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Dietary Effect of Selenium-enriched Radish Sprouts, Vitamin E, and *Rhodobacter capsulatus* on Hypocholesterolemia and Immunity of Broiler

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Abstract

The study was designed to investigate the effects of dietary Selenium-enriched radish sprouts (Se-RS), Vitamin E (Vit E), and *Rhodobacter capsulatus* (*RC*) on immunity, cholesterol concentration, and fatty acid composition in broiler meat. A total of 100 two-week-old male broiler chicks were randomly assigned into five dietary groups: I) Control; II) Se-RS (5 µg/kg Se-RS); III) Se-RS+*RC* (5 µg/kg Se-RS + 0.2 g/kg *RC*); IV) Se-RS+Vit E (5 µg/kg Se-RS + 50 mg/kg Vit E) and V) Se-RS+*RC*+Vit E (5 µg/kg Se-RS + 0.2 g/kg *RC* + 50 mg/kg Vit E). Diets and clean drinking water were offered *ad libitum*. After the end of 3-wk of feeding period, serum cholesterol and triglycerides concentrations were lower ($P < 0.05$) in broilers fed Se-RS + *RC* + Vit E supplemented diet compared to the control diet. At the end of the 6-wk feeding period, birds fed the Se-RS+*RC*+Vit E diet significantly ($P < 0.05$) reduced cholesterol and triglycerides concentrations and improved the ratio of unsaturated fatty acids to saturated fatty acids in broiler meat. The highest ($P < 0.05$) number of leukocytes was observed in broilers fed Se-RS+*RC*+Vit E supplemented diet. Foot web index and weights of spleen, bursa, and thymus were significantly ($P < 0.05$) higher in birds fed Se-RS+*RC*+Vit E compared to the control diet. Our findings suggest that there are dual benefits of supplementing broiler diets with Se-RS+*RC*+Vit E because of improvements in the bird's immunity and meat quality that is important for health conscious consumers.

Introduction

Selenium (Se) is an essential mineral that can supplement poultry diets as a mineral premix. Compared to inorganic Se, organic Se can increase the concentration of Se in tissues but not affect growth performance, carcass traits, or plasma glutathione peroxidase (*GPx*) activity (Yoon *et al*., 2007). There is a synergistic effect of Se and vitamin E (Vit E) supplementation together compared to the use of either alone. Se has been reported to interact synergistically with other bioactive ingredients as well. The addition of 0.3 or 6.0% distilled fatty acids to diet increased growth of poultry, and this effect would have been higher if diet had been supplemented with Vit E and Se as well (Attia *et al*., 2006).

The *Rhodobacter capsulatus* (*RC*; ATCC 11166) is a photosynthetic purple bacteria involved in the production of single-cell protein, water purification, and fish culture (Kobayashi and

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Kurata, 1978). *RC* is also known as a hypocholesterolemic agent that reduces serum cholesterol and triglycerides concentrations in rats and pigs (Tsujii *et al*., 2007; 2008, 2016). It is also a potential agent for lowering cholesterol in egg-yolk of laying hens and Japanese quails, as well as meat in broilers (Salma *et al*., 2007a, b, c). The dietary supplementation of *RC* improved the ratio of unsaturated fatty acids (UFA) to saturated fatty acids (SFA) in egg-yolk of laying hens and Japanese quails and in broiler meat (Salma *et al*., 2007a, b, c; Afrose *et al*., 2010; Salma *et al*., 2011).

Hypercholesterolemia and immunity are interesting factors for commercial chicken producers, but still there is no breed or strain developed with superior immune-competency. Martens *et al*., (2008) reported that there was a strong relationship between hypercholesterolemia and the immune status in mice. In birds, yolk antibodies transfer to individual eggs on the basis of offspring sex and egg-laying sequence, and was suggested to be a strategy by which a mother may enhance the performance of the more vulnerable offspring (Hargitai *et al*., 2006).

Dietary supplementation of selenium-enriched radish sprouts (Se-RS) and *RC* in diets together decreased egg-yolk cholesterol but developed immunity in laying hens (Hossain *et al*., 2010). Since immune response and meat cholesterol are crucial factors for commercial broiler producers, this study was designed to investigate the effect of Se-RS, Vit E and *RC* on immune response and hypocholesterolemic functions in broilers.

Materials and Methods

Radish sprouts (*Raphanus sativas*) and *Rhodobacter capsulatus*

Radish sprouts were grown according to methods described by Yamanoshita *et al*. (2007) using Se-added liquid fertilizer (MI Tech Co., Ltd., Nagano, Japan). After harvesting, Se concentrations were analyzed using microwave induced plasma mass spectrometry (PIM-MS) and were determined to be 600 ppm Se by dry weight (Hossain *et. al*., 2010). Dried *RC* was obtained from Matsumoto Institute of Microorganism, Ltd., Matsumoto, Japan. The *RC* cell was grown in outdoor culture under natural illumination using previously described methods (Tsujii *et al*., 2007). In brief, the cells of *RC* were collected by centrifugation and spray-dried. The dried *RC* powder was mixed with high soft mineral mix (MIM Co., Ltd., Matsumoto, Japan) as 1:10 and stored at 4°C until use. The nutrient composition is shown in Table 1.

Table 1. Nutrient composition of *Rhodobacter capsulatus*[1] (on dry matter basis)

General composition	**(%)**
ME (Kcal/kg)	2050
Crude protein	59.80
Crude fat	9.40
Crude fiber	0.90
Crude ash	9.40
Mineral content	**(%)**
Na	1.59
P	1.39
Fe	0.05
Ca	0.10
K	1.03
Mg	0.36
Zn	0.01
Pigment content	**(%)**
Carotenoids	4.17
Bacteriochlorophyll	5.61
Vitamin content	**(mg/100g)**
Thiamin	8.70
Riboflavin	6.20
Pyridoxine	8.30
Biotin	0.20
Pentothenic acid	4.30
Niacin	10.00
Vitamin E	31.20

[1]Source: MIM Co. Ltd., Matsumoto, Japan.

Amino acid content	**(%)**
Arginine	3.42
Lysine	2.32
Histidine	1.14
Phenylalanine	2.29
Tyrosine	1.90
Leucine	4.15
Isoleucine	2.13
Methionine	1.52
Valine	3.28
Alanine	4.61
Glycine	2.91
Proline	2.22
Glutamic acid	5.65
Serine	1.86
Threonine	2.64
Aspartic acid	4.53
Tryptophan	1.34
Cysteine	0.38
Fatty acid content	**(%)**
Sweet cicely acid	0.17
Palmitic acid	0.68
Palmitoleic acid	0.17
Stearic acid	1.03
Octadecenoic acid	5.13
Phosphatide	9.32

Birds, management and diets

A total of 150 newly hatched male Chunky broiler chicks were obtained from Mori Hatchery, Fukuoka, Japan. Chunky broiler is one of the most famous broiler strains developed in Japan and uses Cornish line (Shen *et al*., 2002). Standard care and uniform management were employed in this experiment in accordance with "Guidelines for Regulation of Animal Experimentation, Shinshu University". The chicks were placed in a battery brooder and raised on a commercial starter diet until two weeks of age. At two weeks of age, 100 chicks with similar body weight (370−375 g) were selected and randomly assigned to five dietary groups (20 chicks per group). They were housed individually in wire cages (40 × 40 cm) with individual feed-troughs and common water-troughs. Room temperature was maintained at 20−24°C, and continuous lighting was provided throughout the experimental period. The basal finisher diet (Table 2; purchased from Toyohashi Shiryo, Kabushiki Gaisha, Aichi, Japan) was supplied as I) Control diet or supplemented diets with II) Se-RS (5 µg/kg Se-RS); III) Se-RS+*RC* (5 µg/kg Se-RS + 0.2 g/kg *RC*); IV) Se-RS+Vit E (5 µg/kg Se-RS + 50 mg/kg Vit E) and V) Se-RS+*RC*+Vit E (5 µg/kg Se-RS + 0.2 g/kg *RC* + 50 mg/kg Vit E). Vit E (alpha tocopherol) was purchased from Eisai Co., Ltd. 4-6-10 Koishikawa Bunkyo-ku, Tokyo, Japan. The supplementation levels of these feed additives were selected based on the individual effects of 5 µg/kg Se-RS on layer hen (Hossain *et al*., 2010) and 0.2 g/kg *RC* on broiler (Salma *et al*., 2007c). Clean drinking water and the experimental diets were supplied *ad libitum* for six weeks.

Table 2. Composition of basal diet (on dry matter basis)

Ingredient composition	**(%)**
Ground corn	58.00
Soybean meal	30.00
Soybean oil	3.30
Corn gluten meal	3.75
Fish meal	2.00
Limestone	1.00
DL-Methionine	0.20
Dicalcium phosphate	1.30
Sodium chloride	0.20
Vit. mix[1]/mineral mix[2]	0.25
Calculated nutrient	**(%)**
ME (Kcal/kg)	3100
Calcium	0.80
Total phosphorus	0.56
Lysine	0.90
Methionine	0.55
Carotenoids (mg/kg)	10.20

Analyzed nutrient	**(%)**
Crude protein	20.50
Crude fiber	3.60
Crude ash	6.20
Crude fat	6.50
Cholesterol (µg /kg)	84.50
Fatty acid content[3]	**(%)**
Palmitic acid	17.35
Stearic acid	4.11
Oleic acid	37.20
Linoleic acid	36.98
Linolenic acid	3.43
Unidentified fatty acids	0.72

[1]Vitamin mix provided per kilogram of diet: Vitamin A, 5,000 IU; cholecalciferol, 2000 IU; Vitamin E, 11 IU; Vitamin K_3, 4.0 mg; Vitamin B_1, 1.5 mg; Vitamin B_2, 4.3 mg; nicotinic acid, 44 mg; Ca pantothenate, 12 mg; pyridoxine, 4.0 mg, choline Cl, 220 mg; folic acid, 0.5 g; biotin, 220 µg; Vitamin B_{12}, 10 µg.

[2]Mineral mix was replaced by high soft mineral mix (4 g per kilogram of diet), in which experimental *R. capsulatus* were mixed. High soft mineral: SiO_2, 55.26%; CaO, 5.08%; MgO, 1.53%; Fe, 4.14%; Al, 7.67%; S, 1.74%; Na, 0.84%; C, 1.11%; Cl, 50 (mg/kg); MnO, 550 (mg/kg); B_2O_2, 35 (mg/kg); Cu, 19 (mg/kg); Zn, 80 (mg/kg); Co, 12 (mg/kg); Se, 1.6 (mg/kg); Ni, 19 (mg/kg); V, 14 (mg/kg); Mo, 3.6 (mg/kg); I, 10 (mg/kg).

[3]Percentage fatty acid methyl ester of total fatty acid methyl esters.

Record keeping and data

The weights of the birds were recorded at the beginning and end of the experimental period. Daily feed intake per bird and mortality were recorded during the experimental period. Feed efficiency was calculated by dividing body weight gain with feed intake and multiplying the quotient with 100.

Blood collection

Blood samples from each broiler were collected on the first day, third week and sixth week. Birds were fasted overnight and blood was collected from the brachial vein using sterilized syringes and needles. After 1 hr in room temperature, serum was isolated by centrifugation at 1,000 × *g* for 10 min. Serum samples were stored at –80°C until analysis.

White blood cell differentiation

The total number of leukocytes in the blood was assessed by hemocytometry. Approximately 100 µl of citrate-stabilized blood was analyzed in a Cell-Dyn 3500 hemocytometer (Abbott Laboratories, Abbott Park, IL) using a specialized configuration for chicken blood. The apparatus was standardized daily using Cell-Dyn 22 controls. Leukocytes were measured as number of cells × 10^9/L. Cell deposits consisting of 98% or more lymphocytes were smeared on a slide and stained with Wright's or May-Grunwald-Giemsa stain, and then scanned for atypical cells. Through the use of a fixed-size wire loop (1.0 mm in outer diameter, Brown and Sharpe Gauge No. 24), four loopfuls of cell deposits were used to deliver approximately 5.0 × 10^5 cells for each smear.

Liver, muscle and abdominal fat collection

At the end of the 6-wk feeding period, broilers were decapitated and the weights of carcass and edible meat were recorded. Left liver lobe, left side thigh (biceps femoris), and breast (pectoralis major) muscles without skin and adipose tissues were collected and washed with normal saline, blotted dry on filter paper, chopped, ground, and stored at –40°C. Muscle was dissected free of surface (non-intrinsic) fat. Abdominal fat content was measured by removing and weighing all adipose tissues surrounding the gizzard, cloaca, and adjacent muscles (Kubena *et al*., 1974).

Liver and muscle sample preparation

Total lipid content in liver and muscle samples were extracted following methods described by Elkin and Rogler (1990). In brief, liver and muscle samples (~1 g each) were homogenized in 12 mL of chloroform-methanol 2:1 (by volume) and filtered directly into a 50-mL volumetric flask using a glass microfiber filter. Following re-homogenization and refiltration, the liver and muscle filtrates were diluted to a final volume of 50 mL with chloroform-methanol 2:1 (by volume). In addition, to increase the concentration of lipid extract of the muscle samples, chloroform-methanol was removed by rotary evaporator (Virtis, Gardiner, NY) following centrifugation (1,000 × *g* for 10 min). The dried extract was dissolved in 5 mL of chloroform-methanol 2:1 (by volume). The lipid extract samples were stored at –80°C until analysis.

Enzymatic analysis

Total cholesterol, triglyceride, and high-density lipoprotein cholesterol (HDL-c) concentrations in serum were determined enzymatically using commercially available reagent kits (Wako Pure Chemical Industries Ltd., Tokyo, Japan) as described by Salma *et al*. (2007b). Cholesterol and triglyceride concentrations in total lipid extracts were obtained from thigh and breast muscle samples using the same reagent kits as those used for serum analysis.

Fatty acid determination

Total lipid extracts from muscle samples were transmethylated into fatty acid methyl esters and separated using gas chromatography (Simadzu, GC14B, Kyoto, Japan). Aliquots of 2 µL were injected into an Omegawax 250 capillary column (30 m × 0.25 mm i.d, 0.25-µm thickness; Supelco, Bellefonte, PA) with cyanopropyl methyl silicone as the stationary phase. Helium was used as the carrier gas at a constant flow rate of 4.7 mL/min. The following oven temperature program was used: 100°C for 1 min, 160°C at 40°C/min, then 240°C at 7°C/min, and 240°C held for 10 min. Peaks were separated using a flame-ionization detector and were quantified with an electric integrator (Shimadzu, CR-7A, Kyoto, Japan) using pure standard mixtures (Sigma, St. Louis, MO, USA). The weight of each fatty acid in all detected fatty acids was determined as a measurement value.

Lymphoid organs and cutaneous hypersensitivity

At the end of the experiment, four birds from each treatment were randomly selected and killed to determine the relative weights of the liver and lymphoid organs. The thymus tissue was carefully dissected from each side of the neck to ensure complete removal. Relative weights of organs were measured to the nearest 0.1 mg. The foot web index (FWI) was used as an index of the cell-mediated immune response. Another four birds from each treatment were selected and 0.1 mL PHA-P mitogen (1 mg/mL PBS) was intradermally injected into the left foot web. Sterile PBS (0.1 mL) was injected into the right foot web to serve as the control. Measurements were done with a constant tension caliper at 0 and 24 hrs after the injection. Foot web swelling was calculated by subtracting skin thickness at 24 hrs post-injection from that at 0 hr pre-injection. The FWI was converted into

absolute percentage values over that of initial 0-hr values (mean of left and right foot-web thickness).

Statistical Analysis

Data were analyzed using Fisher's protected least significant difference test. The NCSS (Number Cruncher Statistical System, NCSS Statistical Software, Kaysville, UT) Version 5.01 computer software package was used for all statistical analyses. All data are expressed as mean ± SEM. Differences were considered significant at the level of $P < 0.05$.

Results

Body weight gain, feed intake, and feed efficiency in broilers of five dietary groups are shown in Table 3. Body weight gain in broilers fed diets supplemented with Se-RS+*RC*, Se-RS+Vit E and Se-RS+*RC*+Vit E were higher ($P < 0.05$) than those in the control group. Feed efficiency also significantly improved ($P < 0.05$) in these broilers, though feed intake did not differ ($P < 0.05$). Among the treatments, broilers fed diet supplemented with Se-RS+*RC*+Vit E had the highest body weight gain and highest feed efficiency.

Table 3. Dietary effects of Se-enriched radish sprout, Vit E and *R. capsulatus* on body weight (BW) gain and feed efficiency of broilers for 6-wk feeding period[1]

Performance	Control	Se-RS	Se-RS+*RC*	Se-RS+Vit E	Se-RS+*RC*+Vit E
Initial BW (g)	370±0.85	369±1.49	374±2.95	368±2.54	372±2.29
Final BW (g)	2650±64.6^{c}	2875±47.9^{bc}	3125±72.2^{ab}	2975±47.9^{b}	3325±103.1^{a}
BW gain (g/d)	37.6±0.85^{c}	43.3±0.76^{bc}	45.1±1.30^{b}	45.5±0.42^{b}	47.6± 0.64^{a}
Feed intake (g/d)	161.7±1.86	161.2± 2.64	155.2±3.17	162.0±1.74	136.9± 2.86
Feed efficiency	23.2±0.74^{c}	26.9±0.36^{bc}	29.1±0.99^{b}	28.2±0.33^{b}	30.4±0.70^{a}

$^{a\text{-}c}$Means within a row without common superscripts differ significantly ($P < 0.05$).

[1]All measurements were done as fresh basis; values are mean ± SEM for 20 broilers per treatment group. There were five dietary groups: I) Control; II) Se-RS (5 µg/kg Se-enriched radish sprout); III) Se-RS+*RC* (5 µg/kg Se-enriched radish sprout + 0.2 g/kg *R. capsulatus*); IV) Se-RS+Vit E (5 µg/kg Se-enriched radish sprout + 50 mg/kg vitamin E) and V) Se-RS+*RC*+Vit E (5 µg/kg Se-enriched radish sprout + 0.2 g/kg *R. capsulatus* + 50 mg/kg vitamin E).

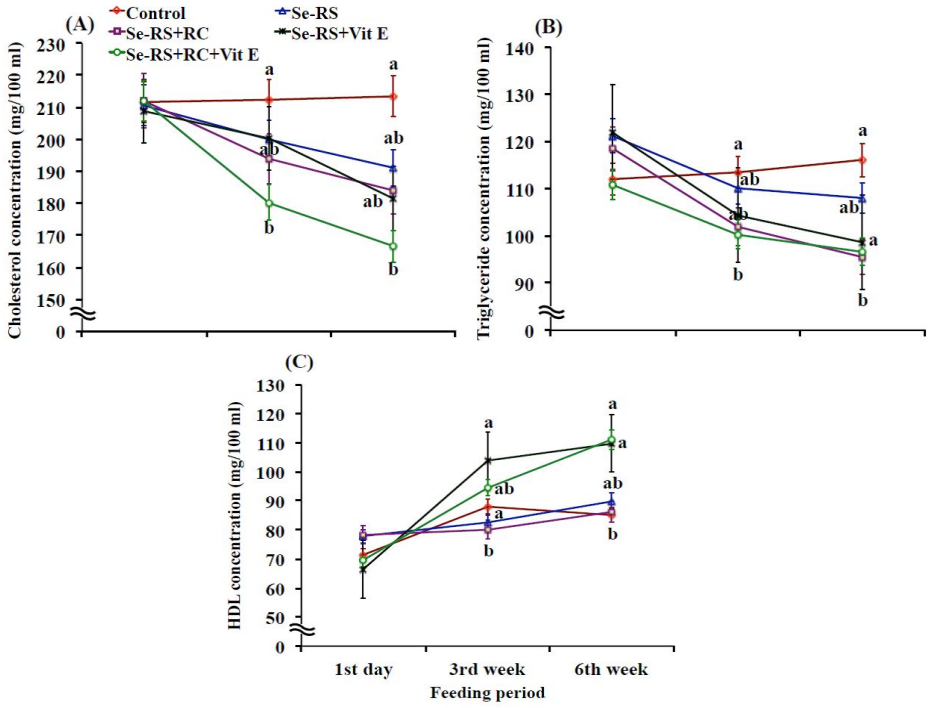

Figure 1. Effects of dietary Se-enriched radish sprout, vitamin E and *R. capsulatus* on (A) cholesterol, (B) triglyceride and (C) HDL-c concentration in serum.

Each line with error bar represents the mean ± SEM values; different letters above the error bars of the same feeding period indicate significant differences ($P < 0.05$).

The effects of dietary Se-RS, Vit E and *RC* on cholesterol, triglycerides and HDL-c concentration in serum are shown in Fig 1 (A, B & C, respectively). After the 6-wk feeding period, the concentrations of cholesterol and triglycerides in serum were significantly ($P < 0.05$) lower in broilers fed diet supplemented with Se-RS+*RC*+Vit E than broilers in the control group. The concentration of HDL-c in serum was significantly ($P < 0.05$) higher in the broilers fed Se-RS+*RC*+Vit E compared to the control diet.

Cholesterol and triglyceride concentration in breast and thigh muscles were significantly ($P < 0.05$) lower in broilers fed Se-RS+*RC* and Se-RS+*RC*+Vit E compared to the control diet (Fig. 2. A & B, respectively). The concentration of cholesterol and triglycerides in breast muscles were decreased by 38 and 41%, respectively, in broilers fed diet supplemented with Se-RS+*RC*+Vit E compared to the diet.

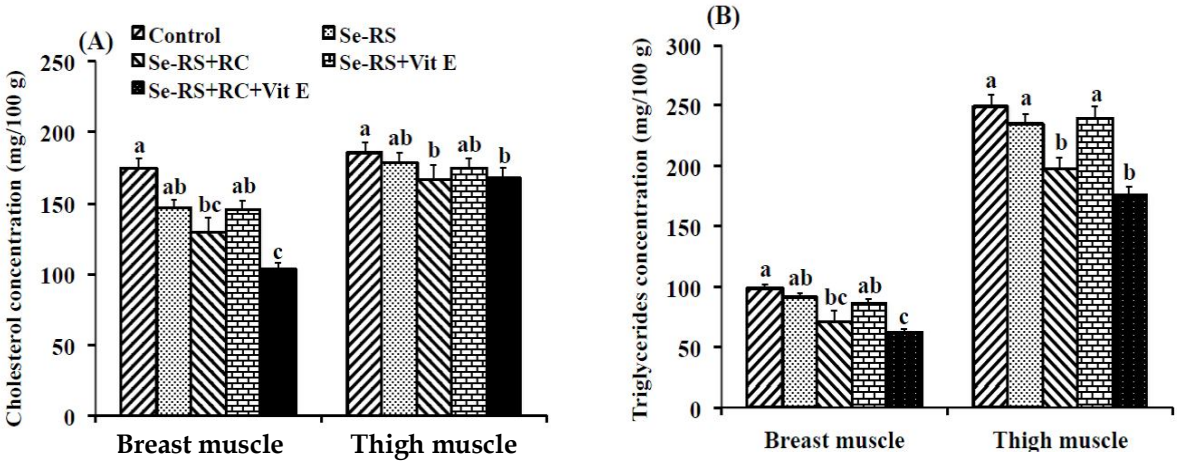

Figure 2. Effects of dietary Se-enriched radish sprout, vitamin E and *R. capsulatus* on (A) cholesterol and (B) triglycerides concentration in breast and thigh muscles.
Each line with error bar represents the mean ± SEM values; different letters above the error bars of the same feeding period indicate significant differences ($P < 0.05$).

Dietary effects of Se-RS, Vit E and *RC* on fatty acid composition in thigh muscle (Biceps femoris) of broilers are shown in Table 4. Among the fatty acids, saturated fatty acids decreased and unsaturated fatty acids increased in thigh muscle of the broilers fed diets supplemented with Se-RS+*RC*+Vit E compared to the control diet. The concentrations of oleic acid (18:1) in thigh muscle were higher in the broilers fed diets supplemented with Se-RS+*RC* and Se-RS+*RC*+Vit E than the control diet. Among the SFA, palmitic acid (C16:0) in thigh muscle slightly decreased with Se-RS+*RC* and Se-RS+*RC*+Vit E supplemented diets. The Se-RS+*RC* and Se-RS+*RC*+Vit E diets did not have significant effects on stearic acid (C18:0) concentration in thigh muscle. The concentration of monounsaturated fatty acids (MUFA) increased in the broilers fed diets supplemented with Se-RS+*RC* and Se-RS+*RC*+Vit E compared with the control diet. The ratio of UFA/SFA was greater in thigh muscle of broilers fed Se-RS+*RC*, Se-RS+Vit E and Se-RS+*RC*+Vit E.

The effects of dietary Se-RS, Vit E, and *RC* on total and differential leucocyte counts of broilers are shown in Table 5. At the end of the 3-wk feeding period, broilers had significantly ($P < 0.05$) higher amounts of total leucocytes, lymphocytes, eosinophils, and neutrophils with Se-RS+Vit E and Se-RS+*RC*+Vit E diets. Among the treatments, the broilers fed Se-RS+*RC*+Vit E showed the highest ($P < 0.05$) numbers of leucocytes, lymphocytes, and neutrophils. Basophils were significantly ($P < 0.05$) lower in broilers fed diets supplemented with Se-RS+*RC*+Vit E than broilers fed other diets. The number of monocytes was highest in the broilers fed Se-RS+Vit E and second highest in broilers fed Se-RS+*RC*+Vit E.

Table 4. Dietary effects of Se-enriched radish sprout, Vit E and *R. capsulatus* on fatty acid composition (% of total fatty acids) in thigh muscle (Biceps femoris) of broilers for 6-wk feeding period[1]

Fatty acid	Control	Se-RS	Se-RS+*RC*	Se-RS+Vit E	Se-RS+*RC*+Vit E
16:0	0.13±0.01	0.12±0.03	0.14±0.02	0.12±0.02	0.12±0.02
18:0	0.11±0.01	0.11±0.03	0.13±0.02	0.12±0.02	0.10±0.01
18:1	0.06±0.01^{b}	0.08±0.04^{a}	0.06±0.01^{b}	0.07±0.02^{ab}	0.08±0.00^{a}
18:2	0.09±0.02	0.09±0.02	0.07±0.01	0.08±0.01	0.08±0.01
20:4	0.07±0.01^{b}	0.06±0.01^{bc}	0.10±0.02^{a}	0.06±0.01^{bc}	0.05±0.01^{c}
SFA	0.23±0.02	0.23±0.06	0.27±0.04	0.24±0.04	0.22±0.03
MUFA[2]	0.06±0.01^{b}	0.08±0.04^{a}	0.06±0.01^{b}	0.07±0.02^{ab}	0.08±0.02^{a}
PUFA[3]	0.17±0.01	0.15±0.03	0.17±0.03	0.14±0.02	0.11±0.03
UFA/SFA	1.01±0.04^{b}	0.99±0.03^{b}	1.20±0.08^{a}	1.18±0.02^{a}	1.17±0.08^{a}

$^{a-c}$Means within a row without common superscripts differ significantly ($P < 0.05$).
[1]All measurements were done as fresh basis; values are mean ± SEM for 20 broilers per treatment group. There were 5 dietary groups: I) Control; II) Se-RS (5 µg/kg Se-enriched radish sprout); III) Se-RS+*RC* (5 µg/kg Se-enriched radish sprout + 0.2 g/kg *R. capsulatus*); IV) Se-RS+Vit E (5 µg/kg Se-enriched radish sprout + 50 mg/kg vitamin E) and V) Se-RS+*RC*+Vit E (5 µg/kg Se-enriched radish sprout + 0.2 g/kg *R. capsulatus* + 50 mg/kg vitamin E).
[2]MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; SFA: saturated fatty acids.

Table 5. Dietary effects of Se-enriched radish sprout, Vit E and *R. capsulatus* on leukocytes counts (µL) after 6-wk feeding period[1]

Parameter	Control	Se-RS	Se-RS+*RC*	Se-RS+Vit E	Se-RS+*RC*+Vit E
Leukocytes	16062±231^{b}	18993±973^{ab}	20269±1240^{ab}	19594±1351^{ab}	22188±407^{a}
Lymphocyte	11368±969^{c}	15308±300^{bc}	16540±1203^{ab}	16751±846^{ab}	18398±618^{a}
Eosinophil	386±94^{c}	1016±145^{b}	1346±379^{ab}	997±242^{b}	1423±231^{a}
Basophil	366±152^{b}	516±80^{a}	370±112^{b}	426±116^{b}	279±65^{c}
Neutrophil	2059±230^{b}	1425±185^{bc}	1594±228^{bc}	1178±102^{c}	2635±572^{a}
Monocyte	520±55^{bc}	347±10^{c}	670±93^{b}	1112± 288^{a}	793± 89^{b}

$^{a-c}$Means within a row without common superscripts differ significantly ($P < 0.05$).
[1]All measurements were done as fresh basis; values are mean±SEM for 20 broilers per treatment group. There were 5 dietary groups: I) Control; II) Se-RS (5 µg/kg Se-enriched radish sprout); III) Se-RS+*RC* (5 µg/kg Se-enriched radish sprout + 0.2 g/kg *R. capsulatus*); IV) Se-RS+Vit E (5 µg/kg Se-enriched radish sprout + 50 mg/kg vitamin E) and V) Se-RS+*RC*+Vit E (5 µg/kg Se-enriched radish sprout + 0.2 g/kg *R. capsulatus* + 50 mg/kg vitamin E).

Table 6. Dietary effects of Se-enriched radish sprout, Vit E and *R. capsulatus* on immune and internal organ weight (g/kg body weight) of broilers at end of the 6-wk feeding period[1]

Parameter	Control	Se-RS	Se-RS+*RC*	Se-RS+Vit E	Se-RS+*RC*+Vit E
Liver	15.27±0.73^{b}	15.07±0.09^{b}	16.65±0.70^{ab}	18.50±0.54^{a}	17.44±0.52^{a}
Spleen	1.27±0.03^{b}	1.36±0.08^{b}	1.45±0.09^{ab}	1.41±0.06^{ab}	1.65±0.08^{a}
Gallbladder	1.23±0.05	1.19±0.04	1.19±0.03	1.24±0.01	1.29±0.02
Thyroid gland	0.31±0.06^{c}	0.55±0.05^{a}	0.31±0.0^{c}	0.36±0.03^{b}	0.37±0.03^{b}
Thymus	0.44±0.06^{b}	0.69±0.05^{a}	0.47±0.06^{b}	0.71±0.09^{a}	0.76±0.11^{a}
Preen gland	0.72±0.07^{b}	0.72±0.11^{b}	0.96±0.14^{a}	0.89±0.10^{a}	0.73±0.11^{b}

$^{a-c}$Means within a row without common superscripts differ significantly ($P < 0.05$).
[1]All measurements were done as fresh basis; values are mean ± SEM for 20 broilers per treatment group. There were 5 dietary groups: I) Control; II) Se-RS (5 µg/kg Se-enriched radish sprout); III) Se-RS+*RC* (5 µg/kg Se-enriched radish sprout + 0.2 g/kg *R. capsulatus*); IV) Se-RS+Vit E (5 µg/kg Se-enriched radish sprout + 50 mg/kg vitamin E) and V) Se-RS+*RC*+Vit E (5 µg/kg Se-enriched radish sprout + 0.2 g/kg *R. capsulatus* + 50 mg/kg vitamin E).

The effects of dietary Se-RS, Vit E and *RC* on internal organ weights (including organs of the immune system) are shown in Table 6. Liver and thyroid gland weights were significantly ($P < 0.05$) greater in broilers fed diets supplemented with Se-RS+Vit E and Se-RS+*RC*+Vit E compared to the control diet. Broilers fed Se-RS had the highest thyroid gland weight. Spleen weight was significantly greater ($P < 0.05$) in broilers fed Se-RS+*RC*+Vit E than those fed

control or Se-RS diets. The weight of the gallbladder was similar ($P > 0.05$) among the treatment groups, but the weights of the thymus and preen gland were significantly ($P < 0.05$) differed between experimental treatments.

Foot web index (FWI) was significantly ($P < 0.05$) higher in all of the dietary treatments than control group, and the highest response was in broilers fed Se-RS+*RC*+Vit E (Fig. 3). There was no mortality in all treatments during the experimental period.

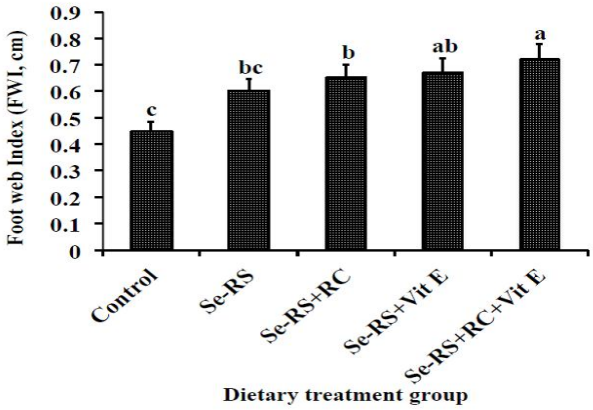

Figure 3. Effects of dietary Se-enriched radish sprout, vitamin E and *R. capsulatus* on foot web index. Each line with error bar represents the mean ± SEM values; different letters above the error bars of the same feeding period indicate significant differences ($P < 0.05$).

Discussion

It has been reported that supplementation of Se in diets at levels above 0.25 ppm improves growth performance of chicks (Colnago *et al*., 1984). On the contrary, Yoon *et al*. (2007), Deniz *et al*. (2005) and Payne and Southern (2005) reported that the growth performance of broilers was not affected by the source or level of Se supplementation. In contrast, the addition of Vit E to bird diet improves growth, viability (Serman *et al*., 1992), and productivity, and also provides a source of Vit E useful for human nutrition and reproductive health (Grau *et al*., 2001). Kim *et al*., (2010) reported that the combination of Se and the Vit E in broiler diets did not influence weight gain, feed intake, and feed efficiency. However, in this study, body weight gain and feed efficiency improved in the broilers receiving Se-RS, and the highest improvement was in the broilers receiving concurrent supplementation of Se-RS, *RC*, and the Vit E. Similar results were observed in a previous study (Hossain *et al*., 2010).

Selenium exists in several chemical forms. Feed efficiency was higher when broilers were fed organic Se than when broilers were fed inorganic or no Se (Deniz *et al*., 2005). Sunde (1997) reported that selenomethionine could be incorporated into protein at a rate similar to methionine, because Se and selenomethionine have similar atomic properties. In this study, Se-RS contained 80% Se-methylseleno-cysteine (MeSeCys) as the major chemical form of Se (Yamanoshita *et al*., 2007). Regardless of form, Se must be converted to selenocysteine before it can be incorporated into plasma *GPx* (Forstrom *et al*., 1978). Sunde and Hoekstra (1980) reported that inorganic sodium selenite can efficiently metabolize into selenocysteine, whereas Henry and Ammerman (1995) indicated that Se converts to selenocysteine at a lower rate of efficiency.

In this study, supplementation of Se-RS and/or Vit E did not lower cholesterol and triglyceride in the serum and meat after the 6-wk feeding period. Ryu *et al*. (2005) reported that selenium supplementation did not reduce cholesterol oxidation products, though Vit E did affect cholesterol oxidation. In this study, concurrent supplementation of Se-RS, *RC*, and Vit E was shown to reduce cholesterol and triglyceride concentration. Supplementation of *RC* also reduced serum and meat cholesterol and triglycerides in broilers (Salma *et al*., 2007b). The regulatory mechanisms that maintain a relatively constant serum cholesterol level include efficiency of intestinal cholesterol absorption, adjustments in the rates of

cholesterol biosynthesis, LDL receptor activity, secretion of cholesterol into bile, and hepatic conversion of cholesterol into bile acids (Kern, 1991). Dietary *RC* caused a similar hepatic bile acid synthesis in rats (Tsujii *et al*., 2007). The liver plays a key role in cholesterol homeostasis and is involved in the metabolism of LDL-c. The conversion of cholesterol to bile acids in the liver is the principal metabolic pathway of cholesterol, and is critical for the digestion and absorption of lipid nutrients and excreting excess cholesterol from the body (Russell, 2003). The accelerated fecal excretion of cholesterol by *RC* supplementation is also a consequent hypocholesterolemic effect. However, the mechanism(s) involved in the hypocholesterolemic effect is not fully understood as there is a scarcity of information on 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) enzyme activity and Farnesoid X receptor α (FXRα) gene expression associated with *RC* supplementation.

Poultry meat is sensitive to oxidation because of its high content of polyunsaturated fatty acids (PUFA) (Zhao *et al*., 2008). Supplementation of Se in diets can be a simple method for improving lipid oxidation of poultry meat (Ryu *et al*., 2005). Supplementation of α-tocopherol in poultry diet results in an increase of Vit E concentration in the tissue and increases the lipid oxidative stability of poultry meat (Grau *et al*., 2001). In this study, the ratio between the diversified unsaturated fatty acids (UFA) and SFA increased in the broiler meat by supplementation of Se-RS+*RC* and Se-RS+ Vit E+*RC*, but not with Se supplemented alone in diet. It may be possible that the intake of Se, Vit E, and *RC* needed for saturation of antioxidative selenoenzymes in muscle cells (*GPx-1*, *GPx-4*, thioredoxin reductase and selenoprotein) is higher than what is needed in blood plasma and blood cells. High contents of MUFA in animal products may be beneficial for human health. Several nutritional studies strongly support a relationship between SFA and risk for cardiovascular heart diseases, and hence there is a need to reduce consumption of SFA and increase consumption of MUFA (Mozaffarian and Clarke. 2009). Dietary Se-RS+*RC* and Se-RS+Vit E+*RC* increased MUFA and decreased SFA in thigh muscle. Foods rich in PUFA but low in cholesterol are helpful in reducing the incidence of cardiovascular diseases.

In this study, administering Se-RS and the concurrent supplementation of Se-RS, *RC*, and Vit E increased total leukocytes and lymphocytes. Similar types of results were also observed by Hossain *et al*. (2010). Kiremidjian-Schumacher *et al*. (1994) stated that Se supplementation in diets enhanced T-cell responses and antibody production, and also protected immune cells from oxidative stress. Similar to findings of Hossain *et al*. (2010), the lymphoid organs weight increased with Se-RS supplementation as well as the commingled supplementation of Se-RS, *RC*, and Vit E. Weights of the bursa and spleen increased in broilers with supplementation of Se and Vit E (Singh *et al*., 2006). The thymus is an important lymphoid organ involved in the development and differentiation of T lymphocytes (Eerola *et al*., 1987). The increase in bursal weight after supplementation of Se-RS or the commingled supplementation of Se-RS, *RC*, and Vit E, along with the increased production of circulatory immunoglobulins and immune complexes, suggest that there may be greater proliferation of bursal B cells, possibly due to decreased oxidative stress, with enhanced production of immunoglobulins and improved antibody responses. One possibility is that Se-RS along with *RC*, and Vit E may be associated with immune response mechanism by increasing membrane fluidity of lymphoid cells. Selenium is involved in antibody production, and stimulates phagocytosis and chemotaxis of macrophages and neutrophils, depending on the pathogen and on the levels of Vit E in the diet. It is an essential component of *GPx*, and plays a major role against diseases (Kidd, 2004).

Conclusion

Supplementation of Se-RS+*RC* and Se-RS+Vit E+*RC* to broiler diets improved body weight gain, feed efficiency, and weights of immune organs. Supplementation of Se-RS, *RC*, and Vit E lowered meat cholesterol and triglycerides. Therefore, this study suggests that there are dual benefits of concurrent supplementation of Se-RS, *RC*, and Vit E in broiler diets improved immunity and meat quality for health conscious consumers.

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