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Effect of *Rubus coreanus* Miquel Byproducts on Performance and Hormone Secretion of Crossbred Chicks

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ABSTRACT

The present study was undertaken to investigate the feeding *Rubus coreanus* Miquel byproducts (RCMB) on the performance, blood composition and hormone concentrations of crossbred chicks. Day-old male crossbred chicks (384) were allocated to four RCMB (0, 0.25, 0.5, 1.0%) level in the diets with six replication having 64 chicks in each treatment. Four dietary energy (3,000, 3,100, 3,100 and 3,200 kcal kg^{-1} ME) and CP (22, 21, 19 and 17%) levels were provided to the starting (0-2 weeks), growing (3-5 weeks), growing-finishing (6-8 weeks) and finishing (9-10 weeks) period. No significant performances were observed during the starting and growing periods but weight gain and feed intake were increased in the growing-finishing and finishing period. Thus the total weight gain and feed intake were significantly higher in RCMB treatment group as compared to those of the control ($p<0.05$), except feed conversion. Triglycerides were significantly lower in birds fed 1.0% RCMB compared to those in the controls and in the 0.25% RCMB fed birds ($p<0.05$). Total protein concentrations were not different among the treatments, but albumin concentration was increased in the 1.0% RCMB treatment compared to those of the other treatments ($p<0.05$). The melatonin, growth hormone and testosterone concentration was increased significantly ($p<0.05$) in RCMB treatment than that of the control. Therefore, the inoculation of 1% level of RCMB in the diet of crossbred chicks appeared to enhance the performance and hormone secretion. Meanwhile, further follow-up studies should be conducted to investigate RCMB additions of more than 1% in chicken diets.

Key words: Crossbred chicks, hormone, *Rubus coreanus*, Miquel byproducts, performance

INTRODUCTION

Rubus coreanus Miquel is a perennial wild berry type of plants that belongs to the Rosaceae family and its cultivation is limited to the southern parts of the Korean peninsula, China and Japan. During 2001 to 2005, *Rubus coreanus* Miquel production have increased from 1510 to 2733 tons (Ku and Mun, 2008) and which influenced the development of various Bokbunja products (Jin *et al*., 2008). It is a rich source of flavonoids, tannins, triterpenoids and phenolic compounds (Ju *et al*., 2009). It composed mainly of triterpenoids that include coreanoside F_1 suavissimoside and nigaichigoside F1 and F2 (Kim and Kang, 1993). The presence of antioxidative agents such as phenolic acid and organic acids has been reported (Lee, 1995; Yoon *et al*., 2002) along with the presence of Quercetin (Yoon *et al*., 2003). In the berries of *Rubus coreanus* Miquel, hydrolyzable

tannins of gallotannin and ellagitannin were found along with gallic acid, 2, 3-(S)-HHDP-D-glucopyranose, sanguiin H-4 and sanguiin H-6 (Lee and Lee, 1995; Pang *et al*., 1996).

Recently, it was reported that weight gain and feed conversion ratio in crossbred chicks had increased by inoculation of *Rubus coreanus* Miquel extracts with the diet (Kim *et al*., 2009). Several studies have been conducted to create high value products by agri-byproducts such as *Rubus coreanus* Miquel byproducts (Isci and Demirer, 2007). From 2001 to 2005, the growth area in South Korea has increased about 13 times and the harvest quantity increased nearly two times (Ku and Mun, 2008). Most Bokbunja seeds are byproducts from wine processing and thus, the search for other applications may contribute to minimize waste disposal problems, improving the value of Bokbunja fruit and enhancing the agricultural economy (Ku and Mun, 2008). Therefore, the potentialities of the RCM byproducts started to draw researcher's attention; the usefulness and feasibility of using RCM byproducts for various applications becomes the subject of studies. Researchers generally focused on the application of RCM in human and experimental rodents, but there have not been studied on the subject to the inoculation of RCM byproducts to the poultry yet.

Thus, the present study investigated the effects of adding different levels of *Rubus coreanus* Miquel byproducts to the crossbred chick's diet and reviewed overall performance, hematology and the differentiation of hormone secretion levels of the chicks to confirm the potential value of *Rubus coreanus* Miquel byproduct as a feed for crossbred chicks.

MATERIALS AND METHODS

Preparation and analysis of RCMB: The fruit flesh and residues containing seeds of the *Rubus coreanus* Miquel, which were acquired after manufacturing *Rubus coreanus* Miquel wine (Ku and Mun, 2008). The RCM byproduct were separated and dried at room temperature and then crushed and mixed with the basal diet.

The *Rubus coreanus* Miquel byproducts (RCMB) was dried in the oven at 60°C for 24 h and powdered by a roller mill. Thereafter, moisture, crude protein and crude ash were analyzed by the AOAC (1995) methods. The dried RCMB was contained 4.98% moisture, 12.99% CP, 1.48% crude ash and 13.61% crude fat. Oil content was extracted by Soxhlet extraction and amino acids were analyzed as follows. One gram RCMB was added into 10 mL of conical flask and mixed with 5 mL of 1% boric acid solution and kept at 4°C for 24 h. It was centrifuged at 5,000 rpm for 30 min and 0.5 mL of supernatant were separated from the solution and eluted through the column (Sephadex G-50) and then 200 μm eluted sample added with 200 μm chloroform and mixed by vortex. The mixture was again centrifuged at 15,000 rpm for 5 min. Supernatant liquid was filtered by 0.22 μm syringe filter and reacted derivatization by kit (AccQ-Flour Reagent, Water). Amino acid compositions were determined by amino acid analyzer (water 2690, Waters Co., USA) equipped with a Waters 747 scanning fluorescence detector (Water Co., USA) and a AccQ-Tag column (3.9×150 mm, Waters) where the sample injection volume was 10 μm with flow rate was 1.0 mL min^{-1}. Fatty acids in RCMB were analyzed as described by Ku and Mun (2008).

Feeding trials: In a 70 days (March-May, 2007) feeding trial, 384, one-day-old male crossbred chicks (Hanhyup 3 Ho) was placed into floor pens for ten weeks. A basal diet was prepared by mixing corn and soybean meals and the prepared feeds were provided in the starting (0-2 weeks), growing (3-5 weeks), growing-finishing (6-8 weeks) and finishing (9-10 weeks) periods to meet the metabolic energy levels of 3,000, 3,100, 3,100 and 3,200 kcal kg^{-1}, respectively. The crude protein levels of the feeds were prepared at 22, 21, 19 and 17%, respectively, as shown in Table 1. *Rubus*

Table 1: Basal diet composition

Ingredients	0-2 week	3-5 week	6-8 week	9-10 week
	(%)			
Corn	60.057	62.095	66.280	69.853
Soybean meal	28.588	25.732	25.430	22.636
Corn gluten meal	6.930	7.064	3.616	1.832
Soybean oil	1.000	2.000	2.000	3.000
Limestone	0.929	1.132	1.166	1.077
Tri calcium phosphate	1.750	1.228	0.907	1.002
Salt	0.400	0.400	0.400	0.400
Lysine	0.054	0.125	0.000	0.000
DL-methionine	0.092	0.024	0.000	0.000
Vitamin premix[1]	0.100	0.100	0.100	0.100
Mineral premix[2]	0.100	0.100	0.100	0.100
Composition (calculated)				
ME (kcal kg^{-1})	3,000	3,100	3,100	3,200
CP (%)	22.00	21.00	19.00	17.00
Lysine (%)	1.05	1.05	0.90	0.80
Methionine (%)	0.48	0.40	0.34	0.30
Ca (%)	1.00	0.90	0.80	0.80
Available phosphate (%)	0.45	0.35	0.30	0.30

Provide per kilogram of diet: vit. A: 12,000 IU: vit D3: 5,000 IU, vit E: 50 mg, vit K3: 3.0 mg, vit B1: 2.0 mg, vit B2: 6.0 mg, vit B6: 4.0 mg, vit B12: 0.025 mg, Biotin: 0.15 mg, Pantothenic acid: 20.0 mg, Folic acid: 2.0 mg, Nicotinic acid: 7.0 mg, [2]Contents per kg; Fe: 66.72 mg, Cu: 41.7 mg, Mn: 83.4 mg, Zn: 66.72 mg, I: 0.834 mg, Se: 0.25 mg

coreanus Miquel byproducts (RCMB) were added at the rate of 0, 0.25, 0.5 and 1.0% with the basal diets and each treatment was replicated six times, with 96 chicks assigned to each treatment and a total of 384 chicks allocated with similar body weight. Chicks were provided feed and water without restriction and 24 h continuous lighting was available during the entire trial period.

Weight gain, feed intake and feed conversion ratio: The body weights of the chicks were measured per week and the feed intake was calculated by deducting the residual amount of feed from the total amount of feed provided. The feed conversion ratio was calculated by dividing the feed intake amount by the weight gain.

Blood test and hormone assay: At the end of the feeding trial, the blood tests were conducted by selecting ten chicks from each treatment group. Blood was collected from the brachial vein into a vacuum tube using a 5 mL syringe. After blood collecting, serum was separated and stored at -70°C until analysis. Blood total cholesterol, triglyceride, HDL-cholesterol and LDL-cholesterol concentrations were measured using a blood analysis kit that used an enzymatic colorimetric method (AM 202-K; Asan Pharm Co., LTD; Korea) and total protein, albumin and glucose levels were measured using an automatic blood analyzer (Minos BAT; France).

The blood growth hormone level was measured using the RIA test kits manufactured by Linco Research (USA) to measure the blood IGF-1 level. Two hundred microlitres of blood plasma was mixed with 800 μL acid-ethanol (2 M HCl: ethanol in the ratio of 1:7) and centrifuged at 4°C for 30 min at a speed of 3,000 rpm. Then, 500 μL of the supernatant fraction containing the free IGF-1 was mixed with 200 μL 0.855 M trizma base and the 100 μL of the reactant was added to 100 μL of the [^{125}I]-IGFs (20000 cpm/100 μL) prepared using the Chloramin-T method. To this, 50 μL of

1000 time's diluted polyclonal anti-IGFs (Gro-pep, Australia) was added to react for 18 h at 4°C. After the reaction, 50 μL serum and 1 mL 12% polyethylene glycol # 8000 (PEG) were added and the mixture was centrifuged for 30 min at a speed of 3,000 rpm in order to separate the fraction into the bound and free forms of IGF. The radioactivity of the bound form was measured using a gamma counter (Packard, ILL, USA). The measurement of blood testosterone level was performed by applying the radioimmunoassay method using the Immuchem testosterone kit (ICN Biomedicals, INC. Diagnostics, U.S.A.). Specifically, 100 μL serums and the same amount of testosterone standard were contained within anti-testosterone coated tables and 1.0 mL ^{125}I-testosterone was placed onto the total tube and gently shaken and incubated at 37°C for 90 min. After incubation, the liquid fractions within the tube were discarded and radioactivity was measured for 60 sec using the gamma counter. The blood melatonin concentration was measured using the RIA test kits manufactured from Buhlmann Laboratories AG (Switzerland). The RIA test kits utilize two types of G280 anti-melatonin antibodies (primary antibody and secondary antibody) to measure the melatonin concentration. The analysis procedure of the RIA includes the initial pre treatment procedure with a column preparation step, the control procedure in which 1 mL of methanol and the same amount of distilled water are injected and a washing step in which 1 mL of 10% methanol was injected two times along with 1 mL of hexane. Next, the sample was diluted five-fold and 1 mL methanol was added for the final extraction step. After the pretreatment procedure, samples were reacted with 100 μL primary antibodies and 100 μL I-125 for 20±4 h and 100 μL secondary antibodies was injected before measuring the radioactivity using the gamma counter.

Statistical analysis: Statistical analysis of all data was performed using analysis of variance on the GLM program of SAS (version 9.0). The post-hoc tests were performed using Duncan's multiple range tests (Steel and Torrie, 1980) and the statistical significance between the treatment intervals was determined at the 5% level.

RESULTS

Chemical and nutritional properties of RCMB: Moisture, Crude protein, ash and oil contents of RCMB were 4.98, 12.99, 1.48 and 13.61%, respectively. The amino acids composition of RCMB is presented in Table 2 where proline, leucine and lysine were showed highest concentration (up to 30 μg g^{-1}) and aspartic acid, glutamic acid, alanine and phenyl alanine were shown to be 20 μg g^{-1}. Furthermore, Serine, threonine, arginine, tyrosine, valine, methionine and lsoleucine concentration ranged from 10 to 20 μg g^{-1}. The values of glycine and histidine were 4.28 and 4.78 μg g^{-1}, respectively. The concentration of fatty acids, palmitic, stearic, oleic, linoleic and linolenic acid composition of RCMB are presented in Table 3. The linoleic acid content was much higher than that of others fatty acids. Out of total fatty acid, saturated and unsaturated fatty acids content in RCMB was approximately 5 and 95%, respectively.

Weight gain, feed intake and feed conversion ratio: The productivities of the crossbred chicks fed with RCMB are shown in Table 4. In the starting period, weight gains, feed intake and feed conversion ratios did not show any statistical differences among the treatments, but weight gain of the treatment groups (194, 193 and 195 g in 0.25, 0.5 and 1.0% RCMB, respectively) were increased than that of control group (187 g). During the growing period, the performance trends of the crossbred chicks were similar to those of starting period. Though, the productivities of the growing-finishing and finishing periods were non-significant but higher feed intake and weight

Table 2: Amino acid composition in *Rubus coreanus* Miquel byproducts

Amino acids	Concentration (μg g^{-1})
Aspartic acid	22.60
Glutamic acid	25.86
Serine	10.98
Glycine	4.28
Histidine	4.78
Threonine	17.70
Arginine	19.20
Alanine	24.50
Proline	30.98
Tyrosine	18.58
Valine	16.87
Methionine	12.03
Isoleucine	12.26
Leucine	30.83
Lysine	30.23
Phenylalanine	23.37

Values are presented as means of tri-replicated analysis

Table 3: Fatty acid compositions in *Rubus coreanus* Miquel byproducts

Fatty acids	Composition (%)
Palmitic acid (C16:0)	3.85
Stearic acid (18:0)	1.26
Oleic acid (C18:1)	10.75
Linoleic acid (C18:2)	54.21
Linolenic acid (C18:3)	30.15
SFA	5.09
UFA	95.11
Mono UFA	10.75
Poly UFA	84.36
SFA/UFA	0.05

Values are presented as means of tri-replicated analysis, SFA: Saturated fatty acids, UFA: Unsaturated fatty acids

gain was obtained in treatment groups. Meanwhile, weight gain and feed intake pattern of the 1.0% RCMB fed group (2203 and 5251 g) was significantly ($p<0.05$) higher in the whole experimental period as compared to the control (2093 and 4951 g) but the feed conversion ratios were not influenced by the treatments.

Blood cholesterol and hormone: Blood total cholesterol and LDL-cholesterol concentrations was decreased in RCMB feeding chicks compared to those of the control, but the HDL-cholesterol concentration increased with the RCMB level in the diets (Table 5). The triglyceride concentration was decreased significantly ($p<0.05$) with the increasing level of RCMB in the diets (13.6, 10.9 and 8.6 mg dL^{-1} in 0.25, 0.5 and 1.0% RCMB treatments, respectively). Thus, the triglyceride concentrations in chick's blood were significantly higher in 1% RCMB treatment group than that of control and 0.25% RCMB feeding group. Though, the glucose level and total protein content was non-significant, but blood albumin concentration in 1.0% RCMB treatment (1.3 g dL^{-1}) group was significantly higher ($p<0.05$) than those of other treatments.

Table 4: Effects of *Rubus coreanus* Miquel byproducts on performance in crossbred chicks

Treatment (%)	Weight gain (g)	Feed intake (g)	FCR[1] 0-2 weeks
0[2]	187±2.27	281±3.44	1.503±0.01
0.25	194±3.34	291±5.20	1.504±0.01
0.50	193±2.41	290±4.64	1.506±0.01
1.00	195±4.64	294±4.92	1.511±0.01
3-5 weeks			
0	692±9.01	1,325±10.51	1.917±0.02
0.25	704±5.39	1,344±8.88	1.908±0.01
0.50	706±3.83	1,357±21.62	1.922±0.03
1.00	711±15.28	1,369±21.54	1.926±0.04
6-8 weeks			
0	720±25.30	1,673±26.64	2.333±0.05
0.25	737±14.26	1,714±45.10	2.325±0.02
0.50	735±9.26	1,720±35.55	2.340±0.04
1.00	750±9.08	1,760±22.99	2.347±0.04
9-10 weeks			
0	505±15.62	1,730±47.90	3.429±0.07
0.25	522±10.16	1,771±26.13	3.400±0.07
0.50	526±17.70	1,825±57.58	3.468±0.02
1.00	552±27.75	1,898±65.16	3.459±0.08
Whole period			
0	2,093±11.93^{b}	4,950±80.71^{b}	2.365±0.04
0.25	2,131±18.37^{ab}	5,040±42.20^{ab}	2.366±0.03
0.50	2,151±29.10^{ab}	5,130±76.22^{ab}	2.385±0.01
1.00	2,203±36.11^{a}	5,251±90.18^{a}	2.385±0.04

Values are Mean±SD, ^{a,b}Values with the same letters in the column are not significantly different at the 5% level, [1]Feed conversion ratio, [2]*Rubus coreanus* Miquel byproduct levels in the diets

Table 5: Effects of feeding *Rubus coreanus* Miquel byproducts on blood composition in crossbred chicks

Treatment (%)	Total cholesterol (mg dL^{-1})	HDL-cholesterol (mg dL^{-1})	LDL-cholesterol (mg dL^{-1})	Triglyceride (mg dL^{-1})	Glucose (mg dL^{-1})	Total protein (g dL^{-1})	Albumin (g dL^{-1})
0[1]	140.8±3.20	71.8±2.13	64.5±2.53	15.4±1.73^{a}	206.9±9.75	3.5±0.11	1.1±0.04^{b}
0.25	126.1±4.75	72.2±2.45	52.2±5.40	13.6±1.13^{a}	190.0±5.70	3.5±0.09	1.1±0.02^{b}
0.50	139.9±4.58	76.3±6.67	55.5±3.40	10.9±1.83^{ab}	197.1±9.68	3.6±0.10	1.1±0.02^{b}
1.00	133.8±4.05	80.3±2.34	51.7±4.65	8.6±1.18^{b}	199.6±2.87	3.5±0.11	1.3±0.05^{a}

Values are means±standard error, ^{a,b}Values with the same letters in the column are not significantly different at the 5% level, [1]*Rubus coreanus* Miquel byproduct levels in the diet

In the present experiment, growth hormone level in the blood (28.80, 62.60, 66.20 and 77.00 ng mL^{-1} for 0, 0.25, 0.5 and 1.0% RCMB, respectively) was increased ($p<0.05$) with dietary inoculation of RCMB in the diet (Table 6). Consequently, the blood IGF-1 concentration also tended to increase with increasing level of RCMB in the diet, but the differences was non-significant (Table 6). The blood testosterone concentration rapidly increased with increased RCMB feeding levels, with concentrations of 68.60±8.30, 231.80±19.02, 664.20±15.03 and 753.60±35.97 ng mL^{-1} for 0, 0.25, 0.5 and 1.0% RCMB, respectively. On the other hand, the blood melatonin concentration of the control group was 65.20±3.31 pg mL^{-1} and the 0.25, 0.5 and 1.0% RCMB treatments were 92.00±4.91, 131.60±14.43 and 209.80±19.00 pg mL^{-1}, respectively (Table 6). Therefore, these results demonstrated that blood melatonin concentration was sharply responsive ($p<0.05$) to the RCMB treatment (0.5 and 1.0% level in the diet).

Table 6: Effect of feeding *Rubus coreanus* Miquel byproducts on blood hormone levels in crossbred chicks

Items	Melatonin (pg mL^{-1})	Growth hormone (ng mL^{-1})	Testosterone (ng mL^{-1})	IGF-1 (ng mL^{-1})
Control	65.20±3.31^{c}	27.00±3.30^{b}	68.60± 8.30^{d}	23.60±1.99
RCMB 0.25%[1]	92.00±4.91^{c}	62.60±6.35^{a}	231.80±19.02^{c}	28.60±5.73
RCMB 0.5 %	131.60±14.43^{b}	66.20±7.02^{a}	664.20±15.03^{b}	32.20±3.34
RCMB 1.0 %	209.80±19.00^{a}	81.20±6.60^{a}	753.60±35.97^{a}	32.40±2.50

Value are means±Standard error, ^{a,b,c,d} Value with the same letters in the column are not significantly different at 5% level, *Rubus coreanus* Miquel by-products feeding levels in diet

DISCUSSION

Fruits of *Rubus coreanus* have been used in oriental traditional medicine as the remedies for impotence, pollution, premature ejaculation and frequency of urination (Pang *et al*., 1996). The physiological activity and antioxidative effects of *Rubus coreanus* Miquel have been mainly performed on human and experimental animals. No other studies have ever been conducted to know the physiological effects of feeding *Rubus coreanus* Miquel byproducts on poultry. Therefore, the present study was aimed to investigate the effect of *Rubus coreanus* Miquel byproduct (RCMB) on the growth-related hormonal changes in crossbred chicks.

In composition of the RCMB, fat, crude protein content was higher than that of moisture and crude ash content. But, in the previous observation Cha *et al*. (2007) determined that *Rubus coreanus* Miquel fruit with seed and fresh pulp contained lower level of crude protein, fat and ash and higher level of moisture which contradicts with the present findings. Likewise amino acid compositions also have differences. This disparity attributed due to the sample state between the present study and previous findings. The major fatty acid were palmitic, stearic, oleic, linoleic and linolenic acid in RCMB where linoleic acid was the most general, accounting for up to 50% of the total fat in RCMB and which agreed with the previous findings (Ku and Mun, 2008). Therefore, the linolenic acid may become an important indicator of adulteration of RCMB. The present results were indicated that unsaturated fatty acid contained up to 95% of the total fatty acids in RCMB which is higher than the previous results (Ku and Mun, 2008). However, chemical and nutritional values in *Rubus coreanus* Miquel fruit are changed, according to maturating level or collecting time (Cha *et al*., 2007). Thus, the author suggested that the RCMB fruit residues needed to analyze before starting of the experiment.

During the entire period of the experiment, weight gain and feed intake were increased significantly with 1.0% RCMB treatment ($p<0.05$). Due to the lack of previous studies regarding the effect of RCMB treatment in poultry productivity, a direct comparison was not possible with the present results; however, our results were some what similar to those of a previous report (Kim *et al*., 2009) who showed that the improvements of body weight gain and feed conversion ratio of birds fed RCMB. In the berries of *Rubus coreanus* Miquel, contain 52 types of volatile flavor components that produce its unique flavor (Lee and Do, 2000) and which increase the favorability of feeds and correspondingly stimulates the feed intake and which reflect on weight gain of chicks. Since the body weight gain of the birds showed a linear increase up to the 1% RCMB treatment level in this experiment, follow-up studies should be conducted to investigate RCMB additions of more than 1% in chicken diets. In the peel of *Rubus coreanus* Miquel, a polyphenolic antioxidative agent of anthocyanin has been reported as a preventive effect on the occurrence of cardiovascular diseases (Toufektsian *et al*., 2008). Although, no statistically significant difference was observed between the RCMB treatments and the control, the HDL-cholesterol concentrations tended to increase with RCMB treatment, while the LDL-cholesterol concentration tended to decrease. Similar

results also obtained by Seo *et al.* (2011). Several studies have shown that an RCM extract has higher electron donation ability and prevents low-density lipoprotein (LDL) oxidation in *in vitro* studies (Yoon *et al.*, 2003; Cho *et al.*, 2005; Lee and Do, 2000). In the case of triglyceride concentration, a significant reduction was observed in the 1.0% RCMB treatment compared to that of the control ($p<0.05$). Given the reduction in triglyceride concentration in the RCMB treatments, the present study suggests that *Rubus coreanus* Miquel byproducts may play a role in internal lipid metabolism. Synthesized in the liver, blood albumin has been widely used as an important indicator of the synthesis activities and abnormality of the liver. The albumin concentration in blood was highest with 1% RCMB treatment, showing the same pattern as the weight gains of the chicks. In the present study, the growth hormone level was increased ($p<0.05$) with the inoculation of RCMB to the diet. Brooks *et al.* (2006) reported that growth hormones act on most growing cells to affect cell size, as well as to increase the cell population through mitosis, which will have a potentially positive effect on growth. In the present study, the levels of IGF-1 were numerically increased with the inoculation of RCMB in the crossbred chick's diet. Thereafter, *Rubus coreanus* Miquel byproducts was increased the blood testosterone concentrations of male crossbred chicks ($p<0.05$). This result is thought to be attributable to the flavonoids present in *Rubus coreanus* Miquel, which enhance the red coloring and male sexual performance. In addition, Chen *et al.* (1996) reported that the administration of *Rubus coreanus* Miquel increased the level of testosterone secretion and sperm count. It also activate the sexual performances of males (Lee, 1966; Kim *et al.*, 2005; Oh *et al.*, 2007), by increasing in testis weight, sperm count, activity and expression rate of the cAMP-responsive element modulator (CREM) gene, along with a significant increase in CREM protein expression rate. In the present study, the melatonin concentration was increased significantly ($p<0.05$) with increasing level of RCMB in the diet. On the other hand, melatonin is a hormone secreted from the pineal gland to control reproductive function and is known to act effectively on life span extension through the prevention of aging (Tan *et al.*, 1993). Although *Rubus coreanus* Miquel has been reported to have both stimulatory and inhibitory effects on the reproduction capacities of animals (Herbert, 1981). The present study revealed that the feeding of RCMB increased the testosterone levels and melatonin concentrations in the crossbred chicks, suggesting mutual dependence.

CONCLUSIONS

The present study indicated that the addition of 1% RCMB into the diet of crossbred chicks resulted in increases of feed intake, weight gain and blood albumin concentration, but decrease in triglyceride level in the blood. The inoculation of RCMB also increased the levels of growth hormone, testosterone and melatonin hormones in crossbred chick's blood. Therefore, the inoculation of 1% level of RCMB in the diet of crossbred chicks appeared to enhance performance and hormone secretion. Meanwhile, further follow-up studies should be conducted to investigate RCMB additions of more than 1% in chicken diets.

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