JWPR
Journal of World's Poultry Research

2022, Scienceline Publication

J. World Poult. Res. 12(3): 181-191, September 25, 2022

Research Paper, PII: S2322455X2200021-12

DOI: https://dx.doi.org/10.36380/jwpr.2022.21

The Long-term Effects of Dietary Replacement of Fish Meal with Black Soldier Fly (*Hermetia illucens*) Larvae on Nutritional Content and Eggshell Quality in Layer Chickens

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Received: 09 July 2022
Accepted: 01 September 2022

ABSTRACT

Although the egg is one of the foods offering nutrients of high biological value, the diet of layer chickens can change these characteristics. The aim of this study was to evaluate the effect of a long-term dietary replacement of fish meal with maggot meal of black soldier fly larvae on egg quality of hens. A total of 480 one-day-old Isa brown chicks were randomly assigned to 4 dietary treatment groups. The groups were named T0 (8% fish meal), T1 (4% maggot meal and 4% fish meal), T2 (6% maggot meal and 2% fish meal), and T3 (8% maggot meal). Each treatment group had 6 replicates of 20 chicks each. Data were collected on the eggshell quality parameters between 22 and 56 weeks of age. The results indicated that egg weight, shell weight, shape index, shell index, egg surface area, egg volume, density, yolk pH, albumen pH, yolk and albumen moisture content, yolk color, and yolk height were not influenced by the use of larval meals. Although the proportion of the yolk increased with age, there was no interaction between the use of fly larvae and the duration of its use for the collected parameters. However, the proportion of albumen, Haugh's unit in T1 and T3 treatments were higher than those of T0 and T2. The proportion of egg yolk, the yolk to albumen ratio, and the count of cracked eggs of T0 and T2 varied significantly compared to T1 and T3. Total egg fat decreased significantly as a result of the use of maggot meal. Total cholesterol, High-density lipoprotein (HDL) cholesterol, Low-density lipoprotein (LDL) cholesterol, and LDL/HDL ratio were lower in groups fed larvae meal, compared to the control group. It was concluded that the use of black soldier fly larvae meal during the entire rearing cycle and period of layers did not adversely affect the eggshell quality and nutritional content of the eggs.

Keywords: Black soldier fly, Cholesterol, Egg quality, Haugh unit, Larvae meal, Lipid

INTRODUCTION

The main characteristic of the egg among human foods is its richness in proteins and essential fatty acids (FAO, 2015). Feeding laying hens is crucial to optimize the excellent genetic potential of modern lines in terms of production performance and egg quality (Roberts, 2004; Leeson, 2011). Proteins and fatty acids are vital nutrients that can modulate not only the growth of hens but also the quality of the egg. The main concern of producers is the reduction of production costs through the high quality of the ingredients that ensure efficient production with the best possible profitability. In sub-Saharan Africa, particularly in Togo, the low availability in quantity and quality of feed combined with the high cost of animal proteins, such as fish meal, is one of the constraints to the growth of the poultry sector, which remains highly dependent on imports. Therefore, crucial to explore other unconventional and economically more profitable feed ingredients, available and not in competition with human food (Heuel et al., 2021). Also, in terms of quality, the fatty acid profile of the egg, whether in triglycerides or phospholipids, directly reflects the hen's food consumption of fatty acids (Chambers et al., 2017; Kralik et al., 2021). Additionally, the fat and cholesterol contents of meat are major characteristics of nutritional quality and health value due to the well-established relationship between diet, health, and well-being (Scollan et al., 2014). A reduced fat and cholesterol intake is associated with a reduced risk of chronic diseases, such as obesity, hypercholesterolemia, and cardiovascular disease (Chizzolini et al., 1999). According to Lessire (1995), chickens tend to incorporate dietary fatty acids, including long-chain monounsaturated fatty acids, into their tissues. This report implies that feed producers should use raw materials rich in essential fats to improve their nutritional values (Lessire, 1995).

There has been growing interest in using insects as a suitable source of protein in animal feed, and many studies have shown the impact of incorporating partially or totally defatted insect meals into poultry feed (Al-Qazzaz et al., 2016; Maurer et al., 2016; Marono et al., 2017). Although some studies have looked at substitution, they have only been short-lived and or focused on substituting a vegetal ingredient (soy) with an animal (insect) component (Kawasaki et al., 2019). Also, most of these studies have mainly identified only the protein or lipid potential of the maggots in the diet of laying hens on performance parameters (Al-Qazzaz et al., 2016; Maurer et al., 2016; Schiavone et al., 2017). However, few studies have shown interest in evaluating the effect of black soldier larvae meal, including in laying hen diet, on the quality values of egg, which will allow a better evaluation of this new feed ingredient in poultry production. One of the important concerns that remain with regard to the use of insect meal in the laying hen diet is whether the health of consumers will be affected by the extensive use of this ingredient. In this context, the aim of this study was to assess the impact of the long-term partial or total replacement of fish meal by undefeated maggot meal on the quality and safety of the layer eggs.

MATERIALS AND METHODS

Ethical approval

This study was carried out in strict compliance with the recommendations of the Guide for the Care and Use of Experimental Animals of the University of Lomé, Togo (008/2021/BC-BPA/FDS-UL). The protocol was approved by the Animal Experimentation Ethics Committee of the same University. All efforts were made to minimize chicken discomfort.

Animals and food

Maggot production

The meal of black soldier fly larva used in this study was produced at the Regional Centre of Excellence in Avian Sciences (CERSA), University of Lomé, Lomé, Togo. *Hermetia illucens* larvae were produced by Sheppard et al. (2002) modified method. Freshly laid eggs on stacked wooden sticks were transferred to plastic dishes for incubation, where the larvae hatched between three and four days. The larvae were initially fed a diet of local brewers' grains. After a maximum development time of 6 days, the larvae were transferred to growth units on substrates composed of 1/3 brewers' grains and 2/3 palm kernel cakes. The larvae were fed these vegetarian by-products until day 14.

The larvae were harvested before they reached the pre-pupal stage to have a low degree of sclerotization of the cuticle and the resulting higher digestibility (Bosch et al., 2014). They were washed, followed by killing, and dried at 70°C for 48-72 hours, depending on the water content. Once dried, the maggot larvae were mechanically ground into a fine powder. The resulting product was a *Hermetia* larvae meal with 25% fat, 9.57% ash, and 41.1% crude protein (Table 1).

Table 1. Chemical-nutritional characteristics of the black soldier fly larvae meal and commercial fish meal

Chemical nutritional characteristics	Black soldier fly larvae	Fish meal 40%
Dry mater (%)	91.3	90.4
Crude protein (%)	41.1	40
Crude fat (%)	25	10
Cholesterol (% Ether extract)	79.42	22.10
Ash (%)	9.57	18.2
Crude fibre (%)	7	1.5
Lys (%)	4.90	1.91
Met (%)	1.84	1.54
Met+ Cys (%)	1.42	2
Tryptophan (%)	0.47	0.04
Threonine (%)	1.20	1.53
Calcium (%)	6.50	4
Total phosphor (%)	1.05	2.5
Metabolisable Energy (MJ/kg)	13	11

DM: Dry mater, EE: Ether extract, CF: Crude fibre, Lys: Lysine, Meth: Methionine, Cys: Cystine, Tryp: Tryptophan, Threo: Threonine, Ca: Calcium, P: Phosphore, ME: Metabolism energy, CNC: Chemical nutritional characteristics and AAS, Met+ Cys: Methionine + Cysteine

Animals

A total of 480 one-day-old Isa brown laying chicks were randomly assigned to four treatment groups; each treatment group had 6 replicates of 20 chicks each. The groups were named T0 (8% fish meal), T1 (4% maggot meal and 4% fish meal), T2 (6% maggot meal and 2% fish meal), and T3 (8% maggot meal, Table 2). Feed was supplied in mash form in all four diets by CERSA feed unit (University of Lomé, Togo). The chicks were purchased from Incubel SA. (Hoogstraten, Belgium) and the experiment lasted 56 weeks (August 2020- September 2021). The chicks were reared on the floor on wood shavings in 24 cages of surface 4 m^2. The chickens were randomly allocated to an open hen house on deep litter with a natural environment, ventilation, temperature (21°C-27°C, and humidity (78%) to avoid position effects. The lighting program consisted of continuous light from weeks 1 to 8, followed by 18 hours of light from weeks 9 to 56.

Experimental device

The eggs used were collected from layer chickens in each group. The external and internal qualities of the eggs were determined at a 14-day interval throughout the experimental period. Parameters were collected between weeks 22 and 56 of age. The data collected on the two laying phases (between weeks 22 and 39, then weeks 40 and 56) made it possible to evaluate the interaction effects between the treatment and the age of the layers.

Table 2. Composition of experimental diet fed Isa brown laying chicks during the starter and grower and laying period

Treatments / Ingredients (kg/100kg)	Starter (1-8 weeks of age)				Grower (9-20 weeks of age)				Laying period (21-68 weeks of age)			
	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3
White maize	52.8	51.8	51.6	51.1	55	54	54	54	54.5	54	54	53.9
Wheate bran	10	11	12	12.5	15	16	16.5	16.5	9.5	10.3	10.9	11
Fish meal	8	4	2	0	8	4	2	0	8	4	2	0
Soya seed	20	20	19	19	13	13	12.5	12	14.5	14	13.3	12.8
Oyster shell	2	2	2	2	5	5	5	5	8	8	8	8
Concentrate	7	7	7	7	4	4	4	4.5	5	5	5	5.5
Lysine	0.1	0.1	0.2	0.2	0	0	0	0	0.2	0.3	0.4	0.4
Methionine	0.1	0.1	0.2	0.2	0	0	0	0	0.3	0.4	0.4	0.4
Maggot meal	0	4	6	8	0	4	6	8	0	4	6	8
Total (Kg)	100	100	100	100	100	100	100	100	100	100	100	100
Chemical nutritional characteristics												
DM[1] (%)	98.68	98.55	98.42	98.35	98.12	97.89	97.82	97.82	98.75	98.64	98.56	98.55
CP (%)	20.26	20.37	20.25	20.30	17.12	17.23	17.11	17.07	18.29	18.35	18.25	18.29
EE (%)	7.34	7.94	8.06	8.36	6.15	6.75	6.96	7.16	6.38	6.89	7.06	7.27
Ash (%)	1.56	1.62	1.62	1.65	1.49	1.55	1.55	1.52	1.26	1.28	1.28	1.26
CF (%)	4.63	4.90	5.04	5.18	4.82	5.10	5.23	5.31	4.49	4.59	4.72	4.80
Lys (%)	1.22	1.33	1.45	1.50	0.95	1.07	1.11	1.17	1.15	1.33	1.45	1.51
Meth (%)	0.63	0.65	0.76	0.77	0.43	0.45	0.46	0.49	0.72	0.84	0.85	0.87
AAS (%)	0.86	0.80	0.80	0.88	0.62	0.60	0.58	0.58	0.93	1.00	0.98	0.98
Tryp (%)	0.11	0.11	0.11	0.11	0.09	0.09	0.09	0.09	0.09	0.09	0.08	0.08
Threo (%)	0.33	0.33	0.33	0.33	0.26	0.26	0.26	0.25	0.25	0.25	0.24	0.23
Ca, (%)	1.71	1.81	1.86	1.91	2.29	2.39	2.44	2.53	2.92	3.02	3.07	3.13
Total P, (%)	0.58	0.53	0.51	0.47	0.60	0.55	0.53	0.50	0.67	0.62	0.59	0.57
ME (MJ/kg)	12.1	12.1	12.1	12.1	11.5	11.5	11.5	11.5	11.5	11.5	11.5	11.5

EE: Ether extract, CF: Crude fibre, Lys: Lysine, Meth: Methionine, Cys: Cystine, Tryp: Tryptophan, Threo: Threonine, Ca: Calcium, P: Phosphore, ME: Metabolism energy, AAS: methionine + cysteine. NB: Chemical nutritional characteristics are calculated based on the feed

External egg quality

Six eggs per replicate (48 eggs per treatment) were collected every 4 weeks. The egg weight (MEW) per treatment was calculated according to the formula MEW = ∑W/N. Where W is egg mass and N denotes the number of eggs. The width and height of each egg were measured (Breadth-B; Length-L) with a digital caliper with an accuracy of 0.01 mm. The following parameters were calculated using the weight of the eggs.

Shape index = B/L (Panda, 1996), Shell index (SI g/cm2) = (Shell weight / Shell surface area, Rodriguez-navarro et al., 2002), and the volume V (cm^3) is $\pi/6 \times L$ (cm) $\times B^2$ (cm^2, Bonnet and Mongin, 1965). Moreover, the density was calculated as density (g/cm^3) = MEW/V, where MEW(g) is egg weight and V (cm^3) is the volume of the egg. The acoustic test was repeated for all eggs using the same device (Digital caliper blet, China). Each egg was classified as intact or cracked.

Internal egg quality

The weight of the yolk and that of the albumen were measured using an electronic balance with a sensitivity of 0.01 g. Albumen height and Haugh unit (HU) were measured using an electric micrometric (Futura, Lohne, Germany) tripod with an accuracy of 0.01 micrometer. Egg yolk color was measured using the Roche color scale (RCF), which is an industrial color scale with visual ratings ranging from 1 (light yellow) to 16 (dark orange). The pH measurement (pH digital meter OAKTON pH700) was taken individually for each egg in albumen and egg yolk by immersing the probe inside the sample solution. Between two measurements, the electrode was cleaned with distilled water and then recalibrated using a buffer solution (Silversides and Budgell, 2004).

Yolk total fat

At 38-41weeks of age, lipid and cholesterol content were measured. The materials used for the extraction of fat were the Soxhlet and the oven. The reagent used for this analysis was hexane. The test portion (M_1) of the sample was about 0.5 g to 1 mg and the time of analysis was about 1 hour. The samples were weighed and placed in cellulose cartridges. These cartridges were inserted into crucibles containing 30 ml of hexane previously placed under vacuum mass, M_2, in the oven for 10 minutes and cooled in a desiccator. The crucibles containing the hexane and the cartridges containing the samples were placed in the Soxhlet. The fat extraction took place in three stages for one hour: boiling, rinsing, and hexane recovery. The crucibles were then recovered, kept in an oven for 15

minutes, cooled in a desiccator, and weighed (M_3.) The fat content was obtained using the following formula:

$$\text{Fat contain} = \frac{M_3\text{-}M_2}{M_1} \times 100$$

Cholesterol profiling

Total cholesterol and High-density lipoprotein (HDL) cholesterol were determined using the respective cholesterol assay kit (Fortress Diagnostics, UK). The kit contained the standards, assay reagent, and color development reagent (Pasin et al., 1998).

Total cholesterol

The lipid extract obtained was used for the determination of the cholesterol profile. Therefore, 10 µl of the sample/standard and 1000 µl of cholesterol reagent were added. The solution was vortexed and incubated for 5 minutes at 37°C. The absorbance of the color developed by the sample or standard was measured against the reagent blank at 505 nm.

$$\text{Concentration of Cholesterol} = \frac{\Delta \text{ absorbance } of\ sample}{\Delta \text{ absorbance } of\ Standard} \times standard\ concentration.$$

HDL and LDL

A portion of 0.4 ml of the precipitation reagent was piped into a centrifuge tube. 0.2 ml of sample was taken, mixed, and allowed to stand at room temperature (25°C) for 5 minutes. The solution was centrifuged at 4000 rpm for 10 minutes. 100 µl of the sample/standard and 1ml of cholesterol reagent were added. The solution was vortexed and incubated for 5 minutes at 37°C. The absorbance of the developed color of the sample or standard against the reagent blank was then measured (Pasin et al., 1998).

$$\text{Concentration HDL} = \frac{\Delta \text{ absorbance of sample}}{\Delta \text{ absorbance of Standard}} \text{ x standard concentration}$$

Statistical analysis

All external and internal egg quality data were analyzed using graph pad prism 9.0 software. One-way analysis (ANOVA one-way) was used for data analysis. The comparison of the means was made using the Tukey test with $p < 0.05$ as the significance threshold. For the assessment of the interaction between age and treatment, a two-way factorial analysis of variance (ANOVA two-way) was used. Principal Component Analysis (PCA) was performed using XLSTAT software (version 2021) to assess the correlation between parameters. The results are presented as mean ± standard error of the mean.

RESULTS

Larvae meal and external egg quality

The physical parameters of the collected eggs showed no significant difference among the treatment groups; Egg weights, shell weight, shape index, shell index, egg surface, egg volume, and density were not influenced by the use of larva meal ($p > 0.05$). However, cracked eggs in the control group were significantly higher ($p < 0.05$) than those in the treatment which received the maggot meal (Table 3).

Larvae meal and internal eggs quality

The results showed a significant influence on the percentage of albumen, the albumen height, and the Haugh unit of the treatments T1, T2, and T3, which were greater than those of the control group ($p < 0.05$). The proportion of egg yolk and the ratio between the yolk and the albumen of the control hens were statistically higher. However, yolk pH, albumen pH, yolk and albumen moisture content (Mc), and yolk color and height were not affected by the treatments ($p > 0.05$, Table 4).

Egg quality parameters of the laying phases

Egg weight

The egg weights of the second phase of laying were greater than the weight of the first phase (Table 5). There was no interaction between maggot meal feeding and hen age on egg weights. The proportion of the egg albumen in the second phase of laying was statistically lower than the proportion of the first phase ($p < 0.05$). There was no interaction between maggot diet and hen age on the percentage of albumen ($p > 0.05$). The proportion of yolk in the second phase of laying was Significantly higher than the proportion of the first phase ($p < 0.05$). The treatments showed no interaction between maggot meal feeding and hen age on egg yolk percentage ($p > 0.05$). The Haugh unit of the egg albumen of the second phase of laying was significantly lower than those of the first phase ($p < 0.05$). The treatments showed no interaction between maggot meal feeding and the age of hens in the Haugh unit ($p > 0.05$).

Total lipid level of egg yolk

The fat content of eggs in the control group was significantly higher than that of hens fed maggot meal ($p < 0.05$, Figure 1). This rate was statistically similar for the yolk of T1 and T3 eggs. Hens received 75% maggot meal showed yellows with a low proportion of fat (Table 4).

Total cholesterol level of egg yolk

The total yolk cholesterol level of the control group hens fed fish meal was significantly higher than T1 hens ($p < 0.05$, Figure 2). This level was higher in treatment T0 (445.6 mg/100 g of egg yolk), compared to 340, 393.8, and 410.7 mg/100g for T1, T2, and T3), respectively. The proportion of cholesterol in the yolk increased significantly with the increase in the level of larvae meal in the feed ($p < 0.05$) however remained statistically similar for the yolk of T2 and T3 eggs ($p > 0.05$).

High-density lipoprotein of egg yolk

The egg yolk HDL cholesterol level of the control group hens fed fish meal was significantly higher than maggot meal ($p < 0.05$, Figure 3). The proportion of HDL cholesterol in yolk increased with the increase in the level of maggot meal in the feed. It was significantly higher for the yolk of T2 and T3 eggs, compared to T1 ($p < 0.0001$). This rate was very high for batch T0 (349.4 mg/100 g of

egg yolk against 280.3, 331.6, and 330.3 mg/100g for T1, T2, and T3, respectively).

Low-density lipoprotein of egg yolk

The yolk low-density lipoprotein (LDL) cholesterol level of the control group hens fed fish meal was significantly higher than maggot meal ($p < 0.05$). The proportion of LDL cholesterol in yolk increased with the increase in the level of maggot meal in the feed. It was significantly higher for egg yolk T3 compared to lower T1 and T2 and statistically comparable ($p < 0.05$, Figure 4). This rate was very high for T0 treatment (96.19 mg/100 g of egg yolk against 59.73, 62.24, and 98.2 mg/100 g for T1, T2, and T3, respectively).

Atherogenicity index

The LDL/HDL ratio appeared to increase with the incorporation rate of maggot meal. It was significantly low compared to the control for 4% and 6% incorporation; however statistically comparable between 8% larvae meal inclusion and the control group ($p < 0.05$, Figure 5).

Principal component analysis

Correlation circle

Figure 6 shows the degree of connection between the variables. Yolk height, yolk color, albumen percentage, and Haugh unit were positively correlated. Similarly, total fat content, total cholesterol level , and HDL level on the one hand, then shell area, albumen moisture content, volume, and egg weight on the other were closely related and positively correlated. The shell area and albumen moisture content were negatively correlated with albumen pH ($r = -0.97$ and $r = -0.99$, respectively).

Treatment

The use of soldier fly larvae meal showed two different groups of characteristics. The eggs of groups T1, T2 and T3 from hens fed with soldier fly larvae meal had comparable characteristics. The eggs of the control groups (T0) showed different characteristics (Figure 7).

Combination of correlation circle and treatment

Combining the correlation circle with the treatment map showed that T1 and T3 were grouped by moisture content, albumen height, egg weight, shell area, volume, and Haugh unit on axis 1 (Figure 8). However, on axis 2, the T3 treatment was characterized as the T0 eggs by the high values of LDL level and egg density. The T0 treatment was distinguished from T3 by the yolk percentage, the total cholesterol level, and the yolk/albumen ratio.

Table 3. Effect of larvae meal on the external quality of the egg (mean values)

Parameters \ Treatments	T0	T1	T2	T3	P-Value
Egg weight (g)	53.01	53.34	53.11	54.87	0.2284
Shell weight (g)	7.06	7.08	7.04	7.35	01744
Shape index	0.76 ± 0.003	0.77 ± 0.003	0.76 ± 0.002	0.77 ± 0.003	0.3753
Shell index	0.087 ± 0.001	0.087 ± 0.001	0.087 ± 0.001	0.088 ± 0.001	0.5323
Shell proportion (%)	11.98	11.91	12.23	12.07	0.6381
Cracked eggs (%)	0.44	0.16	0.17	0.05	0.0186
Egg area (Cm^2)	80.60 ± 0.67	81.03 ± 0.60	80.83 ± 0.80	82.22 ± 0.74	0.3729
Volume (Cm^3)	48.81 ± 0.52	49.06 ± 0.46	49.36 ± 0,65	50.47 ± 0.63	0.1837
Density	1.08 ± 0,01	1.07 ± 0.01	1.08 ± 0.01	1.09 ± 0.01	0.8541

[a,b,c] The mean values within the same row with different superscript differ significantly ($p < 0.05$). T0: 8% fish meal, T1: 4% maggot meal and 4% fish meal, T2: 6% maggot meal and 2% fish meal, and T3: 8% maggot meal

Table 4. Effect of larvae meal on the internal composition of the egg (mean values)

Parameters \ Treatments	T0	T1	T2	T3	P-value
Albumen (%)	60.95	62.75	62.32	62.35	0.0045
Albumen height (mm)	6.79 ± 0.06^b	7.34 ± 0.07^a	6.88 ± 0.06^b	7.28 ± 0.86^a	0.0001
Albumen moisture content (%)	83.10	83.40	83.21	83.91	0.3659
Yolk (%)	25.01	24.04	24.14	24.14	0.0071
Yolk height (mm)	14.11 ± 0.10^b	14.49 ± 0.08^a	14.22 ± 0.09^b	14.30 ± 0.11^b	0.0431
Yolk index	0.44 ± 0.01	0.44 ± 0.01	0.43 ± 0.01	0.44 ± 0.01	0.3080
Color of yolk	7.75 ± 0.27	8.03 ± 0.39	8.13 ± 0.39	8.04 ± 0.30	0.8830
Yolk moisture content (%)	60.92	58.95	57.34	58.82	0.1652
Yolk/albumen	0.41 ± 0.00^a	0.39 ± 0.01^b	0.40 ± 0.01^a	0.40 ± 0.01^a	0.0003
Haugh unit	84.18 ± 0.84^b	87.61 ± 0.83^a	85.71 ± 0.83^b	87.69 ± 1.1^a	0.0008
Albumen pH	7.76 ± 0.01	7.73 ± 0.01	7.75 ± 0.01	7.70 ± 0.02	0.0623
Yolk pH	5.34 ± 0.04	5.35 ± 0.06	5.34 ± 0.07	5.33 ± 0.02	0.9195

[a,b,c] The mean values within the same row with different superscript differ significantly ($p < 0.05$). T0: 8% fish meal, T1: 4% maggot meal and 4% fish meal, T2: 6% maggot meal and 2% fish meal and T3: 8% maggot meal, Mc: Moisture content.

Table 5. Effect of maggot meal and age of layers on egg weight, shell proportion, albumen proportion, Haugh unit, and yolk/albumen ratio (mean values)

Parameters	Laying period								P-value		
	22-39 weeks				40-56 weeks						
	T0	T1	T2	T3	T0	T1	T2	T3	Diet	Age	Diet×age
Egg weight (g)	50.22±4.58^{b}	51.42±4.1^{b}	49.97±5.20^{b}	51.42±4.86^{b}	56.36±3.97^{a}	55.75±3.83^{a}	57.11±4.59^{a}	58.58±4.99^{a}	0.1088	<0.0001	0.2145
Shell (%)	12.40	12.20	13.00	12.39	11.58	11.63	11.47	11.76	0.4762	<0.0001	0.0987
Albumen (%)	62.38	64.25	62.18	64.11	61.03	61.24	60.25	61.01	0.0003	<0.0001	0.0839
Haugh unit	85.36±3.33^{b}	88.97±3.64^{a}	85.50±3.34^{b}	88.09±3.97^{a}	83.01±3.53^{c}	85.81±3.42^{d}	83.79±3.91^{c}	85.05±3.20^{d}	<0.0001	<0.0001	0.4620
Yolk (%)	24.56	23.44	23.67	23.50	25.45	24.64	24.61	24.68	0.0004	<0.0001	0.9096
Yolk/Albumen	0.39±0.04^{c}	0.36±0.04^{d}	0.38±0.04^{c}	0.36±0.04^{d}	0.42±0.04^{a}	0.40±0.05^{b}	0.41±0.02^{a}	0.40±0.04^{b}	0.0007	<0.0001	0.6386

^{a,b,c,d,e} The mean values within the same row with different superscript differ significantly ($p < 0.05$). T0: 8% fish meal, T1: 4% maggot meal and 4% fish meal, T2: 6% maggot meal and 2% fish meal, and T3: 8% maggot meal.

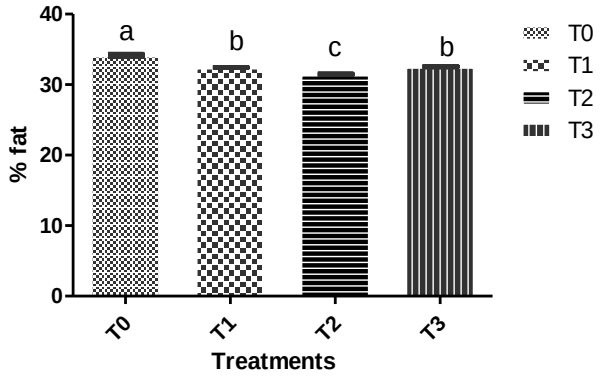

Figure 1. Effect of different level of larvae meal on the variation of the total fat content of the Isa brown laying chicks' eggs. ^{a,b,c} The mean values within the same row with different superscripts differ significantly ($p < 0.05$). T0 (8% fish meal), T1 (4% maggot meal and 4% fish meal), T2 (6% maggot meal and 2% fish meal) and T3 (8% maggot meal).

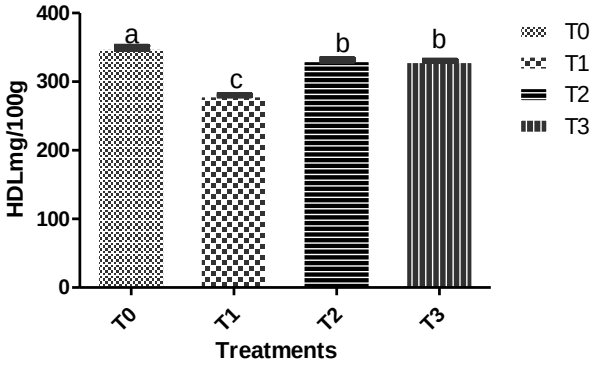

Figure 3. Effect of different level of larvae meal on the variation of the HDL cholesterol level of the Isa brown laying chicks' eggs. ^{a,b,c} The mean values within the same row with different superscripts differ significantly ($p < 0.05$). T0 (8% fish meal), T1 (4% maggot meal and 4% fish meal), T2 (6% maggot meal and 2% fish meal) and T3 (8% maggot meal).

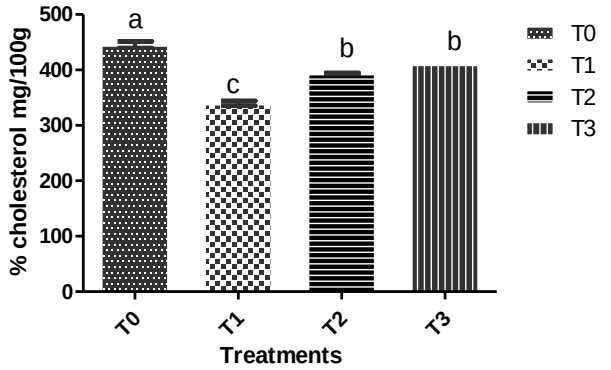

Figure 2. Effect of different level of larvae meal on the adequacy of total cholesterol levels in the Isa brown laying chicks' eggs. ^{a,b,c} The mean values within the same row with different superscripts differ significantly ($p < 0.05$). T0 (8% fish meal), T1 (4% maggot meal and 4% fish meal), T2 (6% maggot meal and 2% fish meal) and T3 (8% maggot meal).

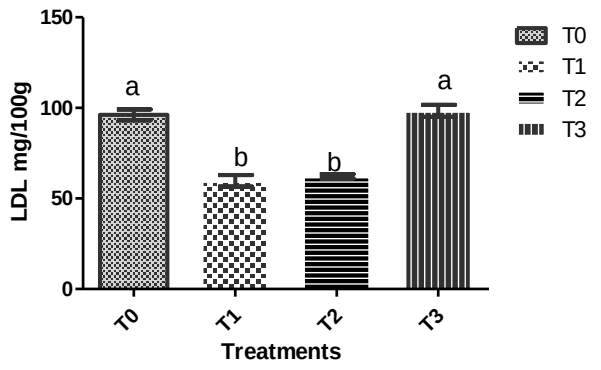

Figure 4. Effect of different level of maggot meal on the level of HDL cholesterol levels in the Isa brown laying chicks' eggs. ^{a,b,c} The mean values within the same row with different superscript differ significantly ($p < 0.05$). T0 (8% fish meal), T1 (4% maggot meal and 4% fish meal), T2 (6% maggot meal and 2% fish meal) and T3 (8% maggot meal).

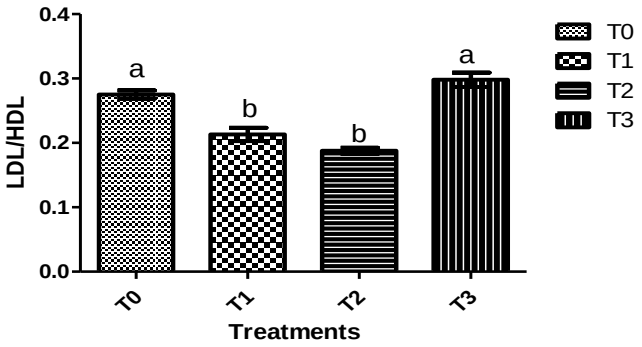

Figure 5. Effect of different level of maggot meal on the level of HDL cholesterol levels in the Isa brown laying chick's eggs. [a,b,c] The mean values within the same row with different superscripts differ significantly ($p < 0.05$). T0 (8% fish meal), T1 (4% maggot meal and 4% fish meal), T2 (6% maggot meal and 2% fish meal) and T3 (8% maggot meal).

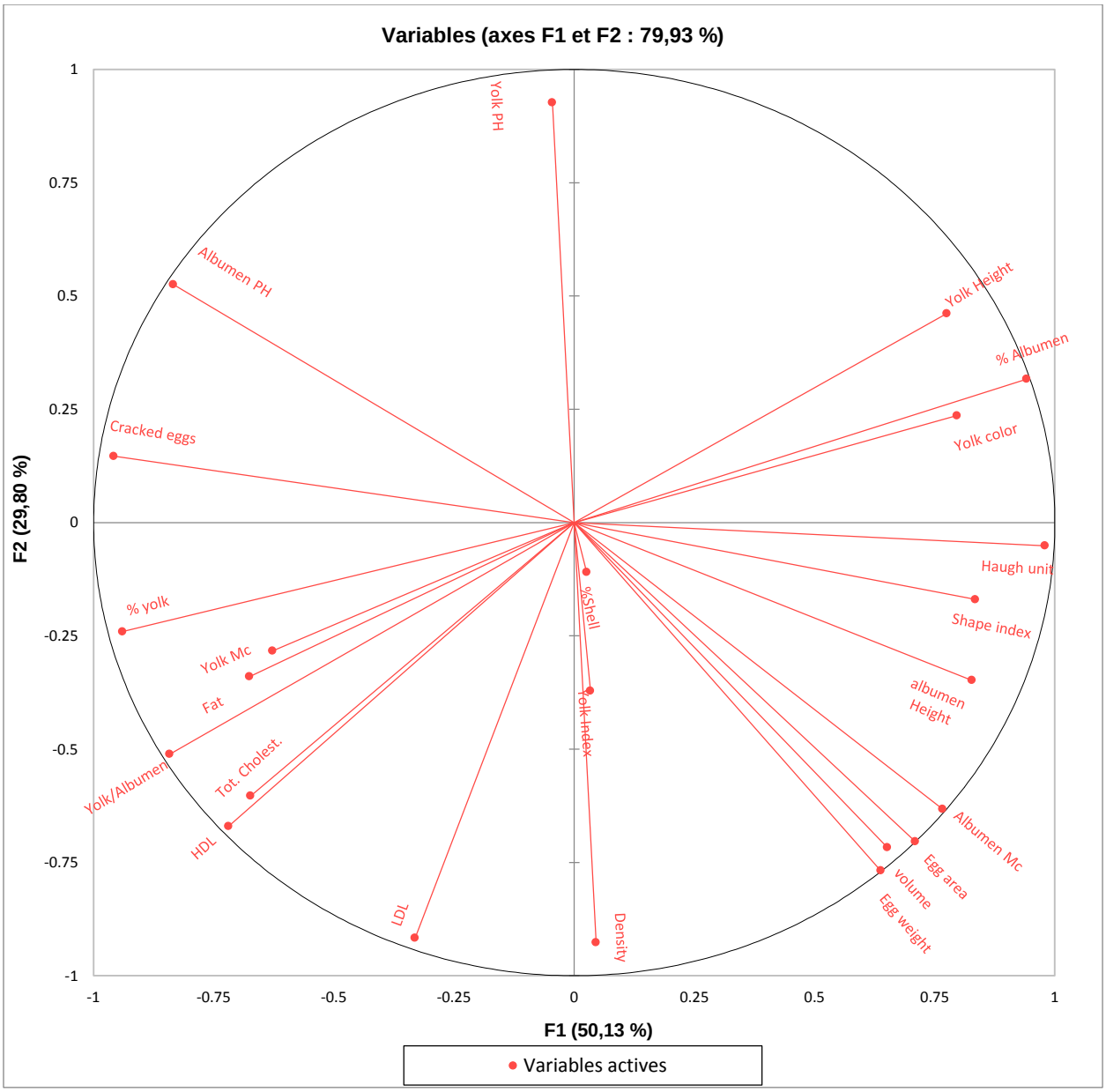

Figure 6. Correlations between measured parameters. Haugh unit, Shape index, Volume, Albumen height, Albumen moisture content, Egg surface area, Egg weight, Egg shell proportion (%), Yolk index; Density, Total cholesterol, HDL, LDL, Yolk/albumen ratio, Yolk proportion (%), Cracked eggs, Albumen pH, Yolk pH, Yolk height, Yolk color, Albumen proportion (%).

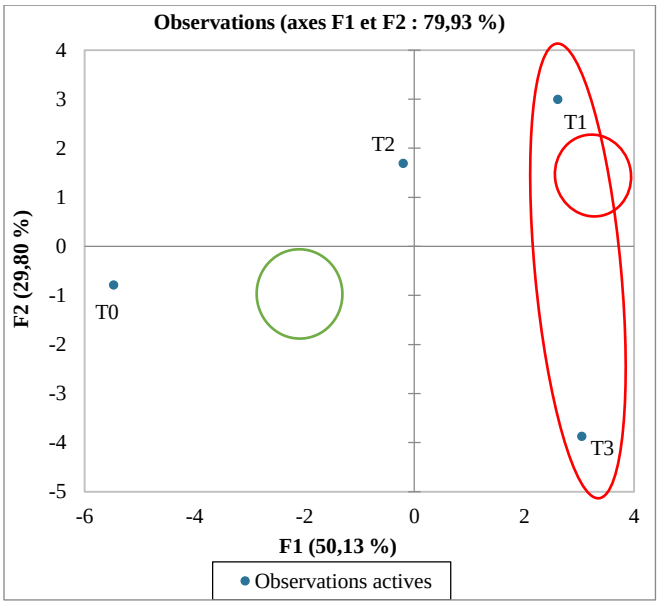

Figure 7. The differences and similarities of the treatments

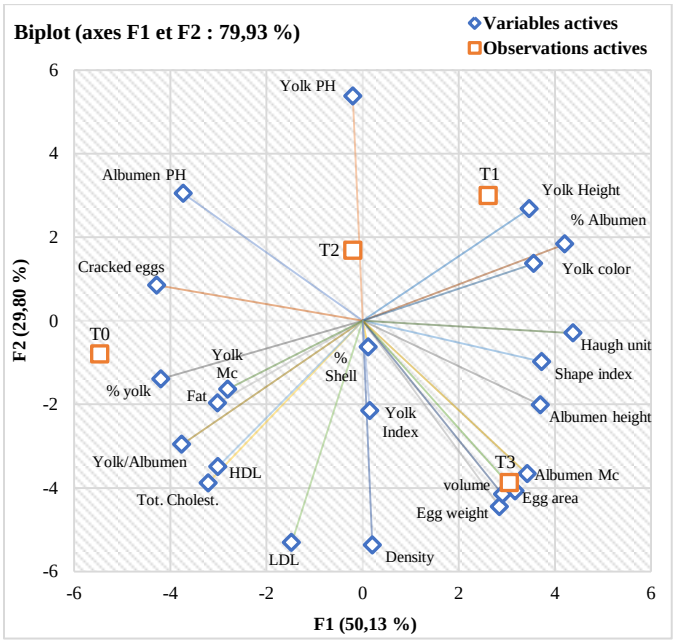

Figure 8. Combination of correlation circle and similarities of the treatments

DISCUSSION

In this study, the mean value of the Haugh units was between 84.18 and 89.69 (Table 4), which is similar to the values observed by Akpodiete et al. (1998), indicating that fresh eggs had an HU value of more than 72. Although age and storage time are the main factors influencing egg freshness, the source of dietary protein and its amino acid profile can also modulate the albumen height and, thus, the Haugh unit of the egg. Therefore, the improved Haugh unit of eggs from hens fed soldier fly larvae meal is explained by the higher lysine content (Roberts, 2004). The significant values of albumin height and Haugh's unit are in agreement with the results observed by Brah et al. (2017) and Park et al. (2017) using grasshopper meal and black soldier fly pupa meal, respectively, in laying hen feed. Present results showed that the albumen is the part of the egg most affected by the larvae meal (Akpodiete et al., 1998; Irawan et al., 2019).

The yolk/albumen ratio was higher in control eggs receiving 8% fish meal. The increase in the proportion of yolk in the eggs of hens fed with the control feed (T0) was due to the reduction in the proportion of albumen. Larvae meal had no effect on the yolk color in the present study. This result is consistent with those found in previous studies (Agunbiade et al., 2007; Amao et al., 2010). Dietary treatment did not affect egg and shell weight ($p > 0.05$). The results of this study are consistent with the findings of Akpodiete et al. (1998), who showed that the use of soldier fly larvae meal does not influence egg weight and yolk color ($p > 0.05$). Similarly, the results of this study confirmed the deduction of Olteanu et al. (2012), who concluded that feeding insect meals has no significant effect on egg weight. The yolk color of hens fed black soldier fly larvae meal showed no significant difference from the yolk of the control group.

Although the larvae used were raised on plant substrates, this result indicates that there was no possible transfer of carotenoids or xanthophyll pigments from these plant residues by the larvae into the coloration of the yolk (Suparman et al., 2020). The average value of the egg shape index and egg surface area for hens fed the larvae meal were 0.76-0.77 and 80.83-82.22 cm^2, respectively. These values were not significantly different from the control group ($p > 0.05$). Similar results were also observed by the report of Agunbiade et al. (2007) who showed that the egg shape index was not affected significantly with the use of maggot meal in a layer diet. The egg shape index determines the physical quality of the egg and provides information on the functional state of the isthmus and its diameter which it is closely dependent. The results of the present study indicated that larvae meal had no negative effect on shell gland muscle tone. Similarly, egg density, egg volume, albumen pH, and yolk pH were not influenced by larvae meal. However, the cracked egg content of the T1 and T3 groups was relatively low. Reducing these non-integrity defects of the shell that promote the penetration of pathogenic bacteria into the egg would reduce the risk of food poisoning linked to the consumption of eggs from this production.

In the current study, the increase in egg weight, yolk proportion, and decrease in Haugh unit during the second phase of egg laying would be due to the effect of age (Table 5). Indeed, as the hens' age increases, lipid catabolism increases due to a greater secretion of bile. This allows for better yolk formation. The improvement in yolk proportion is consistent with the results of Bejaei and

Cheng (2020). This study also showed that the large variations in the results obtained in several previous studies with the use of fly larvae for the assessment of egg parameters would depend on the age or laying phase of the layers used for the experiment concerned. In this study, the age of the hen and the level of fly larvae meal incorporated into the feed had effects on the proportion of albumin and the Haugh unit, but statistical analysis showed no interaction between these two factors. This observation is contrary to the results of Bejaei and Cheng (2020), who found no improvement in albumin weight and no significant effect on the Haugh unit. This contradiction would be due to the very high level of larvae meal incorporated and their method of analysis. In a recent study, Star et al. (2020) found no significant effect on the Haugh unit with the inclusion of soldier fly larvae in the hen's diet but found the period of their experiment too short. The same authors supported their results by reporting the age and laying phase of their laying hens. The results of the current study are in agreement with Akpodiete et al. (1998), who conducted a 56-day experiment to determine the effect of maggot meal supplementation in chicken diets and found that albumin weight was significantly affected. This observation was different from those of Shah (2020), who explained their result by the difference in age of the laying hens.

The chemical composition of the eggs obtained in this study is in agreement with those of Shin et al. (2013), who observed depletion of the lipid content of the egg yolk of hens fed with black soldier fly larva meal. Total lipid levels decreased with the replacement of fish meal with larvae meal (Figure 1). Eggs from hens fed larvae meal had significantly lower fat levels than control eggs ($p < 0.05$). According to Hossain and Blair (2007), chitin causes a decrease in triglyceride levels, which is the main component of total lipids. These authors showed a reduction in serum cholesterol and triglycerides by incorporating commercial chitin in the diet of broilers from 1 to 21 days of age at zero, 25, 50, and 75 g/kg. The effect of chitin on triglyceride reduction could be attributed to its positive charge capable of attracting negatively charged bile acids and free fatty acids during digestion (Prajapati and Patel, 2010). The lipid depletion was 1.6 g less fat in 100 g of egg yolk from hens receiving only larvae meal (T3) compared to the control. These results are also consistent with the results of Secci et al. (2018), who showed a decrease in yolk lipid levels with the use of maggot meal. Eggs from layers fed with larvae meal have reduced fat content, which could be advantageous for consumers who increasingly desire less fat in their food for their health and safety. Cholesterol levels in the yolk are known to be more regulated by endogenous metabolism than by dietary cholesterol. The average cholesterol level obtained during the current test was 445.6 mg/100g for T0. This was between 340 mg/100 g and 410, 7 mg/100 g or 3.4 to 4.1 mg/g of egg yolk for the eggs of layers fed larvae meal (Figure 2). The mean egg yolk cholesterol level in this study was lower than the values obtained by Irawan et al. (2019) and Cayan and Erener (2015), who found cholesterol levels of 5.20-5.90 mg/g and 8.34-9.24 mg/g for egg yolk, respectively.

Fat and cholesterol contents were positively correlated with the egg yolk proportion ($r = 0.79$, Figure 5). Cholesterol level in the egg yolk depends mainly on the balance between cholesterol biosynthesis and excretion (Griffin, 1992). The reduction in cholesterol levels in eggs in groups fed larvae meal is thought to be due to the fatty acid profile of larvae meal (Mazalli et al., 2004). Previous research testing meal from black soldier fly larvae as a feed ingredient in laying hens (100% replacement of soybean meal), observed an 11.7% reduction in yolk cholesterol content in hens receiving the experimental diet comprising the black soldier fly larvae meal compared to a control group fed soybean meal (Secci et al., 2018). This study shows that black soldier fly larvae meal resulted in a decrease in the cholesterol level of the eggs. As well as the triglycerides, this reduction in cholesterol level would also depend on chitin, a natural component of the insect exoskeleton, which exerts an attraction on negatively charged bile acids and free fatty acids (Prajapati and Patel, 2010). The reduction observed was 105mg/100g, 51.8 mg/100g, and 35mg/100g for cholesterol in egg yolk T1, T2, and T3, respectively (Shin et al., 2013). Cholesterol and its esters are found only in egg yolk, where they form an emulsion of LDL and HDL. HDL and LDL cholesterol positively correlated with total cholesterol ($r = 0.97$ and $r = 0.81$, respectively), increased with the black soldier fly larvae meal use rate. This study showed that larvae meal would have induced an important secretion of vitellogenin, which oocytes represent the principal target of accumulation (Voet and Voetová, 1995). Indeed, egg yolk lipoproteins known as vitellogenin have biochemical qualities similar to mammalian serum lipoproteins (Voet and Voetová, 1995). They are synthesized in the liver and secreted into the blood, where they are captured by oocytes. The atherogenic index (LDL/HDL) is a relevant factor in assessing the nutritional quality of the egg for consumer protection. Eggs with a low atherogenic index are good for delaying atherosclerosis and, therefore, the risk of cardiovascular disorders (EL-Wakf et al., 2010).

CONCLUSION

Black soldier flies maggot meal had no negative influence on the eggs' external, internal, and nutritious quality. It improved the proportion of albumen, a valuable source of high-quality protein. Egg freshness and chemical quality of 4% and 8% incorporation eggs have been improved. Meal from soldier fly larvae can reduce egg yolk fat and cholesterol content. In addition, larvae meal can be used at an incorporation rate of 4% or 8% in the feed throughout the rearing cycle of layers. However, dietary cholesterol that increases the sensitivity of LDL to oxidation and potentiates the adverse effects of dietary saturated fats in

humans remains very high in the egg. Following dietary recommendations that indicate the need to limit cholesterol intake to less than 300 mg/day, it would be desirable to consume eggs in moderation. Based on the results of the current study, the authors conclude that the long-term inclusion of black soldier fly larvae meal in laying hen diets is safe and feasible.

DECLARATIONS

Acknowledgments

The authors thank World Bank Grant International Development of Association (IDA) 5424, "Centre d'Excellence Regional sur les Sciences Aviaires (CERSA)" of the University of Lome, Togo, for financially supporting this study.

Authors' contribution

Kodjo Gnatépé Mlaga designed, performed the experiment (project administration Methodology; analysis), and wrote the manuscript. Komi Attivi participated in the manipulations and reading of the manuscript; Komi Agboka and Kokou Tona participated in the funding acquisition and reading of the manuscript. Elolo Osseyi supervised, analyzed data, and approved the final manuscript. All authors read and approved the final version of the manuscript for publishing in the present journal.

Competing interests

The authors declare no conflict of interest.

Ethical consideration

The authors have verified the ethical issues related to plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy).

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