

FATTY ACID-BASED NUTRITIONAL INDICES/RATIOS OF EGG YOLK FROM PHILIPPINE NATIVE AND COMMERCIAL BREEDS OF CHICKENS

Orville L. Bondoc¹, Ana Rose Ramos¹ and Aldrin O. Ebron¹

ABSTRACT

The nutritional value of chicken egg yolk is important as people now are more interested on its effects on cardiovascular health and disease. This study compared the fatty acid (FA) profile and nutritional indices/ratios of egg yolk from different breeds – Philippine native chickens (Banaba, Joloano, Paroakan, and Palawan lasak), egg-type (White Leghorn), meat-type (White Rock), dual-purpose breeds (Barred Plymouth Rock, Black Australorp, Nagoya, New Hampshire, Rhode Island Red, and Taiwan Yellow), and fancy-type breeds (Black Silkies and White Silkies) raised in a government poultry research facility at Tiaong, Quezon. Among the native breeds, the egg yolk from Joloano was healthier from the nutritional point of view, in terms of the highest polyunsaturated FA to saturated FA ratio (PUFA/SFA = 0.49), highest health-promoting index (HPI = 2.01) and hypocholesterolemic/ hypercholesterolemic ratio (h/H = 2.12); and lowest omega-6 to omega-3 ratio (n-6/n-3 = 20.89), atherogenicity index (IA = 0.50), and thrombogenicity index (IT = 1.05). Overall, the yolk from Rhode Island Red had the highest monounsaturated FA to saturated FA ratio (MUFA/SFA = 1.33), HPI (2.10), and h/H ratio (2.18); and lowest IA (0.48) and IT value (1.04). The results of the study not only contribute to the local information on the egg yolk's nutritional quality and their possible effects on human cardiovascular disease but also provide justifications to conserve the Philippine native chickens.

Keywords: Egg yolk, fatty acids, native chickens, nutritional indices

INTRODUCTION

The annual per capita consumption of chicken eggs in the Philippines in 2018 was 5.78 kg or 85 eggs (PSA, 2021). This was highest among individuals in the higher income groups such as those in the National Capital Region (118 eggs), Central Luzon (102 eggs), CALABARZON (101 eggs) and lowest in the Bangsamoro Autonomous Region in Muslim Mindanao (30 eggs). In 2021, the chicken egg industry in the Philippines produced 661.39 thousand metric tons of eggs from commercial layer farms, worth 77.27 billion pesos (PSA, 2022). Unfortunately, no data is available on the production and consumption of eggs from

¹Institute of Animal Science, College of Agriculture and Food Science, University of the Philippines Los Baños, College, Laguna 4031 Philippines (email: olbondoc@up.edu.ph).

native/improved native chickens raised in backyard or poor smallholder farms.

Other than a source of protein in the human diet, the egg yolk also contains fats that may have different nutritional qualities and effects on human health. Yolk fats may be characterized by its fatty acid (FA) composition including the saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and omega-3 and omega-6 polyunsaturated fatty acids (PUFA) – which can be influenced by the genetic strain (Rey *et al.*, 2021) and the nutrient composition of the hen's diet (Goldberg *et al.*, 2013).

While breed variations in the characteristics and composition of chicken eggs have recently been reported (Bondoc *et al.*, 2020 and 2021), there is very little information on the measurement of the nutritional quality of egg yolk from locally adapted chicken breeds that would signify the influence of FAs on human cardiovascular health and disease (Chen and Liu, 2020). Such information is important not only for those who have limited access to eggs from commercial layer farms but also for describing distinct breed characteristics that may be used to justify the conservation and improvement of Philippine native chickens. In this regard, this study evaluated yolk weight and color, fat and protein content, FA composition and related nutritional indices/ratios of the egg yolk from fresh eggs produced by four native chicken breeds (Banaba, Joloano, Paraoakan, and Palawan lasak) in comparison with those from adapted egg-type, meat-type, dual-purpose, and fancy-type breeds.

MATERIALS AND METHODS

Data

Newly laid chicken eggs (N=224) were randomly collected from four Philippine native breeds (Banaba, Joloano, Paraoakan, and Palawan lasak), egg-type (White Leghorn), meat-type (White Rock), and six dual-purpose breeds (Black Australorp, Barred Plymouth Rock, Nagoya, New Hampshire, Rhode Island Red, and Taiwan Yellow), and fancy-type (Black Silkies and White Silkies) at the National Swine and Poultry Research and Development Center (NSPRDC), Bureau of Animal Industry – Department of Agriculture (BAI-DA) in Tiaong, Quezon.

Each egg was recorded for its weight, yolk weight, and yolk color (measured using a 16 scales color index DSM yolk color fan –formerly Roche Yolk Color Fan, USA) within 24 hours after its collection. Four (4) yolk samples from the same breed and collected on the same day were pooled and placed in 200 ml plastic bottles and immediately frozen at -20°C . Fifty-six (56) pooled samples comprised of 16 eggs per breed were analyzed for fat content and fatty acid composition.

Individual FAs were analyzed as a percentage of total FAs (g/100 g), i.e., 8 saturated FAs (SFA) – C12:0 (lauric acid), C14:0 (myristic acid), C15:0 (pentadecylic acid), C16:0 (palmitic acid), C17:0 (margaric acid), C18:0 (stearic acid), C20:0 (arachidic acid), C22:0 (behenic acid); 6 monounsaturated FAs (MUFA) – C14:1n-5 (myristoleic acid), C16:1n-7 (palmitoleic acid), C18:1n-9 (oleic acid), C18:1n-7 (trans-vaccenic acid), C20:1n-11 (eicosenoic acid), C22:1n-9 (erucic acid); and 5 polyunsaturated FAs (PUFA) – C18:2c9t11 (conjugated linoleic acid or CLA), C18:2n-6 (linoleic acid or LA), C18:3n-3 (*α* -linolenic acid or ALA), C20:4n-6 (arachidonic acid or AA), C22:6n-3 (docosahexaenoic acid or DHA).

Six FA groups (i.e., SFA, MUFA, PUFA, UFA = MUFA + PUFA, omega-3 FA = C18:3n-3 and C22:6n-3, and omega-6 FA = C18:2n-6 + C20:4n-6) and eight FA-based

nutritional indices/ratios with health implications were also determined. The FA-based nutritional indices/ratios were: PUFA/SFA ratio, MUFA/SFA ratio, omega-6/omega-3 (n-6/n-3) ratio, linoleic acid / α -linolenic acid (LA/ALA) ratio, atherogenicity index, thrombogenicity index, health-promoting index, and hypocholesterolemic/ hypercholesterolemic (h/H) ratio

The index of atherogenicity (IA) and index of thrombogenicity (IT) were estimated using the equations of Ulbricht and Southgate (1991) as follows:

$$IA = [C12:0 + (4 \times C14:0) + C16:0] / \Sigma UFA$$

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

The health-promoting index (HPI) used by Chen *et al.* (2004) was

$$HPI = UFA / [C12:0 + (4 \times C14:0) + C16:0].$$

The hypocholesterolemic/ hypercholesterolemic (h/H) ratio used by Mierlita (2018) was $h/H = (C18:1n-9 + PUFA) / (C12:0 + C14:0 + C16:0)$.

All breeds were managed equally and fed with chicken layer mash containing 3.36% crude fat and total FAs consisting of 19.84% SFA – C12:0 (1.29%), C14:0 (1.25%), C16:0 (12.51%), C18:0 (3.90%), C20:0 (0.47%), C22:0 (0.42%); 15.90% MUFA – C16:1n-7 (0.59%), C18:1n-9c (15.31%); and 11.18% PUFA – C18:2n-6 (11.00%), and C18:3n-3 (0.18%).

Fat content and fatty acid (FA) analysis

The fat content (%) in the yolk from chicken eggs was determined using the Mojonnier method (AOAC Official Method 925.32: Fats in Eggs). In addition, percent protein in the yolk was determined using the Kjeldahl method (AOAC Official Method 932.08 Nitrogen (Water-Soluble and Crude Albumin) in Liquid Eggs).

Yolk fat was extracted following the method presented by Folch *et al.* (1957). The fatty acid methyl esters (FAMES) were prepared following the rapid methanolysis/methylation procedure used by Ichihara and Fukubayashi (2010). The FAMES were quantified using a Shimadzu GC 2010 Plus - Capillary Gas Chromatograph System (Shimadzu Corporation, Kyoto, Japan). The FAMES were identified using the LabSolutions software by comparing the retention times of sample peaks with known FAME standards – Grain FAME Mix (CRM47801), arachidonic acid (A3611), docosahexaenoic acid (D2534), trans-vaccenic acid (V1131), and conjugated linoleic acid (16413) obtained from Sigma Aldrich.

Statistical analysis

Differences in egg weight, yolk weight, yolk color, and yolk composition among the chicken breeds were initially determined using ANOVA (SAS Ver. 9.2, 2009). The general least squares procedures for unbalanced data were then used to analyze each FA in egg yolk. Statistical significance was set at P value <0.05. The final mathematical model was $y_{ijklmn} = \mu + \text{Breed}_i + \text{Age}_j + \text{YolkWt}_k + \text{YColor}_l + \text{PFat}_m + e_{ijklmn}$, where y_{ijklmn} is the proportion of FA by weight of total identified FAs (g/100 g), μ is the overall mean, Breed_i is the fixed effect of the i^{th} breed, Age_j is the j^{th} covariate effect of hen's age at lay (years), YolkWt_k is the k^{th} covariate effect of yolk weight (g), YColor_l is the l^{th} covariate effect of yolk color (i.e., 1-16), PFat_m is the m^{th} covariate effect of fat content (%), and e_{ijklmn} is the error term. The least square

mean for various FA in yolk fats were used to compute the FA-based nutritional indices/ratios. Regression coefficients (no intercept model) were also reported for FAs found to be significantly associated with hen's age at lay, yolk weight, yolk color, and fat content.

RESULTS AND DISCUSSION

Factors affecting fatty acid (FA) composition in chicken egg yolk

Among the major FAs with the highest proportion, stearic acid was the most variable (i.e., coefficient variation (CV) = 9.29%), followed by linoleic acid (CV= 8.82%), palmitic acid (CV= 4.95%) and oleic acid (CV= 3.63%), see Table 1.

Palmitic acid was lower in the egg yolk from older hens (i.e., lower by 2.83% for every additional year of age). Stearic acid was also lower by 0.75% in older hens. Similarly, Zita *et al.* (2022) reported that C16:0 and C18:0 significantly decreased with the age of organically reared hens and were lower in the second laying cycle by 1.25 and 0.71 percentage points.

Palmitic acid was higher in egg yolks having higher percent fat, i.e., higher by 0.16% for every increase in percent fat. Linoleic acid was also higher by 0.07% for every increase in fat percentage. However, the amount of yolk fat may vary in different breeds. For example, Rey *et al.* (2021) reported that yolks from Rhode Island Red hens had higher fat content and C16:0 than those from White Leghorn.

Yolk weight and yolk color had significant effects on other FAs, which were found in very low amounts (less than one percent).

Yolk characteristics and composition

Yolk weight was not significantly different among the four native chicken breeds, ranging from 14.5 g to 17.4 g and similar with that from fancy-type breeds such as Black Silkies (14.7 g) and White Silkies (13.9 g), see Table 2. Among the dual-purpose breeds, yolk weight was highest in Nagoya (20.5 g), followed by New Hampshire (18.5 g), Black Australorp (17.5 g), Barred Plymouth Rock (17.0 g), Taiwan Yellow (16.7 g), and lowest in Rhode Island Red (16.1 g), see Table 3. The yolk weight in egg-type White Leghorn (17.4 g) was higher than in White Rock – a meat-type breed (16.2 g). Although not correlated with an egg's quality and nutritional value, the yolk color score in native chicken breeds (6.4 to 7.1) was considerably lower than Black Silkies (9.2) and some dual-purpose breeds – Nagoya (9.8), Black Australorp (8.1), and New Hampshire (8.1). The yolk weights in this study were within the range of the normal size of yolks reported by Bondoc *et al.* (2021) for different chicken breeds under Philippine conditions.

The moisture content of egg yolk was significantly higher in Joloano (50.5%), Banaba (50.2%), and Paraoakan (49.2%) compared to Palawan lasak (47.6%). For other breeds, the moisture content in egg yolk was highest in Nagoya (52.6%) and lowest in White Leghorn (47.8%). The protein content of egg yolk in the native breeds (16.0% to 16.4%) was higher than in Nagoya (14.5%) but lower than White Silkies (16.7%). The fat content of egg yolk in the native breeds (29.0% to 30.4%) was also lower than White Silkies (31.8%). This implies that egg yolk from Philippine native chickens which contain more protein but less fat, may be preferred by consumers over other locally available chicken breeds.

Table 1. Mean square F tests for the effects of breed and covariate effects of age at lay, yolk weight, yolk color, and percent fat on fatty acids (g/100 g of total identified FAs) in egg yolk from different chicken breeds.

	Breed	Age at lay	Yolk weight	Yolk color	% Fat	Coefficient of variation (%)
Saturated FAs						
C12:0	**	** -0.04 ± 0.01	ns	** -0.003 ± 0.000	ns	22.47
C14:0	**	** -0.26 ± 0.06	ns	** 0.015 ± 0.003	* 0.010 ± 0.004	16.31
C15:0	**	* -0.01 ± 0.00	ns	ns	** 0.001 ± 0.000	13.64
C16:0	**	** -2.84 ± 0.47	ns	ns	** 0.161 ± 0.033	4.95
C17:0	**	ns	ns	** 0.004 ± 0.002	ns	59.71
C18:0	**	* -0.75 ± 0.24	ns	ns	ns	9.29
C20:0	**	ns	** -0.004 ± 0.001	ns	ns	19.98
C22:0	**	** -0.10 ± 0.02	ns	ns	ns	>100
Monounsaturated FAs						
C14:1n-5	**	** -0.05 ± 0.01	ns	ns	ns	25.92
C16:1n-7	**	** -0.45 ± 0.17	ns	ns	** 0.045 ± 0.012	14.73
C18:1n-9	**	ns	ns	ns	* 0.086 ± 0.040	3.63
C18:1n-7	**	ns	ns	** 0.014 ± 0.005	ns	22.04
C20:1n-11	**	* -0.04 ± 0.02	ns	ns	** 0.004 ± 0.001	33.98
C22:1n-9	**	* 0.05 ± 0.00	ns	ns	** 0.005 ± 0.002	>100
Polyunsaturated FAs						
C18:2c9 t11, CLA	**	* 0.07 ± 0.03	ns	** -0.005 ± 0.001	* -0.006 ± 0.002	31.88
C18:2n-6, LA	**	ns	ns	ns	** 0.071 ± 0.025	8.82

Table 1. Continuation...

	Breed	Age at lay	Yolk weight	Yolk color	% Fat	Coefficient of variation (%)
Polyunsaturated FAs						
C18:3n-3, ALA	**	ns	*-0.001 ± 0.000	ns	ns	10.81
C20:4n-6, AA	**	*0.22 ± 0.10	ns	ns	ns	96.69
C22:6n-3, DHA	**	**0.18 ± 0.06	ns	**0.015 ± 0.004	ns	94.94

Note: ns -No significant differences (P>0.05); * -Significant differences (P<0.05); ** -Highly significant differences (P<0.01).

Numbers in covariate columns are the regression coefficients and corresponding standard errors.

Table 2. Least square means for yolk weight, yolk color, percent moisture, protein, and fat, and proportion of major fatty acids in egg yolk from native, egg-type (White Leghorn), and fancy-type chicken breeds.

	Banaba	Joloano	Paraoakan	Palawan lasak	White Leghorn	Black Silkies	White Silkies
Yolk weight, g	16.23 ± 1.89 ^b	14.53 ± 1.89 ^b	14.88 ± 1.95 ^b	15.78 ± 1.89 ^b	17.40 ± 1.89 ^{ab}	14.67 ± 1.95 ^b	13.94 ± 1.89 ^b
Yolk color	7.12 ± 0.48 ^c	6.44 ± 0.48 ^{cd}	7.12 ± 0.48 ^c	6.38 ± 0.48 ^{cd}	6.06 ± 0.48 ^d	9.20 ± 0.50 ^a	6.94 ± 0.48 ^{cd}
% Moisture	50.24 ± 0.40 ^{bc}	50.48 ± 0.40 ^{bc}	49.22 ± 0.40 ^c	47.60 ± 0.40 ^c	47.78 ± 0.40 ^c	49.87 ± 0.42 ^{bc}	49.80 ± 0.40 ^c
% Protein	15.98 ± 0.13 ^c	16.00 ± 0.13 ^c	16.40 ± 0.13 ^b	16.25 ± 0.13 ^{bc}	16.50 ± 0.13 ^{ab}	14.85 ± 0.13 ^d	16.70 ± 0.13 ^a
% Fat	29.21 ± 0.39 ^{cd}	29.05 ± 0.39 ^{cd}	29.48 ± 0.39 ^c	30.42 ± 0.39 ^b	30.10 ± 0.39 ^b	30.44 ± 0.40 ^b	31.78 ± 0.39 ^a
Saturated FAs							
C12:0	0.09 ± 0.01 ^{ab}	0.07 ± 0.01 ^b	0.06 ± 0.01 ^b	0.09 ± 0.01 ^{ab}	0.12 ± 0.01 ^a	0.05 ± 0.01 ^b	0.04 ± 0.01 ^c
C14:0	1.11 ± 0.04 ^b	1.06 ± 0.04 ^b	0.93 ± 0.04 ^c	1.21 ± 0.04 ^a	1.19 ± 0.04 ^{ab}	0.82 ± 0.04 ^d	0.80 ± 0.04 ^d
C15:0	0.05 ± 0.00 ^b	0.07 ± 0.00 ^a	0.06 ± 0.00 ^{ab}	0.05 ± 0.00 ^b	0.04 ± 0.00 ^b	0.07 ± 0.00 ^a	0.05 ± 0.00 ^b

Table 2. Continuation...

	Banaba	Joloano	Paraoakan	Palawan lasak	White Leghorn	Black Silkies	White Silkies
Saturated FAs							
C16:0	26.40 ± 0.34 ^b	24.97 ± 0.33 ^{cd}	24.86 ± 0.35 ^c	25.50 ± 0.33 ^c	25.46 ± 0.35 ^c	24.58 ± 0.36 ^d	24.76 ± 0.36 ^d
C17:0	0.15 ± 0.02 ^b	0.20 ± 0.02 ^a	0.16 ± 0.02 ^{ab}	0.14 ± 0.02 ^b	0.09 ± 0.02 ^c	0.15 ± 0.02 ^b	0.12 ± 0.02 ^{bc}
C18:0	7.95 ± 0.18 ^b	7.12 ± 0.17 ^{cd}	7.34 ± 0.18 ^c	7.87 ± 0.17 ^{bc}	7.70 ± 0.18 ^{ab}	7.38 ± 0.19 ^{cd}	7.70 ± 0.19 ^{ab}
C20:0	0.20 ± 0.01 ^b	0.21 ± 0.01 ^{ab}	0.24 ± 0.01 ^a	0.19 ± 0.01 ^b	0.20 ± 0.01 ^b	0.20 ± 0.01 ^b	0.17 ± 0.01 ^c
C22:0	0.00 ± 0.02 ^c	0.01 ± 0.02 ^c	0.00 ± 0.02 ^c	0.00 ± 0.02 ^c	0.08 ± 0.02 ^b	0.09 ± 0.02 ^b	0.08 ± 0.02 ^b
Monounsaturated FAs							
C14:1n-5	0.22 ± 0.01 ^a	0.17 ± 0.01 ^{bc}	0.17 ± 0.01 ^{bc}	0.23 ± 0.01 ^a	0.23 ± 0.01 ^a	0.18 ± 0.01 ^b	0.17 ± 0.01 ^{bc}
C16:1n-7	2.86 ± 0.12 ^b	2.16 ± 0.12 ^d	2.46 ± 0.12 ^c	2.64 ± 0.12 ^c	2.94 ± 0.13 ^b	2.65 ± 0.12 ^{bc}	2.87 ± 0.13 ^{bc}
C18:1n-9	39.70 ± 0.40 ^a	38.07 ± 0.39 ^c	37.71 ± 0.41 ^{cd}	39.65 ± 0.36 ^{ab}	36.72 ± 0.42 ^d	38.97 ± 0.43 ^b	40.10 ± 0.43 ^a
C18:1n-7	1.19 ± 0.07 ^b	1.18 ± 0.07 ^b	1.32 ± 0.07 ^{ab}	1.24 ± 0.07 ^b	1.19 ± 0.07 ^b	0.95 ± 0.07 ^c	1.18 ± 0.07 ^b
C20:1n-11	0.02 ± 0.01 ^c	0.04 ± 0.01 ^{de}	0.19 ± 0.01 ^{ab}	0.16 ± 0.01 ^b	0.18 ± 0.01 ^b	0.05 ± 0.01 ^d	0.06 ± 0.01 ^d
C22:1n-9	0.00 ± 0.02 ^c	0.00 ± 0.02 ^c	0.00 ± 0.02 ^c	0.00 ± 0.02 ^c	0.05 ± 0.01 ^b	0.02 ± 0.02 ^c	0.02 ± 0.02 ^c
Polyunsaturated FAs							
C18:2c9 t11, CLA	ND	ND	0.26 ± 0.02 ^a	0.08 ± 0.02 ^c	0.09 ± 0.02 ^c	0.16 ± 0.03 ^b	0.15 ± 0.02 ^b
C18:2n-6, LA	13.05 ± 0.25 ^d	15.89 ± 0.24 ^a	14.15 ± 0.26 ^b	13.32 ± 0.25 ^c	13.30 ± 0.26 ^d	11.39 ± 0.27 ^e	10.21 ± 0.26 ^f
C18:3n-3, ALA	0.18 ± 0.01 ^c	0.20 ± 0.01 ^e	0.31 ± 0.01 ^a	0.36 ± 0.01 ^b	0.43 ± 0.01 ^a	0.26 ± 0.01 ^d	0.33 ± 0.01 ^{bc}
C20:4n-6, AA	0.36 ± 0.07 ^b	0.58 ± 0.07 ^a	0.16 ± 0.07 ^c	0.06 ± 0.07 ^c	0.29 ± 0.07 ^b	0.27 ± 0.08 ^b	0.26 ± 0.07 ^b
C22:6n-3, DHA	0.38 ± 0.04 ^b	0.59 ± 0.04 ^a	0.08 ± 0.05 ^d	0.03 ± 0.04 ^d	0.14 ± 0.05 ^{cd}	0.16 ± 0.05 ^{cd}	0.66 ± 0.05 ^a

Note: ND - Not detected.

Least square means within a row without common letter superscripts are significantly different (P<0.05).

Table 3. Least square means for yolk weight and color, percent moisture, protein, and fat, and proportion of major fatty acids in egg yolk from meat-type (White Rock) and dual-purpose chicken breeds.

	White Rock	Barred Plymouth Rock	Black Australorp	Nagoya	New Hampshire	Rhode Island Red	Taiwan Yellow
Yolk weight, g	16.23 ± 1.89 ^b	17.04 ± 1.89 ^{ab}	17.48 ± 1.89 ^b	20.52 ± 1.89 ^a	18.51 ± 1.89 ^{ab}	16.09 ± 1.89 ^b	16.70 ± 1.89 ^b
Yolk color	7.06 ± 0.48 ^c	7.88 ± 0.48 ^{bc}	8.12 ± 0.48 ^b	9.81 ± 0.48 ^a	8.06 ± 0.48 ^b	6.94 ± 0.48 ^{cd}	6.81 ± 0.48 ^{cd}
% Moisture	50.78 ± 0.40 ^b	49.65 ± 0.40 ^c	49.75 ± 0.40 ^c	52.60 ± 0.40 ^a	48.30 ± 0.40 ^d	48.78 ± 0.40 ^d	49.50 ± 0.40 ^c
% Protein	16.52 ± 0.13 ^{ab}	15.88 ± 0.13 ^c	16.25 ± 0.13 ^{bc}	14.52 ± 0.13 ^c	16.38 ± 0.13 ^b	16.60 ± 0.13 ^{ab}	16.35 ± 0.13 ^b
% Fat	31.40 ± 0.39 ^{ab}	29.25 ± 0.39 ^{cd}	28.50 ± 0.39 ^d	27.95 ± 0.39 ^a	30.65 ± 0.39 ^b	28.85 ± 0.39 ^{cd}	30.15 ± 0.39 ^b
Saturated FAs							
C12:0	0.09 ± 0.01 ^{ab}	0.07 ± 0.01 ^b	0.08 ± 0.01 ^b	0.09 ± 0.01 ^{ab}	0.12 ± 0.01 ^a	0.06 ± 0.01 ^b	0.05 ± 0.01 ^b
C14:0	1.11 ± 0.06 ^{ab}	1.02 ± 0.05 ^c	1.11 ± 0.06 ^{ab}	1.20 ± 0.06 ^{ab}	1.22 ± 0.06 ^a	0.95 ± 0.05 ^c	0.79 ± 0.04 ^d
C15:0	0.05 ± 0.00 ^b	0.06 ± 0.00 ^{ab}	0.05 ± 0.00 ^b	0.05 ± 0.00 ^b	0.05 ± 0.00 ^b	0.06 ± 0.00 ^{ab}	0.05 ± 0.00 ^b
C16:0	26.28 ± 0.47 ^b	26.16 ± 0.38 ^b	26.83 ± 0.46 ^{ab}	27.35 ± 0.47 ^a	26.08 ± 0.48 ^b	23.36 ± 0.39 ^e	23.31 ± 0.36 ^e
C17:0	0.15 ± 0.03 ^{ab}	0.10 ± 0.02 ^{bc}	0.15 ± 0.27 ^{abc}	0.17 ± 0.03 ^{ab}	0.15 ± 0.03 ^{ab}	0.12 ± 0.02 ^{bc}	0.12 ± 0.02 ^{bc}
C18:0	7.73 ± 0.25 ^{bcd}	7.30 ± 0.20 ^{cd}	7.71 ± 0.24 ^{bc}	8.73 ± 0.24 ^a	7.01 ± 0.25 ^{cd}	6.55 ± 0.20 ^d	6.79 ± 0.19 ^d
C20:0	0.21 ± 0.01 ^{ab}	0.20 ± 0.01 ^b	0.21 ± 0.01 ^{ab}	0.22 ± 0.01 ^{ab}	0.24 ± 0.01 ^a	0.20 ± 0.01 ^b	0.21 ± 0.01 ^{ab}
C22:0	0.00 ± 0.02 ^c	0.07 ± 0.01 ^b	0.00 ± 0.02 ^c	0.00 ± 0.02 ^c	0.00 ± 0.03 ^c	0.07 ± 0.02 ^a	0.14 ± 0.02 ^a
Monounsaturated FAs							
C14:1n-5	0.21 ± 0.01 ^a	0.22 ± 0.01 ^a	0.21 ± 0.01 ^a	0.22 ± 0.01 ^a	0.21 ± 0.01 ^a	0.15 ± 0.01 ^c	0.14 ± 0.01 ^c
C16:1n-7	2.52 ± 0.17 ^{bc}	3.28 ± 0.14 ^a	2.70 ± 0.17 ^{bc}	2.63 ± 0.17 ^{bc}	2.74 ± 0.17 ^{bc}	2.17 ± 0.14 ^a	2.09 ± 0.13 ^d
C18:1n-9	38.15 ± 0.56 ^{bc}	34.64 ± 0.45 ^f	37.62 ± 0.55 ^{cd}	36.31 ± 0.36 ^d	37.74 ± 0.17 ^{cd}	37.67 ± 0.46 ^{cd}	35.33 ± 0.43 ^e

Table 3. Continuation...

	White Rock	Barred Plymouth Rock	Black Australorp	Nagoya	New Hampshire	Rhode Island Red	Taiwan Yellow
Monounsaturated FAs							
C18:1n-7	1.50 ± 0.10 ^a	0.93 ± 0.08 ^c	1.32 ± 0.09 ^{ab}	1.21 ± 0.09 ^b	1.51 ± 0.10 ^a	1.36 ± 0.08 ^a	1.26 ± 0.07 ^b
C20:1n-11	0.14 ± 0.02 ^{bc}	0.16 ± 0.01 ^b	0.16 ± 0.01 ^b	0.10 ± 0.02 ^c	0.12 ± 0.02 ^c	0.22 ± 0.01 ^a	0.18 ± 0.01 ^b
C22:1n-9	0.00 ± 0.02 ^c	0.05 ± 0.02 ^b	0.00 ± 0.03 ^{bc}	0.00 ± 0.02 ^b	0.00 ± 0.02 ^b	0.03 ± 0.02 ^{bc}	0.09 ± 0.02 ^a
Polyunsaturated FAs							
C18:2c9 t11, CLA	0.14 ± 0.03 ^{bc}	0.10 ± 0.02 ^c	0.17 ± 0.03 ^b	0.14 ± 0.03 ^{bc}	0.15 ± 0.03 ^{bc}	0.28 ± 0.29 ^{abc}	0.13 ± 0.02 ^{bc}
C18:2n-6, LA	12.95 ± 0.35 ^d	12.99 ± 0.28 ^d	13.66 ± 0.34 ^{cd}	14.09 ± 0.35 ^b	14.70 ± 0.36 ^b	14.32 ± 0.29 ^c	13.86 ± 0.27 ^d
C18:3n-3, ALA	0.26 ± 0.01 ^d	0.43 ± 0.01 ^a	0.29 ± 0.01 ^c	0.36 ± 0.01 ^b	0.28 ± 0.01 ^{cd}	0.30 ± 0.01 ^c	0.42 ± 0.01 ^a
C20:4n-6, AA	0.14 ± 0.10 ^c	0.28 ± 0.08 ^b	0.12 ± 0.10 ^c	0.00 ± 0.10 ^c	0.10 ± 0.10 ^c	0.33 ± 0.08 ^b	0.42 ± 0.08 ^b
C22:6n-3, DHA	0.01 ± 0.06 ^d	0.18 ± 0.02 ^c	0.03 ± 0.06 ^d	0.01 ± 0.06 ^d	0.02 ± 0.06 ^d	0.23 ± 0.05 ^{bc}	0.32 ± 0.05 ^b

Note: Least square means within a row without common letter superscripts are significantly different ($P < 0.05$).

Major FAs in chicken egg yolk

The four major FAs in the egg yolk of native breeds – representing about 83% to 90% of total FAs were oleic acid C18:1n-9 (37.1–39.7%), followed by palmitic acid C16:0 (24.9–26.4%), linoleic acid C18:2n-6 (13.0–15.9%), and lowest in stearic acid C18:0 (7.1–8.0%). In particular, the egg yolk from Banaba had the highest oleic acid (39.7%), palmitic acid (26.4%), and stearic acid (8.0%), while Joloano had the highest linoleic acid (15.9%). In other breeds, oleic acid was highest in the White Silkies (40.1%); The Nagoya breed had the highest palmitic acid (27.35%) and stearic acid (8.73%). Linoleic acid was highest in New Hampshire (14.70%), see Tables 2 and 3. In this regard, many studies have shown that the use of different hen genotypes significantly affects the fatty acid profiles of egg yolks. For example, Rey *et al.* (2021) reported that yolks from Rhode Island Red hens had lower C16:0 and C18:0 0 but higher fat content and C18:2n-6, when compared to White Leghorn.

Fatty acid-based nutritional indices/ratios for chicken egg yolk

The differences in nutritional indices/ratios of egg yolk among chicken breeds are shown in Tables 4 and 5.

PUFA/SFA ratio. The PUFA/SFA ratio measures the amount of PUFAs that are known to reduce low-density lipoprotein cholesterol and depress the levels of serum cholesterol, in relation to all SFAs that may add to high levels of serum cholesterol (Chen and Liu, 2020). A higher PUFA/SFA ratio suggests a beneficial effect in protecting the cardiovascular system from the harmful effects of atherosclerotic lesions (Naeini *et al.*, 2020). The PUFA/SFA ratio in dietary fats from meat and milk ranged from 0.11–1.29 and 0.06–0.18, respectively (Chen and Liu, 2020). By comparison, the PUFA/SFA ratio for egg yolk in different chicken breeds (0.37–0.51) was comparable to that of meat, but higher (more beneficial to human health) than that of milk. The PUFA/SFA ratio for egg yolk from native chickens was highest in Joloano (0.51), followed by Paraoakan (0.44) and lowest in Palawan lasak (0.39) and Banaba (0.39). These were comparable to the that of White Leghorn (0.41), and other dual-type breeds (0.39–0.49), but higher than that of White Rock (0.38) and fancy-type breeds (0.34–0.37).

MUFA/SFA ratio. The MUFA to SFA ratio is a measure of all MUFAs, especially oleic acid, 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 can have beneficial effects to human health as MUFAs had been associated with a lower risk of cardiovascular disease (CVD) and death (Guasch-Ferre *et al.*, 2015). In this study, the MUFA/SFA ratio for egg yolk from native chickens was highest in Palawan lasak (1.25), closely followed by Paraoakan (1.24), Joloano (1.23), and Banaba (1.22). In all breeds, the yolk from the Rhode Island Red had the highest MUFA/SFA ratio (1.33) and lowest in Nagoya (1.07).

LA/ALA ratio. The linoleic acid to α -linolenic acid (LA/ALA) ratio measures the balance between LA and ALA, both of which cannot be synthesized by humans. It was developed as a guide to improve the nutritional value of baby food or infant formula (milk), with a minimum reference value usually set within 5–15: 1 (Chen and Liu, 2020). A higher LA/ALA ratio implies faster rates of synthesis of ALA. In this study, the LA/ALA ratio for egg yolk from different chicken breeds was considerably higher than that in cow's milk fat which ranged from 2.46–3.45 (Chen and Liu, 2020). The LA/ALA ratio for egg yolk from native chickens was highest in Joloano (79.84), followed by Banaba (70.93), Paraoakan

Table 4. FA groups and FA-based nutritional indices/ratios for egg yolk from native, egg-type (White Leghorn), and fancy-type chicken breeds.

FA groups	Banaba	Joloano	Paraoakan	Palawan lasak	White Leghorn	Black Silkies	White Silkies
SFA	35.95	33.72	33.64	35.06	34.88	33.34	33.70
UFA	57.95	58.87	56.82	57.75	55.55	55.05	56.01
MUFA	43.98	41.61	41.85	43.91	41.31	42.82	44.40
PUFA	13.98	17.26	14.97	13.84	14.25	12.24	11.61
n-3 (ALA + DHA)	0.57	0.79	0.40	0.38	0.57	0.42	0.99
n-6 (LA + AA)	13.41	16.47	14.31	13.38	13.59	11.66	10.47
Nutritional indices/ratios							
PUFA/SFA ratio	0.39	0.51	0.44	0.39	0.41	0.37	0.34
MUFA/SFA ratio	1.22	1.23	1.24	1.25	1.18	1.28	1.32
LA/ALA ratio	70.93	79.84	45.22	37.53	31.30	44.48	31.25
n-6/n-3 ratio	23.69	20.89	36.23	35.13	24.02	28.09	10.56
Atherogenicity index	0.53	0.50	0.50	0.53	0.55	0.51	0.50
Thrombogenicity index	1.17	1.05	1.13	1.16	1.18	1.15	1.09
Health-promoting index	1.87	2.01	1.98	1.90	1.83	1.97	2.00
Hypo/hypercholesterolemic ratio	1.94	2.12	2.04	2.00	1.90	2.01	2.02

Note: Least square means with different superscript letters within a row are significantly different ($P < 0.05$).

Abbreviations: SFA, saturated fatty acids; UFA, unsaturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; LA, linoleic acid (C18:2n-6); ALA, α -linolenic acid (C18:3n-3); AA, arachidonic acid (C20:4n-6); DHA, docosahexaenoic acid (C22:6n-3); n-3, Omega-3 fatty acids; n-6, Omega-6 fatty acids

Table 5. FA groups and FA-based nutritional indices/ ratios for egg yolk from meat-type (White Rock) and dual-purpose chicken breeds.

FA groups	White Rock	Barred Plymouth Rock	Black Australorp	Nagoya	New Hampshire	Rhode Island Red	Taiwan Yellow
SFA	35.62	34.97	36.13	37.81	34.86	31.39	31.47
UFA	56.01	53.26	56.26	55.06	57.57	57.09	54.23
MUFA	42.52	39.28	42.00	40.47	42.32	41.64	39.09
PUFA	13.49	13.98	14.26	14.60	15.26	15.45	15.15
n-3 (ALA + DHA)	0.26	0.62	0.31	0.36	0.30	0.52	0.74
n-6 (LA + AA)	13.09	13.27	13.78	14.10	14.80	14.65	14.28
Nutritional indices/ratios							
PUFA/SFA ratio	0.38	0.40	0.39	0.39	0.44	0.49	0.48
MUFA/SFA ratio	1.19	1.12	1.16	1.07	1.21	1.33	1.24
LA/ALA ratio	50.80	29.92	47.75	39.47	52.30	48.21	33.09
n-6/n-3 ratio	50.36	21.47	44.02	38.83	49.17	28.07	19.27
Atherogenicity index	0.55	0.57	0.56	0.59	0.54	0.48	0.49
Thrombogenicity index	1.23	1.22	1.24	1.31	1.16	1.04	1.07
Health-promoting index	1.82	1.76	1.80	1.71	1.85	2.10	2.05
Hypo/hypercholesterolemic ratio	1.88	1.78	1.85	1.78	1.93	2.18	2.09

Note: Least square means with different superscript letters within a row are significantly different ($P < 0.05$).

Abbreviations: SFA, saturated fatty acids; UFA, unsaturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; LA, linoleic acid (C18:2n-6); ALA, α -linolenic acid (C18:3n-3); AA, arachidonic acid (C20:4n-6); DHA, docosahexaenoic acid (C22:6n-3); n-3, Omega-3 fatty acids; n-6, Omega-6 fatty acids

(45.22), and lowest in Palawan lasak (37.53). These were comparable to that of White Rock (50.80), Black Silkies (44.48), and some dual-type breeds (39.47–52.30) – Black Australorp, Nagoya, New Hampshire, and Rhode Island Red, but higher than from Taiwan Yellow (33.09), White Leghorn (31.30), and White Silkies (31.25).

n-6/n-3 ratio. The omega-6 FAs / omega-3 FA or n-6/n-3 ratio is used as a measure of the dietary contribution of omega-6 PUFAs (i.e., C18:2n-6 and C20:4n-6) that are generally pro-inflammatory, in relation to omega-3 PUFAs (i.e., C18:3n-3 and C22:6n-3) that are anti-inflammatory. According to Patterson *et al.* (2012), the increased dietary intake of n-6 PUFA and decreased dietary intake of n-3 PUFA can change the production of important mediators and regulators of inflammation and immune responses towards a proinflammatory profile associated with chronic inflammatory diseases. Hence, a lower n-6/n-3 ratio (e.g., 1–2:1) suggests a favorable effect to alleviate the effects of inflammatory diseases and reduce the risk of many chronic diseases such as nonalcoholic fatty liver disease, cardiovascular disease, obesity, inflammatory bowel disease, rheumatoid arthritis, and Alzheimer’s disease. In this study, the n-6/n-3 ratio for egg yolk from native chickens was highest in Paraoakan (36.23) and Palawan lasak (35.13), followed by Banaba (23.69), and lowest in Joloano (20.89). These were lower than that of White Rock (50.36), and some dual-type breeds (38.83–49.17) – Black Australorp, Nagoya, and New Hampshire, but higher than from Taiwan Yellow (19.27) and White Silkies (10.56).

Atherogenicity index. The index of atherogenicity (IA) measures the dietary contribution of some SFAs that are pro-atherogenic (i.e., lauric acid, myristic acid, and palmitic acid, except stearic acid), in relation to total MUFA and PUFA that are anti-atherogenic (Ulbricht and Southgate, 1991). The pro-atherogenic FAs favor the adhesion of lipids to cells of the circulatory and immunological systems, while the anti-atherogenic FAs inhibit the accumulation of fatty plaque and decrease the levels of phospholipids, cholesterol, and esterified FAs. Hence, a lower IA value in dietary fat implies lower likelihood to form fatty plaques in the arteries (Chen and Liu, 2020). The IA values in dietary fats reported for meat and milk ranged from 0.27–1.32 and 1.42–5.13, respectively (Chen and Liu, 2020). By comparison, the atherogenicity for egg yolk in different chicken breeds (0.48–0.59) was comparable to that of meat but lower (more beneficial to human health) than that of milk. The IA value for egg yolk from native chickens was lowest in Joloano (0.50) and Paraoakan (0.50) and highest in Banaba (0.53) and Palawan lasak (0.53). These were comparable to the that of Black Silkies (0.51), White Silkies (0.50), and other dual-type breeds (0.39–0.49), higher than Rhode Island Red (0.48) and Taiwan Yellow (0.49), but lower than that from White Leghorn (0.55), White Rock (0.55) and some dual dual-type breeds (0.56–0.59) – Barred Plymouth Rock, Black Australorp, and Nagoya.

Thrombogenicity index. The index of thrombogenicity (IT) measures the dietary contribution of prothrombogenic SFAs (i.e., lauric acid, myristic acid, and palmitic acid) in relation to total MUFA and PUFA that are anti-thrombogenic (Ulbricht and Southgate, 1991). A lower IT value suggests lower likelihood to form clots in blood vessels. The IT values in dietary fats from meat and milk ranged from 0.29–1.69 and 1.00–5.04, respectively (Chen and Liu, 2020). By comparison, the thrombogenicity for egg yolk in different chicken breeds (1.04–1.31) was comparable to that of meat and milk. The IT value for egg yolk from native chickens was lowest in Joloano (1.05), followed by Paraoakan (1.13), Palawan lasak (1.16), and highest in Banaba (1.17). These were comparable to the that of the fancy-type breeds (1.09–1.15), New Hampshire (1.16), Taiwan Yellow (1.07), and Rhode Island Red

(1.04), but lower than that from White Leghorn (1.18), White Rock (1.23), and some dual-type breeds (1.22–1.31) – Barred Plymouth Rock, Black Australorp, and Nagoya.

Health-promoting index. The health-promoting index (HPI) is the inverse of the atherogenicity index (Chen *et al.*, 2004), with a higher HPI value suggesting more benefits for human health. While the HPI values ranged from 0.16–0.68 in dairy products such as milk and cheese (Chen and Liu, 2020), this study reveals a higher HPI value (more beneficial for human health) for egg yolk from different chicken breeds (1.71–2.10). The HPI for egg yolk from native chickens was highest in Joloano (2.01), followed by Paraoakan (1.98), Palawan lasak (1.90), and lowest in Banaba (1.87). These were comparable to that from White Leghorn (1.90), fancy-type breeds (1.97–2.00), lower than in Taiwan Yellow (2.05), and Rhode Island Red (2.10), but higher than that from White Rock (1.82), and some dual-type breeds (1.71–1.85) – Barred Plymouth Rock, Black Australorp, Nagoya, and New Hampshire.

h/H ratio. The hypocholesterolemic / hypercholesterolemic or h/H ratio reflects the level of hypocholesterolemic FAs (i.e., C18:1n-9 and PUFAs) relative to hypercholesterolemic FAs (i.e., C12:0, C14:0, and C16:0). A higher h/H value implies lower levels of cholesterol that may possibly contribute to a decrease in the incidence of cardiovascular disease (Mierlita, 2018). The h/H ratio in dietary fats from meat and dairy products ranged from 1.27–2.79 and 0.32–1.29, respectively (Chen and Liu 2020). By comparison, the h/H ratio for egg yolk in different chicken breeds (1.78–2.18) was higher (more beneficial to human health) than that of meat and milk. The h/H ratio for egg yolk from native chickens was highest in Joloano (2.12), followed by Paraoakan (2.04), Palawan lasak (2.00), and highest in Banaba (1.94). These were comparable to the that of the fancy-type breeds (2.01–2.02), and Taiwan Yellow (2.09), lower than in Rhode Island Red (2.10), but higher than that from White Leghorn (1.90), White Rock (1.88), and some dual-type breeds (1.78–1.93) – Barred Plymouth Rock, Black Australorp, Nagoya, and New Hampshire.

CONCLUSION

The egg yolk from the Joloano breed had the highest FA-based nutritional value (i.e., highest PUFA/SFA ratio, HPI, and h/H ratio; and lowest n-6/n-3 ratio, IA, and IT value) compared to those from Banaba, Paraoakan, and Palawan lasak native chickens. The egg yolk from the Paraoakan consistently ranked second to Joloano in terms of PUFA/SFA ratio, IA, IT, HPI values, and h/H ratio. Overall, the egg yolk from Rhode Island Red appears to be most beneficial for human cardiovascular health (i.e., highest MUFA/SFA ratio, HPI value, and h/H ratio; and lowest IA and IT values). The results of the study, however, may be used to provide justifications conserving the local breeds of chickens in the Philippines.

ACKNOWLEDGMENT

The authors are grateful for the financial support to the Philippine Agriculture and Fisheries Biotechnology Program under the Department of Agriculture Biotechnology Program (DA-BIOTECH). The authors are thankful to Rene C. Santiago, Vea Roven E. Arellano, and Mat M. San Agustin of NSPRDC, BAI-DA, Tiaong, Quezon and Alyssa T. Odiner and Mark C. Agsunod of IAS, CAFS, UPLB for their valuable assistance in collecting, handling, and analyzing the chicken egg samples.

REFERENCES

- Bondoc OL, Ebron AO, Ramos AR and Santiago RC. 2020. Comparison of egg quality traits in different poultry species and breeds. *Philipp J Vet Med* 57(2): 220-235.
- Bondoc OL, Santiago RC, Bustos AR, Ebron AO and Ramos AR. 2021. Grading and weight classification of chicken eggs produced by native, egg-type, meat-type, dual-purpose and fancy-type chicken breeds under Philippine conditions. *Int J Poult Sci* 20(2): 87-97.
- Chen J and Liu H. 2020. Nutritional indices for assessing fatty acids: a mini-review. *Int J Mol Sci* 21(16): 5695.
- Chen S, Bobe G, Zimmerman S, Hammond EG, Luhman CM, Boylston TD, Freeman AE and Beitz DC. 2004. Physical and sensory properties of dairy products from cows with various milk fatty acid compositions. *J Agric Food Chem* 52(11): 3422–3428.
- Folch J, Lees M and Sloane Stanley GH. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* 226(1): 497-509.
- Goldberg EM, Ryland D, Gibson RA, Aliani M and House JD. 2013. Designer laying hen diets to improve egg fatty acid profile and maintain sensory quality. *Food Sci Nutr* 1(4): 324-335.
- Guasch-Ferre M, Babio N, Martinez-Gonzalez MA, Corella D, Ros E, Martin-Pelaez S, Estruch R, Aros F, Gomez-Gracia E, Fiol M, Santos-Lozano JM, Serra-Majem L, Bullo M, Toledo E, Barragan R, Fito M, Gea A and Salas-Salvado J. 2015. Dietary fat intake and risk of cardiovascular disease and all-cause mortality in a population at high risk of cardiovascular disease. *Am J Clin Nutr* 102(6): 1563-1573.
- Ichihara K and Fukubayashi Y. 2010. Preparation of fatty acid methyl esters for gas-liquid chromatography. *J Lipid Res* 51: 635-640.
- Mierlita D. 2018. Effects of diets containing hemp seeds or hemp cake on fatty acid composition and oxidative stability of sheep milk. *S Afr J Anim Sci* 48(3): 504-515.
- Naeini Z, Toupehian O, Vatannejad A, Sotoudeh G, Teimouri M, Ghorbani M, Nasli-Esfahani E and Koohdani F. 2020. Effects of DHA-enriched fish oil on gene expression levels of p53 and NF- κ B and PPAR- γ activity in PBMCs of patients with T2DM: a randomized, double-blind, clinical trial. *Nutr Metab Cardiovasc* 30(3): 441-447.
- Patterson E, Wall R, Fitzgerald GF, Ross RP and Stanton C. 2012. Health implications of high dietary omega-6 polyunsaturated fatty acids. *J Nutri Metab* 2: 539426.
- Philippine Statistics Authority [PSA]. 2021. PSA 2021 Special Release, Average Annual Per Capita Consumption of Selected Agricultural Commodities, 2018. 18 pp. Retrieved on 15 June 2022 from <https://psa.gov.ph/content/average-annual-capita-consumption-selected-agricultural-commodities-2018>
- Philippine Statistics Authority [PSA]. 2022. Chicken Situation Report (January – December 2021). Philippine Statistics Authority (PSA). 47 pp. Retrieved on 15 June 2022 from https://psa.gov.ph/sites/default/files/1_Chicken Annual Situation Report_ONS-signed.pdf
- Rey AI, De-Cara A, Rebole A and Arija I. 2021. Short-term Spirulina (*Spirulina platensis*) supplementation and laying hen strain effects on eggs' lipid profile and stability. *Animals* 11(7): 1944.
- Statistical Analysis System [SAS]. 2009. SAS/STAT $\text{\textcircled{R}}$ 9.2 User's Guide. 2nd ed. USA: SAS Institute, Inc. Cary, NC USA.

- Ulbricht TLV and Southgate DAT. 1991. Coronary heart disease: Seven dietary factors. *Lancet* 338(8773): 985-992.
- Zita L, Okrouhla M, Krunt O, Kraus A, Stadnik L, Citek J and Stupka R. 2022. Changes in fatty acids profile, health indices, and physical characteristics of organic eggs from laying hens at the beginning of the first and second laying cycles. *Animals* 12(1):125.