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Fat Content, Fatty Acid Composition, and Nutritional Indices/Ratios of Balut from Itik-Pinas Mallard Ducks in the Philippines

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ABSTRACT

Fatty acid (FA) composition is the principal measure of the nutritional quality of fats in balut (i.e., boiled fertilized eggs from mallard ducks) that may affect human cardiovascular health. This study aimed to compare the fat content, FA composition, and nutritional indices/ratios concerning the edible components of 15-day old balut (B15d) and 18-day old balut (B18d) produced by Itik Pinas duck breeds (IP-Itim, IP-Khaki, and Kayumanggi-IP– an "IP-Khaki × IP-Itim" F1 cross) in the Philippines. A total of 275 pooled samples of the embryo, yolk, albumen, and fluid portion from 154 B15d and 175 B18d balut eggs were analyzed for fat content and FA composition by gas chromatography. Fat content was highest in the yolk (29.59%), followed by the embryo (1.63%) and negligible in both albumen and fluid portions. The major FAs with the highest proportion by weight of total FAs in the solid components of balut were oleic acid C18:1n-9 (20.7%–43.8%), palmitic acid C16:0 (12.0%–24.5%), stearic acid C18:0 (2.7%–8.9%), and linoleic acid C18:2n-6 (3.5%–6.9%). The fluid portion was dominated by arachidonic acid C20:4n-6 (18.8%), trans-vaccenic acid C18:1n-7 (17.6%), oleic acid (9.0%), and palmitic acid (8.3%). Total saturated FAs (SFA) were higher in the embryo than in the yolk. However, monounsaturated FAs (MUFA) and polyunsaturated FAs (PUFA) were higher in the yolk than in the embryo. Total SFA was higher, while total MUFA and PUFA were lower in B15d balut than those in B18d balut. Since SFAs are considered unhealthy compared to MUFAs and PUFAs, the yolk from B18d balut produced by Kayumanggi-IP ducks appears to have greater health benefits due to its lower atherosclerotic and thrombotic potential and higher health-promoting index and hypocholesterolemic/ hypercholesterolemic ratio.

Keywords: balut; fatty acids; nutritional indices/ratios

INTRODUCTION

Balut is a popular Filipino food made from boiled incubated eggs from mallard ducks (*Anas platyrynchos*). The technical characteristics of balut have not been reported until recently (Bondoc *et al.* (2022), i.e., balut is comprised of the embryo (23.70%), yolk (32.54%), albumen (16.24%), fluid portion (9.87%) and shell (17.73%). In balut production, fertilized eggs are allowed to grow in the incubator until 15 days (B15d balut) or 18 days (B18d balut), corresponding to embryo stage No. 38 and 41, respectively, according to the HH embryonic staging system for ducks developed by Li *et al*. (2019). The younger B15d balut had a lower embryo weight compared to the older B18d balut. However, albumen weight and the amount of embryonic fluids were higher in B15d balut than in B18d balut (Bondoc *et al*., 2022).

Generally perceived to be rich in protein, balut may also contain fats that may have different nutritional qualities and effects on human cardiovascular health. By comparison, the fat and protein content of the yolk in fresh (unfertilized) eggs from mallard ducks was 31.8% and 16.1%, respectively (Bondoc *et al.*, 2023). The albumen from fresh mallard duck eggs contains 8%-12% protein and less than one percent fat (unpublished data). Unfortunately, local studies to assess the nutritional and/or medicinal values of the edible components of balut (i.e., embryo, yolk, albumen, and fluid portion) balut are uncommon. Information on the fat (and protein) content and fatty acid (FA) composition (Chen & Liu, 2020) may contribute to a better understanding of the nutritional value of balut as a functional food, which in turn, may be used to justify the genetic conservation of local mallard breeds and improve balut properties according to consumer preference.

In this regard, this study evaluated the fat (and protein) content, fatty acid (FA) profile, and corresponding nutritional indices/ratios of the edible components of B15d and B18d balut. These were then compared among the Itik Pinas mallard breeds (IP-Itim, IP-Khaki, and Kayumanggi-IP).

MATERIALS AND METHODS

Data

A total of 329 balut eggs (154 B15d balut and 175 B18d balut) were produced in seven batches from three Itik Pinas (IP) mallard breeds (IP-Itim, IP-Khaki, and Kayumanggi-IP) developed by the National Swine and Poultry Research and Development Center (NSPRDC), Bureau of Animal Industry - Department of Agriculture (BAI-DA), in Tiaong, Quezon, Philippines (Table 1). This study was approved by the Institutional Animal Care and Use Committee of the College of Veterinary Medicine, University of the Philippines Los Baños, Laguna, Philippines (approval number 2019-0034).

The brown-feathered Kayumanggi-IP are F1 crossbred progenies of brown-feathered IP-Khaki males crossed with black-feathered IP-Itim females (to allow feather sexing). At the time of the study, the available IP-Itim and IP-Khaki purebred flocks were about four times larger than the Kayumanggi-IP crosses. This was because Kayumanggi-IP ducks produced at NSPRDC were mostly promoted for use by local smallholder duck raisers as part of its livelihood support program.

The Itik Pinas ducks were raised in group pens by breed, each consisting of about 4 males and 20 females, and fed with the same duck layer mash. (The nutritional content of the duck layer mash was analyzed to contain 6.89% moisture, 18.90% crude protein, 3.84% crude fat, 4.15% crude fiber, 12.23% ash, 3.32% calcium, and 0.72% phosphorus.) Newly collected eggs were pencil-marked with the collection date, breed, and pen/line number and immediately placed in an artificial incubator. The incubated eggs in a batch were initially candled on day 7 to separate the infertile and fertilized eggs. The fertilized eggs were then allowed to grow in the incubator until 15 days old (i.e., B15d balut) or 18 days old (i.e., B18d balut). During the second candling on day 15, balut eggs of the same breed were divided into equal lots, i.e., about half were immediately evaluated as B15d balut. In contrast, the other half remained in the incubator for three more days and was evaluated as B18d balut. Depending on the number of balut produced in a batch of incubated eggs, the number of replications may not be the same between balut types and between breeds.

Balut eggs were boiled to 100 °C or above for 30 min and cooled prior to dissection. The edible components of each boiled balut were manually separated into the embryo, yolk, albumen, and fluid portion samples. The yolk refers to the solidified yolk and yolk sac membrane, while the hard solid-textured albumen is the solidified albumen and sub-embryonic fluid in the albumen sac. The fluid portion is embryonic fluid consisting of amniotic fluid and allantois fluid.

Each balut component extracted on the same day (batch) and belonging to the same Itik Pinas breed and balut type (i.e., B15d or B18d) from about 2–8 balut eggs were pooled and placed in 250 mL plastic bottles and immediately frozen at –20 °C until further analysis. A total of 275 pooled samples of the embryo, yolk, albumen, and fluid portion were analyzed for fat, protein, and moisture content, and FA composition.

Analysis of Fat (and Protein) Content

The fat content in the embryo and yolk of boiled balut (and the duck layer mash) was analyzed using the Soxhlet extraction method (AOAC, 2006). Negligible amounts of fat (less than one percent) were found in the fluid samples using the Mojonnier method (AOAC, 2016a). In addition, the protein percentage was analyzed for the embryo, yolk, and albumen using the Kjeldahl method (AOAC, 2016b).

Table 1. Number of samples and distribution of balut eggs and their edible components by type of balut and by breed of Itik Pinas (IP) mallard ducks

Items	Itik Pinas (IP) breeds			Total
	IP-Itim	IP-Khaki	Kayumanggi -IP	
No. of balut eggs				
B15d balut	56	83	15	154
B18d balut	66	91	18	175
Total balut eggs	122	174	33	329
No. of samples				
B15d balut				
Embryo	56	83	15	154
Yolk	56	83	15	154
Albumen	56	83	15	154
Fluid portion	56	83	15	154
Sub-total	224	332	60	616 (125)
B18d balut				
Embryo	66	91	18	175
Yolk	66	91	18	175
Albumen	66	91	18	175
Fluid portion	66	91	18	175
Sub-total	264	364	72	700 (150)
Total samples	488 (132)	696 (111)	132 (32)	1,316 (275)

Note: Numbers in parenthesis are the total number of pooled samples by breed and by type of balut.

Fatty Acid (FA) Analysis

Fat was extracted from the edible components of balut, and the fatty acid methyl esters (FAMEs) were prepared following the methods used by Bondoc *et al.* (2023) for the yolk of fresh eggs from various poultry species/breeds. A mixture of 3 mL 8% methanolic HCl solution, 1 mL n-hexane, and 3 mL distilled water was added to each pooled sample. These were placed in a screw-capped glass test tube and centrifuged for 5 min at 8000 rpm. The upper organic hexane layer was transferred into 2 mL amber gas chromatography (GC) vials and cleared with ultra-pure nitrogen gas for 20 s before storage in the refrigerator (–20 °C).

The FAMEs were quantified using a Shimadzu GC 2010 Plus capillary GC system with a flame ionization detector (FID) and AOC-20i autosampler (Shimadzu Corporation, Kyoto, Japan). An aliquot μL of the hexane phase was injected in split mode (50:1) onto a FAMEWax (USP G16) capillary column (30 m, 0.32 mm ID, and 0.25 μm film thickness, Restek Corporation, U.S.). The injector port and FID temperatures were initially set to 125 °C, then increased to 240 °C at 3 °C/min and maintained for 5 min. Hydrogen was used as a carrier gas at 40 mL/min, while nitrogen was used as a makeup gas at 30 mL/min. The FAME peaks were identified with known FAME standards.

Individual FAs were determined as a percentage of total identified FAs (g/100 g) for eight saturated fatty acids (SFAs) – lauric acid (C12:0), myristic acid (C14:0), pentadecylic acid (C15:0), palmitic acid (C16:0), margaric acid (C17:0), stearic acid (C18:0), arachidic acid (C20:0), and behenic acid (C22:0); six monounsaturated fatty acids (MUFAs) – myristoleic acid (C14:1 n-5), palmitoleic acid (C16:1 n-7), oleic acid (C18:1 n-9), transvaccenic acid (C18:1 n-7), eicosenoic acid (C20:1 n-11), and erucic acid (C22:1 n-9); and four polyunsaturated fatty acids (PUFAs) – conjugated linoleic acid or CLA (C18:2 c9tll), linoleic acid or LA (C18:2 n-6), α-linolenic acid or ALA (C18:3 n-3), arachidonic acid or AA (C20:4 n-6), and docosahexaenoic acid or DHA (C22:6 n-3).

Seven fatty acid - based nutritional indices/ratios with health implications (Chen & Liu, 2020) were calculated, including PUFA/SFA ratio, MUFA/SFA ratio, omega-6 FA to omega-3 FA (n-6/n-3) ratio, atherogenicity index (IA) and thrombogenicity index (IT) according to Ulbricht & Southgate (1991), health-promoting index (HPI) by Chen *et al*. (2004), and hypocholesterolemic/hypercholesterolemic (h/H) ratio by Mierlita (2018).

The equations for the nutritional indices/ratios were as follows:

PUFA/SFA ratio= (C18:2 c9tll + C18:2 n-6 + C18:3 n-3 + C20:4 n-6 + C22:6 n-3) / (C12:0 + C14:0 + C16:0 + C17:0 + C18:0 + C20:0 + C22:0)

MUFA/SFA ratio= (C14:1 n-5 + C16:1 n-7 + C18:1 n-9 + C18:1 n-7 + C20:1 n-11 + C22:1 n-9) / (C12:0 + C14:0 + C16:0 + C17:0 + C18:0 + C20:0 + C22:0);

n-6/n-3 ratio= (C18:2 n-6 + C20:4 n-6) / (C18:3 n-3 + C22:6 n-3)

IA= [C12:0 + (4 × C14:0) + C16:0] / (MUFA + PUFA)

IT= (C14:0 + C16:0 + C18:0) / [(0.5 × MUFA) + (0.5 × n-6 PUFA) + (3 × n-3) + (n-3 / n-6)]

HPI= (MUFA + PUFA) / [C12:0 + (4 × C14:0) + C16:0]

h/H ratio= (C18:1 n-9 + PUFA) / (C12:0 + C14:0 + C16:0).

Statistical Analysis

Differences in the weight of balut components and their fat (and protein) contents between B15d and B18d balut and between Itik-Pinas breeds were determined using ANOVA with duck hen age, egg weight, and balut component weight as covariates. Statistical significance was set at $p<0.05$.

The general least squares procedures for unbalanced data (SAS Ver. 9.2, 2009) in a factorial design were used to evaluate each FA according to the mathematical model as follows: $y_{ijklmno} = \mu + Bcomp_i + Btype_j (Bcomp_i) + (Breed_k \times Btype_j \times Bcomp_i) + Age_l + WtEgg_m + WtComp_n + e_{ijklmno}$ where $y_{ijklmno}$ is the proportion of the FA (g/100 g), μ is the overall mean, $Bcomp_i$ is the fixed effect of the i^{th} edible component in balut (i.e., embryo, yolk, albumen, and fluid portion), $Btype_j (Bcomp_i)$ is the fixed effect of the j^{th} type of balut (*i.e.*, B15d and B18d) nested within the i^{th} balut component, $(Breed \times Btype \times Bcomp)_{ijk}$ is the three-factor interaction effect of the k^{th} breed (IP-Itim, IP-Khaki, and Kayumanggi-IP), j^{th} balut type, and i^{th} balut component, Age_l is the l^{th} covariate effect of duck hen's age at lay (weeks), $WtEgg_m$ is the m^{th} covariate effect of egg weight (g), $WtComp_n$ is the n^{th} covariate effect of the weight of balut component (g), and $e_{ijklmno}$ is the error term assumed to be normally distributed with the variance of errors as constant across observations.

The least-square means and standard error were used to compare differences in FA composition between balut components and between B15d and B18d balut. The FA groups and FA-based nutritional indices/ratios were calculated based on the least-square means of the "Breed × Btype × Bcomp" interaction effects and used to compare between Itik Pinas breeds. Regression coefficients (no intercept model) were also determined for major FAs that were significantly associated with duck hen's age at lay, egg weight, and balut component weight.

RESULTS

Major FAs in the Edible Components of Balut

The four major FAs with the highest proportions by weight of total FAs in the solid edible components of balut were oleic acid C18:1n-9, palmitic acid C16:0, stearic acid C18:0, and linoleic acid C18:2n-6 – representing about 69.60%, 79.73%, and 38.99% of total FAs in the embryo, yolk, and albumen, respectively (Table 2). Except for C16:1n-7, several FAs which comprised less than one percent of total FAs in the solid components of balut included the SFAs (C12:0, C14:0, C15:0, C17:0, C20:0, and C22:0); MUFAs (C14:1n-5, C20:1n-11, and C22:1 n-9); and PUFAs (C18:3n-3, C20:4n-6, and C22:6n-3). Some FAs were not found in all balut components, such as C15:0, C14:1n-5, C18:1n-7, C20:4n-6, C22:6n-3 in the embryo; C18:1n-7 in the yolk; and C12:0, C15:0, C17:0,

C20:0, C22:0, C14:1n-5, C18:1n-7, C20:1n-11, C22:1 n-9; C18:3n-3, C20:4n-6, and C22:6n-3 in the albumen. In the fluid portion, the four major FAs were arachidonic acid C20:4n-6, trans-vaccenic acid C18:1n-7, oleic acid, and palmitic acid, representing about 53.75% of total FAs found in embryonic fluids. The conjugated linoleic acid C18:2 c9tll, which was reported to be an anticarcinogenic, antiatherogenic, anti-inflammatory, and weight-reducing substance found in milk fat (Rodriguez-Alcala *et al*., 2013; Cichosz *et al*., 2020) was not detected in balut.

The same major FAs were found in the duck layer feeds, although oleic acid in the feeds (26.86%) was lower than in the embryo and yolk but higher than in the albumen. Palmitic acid in the feeds (16.65%) was also lower than in the embryo and yolk but higher than in the albumen. Stearic acid in the embryo, yolk, and albumen were higher than in the feeds (2.13%). Linoleic acid which is synthesized in the embryo, yolk, and albumen, was lower than that fed linoleic acid (38.95%), which is metabolized.

By comparison, the same major FAs found in balut were also reported, albeit in varying proportions, in the yolk of fresh eggs from various breeds of chickens, mallard, quail, Muscovy, Guinea fowl, turkey, and ostrich (Bondoc *et al*., 2023).

Factors Affecting FA Composition in Balut

Among the major fatty acids in balut, stearic acid C18:0 was the most variable, with a coefficient of variation (CV) of 28.78%, followed by oleic acid C18:1n-9 (CV= 25.78%), linoleic acid C18:2n-6 (CV= 25.37%), and palmitic acid C16:0 (CV= 23.10%). The proportion of each major FAs was significantly affected ($p<0.01$) by the edible component of balut (embryo, yolk, albumen, and fluid portion), type of balut (B15d vs. B18d balut), and Itik Pinas duck breed (IP-Itim, IP-Khaki, and Kayumanggi-IP), are presented in Table 3.

The proportion of C18:1n-9, a MUFA, was the highest in the yolk (43.82%), embryo (32.10%), albumen (20.72%), and the lowest in the fluid portion (9.01%). C16:0 was similar in the embryo (24.29%) and yolk (24.47%) of balut, which were higher than that in the albumen (12.02%) and fluid portion (8.33%). C18:0 was the highest in the embryo (8.91%), followed by the yolk (4.54%), fluid portion (3.93%), and albumen (2.71%). Linoleic acid C18:2n-6 was higher in the yolk (6.90%) than in the embryo (4.30%) and albumen (3.54%) but not found in the fluid portion (Table 2).

Oleic acid in balut was lower in older duck hens (i.e., lower by 0.07% per additional week of age at lay). It increased with the weight of the edible component (i.e., higher by 0.20% for every gram increase in component weight). Palmitic acid and stearic acid were also lower in older duck hens (i.e., lower by 0.15% and 0.09%, respectively, per additional week of age). Linoleic acid was higher in older duck hens (i.e., higher by 0.03% per additional week of age), increases with component weight (i.e., higher by 0.06% for every gram increase in component weight), but decreases with egg weight (i.e.,

Table 2. Least square means of moisture, protein, and fat content, and proportion of fatty acid (g/100 g of total fatty acids) for the edible components of balut from Itik Pinas ducks

Variables	Edible components of balut			
	Embryo	Yolk	Albumen	Fluid
Moisture content, %	88.33 ± 3.02^{a}	52.92 ± 2.42^{c}	63.42 ± 2.60^{b}	n.a.
Protein content, %	8.71 ± 2.24^{c}	14.00 ± 1.18^{b}	31.57 ± 2.22^{a}	n.a.
Fat content, %	1.63 ± 0.38^{b}	29.59 ± 1.64^{a}	n.a.	n.a.
Saturated FAs, %				
C12:0	0.10 ± 0.00	0.11 ± 0.00	n.d.	n.d.
C14:0	0.61 ± 0.02^{c}	0.83 ± 0.02^{b}	0.55 ± 0.03^{d}	2.17 ± 0.06^{a}
C15:0	n.d.	0.03 ± 0.00	n.d.	n.d.
C16:0	24.29 ± 0.29^{a}	24.47 ± 0.43^{a}	12.02 ± 0.31^{b}	8.33 ± 0.41^{c}
C17:0	0.13 ± 0.00^{a}	0.08 ± 0.00^{b}	n.d.	n.d.
C18:0	8.91 ± 0.11^{a}	4.54 ± 0.16^{b}	2.71 ± 0.11^{d}	3.93 ± 0.15^{c}
C20:0	0.14 ± 0.00^{c}	0.17 ± 0.01^{b}	n.d.	1.41 ± 0.03^{a}
C22:0	0.14 ± 0.00^{a}	0.03 ± 0.00^{b}	n.d.	n.d.
Monounsaturated FAs, %				
C14:1 n-5	n.d.	0.08 ± 0.00	n.d.	n.d.
C16:1 n-7	1.29 ± 0.03^{b}	2.57 ± 0.04^{a}	1.23 ± 0.07^{b}	1.37 ± 0.10^{b}
C18:1 n-9	32.10 ± 0.50^{b}	43.82 ± 0.73^{a}	20.72 ± 0.52^{c}	9.01 ± 0.68^{d}
C18:1 n-7	n.d.	n.d.	n.d.	17.57 ± 1.35^{a}
C20:1 n-11	0.29 ± 0.00^{a}	0.03 ± 0.00^{c}	n.d.	0.17± 0.01^{b}
C22:1 n-9	0.21 ± 0.01^{a}	0.02 ± 0.01^{b}	n.d.	n.d.
Polyunsaturated FAs, %				
C18:2 n-6, LA	4.30 ± 0.08^{b}	6.90 ± 0.12^{a}	3.54 ± 0.11^{c}	n.d.
C18:3 n-3, ALA	0.47 ± 0.00^{a}	0.37 ± 0.00^{b}	n.d.	n.d.
C20:4 n-6, AA	n.d.	0.81 ± 0.05^{b}	n.d.	18.84 ± 1.54^{a}
C22:6 n-3, DHA	n.d.	0.35 ± 0.01	n.d.	n.d.

Note: Means in the same row with different superscripts differ significantly ($p<0.05$). n.a.= Not analyzed; n.d.= Not detected.

lower by 0.02% per every gram increase in egg weight). The weight of the egg and edible components of balut were not related to the proportion of palmitic acid and stearic acid.

DISCUSSION

Comparisons between B15d and B18d Balut

Edible components of balut. Consumer preferences for a larger yolk, smaller embryo, and an ample amount of the fluid portion (except the smaller albumen) mainly corresponded to the compositional characteristics of B15d balut. That is, the embryo was smaller in B15d balut (8.0 g) than in B18d balut (21.6 g). More embryonic fluids were obtained from B15d balut (8.2 mL) than from B18d balut (4.4 mL). The yolk in B15d balut (21.7 g) was slightly bigger than in B18d balut (20.1 g). However, the albumen was larger in B15d balut (13.8 g) than in B18 balut (6.5 g) (Table 4).

Fat (and protein and moisture) content. In general, yolk fat in balut was about 15 times higher than fat in the embryo (Table 2). This is because the yolk is the only source of lipids for embryo tissue growth (Sahan *et al.*, 2014). By contrast, the amount of fats in the albumen and fluid portion of balut was negligible. The differences in fat content between B15d and B18d balut for the embryo (1.43% vs 1.92%) and yolk (29.74% vs 28.97%) were small (less than one percent) (Table 4).

The protein content of balut was usually highest in the albumen, which is about 1.3 times higher than proteins in the yolk or about 3.4 times higher than proteins found in the embryo (Table 3). This is because the albumen is the main source of proteins for tissue synthesis in the developing embryo (Willems *et al.*, 2014). Although protein content in the fluid portion of balut was not determined in this study, the embryonic fluids, especially the allantoic fluid, had been reported to contain not only wastes that result from the embryo's metabolism but also proteins involved in lipid, vitamin metabolisms, and metal ion transport (Da Silva *et al.*, 2017). Table 4 shows that proteins in the embryo were lower in B15d balut than in B18d balut (8.06% vs. 10.32%). Protein percentage in the yolk was similar in B15d and B18d balut (24.34% vs. 24.60%). In contrast, the amount of protein in the albumen was higher in B15d balut than in B18d balut (32.18% vs. 30.07%).

Moisture percentage was highest in the embryo, about 1.7 times higher than in the yolk and 1.4 times higher than in the albumen (Table 2). The decrease in moisture content in the albumen is due to the continuous removal of water into the yolk to form sub-embryonic fluid. The water loss in the yolk can be attributed to the excretion of water into the allantois as the yolk decreases in size on the 18th day of incubation (Li *et al.*,

Table 3. Mean square F tests for the effects of balut component, balut type (within each balut component), breed × type × component interaction, and covariate effects of age at lay, egg weight, and component weight on the proportion of fatty acids in balut

Fatty acids	Balut compo-nent	Balut type within component	Breed × Type × Component	Age at lay	Egg weight	Com-ponent weight	CV (%)
Saturated FAs							
C12:0	**	ns	**	**	ns	ns	22.35
C14:0	**	ns	**	**	ns	ns	32.32
C15:0	**	ns	**	**	ns	ns	15.23
C16:0	**	**	**	** b =-0.15	ns	ns	23.1
C17:0	**	*	**	*	ns	*	49.81
C18:0	**	**	**	** b =-0.09	ns	ns	28.78
C20:0	**	ns	**	ns	*	ns	51.01
C22:0	**	**	**	**	ns	ns	39.66
Monounsaturated FAs							
C14:1 n-5	**	*	**	**	ns	ns	13.97
C16:1 n-7	**	**	ns	*	ns	**	25.72
C18:1 n-9	**	**	**	* b =-0.07	ns	** b =0.20	25.78
C18:1 n-7	**	**	*	**	ns	*	96.6
C20:1 n-11	**	**	**	**	ns	**	33.17
C22:1 n-9	**	ns	**	ns	*	ns	93.95
Polyunsaturated FAs							
C18:2 n-6	**	**	**	** b =0.03	** b =-0.02	** b =0.06	25.37
C18:3 n-3	**	*	*	**	ns	**	12.18
C20:4 n-6	**	ns	**	**	**	**	>100
C22:6 n-3	**	**	**	*	ns	ns	34.53

Note: ns= No significant differences (p>0.05); * = Significant differences (p<0.05); ** = Highly significant differences (p<0.01); b= Regression coefficient; CV= Coefficient of variation. Numbers in the covariate columns are regression coefficients for the major FAs.

2019). Moisture content was higher in B15d balut than in B18d balut, especially for the embryo (89.27% vs. 86.39%) and yolk (54.08% vs. 52.20%). However, moisture content in the albumen was lower in B15d balut than in B18d balut (62.62% vs. 65.10%) (Table 4).

Major FAs. For the major SFAs, C16:0 in the embryo, yolk, and fluid portion of B15d balut was like those of B18d balut. However, C18:0 in the embryo, yolk, and albumen of B15d balut was significantly higher than those in B18d balut. Meanwhile, the major unsaturated FAs (C18:1n-9 and C18:2n-6) in the embryo and yolk in the B18d balut were slightly higher than those in the B15d balut. C18:2n-6 in the albumen and fluid portion of B15d balut were significantly higher than those in B18d balut (Table 4).

Fatty acid groups. Total SFA in the different components of B15d balut were higher than those in B18d balut (Table 5). Total SFA in B15d balut was the highest in the embryo (35.08%), followed by the yolk (30.41%), albumen (17.59%), and embryonic fluids (15.38%).

Total MUFA in the edible components (except albumen) of B18d balut were higher than those in B15d balut. Total MUFA in B15d balut was the highest in the yolk (47.73%), followed by the embryo (35.36%) and embryonic fluids (31.70%).

Total PUFA in the embryo and yolk of B18d balut were higher than that in B15d balut. On the other hand, total PUFA in the albumen and fluid portion of B15d balut was higher than in B18d balut. Total PUFA in B18d balut was the highest in the yolk of balut (7.91%), which was about 1.52 times, 2.46 times, and 5.57 times higher than in the embryo, albumen, and embryonic fluids, respectively.

Omega-3 fatty acids or n-3 PUFAs (i.e., α-linolenic acid C18:3n-3 and docosahexaenoic acid or C22:6n-3) in the embryo and yolk of B18d balut were slightly higher compared to that in B15d balut. Omega-3 fatty acids in B18d balut were higher in the yolk (0.75%), about 1.56 times higher than in the embryo. C18:3n-3 and C22:6n-3 were not detected in the albumen and fluid samples. The n-3 PUFAs are known to help prevent atherosclerosis cardiovascular disease through multiple mechanisms, including lowering plasma triglyceride levels, anti-inflammatory effects, antithrombotic effects, and effects on endothelial function (Wu *et al*., 2020).

Omega-6 fatty acids or n-6 PUFAs (i.e., linoleic acid C18:2n-6 and arachidonic acid C20:4n-6) in the embryo and yolk of B18d balut were slightly lower than those in

Table 4. Least square means of protein and fat content, and proportion of major fatty acids (g/100 g of total fatty acids) in the edible components of B15d and B18d balut from different Itik Pinas (IP) mallard breeds

Variables	B15d balut			B18d balut		
	IP-Itim	IP-Khaki	Kayumanggi-IP	IP-Itim	IP-Khaki	Kayumanggi-IP
Embryo						
Protein content, %	7.82 ± 0.44^{c}	6.13 ± 0.35^{d}	10.24 ± 0.60^{ab}	10.10 ± 0.34^{b}	9.76 ± 0.36^{b}	11.11 ± 0.56^{a}
Fat content, %	1.47 ± 0.07^{c}	1.83 ± 0.06^{b}	1.32 ± 0.06^{d}	1.75 ± 0.06^{b}	1.51 ± 0.10^{c}	2.16 ± 0.09^{a}
C16:0	24.09 ± 0.60^{ab}	24.72 ± 0.48^{a}	25.32 ± 1.06^{a}	24.00 ± 0.60^{ab}	23.14 ± 0.61^{b}	24.50 ± 1.00^{ab}
C18:0	10.75 ± 0.22^{a}	9.72 ± 0.18^{b}	8.92 ± 0.39^{c}	8.70 ± 0.22^{c}	7.88 ± 0.22^{d}	7.48 ± 0.37^{d}
C18:1 n-9	28.73 ± 1.01^{c}	29.93 ± 0.82^{c}	33.19 ± 1.78^{ab}	33.29 ± 1.02^{ab}	31.78 ± 1.03^{b}	35.66 ± 1.69^{a}
C18:2 n-6	3.48 ± 0.19^{d}	3.77 ± 0.16^{d}	5.30 ± 0.29^{a}	4.72 ±0.17 ^{b}	4.18 ± 0.17^{c}	5.30 ± 0.29^{a}
Yolk						
Protein content, %	12.33 ± 0.16^{c}	12.68 ± 0.13^{b}	12.96 ± 0.29^{b}	15.25 ± 0.14^{a}	15.21 ± 0.13^{a}	15.18 ± 0.28^{a}
Fat content, %	30.11 ± 0.22^{a}	30.25 ± 0.18^{a}	30.12 ± 0.18^{a}	28.63 ± 0.18^{b}	29.00 ± 0.41^{b}	28.02 ± 0.40^{c}
C16:0	24.20 ± 0.56	24.87 ± 0.55	24.45 ± 1.11	24.20 ± 0.56	25.25 ± 0.49	24.35 ± 1.01
C18:0	4.68 ± 0.25^{a}	4.19 ± 0.41^{ab}	4.19 ± 0.41^{ab}	4.56 ± 0.21^{a}	4.32 ± 0.18^{ab}	3.83 ± 0.37^{b}
C18:1 n-9	42.96 ± 1.13^{a}	40.35 ± 0.93^{b}	44.80 ± 1.87^{a}	44.56 ± 0.95^{a}	45.04 ± 0.83^{a}	45.21 ± 1.71^{a}
C18:2 n-6	6.59 ± 0.19^{b}	6.00 ± 0.15^{b}	7.30 ± 0.33^{a}	7.19 ± 0.16^{a}	6.93 ± 0.14^{a}	7.37 ± 0.30^{a}
Albumen						
Protein content, %	32.85 ± 0.43^{a}	33.37 ± 0.34^{a}	30.32 ± 0.60^{b}	30.91 ± 0.32^{b}	30.38 ± 0.32^{b}	28.93 ± 0.56^{c}
Fat content, %	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
C16:0	11.59 ± 0.54^{c}	15.79 ± 0.45^{a}	14.01 ± 1.04^{b}	11.51 ± 0.55^{c}	12.64 ± 0.52^{bc}	6.56 ± 0.99^{d}
C18:0	2.96 ± 0.20^{b}	3.68 ± 0.16^{a}	1.15 ± 0.36^{c}	2.74 ± 0.21^{b}	2.64 ± 0.19^{b}	1.15 ± 0.36^{c}
C18:1 n-9	18.47 ± 0.91^{c}	24.91 ± 0.75^{a}	24.72 ± 1.76^{a}	21.17 ± 0.94^{b}	21.86 ± 0.87^{b}	13.19 ± 1.67^{d}
C18:2 n-6	2.96 ± 0.16^{c}	4.34 ± 0.13^{a}	4.27 ± 0.31^{a}	3.52 ± 0.18^{b}	3.70 ± 0.18^{b}	2.44 ± 0.31^{d}
Fluid portion						
C16:0	7.55 ± 0.58^{b}	9.76 ± 0.48^{a}	6.73 ± 1.06^{b}	23.99 ± 2.91^{a}	26.31 ± 1.78^{a}	16.46 ± 3.06^{b}
C18:0	3.57 ± 0.22^{b}	4.42 ± 0.18^{a}	3.39 ± 0.39^{b}	n.d.	0.88 ± 0.57^{c}	1.96 ± 0.56^{bc}
C18:1 n-9	8.48 ± 0.97^{b}	10.54 ± 0.82^{a}	6.72 ± 1.80^{b}	17.63 ± 1.58^{b}	15.18 ± 1.58^{b}	23.72 ± 2.25^{a}
C18:1 n-7	11.36 ± 1.29^{c}	12.31 ± 1.13^{bc}	14.98 ± 1.93^{b}	8.05 ± 0.62^{b}	7.62 ± 0.57^{b}	10.30 ± 1.01^{a}
C18:2 n-6	2.43 ± 0.29^{b}	4.01 ± 0.33^{a}	2.94 ± 0.86^{b}	3.65 ± 0.23^{b}	3.58 ± 0.22^{b}	4.98 ± 0.37^{a}
C20:4 n-6	7.55 ± 0.58^{b}	9.76 ± 0.48^{a}	6.73 ± 1.06^{b}	8.87 ± 1.04^{a}	9.14 ± 0.96^{a}	10.35 ± 1.71^{a}

Note: Means in the same row with different superscripts differ significantly ($p<0.05$). n.a.= Not analyzed; n.d.= Not detected.

Table 5. Fatty acid-based nutritional indices/ratios for the edible components of B15d and B18d balut from different Itik Pinas (IP) mallard breeds

Nutritional indices/ratios	B15d balut			B18d balut		
	IP-Itim	IP-Khaki	Kayumanggi-IP	IP-Itim	IP-Khaki	Kayumanggi-IP
Embryo						
PUFA/SFA ratio	0.11	0.12	0.16	0.15	0.15	0.18
MUFA/SFA ratio	0.85	0.90	0.99	1.03	1.06	1.13
n-6/n-3 ratio	7.74	8.16	11.68	10.10	9.06	10.62
Atherogenicity index	0.77	0.75	0.68	0.67	0.67	0.62
Thrombogenicity index	1.92	1.81	1.62	1.56	1.55	1.42
Health promoting index	1.30	1.34	1.48	1.49	1.50	1.60
h/H ratio	1.32	1.35	1.50	1.55	1.53	1.65
Yolk						
PUFA/SFA ratio	0.24	0.22	0.27	0.26	0.25	0.28
MUFA/SFA ratio	1.51	1.42	1.58	1.56	1.55	1.63
n-6/n-3 ratio	11.10	8.41	10.15	9.14	10.07	9.44
Atherogenicity index	0.53	0.56	0.50	0.51	0.52	0.49
Thrombogenicity index	1.06	1.11	1.00	1.00	1.03	0.96
Health promoting index	1.89	1.87	2.00	1.97	1.94	2.04
h/H ratio	1.99	1.83	2.09	2.08	2.01	2.11
Albumen						
PUFA/SFA ratio	0.20	0.22	0.27	0.25	0.24	0.32
MUFA/SFA ratio	1.28	1.32	1.67	1.57	1.52	1.85
n-6/n-3 ratio	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Atherogenicity index	0.63	0.58	0.53	0.45	0.47	0.39
Thrombogenicity index	1.35	1.30	1.03	1.10	1.14	0.92
Health promoting index	1.60	1.73	1.88	2.24	2.13	2.54
h/H ratio	1.76	1.80	1.99	2.14	2.02	2.38
Fluid portion						
PUFA/SFA ratio	0.16	0.23	0.25	1.51	1.43	1.68
MUFA/SFA ratio	1.40	1.41	1.86	2.81	3.17	1.75
n-6/n-3 ratio	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Atherogenicity index	0.78	0.58	0.27	0.16	0.15	0.20
Thrombogenicity index	1.18	1.11	0.82	0.46	0.43	0.58
Health promoting index	1.28	1.73	3.66	6.27	6.76	5.10
h/H ratio	1.07	1.27	1.43	3.29	3.31	3.50

Note: SFA= saturated fatty acids; MUFA= monounsaturated fatty acids; PUFA= polyunsaturated fatty acids; n-3= Omega-3 fatty acids; n-6= Omega-6 fatty acids; h/H ratio= hypocholesterolemic/ hypercholesterolemic ratio; n.d.= Not detected.

B15d balut. In contrast, the Omega-6 fatty acids in the albumen and embryonic fluids of B18d balut were slightly higher than those in B15d balut. The omega-6 fatty acids in B18d balut were the highest in the yolk (7.16%), about 1.51 times, 2.22 times, and 5.04 times higher than in the embryo, albumen, and embryonic fluids. Some benefits from n-6 PUFAs include reduced risk of coronary heart disease and type 2 diabetes and reduced total cardiovascular disease, cardiovascular mortality, and ischemic stroke (Wu *et al*., 2020).

Fatty acid-based nutritional indices/ratios. The PUFA to SFA ratio measures all PUFAs that reduce low-density lipoprotein cholesterol and lower serum cholesterol in relation to all SFAs that may increase serum cholesterol (Chen & Liu, 2020). A high PUFA/SFA ratio protects the cardiovascular system from the harmful effects of atherosclerosis or progressive thickening and loss of elasticity of the artery wall (Naeini *et al*., 2020). In this study, the PUFA/SFA ratio was highest in the yolk and albumen but lower in the embryo and embryonic fluids. The PUFA/SFA ratio was higher in the edible components (except albumen) of B18d balut than in B15d balut (Table 5). The high PUFA/SFA ratio in the yolk was mainly due to the relatively high proportion of linoleic acid C18:2n-6.

The MUFA to SFA ratio measures all MUFAs that increase the activity of low-density lipoprotein receptors and decrease the cholesterol concentration in serum in relation to all SFAs that may increase serum cholesterol. A high MUFA/SFA ratio implies greater health benefits as MUFAs have been associated with a lower risk of cardiovascular disease (CVD) and death (Guasch-Ferre *et al*., 2015). Replacing SFAs with MUFAs, especially C18:1n-9, found abundantly in dietary food, and reduced CVD and all-cause mortality (Wang *et al*., 2016). In this study, the MUFA/SFA ratio was highest in the embryonic fluids, followed by the albumen and yolk, and lowest in the embryo. The MUFA/SFA ratio was higher in the edible components of B18d balut compared to that in B15d balut (Table 5).

The omega-6 FAs to omega-3 FAs (n-6/n-3) ratio measures eicosanoids that have important roles in regulating inflammation. The eicosanoids derived from n-6 PUFAs (i.e., linoleic acid and arachidonic acid C20:4n-6) are proinflammatory, while eicosanoids derived from n-3 PUFAs (i.e., ALA C18:3n-3 and DHA C22:6n-3) are anti-inflammatory. Hence, an increase in the n-6/n-3 ratio may intensify the inflammatory processes and consequently aggravate many inflammatory diseases (Patterson *et al*., 2012). The optimal dietary intake of the n-6/n-3 ratio should be around 1–4: 1, although this may vary depending on the degree of severity of the disease. In this study, the n-6/n-3 ratio in the edible components (except the albumen and fluid portion) was lower in B15d balut compared to that in B18d balut. The n-6/n-3 ratio in B15d balut was slightly lower in the embryo (8.68) than in the yolk (9.81), Table 5. The n-6/n-3 ratios for both embryo and yolk in balut were more than double of the recommended optimal dietary intakes. The n-6/n-3 ratio was not determined for the albumen and embryonic fluids since no omega-3 PUFA was detected.

The atherogenicity index (IA) is a measure of some SFAs (C12:0, C14:0, and C16:0, except C18:0) that are pro-atherogenic, relative to MUFAs and PUFAs that are anti-atherogenic (Ulbricht & Southgate, 1991). A lower atherogenicity may help prevent the accumulation of fatty plaque and reduce the levels of phospholipids, cholesterol, and esterified FAs. Hence, the edible components of balut with lower IA values may suggest greater health benefits to reduce the risk of coronary heart disease through reduced levels of total cholesterol and LDL cholesterol, which may accumulate in the artery wall (Salter, 2013). In this study, atherogenicity was lowest in embryonic fluids (0.26), followed by albumen (0.44) and yolk (0.50), and highest in the embryo (0.66). The IA values for the edible components of balut were lower in B18d balut than in B15d balut (Table 5). The generally low IA values (i.e., greater health benefits) for the edible components of balut were mainly due to their lower proportion of the major SFA (palmitic acid) relative to a higher proportion of the major unsaturated FAs (oleic acid and linoleic acid).

The thrombogenicity index (IT) is a measure of some SFAs that are pro-thrombogenic (C14:0, C16:0, and C18:0), with respect to MUFAs and PUFAs that are anti-thrombogenic (Ulbricht & Southgate, 1991). Lower thrombogenicity may mean lower tendency to form clots in blood vessels, which is beneficial for cardiovascular health (Chen & Liu, 2020). In this study, thrombogenicity was lowest in embryonic fluids and yolk, followed by albumen and highest in the embryo. The IT values for the edible components of balut were lower in B18d balut than in B15d balut (Table 5). The low thrombogenicity of the yolk (i.e., greater health benefits) was mainly due to its low proportion of stearic acid C18:0 and relatively high proportion of oleic acid C18:1 n-9.

The health-promoting index (HPI) is the inverse of the atherogenicity index (Chen *et al*., 2004). High HPI values may suggest more benefits for human health. In this study, the HPI was the highest in embryonic fluids (3.83), followed by the albumen (2.26) and the yolk (1.98), and least in the embryo (1.53). The HPI values of the edible components of B18d balut were higher than those in B15d balut (Table 5).

The hypocholesterolemic to hypercholesterolemic (h/H) ratio is a measure of the relationship between hypocholesterolemic FAs (oleic acid and PUFAs) and hypercholesterolemic FAs (C12:0, C14:0, and C16:0) (Mierlita, 2018). The edible components of balut with a higher h/H ratio may suggest greater health benefits. In this study, the h/H ratio was highest in the albumen (2.14), followed by the yolk (2.07) and embryo (1.57) and lowest in the embryonic fluids (1.26). The h/H ratio in the edible components of B18d balut was higher than those in B15d balut (Table 5).

Comparisons among Itik Pinas Mallard Duck Breeds

Fat percentage and major FAs. Kayumanggi-IP had the highest fat percentage in the embryo of B18d balut (2.16%). The fat percentage in the yolk of B15d balut (30.11%–30.25%) and B18d balut (28.02%–29.00%) were similar among the IP-Itim, IP-Khaki, and Kayumanggi-IP breeds (Table 4).

For the major SFAs, the proportion of C16:0 in the embryo (24.09%–25.32%) and yolk (24.20%–25.25%) was similar across Itik Pinas breeds. However, Kayumanggi-IP had the lowest C16:0 in the albumen of B18d balut (6.56%) and fluid portion of B15d balut (6.73%). Kayumanggi-IP also had the lowest C18:0 in the embryo (7.48%), yolk (3.83%), and albumen (1.15%) of B18d balut.

For the major unsaturated FAs, Kayumanggi-IP had the highest proportion of C18:1n-9 in the embryo (35.66%), yolk (45.21%), and fluid portion (23.72%) of B18d balut. Kayumanggi-IP also had the highest proportion of C18:2n-6 in the embryo (5.30%), yolk (7.37%), and fluid portion (4.98%) of B18d balut.

Fatty acid-based nutritional indices/ratios. The edible components of B18d balut from Kayumanggi-IP, compared to IP-Itim and IP-Khaki, seem to be most beneficial to human cardiovascular health. Kayumanggi-IP had the highest PUFA/SFA and MUFA/SFA ratio, the lowest atherogenicity and thrombogenicity potential, and the highest health-promoting index and h/H ratio in each edible component of B18d balut (Table 5).

In summary, this study presents new information on the amount and quality (i.e., nutritional value) of fat in the edible components of balut, which may be used as a potentially beneficial food ingredient or functional food. These findings also provide the technical basis to promote and improve the nutritional value of balut according to consumer preferences in local nucleus and multiplier duck farms.

CONCLUSION

In balut, fat content was higher in the yolk than in the embryo, while fat percentage was negligible in the albumen and embryonic fluids. In contrast, protein content was higher in the albumen than in the

yolk or embryo of balut. The yolk (compared to the embryo), B18d balut (compared to B15d balut), and balut produced by Kayumanggi-IP ducks (compared to IP-Itim and IP-Khaki) seem to be more beneficial for cardiovascular health. The FA-based nutritional indices/ratios for the albumen and fluid portion may not be important since the amount of fat obtained from the albumen and embryonic fluids was negligible.

CONFLICT OF INTEREST

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

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