
7. Dalia AM, Loh TC, Sazili AQ, Jahromi MF, Samsudin AA. Effects of vitamin E, inorganic selenium, bacterial organic selenium, and their combinations on immunity response in broiler chickens. *BMC Veterinary Research*. 2018;14:1-10. [PMID: 30143038] [PMCID: PMC6109295] [DOI]
8. Dalia AM, Loh TC, Sazili AQ, Samsudin AA. Influence of bacterial organic selenium on blood parameters, immune response, selenium retention and intestinal morphology of broiler chickens. *BMC Veterinary Research*. 2020;16:1-10. [PMID: 32993790] [PMCID: PMC7526326] [DOI]
9. Ahmadi M, Ahmadian A, Seidavi AR. Effect of different levels of nano-selenium on performance, blood parameters, immunity, and carcass characteristics of broiler chickens. *Poultry Science Journal*. 2018;6:99-108 [DOI]
10. Bakhshalinejad R, Hassanabadi A, Swick RA. Dietary sources and levels of selenium supplements affect growth performance, carcass yield, meat quality, and tissue selenium deposition in broilers. *Animal Nutrition*. 2019;5(3):256-63. [PMID: 31528727] [PMCID: PMC6737497] [DOI]
11. Khajeh Bami M, Afsharmanesh M, Salarmoini M, Ebrahimnejad H. Effects of selenium-chitosan on intestinal microflora, intestinal histomorphology, and immune response of broiler chickens. *Livestock Science*. 2022;255:104806 [DOI]
12. Marković R, Čirić J, Starčević M, Šefer D, Baltić MŽ. Effects of selenium source and level in diet on glutathione peroxidase activity, tissue selenium distribution, and growth performance in poultry. *Animal Health Research Reviews*. 2018;19(2):166-76. [PMID: 30683170] [DOI]
13. Lu J, Qu L, Shen MM, Wang XG, Guo J, Hu YP, et al. Effects of high-dose selenium-enriched yeast on laying performance, egg quality, clinical blood parameters, organ development, and selenium deposition in laying hens. *Poultry Science*. 2019;98(6):2522-30. [PMID: 30715535] [DOI]
14. Zhao M, Sun Q, Khogali MK, Liu L, Geng T, Yu L. Dietary selenized glucose increases selenium concentration and antioxidant capacity of the liver, oviduct, and spleen in laying hens. *Biological Trace Element Research*. 2021;199:4746-52. [PMID: 33506411] [DOI]
15. Victor H, Zhao B, Mu Y, Dai X, Wen Z, Gao Y. Effects of Se-chitosan on the growth performance and intestinal health of the loach *Paramisgurnus dabryanus* (Sauvage). *Aquaculture*. 2019;498:263-70 [DOI]
16. Wang L, Xiao JX, Hua Y, Xiang XW, Zhou YF, Ye L. Effects of dietary selenium polysaccharide on growth performance, oxidative stress, and tissue selenium accumulation of juvenile black sea bream, *Acanthopagrus schlegelii*. *Aquaculture*. 2019;503:389-95 [DOI]
17. Huang S, Yang W, Huang G. Preparation and activities of selenium polysaccharide from plant such as *Grifola frondosa*. *Carbohydrate Polymers*. 2020;242:116409. [PMID: 32564830] [DOI]
18. Zhou W, Li P, Liu J, Yu L. Kilogram-scale production of selenized glucose. *Industrial & Engineering Chemistry Research*. 2020;59(23):10763-7 [DOI]
19. Yuan D, Zhan XA, Wang YX. Effect of selenium sources on the expression of cellular glutathione peroxidase and cytoplasmic thioredoxin reductase in the liver and kidney of broiler breeders and their offspring. *Poultry Science*. 2012;91(4):936-42. [PMID: 22399733] [DOI]
20. Aviagen. Nutrition Specifications Manual: Ross 308. Scotland, UK: Aviagen Ltd.; 2014.
21. Khajeh Bami M, Afsharmanesh M, Espahbodi M, Esmaeilzadeh E. Effects of dietary nano-selenium supplementation on broiler chicken performance, meat selenium content, intestinal

microflora, intestinal morphology, and immune response. *Journal of Trace Elements in Medicine and Biology*. 2022;69:126897.[[PMID: 34814061](#)] [[DOI](#)]

22. Wegmann TG, Smithies O. A simple hemagglutination system requiring small amounts of red cells and antibodies. *Transfusion*. 1966;6(1):67-73[[DOI](#)]

23. Zhai Q, Cen S, Li P, Tian F, Zhao J, Zhang H. Effects of dietary selenium supplementation on intestinal barrier and immune responses associated with its modulation of gut microbiota. *Environmental Science & Technology Letters*. 2018;5(12):724-30[[DOI](#)]

24. Kasaikina MV, Kravtsova MA, Lee BC, Seravalli J, Peterson DA, Walter J, et al. Dietary selenium affects host selenoproteome expression by influencing the gut microbiota. *The FASEB Journal*. 2011;25(7):2492-9.[[PMID: 21493887](#)] [[PMCID: PMC3114522](#)] [[DOI](#)]

25. Guo FC, Williams BA, Kwakkel RP, Li HS, Li XP, Luo JY. Effects of mushroom and herb polysaccharides, as alternatives for an antibiotic, on the cecal microbial ecosystem in broiler chickens. *Poultry Science*. 2004;83(2):175-82.[[PMID: 14979567](#)] [[DOI](#)]

26. Khan SH, Ansari J, Haq AU, Abbas G. Black cumin seeds as phytogenic product in broiler diets and its effects on performance, blood constituents, immunity and caecal microbial population. *Italian Journal of Animal Science*. 2012;11(4):e77[[DOI](#)]

27. Muhammad AI, Mohamed DA, Chwen LT, Akit H, Samsudin AA. Effect of selenium sources on laying performance, egg quality characteristics, intestinal morphology, microbial population and digesta volatile fatty acids in laying hens. *Animals*. 2021;11(6):1681.[[PMID: 34199988](#)] [[PMCID: PMC8228612](#)] [[DOI](#)]

28. Lv CH, Wang T, Regmi N, Chen X, Huang K, Liao SF. Effects of dietary supplementation of selenium-enriched probiotics on production performance and intestinal microbiota of weanling piglets raised under high ambient temperature. *Journal of Animal Physiology and Animal Nutrition*. 2015;99(6):1161-71.[[PMID: 25900236](#)] [[DOI](#)]

29. Gangadoo S, Bauer BW, Bajagai YS, Van TTH, Moore RJ, Stanley D. In vitro growth of gut microbiota with selenium nanoparticles. *Animal Nutrition*. 2019;5(4):424-31.[[PMID: 31890921](#)] [[PMCID: PMC6920403](#)] [[DOI](#)]

30. Tang D, Li Z, Mahmood T, Liu D, Hu Y, Guo Y. The association between microbial community and ileal gene expression on intestinal wall thickness alterations in chickens. *Poultry Science*. 2020;99(4):1847-61.[[PMID: 32241465](#)] [[PMCID: PMC7587722](#)] [[DOI](#)]

31. Choct M. Managing gut health through nutrition. *British Poultry Science*. 2009;50(1):9-15.[[PMID: 19234925](#)] [[DOI](#)]

32. Boostani A, Sadeghi AA, Mousavi SN, Chamani M, Kashan N. Effects of organic, inorganic, and nano-Se on growth performance, antioxidant capacity, cellular and humoral immune responses in broiler chickens exposed to oxidative stress. *Livestock Science*. 2015;178:330-6[[DOI](#)]

33. Mohammadi E, Janmohammadi H, Olyayee M, Helan JA, Kalanaky S. Nano selenium improves humoral immunity, growth performance and breast-muscle selenium concentration of broiler chickens. *Animal Production Science*. 2020;60(16):1902-10[[DOI](#)]

34. Rao SVR, Prakash B, Raju MVLN, Panda AK, Poonam S, Murthy OK. Effect of supplementing organic selenium on performance, carcass traits, oxidative parameters and immune responses in commercial broiler chickens. *Asian-Australasian Journal of Animal Science*. 2013;26(2):247-.[[PMID: 25049783](#)] [[PMCID: PMC4093145](#)] [[DOI](#)]

35. Cai SJ, Wu CX, Gong LM, Song T, Wu H, Zhang LY. Effects of nano-selenium on performance, meat quality, immune function, oxidation resistance, and tissue selenium content in broilers. *Poultry Science*. 2012;91(10):2532-9.[[PMID: 22991539](#)] [[DOI](#)]

36. Moghaddam AZ, Hamzekolaei MM, Khajali F, Hassanzpour H. Role of selenium from different sources in prevention of pulmonary arterial hypertension syndrome in broiler chickens. *Biological Trace Element Research*. 2017;180:164-70.[[PMID: 28317078](#)] [[DOI](#)]