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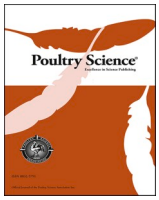

Full-Length Article

Effects of partial replacement of soybean meal with hemp seed (*Cannabis sativa* L.) cake on the growth and meat quality in female three-yellow chickens

Qin He [a], Zongyao Zhang [a,b], Hao Tian [a], Haopeng Wang [a], Xiaoyu Lu [a], Hexuan Deng [a], Fujian Yang [b], Xuemei Tang [b], Jian Wang [a], Zhipeng Li [a], Hui Li [a], Shuibao Shen [a], Yangqing Lu [a], Jieping Huang [a,*]

[a] *State Key Laboratory for Conservation and Utilization of Subtropical Agro-Bioresources, Guangxi Key Laboratory of Animal Breeding, Disease Control and Prevention, Guangxi University, Nanning, Guangxi, 530005, China*
[b] *Guangxi Shenhuang Breeding Group Co. Ltd., Yulin, Guangxi, 537000, China*

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ABSTRACT

Hemp seed cake (**HSC**) (*Cannabis sativa* L.) is a high-quality plant-derived protein source rich in polyunsaturated fatty acid (PUFA). To assess the effects of HSC addition in diets on the growth and meat quality in broiler chicken, a total of 240 female three-yellow chickens (50 days of age) were randomly assigned to four groups and fed with varying levels of HSC (0% (**HSC0**), 5% (**HSC5**), 10% (**HSC10**), and 20% (**HSC20**)) for 9 weeks. As a result, the daily feed intake, weight gain and feed conversion efficiency were significantly increased in the HSC20 group. Moreover, the meat quality traits, including the meat colour, water-holding capacity, intramuscular fat content, and proportion of n-3 PUFA significantly improved, and the expression of lipid synthesis genes, were increased in the HSC20 group. Meanwhile, the development of immune organs and the anti-inflammatory capabilities were enhanced in the HSC20 group. In addition, the blood lipid of chicken was reduced by improving the lipid metabolism in the HSC20 group. Therefore, adding 20% HSC in the feed had a notable effect on the growth, antioxidant and immune capabilities, blood lipid metabolism, and meat performance of the female three-yellow chickens. These findings provide significant information for improving the production performance of broiler chickens through the effective utilization of HSC.

Introduction

The shortage of protein feed is becoming increasingly severe in some Asian countries (Kim et al., 2019). The Asian region is highly dependent on imported soybeans (Tong et al., 2023). The fluctuation of soybean prices and supply stability in the international market directly affect feed costs and the development of the livestock industry in Asia. To reduce dependence on soybean meal, various types of miscellaneous meal substitutes have been developed, such as rapeseed meal, cotton-seed meal, peanut meal, etc (Qu et al., 2025; Yin et al., 2024; Zhan et al., 2024). Hemp (*Cannabis sativa L.*) is an erect herbaceous annual plant of the Cannabis family with a wide-ranging edible and medicinal value. In conventional medicine, hemp has been utilized to reduce cholesterol, treat gastrointestinal disorders, and plays significant roles in anti-inflammatory, antibacterial, and immunomodulatory (Straus, 2000; Cheng et al., 2011). Hemp seeds contain approximately 25%-35% lipids, 20%-25% proteins, 20%-30% carbohydrates, and 4%-7.6% ash content (Montero et al., 2023). Hemp seeds are rich in polyunsaturated fatty acid (PUFA), comprised of 53%-60% linoleic acid (C18:2, n-6), 15%-25% α-linolenic acid (C18:3, n-3), and 3-6% γ-linoleic acid (C18:3, n-6) (Rosso et al., 2024). Additionally, hemp seeds are abundant in vitamins and minerals (Kelley and Rudolph, 2000; Leizer et al., 2000; Callaway, 2004). Various studies have demonstrated the positive effects of hemp seeds on chicken production. Dietary supplementation with hemp seeds can significantly enhance the growth of broiler chicks (Khan et al., 2010), improve meat and bone quality (Skrivan et al., 2020), and reduce blood serum lipid levels (Mahmoudi et al., 2015).

Due to the high lipid content, hemp seeds are primarily used for oil extraction. Hemp seed oil is a naturally rich source with high-quality protein and essential PUFA, including n-6 and n-3 (Callaway, 2004).

* Corresponding author.
E-mail address: huangjieping@gxu.edu.cn (J. Huang).

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The portion remaining after oil extraction from hemp seeds is known as hemp seed cake (**HSC**). HSC contains 10% residual oil and 30%-50% crude protein, which is close to that of soybean meal (35%-50%). In addition, HSC is rich in edestin and albumin, which are easy to be digested and rich in essential amino acids, with unusually high arginine levels (Callaway, 2004). Therefore, HSC is a promising plant-derived protein feed. Studies have shown that dietary HSC supplementation increase the proportion of PUFA in eggs in laying hens (Silversides and Lefrancois, 2005; Halle and Schoene, 2013). Research on the effect of HSC on broiler production is still limited. A recent study showed that dietary HSC supplementation of up to 10% did not affect the growth and slaughter performance while had positive effects on the fatty acid (FA) profile, oxidative status, and gut health in broilers (Tufarelli et al., 2023). The effect of different HSC addition amounts on the production performance of different broiler breeds still needs to be studied.

Three-yellow chicken is a prevalent yellow-feathered broiler breed in Asia, particularly in China. This breed is characterized by yellow feathers, beak, and feet (Huang et al., 2020). Three-yellow chicken is widely raised in China because of its excellent performance in meat production, stress resistance and reproduction (Xiao et al., 2024). To further explore the effect of replacing soybean meal with HSC on broiler production performance, the present study investigated the effects of different HSC addition ratios on the growth performance, carcass traits, meat quality, antioxidant levels, immune modulation, and blood lipid metabolism in female three-yellow chickens. The results of this study can provide a theoretical basis for the effective utilization of HSC, alleviating the supply pressure of soybean meal, and improving the quality of chickens in broiler production.

Materials and methods

Analysis of nutritional composition of hemp seed cake

Fresh HSC, the byproduct of the cold pressing of hemp seeds, was sampled from Bama County (Guangxi Zhuang Autonomous Region, China). The routine nutritional composition and metabolic energy of the HSC were determined by Ulanqab Yima Agriculture and Animal Husbandry Technology Co., Ltd. (Ulanqab, Inner Mongolia, China) using a near-infrared scanning detection method (Tang et al., 2024). Briefly, sample was fully dried and crushed, then scanned by a near-infrared spectrometer. A calibration model was established by the spectral data and the reference data in Dairy One database using the chemometric method. The model was then corrected using artificial neural network and modified partial least squares. The corrected model was used to analyse the spectral data to obtain estimates of each nutrient in the sample.

Analysis of fatty acids

FA analysis was performed by the Qingdao Stande Standard Testing Company (Qingdao, China) (Ponphaiboon et al., 2018;). Firstly, the sample was hydrolysed by 95% ethanol and 8.3 mol/L hydrochloric acid solution at 80°C water bath for 40 min. Then, the sample was cooled to room temperature. The sample was treated with 95% ethanol and the fat was extracted three times by a diethyl ether and petroleum ether mixture. The saponification and esterification for the fat was performed orderly by (1) 2% sodium hydroxide methanol solution at 45°C water bath for 20 min and (2) 14% boron trifluoride methanol solution at 45°C water bath for 20 min. Total FAs were extracted by n-hexane, shaking for 2 min and standing until layering. The supernatant (total FAs) was obtained and treated with a 0.45 μm filter.

Total FAs were tested by a gas chromatograph mass spectrometer (Trace1310 ISQ, Thermo, USA). The main parameters are as follows: (1) chromatographic column, TG-FAM (50 m × 0.25 mm × 0.20 μm); (2) temperature program, maintain 80°C for 1 min, heat up to 160°C at a rate of 20°C/min, maintain for 1.5 min, and then heat up to 230°C at a rate of 5°C/min, maintain for 6 min; (3) ion source temperature, 280°C; (4) solvent delay time, 4 min; (5) ion source, EI source 70eV.

Formula for calculating the content of a kind of FA, $W = (C \times V \times N/m) \times k \times 10^{-4}$. In this formula, W indicates the content of a kind of FA (g/100g), C indicates the concentration of FA methyl esters (mg/L), V indicates the constant volume (mL), N means the dilution ratio, m indicates the weight of sample (g), k indicates the conversion factor for FA methyl esters to FA.

Table 1
Nutritional composition of hemp seed cake.

Items	
Dry matter, %	95.59 ± 0.07
Crude protein, %	35.12 ± 1.18
ADF, %	29.73 ± 0.95
NDF, %	45.50 ± 0.83
NFC, %	0.25 ± 0.09
Fat, %	8.06 ± 0.40
Ash, %	6.67 ± 0.18
TDN, %	74.00 ± 1.00
NEM (Kcal/Kg)	1.78 ± 0.03
NEG (Kcal/Kg)	1.17 ± 0.02
Calcium, %	0.51 ± 0.00
Phosphorus, %	0.86 ± 0.03
Magnesium, %	0.50 ± 0.02
Potassium, %	1.04 ± 0.04
Sulfur, %	0.34 ± 0.07
ME (Kcal/Kg)	2269.67 ± 41.1

[1]ADF = acid detergent fiber; NDF = neutral detergent fiber; NFC = non-fiber carbohydrates; TDN = total digestible nutrients, TDN = digestible nitrogen-free extract + digestible crude fiber + digestible crude protein + (digestible ether extract × 2.25); NEM = net energy for maintenance, NEM = ME - (urinary energy + heat production); NEG = net energy for gain, NEG = DM × efficiency of converting digestible energy to net energy for gain.

Table 2
Ingredients and chemical composition of experimental diets.

Items[1]	HSC0 (%)	HSC5 (%)	HSC10 (%)	HSC20 (%)
Corn	69.67	68.00	67.10	62.08
Soybean	16.95	13.11	8.90	3.70
Hemp meal	-	5.00	10.00	20.00
Fish meal	2.00	2.18	2.78	1.10
Bran	5.00	4.91	4.00	5.00
Soybean oil	3.00	3.34	3.46	4.6
Limestone	0.96	0.96	0.96	0.81
$CaHPO_4$	0.93	0.86	0.93	0.59
NaCl	0.30	0.30	0.39	0.30
L-Lys.HCl	0.10	0.23	0.35	0.63
DL-Met	0.09	0.11	0.13	0.19
Premix[2]	1.00	1.00	1.00	1.00
Total	100	100	100	100
Nutrient levels				
ME/(Kcal/Kg)[3]	3044.93	3044.93	3044.93	3044.93
Crude protein, g	15.37	15.37	15.37	15.37
Crude fiber, g	3.20	4.14	5.02	7.07
Crude fat, g	4.28	4.55	4.87	5.20
Calcium, g	0.89	0.89	0.95	0.74
Total phosphorus, g	0.62	0.62	0.65	0.57
Lysine, g	0.90	0.90	0.90	0.90
Methionine, g	0.34	0.34	0.34	0.34
Cystine, g	0.26	0.24	0.21	0.16

[3]Metabolizable energy.

[1] HSC0 = control diet without hemp seed cake; HSC5 = diet containing 5% of hemp seed cake; HSC10 = diet containing 10% of hemp seed cake; HSC20 = diet containing 20% of hemp seed cake.

[2] The premix provided the following per kg of diets: Cu 10 mg, Fe 80 mg, Mn 60 mg, Zn 70 mg, I 2 mg, Se 0.40 mg, VA 10000 IU, VD 32000 IU, VE 10 mg, VB 2mg, VB 3mg, VB 3.50 mg, nicotinic acid 15 mg, Folic acid 0.50 mg, Pantothenic acid 10 mg, Biotin 0. 15 mg, Cobalt cyanide amine 10 μg.

Management and dietary treatments of the chickens

A total of 240 healthy three-yellow chickens (female, 50-day-old) with the same genetic background were randomly divided into four groups, each group with 6 replicates, each replicate with ten chickens. The control group (**HSC0**) was fed with a basal corn-soybean meal diet, and the three treatment groups were respectively fed with 5% (**HSC5**), 10% (**HSC10**), and 20% (**HSC20**) HSC addition in the essential diet (Table 1) for 9 weeks. The nutritional value of feed compositions was analyzed by Nanning Huagang Agriculture and Animal Husbandry Development Co., Ltd (Nanning, Guangxi, China). Based on the analyzed nutritional value of HSC (Table 1) and feed compositions, the ratio of each composition was determined by referring to the nutritional standard of Broiler Chicken in "Compound Feed for Egg Laying Chickens and Broiler Chickens" (standard number GB/T 5916-2020). The ingredients and chemical composition of the diet are presented in Table 2. All the feed materials except HSC were purchased and produced from Nanning Huagang Agriculture and Animal Husbandry Development Co., Ltd (Nanning, Guangxi, China). Animals were raised under a standard condition with 14 h light, 18-22°C temperature, 55%–65% humidity, and free accessing to food and water. Weekly body weight and feed intake were recorded to calculate the average daily gain (**ADG**), average daily feed intake (**ADFI**), and feed conversion ratio (**FCR**). Chickens were raised in the Guangxi Shenhuang Group Breeding Enterprise (Yulin, Guangxi, China).

At the end of the experiment, ten animals of each group were randomly selected and weighed after fasting for 12 h. Animals were slaughtered by jugular exsanguination postanaesthesia. Meanwhile, the fresh blood in jugular vein was collected. The preslaughter live weight, carcass weight, semi-eviscerated weight, eviscerated weight, breast muscle weight, leg muscle weight, and abdominal fat weight were measured to calculate the slaughter rate, semi-eviscerated rate, eviscerated percentage, percentage of breast muscle, percentage of leg muscle and abdominal fat rate, respectively. The heart, liver, spleen, and stomach were weighed, and the viscera indices were calculated by the following formula: organ index (%) = 100 × fresh organ weight (g)/live weight (g). The fresh blood was left to stand for two hours at room temperature, after which the serum was separated and stored in a refrigerator at -20°C. Fresh breast (left) muscle was sampled for analysis of meat quality. Specifically, for qRT-PCR and FA detection, muscle tissue was immediately stored in liquid nitrogen and brought back to laboratory.

Analysis of meat quality

The fresh breast muscle was used to assess meat colour, pH, shear force, 24-hour dripping loss, cooking loss, triglyceride (**TG**), total cholesterol (**TC**), and for oil red O staining of frozen muscle tissue sections. The meat colour within 1 h after slaughter was measured using a meat colour metre (NS800, 3nh, Shenzhen, China). The pH at 45 min and 24 h after slaughter was measured by a pH meter (pH5, SANXIN, ShangHai, China). All the assaies were performed accoding to the manufacturer's protocols.

Table 3
Composition of total fatty acids in hemp seed cake.

Items	Content (g/100g)	Items	Content (g/100g)
C16:0	0.5852 ± 0.0059	C18:2n6c	4.0103 ± 0.0503
C18:0	0.2445 ± 0.0047	C18:3n6	0.0126 ± 0.0011
C20:0	0.0538 ± 0.0026	C18:3n3	0.7669 ± 0.0190
C22:0	0.0372 ± 0.0005	C20:2	0.0051 ± 0.0002
C23:0	0.0055 ± 0.0001	C20:5n3	0.0110 ± 0.0001
C24:0	0.0126 ± 0.0003	PUFA	4.8059 ± 0.0504
SFA	0.9389 ± 0.0107	FA	7.9023 ± 0.0816
C16:1	0.0056 ± 0.0001	SFA/FA	11.88%
C18:1n9c	0.6400 ± 0.0119	UFA/FA	88.12%
C20:1	1.4865 ± 0.0175	MUFA/FA	27.30%
C22:1n9	0.0254 ± 0.0003	PUFA/FA	60.82%
MUFA	2.1575 ± 0.0270	n-6/n-3	5.18:1

To detect the 24-hour dripping loss, 10 g of freash sample was weighed and then maintained at 4°C for 24 h. The sample was weighed again. The dripping loss was calculated as follows: dripping loss (%) = [(initial weight - final weight)/initial weight] × 100.

To measure the cooking loss, breast muscles (approximately 20 g per sample) was cooked in a 80°C water bath until the internal temperature reached 72°C and then weighed after cooling to room temperature. The cooking loss was calculated as follows: cooking loss (%) = [(weight before cooking - weight after cooking)/weight before cooking] × 100. Meanwhile, the cooked muscle was used to measured the shear force by a tenderness metre (MAQC-12, M-AO, Nanjing, China).

The TG and TC were respectively detected by a triglyceride assay kit (Nanjing Jiancheng, Nanjing, China) and a total cholesterol assay kit (Nanjing Jiancheng, Nanjing, China) according to the manufacturer's instructions.

Before oil red O staining, the frozen sections were made for breast muscle tissue. The sample was fixed in 4% paraformaldehyde for 24 h and then dehydrated in 15% and 30% sucrose, respectively. The sample was then immersed into the paraffin wax and snap frozen. The paraffin block was sectioned into 6 μM slices. The section was stained with fresh oil red O working solution for 8-10 min, washed in 60% isopropyl alcohol and counterstained with haematoxylin. Finally, the section was mounted by a mounting medium of glycerinated gelatin. The section was imaged by a microscope (Cytation5, Biotek, USA).

RNA extraction and qRT-PCR

Total RNA was extracted from breast muscle tissue using TRIzol and reverse transcribed into cDNA using a HiScript III RT SuperMix (Vazyme, Nanjing, China). The qPCR assay was conducted by the ChamQ Universal SYBR qPCR Master Mix (Vazyme, Nanjing, China). All experimental procedures were performed according to the manufacture's protocol. The primers were designed with Primer 5.0 and synthesized by Anhui General Biotechnology Co., Ltd (Anhui, China). The *GAPDH* was used to normalize the expression of target genes. The relative mRNA expression of each gene was calculated using the $2^{-\Delta\Delta Ct}$ method. Details of the primers were presented in Table S1.

Serum lipid metabolism and antioxidant and immunity indicators

The TG and TC in serum were detected as previous described. The high-density lipoprotein cholesterol (**HDL-C**), and low-density lipoprotein cholesterol (**LDL-C**) level, total antioxidant capacity (**T-AOC**), superoxide dismutase (**SOD**), malondialdehyde (**MDA**) in serum were respectively detected by the High-density Lipoprotein Cholesterol Assay Kit (Nanjing Jiancheng, Nanjing, China), the Low-density Lipoprotein Cholesterol Assay Kit (Nanjing Jiancheng, Nanjing, China), the Total Antioxidant Capacity Assay Kit (Nanjing Jiancheng, Nanjing, China), the Total Superoxide Dismutase (T-SOD) Kit (Nanjing Jiancheng, Nanjing, China), and the Malondialdehyde (MAD) Assay Kit (Nanjing Jiancheng, Nanjing, China) according to the manufacturer's instructions.

Serum immune markers included immunoglobulin (**IgG**), interleukin 6 (**IL-6**), and tumour necrosis factor (**TNF-a**) were respectively detected by the Chicken Immunoglobulin G ELISA Kit (Fankewei, Shanghai, China), the Chicken IL-6 ELISA Kit (Fankewei, Shanghai, China), and the Chicken TNF-a ELISA Kit (Fankewei, Shanghai, China) according to the manufacturer's instructions.

Statistical analysis

Data were analyzed by using the SPSS software (version 26.0, SPSS Inc., Chicago). Significant difference was analyzed by one-way ANOVA method. Data are presented as means ± SEM by GraphPad Prism (version 8.0.2). A P-value <0.05 was considered statistically significant.

Table 4
Effects of HSC addition on the growth performance of female three-yellow chickens.

Items[2]	Groups[1]				SEM	*P*-valve
	HSC0	HSC5	HSC10	HSC20		
IBW, g	557.31	552.41	545.00	553.70	5.37	0.896
FBW, g	1983.89^{B}	2030.28^{AB}	2045.00^{AB}	2109.03^{A}	13.52	0.006
ADG, g/d	23.61^{b}	23.94^{b}	24.59^{ab}	25.85^{a}	0.29	0.026
ADFI, g/d	98.10^{B}	100.31^{AB}	103.06^{AB}	105.17^{A}	0.82	0.007
FCR, g/g	4.16	4.20	4.20	4.08	0.05	0.842
Mortality,%	1.67	3.33	0.00	1.67		

Upper case and lower case indicate highly significant difference ($P < 0.01$) and significant difference ($P < 0.05$), respectively.
[1] HSC0 = control diet without hemp seed cake; HSC5 = diet containing 5% of hemp seed cake; HSC10 = diet containing 10% of hemp seed cake; HSC20 = diet containing 20% of hemp seed cake.
[2] IBW = initial body weight; FBW=final body weight; ADG = average daily gain; ADFI =average daily feed intake; FCR = feed conversion ratio.

Table 5
Effects of HSC addition on the carcass traits of female three-yellow chickens[1].

Items	Groups[2]				SEM	*P*-valve
	HSC0	HSC5	HSC10	HSC20		
Live weight,g	1872.50^{C}	1957.50^{AB}	1937.50^{B}	2017.50^{A}	11.64	<0.001
Slaughter rate, %	92.96	92.72	91.87	92.56	0.21	0.323
Half bore rate, %	84.56	85.32	84.26	84.38	0.25	0.442
Full bore rate, %	75.19	75.23	74.97	73.95	0.31	0.440
Abdominal fat, %	7.13	7.38	6.79	8.03	0.22	0.242
Breast muscle rate, %	10.89	10.55	10.88	11.29	0.24	0.797
Leg muscle rate, %	15.17	15.08	15.56	14.08	0.21	0.091

Upper case indicates highly significant difference ($P < 0.01$).
[1] Each value represents the mean of 10 birds per treatment.
[2] HSC0 = control diet without hemp seed cake; HSC5 = diet containing 5% of hemp seed cake; HSC10 = diet containing 10% of hemp seed cake; HSC20 = diet containing 20% of hemp seed cake.

Results

Nutritional and fatty acid composition of hemp seed cake

The nutritional composition of the HSC was analyzed (Table 1). The dry matter content of HSC was up to 95.59%. The crude protein and the crude fat were accounted for 35.12% and 8.06%, respectively. The metabolic energy was 2269.67 kcal/kg. The FA composition of the HSC is shown in Table 3. HSC was highly rich in unsaturated fatty acid UFA content (88.12%). Among which, monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) accounted for 27.30% and 60.82%, respectively. The n-6/n-3 ratio was 5.18:1. In contrast, the ratio of saturated fatty acids (SFA) was only 11.88%.

Effects of HSC addition on the growth performance of female three-yellow chickens

To explore the effect of HSC addition on the growth of broiler, a total of 240 female three-yellow chickens were used and randomly assigned to four groups, which were fed with different HSC addition (0%, 5%, 10%, and 20%) for nine weeks. The growth data were recorded and analyzed. As shown in Table 4, significant difference between groups were found in final body weight (FBW), average daily gain (ADG), and average daily feed intake (ADFI) by HSC addition in the diets. The highest FBW was found in HSC20 group, followed by HSC10 and HSC5 group. The FBW in HSC0 group was the lowest. Similarly, the highest ADG and ADFI were detected in HSC20 group and the lowest ADG and ADFI were detected in HSC0 group. No significant difference was found in feed conversion ratio (FCR) and mortality ratio.

Effects of HSC addition on the carcass traits and organ indices of female three-yellow chickens

The effects of HSC addition on the carcass traits of the three-yellow chickens are presented in Table 5. The highest live weight was found in HSC20 group, followed by HSC10 and HSC5 group. The lowest live weight was identified in HSC0 group. No significant difference was found between groups in slaughter rate, half bore rate, full bore rate, abdominal fat rate, breast muscle rate, and leg muscle rate. The effects of HSC addition on the organ indices of the three-yellow chickens are presented in Table 6. The spleen indices in HSC addition groups were higher than that in HSC0 group. No significant difference was found between HSC addition and HSC0 groups in other indices.

Table 6
Effects of HSC addition on the organ indices of female three-yellow chickens[1].

Items	Groups[2]				SEM	*P*-valve
	HSC0	HSC5	HSC10	HSC20		
Heart	0.46	0.44	0.43	0.40	0.01	0.546
Liver	1.70	1.70	1.83	1.92	0.04	0.249
Spleen	0.11^{c}	0.14^{b}	0.17^{a}	0.13^{bc}	0.01	<0.001
Muscular	1.13	1.03	1.15	1.05	0.03	0.535
Glandular	0.52	0.49	0.49	0.39	0.03	0.561
Stomach	1.66	1.55	1.62	1.43	0.05	0.429

Lower case indicates significant difference ($P < 0.05$).
[1] Each value represents the mean of 10 birds per treatment.
[2] HSC0 = control diet without hemp seed cake; HSC5 = diet containing 5% of hemp seed cake; HSC10 = diet containing 10% of hemp seed cake; HSC20 = diet containing 20% of hemp seed cake.

Effects of HSC addition on the meat quality of female three-yellow chickens

The effects of HSC addition on the quality of breast muscle are shown in Table 7. The meat pH at 24 h in the HSC5 group was significantly higher than that in the HSC0 and HSC10 groups ($P < 0.05$). The meat lightness (L*) in HSC10 and HSC20 groups was considerably lower than that in HSC0 group ($P < 0.05$). The meat redness (a*) in the HSC20

Table 7
Effects of HSC addition on breast muscle quality of female three-yellow chickens[1].

Items	Groups[2]				SEM	*P*-valve
	HSC0	HSC5	HSC10	HSC20		
pH_{45min}	6.21	6.12	6.17	6.25	0.03	0.585
pH_{24h}	5.68^{b}	5.89^{a}	5.73^{b}	5.78^{ab}	0.02	0.015
*L**	53.27^{a}	49.08^{ab}	46.63^{b}	40.48^{c}	1.04	<0.001
*a**	-2.08^{b}	-2.65^{b}	0.28^{ab}	4.08^{a}	0.76	0.004
*b**	11.04^{a}	10.76^{a}	7.12^{ab}	2.71^{b}	0.96	0.004
24h dripping loss, %	6.42	6.29	6.00	5.56	0.24	0.634
shear force, N	24.69	21.12	20.11	19.61	0.81	0.108
cooking loss, %	23.96^{a}	21.60^{ab}	20.09^{b}	19.79^{b}	0.55	0.008

Lower case indicates significant difference ($P < 0.05$).
[1] Each value represents the mean of 10 birds per treatment.
[2] HSC0 = control diet without hemp seed cake; HSC5 = diet containing 5% of hemp seed cake; HSC10 = diet containing 10% of hemp seed cake; HSC20 = diet containing 20% of hemp seed cake. *L**, meat lightness; *a**, meat redness; *b**, meat yellowness.

breast muscle was significantly greater than that in the HSC0 and HSC5 groups ($P < 0.05$). Furthermore, the meat yellowness (b*) in the HSC20 group was substantially lower than that in the HSC0 and HSC5 groups ($P < 0.05$). The cooking losses in the HSC10 and HSC20 groups were significantly lower than those in the HSC0 group ($P < 0.05$). There was a decreasing trend in dripping loss and shear force in the experimental group, but there was no significant difference.

Effects of HSC addition on the intramuscular fat of breast muscle are presented in Fig. 1. TG level in the HSC20 group was significantly higher than that in the HSC0 group ($P < 0.01$) (Fig. 1A). However, no significant difference was identified between groups in TC level (Fig. 1B). Oil red O staining revealed obvious differences in intramuscular fat content between HSC0 and HSC20 (Figs. 1C and D). The relative area and number of lipid droplets in HSC20 were higher than those in HSC0 (Fig. 1E and F, $P < 0.0001$). Further, the FA composition of breast muscle between HSC0 and HSC20 groups was analyzed (Table 8). The MUFAs/FAs ratio in HSC20 group was lower than that in HSC0 group ($P < 0.01$). Meanwhile, the PUFAs/FAs ratio in HSC20 group was higher than that in HSC0 group ($P < 0.01$). Especially, the content of n-3 PUFAs significantly increased in HSC20 group ($P < 0.01$). HSC addition in the diets significantly increased the expression of PPARG, FASN, FABP, ACC, and LPL (Fig. 2).

Effects of HSC addition on antioxidant and immunity indicators of female three-yellow chickens

Effects of HSC addition on antioxidant and immunity indicators are reported in Table 9. Compared with those in the HSC0 group, IL-6 and IgG concentrations in the serum in the treatment groups were significantly lower ($P < 0.01$). Despite the lack of significant difference between groups in T-AOC, SOD, MDA, and TNF-α, in the treatment groups, the serum T-AOC level was greater and the MDA and TNF-α levels were lower.

Effects of HSC addition on the serum lipid metabolism of female three-yellow chickens

Effects of HSC addition on the serum lipid metabolism are shown in Fig. 3. HSC addition in the diets decreased the LDL-C concentration in serum ($P < 0.01$). However, no significant difference was found between HSC0 and HSC treatment groups in TG, TC, and HDL-C concentrations.

Discussion

This study investigated the effects of replacing soybean meal with varying levels of HSC on broiler production performance, meat quality, immune antioxidants, and blood lipid metabolism. The data of this study

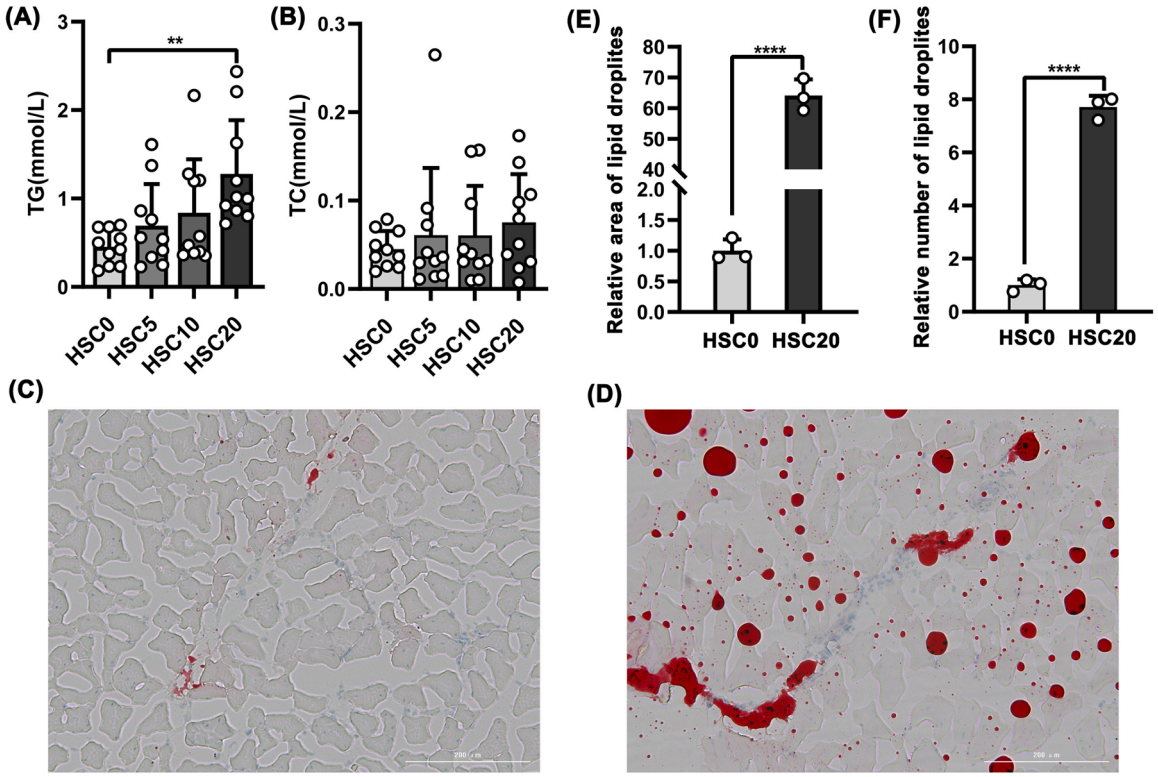

Fig. 1. Effects of HSC on the intramuscular fat content in the muscle of female three-yellow chickens. Triglyceride (TG) (A) and total cholesterol (TC) (B) content in breast muscle. Oil red O staining of the breast muscle in HSC0 (C) and HSC20 (D) groups. Scale bar, 200 μm. Relative area (E) and number (F) of lipid droplets in the breast muscle in HSC0 and HSC20 groups. The data are presented as mean ± SEM (**** and ** indicate $P < 0.0001$ and $P < 0.01$, respectively).

Table 8
Fatty acid composition of breast muscle between HSC0 and HSC20 groups[1].

Items	Groups[2]			
Total fatty acids (g/100 g muscle) [3]	HSC0	HSC20	SEM[4]	*P*-valve[4]
Myristic acid (C14:0)	0.0052	0.0082	0.0011	0.285
Palmitic acid (C16:0)	0.3906	0.4392	0.0530	0.689
Stearic acid (C18:0)	0.1749	0.1961	0.0188	0.624
Docosanic acid (C23:0)	<0.0033	0.0036	-	-
∑SFA	0.5687	0.6409	0.0732	0.667
Myristic dilute acid (C14:1)	<0.0033	0.004	-	-
Palmitoleic acid (C16:1)	0.0579	0.0519	0.0094	0.779
Oleic acid (C18:1n9c)	0.4682	0.5208	0.0651	0.725
Eicocarbon dilute acid (C20:1)	0.0041	0.0057	-	-
Erucic acid(C22:1n9)	0.0238	0.0362	-	-
∑MUFA	0.5334	0.5819	0.0736	0.774
Linoleic acid (C18:2n6c,n-6)	0.2670	0.4623	0.0682	0.188
α-linolenic acid (C18:3n3, n-3)	0.0065	0.0251	0.0049	0.069
Eicosadidilic acid (C20:2)	<0.0033	0.0049	-	-
Eicosatridilic acid (C20:3n6, n-6)	0.0057	0.0065	0.0003	0.234
Eicosatridilic acid (C20:3n3, n-3)	0.0101	0.0132	-	-
Arachidonic acid (C20:4n6, n-6)	0.0558	0.0618	0.0026	0.304
Docosahexahexa dilute acid (C22:6n3, n-3)	0.0132	0.0222	0.0023	0.061
∑PUFA	0.3502	0.5812	0.0738	0.146
∑FA	1.4523	1.8039	0.2148	0.470
SFA/FA	0.3907	0.3621	0.0078	0.076
UFA/FA	0.6093	0.638	0.0078	0.076
MUFA/FA	0.3634^{A}	0.3162^{B}	0.0093	0.005
PUFA/FA	0.2458^{B}	0.3217^{A}	0.0135	0.001
n-3	0.0217^{B}	0.0499^{A}	0.0053	<0.001
n-6	0.3285	0.5293	0.0698	0.182
n-6/n-3	15.70	10.07	1.4971	0.068

Upper case indicates highly significant difference ($P < 0.01$).
[1] Each value represents the mean of 5 birds per treatment.
[2] HSC0 = control diet without hemp seed cake; HSC20 = diet containing 20% of hemp seed cake.
[3] FA, fatty acid; SFA, saturated fatty acid (sum of C14:0+ C16:0+ C18:0+ C23:0); MUFA, monounsaturated fatty acid (sum of C14:1+C16:1+ C18:1+C20:1+C22:1); total n-6 (sum of C18:2n6c + C20:3n6+ C20:4n6); total n-3 (sum of C18:3n3+ C20:3n3+ C22:6n3); PUFA, polyunsaturated fatty acid (sum of n-6 +n-3+C20:2).
[4] The detection value is less than 0.0033, or the number of valid detection samples is less than 3. The SEM and P values cannot be calculated.

indicated that (1) 20% HSC addition in the diet significantly increased the daily feed intake and weight gain and enhanced feed conversion efficiency in female three-yellow chickens; (2) 20% HSC addition in the diet significantly increased the meat colour, water-holding capacity, intramuscular fat content, and n-3 PUFA, and upregulated the expression of genes related to fat synthesis; (3) 10% and 20% HSC addition in the diets enhanced the development of immune organs and inhibited inflammatory mediators, thereby enhancing the anti-inflammatory ability of broilers; (4) 20% HSC addition in the diet significantly reduced blood lipids and improved the lipid metabolism in the chickens.

The nutritional and fatty acid composition of hemp seed cake

HSC is rich in nutrition, especially the PUFA content. The nutritional and FA composition of HSC are significantly influenced by variety, pressing, and seed treatment methods (House et al., 2010; Faugno et al., 2019). Generally, HSC contains 24.8-34.3% protein (St'astnik et al., 2019; Bailoni et al., 2021; Arango et al., 2022) and 8.42-12.7% fat (St'astnik et al., 2019; Bailoni et al., 2021; Arango et al., 2022). In the present study, the crude protein content of HSC was 35.12% (Table 1), which was slightly higher than that in other researches. Generally, the SFA content of HSC ranges from 10%-13.33% (Arango et al., 2022; Banskota et al., 2022), the MUFA content ranges from 10.3%-17.76% (Razmaite et al., 2022; Juodka et al., 2023), the PUFA content ranges from 69.21%-79.69% (Arango et al., 2022; Juodka et al., 2023), and the n-6/n-3 ratio ranges from 3-13.61 (Banskota et al., 2022; Razmaite et al., 2022). In this study, the MUFA content of HSC was higher (27.3%), the PUFA content was slightly lower (60.82%), and the n-6/n-3 ratio was ideal (5.18) (Table 3). Thus, the nutritional and FA composition should be detected before HSC is used in the feed.

HSC addition enhances the growth performance of female three-yellow chickens

To date, studies on the utilize of HSC in broiler diets are limited. Previous studies have shown that incorporating HSC as a partial replacement for soybean meal had no negative effect on the growth performance of broiler chickens. A study demonstrated that adding 20% HSC to the diets of laying hens had no adverse effects on feed intake, BW, or FCR (Silversides and Lefrancois, 2005). Similarly results were obtained by 10% HSC addition in slow-growing broilers and laying hens (Halle and Schöne, 2013; Tufarelli et al., 2023). However, other study observed that 10% and 20% HSC addition enhanced the body weight at 21 and 35 days and effects of 20% HSC addition were better (Eriksson and Wall, 2012). In the present study, broilers fed with 20% HSC addition showed a 9.5% increase in ADG, a 7.2% increase in ADFI, and a 6.3% increase in FBW (Table 4). The positive effects of HSC on the growth performance in this study are mainly attributed to its nutritional composition. The high PUFA content increases the palatability of feed and the ADFI of chicken. Meanwhile, the high quality feed increases the ADG and FBW. However, HSC addition had no significant effect on the slaughter performance except for the live weight in the present study (Table 5). Similar results were reported by other studies (Eriksson and Wall, 2012; Vispute et al., 2019). Thus, 20% HSC addition has significantly positive effects on the growth in female three-yellow chickens.

HSC addition improves the meat quality of female three-yellow chickens

The meat colour, pH, dripping loss, cooking loss, and shear force are essential indicators of meat quality and are closely linked to chicken tenderness and flavour (Yu et al., 2020). The pH of muscle is rapidly decreased after slaughter as the cells generate lactic acid and phosphoric acid through glycolytic reactions. Decrease of pH generally results in juicier and paler meat, reduces water-holding capacity and affects meat colour. Our data indicated that HSC addition in diets enhanced the pH and a* value and decreased the L* value and cooking losses of the meat. Studies indicate that consumers prefer meat with low L* value and high a* value (Kuttappan et al., 2012). Higher levels of HSC addition had positive effects on the meat colour in chickens (St'astnik et al., 2019; Kasula et al., 2021).

The intramuscular fat content is crucial for the tenderness and juiciness of meat (Li et al., 2020), and the fatty acid composition primarily affects the flavour and nutritional quality of meat (Wood et al., 2008). Our findings indicated that HSC addition in the diets significantly increased the fat content and the proportion of PUFAs, especially n-3 PUFAs, in chicken breast muscle (Fig. 1, Table 8). Moreover, the proportions of MUFAs and the n-6/n-3 ratio decreased considerably (Table 8). As a byproduct of oil crops, the fat content of HSC is up to 8.06% (Table 1), and PUFAs accounted for as much as 60.82% (Table 3). A high-fat diet enhances lipid absorption through VLDL and FAT/CD-36 proteins in muscles (Kolditz et al., 2008). In the present study, HSC upregulated the gene expression of essential transcription factors and critical enzymes related to fat synthesis, thereby accelerating intramuscular fat deposition (Fig. 2). The FA concentration in animal products is heavily influenced by the diet (Haug et al., 2007; Bou et al., 2009). Researches on laying hens and Japanese quails demonstrated that increasing dietary hemp seed levels significantly increased the n-3 PUFA content of egg yolks (Konca et al., 2014; Taaifi et al., 2023). In addition, adding 10% hemp meal can dramatically improve the fatty acid profile of muscle, with an increase in n-3 PUFAs and a decrease in the n-6/n-3 ratio in broiler chickens (Tufarelli et al., 2023). The ratio of

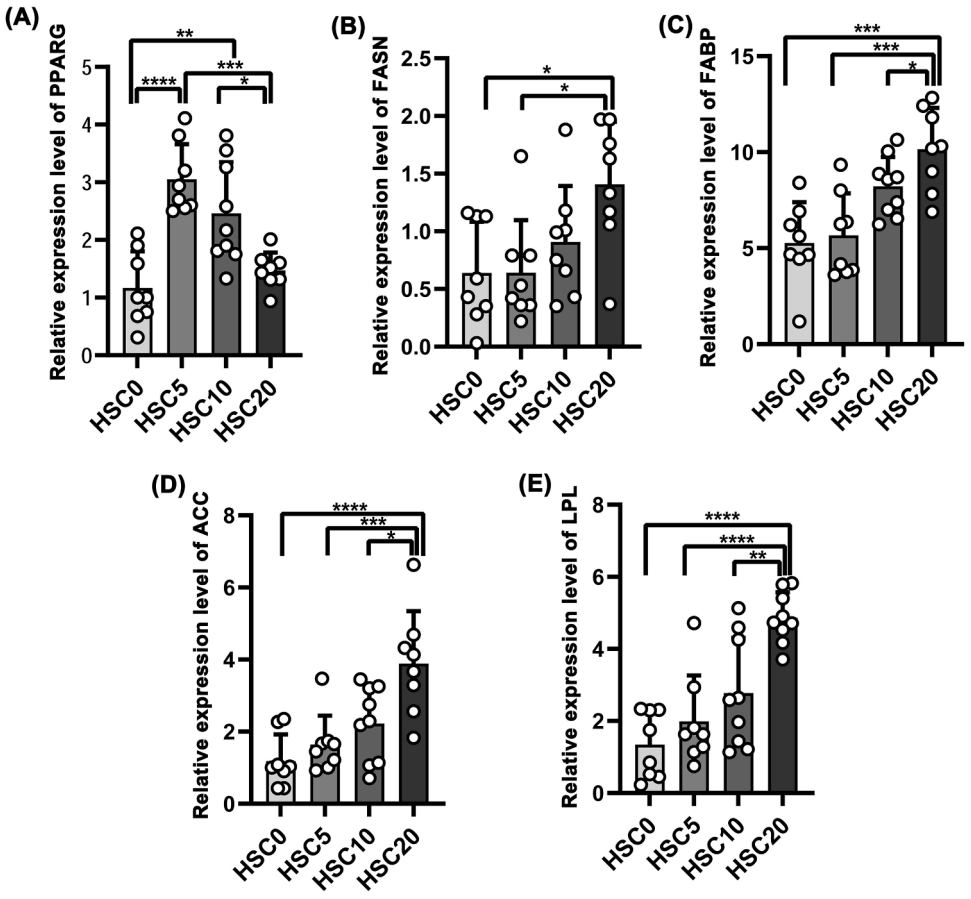

Fig. 2. Effects of HSC on the mRNA expression of lipid metabolism related genes in the muscle of female three-yellow chickens. The mRNA expression of PPARG (A), FASN (B), FABP (C), ACC (D), and LPL (E) in breast muscle was detected by qRT-PCR. The data are presented as mean ± SEM (****, ***, ** and * indicate $P < 0.0001$, $P < 0.001$, $P < 0.01$ and $P < 0.05$, respectively).

Table 9
Serum antioxidant and immune capacity of female three-yellow chickens[1].

Items	Groups[2]				SEM	*P*-valve
	HSC0	HSC5	HSC10	HSC20		
T-AOC, U/mL	2.59	2.98	4.04	4.85	0.34	0.070
SOD, U/mL	109.36	112.91	110.40	108.58	0.66	0.109
MDA, nmol/mL	8.61	6.28	6.17	6.02	0.37	0.041
TNF-a, pg/mL	130.13	118.21	126.11	108.84	3.46	0.154
IL-6, pg/mL	32.44^{A}	31.46^{A}	26.14^{B}	20.73^{C}	5.50	<0.001
IgG, g/L	34.96^{A}	33.13^{AB}	29.48^{B}	21.64^{C}	1.02	<0.001

Upper case indicates highly significant difference ($P < 0.01$).
[1] Each value represents the mean of 10 birds per treatment.
[2] HSC0 = control diet without hemp seed cake; HSC5 = diet containing 5% of hemp seed cake; HSC10 = diet containing 10% of hemp seed cake; HSC20 = diet containing 20% of hemp seed cake.

n-6/n-3 decreased, suggesting that the deposition efficiency of n-3 PUFAs was higher than that of n-6 PUFAs. Other studies have shown a linear relationship between the n-3 PUFA content in muscle and the percentage of hemp seed addition, but this was not observed for n-6 PUFA (Skrivan et al., 2020). Similarly, another study reported that the deposition efficiency of n-3 PUFAs was greater than that of n-6 PUFAs (Skiba et al., 2015). This is probably due to the level of α-Linolenic acid in HSC and the strong ability to convert α-Linolenic acid to n-3 PUFAs in hens (Howe et al., 2002). Meanwhile, n-3 PUFAs inhibites the activity of Δ-9 desaturase, an enzyme crucial for MUFA formation (Jing et al., 2017). Thus, MUFAs content in muscle was decreased by HSC addition.

HSC addition enhances the antioxidant and immunity of female three-yellow chickens

In intensive farming systems, chickens generally suffer from immunological and oxidative stresses, which disrupts their redox balance and results in immune responses and excessive nutrient and energy consumption (Kuo et al., 2011; Chen et al., 2019; Lauridsen, 2019; Liu et al., 2021). Enhancing the immunity and antioxidant capacity is of advantage to the growth and health in chickens. In the present study, HSC addition in the diets increased spleen index (Table 6) and the T-AOC level in serum in chickens (Table 10). HSC is rich in natural polyphenols such as tocopherol and cannabidiol, which are known for their antioxidant properties (Jiang et al., 2001). Cannabidiol also has anti-inflammatory effects (Fernandez-Ruiz et al., 2013). This is evidenced by the decrease of proinflammatory cytokines in serum in the present study (Table 9). Cannabinoids can relieve the inflammation caused by the release of cytokines such as TNF-a (Shohami and Mechoulam, 2000). However, an excessive hemp or cannabinoid supplementation suppresses immune functions and reduces productive performance (Hassan et al., 2023). Thus, adding HSC in the diets in moderation will enhance the immunity in female three-yellow chickens.

HSC addition enhances the serum lipid metabolism of female three-yellow chickens

Serum lipid metabolism plays a crucial role in maintaining overall lipid balance in the body in response to varying nutrient levels and physiological conditions. Our data demonstrated that HSC supplementation significantly decreased the LDL-C level in the serum of three-

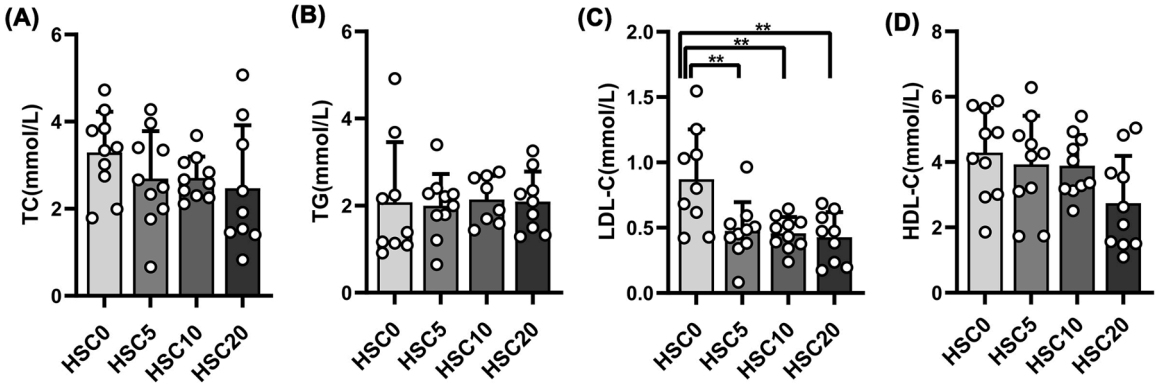

Fig. 3. Effects of HSC on the serum biochemical indicators regarding lipid metabolism in female three-yellow chickens. The changes in serum biochemical indicators regarding lipid metabolism. (A) Serum total cholesterol (TC) content. (B) Serum triglyceride (TG) content. (C) Serum low-density lipoprotein cholesterol (LDL-C). (D) Serum high-density lipoprotein cholesterol (HDL-C). The data are presented as mean ± SEM (** indicates $P < 0.01$).

yellow chickens (Fig. 3C). TC level also tended to decrease, though no significant difference was identified (Fig. 3A). Similar results have been revealed by previouse studies (Mahmoudi et al., 2015; McKenney and Sica, 2007; Gakhar et al., 2012). These results indicate that HSC addition can decrease the lipid levels by enhancing the lipid metabolism in serum in female three-yellow chickens.

Conclusions

The data of this study illustrated that 20% HSC addition in the diet had significantly positive effect on the growth performance, meat quality, antioxidant properties, anti-inflammatory activity, and hypolipidaemia in female three-yellow chickens. Thus, HSC can be served as a sustainable and nutrient-rich protein source for broiler diets, potentially replacing soybean meal.

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Ethics approval and consent to participate

All procedures used in this study were approved by the Institutional Animal Care and Use Committee of Guangxi University.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psj.2024.104466.

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