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Hypocholesterolemic Response to Karaya Saponin and *Rhodobacter capsulatus* in Broiler Chickens

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ABSTRACT : Dietary karaya saponin and *Rhodobacter capsulatus* (*R. capsulatus*) are known to have hypocholesterolemic actions, as reported in our previous studies. This study examined possible synergistic hypocholesterolemic effects of karaya saponin and *R. capsulatus* in broilers. A total of 150 broilers were allocated into 10 treatments: control, saponin 25 mg, saponin 50 mg, saponin 75 mg, saponin 25 mg+*R. capsulatus* 0.2 g, saponin 25 mg+*R. capsulatus* 0.4 g, saponin 50 mg+*R. capsulatus* 0.2 g, saponin 50 mg+*R. capsulatus* 0.4 g, saponin 75 mg+*R. capsulatus* 0.2 g and saponin 75 mg+*R. capsulatus* 0.4 g. Feed intake and feed efficiency were improved when karaya saponin and *R. capsulatus* were synergistically supplemented in the diet. Combinations of karaya saponin, especially supplementation of karaya saponin 50 mg+*R. capsulatus* 0.4 g were shown to have potential hypolipidemic actions in breast and thigh muscle cholesterol and triglycerides, serum cholesterol, low density lipoprotein-cholesterol and triglycerides, as well as improved high density lipoprotein (HDL)-cholesterol ($p<0.05$). Compared to the control, almost all the treatments significantly increased serum, liver and fecal concentrations of bile acids ($p<0.05$). Supplementation of both karaya saponin (75 mg) and saponin 50 mg+*R. capsulatus* 0.4 g reduced palmitic acid (C16:0) and stearic acid (C18:0) in a similar fashion ($p<0.05$). The ratios of PUFA:SFA or PUFA+MUFA:SFA in the thigh and breast muscle of broilers were greater in karaya saponin and *R. capsulatus* supplemented groups than in the control group. Thus, our study concluded that supplementation of karaya saponin synergistically with *R. capsulatus* in the diet of broilers is an effective way to obtain low-cholesterol, low-triglyceride and high HDL-cholesterol enriched poultry meat with a unique fatty acid balance. (**Key Words :** Broilers, Cholesterol, Karaya Saponin, *Rhodobacter capsulatus*, Triglycerides)

INTRODUCTION

Saponins are natural detergent forms of a heterogenous group of triterpene or steroid glycosides that occur in many hundreds of plant species (Sidhu and Oakenfull, 1986). A number of studies have shown that different kinds of saponins lower serum cholesterol levels in a variety of animals and human subjects (Southon et al., 1988; Potter et al., 1993; Matsuura, 2001). However, there is no agreement on the specific hypocholesterolemic activity of all saponins and the search for new saponin sources continues (Li et al., 2007; Son et al., 2007; Zhang et al., 2008; Zhao et al., 2008). Recently, we conducted a study to compare different kinds of saponins as a hypocholesterolemic agent in rats. It was observed that karaya saponin was the best hypocholesterolemic substrate, which decreased serum cholesterol concentration in rats by 34% while a less than 20% reduction was obtained with tea, quillaja or soyabean saponins (Afrose et al., 2009a). In our other study, karaya saponin successfully reduced serum (23%) and egg yolk cholesterol (15%) in laying hens, in a time-dependant manner (Afrose et al., 2009b). Further, to achieve a more effective cholesterol reduction in laying hens, we used combinations of karaya saponin and *R. capsulatus* as supplements in the diet of hens, where we observed a much higher degree of serum (32%) and egg yolk cholesterol (18%) reduction than that with the single saponin (Afrose et al., 2009c). In these studies, karaya saponin not only reduced cholesterol, but also decreased serum triglycerides and elevated high-density lipoprotein (HDL)-cholesterol levels (Afrose et al., 2009abc). *R. capsulatus* is a photosynthetic bacterium reported by our research group to have hypocholesterolemic actions in laying hens (Salma et al., 2007a), Japanese quails (Salma et al., 2008), rats (Tsujii et al., 2007) and pigs (Tsujii et al., 2008). Thus, we assumed

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that karaya saponin might be an effective hypocholesterolemic substrate to reduce broiler meat cholesterol, either individually or synergistically with *R. capsulatus*. In addition, the use of these two substrates in combination may allow lower doses of both agents. These two natural cholesterol-lowering agents may enable some significant molecular changes associated with the cholesterol-lowering mechanism to occur. Karaya saponin is a novel hypocholesterolemic substrate; there are no previous reports on its effect in broiler chickens in reducing meat cholesterol.

Thus, the objective of this study was to explore the effect of karaya saponin, alone or in combination with *R. capsulatus*, on meat and serum cholesterol, triglycerides and HDL-cholesterol, as well as on hepatic and fecal levels of bile acids in broiler chickens.

MATERIALS AND METHODS

Birds, management, and diets

A total of 200 newly hatched male Chunky broiler chicks were obtained from a commercial hatchery (Mori Hatchery, Fukuoka, Japan). Chunky broiler is one of the most famous broiler strains developed in Japan and belongs to the Cornish line. The conditions and standards of care employed in this experiment were in accordance with "Guidelines for Regulation of Animal Experimentation, Faculty of Agriculture, Shinshu University" and approved by the committee. The chicks were placed in a battery brooder and raised on a commercial starter diet until 2 weeks of age. At 2 wk of age, 150 chicks with similar body weight (370-375 g) were randomly divided into ten groups (n = 15). They were housed individually in wire cages (40×40 cm) with individual feed-troughs and a common water-trough. Room temperature was maintained at 20-24°C, and lighting was provided continuously throughout the experimental period. The experimental diet was provided for a period of 6 weeks and drinking water was given *ad libitum*. The basal finisher diet (Toyohashi Shiryo, Kabushiki Gaisha, Aichi, Japan) was supplemented with karaya saponin (Nacalai Tesque, Kyoto, Japan), *R. capsulatus*, and their combinations. The dried *R. capsulatus* was obtained from Matsumoto Institute of Microorganism, Ltd., Matsumoto, Japan. The composition of the basal diet is shown in Table 1. The dietary treatments were as follows: i) Control, ii) Saponin 25 mg, iii) Saponin 50 mg, iv) Saponin 75 mg, v) Saponin 25 mg+*R. capsulatus* 0.2 g, vi) Saponin 25 mg+*R. capsulatus* 0.4 g, vii) Saponin 50 mg+*R. capsulatus* 0.2 g, viii) Saponin 50 mg+*R. capsulatus* 0.4 g, ix) Saponin 75 mg+*R. capsulatus* 0.2 g, and x) Saponin 75 mg+*R. capsulatus* 0.4 g. As the individual effect of 0.2 g *R. capsulatus* and 0.4 g *R. capsulatus* on cholesterol was studied (Salma et al., 2007) by us previously, these treatments were excluded from this study.

Table 1. Composition of basal diet

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
Vitamin mix[1]/mineral mix[1]	0.25
Calculated nutritent	%
ME (kcal/kg)	3,100
Calcium	0.80
Total phosphorus	0.56
Lysine	0.90
Methionine	0.55

[1] Provided per kilogram of diet: vitamin A, 5,000 IU; cholecalciferol, 2,000 IU; vitamin E, 11 IU; vitamin K_3, 4.0 mg; thiamin, 1.5 mg; riboflavin, 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: 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).

Data collection

The weights of the birds were recorded at the beginning (370-375 g) and at the end of the experimental period. During the experimental period, daily feed intake per bird and mortality were recorded. Feed conversion efficiency was calculated from body weight gain and feed intake up during the 6-wk experimental period.

Blood collection

A blood sample from each individual broiler was collected at the end of the experimental period. Blood was collected from the brachial vein of overnight-fasted broilers using sterilized syringes and needles. After 1-h standing at room temperature, serum was isolated by centrifugation at 1,000×g for 10 minutes. Serum samples were stored at -80°C until analyzed.

Liver, muscle, and abdominal fat collection

At the end of the 6-week 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 from the same location in

each bird and washed with normal saline, blotted dry on filter paper, chopped, ground, and stored at -80°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 in liver and muscle samples was extracted following the method described by Elkin and Rogler (1990). Briefly, about 1 g of each liver and muscle sample was homogenized with 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 re-filtration, 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 extracted from muscle samples, the chloroform-methanol was removed by rotary evaporator (Virtis, Gardiner, NY, USA) following centrifugation (1,000×g for 10 min) and filtration; finally the dried extract was dissolved in 5 ml of chloroform-methanol 2:1 (by volume). The lipid samples were stored at -80°C until analyzed.

Enzymatic analysis

Total cholesterol, triglycerides, HDL-cholesterol, and glucose concentrations in serum were determined enzymatically using commercially available reagent kits, (Wako Pure Chemical Industries, Ltd., Tokyo, Japan) as described previously (Afrose et al., 2009a). Cholesterol and triglyceride concentrations in total lipid extracts obtained from liver and muscle (thigh and breast) samples were determined using the same reagent kits as those used for serum analysis. Low density lipoproteins (LDL)-cholesterol was measured using a cholesterol LDL kit (Daiichi Pure Chemicals Co. Ltd, Tokyo, Japan). The atherogenic index (AI) was calculated as the ratio of (total cholesterol–HDL-cholesterol)/HDL-cholesterol.

Fatty acid determination

Fatty acids in the muscles were determined for control, Saponin 75 mg and Saponin 50 mg+PSB 0.4 g treatments because of their high hypocholesterolemic activity. Total lipid extracts of muscle samples were transmethylated into fatty acid methyl esters and separated by gas chromatograph (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, USA) with cyanopropyl methyl silicone as 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 held for 1 min, increased to 160°C at 40°C/min, then to 240°C at 7°C/min, and held at 240°C for 10 min. Peaks were separated using a flame-ionization detector, quantified with an electronic integrator (Shimadzu, CR-7A, Kyoto, Japan) using pure standard mixtures (Sigma, St. Louis, MO, USA) and identified. The percentage weight of each fatty acid in total detected fatty acids was adopted as a measurement value.

Statistical analysis

Data were analyzed as a one-way ANOVA by the general linear model procedures of SAS (SAS Institute, 1996). The means for treatments showing significant differences in the ANOVA were compared using Fisher's protected least significant difference procedure. Values were expressed as mean±SD. Differences were considered significant at the level of $p<0.05$.

RESULTS

The effects of dietary karaya saponin with *R. capsulatus* on body weight, feed intake, feed efficiency and abdominal fat deposition are summarized in Table 2. Dietary treatments had no significant effect on final body weight, but when 0.4 g *R. capsulatus* was combined with karaya saponin (75 mg) there was a tendency to increase body weight. Compared to the control, feed intake was significantly reduced by the karaya saponin 25 mg+*R. capsulatus* 0.2 g, karaya saponin 25 mg+*R. capsulatus* 0.4 g, karaya saponin 50 mg+*R. capsulatus* 0.2 g and karaya saponin 75 mg+*R. capsulatus* 0.2 g treatments ($p<0.05$). In contrast, feed efficiency was improved by the karaya saponin 50 mg, karaya saponin 25 mg+*R. capsulatus* 0.2 g, karaya saponin 50 mg+*R. capsulatus* 0.4 g and karaya saponin 75 mg+*R. capsulatus* 0.2 g treatments when compared with the control diet. Abdominal fat deposition was significantly decreased by all the treatments except karaya saponin 25 mg and 50 mg ($p<0.05$). Mortality was 0% in all groups during the experimental period.

The effects of dietary karaya saponin with *R. capsulatus* on breast muscle, thigh muscle and liver cholesterol and triglyceride concentrations are shown in Table 3. Compared with the control, breast muscle cholesterol was significantly reduced by all the treatments except the karaya saponin 25 mg+*R. capsulatus* 0.2 g ($p<0.05$). The highest reduction of cholesterol was observed with karaya saponin 50 mg+*R. capsulatus* 0.4 g treatment ($p<0.05$). The highest reduction of thigh muscle cholesterol was observed for karaya saponin 50 mg+*R. capsulatus* 0.4 g and karaya saponin 75 mg+*R. capsulatus* 0.4 g treatments ($p<0.05$), compared with the control. Hepatic cholesterol and triglyceride concentrations were lower in broilers fed the saponin 50 mg +*R. capsulatus* 0.4 g supplemented diet than on the control diet ($p<0.05$).

Table 2. Effect of dietary karaya saponin combination with *R. capsulatus* on body weight, feed intake, feed efficiency and abdominal fat deposition in broiler chickens

Treatment	Production parameters			
	Final body weight (g)	Feed intake (g/bird/d)	Feed efficiency	Abdominal fat (%)
Control	2,911±98.3^{ab}	135.47±2.9^{a}	2.10±0.09^{a}	16.55±2.20^{a}
Saponin 25 mg	2,880±172.0^{ab}	130.36±4.6^{ab}	2.00±0.23^{a}	14.37±2.24^{ab}
Saponin 50 mg	3,097±125.7^{ab}	128.30±1.6^{ab}	1.62±0.20^{b}	16.97±3.08^{a}
Saponin 75 mg	2,810±111.4^{a}	128.35±3.3^{ab}	1.75±0.13^{ab}	9.87±1.70^{b}
Saponin 25 mg+SB 0.2 g	3,067±189.7^{ab}	125.75±3.2^{b}	1.60±0.09^{b}	10.70±0.98^{b}
Saponin 25 mg+SB 0.4 g	2,994±180.3^{ab}	119.32±1.8^{b}	1.82±0.16^{ab}	10.30±2.25^{b}
Saponin 50 mg+SB 0.2 g	3,119±140.8^{b}	123.62±2.7^{b}	1.97±0.17^{a}	12.55±1.23^{b}
Saponin 50 mg+SB 0.4 g	2,855±108.3^{ab}	126.05±2.9^{ab}	1.62±0.06^{b}	10.25±0.84^{b}
Saponin 75 mg+SB 0.2 g	2,950±109.1^{ab}	119.95±1.8^{b}	1.67±0.15^{b}	10.47±1.44^{b}
Saponin 75 mg+SB 0.4 g	3,007±153.0^{ab}	133.57±1.2^{a}	1.82±0.13^{ab}	12.10±2.04^{b}

$^{a-b}$ Values with different superscripts in the same column differ significantly ($p<0.05$) by the Duncan multiple-range test. Values are mean±SD for 15 broilers per group.

Feed efficiency = Feed intake:weight gain; PSB = *Rhodobacter capsulatus.*

The effect of dietary karaya saponin combination with *R. capsulatus* on breast muscle fatty acids is shown in Table 4. Of the fatty acid content within the meat muscle, palmitic acid (C16:0), oleic acid (C18:1), and linoleic acid (C18:2) were present in the higher concentration. Compared to the control, supplementation of karaya saponin (75 mg) and karaya saponin 50 mg+*R. capsulatus* 0.4 g significantly reduced saturated fatty acids palmitic (C16:0) and stearic (C18:0) and increased unsaturated fatty acids palmitoleic (C16:1), oleic (C18:1) and linoleic (C18:2) ($p<0.05$). The combined application of karaya saponin with *R. capsulatus* elevated the ratio between unsaturated and saturated fatty acids in breast muscle ($p<0.05$).

The effect of dietary karaya saponin with *R. capsulatus* on thigh muscle fatty acids is shown in Table 5. Compared to the control, supplementation of both karaya saponin (75 mg) and saponin 50 mg+*R. capsulatus* 0.4 g reduced palmitic (C16:0) and stearic (C18:0) acids in a similar fashion ($p<0.05$). Among the unsaturated fatty acids, oleic (C18:1) and linoleic (C18:2) acids were significantly enhanced by both saponin (75 mg) and saponin 50 mg+*R. capsulatus* 0.4 g, whereas palmitoleic acid (C16:1) was increased only by saponin (75 mg) supplementation. Neither saponin (75 mg) nor saponin 50 mg+ *R. capsulatus* 0.4 g had any influence on linolenic acid (C18:2), though total mono-unsaturated (MUFA) and poly-unsaturated (PUFA) fatty acids were increased by the saponin 50 mg+*R. capsulatus* 0.4 g treatment. Inversely, total saturated fatty acid (SFA) content was reduced by both treatments. The ratios of PUFA:SFA or PUFA+MUFA:SFA in the thigh and

Table 3. Effect of dietary karaya saponin combination with *R. capsulatus* on muscle and liver cholesterol and triglycerides in broiler chickens (mg/100 ml)

Treatment	Breast muscle lipid		Thigh muscle lipid		Liver lipid	
	Cholesterol	Triglycerides	Cholesterol	Triglycerides	Cholesterol	Triglycerides
Control	74.47±3.0^{a}	91.23±5.5^{a}	101.48±5.0^{a}	107.23±6.3^{a}	7.92±0.13^{a}	44.12±3.9^{a}
Saponin 25 mg	62.13±4.4^{b}	83.26±4.4^{ab}	75.77±4.2^{b}	93.27±3.7^{b}	7.42±0.38^{a}	30.25±1.1^{b}
Saponin 50 mg	58.71±1.8^{b}	82.66±3.4^{b}	88.35±4.6^{ab}	99.51±4.6^{ab}	7.00±0.19^{ab}	31.01±2.1^{b}
Saponin 75 mg	57.11±4.3^{b}	79.68±.8^{b}	72.47±5.5^{b}	90.51±5.6^{b}	6.97±0.27^{b}	29.00±2.2^{b}
Saponin 25 mg+PSB 0.2 g	68.73±1.5^{ab}	93.49±3.0^{a}	75.21±4.2^{b}	91.55±2.6^{b}	7.77±0.24^{a}	38.52±3.2^{ab}
Saponin 25 mg+PSB 0.4 g	60.45±2.1^{b}	80.70±1.8^{b}	71.24±3.8^{b}	88.54±6.8^{b}	6.37±0.40^{b}	35.00±3.5^{b}
Saponin 50 mg+PSB 0.2 g	49.67±2.4^{c}	81.13±2.6^{b}	73.01±3.6^{b}	86.24±5.2^{b}	6.72±0.28^{b}	35.51±2.5^{b}
Saponin 50 mg+PSB 0.4 g	50.24±1.3^{c}	75.65±3.4^{b}	61.54±3.3^{c}	91.22±4.0^{b}	5.47±0.37^{c}	29.74±4.6^{b}
Saponin 75 mg+PSB 0.2 g	63.50±4.7^{b}	78.21±1.4^{b}	71.71±2.6^{b}	92.24±6.8^{b}	6.67±0.37^{b}	32.30±2.9^{b}
Saponin 75 mg+PSB 0.4 g	61.10±3.2^{b}	78.42±3.4^{b}	63.75±2.9^{c}	96.22±5.8^{ab}	6.08±0.40^{bc}	32.53±3.8^{b}

$^{a-b}$ Values with different superscripts in the same column differ significantly ($p<0.05$) by the Duncan multiple-range test. Values are mean±SD for 15 broilers per group.

PSB = *Rhodobacter capsulatus.*

Table 4. Effect of dietary karaya saponin combination with *R. capsulatus* on breast muscle fatty acids

Fatty acid	Treatment		
	Control	Saponin 75 mg	Saponin 50 mg+PSB 0.4 g
16: 0	27.50±0.5[a]	22.61±1.35[b]	20.01±0.72[b]
16: 1	1.67±0.15[a]	2.15±0.23[b]	1.97±0.41[ab]
18: 0	17.96±0.33[a]	13.43±0.56[b]	11.43±0.48[b]
18: 1	28.06±0.31[a]	31.63±0.73[ab]	33.66±1.14[b]
18: 2	21.42±0.43[a]	25.90±0.85[b]	27.73±0.58[b]
18: 3	1.53±0.08[a]	2.13±0.07[b]	2.70±0.15[b]
Unidentified	1.85±0.22	2.08±2.20	2.15±0.21
MUFA	29.46±0.45[a]	33.46±1.06[b]	35.61±1.41[b]
PUFA	22.63±0.56[a]	27.89±0.63[b]	30.12±0.42[b]
SFA	45.61± 1.25[a]	36.22±2.41[b]	31.05±1.72[b]
PUFA:SFA	0.49 ±0.05[a]	0.77±0.08[b]	0.97±0.03[b]
(PUFA+MUFA):SFA	1.14±0.04[a]	1.68±0.06[b]	2.12±0.04[b]

[a-b] Values with different superscripts in the same row differ significantly ($p<0.05$) by the Duncan multiple-range test.
Values are mean±SD (mg/g) for 15 broilers per group.
PSB = *Rhodobacter capsulatus*. MUFA = Monounsaturated fatty acids, PUFA = Polyunsaturated fatty acids, SFA = Saturated fatty acids.

breast muscle of broilers were greater in karaya saponin and *R. capsulatus* supplemented groups than in the control group.

The effects of dietary karaya saponin with *R. capsulatus* on serum lipid parameters are shown in Table 6. Compared to the control, serum cholesterol was significantly decreased by the supplementation of karaya saponin (75 mg) and combinations of karaya saponin and *R. capsulatus*, while the highest degree of reduction was observed with karaya saponin 50 mg+*R. capsulatus* 0.4 g treatment ($p<0.05$). In contrast, a similar degree of reduction of triglycerides was observed with all the treatments ($p<0.05$). Single karaya saponin had no influence on HDL-cholesterol; extreme elevation of HDL-cholesterol (48%) was observed with karaya saponin 75 mg+*R. capsulatus* 0.2 g, while marginal increase was observed with other combinations. The greatest decrease in LDL-cholesterol was exhibited by saponin 50 mg+*R. capsulatus* 0.4 g treatment ($p<0.05$) compared with the control. Subsequently, the AI was significantly decreased by all the combinations of karaya saponin and *R. capsulatus* ($p<0.05$), but not by the single karaya saponin.

The effect of dietary karaya saponin with *R. capsulatus* on serum, liver and fecal concentrations of bile acids in broiler chickens is shown in Table 7. Compared to the control, almost all the treatments significantly increased

Table 5. Effect of dietary karaya saponin combination with *R. capsulatus* on thigh muscle fatty acids

Fatty acid	Treatment		
	Control	Saponin 75 mg	Saponin 50 mg+PSB 0.4 g
16: 0	25.93±0.27[a]	22.82±0.38[b]	22.66±0.21[b]
16: 1	2.65±0.27[a]	3.69±0.31[b]	3.11±0.26[ab]
18: 0	11.03±0.38[a]	8.73±0.18[b]	7.13±0.27[b]
18: 1	28.80±0.51[a]	31.96±0.67[b]	32.46±0.89[b]
18: 2	25.63±0.97[a]	28.40±0.60[b]	30.63±0.72[b]
18: 3	2.26±0.21	2.53±0.33	2.69±0.38
Unidentified	2.13±0.22	2.20±0.28	2.56±0.19
MUFA	31.36±1.24[a]	35.54±0.96[b]	35.48±0.86[b]
PUFA	28.61±0.87[a]	30.43±0.75[ab]	32.02±0.64[b]
SFA	36.80±1.23[a]	31.39±1.05[b]	29.81±1.26[b]
PUFA:SFA	0.77±0.09	0.87±0.07	1.07±0.05
(PUFA+MUFA):SFA	1.63±0.06[a]	2.10±0.08[b]	2.26±0.03[b]

[a-b] Values with different superscripts in the same row differ significantly ($p<0.05$) by the Duncan multiple-range test.
Values are mean±SD (mg/g) for 15 broilers per group.
MUFA = Monounsaturated fatty acids, PUFA = Polyunsaturated fatty acids, SFA = Saturated fatty acids, PSB = *Rhodobacter capsulatus*.

Table 6. Effect of dietary karaya saponin combination with *R. capsulatus* on serum lipid parameters in broiler chickens

Treatment	Serum lipid concentration (mg/100 ml)				
	Cholesterol	Triglycerides	HDL-cholesterol	LDL-cholesterol	AI
Control	136.31±4.8^{a}	93.49±1.3^{a}	32.48±2.5^{a}	60.21±4.5^{a}	3.08±0.7^{a}
Saponin 25 mg	118.25±3.3^{ab}	78.23±3.7^{b}	31.25±1.1^{a}	53.11±1.9^{ab}	2.86±0.5^{ab}
Saponin 50 mg	120.10±5.1^{ab}	73.00±.2^{b}	37.74±1.0^{ab}	48.53±2.9^{b}	2.12±0.4^{ab}
Saponin 75 mg	102.24±3.9^{bc}	72.12±2.1^{b}	38.50±2.7^{ab}	42.49±1.4^{c}	1.70±0.6^{b}
Saponin 25 mg+PSB 0.2 g	96.75±6.1^{c}	76.52±1.7^{b}	42.51±2.0^{b}	52.68±3.9^{ab}	1.44±0.6^{b}
Saponin 25 mg+PSB 0.4 g	108.17±6.0^{b}	77.30±4.1^{b}	34.00±2.3^{ab}	49.12±2.6^{b}	1.52±0.8^{b}
Saponin 50 mg+PSB 0.2 g	109.26±3.1^{b}	63.50±2.5^{c}	40.76±2.0^{b}	39.10±1.7^{c}	1.71±0.4^{b}
Saponin 50 mg+PSB 0.4 g	92.32±4.1^{c}	71.22±1.8^{b}	44.27±2.8^{b}	38.40±1.8^{c}	1.06±0.3^{c}
Saponin 75 mg+PSB 0.2 g	109.25±6.4^{b}	65.25±4.1^{c}	48.22±4.5^{b}	47.68±2.4^{bc}	1.28±0.8^{bc}
Saponin 75 mg+PSB 0.4 g	101.00±4.0^{bc}	77.29±3.0^{b}	43.14±1.9^{b}	56.23±2.3^{ab}	1.40±0.5^{b}

$^{a-b}$ Values with different superscripts in the same column differ significantly ($p<0.05$) by the Duncan multiple-range test. Values are mean±SD for 15 broilers per group.
LDL-cholesterol = Low density lipoprotein; HDL = High density lipoprotein; PSB = *Rhodobacter capsulatus.*
AI = (Total cholesterol-HDL-cholesterol)/HDL-cholesterol.

serum, liver and fecal concentrations of bile acids ($p<0.05$). Fecal cholesterol concentration was enhanced by karaya saponin 75 mg, karaya saponin 25 mg+*R. capsulatus* 0.4 g, karaya saponin 50 mg+*R. capsulatus* 0.2 g, karaya saponin 50 mg+*R. capsulatus* 0.4 g, and karaya saponin 75 mg+*R. capsulatus* 0.4 g treatments ($p<0.05$) when compared with the control. None of the treatments had any influence on serum glucose concentration. Liver weight remained almost unchanged although significant reduction was observed in broilers treated with karaya saponin 50 mg+*R. capsulatus* 0.4 g ($p<0.05$).

DISCUSSION

In recent years, due to the risk of cardiovascular diseases, consumers have to pay particular attention to reducing their consumption of cholesterol and saturated fatty acids, and increasing the intake of unsaturated fatty acids. Although previously some initiatives were taken with dietary chitin (Hossain and Blair, 2007), garlic (Konjufca et al., 1997) or chitooligosaccharide (Zhou, 2009) to reduce broiler meat cholesterol, the vast majority of these experiments elicited, at best, only a limited reduction in cholesterol. In this study, adding karaya saponin 50 mg+*R. capsulatus* 0.4 g to the diet of broilers, caused a significant reduction in the content of cholesterol (35%) and triglycerides (17%) in the broiler meat, and the ratio between the diversified unsaturated and saturated fatty acids was increased.

Our previous observations showed that *R. capsulatus* alone can reduce meat cholesterol in broilers by 19% (Salma et al., 2007b). In this study, we observed a 19%

Table 7. Effect of dietary karaya saponin combination with *R. capsulatus* on serum, liver and fecal concentrations of bile acids in broiler chickens

Treatment	Serum		Liver		Feces	
	Glucose (mg/dl)	Bile acid (mM/L)	Weight (% bwt)	Bile acid (nmol/g)	Cholesterol (mg/g)	Bile acid (μmol/g)
Control	301.5±17	0.017±0.003^{a}	2.07±0.24^{a}	48.22±4.6^{a}	0.63±0.06^{a}	27.3±2.3^{a}
Saponin 25 mg	299.6±20	0.023±0.002^{ab}	2.12±0.16^{a}	62.50±3.5^{b}	0.68±0.05^{ab}	36.2±4.2^{ab}
Saponin 50 mg	306.1±23	0.015±0.007^{a}	1.96±0.32^{ab}	51.36±4.1^{ab}	0.65±0.07^{a}	34.5±3.2^{ab}
Saponin 75 mg	296.0±13	0.036±0.009^{b}	1.86±0.22^{ab}	60.64±3.2^{b}	0.72±0.04^{b}	39.8±2.5^{b}
Saponin 25 mg+PSB 0.2 g	289.8±26	0.030±0.005^{b}	2.06±0.13^{a}	75.84±2.8^{b}	0.70±0.09^{ab}	42.6±3.4^{b}
Saponin 25 mg+PSB 0.4 g	315.7±41	0.021±0.004^{ab}	2.11±0.25^{a}	70.21±2.7^{b}	0.81±0.08^{b}	45.7±3.5^{b}
Saponin 50 mg+PSB 0.2 g	297.4±35	0.025±0.003^{b}	1.86±0.20^{ab}	73.36±4.6^{b}	0.78±0.05^{b}	43.1±4.4^{b}
Saponin 50 mg+PSB 0.4 g	303.2±21	0.041±0.008^{b}	1.63±0.18^{b}	68.56±3.4^{b}	0.84±0.07^{b}	50.4±2.6^{b}
Saponin 75 mg+PSB 0.2 g	295.8±22	0.026±0.005^{b}	1.87±0.06^{ab}	71.23±3.7^{b}	0.69±0.03^{ab}	42.6±3.7^{b}
Saponin 75 mg+PSB 0.4 g	290.9±18	0.022±0.002^{ab}	1.98±0.27^{ab}	68.71±2.8^{b}	0.76±0.08^{b}	46.3±3.4^{b}

$^{a-b}$ Values with different superscripts in the same column differ significantly ($p<0.05$). Values are mean±SD for 15 broilers per group.
PSB = *Rhodobacter capsulatus.*

reduction of meat cholesterol with saponin alone, whereas a much higher rate of cholesterol reduction (35%) in meat was obtained when a combination of karaya saponin and *R. capsulatus* was added to the diet. The muscle cholesterol pool comprises a slow turnover pool which equilibrates slowly with the serum cholesterol pool (Chobanian and Hollander, 1962). In the present study, the greater reduction of cholesterol concentration seen in thigh muscle may have been due to the presence of higher cholesterol concentration initially in thigh muscle than in breast muscle. Also, thigh muscles have a much greater content of slow-twitch fibres than breast muscles, which resulted in a quick cholesterol reduction. Further, the results of this study clearly demonstrate that the combination of karaya saponin 50 mg +*R. capsulatus* 0.4 g produces a significantly greater decrease in serum total cholesterol and LDL-cholesterol than saponin alone. A similar magnitude of total cholesterol and LDL-cholesterol reduction in rats (Afrose et al., 2009a) and laying hens (Afrose et al., 2009b) by karaya saponin was observed in our previous studies. Behall et al. (1984) pointed out that when a comparison of karaya gum with carboxymethycellulose gum or locust bean gum as hypocholesterolemic agents was made in human beings, karaya gum showed a similar function to that seen in our study in reducing cholesterol, triglycerides and LDL-cholesterol. High-density lipoproteins form a class of lipoproteins that vary somewhat in size (8 to 11 nm in diameter). These lipoproteins carry fatty acids and cholesterol from the body tissues to the liver. In this study, karaya saponin with *R. capsulatus* increased HDL-cholesterol, which can clear the surplus cholesterol from plasma, transport it, and excrete it in feces. In this study, the concentration of triglycerides in blood decreased in response to combined treatment with karaya saponin and *R. capsulatus*. This result agrees with the finding of our previous studies in laying hens in which individual supplementation of karaya saponin (Afrose et al., 2009b) and *R. capsulatus* (Salma et al., 2007b) decreased serum and meat cholesterol, respectively. Triglycerides are secreted from the liver into the serum by triglyceride-rich lipoproteins; therefore, impaired hepatic lipogenesis results in decreased triglyceride concentration in plasma. Although it is generally accepted that the principal action of saponins on serum cholesterol is by sequestration of cholesterol and bile acids in the intestine, another possible mode of action is via increased intestinal cell turnover rate. It was observed that an increased rate of exfoliation of intestinal cells caused by the membranolytic action of saponins could result in increased loss of cell membrane cholesterol contained in the exfoliated cells (Gee and Johnson, 1988; Milgate and Roberts, 1995).

The regulatory mechanisms that maintain a relatively constant serum cholesterol level include efficiency of intestinal cholesterol absorption, as well as 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). Our results indicate that hepatic bile acid and fecal excretion of bile acid was enhanced by 48% and 85%, respectively, by the combined supplementation of karaya saponin and *R. capsulatus*. Karaya saponin caused a similar hepatic bile acid synthesis in rats to that shown in our previous study (Afrose et al., 2009a). The liver plays a key role in cholesterol homeostasis, involving the metabolism of LDL cholesterol. In the liver, the conversion of cholesterol to bile acids is a principle pathway of cholesterol catabolism, providing sufficient amounts of bile acids as detergents for the digestion and absorption of lipid nutrients and removing excess cholesterol from the body (Russell, 2003). The accelerated fecal excretion of cholesterol by saponin supplementation is also a consequent hypocholesterolemic effect. However, the mechanism(s) involved in the hypocholesterolemic effect is not fully understood as there is a paucity of information regarding HMG-CoA enzyme activity and FXRα gene expression associated with saponin or *R. capsulatus* supplementation. These are considered as principle regulatory mechanisms of cholesterol homeostasis, although we think that, in this study, karaya saponin and *R. capsulatus* synergistically facilitated these pathways to reduce cholesterol.

The hypercholesterolemic effect of saturated fatty acids and the hypocholesterolemic effect of some unsaturated fatty acids are well established (Chan et al., 1991). In this study, the total SFA concentration of fatty acids in breast and thigh meat decreased in response to combined treatment of karaya saponin and *R. capsulatus* in the basal diet. Dietary SFA are an independent risk factor associated with cardiovascular diseases; their negative effects on LDL-cholesterol are stronger than the effects of dietary cholesterol (American Heart Association, 1988; Hornstra et al., 1998). Our study showed that this decrease was primarily due to the decrease in palmitic (C16:0) and stearic (C18:0) acids. However, the total MUFA as well as PUFA were improved in response to treatment with saponin+*R. capsulatus*. This result was primarily caused by the increase in oleic (C18:1) and linoleic (C18:1) acids. Other studies have reported oleic acid (C18:1) to be as effective as linoleic acid (C18:2) in lowering serum cholesterol concentrations (Mattson and Grundy, 1983; McDonald et al., 1989). This observation was supported by Chan et al. (1991) who observed that oleic (C18:1), linoleic (C18:2) and linolenic (C18:2) acids were equally hypocholesterolemic and that unsaturated fatty acids exerted their effects on plasma cholesterol through a common mechanism. The decrease of SFA in the tissues of chicken as a result of dietary karaya saponin with *R.*

capsulatus supplementation may have an effect on the cholesterol concentration in plasma. Keys et al. (1957) and Hegsted et al. (1965), however, suggested that polyunsaturated fatty acids have a cholesterol-lowering effect beyond that associated with the replacement of SFA in the diet. In addition, clinical data strongly support a relationship between cardiovascular diseases and the dietary intake of cholesterol and SFA. Although PUFA protects against coronary heart disease by providing more membrane fluidity than MUFA, they are more vulnerable to lipid peroxidation. The present results are similar to our previous reports (Salma et al., 2007b; Tsujii et al., 2008; Afrose et al., 2009c). The increase in oleic acid (C18: 1) observed in this study may have been the result of an enhanced effect of desaturase activity. This would agree with the result of a study conducted by Brenner (1989), in which desaturase was found to be the key enzyme required for the conversion of palmitic to palmitoleic acid and stearic to oleic acid.

Feed intake and feed efficiency were significantly improved while final body weight remained almost unchanged; this indicates that karaya saponin with *R. capsulatus* can potentially reduce meat cholesterol without any adverse effect on production performance. Interestingly, we observed that although feed intake decreased, there was an increase of body weight and it did not adversely affect any of the production traits and physiological condition. It was reported that dietary saponins depressed feed consumption in gerbils (Potter et al., 1993) and increased growth rate in broilers (Miah et al., 2004), tilapias (Francis et al., 2001) and rabblts (Morehouse et al., 1999). Thus, these results are agreement with our present findings. Seeman et al. (1973) and Seeman (1974) stated that saponin would help to increase absorption of nutrients from the intestine by increasing the diameter of villi which are permeable to large molecules like ferritin, and this mechanism may be responsible for better growth rate. Deposits of fat in the abdominal area of broilers are considered to be associated with cardiovascular disease, as well as sources of waste in the poultry industry. Not only does abdominal fat represent a loss in the market, but it also represents an added expense during the treatment of effluent produced when processing broilers. Thus, the results of this study revealed that this type of waste could be reduced by decreasing the fat content in the abdominal area of broilers by dietary supplementation with karaya saponin plus *R. capsulatus*.

In conclusion, the results of our experiment show that feeding karaya saponin synergistically with *R. capsulatus* in broilers is an effective way to obtain low-cholesterol, low-triglycerides and high HDL-cholesterol enriched poultry meat. Further investigation will be necessary to determine the long-term efficacy and mechanism of action of karaya saponin and *R. capsulatus* in prevention of hypercholesterolemia.

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