Effects of dietary inclusion of cassava starch-extraction-residue meal on egg production, egg quality, oxidative status, and yolk fatty acid profile in laying ducks

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ABSTRACT This study was designed to evaluate the effects of different dietary levels of cassava starch extraction residue meal (**CReM**) on egg production, egg quality, oxidative status, egg yolk fatty acid profile, and hepatic expression of fatty acid metabolism-related genes. In total, 288 Longyan laying ducks aged 21 wk with similar BW were randomly assigned to 4 dietary treatments, each consisting of 6 replicates of 12 birds. The birds were fed a typical corn-soybean meal diet, which contained 0% (control), 5%, 10%, and 15% CReM, mainly replacing wheat bran, and the experiment lasted for 16 wk. The tested CReM levels did not show significant effects on the egg production, nonmarketable egg percentage, egg weight, daily egg mass, and FCR (g feed: g egg), but daily feed intake was reduced with increased CReM level (linear $P < 0.001$, quadratic $P < 0.05$). Yolk color increased (linear and quadratic, $P < 0.01$) with the increase in CReM level, but the Haugh unit, yolk proportion, albumen proportion, shell proportion, eggshell thickness, and eggshell strength were unaffected. Yolk contents of C11:0 and C12:0 (linear, quadratic, $P < 0.01$) and total saturated fatty acids increased, and the C22:1 level decreased (linear $P < 0.01$, quadratic $P < 0.05$) with the increase in CReM level, but the total monounsaturated fatty acids, the individual and total polyunsaturated fatty acids and n−6 and n−3 fatty acids, triglycerides, and total cholesterol in egg yolk were not affected. Hepatic gene expression revealed a significant increase in peroxisome proliferators-activated receptors γ (linear, quadratic, $P < 0.001$), but the expression of fatty acid synthase, sterol regulatory element binding protein 1 and apolipoprotein A1 genes were unaffected by CReM level. In conclusion, the results of the current study indicated that the CReM could be included up to 15% in laying duck diets without negative effects on the egg-laying rate, egg quality, and oxidative status. Dietary inclusion of CReM increased the yolk content of total saturated fatty acids and SOD activity in the liver.

Key words: cassava starch-extraction-residue meal, laying duck, productive performance, yolk fatty acids, lipid metabolism

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INTRODUCTION

In the poultry industry, there is need to evaluate and introduce new energy sources as alternatives to partially or completely substitute for the expensive traditional ingredients used in poultry diets. Cassava pomace (starch extraction residue meal, **CReM**), the agricultural by-product rendered after starch extraction from cassava roots, is of interest in the present study. Raw, nonextracted, cassava root meal (**CRM**) has been seen as a potent energy source for productive animals, mainly because of its high energy content and abundant availability worldwide. Recently, a major part of cassava production has been used for starch extraction, leaving enormous amounts of root residues (Morgan and Choct, 2016). These residues are unexploited and wasted and

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were reported to increase environmental pollution during the harvesting and processing season (Huyen et al., 2007).

To the authors' knowledge, a considerable number of studies have assessed the nutritive value of the raw cassava root meal (CRM) as a nontraditional energy source for poultry (Yang et al., 2010; Saree et al., 2012, Sahoo et al., 2014), but little effort made in evaluating the CReM (Huyen et al., 2007; Abouelezz et al., 2018). CRM incorporation up to 50% in poultry diets is acceptable (Yang et al., 2010; Saree et al., 2012; Sahoo et al., 2014). The CRM contains 3,000 to 3,100 kcal ME/kg (Buitrago et al., 2002; Khajarern and Khajarern, 2007), 70% starch, 5% crude fiber (Balagopalan, 2002; Nguyen et al., 2007), 2% CP (Stupak et al., 2002; Chauynarong et al., 2009), and 1% to 2% lipids (Olugbemi et al., 2010). Compared to nutrient levels in the CRM, the CReM contains lower energy levels (2,109 Kcal MEn/kg), lower starch content (50%) by about 20%, and higher fiber content (14%) due to the starch extraction process of cassava roots, and some changes in the other nutrients (Abouelezz et al., 2018). These negative alterations in nutrient composition put the potential use of CReM as an energy source for poultry in question, particularly from the decreased energy content and increased fiber. Diarra and Devi (2015) reported that the major limitations to the utilization of CReM in poultry diets are the high fiber, low protein, and minor content of cyanide (**HCN**).

Both CRM and CReM have a low crude protein content (2%) compared to that of corn (8.7%), and extremely poor essential amino acid profile; it contains a high concentration of arginine, but low levels of methionine, tryptophan, threonine, cystine, phenylalanine, isoleucine, and proline (Nassar and Sousa, 2007; Olugbemi et al., 2010; Abouelezz et al., 2018). These lower contents of essential amino acids in the CReM mean that cassava-based diets require supplementation with appropriate amino acids (Ngiki et al., 2014). The hydrocyanide (HCN), which is toxic for poultry, can be eradicated from raw cassava roots by boiling, sun-drying, or oven drying before offering to animals (Oguntimein, 1988; Ngiki et al., 2014). Screening the levels of blood metabolites and the anti-oxidative status in birds fed CReM can expose potential HCN toxicity (Leeson and Summers, 1988; Yang et al., 2010). In poultry, high-level carbohydrate diets accelerate the rate of digestion and absorption, activating the carbohydrate-insulin axis, promoting the transcription of related genes, and resulting in increased deposition of fat (Zhang, 2009). Increased dietary fiber can reduce liver and body fat deposition in meat-type ducks by regulating the expression of genes related to hepatic lipid metabolism (Han, 2016). Therefore, the yolk FA profile as well as expression of genes related to lipid metabolism were assayed in the present study. The relatively high contents of starch and fiber in CReM may affect lipid metabolism and properties of egg yolk in poultry.

The present study aimed to evaluate the effects of dietary incorporation of CReM on egg production, egg quality, oxidative status, yolk fatty acid profile, and expression of lipid metabolism genes in Longyan laying ducks.

MATERIALS AND METHODS

Experimental Design, Animals, and Housing

The experimental protocol was approved by the Animal Care and Use Committee of Guangdong Academy of Agricultural Sciences with the approval number of "GAASIAS-2016-017." In total, 288 Longyan laying ducks aged 21 wk, having a comparable BW (1,405 ± 12 g) and the same genetic background, were randomly allocated into 4 dietary treatments, each containing 6 replicates of 12 birds. The experiment lasted for 16 wk. The amount of daily feed allowed was 160 g/bird, offered in 2 equal portions at 07:00 am and 15:00 pm. In the 4 experimental diets (Table 1), CReM was incorporated at 0% (control), 5%, 10% or 15%, as a partial

Table 1. Incorporation of cassava starch extraction residue meal (CReM) in laying duck diets.

	CReM levels			
	0%	5%	10%	15%
Ingredients, g/kg				
CReM	0	50	100	150
Corn	539.71	516.71	493.28	447.51
Wheat bran	84.02	42	1.15	0
Soybean meal	262.5	279	289.3	230
Corn cob protein powder	0	0	5	59.51
Calcium hydrogen phosphate	13.85	14.18	14.6	14.8
Limestone	83	81.46	80	78.75
Premix[1]	10	10	10	10
Salt	3	3	3	3
Methionine	2.02	2.05	2.07	1.98
Lysine	0.18	0	0	1.45
Arginine	1.72	1.6	1.6	3
Total	1000	1000	1000	1000
Calculated analysis,[2] %				
ME, Mcal/kg	2.50	2.50	2.50	2.50
CP	17.01	17.00	17.01	17.00
EE	2.77	2.55	2.37	2.51
CF	2.98	3.35	3.71	4.14
NDF	8.7	8.4	9.7	9.9
Ash	2.77	2.93	3.07	3.05
Ca	3.80	3.80	3.81	3.80
Available P	0.35	0.35	0.35	0.35
Met + Cys	0.78	0.78	0.78	0.78
Lys	0.89	0.89	0.89	0.89
Thr	0.70	0.70	0.70	0.70
Arg	1.30	1.30	1.30	1.30
Fatty acid composition[3] (g of fatty acid/100 g of feed)				
C11:0	0.199	0.199	0.195	0.199
C16:0	0.288	0.291	0.271	0.275
C17:0	0.036	0.034	0.036	0.035
C18:0	0.049	0.050	0.051	0.052
C18:1n9	0.174	0.182	0.177	0.178
C18:2n6	0.464	0.472	0.391	0.400
C18:3n3	0.044	0.044	0.037	0.036
C24:0	0.024	0.020	0.018	0.023

[1]Provided per kg of diet: VA 12,000 IU, VD_3 2,000 IU, VE 38 mg, VK_3 1.0 mg, VB_1 3.0 mg, VB_2 9.6 mg, VB_6 6.0 mg, VB_{12} 0.03 mg, choline chloride 500 mg, nicotinic acid 25 mg, D-pantothenic acid 28.5 mg, folic acid 0.6 mg, biotin 0.15 mg, Fe 50 mg, Cu 10 mg, Mn 90 mg, Zn 90 mg, I 0.5 mg, Se 0.4 mg.

[2]Measured values of EE and NDF content. Other nutrient levels are calculated values.

[3]Values are the means of duplicates.

Table 2. Nutrient content of cassava starch extraction residue meal (%, as fed basis).

Nutrient	Content
Chemical analysis	
DM, %	89.36
GE, J/G	14884
ME_n, kcal/kg[1]	2123.75
CP, %	2.73
EE, %	0.498
CF, %	14.0
NDF, %[2]	23.6
Ca, %	0.96
P, %	0.018
Ash, %	5.94
HCN, mg/kg	13.2
Soluble fiber, %	0.653
Insoluble fiber, %	16.5
Amino acid profile	
Asp, %	0.116
Thr, %	0.061
Ser, %	0.062
Glu, %	0.131
Gly, %	0.068
Ala, %	0.090
Cys, %	<0.001
Val, %	0.076
Met, %	0.003
Ile, %	0.060
Leu, %	0.093
Tyr, %	0.019
Phe, %	0.053
Lys, %	0.077
His, %	0.019
Arg, %	0.044
Pro, %	0.060

[1]Calculated according to the following equation: MEn = [39.14 × DM - 39.14 × ash - 82.78 × CF] (Janssen, 1989) Dried roots Tapioca meal (e.g., cassava) Page 113, Table B1, National Research Council (1994).
[2]Analyzed.

replacement of corn and wheat bran, with a small change in soybean meal or corn cob protein, to obtain iso-caloric iso-nitrogenous diets. The diets were formulated to provide nutrient requirements of Longyan ducks determined for this breed (Xia et al., 2019a,b; Xia et al., 2020). CReM was purchased from a local feed supplier (HAID, Guangzhou China). The nutrient content of CReM used here is presented in Table 2. Each new batch of diets was mixed and pelleted. All ducks were housed in individual cages (40 × 40 × 40 cm) in the same room, with artificial lighting (10 lx) for 15 h and 9 h of dark daily.

Nutrient Contents of CReM and Diets

Proximate analyses of CReM were performed in duplicate following procedures of AOAC (2000). CReM was dried in a forced air oven at 60°C for 48 h to determine its DM content (index no. 934.01, AOAC, 2000). Crude protein (**CP**) content was estimated by assaying the N content using the Kjeldahl method, and by multiplying N × 6.25 (index no. 968.06, AOAC, 2000). Ash was measured by burning the samples in a muffle furnace at 550°C for 3 h (index. 942.01, AOAC, 2000). Ether extract (**EE**) content was measured with a Goldfisch fat extraction Soxhlet unit (index. 920.39, AOAC, 2000). Gross energy was measured with a bomb calorimeter (model HWR-15C, Shanghai Instruments, Shanghai, China). Neutral detergent fiber, aNDFom, was assayed with a heat-stable amylase and expressed exclusive of residual ash (Van-Soest et al., 1991). The calculation of metabolizable energy nitrogen corrected (**MEn**) content was obtained by applying the equation: MEn = 39.14 × DM − 39.14 × ash − 82.78 × CF, (National Research Council, 1994, Table B1, pp. 113). The complete profile of amino acids was assayed using a Biochrom Amino Acid Analyzer (Biochrom 30+, UK, Cambridge). Calcium (Ca) content was determined (procedure 4.8.03, AOAC, 2000), and total phosphorus was measured (index. 3.4.11, AOAC, 2000). The HCN levels in the CReM was measured according to the determination of cyanide in the feed (Zhai et al., 2019). Soluble and insoluble fiber were measured with kits of Beijing Zhongjian emping biological technology CO. LTD. (Beijing,China). The Starch level in the CReM was measured according to Mccleary et al. (1997) procedure. Fatty acid composition of the diet was determined as previously described (Li et al., 2017).

Tissue Sampling and Storage

After 16 wk of feeding the experimental diets, 2 ducks were randomly selected from each replicate. Blood samples were collected from the wing vein into 5-mL heparinized tubes and centrifuged at 4°C (1,200 × *g*) for 10 min to obtain plasma, which was held at −20°C until analysis. The birds were then slaughtered, and samples of reproductive organs and liver were obtained. The liver samples were rinsed quickly in PBS, and snap-frozen in liquid nitrogen, then held at −80°C for further analysis. The ovaries and oviducts were dissected, weighed, and expressed as proportions of live body weight. Small yellow follicles (3 mm < diameter < 8 mm), and large yellow follicles (diameter ≥ 8 mm) were dissected, counted, and weighed; each was expressed as a proportion of ovarian weight (Xia et al., 2019a).

Productive Performance

Offered feed and refusals were recorded daily, on a per replicate basis, to calculate feed intake. The number of total eggs as well as the nonmarketable eggs (broken, small, large, or shell-less eggs) were recorded for each replicate. All produced eggs were weighed individually. Egg production (%), egg weight, ADFI, egg mass (**EM**), and FCR (g feed: g egg) were calculated daily on a per replicate basis and presented as averages for the experimental period (16 wk).

Egg Quality

Thirty-six eggs from each treatment (3 eggs/replicate at 30 and 36 wk of age) were collected randomly for measuring egg quality, and the averages for each replicate were calculated, then pooled for the 6 replicate values per treatment. The measured variables were yolk color, Haugh unit (using an Egg Analyzer - model EA - 01, ORKA Food

Technology, Ramat HaSharon, Israel), eggshell strength (using an Egg Force Reader - model EFR-01, ORKA), and thickness of eggshell (using a digital micrometer); in addition, the weights of yolk, shell (after air drying for 24 h), and albumen were recorded individually and expressed as percentages of egg weight.

Plasma and Liver Biochemical Variables

Plasma concentrations of reduced glutathione (**GSH**) and malondialdehyde (**MDA**), as well as activities of alanine aminotransferase (**AST**), aspartate aminotransferase (**AST**), lactate dehydrogenase (**LDH**), and glutathione peroxidase (**GSH-PX**) were measured colorimetrically with commercial kits purchased from Nanjing Jiancheng Inst. of Bioengineering, (Nanjing, China). Additionally, plasma concentrations of LH and FSH were assayed by radioimmunoassay according to the procedure of Yang et al. (2005), and using kits purchased from Beijing North Institute of Biological Technology (Beijing, China). Forty mg of frozen liver samples were homogenized on ice in tubes containing 4-mL homogenization buffer (0.05 M Tris-HCl, pH 7.4, 0.25 M sucrose, 1-mM EDTA) using an Ultra-Turrax (version: T8, IKA-Labortechnik, Staufen, Germany) for 5 s at 13,500 rpm. The resulting homogenates were centrifuged (3,000 × *g*) for 10 min at 4°C, and the supernatant was collected and stored at −80°C. The hepatic content of MDA and activities of total superoxide dismutase (**T-SOD**) were measured with kits (Nanjing Jiancheng Inst. of Bioengineering). All samples were assayed in duplicate. Protein content of supernatants was estimated following the procedure of Bradford (1976), using bovine serum albumin as the standard and Coomassie Brilliant Blue G250 (Sigma Chemical, St. Louis, MO).

Lipid Analysis

Yolks were isolated from the sampled eggs, and used to measure the content of total cholesterol (**TCH**) and triglycerides (**TG**) using kits purchased from Nanjing Jiancheng Inst. of Bioengineering. The total lipid content was extracted by mixing 0.5 g yolk with 20 mL of chloroform: methanol (2: 1, vol/vol) solution in a 50 mL falcon tube, then homogenized with a Polytron for 5 to 10 s (Folch et al., 1957). The resulting homogenate was filtered over Whatman filter paper into a graduated cylinder (100-mL) containing 5 mL NaCl solution (0.88%). After separation, the top layer was aspirated and the lipid layer was collected, and its volume recorded. The separated total lipids were mixed with boron trifluoride, hexane, and methanol (35:20:45, vol/vol) to convert lipids to fatty acid methyl esters (**FAME**) (Metcalfe et al., 1961). The FAME were quantified by an automated gas chromatograph using a fused silica capillary column (30 m × 0.32 mm internal diameter), according to Cherian and Sim (1991). A Shimadzu EZChrom chromatography (2010 type) data system was used to integrate peak areas. Fatty acid calibration and identification was performed by comparison with retention times of standards, and fatty acid composition was expressed as weight percentages.

Hepatic Expression of Genes Related to Lipid Metabolism

The total RNA in frozen liver samples was isolated using Trizol reagent (Invitrogen, Carlsbad, CA) after removing of genomic DNA by DNase; the RNA was dissolved at 1 μg/μL and stored at −80°C. Total RNA (2.5 μg) was used to generate cDNA in a final volume of 25 μL according to the manufacturer's instructions (Promega, Madison, WI). PCR was conducted in the presence of 200 μM dNTP mixtures, 1.5 mM $MgCl_2$, 10 pmol each of forward and reverse primers, and 1.5 IU Taq polymerase, in a final volume of 50 μL. Primer designs (shown in Table 3) were prepared from GenBank sequences, using Primer Premier 5.0, and obtained from Shanghai Shenggong Biological Company (Shanghai, China). The steps of PCR consisted of denaturation for 5 min at 94°C, then 35 cycles of 30 s at 94°C; 30 s at 60°C, and 30 s at 72°C, followed by a final extension for 10 min at 72°C. Aliquots of the PCR product were assessed by electrophoresis using 1.5% agarose gels, and the resulting products were excised from the gels and sequenced.

The same primers listed in Table 3 were used to quantify mRNA by real time quantitative PCR. In total volumes of 25 μL, 1 μL cDNA was mixed with 0.5 μL (10 mM) of each primer, 12.5 μL 2X iQTM SYBR Green Supermix, water to volume. These reaction mixtures were incubated in an iCycler iQ Real-time Detection system (Bio-Rad, Hercules, CA) using 40 cycles (95°C for 15 s and 60°C for 35 s). A standard curve was designed using 10-fold serial dilutions of cDNA to quantify the

Table 3. Primers for quantitative real-time PCR.

Gene[1]	Gene bank accession	Primer sequences (5′-3′)	Products (bp)	Annealing temperature (℃)
PPARγ	EF546801.2	F:GCAGGAGCAGAACAAAGAGGT R: TCATCAGAGAAGCCAGGAGAGT	194	58
FAS	AY613443.1	F:CAGCGGCAGTTGGTCAGTT R:GGCTCTCTCTCACATTGGCAG	152	59
SREBP1	55793104	F:ACCGCTCATCCATCAACGA R:GGCTGAGGTTCTCCTGCTTC	156	59
APOA-1	XM005009561.1	F:GCTGAGTACCAGGCCAAGGT R:GATGAAGCGGGTCTTGAGGT	123	59

[1]*PPAR*γ = peroxisome proliferators-activated receptors γ; *FAS* = fatty acid synthase; *SREBP1* = sterol regulatory element binding protein 1; *APOA-1* = apolipoprotein A1.

Table 4. Effect of dietary inclusion of cassava starch extraction residue meal (CReM) on egg production variables of laying Longyan ducks aged from 21 to 36 wk.[1]

Variables	Dietary CReM levels				SEM[2]	*P*-value		
	Control (0)	5%	10%	15%		CReM	Linear	Quadratic
Egg production, %	93.59	95.47	93.80	94.57	0.59	0.709	0.963	0.304
Non-marketable eggs[3], %	0.77	1.06	0.73	0.53	0.09	0.709	0.963	0.304
Egg weight, g	62.49	62.61	62.46	61.97	0.22	0.754	0.719	0.688
Daily egg mass, g/bird	59.09	59.74	58.61	59.09	0.36	0.803	0.573	0.494
Daily feed intake, g	154.79	155.35	154.00	150.92	0.43	<0.001	0.008	0.024
Feed conversion ratio, g feed: g egg	2.62	2.61	2.60	2.59	0.01	0.842	0.533	0.721

[1]Each value is the mean of 6 replicates.
[2]Pooled standard error of mean.
[3]Non-marketable eggs (broken, small, large, or shell-less eggs).

transcript amounts. Additionally, a melting curve was made to assure that a single product was amplified. Samples were assayed in triplicate with standard deviations of threshold cycle (**CT**) values not exceeding 0.5. The relative expression of analyzed genes was determined using the ΔCt method ($R = 2^{-\Delta\Delta\ Ct}$), where R is the relative expression of the required gene and ΔCt is the value obtained by subtracting the Ct value for β-actin mRNA from the Ct value for the target mRNA.

Statistical Analysis

Replicate was used as the experimental unit (n = 6), except when noted otherwise. The 2 sampled birds per replicate were used, but averaged to give 6 replicates per treatment. Similarly, the egg quality variables were measured on 6 eggs/replicate; they were averaged for each replicate. The effect of CReM dietary incorporation level was estimated by one-way ANOVA (SAS 9.1. 2004, SAS Institute, Cary, NC). Orthogonal polynomial contrasts were used to estimate the linear and quadratic effects of the increasing dietary CReM level, and probability level at 0.05 was adopted to identify significance. Data for each variable are presented as means, along with the SEM for n = 6, based on the ANOVA error mean square.

RESULTS

Productive Performance

The results presented in Table 4 show the egg laying performance of Longyan ducks as affected by the dietary CReM level. There were no significant effects on the averages of egg production rate (%), nonmarketable egg percentage (%), egg weight (g), daily egg mass (g), and FCR (g feed: g egg) due to CReM level, but daily feed intake decreased with increased CReM in the diet (linear $P < 0.001$, quadratic $P < 0.05$). Finally, no mortality was recorded.

Egg Quality Indices

The egg quality indices are presented in Table 5. Of the measured egg quality variables, only yolk color was affected by CReM dietary inclusion, which increased significantly (linear or quadratic, $P < 0.01$) with the increase in dietary CReM level. The Haugh unit, yolk proportion (%), albumen proportion (%), eggshell proportion (%), eggshell thickness, and eggshell strength were not affected by the CReM level.

Plasma and Liver Biochemical Analysis

As shown in Table 6, there were no significant effects of dietary CReM levels on plasma contents of GSSH, MDA, LH and FSH, plasma activities of AST, ALT, LDH, and GSH-PX, nor the hepatic MDA content. The dietary CReM showed significant effect on hepatic SOD activity, but the response did not prove linearity or deviation from linearity.

Table 5. Effect of dietary inclusion of cassava starch extraction residue meal (CReM) on egg quality variables of laying ducks at 30 and 36 wks.

Variables	Dietary CReM levels				SEM[2]	*P*-value		
	Control (0)	5%	10%	15%		CReM	Linear	Quadratic
Yolk color	5.11	5.39	5.50	7.72	0.12	<0.001	<0.001	<0.001
Haugh Unit	80.91	82.62	81.51	82.72	0.57	0.516	0.678	0.149
Yolk weight, %	29.78	29.38	29.96	29.92	0.16	0.453	0.396	0.431
Albumen weight, %	60.32	60.92	60.14	60.33	0.15	0.246	0.383	0.171
Shell weight, %	9.90	9.69	9.89	9.75	0.06	0.470	0.933	0.122
Shell thickness, μm	328.17	331.74	337.43	334.42	2.17	0.450	0.106	0.947
Shell strength, N	43.28	43.80	43.62	45.92	0.54	0.239	0.489	0.162

[1]Each value is the mean of 6 replicates.
[2]Pooled standard error of mean.

Table 6. Effect of dietary inclusion of cassava starch extraction residue meal (CReM) on blood and liver analysis of laying ducks at peak production.[1]

Variables[2]	Dietary CReM levels				SEM[3]	*P*-value		
	Control (0)	5%	10%	15%		CReM	Linear	Quadratic
Plasma								
AST, U/L	414.03	355.66	441.78	550.67	32.50	0.309	0.677	0.046
ALT, U/L	124.30	123.99	91.37	113.68	5.90	0.054	0.375	0.971
GSSH, μmol/L	5.46	5.15	5.15	4.68	0.11	0.157	0.078	0.249
MDA, nmol/mL	10.78	11.25	11.56	11.00	0.63	0.710	0.971	0.784
GSH-PX, U/mL	51.14	42.46	17.77	77.69	9.58	0.413	0.153	0.085
LDH, U/L	352.12	340.38	340.54	370.96	10.47	0.963	0.645	0.258
FSH, mIU/mL	1.26	1.27	1.30	0.87	0.09	0.733	0.281	0.169
LH, mIU/mL	3.48	3.54	3.43	3.37	0.20	0.872	0.997	0.798
Liver								
MDA, nmol/mg prot	1.44	1.59	2.04	1.88	0.15	0.200	0.478	0.470
SOD, U/mg prot	98.00	152.41	190.68	133.21	14.09	0.038	0.955	0.218

[1]Each value is the mean of 6 replicates.
[2]GSH = reduced glutathione; MDA = malondialdehyde; GSH-PX = glutathione peroxidase; T-SOD = total superoxide dismutase; CAT = catalase.
[3]Pooled standard error of mean.

Ovarian Indices

The results of ovarian indices of laying Longyan duck fed diets containing different levels of CReM are presented in Table 7. Of the measured ovarian indices, only the relative weights of small and large follicles were affected; they increased linearly ($P < 0.05$) with the increase in CReM level in the diet. The numbers of large and small ovarian follicles and relative weights of ovary and oviduct were not affected by the dietary CReM level.

Fatty Acid Composition of Egg Yolk

The fatty acid compositions of egg yolk lipids are presented in Table 8. Dietary CReM level did not affect egg yolk contents of TG or TCH. A significant increase in proportions of C11:0 and C12:0 (Quadratic, $P < 0.001$) and total saturated fatty acids (**SFA**) occurred (linear, $P < 0.05$; quadratic, $P < 0.01$) with increasing CReM level, and a significant decrease was obtained with C22:1 (linear, $P < 0.01$; quadratic, $P < 0.05$) but the total monounsaturated fatty acid (**MUFA**) was unaffected. The contents of the individual and total polyunsaturated fatty acids (**PUFA**) and n-6 and n-3 fatty acids in egg yolk were not affected.

Hepatic Expression of Genes Related to Fatty Acid Metabolism

As shown in Table 9, the relative expression of hepatic genes revealed a significant increase in *PPARγ* (linear or quadratic, $P < 0.01$), but the relative expressions of *FAS, SREBP1* and *APOA-1* genes were unaffected by the diet.

DISCUSSION

The dietary incorporation of up to 15% CReM did not show negative effects on egg production or egg quality indices, except that the average daily feed intake of the laying ducks linearly decreased. The decrease in feed intake is attributable to the relative increase in crude fiber content in CReM diets. In broiler chickens, Oso et al. (2014) found that dietary incorporation of cassava root meal at 10% and 20% of the diet reduced feed intake and growth rate, and reduced feed conversion ratio and crude protein digestibility; these negative effects were attributed to the fibrous nature of CRM, which increased feed bulkiness, decreased feed intake, and consequently depressed growth performance. The same results and explanation were reported in laying hens fed ingredients high in fiber (Abou-Elezz et al., 2011; Mohammed et al., 2012; Abouelezz et al., 2019). The

Table 7. Effect of dietary inclusion of cassava starch extraction residue meal (CReM) on ovarian indices of laying ducks at peak production.[1]

Variables	Dietary CReM levels				SEM[2]	*P*-value		
	Control (0)	5%	10%	15%		CRM	Linear	Quadratic
Large follicle number	4.75	5.33	5.25	5.25	0.22	0.582	0.342	0.381
Small follicle number	42.50	33.83	39.83	41.17	3.40	0.650	0.977	0.481
Oviduct weight, %	3.52	3.39	3.21	3.49	0.09	0.259	0.124	0.520
Ovary weight, %	4.81	4.61	4.17	4.03	0.17	0.079	0.022	0.461
Large follicle weight, %	72.06	79.62	85.11	82.18	2.51	0.071	0.012	0.513
Small follicle weight, %	5.07	4.46	6.18	6.86	0.49	0.046	0.049	0.957

[1]Each value is the mean of 6 replicates.
[2]Pooled standard error of mean.

Table 8. Effect of dietary inclusion of cassava starch extraction residue meal (CReM) on triglyceride, total cholesterol content and fatty acid profile in egg yolk of laying ducks 30 and 36 wks[1].

Variables[2]	Dietary CReM levels				SEM[3]	*P*-value		
	Control (0)	5%	10%	15%		CReM	linear	quadratic
TCH, mg/g	24.02	27.28	24.35	25.53	1.26	0.817	0.891	0.917
TG, mg/g	207.84	194.90	194.76	208.62	3.18	0.222	0.941	0.105
Yolk Fatty acid content, g/100g								
C11:0	0.411	0.444	0.875	0.888	0.039	<0.001	0.533	<0.001
C12:0	0.005	0.005	0.006	0.007	0.0003	<0.001	0.107	<0.001
C14:0	0.111	0.119	0.117	0.128	0.003	0.262	0.066	0.186
C15:0	0.008	0.008	0.008	0.008	0.0002	0.968	0.857	0.885
C16:0	6.481	7.138	7.067	6.823	0.115	0.170	0.365	0.090
C17:0	0.022	0.023	0.022	0.024	0.0004	0.245	0.118	0.303
C18:0	1.463	1.668	1.628	1.561	0.300	0.070	0.355	0.040
C22:0	0.011	0.012	0.010	0.010	0.0003	0.147	0.382	0.667
SFA	8.510	9.418	9.733	9.448	0.155	0.020	0.021	0.007
C14:1	0.010	0.011	0.011	0.011	0.0004	0.429	0.105	0.253
C16:1	0.592	0.646	0.688	0.665	0.016	0.167	0.061	0.081
C18:1(trans-9)	0.072	0.081	0.080	0.083	0.002	0.159	0.047	0.096
C18:1(cis-9)	11.988	13.523	13.565	13.459	0.252	0.062	0.045	0.029
C20:1	0.097	0.104	0.102	0.109	0.002	0.178	0.046	0.143
C22:1	0.086	0.089	0.071	0.061	0.004	0.047	0.009	0.025
C24:1	0.013	0.016	0.015	0.015	0.0004	0.174	0.253	0.098
MUFA	12.858	14.469	14.532	14.402	0.266	0.063	0.045	0.029
C18:2 n−6	1.824	1.856	1.721	1.684	0.038	0.345	0.106	0.251
C18:3 n−3	0.099	0.103	0.100	0.096	0.002	0.825	0.563	0.648
C20:2 n−6	0.050	0.048	0.043	0.045	0.001	0.131	0.047	0.107
C20:3 n−3	0.094	0.104	0.101	0.100	0.002	0.530	0.508	0.428
C20:4 n−6	0.935	0.999	1.000	0.974	0.018	0.574	0.480	0.369
C22:6 n−3	0.089	0.089	0.094	0.092	0.002	0.789	0.415	0.685
PUFA	3.090	3.199	3.058	2.990	0.059	0.676	0.415	0.550
n−6	2.808	2.903	2.764	2.702	0.054	0.632	0.355	0.509
n−3	0.282	0.296	0.294	0.288	0.005	0.802	0.740	0.615

[1]Each value is the mean of 6 replicates.
[2]TG = triglycerides; TCH = total cholesterol; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.
[3]Pooled standard error of mean.

reduction in feed intake in the present study with laying ducks seems to be tolerable as the egg production variables and egg quality indices were not negatively affected. Additionally, the dietary CReM levels linearly increased the relative weight of small follicles, without any negative effect on the other ovarian indices. These results and those of egg production variables indicate that the tested CReM levels up to 15% of the diet were suitable for laying ducks and the birds obtained adequate nutrient levels in all dietary treatments. Furthermore, the CReM showed beneficial effect on yolk color, which increased over that in the controls, particularly at the highest CReM level. In contrast, Saparattananan et al. (2005) found that egg yolk color score in layers fed a CRM diet was lower than that of those fed a maize diet; however, they observed that diets with maize or CRM showed similar egg laying rate and egg quality indices. Cassava leaf meal possesses high carotene content which enriches the egg yolk color (Morgan and Choct, 2016) while cassava root meal has a low content of carotene and other carotenoids (Kanto and Juttupornpong, 2002). Saparattananan et al. (2005) observed that diets with maize or cassava had similar effects on laying rate and egg quality, but egg yolk color score was lower in layers fed the cassava diet. The reason behind obtaining high yolk color scores in the highest CReM treatments in the present study could be related to modifications in levels of other ingredients in the diet rather than inclusion of CReM level. In the 10% and 15% CReM diets, corn cob protein was included at 5 g/kg and 59 g/kg diet; corn cob protein is a concentrated source of lutein and carotenoids (Yang et al., 2018)

Table 9. Effects of dietary inclusion of cassava starch extraction residue meal (CReM) on relative hepatic expression of genes related to lipid synthesis and metabolism in laying ducks at peak production.[1]

Gene[2]	Dietary CReM levels				SEM[3]	*P*-value		
	Control (0%)	5%	10%	15%		CReM	Linear	Quadratic
PPARγ	0.57	0.48	0.62	1.06	0.066	0.005	0.004	0.001
FAS	0.27	0.26	0.20	0.30	0.032	0.746	0.946	0.715
SREBP1	0.33	0.27	0.31	0.46	0.029	0.116	0.095	0.051
APOA-1	0.43	0.37	0.48	0.65	0.046	0.165	0.059	0.079

[1]Each value is the mean of 6 replicates.
[2]*PPARγ* = peroxisome proliferators-activated receptors γ; *FAS* = fatty acid synthase; *SREBP1* = sterol regulatory element binding protein 1; *APOA-1* = apolipoprotein A1.
[3]Pooled standard error of mean.

The toxicity of HCN present in cassava roots is a major concern for its possible use in poultry diets (Akapo et al., 2014; Morgan and Choct, 2016). There were no signs of toxicity of CReM here, based on the birds' livability, laying performance rate, or plasma metabolites, including plasma MDA, ALT, AST, LDH, GSH, GSH-PX, LH, FSH, or hepatic MDA and SOD. Similar results were reported by Abouelezz et al. (2018), who found that the dietary incorporation of CReM up to 15% in growing duck diets had no adverse effect on duck growth performance, mortality rate, or blood metabolites. They suggested that the tested CReM was processed adequately, thereby eliminating the potential toxicity from HCN. Cyanide content ranged between 1,000 and 2,000 mg/kg DM in cassava leaves, and >2,000 mg/kg DM in root cortex and peel (Cooke et al., 1982). Standards in China (GB 13078-2017) for acceptable levels are <50mg/kg in Cassava, its processed products, and total mixed feed. The HCN values obtained here (13.2 mg/kg CReM) are considered to be safe, and much lower than the reported toxic levels (Zhai et al., 2019). The dietary inclusion of CReM resulted in higher SOD activity in the liver than in the controls. Increased SOD activity is a good indicator of improved antioxidative capacity, where SOD is the first line of defense against pro-oxidant molecules. The increase in hepatic SOD activity with the CReM diets here is possibly an adaptive response to increased oxidative stress. In contrast to this scenario, however, the liver content of MDA, as well as plasma MDA, GSG-PX, GSSH, AST, ALT, and LDH were not affected by dietary CReM level.

The interaction between dietary inclusion of cassava root meal and relative expression of genes related to metabolism has not been studied previously. The relative expression of the 4 genes assessed here in liver revealed that *PPARγ* (a group of nuclear receptor proteins that function as transcription factors regulating the expression of genes) was significantly overexpressed at the highest dietary incorporation level of CReM (15%). On the other hand, the relative expressions of *FAS* (a multienzyme protein that catalyzes fatty acid synthesis), *SREBP1* (the master regulator of lipid homeostasis involved in the biosynthesis of cholesterol and fatty acids), and *APOA1* (a major component of high-density lipoprotein particles, which has a specific role in lipid metabolism) were almost stable and not significantly affected by CReM level in the diet. The change in the relative expression of *PPARγ* due to the CReM treatment may impact hepatic lipid metabolism. There is no existing literature on the effect of CReM incorporation in laying duck's diet on the relative expression of hepatic genes related to lipid synthesis and metabolism. Dietary starch and fiber can affect the expression of *SREBP1* and *FAS* in poultry livers (Zhang, 2009; Han, 2016). In the present experiment, changes in starch and fiber may not be sufficient to cause changes in the expression of these genes.

Similarly, we did not find available literature on the effect of dietary inclusion of CReM on egg yolk fatty acid composition. Indeed, the CReM here was not expected to make major modifications in egg yolk lipids or fatty acid profile due to its low content of crude fat with approx. 0.50%, and the fatty acid profiles of the diets were very similar.

In conclusion, the current study indicated that CReM could be included in laying duck diets up to 15% without effect on the number of eggs produced, egg quality, and oxidative status. Increasing amounts of CReM in the diets of laying ducks increased the yolk content of total SFA, and hepatic SOD activity. The yolk color in CReM treatments increased than the control, particularly at the highest CReM level, but this is suggested to be attributable to modifications in levels of other ingredients in the diet rather than inclusion of CReM.

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DISCLOSURES

The authors declare no conflict of interest.

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