

Effects of dietary rapeseed meal inclusion levels on growth performance, organ weight, and serum biochemical parameters in Cherry Valley ducks

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ABSTRACT The aim of this study was to investigate the effects of the inclusion levels of different types of rapeseed meal (**RSM**) on performance, organ weight, and serum biochemical parameters in Cherry Valley ducks in the starter period and grower-finisher period. In Exp. 1, a total of 750 seven-day-old male ducklings were divided into 5 dietary treatments with 6 replicate pens of 25 birds per pen. The starter diets with the inclusion of 0, 5, 10, 15, or 20% of double-low RSM contained 0, 1.37, 2.15, 3.46, or 5.31 μmol glucosinolates (**GLS**)/g in the finished feed (from day 7 to 21). In Exp. 2, a total of 900 fifteen-day-old male ducklings were divided into 6 dietary treatments with 6 replicate pens of 25 birds per pen. The grower-finisher diets with the inclusion of 0, 5, 10, 15, 20, or 25% of Indian RSM contained 0, 7.67, 15.34, 24.66, 31.21, or 38.44 μmol GLS/g in the finished feed (from day 15 to 42). For

ducklings in the starter period (Exp. 1), body weight gain and feed intake decreased linearly as the dietary double-low RSM inclusion level increased at day 7 to 14, while growth rate was not influenced by dietary double-low RSM inclusion levels at day 15 to 21 and day 7 to 21. For ducks in the grower-finisher period (Exp. 2), growth performance decreased linearly as the dietary RSM inclusion level increased from 5 to 20%. In addition, dietary RSM inclusion levels induced liver enlargement in ducklings at day 21 (5 to 20% double-low RSM with 1.37 to 5.31 $\mu\text{mol/g}$ GLS) and thyroid enlargement accompanied by increased serum AST and ALP activities in ducks at day 42 (5 to 15% Indian RSM with 7.67 to 23.66 $\mu\text{mol/g}$ GLS). Therefore, our results indicated that the upper limit of using RSM sources in feed formulation should consider the anti-nutritional factor of GLS content at different stages of duck growth.

Key words: duck, rapeseed meal, growth performance, organ weight

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INTRODUCTION

Rapeseed meal (**RSM**), which is composed of 35 to 42% crude protein, is a good alternative protein source to replace soybean meal in poultry diets (Swick, 1999). Previous studies in broilers have shown that the optimal inclusion of RSM (150 to 200 g/kg) in diets had no adverse effects on growth performance (Kocher et al., 2000; Maroufyan and Kermanshahi, 2006; Ahmad et al., 2007). However, an excessive level of RSM containing 6 to 10 $\mu\text{mol/g}$ GLS could reduce growth rate, accompanied by hypertrophy and hemorrhage of the thyroid and liver (Griffiths et al., 1980; Karunajewa et al., 1990). The use of RSM in poultry diets has been mainly restricted by the presence of anti-nutritional factors, such as glucosinolates (**GLS**; Tripathi and Mishra,

2007) and sinapine (Qiao and Classen, 2003). Therefore, double-low RSM with low levels of erucic acid (<20 g/kg) and GLS (<30 $\mu\text{mol/g}$) has been well developed in the poultry diet (Maison and Stein, 2014). In addition to the type of RSM and concentrations of anti-nutritional factors, research has shown that the recommended levels for RSM inclusion in diets were also dependent upon the breed and age of the poultry being reared (Griffiths et al., 1980; Pearson et al., 1983). For example, broiler strains at the same age displayed more sensitive responses to the inclusion levels of RSM in diets than layer hens, as determined by the growth rate and trimethylamine oxidase activity in livers (Pearson et al., 1983). Compared to broilers, ducks have a greater digestive capability and appear to have more efficient digestion of RSM (Borin et al., 2006). Although RSM, as an alternative protein source, is used extensively to reduce feed costs of duck production in China, it is unclear whether it would be possible to use a higher incorporation rate of RSM in duck diets than broiler diets (Wickramasuriya et al., 2015).

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Qin et al. (2017) indicated that the maximum limit of dietary RSM containing 7.57 $\mu\text{mol/g}$ GLS was estimated to be 4.27% to avoid growth reduction in ducks in the grower-finisher period. However, the tolerance of anti-nutritional factors from RSM inclusion for ducks may be different between the starter and grower-finisher periods. Therefore, the objective of this study was to determine the effect of inclusion levels of double-low and Indian RSM sources on performance, organ weight, and serum biochemical parameters in Cherry Valley ducks in the starter period and grower-finisher period, respectively.

MATERIALS AND METHODS

Double-low RSM and Indian RSM were used to formulate the experimental diets in ducks in the starter period (Exp. 1, day 7 to 21) and grower-finisher period (Exp. 2, day 14 to 42), respectively. The double-low RSM was obtained from a commercial company using extraction processing (Rongcheng Hongda Feed Trade Co. Ltd., Tianjin, China) and contained 39.8% crude protein, 1.36% ether extract, 0.72% calcium, 1.16% total phosphorus, 0.39% non-phytate phosphorus, 2.02% lysine, 0.73% methionine, and 25.1 $\mu\text{mol/g}$ GLS based on analyzed values. Based on the average value of 166 $\mu\text{mol/g}$ GLS in regular RSM of a different origin (Tripathi and Mishra, 2007), Indian RSM containing 160 $\mu\text{mol/g}$ GLS on analysis from a commercial oil processing plant (Kohinoor Grain Processing Pvt. Ltd., Maharashtra, India) was selected for use in Exp. 2. The Indian RSM contained 39.6% crude protein, 1.02% ether extract, 0.81% calcium, 0.97% total phosphorus, 0.27% non-phytate phosphorus, 1.70% lysine and 0.68% methionine based on analyzed values.

Birds, Management, and Diets

All experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee of South China Agricultural University. This study included 2 experiments with ducks in the starter (day 7 to 21, Exp. 1) and grower-finisher (day 15 to 42, Exp. 2) periods and was conducted on a commercial duck farm (Wens Food Group Co. Ltd., Yunfu, Guangdong). In Exp. 1, a total of 750 seven-day-old male Cherry Valley ducklings were kept in the floor pens in a building with a controlled environment, equipped with central heating at 34°C. The ducklings were fed the same corn-soybean meal diet (12.3 MJ/kg metabolizable energy, 220 g/kg crude protein, 12.0 g lysine/kg, 4.5 g/kg methionine, 8.5 g calcium/kg, and 3.8 g/kg non-phytate phosphorus) during the first week. At day 7, the birds were weighed individually and divided into 5 dietary treatments with 6 replicate pens of 25 birds per pen. During the experimental period from day 7 to 21, the room temperature was subsequently reduced by 2°C per week. Light was provided for 24 h daily

throughout the experiment. The starter diets included a corn-soybean meal basal diet with the inclusion level of 0, 5, 10, 15, or 20% of double-low RSM with low GLS content (25.1 $\mu\text{mol/g}$ on analysis). The 5 experiment diets contained 0, 1.37, 2.15, 3.46, and 5.31 $\mu\text{mol GLS/g}$ diet based on the analyzed values. In Exp. 2, 900 Cherry Valley fifteen-day-old male ducklings were reared in floor pens according to the same management as described in Exp. 1. The birds were fed the same starter diet based on corn-soybean meal (12.3 MJ/kg metabolizable energy, 220 g/kg crude protein, 12.0 g lysine/kg, 4.5 g/kg methionine, 8.5 g calcium/kg, and 3.8 g/kg non-phytate phosphorus) for 14 D. Subsequently, birds were allocated randomly to 6 dietary treatments with 6 replicate pens of 25 birds per pen based on the average BW. The grower-finisher diets included a corn-Distillers Dried Grains with Solubles (DDGS)-cottonseed meal basal diet with the inclusion levels of 0, 5, 10, 15, 20, or 25% Indian RSM with high GLS content (160 $\mu\text{mol/g}$ on analysis). The 6 experiment diets contained 0, 7.67, 15.34, 23.66, 31.21, and 38.44 $\mu\text{mol GLS/g}$ diet calculated from the GLS content of Indian RSM. All experimental diets were isoenergetic and isonitrogenous, and were formulated to meet or exceed NRC (1994) nutrient requirements of ducks in the starter period (Exp. 1, Table 1) and grower-finisher period (Exp. 2, Table 2). Experimental diets and water were provided ad libitum. The ducks' care and management were in accordance with the guidelines approved by Cherry Valley Duck Farm.

Sample Preparations and Analyses

For the 2 experiments, after 12-h feed withdrawal, ducks were weighed, and feed consumption was monitored weekly in each replicate pen to calculate the average daily gain (ADG), average daily feed intake (ADFI), and feed: gain ratio (F: G). At the end of Exp. 1 and 2, 1 bird, based on average BW in each pen, was selected for blood sample collection via the bronchial vein (3.5 mL/bird) before euthanasia. Serum samples were obtained by centrifuging blood samples at 3,000 \times g for 20 min at 4°C, and samples were stored at -20°C for analysis of biochemical indices. Subsequently, the bird was euthanized by CO₂ inhalation and was immediately bled. The liver, kidney, thyroid, spleen, bursa of Fabricius, and thymus were separated from the euthanized bird and weighed. The relative organ index was calculated as the organ weight (g): BW (kg) ratio. Additionally, in Exp. 2, the left breast and leg meats were removed and weighed to determine the percentages breast and leg meats relative to live BW at processing. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) activities, total protein (TP), globulin, albumin, and urea nitrogen content were measured using a HITACHI 7180 automatic biochemical analyzer (Hitachi Ltd., Tokyo, Japan) using detection kits (Nanjing JianCheng Bioengineering Institute, Nanjing,

Table 1. Composition and nutrient levels of the experimental diets for the starter period (as-fed basis).

Ingredient, %	Dietary double-low rapeseed meal level, %				
	0	5	10	15	20
Corn	40.26	40.47	40.66	40.84	40.19
Soybean meal	31.53	27.54	23.53	19.51	14.38
Double-low rapeseed meal	0	5	10	15	20
Wheat middling	10	10	10	10	10
Rice chaff and husk	4.20	3.04	1.90	0.75	0.25
Corn gluten meal	4.0	4.0	4.0	4.0	4.0
Puffed soybean powder	3.16	3.16	3.18	3.21	4.31
Mixed oil	3.00	3.00	3.00	3.00	3.25
Limestone	1.21	1.15	1.11	1.08	1.05
Dicalcium phosphate	0.75	0.73	0.70	0.67	0.63
NaCl	0.45	0.45	0.45	0.45	0.45
L-Lysine sulfate	0.18	0.22	0.25	0.29	0.32
Choline chloride	0.15	0.15	0.15	0.15	0.15
DL-Methionine	0.11	0.09	0.07	0.05	0.02
Vitamin and mineral premix ¹	1	1	1	1	1
Total	100	100	100	100	100
Nutrient levels					
Calculated values					
Metabolizable energy, MJ/kg	11.92	11.92	11.92	11.92	11.92
Crude protein, %	22.0	22.0	22.0	22.0	22.0
Lysine, %	1.20	1.20	1.20	1.20	1.20
Methionine, %	0.43	0.43	0.43	0.43	0.43
Methionine + cysteine, %	0.82	0.82	0.82	0.82	0.82
Calcium, %	0.80	0.80	0.80	0.80	0.80
Non-phytate phosphorus, %	0.30	0.30	0.30	0.30	0.30
Glucosinolates, $\mu\text{mol/g}^2$	0	1.25	2.50	3.75	5.00
Analyzed values					
Crude protein, %	21.7	21.4	21.8	21.5	21.5
Calcium, %	0.82	0.82	0.84	0.81	0.80
Lysine, %	1.17	1.18	1.18	1.19	1.19
Methionine, %	0.46	0.44	0.43	0.42	0.40
Glucosinolates, $\mu\text{mol/g}^3$	–	1.37	2.15	3.46	5.31

¹Provided per kilogram of diet: vitamin A, 4,000 IU; vitamin D₃, 2,000 IU; vitamin E, 24 IU; thiamine, 2.0 mg; riboflavin, 12 mg; pyridoxine, 4.0 mg; vitamin B₁₂, 0.02 mg; calcium pantothenate, 10 mg; folate, 0.15 mg; niacin, 50 mg; biotin, 0.15 mg; choline (choline chloride), 1,000 mg; Cu (CuSO₄ · 5H₂O), 8 mg; Fe (FeSO₄ · 7H₂O), 80 mg; Zn (ZnSO₄ · 7H₂O), 90 mg; Mn (MnSO₄ · H₂O), 70 mg; Se (NaSeO₃), 0.3 mg; I (KI), 0.4 mg.

²Calculated values based on the analyzed value 25.1 $\mu\text{mol/g}$ glucosinolates in double-low RSM.

³“–” represents the undetected glucosinolates value.

China). Serum triiodothyronine (T₃) and thyroxin (T₄) levels were determined by radioimmunoassay using a commercial kit (Beijing North Institute of Biological Technology, Beijing, China). The GLS content in RSM was determined by isocratic liquid chromatography, as described by Quinsac et al. (1991).

Statistical Analyses

All values were subjected to a 1-way ANOVA by using the general linear model procedure of SAS (SAS Institute, Cary, NC). The treatment comparisons for significant differences were tested by the LSD method. Orthogonal polynomials were applied for linear and quadratic effects of dependent variables to independent variables. Each replicate served as the experimental

Table 2. Composition and nutrient levels of the experimental diets for the grower-finisher period (as-fed basis).

Ingredient, %	Dietary Indian rapeseed meal level, %					
	0	5	10	15	20	25
Corn	55.72	57.98	59.84	60.71	61.22	61.10
Flour	4.0	4.0	4.0	4.0	4.0	4.0
Indian rapeseed meal	0	5	10	15	20	25
Cottonseed meal	15	15	15	10.36	4.98	0
DDGS	11.4	12.0	7.2	6.0	6.0	6.0
Feather meal	2.0	2.0	0.68	0.83	0.70	0.75
Wheat bran	8.74	0.92	0.34	0.34	0.34	0.30
Limestone	0.97	1.02	0.96	0.78	0.75	0.85
Dicalcium phosphate	0.55	0.46	0.38	0.38	0.42	0.4
NaCl	0.34	0.34	0.34	0.34	0.34	0.34
L-Lysine sulfate	0.25	0.25	0.24	0.24	0.23	0.25
DL-Methionine	0.03	0.03	0.02	0.02	0.02	0.01
Vitamin and mineral premix ¹	1	1	1	1	1	1
Total	100	100	100	100	100	100
Nutrient levels						
Calculated values						
Metabolizable energy, MJ/kg	11.3	11.3	11.3	11.3	11.3	11.3
Crude protein, %	17.0	17.0	17.0	17.0	17.0	17.0
Lysine, %	0.85	0.85	0.85	0.85	0.85	0.85
Methionine, %	0.30	0.30	0.30	0.30	0.30	0.30
Methionine + cysteine, %	0.65	0.65	0.65	0.65	0.65	0.65
Calcium, %	0.65	0.65	0.65	0.65	0.65	0.65
Non-phytate phosphorus, %	0.25	0.25	0.25	0.25	0.25	0.25
Glucosinolates, $\mu\text{mol/g}^2$	0	8	16	24	32	40
Analyzed values						
Crude protein, %	17.0	17.4	17.5	17.1	17.2	17.2
Calcium, %	0.60	0.65	0.65	0.60	0.60	0.60
Lysine, %	0.84	0.83	0.82	0.84	0.86	0.84
Methionine, %	0.29	0.27	0.28	0.28	0.28	0.29
Glucosinolates, $\mu\text{mol/g}^3$	–	7.67	15.34	23.66	31.21	38.44

¹Provided per kilogram of diet: vitamin A, 8,000 IU; vitamin D₃, 4,000 IU; vitamin E, 20 IU; thiamine, 1.5 mg; riboflavin, 8 mg; pyridoxine, 3.0 mg; vitamin B₁₂, 0.02 mg; calcium pantothenate, 10 mg; folate, 0.15 mg; niacin, 50 mg; biotin, 0.20 mg; choline (choline chloride), 750 mg; Cu (CuSO₄ · 5H₂O), 10 mg; Fe (FeSO₄ · 7H₂O), 60 mg; Zn (ZnSO₄ · 7H₂O), 90 mg; Mn (MnSO₄ · H₂O), 80 mg; Se (NaSeO₃), 0.3 mg; I (KI), 0.2 mg.

²Calculated values based on the analyzed value 25.1 $\mu\text{mol/g}$ glucosinolates in double-low RSM.

³“–” represents the undetected glucosinolates value.

unit for all statistical analyses. Significant differences were set at $P \leq 0.05$.

RESULTS

Growth Performance

No differences ($P > 0.39$) on mortality were observed in ducklings in fed diets with double-low RSM levels at day 7 to 21 (Exp. 1) and ducks fed diets with Indian RSM levels at day 15 to 42 (Exp. 2), respectively. As for ducklings at 7 to 15 D of age, BW, ADG, and ADFI were decreased ($P < 0.0004$) linearly as dietary double-low RSM inclusion levels increased, while F/G was increased quadratically as dietary double-low RSM inclusion levels increased ($P = 0.0002$) (Table 3). The ducklings fed the diet with 15% double-low RSM had the lowest BW, ADG, and ADFI and the highest F/G

Table 3. Effects of dietary double-low rapeseed meal inclusion levels on growth performance and mortality of ducklings during 7 to 14, 15 to 21, and 7 to 21 D of age.

Item	Dietary double-low rapeseed meal level, % ¹					SEM	P-value	Linear	Quadratic
	0	5	10	15	20				
Day 7–14									
BW, g	505 ^a	491 ^{a,b}	494 ^a	474 ^c	478 ^{b,c}	4.9	0.001	0.0001	0.52
ADG, g/d/bird	53.9 ^a	51.9 ^{a,b}	52.3 ^a	49.5 ^c	50.0 ^{b,c}	0.70	0.001	<0.0001	0.52
ADFI, g/d/bird	72.2 ^a	69.7 ^b	70.3 ^{a,b}	69.3 ^b	65.5 ^c	0.88	0.0003	<0.0001	0.21
F: G, g/g	1.34 ^{b,c}	1.34 ^{b,c}	1.35 ^b	1.40 ^a	1.31 ^c	0.011	0.0002	0.88	0.004
Mortality, %	2.00	0.67	2.00	0.00	0.67	0.96	0.50	–	–
Day 15–21									
ADG, g/d/bird	78.5	76.3	77.5	77.8	78.6	1.14	0.61	–	–
ADFI, g/d/bird	152.1	147.9	149.9	152.6	144.4	2.19	0.08	–	–
F: G, g/g	1.94 ^a	1.94 ^a	1.94 ^a	1.96 ^a	1.84 ^b	0.18	0.0003	0.003	0.003
Mortality, %	4.08	4.03	4.81	4.67	5.36	0.59	0.50	–	–
Day 7–21									
BW, g	1,054	1,025	1,036	1,020	1,028	9.8	0.14	–	–
ADG, g/d/bird	61.8	59.8	60.5	59.4	60.0	0.65	0.14	–	–
ADFI, g/d/bird	112.1 ^a	108.9 ^{a,b}	110.1 ^a	110.9 ^a	104.9 ^b	1.35	0.01	0.008	0.26
F: G, g/g	1.82 ^b	1.82 ^b	1.82 ^b	1.87 ^a	1.75 ^c	0.11	<0.0001	0.016	<0.0001
Mortality, %	6.50	4.92	7.40	4.92	6.43	1.46	0.70	–	–

^{a-c}Means within a row lacking a common superscript differ ($P < 0.05$).

¹Data represent the means of 6 replicate pens ($n = 6$).

ADFI, average daily feed intake; ADG, average daily gain; BW, body weight; F: G, feed: gain ratio.

Table 4. Effects of dietary Indian rapeseed meal inclusion levels on growth performance and mortality of ducks during 15 to 28, 29 to 42, and 15 to 42 D of age.

Item	Dietary Indian rapeseed meal level, % ¹						SEM	P-value	Linear	Quadratic
	0	5	10	15	20	25				
Day 15–28										
BW, g	2131 ^a	2087 ^{a,b}	2063 ^b	2071 ^b	2050 ^b	1995 ^c	15.6	<0.0001	<0.0001	0.74
ADG, g/d/bird	100.6 ^a	97.7 ^{a,b}	96.1 ^b	96.6 ^b	95.2 ^b	91.6 ^c	1.05	<0.0001	<0.0001	0.77
ADFI, g/d/bird	223.6 ^a	221.5 ^{a,b}	217.1 ^b	216.9 ^b	211.7 ^b	204.5 ^c	1.99	<0.0001	<0.0001	0.13
F: G, g/g	2.22 ^b	2.27 ^a	2.26 ^a	2.25 ^{a,b}	2.22 ^b	2.24 ^{a,b}	0.011	0.02	0.44	0.03
Mortality, %	0.67	1.33	0.00	0.00	0.67	0.67	0.58	0.60	–	–
Day 29–42										
ADG, g/d/bird	120.6 ^a	108.2 ^b	111.3 ^b	110.6 ^b	108.3 ^b	107.6 ^b	1.78	0.0004	0.0002	0.04
ADFI, g/d/bird	321.98	298.2	306.8	306.3	299.9	289.9	4.89	0.16	–	–
F: G, g/g	2.67 ^b	2.76 ^{a,b}	2.76 ^{a,b}	2.77 ^a	2.77 ^a	2.70 ^{a,b}	0.03	0.004	0.52	0.01
Mortality, %	4.69	5.39	3.33	4.67	5.39	4.69	0.94	0.67	–	–
Day 15–42										
BW, g	3,581 ^a	3,385 ^b	3,399 ^b	3,398 ^b	3,350 ^{b,c}	3,285 ^c	33.7	<0.0001	<0.0001	0.17
ADG, g/d/bird	109.6 ^a	102.3 ^b	102.8 ^b	102.8 ^b	101.1 ^{b,c}	98.7 ^c	1.14	<0.0001	<0.0001	0.16
ADFI, g/d/bird	275.3 ^a	264.0 ^b	267.3 ^b	267.7 ^b	264.9 ^b	256.0 ^c	2.33	0.0003	<0.0001	0.58
F: G, g/g	2.51 ^a	2.58 ^b	2.60 ^b	2.60 ^b	2.62 ^b	2.60 ^b	0.018	0.007	0.001	0.01
Mortality, %	5.68	7.18	3.47	5.05	6.49	5.67	1.24	0.40	–	–

^{a-c}Means within a row lacking a common superscript differ ($P < 0.05$).

¹Data represent the means of 6 replicate pens ($n = 6$).

ADFI, average daily feed intake; ADG, average daily gain; BW, body weight; F: G, feed: gain ratio.

at day 7 to 14 (Exp. 1; Table 3). However, during day 15 to 21 (Exp. 1), dietary inclusion levels did not influence ($P > 0.07$) the BW, ADG, and ADFI of ducklings and significantly decreased F/G linearly and quadratically ($P < 0.01$). The ducklings fed the diet with 20% double-low RSM had lower ($P < 0.01$) F/G than birds fed other 4 diets. During day 7 to 21 (Exp. 1), dietary double-low RSM inclusion levels affected ($P \leq 0.01$) ADFI and F/G but had no effect ($P > 0.13$) on ADG of ducklings. An increase in dietary level of

double-low RSM resulted in a linear decrease in ADFI and a quadratic increase in F/G.

As for ducks at the grower-finisher period presented in Table 4, an increase in dietary Indian RSM level also resulted in the decreases ($P < 0.003$) in BW, ADG, and ADFI of ducks at day 15 to 28 and day 15 to 42 of age (Exp. 2; Table 4). The F/G of ducks at 15 to 28, 29 to 42, and 15 to 42 D of age (Exp.2) were increased ($P < 0.01$) quadratically as dietary Indian RSM inclusion level increased.

Table 5. Effects of dietary double-low rapeseed meals inclusion levels on relative organ weight of ducklings at 21 D of age.

Item	Dietary double-low rapeseed meal level, % ¹					SEM	P-value	Linear	Quadratic
	0	5	10	15	20				
Liver, g/kg	24.4 ^b	28.1 ^a	28.1 ^a	27.5 ^a	27.3 ^a	0.67	0.003	0.02	0.002
Kidney, g/kg	12.19	12.98	13.47	12.15	12.52	0.45	0.22	–	–
Spleen, g/kg	1.94	1.94	1.94	1.96	1.84	0.18	0.49	–	–
Bursa of Fabricius, g/kg	1.08	1.19	1.19	2.07	1.29	0.43	0.35	–	–
Thymus, g/kg	3.99	5.45	4.95	5.38	6.01	0.69	0.08	–	–
Thyroid, g/kg	1.63	2.08	2.47	2.29	1.92	0.21	0.88	–	–

^{a,b}Means within a row lacking a common superscript differ ($P < 0.05$).

¹Data represent the means of 6 replicate birds ($n = 6$).

Table 6. Effects of dietary Indian rapeseed meal inclusion levels on relative organ weight and percentages of breast and leg muscles of ducks at 42 D of age.

Item	Dietary Indian rapeseed meal level, % ¹						SEM	P-value	Linear	Quadratic
	0	5	10	15	20	25				
Liver, g/kg	21.5	23.3	22.1	22.7	23.7	24.1	0.74	0.17	–	–
Kidney, g/kg	6.55	6.65	6.73	6.89	7.23	7.67	0.25	0.91	–	–
Spleen, g/kg	0.89	0.72	0.85	0.66	0.68	0.64	0.07	0.12	–	–
Bursa of Fabricius, g/kg	5.87	5.92	5.48	5.92	5.43	5.21	0.50	0.88	–	–
Thymus, g/kg	1.02	1.06	1.28	1.16	1.00	1.00	0.13	0.46	–	–
Thyroid, g/kg	0.047 ^b	0.077 ^a	0.078 ^a	0.068 ^a	0.062 ^{a,b}	0.058 ^{a,b}	0.007	0.02	0.94	0.004
Breast yield, %	14.05	13.79	12.91	13.26	13.72	12.64	0.60	0.61	–	–
Thigh yield, %	12.38	12.82	12.24	12.09	12.11	12.16	0.34	0.68	–	–

^{a,b}Means within a row lacking a common superscript differ ($P < 0.05$).

¹Data represent the means of 6 replicate birds ($n = 6$).

Table 7. Effects of dietary double-low rapeseed meal inclusion levels on serum parameters of ducklings at 21 D of age.

Item	Dietary double-low rapeseed meal level, % ¹					SEM	P-value	Linear	Quadratic
	0	5	10	15	20				
Total protein, g/L	34.5	34.7	35.6	35.1	35.9	1.24	0.91	–	–
Albumin, g/L	14.2	14.5	15.0	14.1	14.4	0.30	0.32	–	–
Globulin, g/L	20.2	20.2	20.6	21.0	21.5	1.12	0.91	–	–
Urea nitrogen, mol/L	0.79	0.90	0.89	0.86	0.87	0.08	0.86	–	–
Alanine aminotransferase, U/L	49.3	60.5	54.2	60.2	56.5	3.86	0.25	–	–
Aspartate aminotransferase, U/L	36.0	46.0	36.7	40.5	38.2	5.31	0.68	–	–
Alkaline phosphatase, U/L	587	734	666	733	591	46.6	0.08	–	–

¹Data represent the means of 6 replicate birds ($n = 6$).

Organ Weight and Percentages of Breast and Leg Muscles

Data on the effects of dietary inclusion levels of RSM on relative weights of liver, kidney, thyroid, spleen, thymus, and bursa of Fabricius of ducks at day 21 (Exp. 1, double-low RSM) and day 42 (Exp. 2, Indian RSM) were shown in Tables 5 and 6, respectively. At day 21, the relative liver weight was increased ($P < 0.003$) linearly with the increase of double-low RSM inclusion levels in diets. The ducks fed the diets with double-low RSM inclusion had higher ($P < 0.002$) relative liver weight than birds fed the control diet with no double-low RSM inclusion (Table 5). At day 42, the relative thyroid weight was increased ($P < 0.03$) quadratically with the increase of double-low RSM inclusion levels in

diets (Table 6). The ducks fed the diets with 5 and 10% Indian RSM inclusion had higher ($P < 0.01$) relative thyroid weight than birds fed the diet with 0% Indian RSM inclusion. The percentages of breast and leg muscles of ducks at day 42 were not influenced ($P > 0.60$) by dietary Indian RSM inclusion levels.

Serum Biochemical Parameters

Data of ALT, AST, and ALP activities, and TP, albumin, globulin, urea nitrogen, T₃ and T₄ levels in serum of ducks at days 21 and 42 were presented in Tables 7 and 8, respectively. Dietary inclusion levels of double-low RSM had no effects ($P > 0.60$) on the above-mentioned serum indices of ducks on day 21

Table 8. Effects of dietary Indian rapeseed meal inclusion levels on serum parameters of ducks at 42 D of age.

Item	Dietary Indian rapeseed meal level, % ¹						SEM	P-value	Linear	Quadratic
	0	5	10	15	20	25				
Total protein, g/L	34.0	36.0	34.4	33.9	35.5	34.6	0.87	0.50	–	–
Albumin, g/L	13.8	14.7	13.6	13.6	14.4	14.3	0.34	0.16	–	–
Globulin, g/L	20.9	20.5	21.9	20.5	21.9	19.0	1.25	0.59	–	–
Urea nitrogen, mol/L	0.49	0.45	0.49	0.50	0.35	0.35	0.05	0.08	–	–
Alanine aminotransferase, U/L	36.7	36.8	38.2	39.3	40.8	39.8	2.02	0.63	–	–
Aspartate aminotransferase, U/L	31.8 ^b	29.6 ^b	33.7 ^b	40.3 ^a	40.7 ^a	34.7 ^{a,b}	2.33	0.01	0.01	0.13
Alkaline phosphatase, U/L	646 ^b	666 ^b	723 ^{a,b}	724 ^{a,b}	814 ^a	847 ^a	46	0.03	0.0009	0.64
Triiodothyronine, nmol/mL	1.15	1.23	1.10	0.90	1.18	1.02	0.09	0.13	–	–
Thyroxin, nmol/mL	0.31	0.31	0.29	0.18	0.18	0.24	0.07	0.46	–	–

^{a,b}Means within a row lacking a common superscript differ ($P < 0.05$).

¹Data represent the means of 6 replicate birds ($n = 6$).

(Table 7). However, dietary Indian RSM inclusion levels affected ($P < 0.04$) serum AST and ALP activities and did not influence other serum biochemical parameters of ducks at day 42 (Table 8). Serum AST and ALP activities were increased linearly as dietary Indian RSM inclusion levels increased at day 42.

DISCUSSION

In developing countries, RSM is a valuable protein source and is used widely to replace soybean meal to reduce feed cost in poultry diets (Swick, 1999). So far, the use of RSM has been limited in poultry diets by the presence of anti-nutritional factors, such as GLS (Tripathi and Mishra, 2007), sinapine (Qiao and Classen, 2003), and NSP (Slominski et al., 1994, 1999). In broiler chickens, the inclusion of 150 and 300 g/kg of RSM in diets was found to have a negative effect on growth performance (Maroufyan and Kermanshahi, 2006). Mawson et al. (1994a) suggested that growth was depressed when the level of GLS increased to 6 to 10 $\mu\text{mol/g}$ in the finished feed, and growth was severely inhibited when the GLS level was above 10 $\mu\text{mol/g}$ in the finished feed. Therefore, double-low RSM due to a low GLS ($<30 \mu\text{mol/g}$ RSM) content has been explored to a greater extent in poultry diets. Compared to broilers, ducks have a greater ability to digest RSM, and duck diets appear to have a higher incorporation rate of RSM (Borin et al., 2006). So far, limited information regarding the feeding value of RSM was available for ducks (Wickramasuriya et al., 2015). In Exp. 1, the usage of double-low RSM was evaluated for ducklings in the starter period from 7 to 21 D of age. As dietary double-low RSM inclusion levels reached up to 15% (3.46 to 5.31 $\mu\text{mol GLS/g}$), the BW, ADG, and ADFI of ducklings at 7 to 15 D of age were decreased and F/G was increased. The growth depression in ducklings was consistent with that seen in broilers fed diets with the inclusion of double-low RSM at 20% (Woyengo et al., 2011). However, in contrast to other previous findings, replacing soybean meal, partly (up to 28%; Salmon et al., 1981) or completely (Leeson et al., 1987), with double-low RSM in broiler diets had

no effects on growth. The reductions in growth and feed intake proved to be directly due to the presence of anti-nutritional factors. First, RSM adversely reduced feed intake due to the low palatability of GLS, as reported previously (Mawson et al., 1993). Moreover, RSM intake could reduce nutrient digestibility due to components such as GLS, phytates, and NSP (Bell, 1993; Kim et al., 2015). Bell (1993) indicated that the available energy levels in RSM were decreased as the first limiting factor, GLS level, was increased. Significant improvements in growth performance were recorded during the starter period with the addition of NSP enzymes and phytase (Ghorbani et al., 2009). It is also speculated that the decreased performance of ducklings might be due to the lower digestibility of amino acids when the soybean meal content was replaced by the increased level of RSM (Kluth and Rodehutschord, 2006; Kong and Adeola, 2010). Although the methionine content decreased from 0.46 to 0.40% as the double-low RSM inclusion levels increased, the methionine values in all diets met or exceeded the requirement of early Peking ducklings according recommendations by the NRC (1994), which recommends 0.40% methionine, as well as by Elkin et al. (1986), who recommends 0.388 to 0.422% methionine. In addition, a previous study demonstrated that growth performance was not affected in ducklings fed diets containing methionine levels between 0.40 and 0.68% from hatch to day 14 (Xie et al., 2004). In Exp. 1, ducks fed the diet with 20% double-low RSM had the lowest F/G at 7 to 21 D of age. On the one hand, diets with 20% double-low RSM reduced feed intake by 4 to 8 g/d/bird. A modest level of feed reduction slowed the digesta passage rate to facilitate better feed efficiency by increasing the time available for nutrient digestion, absorption, and growth of the gut microflora (Scott and Silversides, 2003). On the other hand, some unconventional feed ingredients (such as RSM with a high GLS content) can also reduce the rate of passage, slow gastric emptying, and cause “ileal braking” (Scott, 2005). Then, the principal effect of reflux would be to promote movement of undigested material to environments that promote macromolecule breakdown (gizzard) and

nutrient absorption (upper small intestine) to increase the feed conversion ratio in birds (Sacranie et al. 2005).

In Exp. 1, ducks fed diets with RSM showed compensatory growth and no differences in BW, ADG, and ADFI from day 15 to 21. It is also suggested that ducks in the later period are less sensitive to the anti-nutritional function of RSM compared to birds in the early period, as demonstrated in broilers (Mushtaq et al., 2007). Due to the greater tolerance of RSM usage with the increased age of ducks, Indian RSM containing a higher GLS and lower protein levels was selected to be used in duck diets in the grower-finisher period in Exp. 2. The results showed that an increase in dietary Indian RSM level resulted in a linear decrease in BW, ADG, and ADFI of ducks at day 15 to 28 and 15 to 42 of age. The growth depression in ducks fed up to 5% Indian RSM (25.1 $\mu\text{mol/g}$ GLS) was in agreement with a recent study evaluating ducks fed up to 13.25% RSM (7.57 $\mu\text{mol/g}$ GLS) at day 15 to 35 (Qin et al., 2017). It is implied that, compared to the upper limit of double-low RSM (<15%) in Exp. 1, ducks appear to have lower accepted tolerance levels of traditionally toxic RSM (<5%) containing a high level of GLS, even in the later growth period evaluated in Exp. 2. Therefore, due to the similar feed cost of corn-DDGS-cottonseed meal-RSM diets, a higher economic profit will be obtained from ducks fed a diet with an inclusion level lower than 5% Indian RSM based on growth performance. It should be pointed out that the analyzed Ca content varied from 0.60 to 0.65% in Exp. 2 but met the requirement of 0.60% Ca in ducks from 2 to 7 wk of age according to NRC (1994) recommendations. Xie et al. (2009) also confirmed that no differences were observed in the growth performance of pekin ducks from 3 to 6 wk of age as dietary Ca levels increased from 0.48 to 0.84% when the diets contained a constant non-phytate phosphorus level of 0.33%.

It is clear that GLS content is a primary source of problems when restricting the use of RSM (Tripathi and Mishra, 2007). GLS and GLS hydrolysis products have been identified as being related to damage in various organs and tissues (Mawson et al. 1994b). In this study, the weights of the liver, thyroid, kidney, and immune organs of the experimental ducks were measured as indications of the physiological effects of GLS-containing graded RSM levels. Ducks exhibited liver enlargement, but not thyroid or kidney enlargement, when fed diets with double-low RSM inclusion from 5 to 20%. As reported in broilers (Pearson et al., 1983) and layer broilers (Griffiths et al., 1980) in the starter period, liver size changes and even liver hemorrhage were induced by increasing dietary RSM levels. The above results suggested that a negative reaction may occur in livers when the intake threshold of these anti-nutritional components has been exceeded. The liver enlargement was associated with the hepatotoxin goitricin, as evidenced by the increased cell mitosis and proliferation (Nishie and Daxenbichler, 1982). Despite the high content of

double-low RSM in the diets, we failed to observe increases in the activities of serum ALT, AST, and ALP in ducklings at day 21, which was inconsistent with the results from broilers fed high-GLS RSM (Pearson et al., 1983). Feeding ducks double-low RSM for a period of 21 D was insufficient to induce severe liver damage in the present study. In the grower-finisher period, an increased inclusion of 5 to 15% RSM containing 7.67 to 23.66 $\mu\text{mol/g}$ GLS in the diets resulted in an increase in the relative thyroid weight, which was confirmed in broilers given grower diets containing up to 24% RSM (Kaminska et al., 2000). The results regarding the relative tissue weight from Exp. 1 and 2 demonstrated that there is a tissue-specific reaction in ducks in response to consumption of anti-nutritional factors in RSM between the 2 growth periods. This reaction is possibly due to the different amounts of average feed intake (108 vs. 264 g/d/bird) and ingested GLS (0.15 vs. 2.02 mmol GLS/d/bird) in ducks fed the same 5% double-low and Indian RSM diets at different growth periods, which affect the production, absorption and deposition of GLS or GLS hydrolysis products in different tissues. In addition, the thyroid enlargement was accompanied by a corresponding increase in serum AST and ALP activities in ducks fed the diets with Indian RSM, suggesting that thyroid damage could be induced. The hydrolysis products of GLS, such as goitricin, from RSM have been shown to impair the uptake of iodide and iodide binding to thyroglobulin in the thyroid and reduce the synthesis and release of hormone in the thyroid (Schöne et al., 1993). Reduction of T_4 promotes the release of thyroid-stimulating hormone from the pituitary and increases the size of the thyroid through hyperplasia. However, the results from the present study demonstrated that inclusion of RSM did not affect the serum T_3 and T_4 concentrations at 42 D of age. Clandinin et al. (1966) found that uptake of radio-iodine initially decreased and radio-iodine release increased in the thyroid, but the values returned to normal during prolonged feeding. Our results also suggested that ducks fed RSM diets for 4 wk can restore homeostasis and can reach physiological equilibrium of thyroid hormones by feedback regulation in the endocrine system. In addition, no significant changes in breast and leg muscle percentages were observed in ducks at 42 D, which was in line with the results from broilers (Kaminska et al., 2000) and turkeys (Mikulski et al., 2012).

In conclusion, dietary double-low RSM inclusion levels above 15% (>3.46 μmol GLS/g diet) impaired the growth performance of ducklings from day 7 to 14, and these birds had compensatory growth from day 15 to 21. For ducks in the grower-finisher period, body gain and feed intake were decreased linearly as dietary inclusion of RSM increased from 5 (7.67 μmol GLS/g diet) to 20% (23.66 μmol GLS/g diet). In addition, dietary RSM inclusion levels induced liver enlargement in ducklings at day 21 (5 to 20% double-low RSM with 1.37 to 5.31 $\mu\text{mol/g}$ GLS), and thyroid enlargement accompanied by an increase serum AST and ALP

activities in ducks at day 42 (5 to 15% Indian RSM with 7.67 to 23.66 $\mu\text{mol/g}$ GLS).

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