

Effect of Dietary Inclusion of *Gynura divaricata* (L.) on Growth Performance, Hematology, and Carcass Fat Deposition in Broilers

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The objective of this study was to examine the effect of *Gynura divaricata* (Jakr-Na-Rai, JNR) on the growth performance, hematology, and carcass fat deposition of broilers. A total of 240 male Cobb-500 birds, 22 d old, were randomly allocated into five treatment groups of six replicates. Each group was raised at a high stocking density of 28 kg of body weight/m² until day 43. The treatments consisted of (i) a basal diet, or the basal diet supplemented with (ii) 2.5 mg/kg avilamycin (T2), (iii) JNR crude ethanol extract at a flavonoid level of 1.3 g/kg (T3), and (iv and v) JNR powder at a flavonoid level of 1.3 (T4) or 2.6 g/kg (T5). Dietary supplementation with JNR powder at both flavonoid levels decreased the heterophil/lymphocyte ratio compared to the other groups ($P < 0.001$). Birds in the T5 group presented decreased blood glucose ($P < 0.005$) and cholesterol ($P < 0.002$) levels and a numerically ($P = 0.056$) decreased triglyceride level. The total bile acid concentration increased ($P < 0.001$) in all the JNR-fed groups, but there was no significant effect on the digestibility of ileal protein or fat. At 1.3 g/kg of diet, JNR increased the final body weight and feed intake ($P < 0.05$), but the average daily gain and feed conversion ratio were not different among groups. The carcass and abdominal fat percentages were lowest in chicks fed on the diets supplemented with 2.6 g/kg JNR powder ($P < 0.007$ and $P < 0.025$, respectively). Drip loss and malondialdehyde concentrations in the breast meat did not change. In conclusion, JNR powder improved several hematological parameters, increased total bile acid concentrations, and decreased the percentage of abdominal fat. The powder form of JNR elicited better results than the ethanol extract form at the same flavonoid inclusion level.

Key words: abdominal fat, cholesterol, flavonoid, glucose, heterophil/lymphocyte ratio, polysaccharide

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Introduction

Herbal products are increasingly used as alternatives to antibiotic growth promoters (AGP) in the broiler industry owing to their abundant active ingredients and varied properties (Cheng *et al.*, 2014). Jakr-Na-Rai [JNR, *Gynura divaricata* (L.)], a traditional Chinese medicinal herb (Bai Bei San Qi), is rich in active ingredients and can be easily grown in Thailand and Southeast Asia (Jaiboon *et al.*, 2011). To date, the active ingredients reported for JNR include flavonoids (Wan *et al.*, 2011b; Wu *et al.*, 2011), cerebrosides (Chen *et al.*, 2009), alkaloids (Roeder *et al.*, 1996), polysac-

charides, terpenoids, and β -sitosterol (Chen *et al.*, 2003). The major active substances in JNR are the flavonoids, with kaempferol comprising the main portion (Wan *et al.*, 2011a), and polysaccharides (Deng *et al.*, 2011). Flavonoids can increase bile acid secretion (Crespy *et al.*, 2003), thereby assisting in fat digestion (Lammasak, 2010). Polysaccharides can also lower blood glucose levels (Jiang *et al.*, 2009; Deng *et al.*, 2011). With limited glycogen deposition in the liver, birds survive reductions in blood sugar levels through gluconeogenesis from triglyceride breakdown in adipose tissues (North, 1984; Griffin *et al.*, 1992). Aritajat *et al.* (2008) found that the crude water extract of JNR reduced blood triglyceride levels in chickens, suggesting that JNR may help to enhance fat degradation, resulting in reduced body fat deposition in the carcass. Relatively few studies have reported on the effect of JNR on blood cholesterol levels, and the results are still controversial. In male rats, cholesterol levels increased when they were fed with a water extract from fresh JNR leaves (Aritajat *et al.*, 2008); in rabbits, meanwhile, no effect was found when the animals were fed with JNR from fresh whole plants (Keeratikajorn *et*

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al., 2012). However, there are still no reports on JNR in broilers.

Previous studies on JNR have produced inconsistent results, which may have been due to the different plant parts, extraction methods, and species of test animal used in the experiments. The ethanol extract of JNR yields several flavonoids and phenolic compounds that may be useful as antioxidants and in promoting broiler growth (Xu and Zhang, 2017). However, the composition and active ingredients of the ethanol extract of JNR may be different from that of the powder form of the whole plant. Moreover, the effects elicited by the ethanol extract form and the crude powder form of JNR, which is more practical for use by the farmer, may also differ.

Therefore, the aim of this study was to examine the effect of dietary supplementation with various forms of JNR on the growth performance, total bile acid concentration (TBAC), nutrient digestibility, and blood parameters of broiler chickens. Moreover, changes in lipid peroxidation in the plasma and breast meat, carcass fat deposition, and drip loss in breast meat of the broilers were also investigated.

Materials and Methods

Animals and Management

A total of 300 male Cobb-500 chicks were raised under a normal stocking density (10 chicks/m² or 20 kg/m²) at the starting period in an open-sided housing system, according to the recommendation of the Department of Livestock Development, Thailand (DLD, 2003). At 22 d of age, 240 birds were randomly allocated into five groups (six replicates of eight birds) and raised in floor pens in an open-sided housing system under a high stocking density (14 birds/m² or 28 kg of body weight (BW)/m²) until 43 d of age. All chicks received the Newcastle-infectious bronchitis vaccine at the hatchery and the infectious bursal disease vaccine at 14 d of age. The birds had free access to their respective diets and water throughout the experimental period (22–43 d of age). This experiment was approved by the Animal Care and Use Committee of the Faculty of Veterinary Science, Chulalongkorn University (Approval No. 1431002).

Preparation of JNR Powder and Crude Ethanol Extract Granules

The JNR was cultivated at the veterinary training center in Nakorn Pathom province, Thailand. Plants of JNR at the height approximately of 30 cm were cut from the upper tip of the whole plant, washed with tap water, and used to prepare both the powder and derived crude ethanol extract (CEE) forms. The JNR was first prepared by separating the fresh leaves from the stem, chopping the stem into 1-cm long pieces, and air drying both for 1 d.

For the CEE, the air-dried JNR was extracted with 95% (v/v) ethanol in an Erlenmeyer flask at a 10% (w/v) ratio for 7 d with stirring four times/d. Then, the ethanol solution was filtrated and evaporated in a rotary vacuum evaporator (EYELA rotary vacuum evaporator[®], EYELA, Tokyo, Japan) to yield the CEE (Intajak *et al.*, 2012). To ensure a thorough distribution in the broiler diet, the CEE was then produced

Table 1. Chemical composition of the CEE granule and powder forms of JNR (g/kg DM)

| Nutritional content | CEE ¹ | Powder |
|-------------------------------------|------------------|--------|
| Protein | 87.0 | 184 |
| Crude fat | 43.0 | 34.1 |
| Crude fiber | 17.2 | 144 |
| Ash | 32.2 | 162 |
| Calcium | 1.15 | 15.1 |
| Phosphorus | 2.00 | 5.80 |
| Total flavonoid (mg flavonoid/g DM) | 117 | 31.6 |
| Calculated ME, MJ/kg ² | 14.8 | 10.9 |

¹ Extract from diluted to granule form.

² Metabolizable energy (ME) values were calculated using an equation from AAFCO (2000).

CEE=crude ethanol extract; JNR=Jakr-Na-Rai; DM=dry matter; ME=metabolizable energy.

in granule form. For this, the CEE was diluted with propylene glycol at a 1:1 (w/w) ratio and then mixed with ground corn (0.85 mm particle size) at a ratio of 1:9 (w/w) in a vertical mixer for 30 min. The moist granules were then dried in an oven (Menmert[®] Ule 800, Mannert, Schwabach Germany) at 50°C for 2 h.

The JNR powder was prepared by drying the previously air-dried leaves and chopped stems in an oven at 50°C for 48 h. When cooled, both parts were mixed and ground through a 1-mm diameter sieve blender (Retsch[®], Haan, Germany).

Both the powder and CEE forms of JNR were light-protected in black plastic bags and stored at –20°C until use. In addition, they were randomly sampled and analyzed for moisture, crude protein, ether extract, crude fiber, ash, calcium, and phosphorus contents using AOAC procedures 934.01, 976.05, 920.39, 962.09, 942.05, 927.02, and 964.06, respectively (AOAC, 1990). The metabolizable energy (ME) content of both JNR forms was calculated using an equation from AAFCO (2000), while the total flavonoid content was evaluated as previously described (Wan *et al.*, 2011a). The analytical values of the nutritional composition of the powder and CEE forms of JNR are depicted in Table 1.

Feed Formulation and Broiler Feeding

All the diets were formulated to be isocaloric and isonitrogenous according to the recommendations for the breed (Cobb, 2010). To ensure an even distribution of dietary nutrients, 84% of the basal grower-finisher diet was blended in one batch and used for all the treatment groups. The remaining 16% was formulated by adding JNR (powder or CEE granules) and balancing the nutrients with corn, soybean meal, and Leucaena meal. The quantity of JNR added to the diets was determined by the level of flavonoids added to the diet, and set at 1.3 or 2.6 g/kg of diet. This level was based on a previous finding that 100 mg of flavonoids/kg of BW elicited a beneficial effect on lipid peroxidation in an induced rat model of colorectal cancer (Nirmala and Ramanathan, 2011). Thus, 100 mg of flavonoids/kg of BW, as well as the BW and feed intake of broilers at 42 d, based

Table 2. Composition of the experimental diets (g/kg of diet)

| | Treatment ¹ | | | | |
|------------------------------------|------------------------|-------|-----|------|------|
| | T1 | T2 | T3 | T4 | T5 |
| Corn | 579 | 579 | 459 | 560 | 539 |
| Soybean meal 48.5% CP ⁶ | 227 | 227 | 227 | 218 | 208 |
| Leucaena meal | 50 | 50 | 50 | 32 | 10 |
| Full fat soy | 50 | 50 | 50 | 50 | 50 |
| Rice bran oil | 50 | 50 | 50 | 50 | 50 |
| Mono-DicalPO ₄ | 12 | 12 | 12 | 12 | 12 |
| CaCO ₃ | 18 | 18 | 18 | 18 | 18 |
| NaCl | 4.8 | 4.8 | 4.8 | 4.8 | 4.8 |
| DL-methionine | 2.6 | 2.6 | 2.6 | 2.6 | 2.6 |
| L-lysine | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 |
| L-Threonine | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Choline chloride 60% | 3.3 | 3.3 | 3.3 | 3.3 | 3.3 |
| Vitamin premix ² | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Mineral premix ³ | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Salinomycin 12% | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Surmax, avila 10% | — | 0.025 | — | — | — |
| CEE JNR ^{4,5} | — | — | 12 | — | — |
| JNR powder | — | — | — | 46.1 | 99.2 |

¹ Treatments: T1 control, T2 avilamycin, T3 CEE JNR granules (0.13% flavonoid), T4 JNR powder (0.13% flavonoid), and T5 JNR powder (0.26% flavonoid).

² Vitamin premix/kg of diet contained: A, 12,000 IU; D3, 3,000 IU; E, 15 mg; K3, 1.5 mg; B1, 1.8 mg; B2, 5.5 mg; B6, 2 mg; B12, 0.01 mg; Niacin, 25 mg; Calcium D-pantothenate, 12 mg; folic acid, 0.5 mg; and Biotin, 0.12 mg.

³ Mineral premix/kg of diet contained: Mn, 80 mg; Zn, 60 mg; Fe, 40 mg; Cu, 8 mg; I, 0.5 mg; Co, 0.1 mg; and Se, 0.1 mg.

⁴ CEE=crude ethanol extract; ⁵ JNR=Jakr-Na-Rai; ⁶ CP=crude protein.

Table 3. Chemical analysis of the experimental diets (g/kg diet, unless otherwise specified)

| Nutritional content | Treatments ¹ | | | | |
|-------------------------|-------------------------|------|------|------|------|
| | T1 | T2 | T3 | T4 | T5 |
| Crude protein | 200 | 200 | 198 | 198 | 199 |
| Ether extract | 95.1 | 94.3 | 94.3 | 95.3 | 95.5 |
| Crude fiber | 45.3 | 44.1 | 45.5 | 45.2 | 44.1 |
| Ash | 56.4 | 57.2 | 57.0 | 59.6 | 57.1 |
| Calcium | 8.8 | 8.9 | 8.7 | 8.8 | 8.7 |
| Total-P | 5.8 | 5.8 | 5.7 | 5.7 | 5.7 |
| ME (MJ/kg) ² | 15.2 | 15.1 | 15.1 | 15.1 | 15.2 |

¹ Treatments: T1 control, T2 avilamycin, T3 crude ethanol extract (CEE) of Jakr-Na-Rai (JNR) granules (0.13% flavonoid), T4 JNR powder (0.13% flavonoid), and T5 JNR powder (0.26% flavonoid).

² Metabolizable energy (ME) values were calculated using an equation from AAFCO (2000).

on the recommendation for the breed, were used to calculate the flavonoid doses for inclusion in the diets. Five dietary treatments were evaluated using the standard basal diet for the breed supplemented with (i) nothing (control), (ii) avilamycin at 2.5 mg/kg (SurmaxTM, avila 10%, Eli Lilly, USA), (iii) JNR as CEE granules at a flavonoid level of 1.3 g/kg of diet, and (iv and v) JNR powder at a flavonoid level of 1.3 or 2.6 g/kg, respectively. The composition (ingre-

dients) of each diet is shown in Table 2. After formulation, samples from each diet were analyzed for nutrient and ME composition as described above. The chemical composition of each experimental diet is shown in Table 3.

Data and Sample Collection

The daily temperature and relative humidity (RH) were recorded at 08:00, 12:00, and 16:00. The average temperature and RH in the experimental period were 31.8±1.9°C

and $81.4 \pm 7.2\%$, respectively. The weight and feed intake of the broilers were recorded at 22 and 41 d of age and the values used to evaluate growth performance. Mortality was recorded daily.

On days 39–41, 20 g/kg of acid insoluble ash (AIA) was mixed into the diets as an indigestible marker for analysis of the ileal digestibility of fat and protein. On day 42, two birds were randomly selected from each pen and euthanized by intracardiac injection with sodium pentobarbital (100 mg/kg of BW). The abdomens of the chicks were then opened and the jejunal (from the duodenum to Meckel's diverticulum) and ileal (from Meckel's diverticulum to the ileocecal junction) contents were individually collected by gently squeezing the contents into a plastic bottle. Samples of jejunal or ileal contents from two birds in each pen were equally pooled, forming one sample to represent one replication, and stored at -20°C until analysis. The jejunal contents were analyzed for TBAC as previously reported (Chong, 2006). The ileal content samples, as well as the diets, were analyzed for protein and the ether extract composition using AOAC procedures 976.05 and 920.39, respectively (AOAC, 1990). The AIA was analyzed using the methods of Angkanaporn *et al.* (1996). The analyzed values were used to calculate the nutrient digestibility coefficient.

On day 43, two birds were randomly sampled from each pen and fasted for 3 h. The birds were individually weighed and blood was collected from the wing vein using a 22G, 1-inch needle. Blood droplets from each bird were immediately used to determine blood glucose levels using a digital blood glucose kit (Accu-Check Advantage II[®], Roche, Switzerland) (Meex *et al.*, 2006) and the remainder were harvested into three blood collection tubes. The first tube contained EDTA for blood constituent analysis of white blood cell (WBC), packed cell volume (PCV), heterophil (H), and lymphocyte (L) levels. The second tube contained heparin for blood chemistry analysis (cholesterol and triglyceride levels). Both tubes were sent to the Small Animal Hospital, Faculty of Veterinary Science, Chulalongkorn University, for the respective blood constituent and blood chemistry analyses using the CELL-DYN 3700 system (Interbio-Lab Inc., Orlando, FL, USA). The third tube also contained heparin, but the plasma was centrifuged at $300 \times g$ for 5 min (NF 800R, Nüve Sayani Malzemeler, Istanbul, Turkey) and then stored at -80°C for subsequent analysis of the concentrations of thiobarbituric acid-reactive substance (TBARS). Blood samples were collected from two additional birds from each replicate after fasting overnight for 8 h; the birds were subsequently euthanized by cervical dislocation, bled, dipped in hot water for 1 min, the feathers removed, and the carcass immersed in an icebox for 30 min. The carcass was then opened and both the giblets and abdominal fat pad were removed. The carcass and the abdominal fat pad were weighed and the values used to calculate the percentage of carcass and abdominal fat pad based on the relative live weight, as previously described (Dong *et al.*, 2007). The breast meat was separated into left and right parts; the left part was used to measure drip loss

(Honikel, 1998), while the right part was stored in a plastic bag at -80°C for subsequent TBARS analysis.

Sample Analysis

The total flavonoid content was analyzed as described by Wan *et al.* (2011a). In brief, approximately 0.3 mL of the CEE of JNR was pipetted into a test tube and 8 mL of 10% (w/v) aluminum chloride and 4 mL of 0.2 M sodium acetate were added. The mixture was vortexed and immediately diluted with 12.7 mL of deionized distilled water with thorough mixing and then left at room temperature for 30 min. The absorbance of the reaction mixture was monitored at 350 nm (A_{350}) with a UV-VIS double-beam spectrophotometer (UV-VIS 160A, Shimadzu, Tokyo, Japan) and compared with a kaempferol standard curve.

Plasma TBARS was determined as previously described (Feix *et al.*, 1991), with some modifications. Samples (600 μL each) were mixed with 120 μL of 50 nmol/L butylated hydroxytoluene (BHT) and 1.8 mL of 10% (w/v) trichloroacetic acid and incubated for 10 min at 4°C before being centrifuged at $300 \times g$ for 10 min. Then, 1.5 mL of the harvested supernatant was mixed with 1.5 mL of 5% (w/v) TBA and heated for 15 min in boiling water. The absorbance was read at 532 nm (A_{532}) using a UV-VIS spectrophotometer and compared to a standard curve derived from 0, 2, 4, 6, 8, and 10 nM 1,1,3,3-tetraethoxypropane. The TBARS values were expressed as nmol of malondialdehyde (MDA)/mL.

The TBAC was determined as described by Chong (2006). The jejunal content was freeze-dried at -60°C using a lyophilizer (Labconco[®], Kansas city, MO, USA), ground with a pestle and mortar, and weighed, following which distilled water was added at 0.3 mL/mg. The mixed solution was then clarified by centrifugation ($2,000 \times g$ at 4°C for 5 min) and the harvested supernatant was used to determine the TBAC using a test kit (DZ042A, Diazyme Laboratories, Poway, CA, USA). For this, 270 μL of 0.1 mmol Thio-NAD was pipetted into a cuvette, 4 μL of supernatant or distilled water (as blank) was added, followed by incubation at 37°C for 3 min. Then, 90 μL of 0.1 mmol of a mixture of 3 α -HSD and NADH was added and the absorbance was immediately monitored at 405 nm (A_{405}) for 2 min. The ΔA_{405} (nm/min) for the sample, standard, or blank was obtained by subtracting the A_{405} at 60 s from that at 120 s.

The TBAC was calculated using Equation (1).

$$\text{TBAC (mmol/L)} = \left(\frac{\Delta A_{405\text{sample}} - \Delta A_{405\text{blank}}}{\Delta A_{405\text{standard}} - \Delta A_{405\text{blank}}} \right) \times \text{standard.} \quad (1)$$

The TBARS value of the breast meat was determined as previously described (Cherian *et al.*, 1996). In brief, breast meat was thawed, 2 g of tissue was placed in a 50-mL test tube, and 18 mL of 3.86% (v/v) perchloric acid was added; the tissue was homogenized with a Polytron (Poly PT-MR3100, Kinnematica AG, Littau, Switzerland) for 30 s at 5,000 rpm, with 1 mL of BHT solution being added during homogenization to control the lipid oxidation reaction. The homogenate was then filtered through Whatman filter paper No. 1, and 2 mL of the filtrate was mixed with 2 mL of a 20

mM TBA reagent, incubated in a boiling water bath for 30 min, and then left to cool for 45 min. The absorbance of the filtrate at 532 nm (A_{532}) was monitored using a double-beam UV-VIS spectrophotometer (UV-160 A, Shimadzu) and compared with a malondialdehyde tetrabutylammonium salt solution standard curve. The TBARS values were expressed as mg of MDA/kg of tissue.

Statistical Analysis

For dependent variables (blood constituents and blood chemistry parameters, TBAC, ileal nutrient digestibility coefficient, carcass and abdominal fat percentage, and drip loss and MDA in plasma and breast meat), two birds per pen from the grower-finisher period were used. Growth performance variables were analyzed per pen. Statistical analysis for all the dependent variables was performed as a completely randomized design using one-way analysis of variance (ANOVA) to determine treatment effects (SAS, 2002). Differences among means were compared by the Bonferroni method; P -values <0.05 were considered significant.

Results

The nutrient composition and flavonoid levels of the JNR powder and CEE granule forms are shown in Table 1. The JNR powder had higher protein (2.1-fold), fiber (8.4-fold), calcium (13.1-fold), and phosphorus (2.9-fold) contents than the CEE granule form, but lower fat (1.3-fold), total flavonoid (3.7-fold), and ME (1.4-fold) contents. However, as stated above, the inclusion level of JNR in the diets (Table 2) was calculated based on total flavonoid levels. The analytical value of all the nutrients and the calculated ME of all the diets were near identical (Table 3).

Blood Constituents, Blood Chemistry, and Plasma Lipid Peroxidation

For the broiler blood constituent parameters, the PCV and WBC count did not differ among the five treatment groups, but the levels of H and L were significantly decreased ($P=0.005$) and increased ($P=0.028$), respectively; this led to a significantly smaller H/L ratio in birds fed the diet with JNR powder at 1.3 g/kg compared to the control and antibiotic groups (Table 4). However, increasing the JNR powder level from a 1.3 to 2.6 g/kg flavonoid dosage did not result in significantly different broiler blood parameters.

Blood chemistry analysis showed that glucose and cholesterol levels were significantly decreased ($P=0.005$ and 0.002 , respectively) in the diets containing powder JNR at a 2.6 g/kg flavonoid dose. Triglyceride concentrations in the broilers from all three JNR-fed groups were considerably lower than those in the control and antibiotic fed groups ($P=0.056$), while there was no significant difference in plasma lipid peroxidation levels (as MDA) among all the treatment groups ($P>0.05$).

TBAC and Ileal Nutrient Digestibility Coefficient

The TBAC (Table 5) increased significantly in birds fed with diets containing the powder or CEE forms of JNR ($P=0.001$) compared to the control or antibiotic groups. However, the crude protein and ether extract digestibility coefficients were not different among all the groups.

Growth Performance

The average BW of the birds at the start of the trial (at 22 d of age) was not significantly different among the five groups, but by 41 d of age, the average BW of birds fed with either the CEE or powder forms of JNR at a flavonoid level of 1.3 g/kg of diet was significantly higher than that in the control

Table 4. Effect of treatments on blood constituents, blood chemistry, and thiobarbituric acid-reactive substances in the plasma (expressed as MDA levels) on day 43

| Observations | Treatments ^{1,2} | | | | | SEM | P -value |
|----------------------------------|---------------------------|-------------------|-------------------|-------------------|--------------------|-------|------------|
| | T1 | T2 | T3 | T4 | T5 | | |
| <i>Blood constituents</i> | | | | | | | |
| PCV ³ (%) | 31.5 | 30.9 | 31.1 | 28.1 | 31.3 | 0.514 | 0.181 |
| WBC ⁴ (cell/ μ L) | 17,500 | 21,110 | 23,221 | 30,200 | 25,175 | 1,548 | 0.094 |
| Heterophil (%) | 41.3 ^{ab} | 50.8 ^a | 50.6 ^a | 24.5 ^b | 33.4 ^{ab} | 2.719 | 0.003 |
| Lymphocyte (%) | 43.4 ^b | 45.1 ^b | 43.6 ^b | 56.7 ^a | 57.8 ^a | 1.968 | 0.012 |
| H/L ratio ⁵ | 0.95 ^{ab} | 1.13 ^a | 1.17 ^a | 0.44 ^b | 0.59 ^b | 0.076 | 0.001 |
| <i>Blood chemistry</i> | | | | | | | |
| Glucose (mg/dL) | 212 ^a | 201 ^a | 198 ^{ab} | 183 ^{ab} | 162 ^b | 4.892 | 0.005 |
| Triglyceride (mg/dL) | 38.6 | 32.6 | 26.7 | 24.1 | 26.8 | 4.892 | 0.056 |
| Cholesterol (mg/dL) | 124 ^a | 130 ^a | 129 ^a | 107 ^{ab} | 99.9 ^b | 3.217 | 0.002 |
| MDA ⁶ (nmol/mL) | 3.03 | 3.00 | 2.97 | 2.94 | 2.94 | 0.014 | 0.233 |

¹ Treatments: T1 control, T2 avilamycin, T3 crude ethanol extract (CEE) of Jakr-Na-Rai (JNR) granules (0.13% flavonoid), T4 JNR powder (0.13% flavonoid), and T5 JNR powder (0.26% flavonoid).

² Mean value from six replications per treatment.

³ Packed cell volume

⁴ White blood cell count

⁵ H/L ratio was calculated by dividing % Heterophils by % Lymphocytes.

⁶ Malondialdehyde concentrations

^{a-b} Means in the same row with different superscript letters are significantly different ($P<0.05$).

Table 5. Effect of treatments on the total bile acid concentrations (TBAC) and ileal nutrient digestibility coefficients on day 41

| Observation | Treatment ¹ | | | | | SEM | P-value |
|--|------------------------|-------------------|-------------------|-------------------|-------------------|-------|---------|
| | T1 | T2 | T3 | T4 | T5 | | |
| TBAC (mmol/L) ² | 29.1 ^b | 26.6 ^b | 40.6 ^a | 43.3 ^a | 43.8 ^a | 1.583 | 0.001 |
| Ileal digestibility coefficients ^{2,3} (DM ⁴) | | | | | | | |
| Crude protein | 0.75 | 0.78 | 0.74 | 0.79 | 0.75 | 0.007 | 0.193 |
| Ether extract | 0.78 | 0.79 | 0.79 | 0.79 | 0.79 | 0.008 | 0.987 |

¹ Treatments: T1 control, T2 avilamycin, T3 crude ethanol extract (CEE) of *Jakr-Na-Rai* (JNR) granules (0.13% flavonoid), T4 JNR powder (0.13% flavonoid), and T5 JNR powder (0.26% flavonoid).

² Mean value from six replications per treatment on day 41.

³ Pooled ileal content samples from two birds per replication on day 41.

^{a-b} Means in the same row with different superscript letters are significantly different ($P < 0.05$).

⁴ DM=dry matter.

Table 6. Effect of treatments on the growth performance between days 22 to 41

| Observation | Treatment ^{1,2} | | | | | SEM | P-value |
|--------------------------------|--------------------------|-------------------|-------------------|-------------------|--------------------|------|---------|
| | 1 | 2 | 3 | 4 | 5 | | |
| Body weight, kg/bird | | | | | | | |
| On day 22 | 0.82 | 0.81 | 0.82 | 0.84 | 0.82 | 0.01 | 0.866 |
| On day 41 | 2.45 ^b | 2.46 ^b | 2.61 ^a | 2.59 ^a | 2.53 ^{ab} | 0.02 | 0.038 |
| ADG ³ , g/(bird·d) | 83.6 | 84.5 | 91.7 | 89.9 | 87.5 | 1.07 | 0.066 |
| ADFI ⁴ , g/(bird·d) | 154 ^b | 155 ^{ab} | 166 ^a | 165 ^a | 160 ^{ab} | 1.50 | 0.016 |
| FCR ⁵ | 1.85 | 1.84 | 1.82 | 1.84 | 1.83 | 0.01 | 0.968 |
| Mortality rate (%) | 2.1 | 0.0 | 0.0 | 0.0 | 2.1 | 0.68 | 0.567 |

¹ Treatments: T1 control, T2 avilamycin, T3 crude ethanol extract (CEE) of *Jakr-Na-Rai* (JNR) granules (0.13% flavonoid), T4 JNR powder (0.13% flavonoid), and T5 JNR powder (0.26% flavonoid).

² Mean value from six replications per treatment.

³ Average daily gain

⁴ Average daily feed intake

⁵ Feed conversion ratio

^{a-b} Means in the same row with different superscript letters are significantly different ($P < 0.05$).

or antibiotic groups ($P=0.038$), but this was not significantly altered by the inclusion of the higher level of JNR powder (Table 6). The average daily gain (ADG) of the birds showed only a numerical ($P=0.066$) increase with the JNR diets, presenting the same trend as the average final BW. The average daily feed intake (ADFI) of broilers fed with diets containing the CEE form of JNR was higher than that in the control group ($P=0.016$), while those fed with the powder form of JNR presented only a numerical increase in the ADFI, which was not affected by inclusion of the higher flavonoid level (2.6 g/kg of diet) in both average final BW and ADFI. Finally, the feed conversion ratio (FCR) and mortality rate were not significantly different among all the groups.

Carcass Fat Deposition and Lipid Peroxidation in the Breast Meat

The live BW of fasted birds in all the treatment groups was not significantly different, but tended to decrease in the antibiotic and powder JNR diet groups, as did the carcass weight (Table 7), except that for the higher JNR powder dose (flavonoid level of 2.6 g/kg of diet), which showed signifi-

cantly lower carcass weight than in the control, antibiotic, and CEE JNR diet groups ($P=0.007$). The percentage of abdominal fat was significantly lower in broilers fed with diets containing JNR powder at either dose compared to the control ($P=0.025$); however, no significant difference in the percentage of abdominal fat was found in broilers fed with the CEE form of JNR compared to the control and antibiotic-fed groups. There was no significant effect of the treatments on drip loss or lipid peroxidation (MDA) in breast meat.

Discussion

The nutrient composition of all the experimental diets was similar, indicating that the results likely reflect the effect of dietary JNR. The blood PCV was used as a general health status indicator (Goodwin *et al.*, 1991), while the WBC count (Al-Saffar and Al-Mawla, 2008) and H/L ratio (Post *et al.*, 2003) were used as an indicator of stress. Bounouns and Stedman (2000) reported that the PCV and WBC count in normal broilers at 42 d of age were 22–35% and 12,000–30,000 cells/ μ L, respectively. The values for PCV and WBC count recorded in this study were within these ranges,

Table 7. Effect of treatments on the abdominal fat pad, drip loss, and thiobarbituric acid-reactive substances in breast meat (expressed as MDA levels) on day 43

| | Treatment ^{1,2} | | | | | SEM | P-value |
|---|--------------------------|--------------------|--------------------|--------------------|-------------------|-------|---------|
| | 1 | 2 | 3 | 4 | 5 | | |
| Live weight (kg/bird) | 2.63 | 2.47 | 2.61 | 2.42 | 2.39 | 0.04 | 0.195 |
| Carcass weight ³ (%) | 84.2 ^a | 83.5 ^a | 84.5 ^a | 82.3 ^{ab} | 80.4 ^b | 0.44 | 0.007 |
| Abdominal fat (%) | 1.93 ^a | 1.63 ^{ab} | 1.76 ^{ab} | 1.40 ^b | 1.41 ^b | 0.06 | 0.025 |
| Drip loss (%) | 0.96 | 0.97 | 0.97 | 0.97 | 0.97 | 0.002 | 0.539 |
| MDA ⁴ in breast meat (mg/kg) | 0.43 | 0.42 | 0.43 | 0.44 | 0.44 | 0.008 | 0.933 |

¹ Treatments: T1 control, T2 avilamycin, T3 crude ethanol extract (CEE) of Jakr-Na-Rai (JNR) granules (0.13% flavonoid), T4 JNR powder (0.13% flavonoid), and T5 JNR powder (0.26% flavonoid).

² Mean value from six replications per treatment.

³ Carcass weight without feathers, blood, and giblets.

⁴ Malondialdehyde concentrations

^{a-b} Means in the same row with different superscript letters are significantly different ($P < 0.05$).

indicating that the birds in all the groups were likely to be healthy. However, the H/L ratio was significantly lower in birds fed on JNR powder at both levels compared to those fed with the other diets. Gross and Siegel (1983) reported that the H/L ratio can be used as a more reliable stress indicator, showing less variation than the individual WBC profile, because stress stimulates corticosterone secretion and induces decreased L and increased H levels (Post *et al.*, 2003; Thaxton *et al.*, 2006). The JNR in granule form could not lower the H/L ratio compared to the powder form at the same flavonoid level (1.3 g/kg of diet), indicating that JNR likely contains other active ingredients that were not extracted by ethanol. Yuchan *et al.* (2011) reported that JNR contained 2.52 g/kg ascorbic acid, while Peña *et al.* (2008) reported a synergistic action between flavonoids and ascorbic acid in reducing stress.

The MDA level, as a product of lipid peroxidation, was used as an indicator for lipid peroxidation (Halliwell and Chirico, 1993). There was no significant difference in plasma MDA levels among all the groups, even though the H/L ratio differed significantly between them. JNR exhibits prominent antioxidant activity due to chlorogenic acid, one of its phenolic compounds (Rodriguez de Sotillo and Hadley, 2002), but this may be attributed to the reduced peroxidation of pancreatic β -cells (Xu and Zhang, 2017). Damsawang *et al.* (2010) found that flavonoid phenolic compounds had the ability to decrease MDA levels, while Intajak *et al.* (2012) reported that a CEE of fresh JNR leaves exhibited antioxidative properties and could decrease the concentration of free radicals by 50% *in vitro*.

Blood glucose levels were determined after the chicks had fasted for 3 h as their maximum blood glucose response was reported to be from 50–240 min after feeding (Gutierrez del Alamo *et al.*, 2009). The normal blood glucose level in broilers is reported to be in the range of 180–250 mg/dL (Hernawan *et al.*, 2012). Diets supplemented with JNR powder at the 2.6 g/kg flavonoid dose significantly decreased the broiler blood glucose and cholesterol levels. This may be due to active ingredients like flavonoids and polysaccharides

that inhibit the activities of the enzymes α -amylase and α -glycosidase, leading to reduced levels of produced and absorbed glucose (Deng *et al.*, 2011; Wu *et al.*, 2011). Jiang *et al.* (2009) demonstrated that both flavonoids and polysaccharides (i.e., fructooligosaccharides) were the major ingredients in JNR for decreasing blood glucose levels, which was in agreement with Li *et al.* (2009) who found that crude water and 95% (v/v) ethanol extracts of whole JNR plants at 0.4 g/kg of BW could significantly decrease blood glucose levels in both normal and alloxan-diabetic mice. Similarly, a polysaccharide extract from whole JNR plants significantly decreased blood and urine glucose levels when fed to streptozotocin-induced diabetic rats at 0.4 g/kg of BW (Deng *et al.*, 2011). In this study, however, both the powder and the granule forms of JNR at the lower flavonoid level of 1.3 g/kg did not decrease broiler blood glucose levels, which may reflect the lower polysaccharide contents of both forms (Chen *et al.*, 2003; Wan *et al.*, 2011a).

Silva *et al.* (2007) reported that the normal range of blood cholesterol in 42-d-old broilers was 109–150 mg/dL. In this study, blood cholesterol levels were significantly decreased in chicks receiving JNR powder at a flavonoid dose of 2.6 g/kg of BW. Chen *et al.* (2003) reported that JNR contains β -sitosterol, which is known to inhibit cholesterol absorption in the small intestine, thereby reducing blood cholesterol levels (Matsuoka *et al.*, 2008; Shi *et al.*, 2011). The dietary JNR-mediated reduction in cholesterol levels observed in this study is in contrast to other studies (Aritajat *et al.*, 2008; Keeratikajorn *et al.*, 2012), but direct comparisons are not possible owing to the different plant parts, extraction methods, and species of test animal used, as well as the JNR dosage administered. Blood triglyceride levels in all the JNR-fed groups tended to be lower ($P = 0.056$) than those in the control and antibiotic-fed groups. Musa *et al.* (2007) showed that blood cholesterol levels have a positive correlation with blood triglyceride levels. The crude water extract of JNR had obvious effects on reducing triglyceride levels in experimental hyperlipidemia rats (Tong *et al.*, 2012).

Kaempferol is the main flavonoid in JNR responsible for increasing bile acid secretion (Powell *et al.*, 2001; Crespy *et al.*, 2003). In this study, the TBAC in the jejunum was used to represent the quantity of bile salt secreted into the small intestine. Bile salts help to emulsify lipids in the jejunum for micelle formation and increased fat absorption (Orban and Harmon, 2000). The TBAC was increased in chicks fed with a diet supplemented with either the powder or CEE form of JNR, which may have been due to the effect of kaempferol. Increasing the TBAC will likely increase the efficiency of fat emulsification, thereby improving its digestibility (Powell *et al.*, 2001). However, the increased TBAC in chicks observed in this study did not elicit any beneficial effect on the ileal fat and protein digestibility coefficients. The ether extracts of the diets used in this study were moderately high, which required the activity of both pancreatic enzymes and bile acid to assimilate the fat. The kaempferol-mediated increase in the TBAC level would not evoke any beneficial effect on the digestibility coefficients of ileal nutrients as enzyme activity, and not bile salts, would be the limiting factor.

Compared to the control diet, supplementation with an antibiotic (avilamycin at 2.5 mg/kg) did not elicit any beneficial effect on growth performance at the high stocking density used. Accordingly, avilamycin supplementation at a higher dose (10 mg/kg) in the broiler diet did not significantly affect the ADFI and FCR compared to control diet (Bozkurt *et al.*, 2008). The average final BW of the 41-d-old birds fed with either the JNR supplemented diet at a flavonoid level of 1.3 g/kg was higher than that of birds in the control or antibiotic groups. The greater BW of birds in the JNR-fed groups may have been due to the higher ADFI. Although the ADFI is regulated by the energy requirements in birds (Hernawan *et al.*, 2012), the ME was near identical in all the experimental diets. Therefore, theoretically, the ADFI should not be different and the observed higher ADFI may have been due to the lower blood glucose levels resulting from the flavonoid and polysaccharide content in JNR (Jiang *et al.*, 2009). However, the FCR was not different among all the groups, which corresponded with the absence of any significant difference in ileal nutrient digestibility between the groups. No significant effect of JNR on broiler mortality was detected; the highest mortality rate in this study was 2.1%, which lies within the normal range of <4% (Oluremi *et al.*, 2008).

The glucose-lowering effect of JNR was likely responsible for the high ADFI of the chicks, which increased to fulfill their energy needs. The way to provide the required energy was to break down glycogen followed by triglycerides (North, 1984). Since these birds had fewer glycogen deposits, they subsequently lost their deposited body fat. Abdominal fat has been used to represent net fat deposition in poultry (Griffiths *et al.*, 1976). By day 43, the carcass weight and abdominal fat as a percentage of the live weight were significantly lower in the JNR powder-treated groups, whereas the live weight was not significantly lower. The JNR powder at either dose was marginally more effective

than the CEE form at decreasing the percentage of abdominal fat. This may also reflect the higher polysaccharide level in the powder form that elicits increased blood glucose depletion than the CEE (Wan *et al.*, 2011b). The percentage of carcass weight has been reported to correlate well ($r=0.98$) with the live bird weight (Olawumi, 2013). However, the live weight of random birds in both JNR powder-fed groups was smaller than that in the other groups, although the difference was not significant. The decreased carcass weight and fat deposition may be explained by the lighter BW of the live birds. When the live weight was considered for statistical analysis of both carcass and abdominal fat percentages using analysis of covariance (ANCOVA), the same non-significant trend was found for abdominal fat (data not shown), indicating that further investigation on fat deposition is needed.

The increased drip loss was likely due to the detrimental effects of free radicals on long-chain unsaturated fatty acids in the cell membranes, which leads to changes in cell structure and protein stability (Halliwell and Chirico, 1993). Denatured proteins lower the water absorbing and water retention capacity of the muscle (Huff-Lonergan and Lonergan, 2005). In this study, the production of free radicals may not have been sufficient to destroy the cell membranes, and, therefore, no significant difference in drip loss was observed in breast meat. This explanation could also be applied to the observed MDA levels in breast meat. Significant reductions in drip loss and MDA levels in the breast meat of animals with induced high free radical production were recorded with kaempferol dietary supplementation at 0.2 g/kg of BW (Nirmala and Ramanathan, 2011). The positive correlation between drip loss and MDA levels in breast meat was confirmed by Xiao *et al.* (2013).

In conclusion, dietary inclusion of JNR powder at a flavonoid level of 1.3 g/kg of diet in the grower-finisher period (22–43 d of age) of broilers was sufficient to lower the H/L ratio, blood glucose and cholesterol levels. Moreover, JNR powder supplementation increased the TBAC, promoted growth performance, and decreased the percentage of abdominal fat. The powder form of JNR produced better results than the CEE form at the same flavonoid level.

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Conflicts of Interest

The authors declare no conflict of interest.

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