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Effects of dietary substitution of peanut meal for soybean meal on egg production, egg quality, oxidative status, and yolk fatty acid profile in laying ducks

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ABSTRACT

There is an urgent need to evaluate and introduce alternatives for the unsustainable and traditional feed stuffs in poultry. This study evaluated the effects of graded replacement of soybean meal (**SBM**) by peanut meal (**PNM**) on egg production, egg quality, oxidative status, and yolk fatty acid profile in laying ducks. In total, 360 Longyan ducks aged 21 weeks were allocated to five treatments, each containing six replicates of 12 birds. Birds were fed the diets containing PNM replacing 0 (control), 25, 50, 75 or 100% of SBM for 16 weeks. With the increase of PNM level, egg production was improved (quadratic, $P < 0.05$), egg weight and feed consumption were decreased, feed conversion ratio and egg mass were impaired at 100% PNM, and yolk colour was enhanced (quadratic, $P < 0.05$). Plasma malondialdehyde concentration was increased at 100% PNM, while plasma glutathione concentration was decreased (quadratic, $P < 0.01$) as PNM substitution increased. Total cholesterol content in yolk decreased ($P < 0.01$) in response to increased PNM substitution. The contents of saturated fatty acids C20:0 and C22:0 in yolk increased (linear or quadratic, $P < 0.05$) with increased PNM substitution, but the contents of saturated fatty acid C18:0, monounsaturated fatty acid C22:1, and polyunsaturated fatty acids C18:3n-3 and C22:6n-3 in yolk were decreased (linear, $P < 0.05$) as PNM replacement increased. The contents in yolk of polyunsaturated fatty acid C18:2n-6, C20:2n-6, C20:3n-6, C20:4n-6, and total amount of polyunsaturated fatty acids increased with the highest contents obtained at 75% PNM substitution. The ratio between n-6 and n-3 increased (linear, $P < 0.05$) as PNM substitution increased. The transcript abundance in liver of peroxisome proliferators-activated receptors γ and fatty acid synthase showed quadratic ($P < 0.05$) responses with PNM replacement, with the highest expression of both genes being obtained with 75% substitution with PNM. Replacement of dietary SBM with 100% PNM decreased egg production and antioxidant capacity and increased the ratio of omega fatty acid in yolk between n-6 and n-3 in laying ducks. The obtained results indicate that PNM can be used to replace up to 75% of SBM in the diet of laying ducks without negative effects on the egg-laying production or egg quality. The regression model indicated that the maximal egg mass was obtained at no more than 67.6% replacement of SBM with PNM in the diet of laying ducks.

Implications

In poultry industry, the best strategy to reduce feed costs is using alternatives and locally available ingredients to replace the expensive traditional feedstuffs. Soybean meal, the main traditional protein source in poultry diets worldwide, has a continuous increase in price due to increased demand. The current study evaluated the optimal dietary substitution level of soybean meal by peanut meal in laying ducks. The results indicated that peanut meal can be used to replace up to 75% of soybean meal without negative effects on egg production or egg quality. The obtained results are important for feed formulators and poultry producers.

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Introduction

The increasing demand and insufficient supply of the traditional feedstuffs used as protein sources in poultry diets, mainly soybean meal (**SBM**), have resulted in a continuous increase in prices. This situation has prompted an urgent need to introduce and evaluate alternative protein sources to be included as partial or complete substitutes for more expensive traditional ingredients in poultry diets.

Because of its availability worldwide, peanut meal (**PNM**) is gaining extensive attention as a feedstuff for poultry. PNM is a high-protein content residue from the extraction of oil from peanut kernels (cotyledons), often from peanuts unsuitable for direct human consumption. The analysis of PNM obtained from 70 suppliers has been investigated by Batal et al. (2005), which revealed 2 664 kcal (2 273–3 009 kcal/kg) nitrogen-corrected true metabolisable energy (**TMEn**), 45.6% (40.1–50.9%) CP, 2.47% (0.68–5.97%) crude fat, 8.3% (5.77–12.6%) crude fibre, and 5.02% ash (4.31–7.01%). Compared to SBM, PNM has similar protein content and higher energy content. Thus, if PNM is employed as a feedstuff replacing the dietary SBM, a lower content of the energy resource will be needed to obtain the same total energy level (Batal et al., 2005).

The reported nutrient profile of PNM exposes two major concerns: the first being the great variability in nutrient content between different batches, regions, and suppliers, which indicates the need to analyse the PNM before its incorporation into a diet. The second issue is the suboptimal contents in PNM of some essential amino acids, particularly those of lysine and threonine. In previous studies, PNM protein was supplemented with animal proteins such as meat scraps, skim milk, or buttermilk to eliminate the threonine and lysine deficiency and to achieve better performance (Edwards and Massey, 1941). Since synthetic threonine is now available at a reasonable price, the potential use of PNM in poultry diets has increased (Redhead et al., 2021). The latter authors showed that supplementing a PNM-based diet with threonine led to identical egg production as that of SBM-fed hens (Pesti et al., 2003). Similarly, Samuel et al. (2002) demonstrated that broilers fed a diet based on corn-peanut meal supplemented with methionine, lysine and threonine showed satisfactory performance compared to those fed corn-soybean-based diets. Aflatoxicosis, however, remains as a genuine concern regarding the use of PNM as a poultry feedstuff. The PNM is highly susceptible to fungal mould contamination with the production of mycotoxins (Samuel et al., 2002). Thus, mycotoxin content must be monitored as a critical variable before PNM is offered to poultry. For that reason, it is recommended that quality control for mycotoxins be applied to PNM as well as eggs and meat produced from birds fed diets based on PNM (Pesti et al., 2003).

Much is known about the optimal dietary PNM inclusion level in laying hens. For example, dietary inclusion of 20% PNM is acceptable in laying hens with no adverse effects on egg-laying performance and quality (Redhead et al., 2021; Toomer et al., 2019). These available data were recommended for egg production and quality. To date, little information is available on PNM inclusion in the diets of laying ducks. Toomer et al. (2019) found that dietary inclusion of 20% high-oleic peanuts enhanced egg yolk colour and oleic fatty acid content in eggs of laying hens. The previous findings on dietary PNM inclusion in diets of laying hens are not adequate for laying ducks, due to their different genetic capacities for egg production and quality. It is necessary, therefore, to determine the optimal dietary substitution level of PNM for SBM for laying ducks to maximise their productive performance profile. It is hypothesised that PNM is suitable for, partially or totally, replacing SBM in diets of egg-laying ducks. Longyan duck is the most popular breed of laying duck in China. More than 300 million Longyan ducks are raised in southern China, annually producing 80 billion eggs (Xia et al., 2015). To determine the suitability of PNM as a substitute for dietary SBM, this study aimed to evaluate the effects of graded dietary substitution of PNM for SBM on egg production, egg quality, oxidative status, and yolk fatty acid composition in Longyan laying ducks.

Material and methods

Experimental design, animals, feeding and housing

In total, 360 Longyan laying ducks aged 21 weeks, having comparable egg production (86.1 ± 1.6%) and BW (1 205 ± 12 g) according to ANOVA ($P > 0.05$) and the same genetic background (originally descended from crossing between the Longyan breed, Putian White breed, and Putian Black breed), were randomly allocated into five dietary treatments, each containing six replicates of 12 birds (72 bird/treatment), and they were under study for 16 weeks. The birds had a daily feed allowance of 160 g/bird, offered in 2 equal portions (at 0700 and 1500 hours). The first treatment, which served as the controls, was fed a corn-soybean basal diet (containing 0% PNM). The other four treatments used PNM to replace 25, 50, 75 or 100% of the soybean meal of the control diet. All diets were balanced to maintain equal levels of energy, protein, and amino acids (lysine, methionine and threonine) through modest changes in other constituents of the basal diet, as shown in Table 1. The experimental diets were formulated to afford the nutrient levels for laying Longyan ducks based on previous findings from this laboratory (Xia et al., 2019a; Xia et al., 2019b; Xia et al., 2020). All ducks were individually housed in cages measuring 40 × 40 × 40 cm and received artificial lighting (10 lx) for 15 hours and 9 hours of dark. The ducks were purchased at the age of 17 weeks, and from 17 to 20 weeks of age, they were given a corn-soybean meal basal diet containing 10.87 MJ ME/kg, 160 g/kg CP, 9 g/kg Ca, and 4 g/kg available phosphorus.

Nutrient contents of the peanut meal

Proximate analyses, including calcium and phosphorus content, of the PNM were determined in duplicate following the procedures of AOAC (2000). Gross energy in the PNM was analysed using a bomb calorimeter (model HWR-15C, Shanghai Instruments, Shanghai, China). The apparent metabolisable energy of PNM was practically determined following the force feeding method. The content of NDF in PNM and experimental diets was assayed according to Van-Soest et al. (1991). The complete profile of amino acids in the diets was assayed using a Biochrom 30+ Amino Acid Analyzer (Harvard Apparatus, Cambridge, UK). The calculated nutrient contents of the experimental diets are presented in Table 1, and the analysed nutrient contents of PNM and SBM as well as the content of Aflatoxin-B1 in PNM are presented in Table 2.

Blood and tissue sampling

After 16 weeks of feeding the experimental diets, 12 ducks were randomly selected from each treatment (2 birds/replicate). Individual blood samples were collected from the wing vein in 5 mL heparinised tubes and centrifuged for 10 min (1 200*g*) at 4 °C to obtain plasma samples, which were kept at –20 °C until analysis. The birds were then euthanised by cervical dislocation and exsanguinated, and oviduct, ovary, and liver were weighed. Liver samples were collected, washed in PBS, frozen in liquid nitrogen (−196 °C), and held at –80 °C until analysis (Abouelezz et al., 2022).

Table 1
Composition and nutrient values of the experimental diets (as fed basis) of laying duck.

Item	Replacement level of soybean meal with peanut meal, %				
	0	25	50	75	100
Ingredients, g/kg					
Soybean meal	262.5	196.9	131.3	65.63	0
Peanut meal	0	53.28	107.0	160.5	214.3
Corn	539.7	524.0	510.1	496	481.7
Wheat bran	84.0	109.9	133.0	156.8	180.6
Calcium hydrogen phosphate	13.85	14.28	14.80	15.32	15.79
Limestone	83.03	83.04	83.33	83.4	83.45
Premix[1]	10.0	10.0	10.0	10.0	10.0
Sodium chloride	3.00	3.00	3.00	3.00	3.00
DL-Methionine	2.02	2.54	3.09	3.62	4.15
L-Lysine·HCl	0.18	1.22	2.28	3.32	4.36
L-Threonine	0	0.64	1.30	1.98	2.65
L-Arginine	1.72	1.20	0.80	0.43	0
Total	1 000	1 000	1 000	1 000	1 000
Nutrient level[2], %					
DM	90.13	90.42	90.54	90.66	90.78
Metabolisable energy, kcal/kg	2 500	2 500	2 500	2 500	2 500
CP	17.3	17.4	17.2	17.6	17.4
Ether extract	2.77	2.69	2.60	2.52	2.44
Crude fibre	2.98	3.28	3.57	3.86	4.16
NDF	9.90	10.0	10.9	12.3	12.6
Ash	2.77	2.74	2.70	2.67	2.64
Ca	3.80	3.80	3.80	3.80	3.80
Available P	0.35	0.35	0.35	0.35	0.35
Total Methionine + Cysteine	0.78	0.78	0.78	0.78	0.78
Total Lysine	0.89	0.89	0.89	0.89	0.89
Total Threonine	0.70	0.70	0.70	0.70	0.70
Total Arginine	1.30	1.30	1.30	1.30	1.30

[1] The premix provided the following per kg of diet: Vitamin A 12 000 IU, Vitamin D_3 2 000 IU, Vitamin E 38 mg, Vitamin K_3 1.0 mg, Vitamin B_1 3.0 mg, Vitamin B_2 9.6 mg, Vitamin B_6 6.0 mg, Vitamin B_{12} 0.03 mg, chloride choline 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 CP and ether extract content. Other nutrient levels are calculated values.

Table 2
Chemical analysis and amino acid profile of peanut meal and soybean meal (as fed basis) used in the diet of laying duck.

Nutrient, %	Peanut meal	Soybean meal
Chemical analysis		
Gross energy, kcal/kg	4 565	4 152
Metabolisable energy, kcal/kg[1]	3 012	2 350
CP, %	57.05	44.18
Moisture, %	8.78	10.80
Ether extract, %	5.08	1.95
Crude fibre, %	6.55	5.92
Ca, %	0.20	0.33
P, %	0.96	0.62
Ash, %	5.00	6.14
NDF, %	8.40	13.61
Aflatoxin-B_1, μg/kg	2.98	
Amino acid profile		
Asparagine, %	2.02	5.14
Threonine, %	0.41	1.77
Serine, %	0.74	2.28
Glutamine, %	3.41	8.18
Glycine, %	1.02	1.90
Alanine, %	0.70	1.99
Cysteine, %	0.11	0.67
Valine, %	0.67	2.18
Methionine, %	0.05	0.64
Isoleucine, %	0.61	2.09
Leucine, %	1.13	3.34
Tyrosine, %	0.52	1.51
Phenylalanine, %	0.90	2.28
Lysine, %	0.56	2.78
Histidine, %	0.31	1.20
Arginine, %	2.00	3.36
Proline, %	0.65	2.24

[1] Analysed value.

Ovary-related indices

The ovary-related indices were recorded as described by Xia et al. (2020). Small yellow follicles (**SYF**, 3 mm < diameter < 8 mm) and large yellow follicles (**LYF**, diameter ≥ 8 mm) were dissected and weighed. The numbers of SYF and LYF were recorded, and their weights were each expressed as proportions of ovarian weight. The relative ovarian weight was calculated based on live BW.

Productive performance

The offered feed and refusals were recorded daily on a per replicate basis to calculate feed intake. The number of total eggs and the non-marketable eggs (broken, small, or shell-less eggs) was recorded for each replicate. All produced eggs were weighed individually. Egg production (%), egg weight, feed consumption, egg mass (g egg/bird/day), and feed conversion ratio (**FCR**, g feed: g egg) were calculated on a per replicate basis and presented as averages for the experimental period (16 weeks).

Egg quality

Eighteen eggs from each treatment (three eggs/replicate) after 10 and 16 weeks of treatment (at 30 and 36 weeks of age) were collected randomly for egg quality assessment. The measured variables, all by standard methods, were yolk colour, Haugh unit, eggshell strength, and thickness of eggshell. The breaking strength of uncracked eggs was determined on the vertical axis using an Egg Force Reader (model EFR-01, ORKA Food Technology, Ramat HaSharon, Israel). After weighing each egg individually, it was broken onto a flat surface to measure the yolk colour, albumen height, and Haugh units with an Egg Analyzer (model EA-01, ORKA Food Technology, Ramat HaSharon, Israel). This digital system depends on the traditional yolk colour fan (formerly Roche fan), which is based on a scale of 1–16 colour scores. The yolk, albumen, and shell (air dried for 24 hours) were weighed individually and expressed as percentages of total egg weight. Eggshell thickness was measured based on three pieces of shell without membranes from the blunt, mid-length, and pointed ends using a digital micrometer, and averaged.

Plasma and liver biochemical variables

The plasma and liver biochemical variables were detected as described by Abouelezz et al. (2022). Plasma contents of glutathione (**GSH**), malondialdehyde (**MDA**), and activity of glutathione peroxidase (**GSH-Px**) were measured calorimetrically with commercial kits purchased from Nanjing Jiancheng Inst. of Bioengineering (Nanjing, P.R. China). Frozen liver samples (40 mg) were homogenised on ice in tubes containing 4 mL homogenisation 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 five seconds at 13 500 rpm. The resulting homogenates were centrifuged (3 000*g*) for 10 minutes 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 in duplicate with kits from Nanjing Jiancheng Inst. of Bioengineering. Protein concentrations of supernatants were assayed according to Bradford (1976),

using bovine serum albumin as the standard and Coomassie Brilliant Blue G250 (Sigma Chemical, St. Louis, MO).

Yolk lipid analysis

Yolk lipid analysis was performed as described by Abouelezz et al. (2022). Yolks were separated from the sampled eggs to estimate the content of total cholesterol (**T-CHO**) and triglycerides (**TGs**) using kits purchased from Nanjing Jiancheng Inst. of Bioengineering. Total lipids were extracted (Folch et al., 1957) by mixing 0.5 g yolk with 20 mL of chloroform:methanol (2:1, vol/vol) in a 50 mL glass tube and then homogenised with a Polytron for 5–10 seconds. The resulting homogenate was filtered across Whatman filter paper into a graduated cylinder (100 mL) containing 5 mL NaCl solution (0.88%). After mixing and separation, the top layer was siphoned off and the lipid layer was collected, and its volume was recorded. The separated total lipids were mixed with borontrifluoride, hexane, and methanol (35:20:45, vol/vol) to convert lipids to fatty acid methyl esters (**FAMEs**) (Metcalfe et al., 1961). The FAMEs were quantified by automated gas chromatography 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. The fatty acid calibration and identification were performed by comparison with retention times of standards, and the resulting fatty acid composition was expressed in weight percentages.

Relative expression of genes related to hepatic lipid metabolism: RNA extraction and real-time quantitative PCR

The total RNA isolations, complementary DNA generation, and real-time quantitative PCR process were done as described previously by Xia et al. (2019a). The primer sequences (5′–3′) of the targeted genes were as follows: forward primer (**F**): GCAGGAGCAGAACAAAGAGGT and reverse primer (**R**): TCATCAGAGAAGCCAGGAGAGT for *PPARγ*, F: CAGCGGCAGTTGGTCAGTT and R: GGCTCTCTCTCACATTGGCAG for *FAS*, F: ACCGCTCATCCATCAACGA and R: GGCTGAGGTTCTCCTGCTTC for *SREBP1*, and F: GCTGAGTACCAGGCCAAGGT and R: GATGAAGCGGGTCTTGAGGT for *APOA-1*. A standard curve was designed using 10-fold serial dilutions of cDNA to quantify the transcripts. Samples were assayed in triplicate with SD of threshold cycle (**Ct**) values not exceeding 0.5. The relative expression of targeted genes was determined using the ΔΔCt method ($R = 2^{-\Delta\Delta Ct}$), where R is the relative expression of the gene and ΔCt is the value obtained by subtracting the Ct value for β-actin mRNA from the Ct value of the target mRNA.

Statistical analysis

Replicate was used as the experimental unit (n = 6) and except where otherwise noted, two sampled birds per replicate were used. The effect of PNM dietary incorporation level was estimated by the GLM procedure of SAS (SAS 9.1., SAS Institute, 2004). Orthogonal polynomial contrasts were used to estimate the linear and quadratic effects of the dietary PNM level, and a probability level at 0.05 indicated significance. Tukey's tests were used to compare means wherever significant differences were detected. A broken linear regression model was employed to identify the optimal replacement level of SBM with PNM. The broken linear regression model ($Y = \beta1 + \beta2 \times (\beta3 - X)$) was followed. $(\beta3 - X) = 0$ for $X \leq \beta3$ with Y as the dependent variable as a function of the replacement level of SBM with PNM, $\beta1$ is the value of the dependent variable at the plateau, and $\beta2$ is the slope of the line. The replacement level of SBM with PNM at the breakpoint ($\beta3$) was considered the one providing maximum responses.

Results

Productive performance

As shown in Table 3, egg production showed a quadratic response ($P < 0.05$) to the levels of PNM-SBM substitution, which increased at 25 and 50% PNM, and decreased at higher substitution levels. Egg weight (linear, $P < 0.01$), daily egg mass (linear or quadratic, $P < 0.01$), feed intake (linear, $P < 0.01$) were reduced with the lowest values seen at 100% PNM substitution, but FCR increased (linear or quadratic, $P < 0.01$) as the replacement level of SBM by PNM increased, with the least efficient value obtained at 100% PNM substitution.

Egg quality

As shown in Table 4, egg quality indices were affected by the level of substitution of PNM for SBM. Yolk colour increased in a quadratic pattern ($P < 0.05$), but the yolk, albumin, and shell proportion showed a linear response ($P < 0.05$) to increased PNM. Yolk colour was enhanced ($P < 0.01$) at 50, 75, and 100% compared with the controls, while yolk percent was lower ($P < 0.05$) at 50, 75, and 100% SBM substitution. In addition, albumin proportion increased ($P < 0.05$) at 50% while shell proportion increased ($P < 0.05$) at 75% SBM substitution.

Plasma and liver biochemical variables

The results presented in Table 5 revealed that plasma concentration of MDA increased (linear, $P < 0.05$), and plasma concentra-

Table 3
Effects of gradual replacement of dietary soybean meal with peanut meal on egg production and feed conversion ratio of laying duck.[1]

Variables	Replacement level of SBM[2] with PNM[3], %					SEM	*P*-value[4]		
	0	25	50	75	100		PNM	L	Q
Egg production, %	93.6^{ab}	95.6^{a}	93.9^{ab}	92.7^{ab}	91.0^{b}	0.54	0.034	0.450	0.049
Non-marketable eggs, %	0.77	0.93	0.86	0.67	0.55	0.080	0.694		
Egg weight, g	62.5^{a}	61.5^{ab}	61.5^{ab}	60.6^{b}	58.6^{c}	0.33	0.002	0.001	0.182
Egg mass[5], g/bird/d	59.1^{a}	59.7^{a}	58.7^{a}	57.9^{a}	53.6^{b}	0.49	0.001	0.003	0.001
Feed intake, g/d	155^{a}	153^{ab}	151^{b}	147^{c}	145^{c}	0.79	0.001	0.001	0.282
Feed conversion ratio, g feed/g egg	2.62^{b}	2.58^{b}	2.60^{b}	2.56^{b}	2.72^{a}	0.020	0.002	0.009	0.001

[1] Each value is the mean of six replicates.
[2] SBM = Soybean meal.
[3] PNM = Peanut meal.
[4] Linear (L) and quadratic (Q) effects were tested only when PNM levels were significant.
[5] Regression equation based on replacement level of SBM with PNM (%); broken linear equation: Y(egg mass) = 59.2, PNM substitution level ≤ 67.6; Y(egg mass) = 59.2 + 0.172 × (67.6-PNM substitution level), PNM substitution level > 67.6; $R^2 = 0.98$; *P*-value = 0.02 yielded the optimised PNM substitution level with 67.6%.

Table 4
Effects of gradual replacement of dietary soybean meal with peanut meal on egg quality of laying duck.[1]

Variables	Replacement level of SBM[2] with PNM[3], %					SEM	*P*-value[4]		
	0	25	50	75	100		PNM	L	Q
Yolk colour	5.11[b]	5.17[b]	5.61[a]	5.92[a]	5.58[a]	0.070	0.001	0.638	0.048
Haugh unit	80.9	80.4	82.4	80.6	79.4	0.55	0.527		
Shell thickness, μm	328	334	338	345	339	1.88	0.119		
Shell strength, N	43.3	43.5	45.6	46.7	44.3	0.49	0.107		
Yolk, %	29.8[a]	29.3[ab]	28.6[b]	28.6[b]	28.7[b]	0.15	0.026	0.020	0.356
Albumin, %	60.3[b]	60.9[ab]	61.5[a]	61.2[ab]	61.3[ab]	0.16	0.045	0.025	0.367
Shell, %	9.90[ab]	9.76[b]	9.91[ab]	10.2[a]	10.0[ab]	0.06	0.011	0.040	0.852

[1] Each value is the mean of six replicates.
[2] SBM = Soybean meal.
[3] PNM = Peanut meal.
[4] Linear (L) and quadratic (Q) effects were tested only when PNM levels were significant.

Table 5
Effects of gradual replacement of dietary soybean meal with peanut meal on plasma and liver biochemical variables of laying duck.[1]

Variables[2]	Replacement level of SBM[3] with PNM[4], %					SEM	*P*-value[5]		
	0	25	50	75	100		PNM	L	Q
Plasma									
MDA, nmol/mL	10.8[b]	13.3[b]	14.0[ab]	13.1[b]	17.4[a]	0.63	0.015	0.027	0.908
GSH, μmol/L	5.46[a]	4.78[b]	4.76[b]	4.50[b]	3.91[c]	0.100	0.001	0.919	0.001
GSH-Px, U/mL	51.1	46.5	49.1	74.5	64.2	11.10	0.384		
Liver									
MDA, nmol/mg protein	1.44	1.28	1.28	1.30	0.91	0.187	0.643		
T-SOD, U/mg protein	98.0	100	93.4	105	96.5	5.06	0.966		

[1] Each value is the mean of six replicates.
[2] MDA = malondialdehyde; GSH = glutathione; GSH-Px = glutathione peroxidase; T-SOD = total superoxide dismutase.
[3] SBM = Soybean meal.
[4] PNM = Peanut meal.
[5] Linear (L) and quadratic (Q) effects were tested only when PNM levels were significant.

tion of GSH decreased (quadratic, $P < 0.05$) as SBM was increasingly replaced by PNM. There was a gradual numerical increase in MDA concentrations at 25, 50 and 75% PNM which became more pronounced ($P < 0.01$) at 100% substitution. In contrast, plasma GSH decreased ($P < 0.05$) at all levels of SBM replacement by PNM, and especially at 100% PNM. With regard to plasma activities of GSH-Px, there was an insignificant increase at 75 and 100% PNM.

Liver and reproductive organ indices

Except for the relative weight of the ovary, the relative weight of liver and indices of reproductive organ status were not affected by the gradual replacement of SBM by PNM in diets of laying ducks (Table 6). The relative weight of the ovary was lower ($P < 0.05$) at all PNM replacement levels (25, 50, 75, 100%) compared with the controls.

Yolk lipid analysis

As shown in Table 7, amounts of TG in egg yolk were not affected by the dietary treatments, whereas T-CHO decreased ($P < 0.01$) with PNM substitution for SBM and was most pronounced at 100% substitution. With regard to the saturated fatty acids in yolk lipids, the content of C18:0 decreased (linear,

Table 6
Effects of gradual replacement of dietary soybean meal with peanut meal on liver and reproductive organ indices of laying duck.[1]

Variables[2]	Replacement level of SBM[5] with PNM[6], %					SEM	*P*-value[7]		
	0	25	50	75	100		PNM	L	Q
BW, kg	1.20	1.27	1.28	1.24	1.25	0.020	0.682		
LYF number	4.75	5.25	5.00	5.25	4.83	0.110	0.566		
SYF number	42.5	41.4	50.3	42.0	32.9	2.06	0.134		
Relative weights									
Liver, %[3]	2.85	2.64	2.91	2.72	2.61	0.080	0.704		
Oviduct, %[3]	3.52	3.34	3.27	3.46	3.33	0.060	0.628		
Ovary, %[3]	4.81[a]	3.86[b]	3.67[b]	4.23[b]	3.86[b]	0.110	0.002	0.001	0.015
LYF weight, %[4]	72.1	84.6	75.7	82.1	74.8	1.63	0.062		
SYF weight, %[4]	5.07	6.57	7.43	6.24	6.52	0.280	0.104		

[1] Each value is the mean of six replicates.
[2] LYF = large yellow follicle; SYF = small yellow follicle.
[3] Relative to live BW.
[4] Relative to ovarian weight.
[5] SBM = Soybean meal.
[6] PNM = Peanut meal.
[7] Linear (L) and quadratic (Q) effects were tested only when PNM levels were significant.

Table 7
Effects of gradual replacement of dietary soybean meal with peanut meal on triglyceride (TG), total cholesterol content and fatty acid profile in egg yolk of laying duck.[1]

Lipid[2]	Replacement level of SBM[3] with PNM[4], %					SEM	*P*-value[5]		
	0	25	50	75	100		PNM	L	Q
TG, mg/g	24.0	27.3	25.1	25.5	23.9	1.06	0.186		
T-CHO, mg/g	208^{a}	215^{a}	197^{ab}	197^{ab}	188^{b}	4.9	0.008	0.175	0.451
Yolk fatty acid content, g/100 g of FAME									
C12:0	0.019	0.020	0.019	0.019	0.021	0.0010	0.051		
C14:0	0.449	0.445	0.415	0.433	0.445	0.0170	0.633		
C15:0	0.031	0.033	0.031	0.031	0.032	0.0010	0.275		
C16:0	26.5	26.6	26.5	26.6	26.6	0.25	0.979		
C17:0	0.089	0.095	0.094	0.098	0.097	0.0020	0.128		
C18:0	6.08^{a}	5.57^{b}	5.70^{b}	5.65^{b}	5.30^{b}	0.110	0.001	0.001	0.745
C20:0	0.040^{c}	0.042^{c}	0.048^{b}	0.053^{a}	0.054^{a}	0.0010	0.001	0.002	0.559
C22:0	0.036^{c}	0.049^{b}	0.064^{a}	0.067^{a}	0.067^{a}	0.0040	0.001	0.002	0.016
Total SFA	33.3	32.8	32.8	33.0	32.6	0.29	0.627		
C14:1	0.037	0.037	0.035	0.038	0.039	0.0030	0.907		
C16:1	2.37^{b}	2.67^{a}	2.53^{ab}	2.55^{ab}	2.68^{a}	0.070	0.037	0.004	0.496
C18:1(*trans*-9)	0.297	0.265	0.273	0.290	0.285	0.0090	0.104		
C18:1(*cis*-9)	50.3^{a}	50.0^{ab}	49.5^{ab}	48.7^{b}	49.1^{ab}	0.37	0.031	0.339	0.404
C20:1	0.409^{b}	0.414^{b}	0.427^{b}	0.445^{b}	0.493^{a}	0.0140	0.002	0.005	0.091
C22:1	0.394^{a}	0.241^{b}	0.287^{b}	0.224^{b}	0.264^{b}	0.0290	0.005	0.016	0.019
C24:1	0.056^{a}	0.051^{ab}	0.049^{ab}	0.035^{b}	0.043^{ab}	0.0040	0.011	0.380	0.314
Total MUFA	53.9^{a}	53.7^{ab}	53.1^{ab}	52.3^{b}	52.9^{ab}	0.37	0.039	0.598	0.329
C18:2n-6	7.64^{c}	8.27^{b}	8.67^{ab}	9.05^{a}	8.92^{ab}	0.190	0.001	0.007	0.041
C18:3n-3	0.417^{a}	0.418^{a}	0.389^{ab}	0.384^{ab}	0.357^{b}	0.0120	0.012	0.041	0.485
C20:2n-6	0.209^{b}	0.207^{b}	0.229^{ab}	0.239^{a}	0.252^{a}	0.0070	0.001	0.019	0.540
C20:3n-6	0.382^{b}	0.365^{b}	0.394^{b}	0.438^{a}	0.405^{ab}	0.0120	0.005	0.488	0.721
C20:4n-6	3.91^{b}	3.93^{b}	4.14^{ab}	4.39^{a}	4.35^{a}	0.070	0.001	0.093	0.756
C22:6n-3	0.345^{a}	0.331^{a}	0.284^{b}	0.256^{c}	0.248^{c}	0.0090	0.001	0.001	0.358
Total PUFA	12.9^{c}	13.5^{bc}	14.1^{ab}	14.8^{a}	14.6^{a}	0.24	0.001	0.014	0.092
n-6	12.1^{c}	12.8^{bc}	13.4^{ab}	14.1^{a}	13.9^{a}	0.23	0.001	0.006	0.066
n-3	0.762^{a}	0.749^{a}	0.673^{b}	0.640^{bc}	0.605^{c}	0.0150	0.001	0.001	0.988
n-6/n-3	15.9^{e}	17.1^{d}	20.0^{c}	22.1^{b}	23.0^{a}	0.29	0.001	0.001	0.308

[1] Each value is the mean of six replicates.
[2] TGs = triglycerides; T-CHO = total cholesterol; SFAs = saturated fatty acids; MUFAs = monounsaturated fatty acids; PUFAs = polyunsaturated fatty acids.
[3] SBM = Soybean meal.
[4] PNM = Peanut meal.
[5] Linear (L) and quadratic (Q) effects were tested only when PNM levels were significant.

P < 0.01) as PNM substitution increased. With the increase of PNM substitution for SBM, C22:0 and C20:0 contents increased (linear or quadratic, *P* < 0.05), especially at 75 and 100% replacement.

The contents of C22:1 decreased (linear, *P* < 0.05), while the contents of C16:1 and C20:1 increased (linear, *P* < 0.05) as PNM substitution increased. The contents of C18:1(cis-9), C24:1, and total amount of monounsaturated fatty acids in yolk from the treatment of 75% PNM substitution for SBM were lower (*P* < 0.05) than that of the control diet. The contents of C18:2n-6, C20:2n-6, and the total amount of polyunsaturated fatty acids in yolk increased (linear, *P* < 0.05), while yolk contents of C18:3n-3 and C22:6n-3 decreased (linear, *P* < 0.05) as PNM substitution for SBM increased. The proportion of C20:3n-6 and C20:4n-6 in yolk increased by PNM level up to 75%, but the values at 100% PNM were similar to that of 75% PNM.

The total content of n-6 fatty acids and the n-6: n-3 ratio in yolk increased (linear, *P* < 0.05) as PNM substitution for SBM increased. In contrast, n-3 content decreased (linear, *P* < 0.05) as PNM substitution increased.

Relative expression of genes related to hepatic lipid metabolism

The transcript abundance of hepatic peroxisome proliferator-activated receptors γ (***PPARγ***), fatty acid synthase (***FAS***) and apolipoprotein A1 (***APOA-1***) showed quadratic (*P* < 0.05) responses to increased dietary PNM; the highest gene expression of *PPARγ*

Table 8
Effects of gradual replacement of dietary soybean meal with peanut meal on relative expression of genes related to hepatic lipid synthesis and metabolism in laying duck.[1]

Gene[2]	Replacement level of SBM[3] with PNM[4], %					SEM	*P*-value[5]		
	0	25	50	75	100		PNM	L	Q
PPARγ	0.57^{c}	0.78^{abc}	0.73^{bc}	1.07^{a}	0.90^{ab}	0.100	0.012	0.222	0.040
FAS	0.27^{ab}	0.15^{bc}	0.10^{c}	0.33^{a}	0.30^{a}	0.050	0.010	0.493	0.020
SREBP1	0.33	0.37	0.21	0.32	0.26	0.050	0.165		
APOA-1	0.43^{b}	0.86^{a}	0.69^{ab}	0.54^{b}	0.56^{b}	0.100	0.038	0.069	0.034

[1] Each value is the mean of six replicates.
[2] *PPARγ* = peroxisome proliferator-activated receptors γ; *FAS* = fatty acid synthase; *SREBP1* = sterol regulatory element binding protein 1; *APOA-1* = apolipoprotein A1.
[3] SBM = Soybean meal.
[4] PNM = Peanut meal.
[5] Linear (L) and quadratic (Q) effects were tested only when PNM levels were significant.

and *FAS* occurred with 75% PNM replacement of SBM, and the highest expression of *APOA-1* was with 25% PNM (Table 8).

Discussion

Peanut meal is a high-protein feedstuff with CP content usually ranging from less than 40% to more than 60% of the DM. Despite its high-protein content, PNM is relatively deficient in threonine, and low in lysine and methionine. In the present study, graded replacement of dietary SBM by PNM showed slight effects on egg production and egg quality, but some variables, e.g. egg-laying performance, egg weight, egg mass, and FCR, were deteriorated with complete replacement. Gou et al. (2016) reported that broilers can be fed diets formulated with lower CP ingredients if the diets are supplemented with synthetic amino acids. In the present study, egg production improved quadratically as a function of increasing PNM replacement with the best egg production observed at 25% PNM replacement. Egg weight was significantly decreased in a linear manner by higher replacement with PNM, and especially at 100%. This result is in line with that found in laying hens by Toomer et al. (2019), who found reduced egg weights in laying hens fed a PNM replacement of SBM diet, however, egg mass was not affected until the 75% replacement level, but it was reduced significantly with 100% PNM. Feed consumption with more than 25% replacement levels of SBM with PNM decreased, but FCR was significantly deteriorated than in the controls only at 100% replacement. The decreases in egg weight at 75 and 100% replacement probably reflect dietary amino acid content rather than feed consumption.

Yolk colour depends on the consumption of pigmenting substances in the feed, such as carotenoids (Lessire et al., 2017). Due to the high content of β-carotene in PNM, yolk colour was enhanced here at 50, 75, and 100% replacement with PNM. The same result was obtained previously in laying hens fed PNM (Toomer et al., 2019; Redhead et al., 2021). In the present study, the highest albumin proportion was obtained at 50% PNM, and maximal shell proportion occurred at 75% PNM. Compared to the controls, these results clearly indicate that replacing up to 75% of SBM by PNM gave similar egg-laying rate, egg mass, FCR and almost all egg quality traits with a decreased feed consumption and ovarian weight. The non-gradual change in diet at the start of the experiment or the relatively short-term experiment of 16 weeks probably contributed to the reduced feed consumption found here; reduced feed intake interrupted follicular growth and decreased ovarian weight but did not reduce egg production. In laying hens and laying Japanese quail, the use of high dietary fibre level increased bulkiness of the diet, which significantly reduced feed intake and therefore egg production (Abou-Elezz et al., 2011; Mohammed et al., 2012; Abouelezz et al., 2019). Contamination of poultry feeds with aflatoxins is an important concern determining the potential use of feedstuffs like PNM in poultry diets. There were no signs of stress in terms of negative effects on performance or toxicity in broilers or laying hens if the aflatoxin levels were kept less than 20 pbb and 50 pbb, respectively (Yaling et al., 2008, Aly and Answer, 2009). The amount of aflatoxin B1, the most potent naturally occurring aflatoxin, found in PNM in the present study was 2.98 pbb (μg/kg), which was considered to be negligible. The toxicity of aflatoxins may exert severe hepatocyte dysfunction, follicular growth disruption, and vitellogenesis interruption (Yaling et al., 2008, Aly and Answer, 2009). Therefore, based on egg production and egg quality variables, PNM could substitute up to 75% of dietary SBM (16.05% of the diet). This acceptable replacement level of dietary SBM by PNM in laying ducks is comparable to the value previously reported for laying hens (Pesti et al., 2003).

Some of the biochemical variables assessed here in plasma (i.e., GSH-Px, GSH, MDA), and in liver (i.e., MDA, and T-SOD), were affected by the dietary PNM level. Plasma MDA was not affected by PNM except at 100%. Plasma GSH concentration was decreased at all levels of PNM substitution for SBM. MDA is usually used as a biomarker of oxidative stress and as an indicator of lipid peroxidation (Ahmad et al., 2012), so the present finding implies the absence of oxidative stress arising from replacing up to 75% of dietary SBM with PNM. However, the decrease in plasma concentration of GSH (that functions as a co-factor for several antioxidant enzymes) with PNM replacement for SBM warrants further study in laying ducks.

Egg yolk fatty acid profile and lipid composition can be influenced greatly and rapidly (within 1–2 weeks) by the modification of dietary lipids fed to egg-producing birds (Toomer et al., 2019; Wang et al., 2021). Peanuts possess three major fatty acids that are present as acylglycerol esters, consisting of palmitic acid, oleic acid, and lesser concentrations of linoleic acid (Carrin and Carelli, 2010). In the present study, 100% replacement of SBM by PNM reduced cholesterol content in egg yolk, increased the content of saturated fatty acids (arachidic and behenic), and decreased the contents of erucic acids. Changes in polyunsaturated fatty acids were variable with some fatty acids (linoleic, eicosadienoic, eicosatrienoic, arachidonic) increased, and others (linolenic and docosahexaenoic) decreased, especially at 75% or/and 100% replacement levels. Of the omega fatty acids, increased omega-6 and decreased omega-3 were apparent at the two highest replacement levels (75 and 100%) of SBM by PNM, while the ratio n-6:n-3 increased ($\sim$15.9–23:1) at all levels of replacement. Based on the published literature describing practical dietary intakes for most human healthy adults, the n-6:n-3 ratio should be about 6:1 (Wijendran and Hayes, 2004).

The relative expression of the four genes assessed here revealed that *SREBP1*, the master regulator of lipid homeostasis involved in the biosynthesis of cholesterol and fatty acids, was almost stable and not affected by the substitution of PNM for SBM. In contrast, *PPARγ*, a group of nuclear receptor proteins that function as transcription factors regulating the expression of genes, was increasingly expressed by high-level replacement with PNM. The other two genes *FAS* and *APOA−1* showed opposite responses at moderate replacement, but similar responses at 75 and 100%. The relative expression of *FAS*, the multi-enzyme protein of fatty acid synthesis, was lower at 25 and 50% substitution of PNM for SBM but stable at 75 and 100%. In contrast, *APOA−1*, a major component of high-density lipoproteins with a role in lipid metabolism, was higher at 25 and 50% substitution of PNM for SBM but unchanged at 75 and 100%. These different changes in relative gene expression (especially that of *PPARγ*) may impact hepatic lipid metabolism. In general, there has been only scant research dealing with the replacement of SBM with PNM in laying ducks.

Feeding peanut meal has a positive effect on the broiler's growth performance and might be considered to be an excellent protein source in broiler rations (Ata, 2016). In another study, Lu et al. (2013) found no significant difference in egg production and quality of laying hens between PNM and SBM treatments. They found no effect of PNM level in the diet on the yolk concentrations of saturated fatty acids, monounsaturated fatty acids, or polyunsaturated fatty acids except the percentage of docosahexaenoic acid was lower in yolks of birds fed 15.9% PNM. After 42 days of feeding, however, egg yolk cholesterol concentrations were significantly lower than in controls with 5.3 and 10.6% PNM (Lu et al., 2013), similar to the present findings with ducks. Lu et al. (2013) therefore concluded that it was feasible to replace 100% of the SBM with PNM in laying hens (15.9% of the diet), without affecting performance, egg quality, and fatty acid content while reducing

the production cost. The 75 and 100% PNM substitution for SBM used here with laying ducks represented 16.05 and 21.43% of the total diet.

Based on the present results with laying ducks, it is concluded that 100% replacement of dietary SBM with PNM decreased egg production and antioxidant capacity, and increased the n-6:n-3 ratio of omega fatty acids in yolk. The results indicate that PNM can be used to replace up to 75% of SBM, i.e. 16.05% total inclusion of PNM, in the diet of laying ducks without negative effects on egg-laying production or egg quality. The regression model indicated that the maximal egg mass was obtained at no more than 67.6% replacement of SBM with PNM in the diet of laying ducks.

Ethics statement

All of the procedures employed in this study were approved by the Animal Care and Use Committee of Guangdong Academy of Agricultural Sciences with the approval number "GAASIAS-2016-017".

Data and model availability statement

None of the data were deposited in an official repository. The data that support the study findings are available from the authors upon request.

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Declaration of interest

None.

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