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Carcass Traits, Physicochemical Characteristics, Fatty Acid, and Protein Profile of Khiew Phalee, Pradu Hang Dam and Broiler Chicken Meat

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ABSTRACT

This study investigated the carcass traits, physicochemical characteristics, fatty acid profile and protein profile of male Khiew-Phalee native (KP), Pradu Hang Dam (PHD), and commercial broiler chickens (CBR). All samples were collected from farms in Uttaradit province and determined the carcass traits and physicochemical characteristics, including proximate composition, pH, meat color, shear force, drip loss, cooking loss, and also analyzed the fatty acid profile, purine content, and protein profile. The results showed that carcass traits such as live weight, carcass weight, and cutting percentage showed a highly significant decrease ($p<0.01$) in KP and PHD, except for the percentage of thigh, drumstick, and meat quality traits. There were highly significant differences in chemical composition, including moisture, protein, fat, ash and gross energy, lipid content, pH values, color values, drip loss, cooking loss, and shear force among the chicken breeds ($p<0.01$). The fatty acids significantly decreased in myristic acid, myristoleic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, and erucic acid in KP and PHD. Protein profile analysis found three different protein bands based on SDS-PAGE and LC-MS/MS analysis between three different chicken breeds, including 70 kDa proteins (heat shock 70 kDa and albumin OS) and 110 kDa protein (pyruvate kinase PKM) in KP and PHD with higher intensity than CBR. The cholesterol, purine, and uric acid of breast chicken meat were not affected by breed. Importantly, KP and PHD Thai native chickens possess lower amounts of unhealthy fatty acids, which positively affect the consumer and are anticipated to reduce the risk of many cardiovascular diseases.

Keywords: carcass traits; fatty acid profile; Khiew Phalee chicken; physicochemical characteristics; protein profile

INTRODUCTION

The meat of the Thai native chicken was shown to be high in nutritional value and had some unique features and advantages over commercial broiler chickens (Lengkidworraphiphat *et al.*, 2021). To promote native chicken breast meat as a functional food and establish them in modern trade. A previous study reported the effect of a high protein diet comprising breast meat from Thai native chicken on serum uric acid, biochemical parameters, and antioxidant activities in rats (Potue *et al.*, 2022). The Thai native chicken meat fed rats had lower plasma total cholesterol and triglyceride levels than the control rats that received a standard chow diet. They also integrate well with local and processed feed, exhibiting distinct meat texture and flavor. Particularly, the meat of native chicken breeds has lower fat content, denser texture, and better taste compared to meat from commercial broiler chickens (CBR) (Shohei, 2022).

Previous research has shown that the physicochemical composition of muscle in native chicken breeds has a higher protein content than CBR (Tantiyasawasdikul *et al.*, 2023). The functional properties like pH, water holding capacity, cooking loss, drip loss, and protein profile of marinated chicken breast meat during heating (Singh & Deshpande, 2019) and chicken meat by-products (Azman & Shamsudin, 2022). This makes them highly desirable among consumers and potentially lucrative for commercial farming (Tantiyasawasdikul *et al.*, 2023). Furthermore, Thai native chicken is a key aspect of the government's strategy for breed preservation and development, as outlined in the National Indigenous Chicken Strategy Plan for 2018-2022 by the Department of Livestock Development. This initiative aims to enhance market potential by researching and developing indigenous chicken products (Jaturasitha *et al.*, 2016).

Thai native chickens are considered as a part of Thailand's cultural heritage. They exhibit genetic

diversity and possess unique characteristics such as disease resistance, natural foraging ability, adaptability to local farming practices, and the capability to thrive under the care of rural farmers.

The Khiew-Phalee chicken (KP) is a native breed that has been certified by the Department of Livestock Development as a regional Thai breed of Uttaradit Province since 2013 (Yaemkong *et al.*, 2024). The KP is considered a valuable national resource and merits conservation. It can be raised to increase its value, such as for conservation or ornamental purposes, entertainment, or competitive fighting, thus increasing its value and promoting it as an agricultural product that can boost the livelihoods and incomes of the people in Uttaradit Province. However, the conservation-oriented rearing of KP (ornamental or competition chickens) still involves a considerable number of chickens in the population that do not conform to the ideal breed standards required for competition. Therefore, there is a need to create alternative avenues to generate value, such as raising chickens for economic purposes (for meat or eggs) (Chaiwang *et al.*, 2023). Promoting KP as an economical breed requires studying its nutritional value and consumer-preferred characteristics to enhance marketability among consumers who prioritize healthy and tasty food, especially focusing on the functional meat attributes, such as having low purine content, soft and tender texture, high protein, and collagen content, as well as low cholesterol and fat levels (Jaturasitha *et al.*, 2016). Previous studies on KP have focused on their genetic diversity and external characteristics for breed conservation, but no studies have been carried out on the functional meat properties of KP using agricultural biotechnology. The findings will serve as a guideline for promoting the Pradu Hang Dam chicken meat as a functional food as well as breeding, which could help improve the efficiency of breeding programs based on profitable sustainability.

Therefore, the aim of the present study was to evaluate and determine the carcass traits, physicochemical characteristics, including proximate composition, pH, meat color, shear force, drip loss, cooking loss, and also analyze the fatty acid profile, purine content, and protein profile based on SDS-PAGE in Khiew-Phalee native, Pradu Hang Dam, and commercial broiler chicken meat in Uttaradit province, Thailand.

MATERIALS AND METHODS

Ethical Statement

The present experiment was reviewed and approved by the Institutional Review Board (or Ethics Committee) for Institutional Animal Care and Use Committee (IACUC), Kasetsart University (ID: ACKU65-AGK-037).

Experimental Design and Chicken Samples Preparation

The study was carried out to evaluate the physicochemical and functional properties of Thai native chicken (Khiew-Phalee chicken and Pradu Hang Dam chicken) and commercial broiler chicken meat using a completely randomized design (CRD). The treatments were 3 chicken breed genotypes and 6 replications, and one sample per replication.

Each of the three genotypes of chicken in this study, Khiew-Phalee native (KP) as in Figure 1A, Pradu Hang Dam (PHD) as shown in Figure 1B, and commercial broiler chickens (CBR), were reared in one flock on a single farm in Laplae District, Uttaradit Province (Figure 1) under identical conditions and grown free-range. According to their genetic requirements, the chickens were fed formulated diets obtained from commercial diets and feeding schedule management.

Feed and water were provided for *ad libitum* intake. The broilers were fed until they were 5 wk old, whereas Khiew-Phalee native (KP) and Pradu Hang Dam (PHD) were fed until they were 14 wk old. The average live

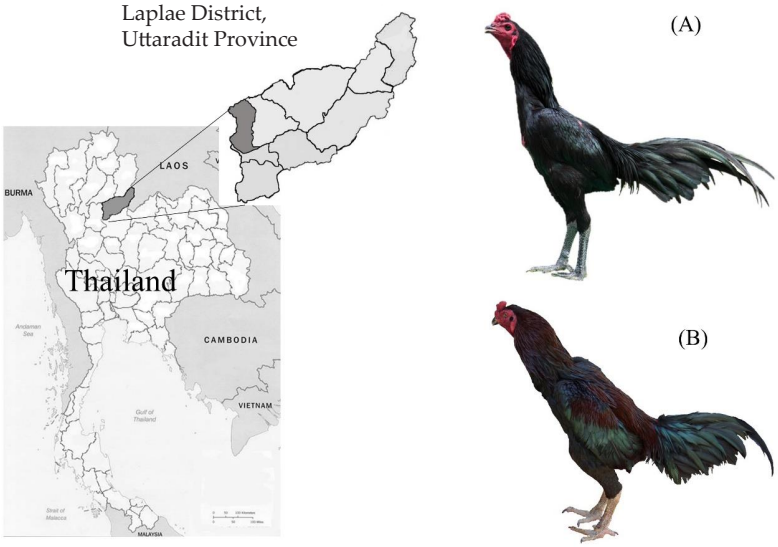

Figure 1. Location of chicken farm in Laplae, Uttaradit Province, Thailand. (A) Khiew-Phalee chicken (KP) and (B) Pradu Hang Dam chicken (PD).

weights of each chicken genotype were 1.5±0.2 kg (KP), 1.2±0.3 kg (PHD), and 2.7±0.3 kg (CBR). At the end of the experiment, six male chickens of each genotype were slaughtered using standard methods. The total 18 carcasses were packed in PE bags and placed in foam boxes with ice during transport to the laboratory. The chicken carcasses were chilled at 4 °C in a refrigerater for 16 hrs before the experiment.

Carcass Traits and Characteristics

The percent of retail cuts and characteristics. Measurement of carcass yield and characteristics were carried out after boneless and skinless thigh meat samples were obtained from each group of chickens and used for meat quality determination as carcass weight, head and neck, leg, breast, wing, fillet, thigh, drumstick, and frame were calculated relative to the slaughter weight and stored in a freezer (-20 °C) for proximate determination and functional properties. All carcasses were dressed according to the method of Jaturasitha (2004), and carcass weight was weighed after removing feathers and blood. The observed variables were the percentage of retail cuts (head and neck, leg, breast, wings, fillet, thigh, drumstick, visceral organ, and frame) as formulation below:
[Retail cut (g) x 100] / Carcass weight (g)

Physicochemical Characteristics

pH determination. The pH of the raw chicken breast and thigh meat were evaluated for 2 positions in pieces with three replicates for each chicken breast and thigh meat samples using a pH meter (Mettler Toledo, SevenGo SG-2 , Switzerland) calibrated at 4.0 and 7.0.

Color meat measurement. Surface color was measured on raw intact chicken breast and thigh meat (three pieces for each genetic breed). Color parameters (L* (Lightness), a* (Redness), and b* (Yellowness)) were evaluated for three replicates of each chicken breast and thigh meat using MiniScan Hunterlab colorimeter (Color Flex, U.S.A.). The replication was applied on the surface of the upper, middle, and lower chicken breast and thigh meat.

Warner-Bratzler shear forces. The measurement of shear forces used chicken breast and thigh meat that was trimmed to three strips of uniform size (1.0 cm wide, 3.0 cm long, and 1 cm thick). The shear force of boiled breast muscle was determined in six 1.27 cm diameter cores using a Warner-Bratzler shear device attached to an Instron universal testing machine (model 3344, Instron Ltd., Buckinghamshire, UK). A crosshead speed of 200 mm/min and a 5 kN load cell calibrated to read over a range of 0-100 N were applied with a method modified from Honikel (1998).

Drip loss. The measurement of drip loss used chicken breast and thigh meat that was cut into uniform pieces. Three strips (1.0 cm wide, 3.0 cm long, and 1 cm thick) from each chicken breast and thigh meat were individually weighed, wrapped with gauze, placed in a sealed polyethylene bag, and storage for 24 h at chilling temperature (1 to 4 °C). After this period, the samples were weighed again, and drip loss was determined as percentage of weight loss by initial sample weight, following the method (Zhang *et al.*, 2008).

Cooking loss. The fresh chicken breast and thigh meat were cut into uniform pieces, weighed, placed on a stainless steel screen and cooked in a water bath at 85 °C for 45 min. The internal temperature applied in this analysis was 85 °C as recommended (Zhang *et al.*, 2008). The chicken breast and thigh meat pieces were then allowed to equilibrate to room temperature and reweighed. Cooking loss was determined as the percentage of weight loss by initial weight of the sample and to calculate the percentage of cooking loss, following the method (Zhang *et al.*, 2008).

Determination of proximate composition. Moisture content (oven method), ash (furnace), crude protein (Kjeldahl method), and crude fat (Soxhlet method) were carried out according to the methodology proposed following in-house method based on AOAC official methods of analysis (AOAC, 2003). The proximate composition analysis was performed at the Department of Animal Science, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, Thailand.

Content of purine and its derivatives. The content of purine derivatives (i.e., adenine, guanine, hypoxanthine, and xanthine) in breast meat was determined using high-performance liquid chromatography (HPLC) following Kaneko *et al.* (2014) with some modifications, and the content of purine derivatives was performed at School of Food Technology, Institute of Agricultural, Technology, Suranaree University of Technology, Nakhon Ratchasima, Thailand. The chicken meat samples were minced, and approximately 500 mg was homogenized in 10 mL of deionized water containing 35% perchloric acid. The homogenate was incubated at 95 °C in a water bath, shaken at 180 rpm for 1 hr, then neutralized with 30% potassium hydroxide immediately and centrifuged at 3,500×g for 15 min at 4 °C. The supernatant was filtered by 0.45-μm filtration membranes and injected into an Agilent Technologies 1260 Infinity HPLC (Agilent Technologies, Santa Clara, CA) for analysis. The column used in the experiment was an Asahipak GS-HQ 320HQ (300 mm×7.5 mm, 6 μm) column (Showa Denko K.K., Tokyo, Japan) at a temperature of 35 °C. HPLC was performed using a mobile phase of 150 mM sodium phosphate buffer (pH 2.5) at a flow rate of 0.6 mL min−1, and the running time was 35 min. All samples were measured twice, and the values were averaged. The total purine content was calculated from the combined amounts of each derivative.

Determination of total lipid content, cholesterol content, and fatty acid profile. Analysis of total lipid content, cholesterol content, and fatty acid profile

was performed at the Institute of Nutrition, Mahidol University at Salaya Campus, Nakhon Pathom, Thailand. Fatty acids were isolated, and the lipid phase was removed from the sample. The esterification of fatty acids was carried out using an in-house method based on AOAC (2019), which was analyzed in a gas chromatograph equipped with a flame ionization detector and fused silica capillary column, with H_2 being used as a carrier gas. Peak identification was obtained by comparing retention times with known composition patterns.

Protein Profile

Protein sample preparation. Approximately 100 g of each chicken breed breast meat was minced and 5 g of minced meat was added to 400 µL of ice-cold lysis buffer and incubated overnight at 4 °C. The sample was centrifuged at 12,000 rpm and 4 °C for 20 min. The supernatant was then transferred to a new tube for protein quantification using a Bradford reagent. The protein content was measured using a microplate reader with agitation for 5 min, and then the absorbance was measured at 595 nm. The protein concentration was calculated using a standard curve and adjusted to equal concentrations. A total of 20 µL of the adjusted protein solution was taken and added to the sample buffer (0.5 M Tris HCl, pH 8, 30% glycerol, 5% SDS, 0.06% BPB, and 2-Mercaptoethanol), then incubated at 90 °C for 5 min followed by cooling on ice for 5 min. The sample was centrifuged at 14,000 rpm at 4 °C for 5 min. The centrifuged sample would be used for SDS-PAGE with a volume of 10 µl per well.

SDS-polyacrylamide gel electrophoresis. Glass plates were rinsed with RO water and wiped with Kimwipes. The glass plates were assembled into the gel casting apparatus. Two parts of gel were prepared: Separating gel (15%) and Stacking gel (5%). The gel assembly was placed into an electrophoresis tank. The tank was filled with a tris-glycine running buffer until the gel was covered. The samples and protein ladder were loaded into the wells, with each sample being 10 µL. The gel was run at 120 volts for 120 min. The gel was removed from the glass plates, and the Stacking gel was discarded. The gel was placed in a staining box, and a coomassie staining solution was added to cover the gel completely. The gel was shaken gently at 50 rpm for 15 min, after which the staining solution was discarded. The methanol-acetic acid destaining solution was added to cover the gel and then shaken gently at 50 rpm for 5 min. The destaining solution was discarded, and the destaining process was repeated twice more for approximately 30 min each, or until the bands on the gel became visible.

LC/MS analysis. The protein bands obtained from SDS-PAGE were examined by sending the chicken protein samples for analysis using LC/MS at the Proteomics Laboratory, Faculty of Medical Technology at Mahidol University.

Statistical Analysis

In this study, a completely randomized design (CRD) was used. Statistical analysis was carried out with analysis of variance (ANOVA) at a 95% confidence level. Duncan's new multiple-range test was employed to compare the treatment means. The data are presented as least square means for the evaluated carcass traits, physicochemical, fatty acid, and protein profile, along with their respective standard errors (SEM) and P values reflecting the error probability. This analysis was conducted using SAS on-demand software (SAS Institute Inc., 2014).

RESULTS

The Percent of Retail Cuts and Characteristics

The physical properties of meat quality from the study are presented in Table 1. The results in Table 1 show no significant difference in carcass weight between KP and PHD, but both differ significantly from CBR ($p<0.05$). Due to the lower carcass weight of KP and PHD compared to CBR, the percentage of breast meat and fillet also differ. CBR has a higher percentage of breast meat and fillet compared to native chickens, with KP and PHD, respectively ($p<0.05$). This study shows that KP has percentages of head, neck, and leg, while PHD has percentages of head, neck, and leg, respectively. These percentages are higher than those of

Table 1. Carcass trait of three different chicken breeds

Variables	Breed of chickens			SEM	p-value
	Khiew-Phalee	Pradu Hang Dam	Broiler		
Carcass weight (g)	1,527.06±193.15[b]	1,238.92±28.68[b]	2,701.88±327.28[a]	317.4	<0.001
Retail cuts (%)					
Head + neck	7.69±0.63[a]	8.15±0.56[a]	4.27±0.53[b]	0.33	<0.001
Leg	5.80 ±0.38[a]	5.38±0.74[a]	3.65±0.23[b]	0.67	<0.001
Breast	9.79±0.79[b]	9.29±1.02[b]	26.20±1.87[a]	2.55	0.01
Wing	11.21±0.71[a]	9.83±0.83[b]	8.15±0.17[c]	0.82	<0.001
Fillet	2.13±0.56[b]	2.69±0.42[b]	3.83±0.49[a]	0.63	<0.001
Thigh	12.03±0.34	11.44±0.45	11.40±0.82	1.37	0.95
Drumstick	12.19±0.67	11.12±0.51	10.41±0.77	2.73	0.97
Frame	23.13±1.11[a]	16.65±0.83[c]	18.74±0.43[b]	1.08	<0.001

Note: [a, b, c] Mean in the same row with different superscripts differ significantly ($p<0.05$).

CBR, with percentages of head, neck, and leg ($p<0.05$). However, regarding wings, KP has a higher percentage than PHD and CBR, respectively ($p<0.05$). Additionally, the KP frame percentage is observed to be higher than that of CBR and PHD, respectively.

pH, color, and shear force. The pH values at 24 h post-mortem for the breast and thigh meat of all three breeds differed significantly ($p<0.05$). Breast meat of CBR had a higher pH value compared to KP and PHD, respectively. Similarly, the thigh meat of CBR had a higher pH value compared to both native breeds, respectively (Table 2). The study involved dissecting meat into sub-components, comprising breast and thigh, to evaluate color indices, which are important factors influencing consumer purchasing decisions. In this study, it was found that the lightness value (L*) of breast meat of KP was similar to that of CBR, respectively, but differed from that of PHD ($p<0.05$). However, the lightness value in the thigh meat did not differ among the three breeds (Table 2). The redness value (a*) of breast meat of PHD was higher than that of KP and CBR ($p<0.05$), respectively. In the thigh meat, the redness value did not differ among the three breeds (Table 2). The yellowness value (b*) of breast meat of CBR was higher than that of KP and PHD, respectively. Similarly, the thigh meat of CBR exhibited higher yellowness values compared to both native breeds, respectively (Table 2).

Shear force values differed significantly in breast meat ($p<0.05$), with higher values in KP and PHD compared to CBR, respectively. Similarly, the thigh meat of KP and PHD had higher shear force values compared to CBR, respectively (Table 2).

Drip loss and cooking loss. The drip loss percentage of KP and PHD in breast meat was higher than CBR ($p<0.05$), respectively. However, there was no significant difference in drip loss percentage among the three breeds in thigh meat ($p>0.05$). Cooking loss percentage in breast meat was higher in PHD compared to KP and CBR ($p<0.05$), respectively. Similarly, the thigh meat of PHD exhibited a higher cooking loss percentage than KP and CBR, respectively ($p<0.05$) as shown in Table 2.

Proximate composition. The moisture content in breast meat ranged from 73.92% to 74.98%. The moisture content of CBR was significantly higher than that of KP and PHD, which did not differ statistically (Table 3). The protein content in the breast meat of CBR was the lowest at 20.60%, significantly lower than that of KP (24.67%) and PHD (24.18%). The fat content in the

Table 2. Meat quality traits of breast muscle and thigh samples obtained from three different chicken breeds at 24h post mortem

Variables	Breed of chickens			SEM	p-value
	Khiew-Phalee	Pradu Hang Dam	Broiler		
Breast					
Meat color (CIE)					
Lightness (L*)	67.20±3.12^{a}	62.35±2.97^{b}	65.88±1.24^{a}	3.34	0.01
Redness (a*)	4.80±0.53^{b}	5.61±0.77^{a}	4.57±0.18^{b}	0.71	0.01
Yellowness (b*)	12.76±0.78^{b}	12.14±1.49^{b}	15.12±1.09^{a}	1.5	0.001
pH value (24 h)	5.69±0.08^{b}	5.60±0.06^{b}	6.05±0.18^{a}	0.16	<0.001
Drip loss (%)	2.62±0.30^{b}	3.50±0.22^{a}	1.08±0.05^{c}	0.28	<0.001
Cooking loss (%)	20.40±0.64^{b}	28.08±0.42^{a}	18.06±0.73^{c}	0.77	<0.001
Shear force (N)	36.07±3.18^{a}	35.81±3.27^{a}	18.53±0.74^{b}	3.44	<0.001
Thigh					
Meat color (CIE)					
Lightness (L*)	63.17±2.15	60.66±2.27	59.86±1.12	4.54	0.13
Redness (a*)	5.52±0.40^{b}	5.75±0.37^{ab}	6.27±0.56^{a}	0.58	0.03
Yellowness (b*)	13.75±1.67^{b}	12.51±0.42^{b}	17.00±1.13^{a}	1.53	<0.001
pH value (24 h)	5.96±0.06^{b}	5.76±0.03^{c}	6.16±0.22^{a}	0.18	<0.001
Drip loss (%)	1.13±0.09	1.16±0.08	1.29±0.19	0.17	0.09
Cooking loss (%)	30.33±0.46^{a}	30.40±0.57^{a}	22.95±1.09^{b}	0.98	<0.001
Shear force (N)	34.59±8.26^{a}	36.65±2.80^{a}	18.19±1.44^{b}	6.58	<0.001

Note: $^{a, b, c}$ Mean in the same row with different superscripts differ significantly ($p<0.05$)

Table 3. The chemical composition in breast muscle samples obtained from different chicken breeds (mean±SD)

Variables	Breed of chickens			p-value
	Khiew-Phalee	Pradu Hang Dam	Broiler	
Moisture (%)	73.94±0.10^{b}	73.92±0.94^{b}	74.98±0.22^{a}	0.007
Protein (%)	24.67±0.11^{a}	24.18±0.79^{a}	20.60±0.22^{b}	<0.001
Fat (%)	0.19±0.01^{c}	0.35±0.03^{b}	3.81±0.04^{a}	<0.001
Ash (%)	1.40±0.03^{a}	1.38±0.06^{a}	1.23±0.03^{b}	<0.001
Gross energy (GE, kcal/kg)	1,417.73±11.55^{b}	1,432.76±50.01^{b}	1,481.02±12.17^{a}	0.007

Note: $^{a, b, c}$ Mean in the same row with different superscripts differ significantly ($p<0.05$). $^{*/}$ Chemical composition values in the form of a percentage of fresh weight (% as feed basis).

breast meat of CBR was the highest, followed by PHD and KP, respectively (Table 3). The ash content in breast meat was higher in KP and PHD compared to CBR, at 1.40% and 1.38%, respectively, compared to 1.23%. This difference was statistically significant ($p<0.05$) (Table 3). The gross energy in breast meat of CBR was higher than PHD and KP, respectively (Table 3).

Purine content and derivatives. Purine contents (i.e., total purine, adenine, guanine, hypoxanthine, and xanthine) of different chicken breeds were determined and are summarized in Table 4. Xanthine was not detectable in all three chicken breeds' meat samples. The breast muscles of PHD, KP, and CRB chickens were shown the hypoxanthine contents as 43.71±3.73, 47.96±12.87, and 52.08±5.94 mg/100 g sample, respectively. Adenosine and guanosine were found to increase in KP and PHD more than CRB chicken. Our results showed total purine content, calculated as uric acid content in different chicken breeds, with non-significant differences ($p>0.05$).

Total lipid content, cholesterol content, and fatty acids profile. Table 5 shows the means of total lipid content (%) and total cholesterol concentrations (mg/100 g) of chicken breasts in three different breeds. There were highly significant differences in total lipid content (%) among three different breeds ($p<0.001$). Total lipid content (%) was higher in BRC than in PHD and KP chicken meat, respectively. The cholesterol concentrations showed non-significant differences when compared with three different chicken breeds ($p=0.103$).

The fatty acids profile (mg/100 g) in breast muscle samples obtained from different chicken breeds are presented in Table 6. As shown in Table 6, fatty acids that were detected in three chicken breeds in breasts differed significantly ($p\leq0.05$). The saturated fatty acids (SFA) of three chicken breeds in breasts showed highly significant differences in the content of palmitic acid (C16:0), stearic acid (C18:0), and myristic acid (C14:0). The highest concentration of palmitic acid (C16:0), stearic acid (C18:0), and myristic acid (C14:0) were found in CBR and lower in KP and PDH ($p<0.001$). However, the concentrations of palmitic acid, stearic acid, and myristic acid in KP and PDH were not different. The content of alpha linolenic acid (C18:3 n-3) was found only in CBR and lauric acid (C12:0) was found only in PHD. Conversely, the concentration of unsaturated fatty acids (USFA) was found in CBR, KP, and PHD and showed highly significant differences in the contents of palmitoleic acid (C16:1), oleic acid (C-18:1), linoleic acid (C18:2n-6) and erucic acid (C22:2). The highest concentrations of palmitoleic acid, oleic acid, linoleic acid and erucic acid were found in CBR and lower in KP and PDH ($p<0.001$). The fatty acid values obtained in the experiment are described in Table 6.

Protein profile analysis. SDS-PAGE analysis of proteins extracted from chicken breast at 20 mg/mL concentration from three different breeds revealed distinct results. At approximately 110 kDa, protein density in CBR meat was notably lower than that in KP and PHD. Additionally, at around 70 kDa, two bands showed higher protein density in CBR meat compared to KP and PHD (Figure 2). Further analysis is required to identify the protein types at 110 kDa and 70 kDa (Table 7). The 110 kDa protein band in Figure 3A was identified as pyruvate kinase PKM, which is involved in the glycolysis process and leads to the production of lactic acid as a final product. The upper protein band at 70 kDa in Figure 3B was identified as heat shock 70 kDa protein 8, related to heat stress, with higher levels observed in the CBR than KP and PHD. The lower protein band at 70 kDa (Figure 3C) was identified as albumin OS, associated with high-energy foods, found at higher CBR levels than KP and PHD.

Table 4. Purine compounds in breast muscle samples obtained from different chicken breeds (mean±SD)

Chicken breeds	Purine compounds (mg/100 g sample (WB))					Classified group#
	Adenine	Guanine	Hypoxanthine	Total purine	Calculated as Uric acid *	
KP	18.14±1.36	15.67±3.10	47.96±12.87	81.77±16.13	99.23±19.65	2
PHD	18.68±1.38	15.36±2.34	43.71±3.73	77.75±7.02	94.31±8.41	2
CBR	16.19±1.57	13.18±2.33	52.08±5.94	81.51±9.23	99.19±11.14	2
P-value	0.120	0.090	0.290	0.930	0.900	

Note: KP = Khiew-Phalee, PHD = Pradu Hang Dam, CBR = commercial broiler chicken, WB = wet basis.
* Calculated as Uric acid (mg/100 g)= (MW. Uric acid (168.1 g/mol) × total purines (μmol/100 g)) / 1,000.
# Classification according to purine content; 1: the very low group: less than 50 mg/100 g (less than 350 μmol/100 g), 2: the low group: 50-100 mg/100 g (350-700 μmol/100 g), 3: the moderate group: 100-200 mg/100 g (700-1400 μmol/100 g), 4: the high group: 200-300 mg/100 g (1400-2050 μmol/100 g), 5: the very high group: more than 300 mg/100 g (more than 2050 μmol/100 g).

Table 5. The total lipid content (%) and total cholesterol concentrations (mg/100 g) in breast muscle samples obtained from different chicken breeds (mean±SD)

Variables	Breed of chickens			p-value
	KP	PHD	CBR	
Total lipid content (%)	0.19±0.01^{c}	0.35±0.03^{b}	3.81±0.04^{a}	<0.001
Total cholesterol concentrations (mg/100g)	54.66±1.15	53.71±0.62	53.48±0.50	0.103

Note: $^{a, b, c}$ Mean in the same row with different superscripts differ significantly ($p<0.05$). KP = Khiew-Phalee, PHD = Pradu Hang Dam, CBR = commercial broiler chicken.

Table 6. The fatty acids profile (mg/100 g) in breast muscle samples obtained from different chicken breeds (mean±SD)

Fatty acids	Breed of chickens			p-value
	KP	PHD	CBR	
Caproic acid (C6:0)	-	-	-	
Caprylic acid (C8:0)	-	-	-	
Capric acid (C10:0)	-	-	-	
Lauric acid (C12:0)	-	40.45±2.47	-	
Myristic acid (C14:0)	10.13±0.76^{b}	19.97±0.49^{a}	20.03±0.50^{a}	0.002
Myristoleic acid (C14:0)	-	-	-	
Palmitic acid (C16:0)	140.32±0.97^{b}	180.38±1.45^{b}	620.38±1.04^{a}	<0.001
Palmitoleic acid (C16:1)	10.08±0.57^{b}	10.11±0.63^{b}	120.22± 0.82^{a}	0.003
Stearic acid (C18:0)	70.14±1.25^{c}	110.08±1.17^{b}	180.32±1.53^{a}	<0.001
Oleic acid (C18:1)	110.38±2.02^{b}	180.44±2.05^{b}	1,080.35±1.86^{a}	0.001
Linoleic acid (C18:2)	40.37±2.45^{b}	30.42±2.38^{b}	370.42±2.52^{a}	0.003
Alpha linolenic acid (C18:0)	-	-	20.67±3.08	
Arachidic acid (C20:0)	-	-	-	
Eicosadienoic acid (cis-C20:2(n-6))	-	-	-	
Dihomo-gamma-linolenic acid (cis-C20:3(n-6))	-	-	-	
Eicosatrienoic acid (cis-C20:3(n-7))	-	-	-	
Arachidonic acid (C20:0)	-	-	-	
Eicosapentaenoic acid (C20:5)	-	-	-	
Behenic acid (C22:0)	-	-	-	
Erucic acid (C22:1)	10.41±2.53^{b}	5.20±2.07^{b}	50.67±3.08^{a}	0.009
Docosadienoic acid (cis-C22:2(n-6))	-	-	-	
Docosahexaenoic acid (C22:6(n−3))	-	-	-	
Lignoceric acid (C24:0)	-	-	-	
Nervonic acid (C24:1)	-	-	-	

Note: $^{a, b, c}$ different letters represent a mean difference of p<0.05. KP = Khiew-Phalee, PHD = Pradu Hang Dam, CBR = commercial broiler chicken.

Table 7. Top five of three protein bands identified by LC-MS/MS and separated by SDS-PAGE in breast muscle samples obtained from different chicken breeds

Band name	Gene symbol	Full protein names	Uniprot ID	Mascot score	Coverage (%)	MW experimental (Da)	MW theoretical (Da)
A	PKM	Pyruvate Kinase	P00548	1144	55	110,000	58,015
	PGM1	Phosphoglucomutase1	F1NN63	942	42	110,000	61,549
	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	P00356	590	45	110,000	35,704
	LOC107050559	Fructose-bisphosphate aldolase	A0A8V0X091	489	46	110,000	32,558
	PYGL	Alpha-1,4 glucan phosphorylase	A0A8V0ZAK2	347	13	110,000	98,272
B	HSPA8	Heat shock 70kDa protein 8	A0A8V1A5Z3	2002	43	71,000	68,961
	PKLR	Pyruvate kinase	A0A8V0ZGA1	529	38	71,000	66,626
	ALB	Albumin	A0A8V0XJ14	505	44	71,000	69,891
	LOC107050559	Fructose-bisphosphate aldolase	A0A8V0X091	299	30	71,000	32,558
	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	P00356	228	34	71,000	35,704
C	ALB	Albumin	A0A8V0XJ14	3075	57	69,000	69,891
	PGM1	Phosphoglucomutase 1	F1NN63	586	39	69,000	61,549
	HSPA8	Heat shock 70kDa protein 8	A0A1D5PFJ6	459	40	69,000	70,331
	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	P00356	452	45	69,000	35,704
	PIT 54	PIT 54	Q98TD1	437	28	69,000	50,822

DISCUSSION

The present experimental data indicate that the carcass weight of CBR is higher than that of KP and PHD because CBR is economically efficient and has a shorter rearing period of up to 8 weeks. The carcass weight of CBR was the highest at 2,701.88±327.28 g, followed by KP at 1,527.06±193.15 g and PHD at 1,238.92±28.68 g, respectively (Table 1.) They are raised with minimal feed and efficiently convert feed into meat (Craig *et al.*, 2016). The carcass proportion of KP and PHD is higher than the CBR because KP and PHD are considered relatively large Thai native chickens (Kanjak *et al.*, 2023; Yaemkong *et al.*, 2024). The breast weight in broilers is much greater than in KP and PHD. In the chicken industry, breeding companies have developed

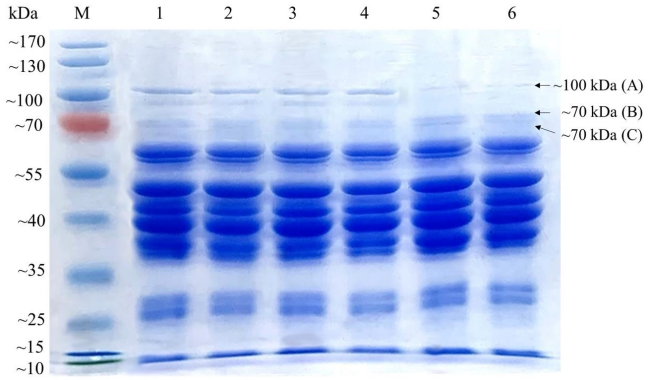

Figure 2. SDS-PAGE run on protein from chicken breast with a protein concentration of 20 mg/mL from 3 chicken breeds. The size of protein band A is ~100 kDa, protein band B is ~70 kDa (the upper band), and the size of protein band C is ~70 kDa (the lower band). M: Protein ladder (10 to 170 kDa), 1-2: Khiew-Phalee chickens 3-4: Pradu Hang Dam chickens 5-6: commercial broiler chicken.

fast-growing CBR strains to produce chicken meat that CBR grows under an intensive rearing regime. It is harvested at 5 wk with live weights of approximately 2.5 kg to provide high meat yields. Native chickens are reared in response to specific requests and are slow-growing. The native chickens have rearing times of approximately 14 to 15 wk and achieve live weights of 1.5 kg (Chumngoen & Tan, 2015). However, this study selected only male chickens because female chickens in the poultry industry are used to produce commercial eggs or hatcheries for chicken keepers.

The pH value in breast and thigh meat of KP and PHD was higher than CBR meat. Chuaynukool *et al.* (2007) reported that broiler breast meat had significantly higher pH values (6.23) compared to Thai native chicken samples (5.93). Chumngoen & Tan (2015) reported that commercial chicken breast meat had significantly higher pH values (5.90) compared to Taiwan native chicken breast meat (5.74). The study of Jaturasitha *et al.* (2004) proposed that the lower pH levels found in native chicken meat are caused by the more aggressive behavior of these chickens. They explained that the increased stress levels experienced by native chickens led to greater glycogen metabolism, which affected post-mortem glycolysis, resulting in lactic acid accumulation and lower pH values in the meat. Concerning redness value (a*), thigh meat exhibited higher overall redness values compared to breast meat, possibly due to a higher accumulation of myoglobin and different muscle structures (Mir *et al.*, 2017; Qamar *et al.*, 2019). The yellowness value (b*) of breast meat and thigh meat of CBR was found to be higher than that of native breeds. This is consistent with a study by Haunshi *et al*. (2022), which reported that higher chicken weight leads to higher fat accumulation, affecting the yellowness of the meat due to intramuscular fat. The drip loss percentage of KP and PHD in breast meat was higher than CBR. It can be affected by various factors, such as the stress-induced during slaughter, affecting the physical properties of the meat, particularly water (Mir

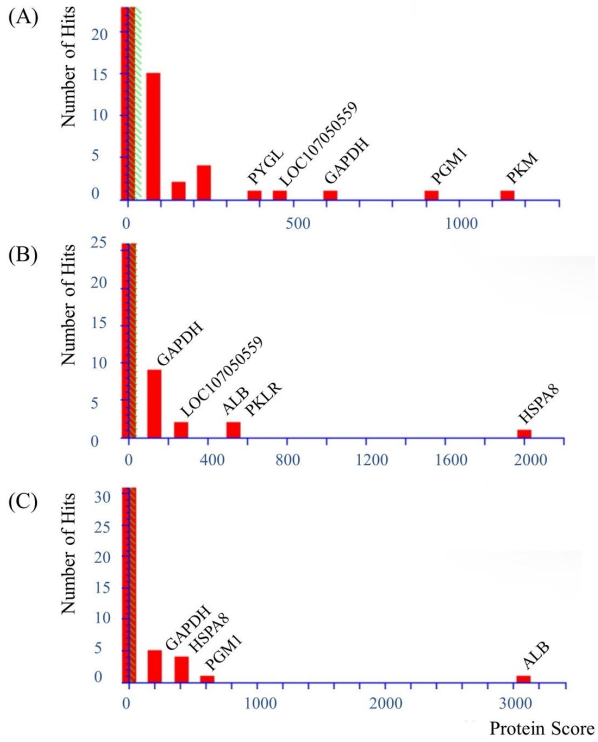

Figure 3. The protein score of protein bands separated by SDS-PAGE and identified by LC-MS/MS in samples of Longissimus thoracis muscle from Thai indigenous chicken. (A) Protein size ~100 kDa, (B) protein size ~70 kDa upper band, and (C) protein size ~70 kDa lower band. PKM (Pyruvate Kinase); PGM1 (Phosphoglucomutase1); GAPDH (Glyceraldehyde-3-phosphate dehydrogenase); LOC107050559 (Fructose-bisphosphate aldolase); PYGL (Alpha-1,4 glucan phosphorylase); HSPA8 (Heat shock 70kDa protein 8); PKLR (Pyruvate kinase) and ALB (Albumin).

et al., 2017). Cooking loss percentage in breast and thigh meat was higher in PHD compared to KP and CBR. This is consistent with the research that reported that native chicken meat has higher cooking loss than CBR meat. Shear force values differed in breast and thigh meat, with higher values in KP and PHD compared to CBR (Mussa *et al.*, 2022). The chicken breed influences the chemical composition of muscles and the effect of shear force. Additionally, shear force is related to the amount of connective tissue and collagen content in the muscles. Muscles that work harder and support more weight have more connective tissue, resulting in higher meat toughness (Jaturasitha *et al.*, 2016).

The protein content in the breast meat of CBR was lower than in KP and PHD. This is consistent with previous experiments that reported protein content in native chicken breast meat at approximately 23.93%-24.53% (Haunshi *et al.*, 2022; Uddin *et al.*, 2021). Native chickens have a high protein content of meat due to the diet and free-range management on a farm; muscles are made up of protein, and when they are worked, there is a gain in size and strength (Uddin *et al.*, 2021; Mussa *et al.*, 2022). The fat content in the breast meat of CBR was higher than in PHD and KP. This finding is consistent with reports which stated that the fat content in the breast meat of CBR is higher than that of native

Thai chickens, with statistically significant differences ($p<0.01$) (Jaturasitha *et al.,* 2016; Montebon *et al.,* 2023). However, KP, one breed of Thai native chicken, had significantly lower fat content in the breast meat compared to PHD, indicating that Thai native chickens have the advantage of possessing lower fat content in their meat compared to CBR. In general, native chickens had less fat content in the meat than boiler chickens because their fat was utilized as energy for unrestricted movements (Uddin *et al.,* 2021; Ali *et al.,* 2021; Montebon *et al.,* 2023). The gross energy in breast meat of CBR was higher than that of PHD and KP. The higher protein content and lower fat content in the breast meat of native chickens compared to CBR can be attributed to the free-range rearing system, which allows chickens to move and exercise, resulting in the increased metabolism of carbohydrates and fats accumulated in the body to provide energy to the muscles.

The analysis of purine compounds revealed that all three chicken breeds were similar to the report that the proportions of hypoxanthine to purine in chicken breast and thigh (weighing 1.55 kg, aged 5 weeks) were 63.53% and 59.18%, respectively (Molee *et al.,* 2022). Similarly, native PHD (weighing 1.51 kg, aged 16 weeks) had 62.76% and 56.48% proportions, respectively. Compared to this study's findings, the proportions of purine compounds in the breast meat of all three breeds were relatively similar, which might be due to the behavior of chickens related to purine accumulation, such as fighting, flying, or other movements involving various muscle groups. Purine compounds are compounds the body can synthesize at approximately 80%, while approximately 20% is obtained from food. The body maintains a balance of nucleotides through synthesis and breakdown mechanisms. Chicken meat contains derivatives of purine compounds, with the highest being hypoxanthine and adenine, which have been found to impact levels of uric acid more than the other phospholipid types (Bednarova *et al.,* 2014; Kaneko *et al.,* 2014; Tantiyasawasdikul *et al.*, 2023).

Cholesterol analysis revealed that all three breeds of chickens were similar. Normally, cholesterol is a substance that the body can synthesize on its own, with synthesis occurring mainly in the liver, intestines, and skin. It is often found in conjunction with circulating fatty acids in the body. The body typically balances cholesterol levels (Chaiwang *et al.,* 2023). Therefore, the cholesterol levels in the breast meat of the three chicken breeds were similar. The fatty acid analysis revealed that no fatty acid was higher in KP and PHD compared to CBR. It is well known that the accumulation of fatty acids in animal bodies is influenced by the types of fatty acids obtained from the diet (Ali *et al.,* 2021; Munyaneza *et al.*, 2024; Molee, 2022). Therefore, the results of this study demonstrate that CBR raised on dense feed have higher quantities of fatty acids than native chickens.

Research on the functional properties of chicken meat among KP, PHD, and CBR breeds revealed that the carcass quality of KP and PHD was similar in each part of the carcass but differed from CBR. The differences in each part of the carcass varied according to the characteristics of each breed. Regarding the chemical composition of chicken breast meat, the protein content in KP and PHD was higher than in CBR. Furthermore, the breast meat of KP and PHD had lower fat content than that of CBR.

Protein profile analysis identified three protein bands with different intensities: A 110 kDa protein in KP and PHD with higher intensity than the CBR, assumed to be protein Pyruvate kinase PKM, which is involved in the glycolysis process, leading to the production of lactic acid as a final product. This can impact the pH of the meat quality and increased acidity in animal meat can affect its water-holding capacity (Jaturasitha *et al.,* 2016). The experimental results stated that when animals die, glycogen in the muscle will be degraded and metabolized through glycolysis to pyruvate (Hong *et al.,* 2017). Pyruvate enters the dehydrogenase process to become lactate. Lactate causes a decrease in pH in chicken meat, affecting the quality of the meat. This is consistent with the research that showed that enzymes in the glycolysis process are related to the characteristics of chicken meat (Linyuan *et al.,* 2022). More acidic meat will affect the meat's ability to hold water (Rajan *et al.,* 2014), which aligns with the physical characteristics of breast meat in terms of having lower pH values in KP partridge and PHD compared to CBR and higher drip loss in KP and PHD compared to CBR. Lactic acid can also enhance the taste of chicken meat (Shohei, 2022), suggesting that KP and PHD may have better taste qualities. A 70 kDa upper band was identified as heat shock 70 kDa protein 8, a protein present in all animals and related to heat stress. According to the experiments, heat shock protein 70kD increases with increasing temperature and rapidly decreases pH during slaughter and after slaughter (Zabolim *et al.,* 2019). This is due to the conversion of glycogen to increase the accumulation of lactic acid at high muscle temperatures and the combination of high temperature with low pH that facilitates sarcoplasmic protein digestion. This causes water in the muscles to decrease, and a lower 70 kDa band is identified as Albumin OS associated with high-energy foods and the growth rate of broiler chicken (Adriani *et al.,* 2021).

The study of chicken meat protein profiles revealed differences, suggesting that KP and PHD meat may have better taste quality than the CBR breed. This quality could motivate consumers to choose chicken meat, considering factors such as color, aroma, taste, and drip loss. This information could be valuable for future animal husbandry planning to meet consumer preferences, offering new options for high-quality protein sources rich in essential nutrients.

CONCLUSION

The carcass properties of the KP and PHD thigh and drumstick were alike except for the cutting percentage. There are significant differences in meat quality traits between three different chicken breeds. The cholesterol, purine, and uric acid of breast chicken meat were not affected by breed. Importantly, KP and PHD Thai native chickens possess lower amounts of unhealthy fatty acids, which positively affect the

consumer and are anticipated to reduce the risk of many cardiovascular diseases. These findings can be used as reference data to promote the consumption of KP and PHD chicken meat and increase consumer awareness of healthier meat choices.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organization related to the material discussed in the manuscript.

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