Effects of Dietary Sanguinarine on Growth Performance, Relative Organ Weight, Cecal Microflora, Serum Cholesterol Level and Meat Quality in Broiler Chickens

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The present study was conducted to investigate whether dietary sanguinarine (Sangrovit®, SGV) could affect growth performance, relative organ weights, gut microbiota, serum cholesterol levels, and malondialdehyde contents of leg meat in broiler chickens. A total of 840 day-old male broiler chicks (Ross breed) was randomly placed on 28 floor pens with rice straw as a bedding and subjected to one of four experimental diets; corn-soybean meal based control diet, the control diet added with avilamycin at 10 ppm as growth promoter (AGP) and SGV at the level of 20 (SGV20) or 50 ppm (SGV50). The final body weight (BW), daily BW gain, and feed conversion ratio (FCR) were significantly improved ($P < 0.05$) in broiler chickens fed either AGP, SGV20 or SGV50 compared with the control diet-fed chickens. Compared with the control group, relative jejunal weight was significantly lowered ($P < 0.05$) in SGV20-fed chickens and relative jejunal or ileal length was significantly increased ($P < 0.05$) in all SGV-fed chickens. Dietary SGV20, but not AGP, altered gut microbiota (especially increase in cecal lactic acid bacteria) compared with the control diet-fed chickens. Total cholesterol of broiler chickens fed on a diet containing SGV20 or SGV50 vs. the control diet was significantly reduced. Finally, the content of malondialdehyde in thigh meats as an indicator of lipid peroxidation was significantly lowered ($P < 0.05$) by dietary SGV compared with that seen in the control chickens. In conclusion, our study clearly reveals that supplementation of SGV into the broilers' diet at 20 or 50 ppm improved growth performance and altered various biological and physiological parameters such as relative organ weights, serum cholesterol levels, gut microbiota, and meat qualities in broiler chickens.

Key words: broiler chickens, growth performance, gut microbiota, meat quality, sanguinarine

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Introduction

World population is expected to increase to more than 9 billion by the year of 2050 and agricultural sectors, specifically global poultry industry, have rapidly responded to meet the growing meat demand, which has already increased to more than 4-fold during last 4 decades (Godfray *et al.*, 2010). In order to sustain the ever growing meat demand, it is true that the poultry production efficiency should be kept as high as possible. This statement however is based on the premises that the genetic potentials of chickens will be achieved and simultaneously any environmental challenges such as climate change, feed security, intensive production,

or ban on antibiotics in poultry industry are effectively controlled (Lillehoj and Lee, 2012).

It is well understood that low-level inclusion of in-feed antibiotics as growth promoters has played a significant role in the growth and development of the poultry industry for more than 50 years. Their efficiency in stimulating growth, improving feed conversion and reducing mortality/morbidity from clinical disease is well recognized (Thomke and Elwinger, 1998). However, the consumers concerns have been raised on the drug residues in meat products and on the occurrence of drug-resistant bacteria due to frequent use of antibiotics in diets (Yang *et al.*, 2009). As a result, the commonly practiced antibiotic inclusion into poultry diets has been banned in Europe since 2006, and this ban is further expected to affect the rest of the world. Consequently, alternative strategies to antibiotics have been mainstream of topics for researches in animal science to sustain the animal production. The most widely accepted alternatives (Lee *et al.*, 2003; Hashemi and Davoodi, 2008; Windisch *et al.*,

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2008; Yang *et al.*, 2009; Grashorn, 2010) include probiotics, prebiotics, exogenous enzymes, and phytochemicals including plant extracts in poultry.

Sanguinarine (SGV) as a phytochemical is a quaternary benzo[c]phenanthridine alkaloid (Dvorak and Simanek, 2007) and is an active component in the powdered stems, leaves, capsules and seeds of *Macleaya cordata*. With the well-established biological properties (Franz *et al.*, 2005), it has been added into diets for bovine, swine and poultry intended to promote growth and to increase feed intake. It is known that sanguinarine possess various biological activities such as analgesic, anti-inflammatory, anti-microbial and immunomodulatory activities (Simanek *et al.*, 2003; Dvorak *et al.*, 2006; Niu *et al.*, 2012). In addition, it is reported that SGV stimulates the secretion of enzymes in the intestine (Franz *et al.*, 2005) which is often regarded as one of characteristic properties of herbs and spices (Lee *et al.*, 2003). Furthermore, SGV is not metabolized into potentially harmful metabolite (e.g., benz[c]acridine) and is excreted without being absorbed through the small intestine (Zdarilova *et al.*, 2008).

It has been reported that dietary SGV affected growth performance (Vieira *et al.*, 2008a,b), cholesterol concentration (Yakhkeshi *et al.*, 2011), fatty acid profiles of breast meats (Juskiewicz *et al.*, 2011), and cecal microflora (Juskiewicz *et al.*, 2011; Yakhkeshi *et al.*, 2011) in broiler chickens and the inclusion levels in diets ranged from 12.5 to 50 ppm. It is of note that dietary sanguinarine stimulated growth at early ages of chickens (Vieira *et al.*, 2008a, b). At this stage, the effect of SGV on meat quality in broiler chickens has not been studied albeit that there is indication on its antioxidant property (Vrba *et al.*, 2004). In addition, the lack of sufficient number of controlled studies may preclude with confidence from concluding various reported biological properties of SGV in broiler chickens (Simanek *et al.*, 2003). Thus, the present study was conducted to investigate whether dietary SGV could affect growth performance, relative organ weights, cecal microflora, serum cholesterol concentration, and lipid peroxidation of leg meats in broiler chickens.

Materials and Methods

Experimental Design

Eight-hundred forty day-old male broiler chicks were obtained from local hatchery. Upon arrival, they were individually weighed and randomly placed 30 chicks per pen, 7 pens per treatment. The ingredients and composition of starter and finisher diets were shown in Table 1 and used as a control diet. The control diet was formulated to meet or exceed the nutrients requirement recommended by National Research Council (NRC, 1994). In order to formulate the experimental diets, avilamycin as growth promoter at the level of 10 ppm and two levels of SGV (Sangrovit®, Phyto-biotics, Eltville, Germany) at 20 (SGV20) and 50 (SGV50) ppm were added into the control diet at the expense of wheat flour. Sangrovit® is a commercial product composed of 1.5 % sanguinarine as an active component. Diet and water were provided on *ad libitum* basis. All experimental protocols

were approved by the Animal Care Committee of Konkuk University. The temperature of facility was maintained at 32°C during the first week posthatch and gradually decreased to reach 25°C at 3 weeks.

Sampling

Feed intake per pen and individual body weight (BW) were measured on a weekly basis and used to calculate feed conversion ratio (FCR). At 5 weeks, blood was sampled from the randomly selected seven chickens per pen after cervical dislocation. Sera were obtained by gentle centrifugation and stored at −20°C prior to use. Immediately after blood sampling, abdominal fat and organs such as spleen and bursa of Fabricius were excised, weighed and expressed as relative weight to BW. In addition, leg and breast meats were sampled and weighed, and the former stored at −20°C for later measurement of malondialdehyde contents. Breast meat was discarded. Whole intestine was sampled and the length and weight of each segment of small intestine were recorded. Duodenum was defined as the pancreatic loop, jejunum as the segment from the end of the duodenum to Meckel's diverticulum and ileum as the region between Meckel's diverticulum and ileocecal junction. Ceca were sampled and stored on ice until used for gut microbiota analysis on the same day.

Total Cholesterol Measurement

Total cholesterol concentration in serum samples was spectrophotometrically measured using the commercially available kit (Cholesterol E kit, Asan Pharmaceutical Co., Seoul, Korea).

Intestinal Microflora

Individual cecal contents were aseptically sampled and kept on ice until use. Cecal contents were then subjected to serial 10-fold (w/v) dilution with ice-cold PBS. Total microbes were counted after grown on total plate agar (Becton Dixon, Franklin Lakes, NJ, USA), lactic acid bacteria on MRS agar (Becton Dixon), and total coli forms on MacConkey agar (Becton Dixon) at 37°C for 24 h. The results were expressed as log base 10 colony-forming unit (cfu) per gram of cecal contents.

Measurement of Malondialdehyde (MDA) in Leg Meat

MDA Standard Curve

A small quantity (73.2 mg) of 1,1,3,3-tetra-ethoxypropane was weighed in a test tube, added with 10 ml 0.1N HCl and immersed into a boiling water bath for 5 min. The test tube was then immediately cooled down under running tap water, diluted to reach 100 ml with distilled water and used as a stock solution (239 µg/ml). The stock solution was used to make serial dilutions which were then measured at 532 nm with UV-visible spectrophotometer (Beckman DU-530, Beckman Counter, Inc., Fullerton, CA, USA). Standard curve was generated and used to calculate unknown samples as described elsewhere (Botsoglou *et al.*, 1994).

MDA Measurement

MDA levels in thigh meats were analyzed as described elsewhere (Botsoglou *et al.*, 2002) with minor modifications. In brief, samples that had been stored at −20°C were thawed at 4°C and used for analysis. Initially, 2 gram samples were

Table 1. The ingredient and composition of the diet

Ingredients	Starter (1–21 days)	Finisher (21–35 days)
Yellow corn, g	569.3	538.2
Wheat flour, g	30.6	31.0
Rice polishing, g	20.0	20.0
Soybean meal, g	152.8	145.4
Rapeseed meal, g	30	45.0
Corn gluten meal (60%), g	54.6	62.2
Distillers grain (CP 28%), g	—	50.0
Dehulled soybean meal (46%), g	50	—
Fish meal (50%), g	28	28.0
Animal fat, g	30	37.4
Limestone, g	8.2	10.8
DL-methionine (99%), g	1.8	1.3
L-lysine HCl (78%), g	2.8	—
Lysine (liquid) (24%), g	—	10.2
Vitamin mineral mixture, g	2.6	2.6
NaHCO ₃ , g	1.0	—
Threonine (99%), g	0.5	1.0
Salt, g	3.0	3.0
Choline Cl, g	0.9	1.2
Dicalcium phosphate, g	13.9	12.7
Total, g	1000	1000
Calculated values		
TMEn, kcal/kg	3100	3100
Crude protein	20.5	20.0
Ca, %	0.9	1.0
Available P, %	0.4	0.4
Lysine, %	1.2	1.1
Total TSAA, %	0.9	0.9

Provided per kg of diet: vitamin A, 18000 IU; vitamin D₃, 3750 IU; vitamin E, 30 IU; vitamin K₃, 2.7 mg; vitamin B₁, 3.0 mg; vitamin B₂, 9.0 mg; vitamin B₆, 4.5 mg; vitamin B₁₂, 30.0 mg; niacin, 37.5 mg; pantothenic acid, 15 mg; folic acid, 1.5 mg; biotin, 0.07 mg; Fe, 75.0 mg; Zn, 97.5 mg; Mn, 97.5 mg; Cu, 7.5 mg; I, 1.5 mg; Se, 0.2 mg.

weighed into a 50 ml/ centrifuge tube, followed by 5 ml/ 0.8% butylated hydroxytoluene (BHT) (dissolved in hexane) and 8 ml/ 5% trichloroacetate (dissolved in distilled water). The mixture was then mechanically homogenized for 30 s and centrifuged (Mage 210, Hanil, Korea) at 3,000 *g* for 5 min. The bottom aqueous layer was carefully filtered (Whatman #1) and made to reach 10 ml/ with 5% trichloroacetate. A 3 ml/ aliquot was transferred to 10 ml/ screw-capped tube and added with 0.8 ml/ 2-thiobarbituric acid (dissolved in distilled water). Following incubation at 70°C for 30 min with gentle shaking, the tube was cooled in ice-cold bath for 7 min and kept at room temperature for another 45 min. The reaction mixture was measured at 532 nm using UV-visible spectrophotometer. All assays were repeated twice.

Statistical Analysis

Pen was considered as an experimental unit. All data were analyzed by analysis of variance using the General Linear Model procedure of SAS program (SAS, 1999). Differences between means were analyzed using Duncan's multiple range test. A statistical significance was preset at $P < 0.05$ unless otherwise stated.

Results

Growth Performance

Final BW and overall BW gain were significantly increased ($P < 0.05$) in all treated groups compared with control group (Table 2). BW gain, feed intake and FCR at days 1–21 were not altered by any of treatments. At days 22–35, broiler chickens fed diets containing either AGP, SGV20 or SGV50 had greater BW gain compared with the control diet-fed chickens. At days 22–35, broiler chickens on a diet added with SGV50 consumed more feed compared with the control group. However, dietary AGP or SGV20 failed to affect feed intake compared with the control chickens. FCR was significantly low ($P < 0.05$) in all treated groups compared with the control group, especially the diets on AGP and SGV20 groups being more efficiently utilized.

Relative Organ, Abdominal Fat and Edible Meat Weights Per 100 g BW

At day 35, relative weight of organs (i.e., spleen and Bursa of Fabricius), abdominal fat, and leg and breast muscles when expressed as 100 g BW were not affected ($P > 0.05$) by any of treatments (Table 3).

Table 2. Effect of sanguinarine on growth performance in broiler chickens¹

	Control	AGP	SGV20	SGV50
Initial BW, g/bird	46.7±0.0	46.8±0.0	46.8±0.0	46.7±0.0
Final BW, g/bird	1836±27 ^c	2104±8 ^a	2067±13 ^{ab}	2047±16 ^b
BW gain, g/d/bird				
d 1-21	45.1±0.4	46.4±0.3	46.4±0.4	45.9±0.4
d 22-35	59.3±2.6 ^b	77.8±0.5 ^a	75.7±1.2 ^a	74.8±1.2 ^a
d 1-35	51.6±1.0 ^b	58.8±0.3 ^a	57.3±0.5 ^a	57.0±0.8 ^a
Feed intake, g/d/bird				
d 1-21	59.0±0.6	58.3±0.2	59.2±0.3	57.3±1.3
d 22-35	127.6±2.5 ^b	138.7±1.1 ^{ab}	136.2±0.7 ^{ab}	145.8±7.0 ^a
d 1-35	86.8±1.2	89.6±0.4	89.9±0.5	93.3±3.9
FCR, feed/gain				
d 1-21	1.31±0.01	1.26±0.01	1.28±0.01	1.25±0.04
d 22-35	2.16±0.07 ^a	1.79±0.02 ^b	1.80±0.03 ^b	1.95±0.08 ^b
d 1-35	1.68±0.01 ^a	1.52±0.00 ^c	1.57±0.02 ^{bc}	1.64±0.05 ^{ab}

^{a-c} Mean±SE values ($n=7$ pens/treatment) in a same row with different superscripts are significantly different ($P<0.05$).

¹ Day-old broiler chicks were fed a corn-soybean meal based control diet or the control diets added with either antibiotic growth promoter (AGP) or sanguinarine at the concentration of 20 (SGV20) or 50 ppm (SGV50) for 35 days. Feed intake per pen and individual body weight (BW) were measured on a weekly basis and used to calculate feed conversion ratio (FCR).

Table 3. Effect of sanguinarine on the relative weights of spleen, bursa of Fabricius, abdominal fats, leg and breast muscles in broiler chicken¹

	Control	AGP	SGV20	SGV50
Spleen ²	0.12±0.01 ³	0.10±0.01	0.13±0.02	0.13±0.01
Bursa of Fabricius ²	0.17±0.01	0.20±0.03	0.23±0.02	0.19±0.03
Abdominal fat ²	1.62±0.08	1.39±0.07	1.51±0.09	1.44±0.08
Leg muscle ²	9.38±0.12	9.32±0.18	9.41±0.19	9.58±0.18
Breast muscle ²	8.64±0.20	9.11±0.23	8.96±0.20	9.07±0.23

¹ Day-old broiler chicks were fed a corn-soybean meal based control diet or the control diets added with either antibiotic growth promoter (AGP) or sanguinarine at the concentration of 20 (SGV20) or 50 ppm (SGV50) for 35 days. At the end of the experiment, organs, abdominal fat, and leg and breast meats per chicken (7 chickens per pen, 7 pens per treatment) were weighed and used to calculate relative weight.

² Values are expressed as relative weight (g per 100 g BW).

³ Values are expressed as mean (±SE).

Relative Weight and Length of Small Intestine

At day 35, relative jejunal weight was significantly lowered ($P<0.05$) in SGV20 or SGV50 groups compared with the control group (Table 4). Dietary AGP failed to affect intestinal weight or length albeit that it marginally lowered ($P>0.05$) the relative jejunal weight and slightly increased ($P>0.05$) jejunal or ileal length in broiler chickens compared with the control group. Relative weight of duodenum or ileum was not altered by any of dietary treatments. Relative intestinal length per 100 g BW tended to be increased when SGV was added into the control diet at the level of 20 or 50 ppm. Especially, relative jejunal length was increased in the SGV20-fed chickens compared with the control diet-fed counterparts. In addition, chickens fed diets containing

SGV20 or SGV50 had longer ileum per 100 g BW compared with the control chickens.

Intestinal Microflora

Total microbes and coli forms in cecal contents were not affected by any of dietary treatments (Table 5). On the other hand, SGV20 significantly increased ($P<0.05$) cecal lactic acid bacteria compared with the control diet-fed chickens.

Total Cholesterol Levels in Serum and MDA Content in Leg Meat

Serum total cholesterol levels were significantly low ($P<0.05$) in SGV20- or SGV50-fed chickens compared with the control group (Table 6). MDA as an indicator of lipid peroxidation was measured in this experiment and found that dietary SGV at all inclusion levels used significantly lowered

Table 4. Effect of sanguinarine on the relative weights of and length of small intestine of broiler chicken¹

	Control	AGP	SGV20	SGV50
Weight ²				
Duodenum	0.34±0.03	0.32±0.02	0.27±0.02	0.30±0.01
Jejunum	0.67±0.05 ^a	0.62±0.03 ^{ab}	0.47±0.01 ^c	0.54±0.02 ^{bc}
Ileum	0.55±0.04	0.56±0.01	0.47±0.04	0.48±0.01
Length ³				
Duodenum	1.13±0.03	1.17±0.03	1.24±0.02	1.21±0.03
Jejunum	3.06±0.10 ^b	3.21±0.11 ^{ab}	3.50±0.08 ^a	3.36±0.10 ^{ab}
Ileum	3.12±0.10 ^c	3.22±0.07 ^{bc}	3.37±0.07 ^{ab}	3.48±0.03 ^a

^{a-c} Mean±SE values ($n=7$ /treatment) in a same row with different superscripts are significantly different ($P<0.05$).

¹ Day-old broiler chicks were fed a corn-soybean meal based control diet or the control diets added with either antibiotic growth promoter (AGP) or sanguinarine at the concentration of 20 (SGV20) or 50 ppm (SGV50) for 35 days. At the end of the experiment, individual small intestine per chicken (7 chickens per pen, 7 pens per treatment) was excised and relative weight and length were recorded.

² Values are expressed as relative weight (g per 100 g BW).

³ Values are expressed as relative length (cm per 100 g BW).

Table 5. Effect of sanguinarine on intestinal microflora in broiler chickens¹

	Control	AGP	SGV20	SGV50
Total microbes, cfu/g	6.06±0.23	5.83±0.21	6.17±0.24	5.63±0.10
Coli forms, cfu/g	5.77±0.21	5.27±0.18	5.46±0.22	5.39±0.47
Lactic acid bacteria, cfu/g	6.01±0.20 ^b	6.01±0.17 ^b	6.62±0.09 ^a	6.24±0.03 ^{ab}

^{a-c} Mean±SE values ($n=7$ /treatment) in a same row with different superscripts are significantly different ($P<0.05$).

¹ Day-old broiler chicks were fed a corn-soybean meal based control diet or the control diets added with either antibiotic growth promoter (AGP) or sanguinarine at the concentration of 20 (SGV20) or 50 ppm (SGV50) for 35 days. At the end of the experiment, individual cecal content per chicken (7 chickens per pen, 7 replicates per treatment) was aseptically sampled for selected microbiota measurement.

Table 6. Effect of sanguinarine on serum cholesterol level and malondialdehyde content of leg muscle in broiler chicken¹

	Control	AGP	SGV20	SGV50
Total cholesterol, mg/dl	144.9±1.1 ^a	136.0±5.3 ^{ab}	128.2±4.5 ^{bc}	117.0±6.1 ^c
Malondialdehyde, µg/g	0.19±0.01 ^a	0.20±0.03 ^a	0.12±0.02 ^b	0.13±0.01 ^b

^{a-c} Mean±SE values in a same row with different superscripts are significantly different ($P<0.05$).

¹ Day-old broiler chicks were fed a corn-soybean meal based control diet or the control diets added with either antibiotic growth promoter (AGP) or sanguinarine at the concentration of 20 (SGV20) or 50 ppm (SGV50) for 35 days. At the end of the experiment, blood and thigh meats per chicken were randomly sampled from 7 chickens per pen (7 pens per treatment) and used for measurement of cholesterol and malondialdehyde levels.

($P<0.05$) MDA levels in leg meats compared with those seen in the control group (Table 6). On the other hand, dietary AGP did not affect MDA levels.

Discussion

The present experiment was conducted to evaluate the novel plant-derived SGV as an alternative to antibiotic growth promoters (AGPs) in broiler chickens. In summary,

BW gain increased while feed conversion ratio (FCR) decreased in AGP- or SGV-treated chickens compared with the control chickens. SGV-fed chickens had decreased relative jejunal weight, but increased relative jejunal or ileal length compared with the control group. In addition, cecal lactic acid bacteria were significantly higher in broiler chickens fed a SGV-supplemented diet compared with those fed on the control diet. Finally, dietary SGV significantly

lowered total cholesterol level in blood samples and MDA concentrations in thigh meats compared with the control group. It is clear from this study that dietary sanguinarine stimulates growth and exhibits various biological activities in broiler chickens of which properties lends its feasibility as the novel alternative to AGPs in poultry.

In this study, dietary AGP and SGV did not affect growth performance of broiler chickens at days 1–21, but significantly increased BW in broiler chickens at days 22–35 or overall 1–35 days. At this stage, clear explanation on why AGP or SGV did not effect on body weight at early days of broiler chicken is not readily available. It is however of note that the growth-enhancing effect associated with the improvement of FCR is prominent at later days of life in broiler chickens. Feed intake was not affected by the treatments except for the SGV50-fed chickens which significantly consumed more at days 22–35 compared with the control group. Thus, it is likely that growth enhancing effect seen in this study is in part associated with the greater feed efficiency offered by AGP or SGV. In line with our result, dietary SGV has been known to increase BW of chickens, the effect being apparent at 21 days (Vieira *et al.*, 2008a, b).

Dietary SGV lowered relative intestinal weight but increased relative intestinal length per 100 g BW. AGP did not affect intestinal morphology although it tended to increase relative intestinal length and to decrease the relative intestinal weight. Thus, it is likely that SGV-mediated increase in growth performance as shown in this study would be the consequence of altered gut morphology (e.g., thinning the intestine), thus facilitating efficient nutrient absorption. Indeed, it is well-known that dietary additives with antibiotic activity have been reported to affect small intestinal morphology, thereby leading to better nutrient digestion and absorption (Jukes *et al.*, 1956; Hill *et al.*, 1957; Humphrey *et al.*, 2002).

Among cecal microflora measured, lactic acid bacteria were significantly increased ($P < 0.05$) by dietary SGV, but not by AGP. Total coli forms marginally lowered ($P > 0.05$) in all treated groups by more than 5% compared with the control group. The antibacterial activity by SGV has been reported *in vitro* (Herrera-Mata *et al.*, 2002) and addition of SGV to broilers' diet lowered cecal fermentation as manifested by low levels of total short-fatty acid concentration (Jankowski *et al.*, 2009). Similarly, Yakhkeshi *et al.* (2011) reported that dietary SGV increased lactic acid bacteria in ileal and cecal contents of broiler chickens at days 21 but this effect was disappeared at day 42. In addition, total coliforms in ileal and cecal contents were not altered by dietary SGV at days 21 and 42 (Yakhkeshi *et al.*, 2011). It seems that dietary SGV favorably increased cecal lactic acid bacteria thus balancing gut microflora which otherwise may lead to lower growth performance. However, a question may arise whether simply increase in lactic acid bacteria is always beneficial to chickens. Further study on gut microbiota profile using molecular tools at the strain levels may help us understand its role in poultry nutrition and poultry health.

There is an indication that various plant-derived compo-

nents may have hypocholesterolemic effect in broiler chickens (Lee *et al.*, 2003), which prompted us to measure cholesterol concentrations in this study. As expected, we clearly observed the SGV-mediated hypocholesterolemic effect in broiler chickens. Earlier, conflicting results by SGV on cholesterol levels have been reported. For example, it is reported that SGV marginally lowered cholesterol in broiler chickens (Yakhkeshi *et al.*, 2011) and pigs (Gudev *et al.*, 2004). In contrast, Jankowski *et al.* (2009) reported a marginal 12% increase ($P = 0.07$) by dietary SGV in total cholesterol concentration compared with the control chickens. However, the underlying hypocholesterolemic mechanism(s) is not readily available at this stage which needs to be addressed.

Meat quality was assessed by monitoring MDA levels in leg meats of broiler chickens. It is often observed that various herbs and spices exhibited *in vivo* antioxidant property in broiler chickens (Botsogou *et al.*, 2003; Rababah *et al.*, 2006; Keokamnerd *et al.*, 2008; Kim *et al.*, 2009). In this study, dietary SGV at the level of 20 or 50 ppm significantly lowered MDA concentrations of thigh meats in broiler chickens. However, dietary AGP did not exhibit antioxidant effect in broiler chickens. It is clear from this study that SGV *vs.* AGP exhibited more systemic biological properties which further lend its advantage over AGP use in broiler chickens. At this stage, question may arise as to how SGV exhibits the antioxidative effect in broiler chickens. The underlying mechanism on the SGV-mediated decrease in MDA concentration would be either its direct protective effect on meat oxidation or via the altered fatty acid composition. The former mechanism is however not likely to occur as SGV is reported to be excreted without being absorbed via intestine (Zdarilova *et al.*, 2008). It is thus more likely that SGV-fed chickens may contain less oxidative fatty acids. Indeed, a previous study (Juskiewicz *et al.*, 2011) showing that dietary SGV altered the fatty acid compositions of breast meats in broiler chickens supports our latter statement on the putative mechanism for its antioxidant property. In any event, further study is needed to clarify the effect of SGV on lipid metabolism and its antioxidant activity in broiler chickens.

In this study, we did observe various biological effects by SGV in addition to its growth enhancing property which is of practical importance to poultry industry. SGV is mainly composed of sanguinarine, a quaternary benzo[c]phenanthridine alkaloid (Dvorak and Simanek, 2007), as active component which derived from the powered stems, leaves, capsules and seeds of *Macleaya cordata*. In traditional Chinese medicine, the aerial part of *M. cordata* has long been used for its analgesic, anti-inflammatory properties, antimicrobial and immunomodulatory activities (Simanek *et al.*, 2003; Dvorak *et al.*, 2006; Niu *et al.*, 2012). Of practical importance, SGV stimulates the secretion of enzymes in the intestine (Franz *et al.*, 2005), which is often regarded as typical characteristic of herbs and spices (Lee *et al.*, 2003). It has been reported that dietary SGV is not metabolized into potentially harmful metabolite (e.g., benz[c]acridine) and is

excreted without being absorbed through the small intestine (Zdarilova *et al.*, 2008). Collectively, it is likely that the primary action mechanism by SGV is via intestine. Indeed, the latter statement supports our finding on the SGV-mediated alteration in relative small intestine per 100 g BW and gut microbiota in broiler chickens, which could lead to efficient nutrient partitioning into growth.

In conclusion, the present study revealed that dietary SGV (sanguinarine as an active component) significantly increased BW gain and improved FCR in broiler chickens. In addition, it altered gut structure, balanced gut microbiota, and lowered serum total cholesterol levels and MDA in thigh meats of 35-d-old broiler chickens. Our study suggests that dietary SGV can favorably use as an alternative to AGP in poultry feeding. Further study is warranted to see whether dietary SGV can exhibit beneficial preventive effect on economically important diseases such as *Clostridium performance* or *Eimeria* spp. in broiler chickens. Indeed, recent study by Pickler *et al.* (2013) who reported that sanguinarine effectively controlled *Salmonella* Enteritis in broiler chickens indicates that dietary sanguinarine could play an important role in reducing economically important enteric diseases.

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