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# Insects as a natural component of pheasant diets: effects of full-fat *Hermetia illucens* meal on egg production and quality, hatchability, and selected physicochemical egg indices

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**ABSTRACT.** The present study aimed to evaluate the effect of supplementing *Hermetia illucens* larval meal in pheasant diets on selected hatchability parameters and physicochemical properties of eggs. A total of 72 pheasants (63 females and 9 males) were randomly allocated to three groups: BSFL0, a basal diet without *H. illucens*, and BSFL10 and BSFL20 diets, with 100 and 200 g/kg *H. illucens* larval meal, respectively. The evaluation of selected egg production indices was based on 1200 properly developed eggs. The BSFL20 group exhibited the highest number of eggs and a reduced percentage of culled eggs compared to the control treatment. The lowest albumen height was determined in BSFL0, and the highest in BSFL20. In addition, the highest Haugh index values were also recorded for the BSFL20 group. Tendencies towards improved survival of chicks were observed up to day 7 and day 14 after hatching, connecting with the incrementing BSFL dose. Egg yolk dry matter and crude protein levels were increased in the BSFL20 group, accompanied by tendencies towards elevated crude ash and reduced cholesterol levels. A significant increase in C12:0 and C14:0 fatty acids in both experimental groups, and a decrease in C18:0 fatty acid levels in the BSFL20 group were observed. A reduction in C16:1 and monounsaturated fatty acid levels was noted in the experimental groups. The atherogenicity index was higher in the BSFL20 group, while the n-6/n-3 ratio was lower compared to the control. In conclusion, *H. illucens* can be implemented in pheasant diets with beneficial effects on the number of eggs and positive trends for selected survival indices. Furthermore, *H. illucens* full-fat meal positively modified the chemical composition of the eggs.

## Introduction

Pheasants, under strict hunting management, breeding, and current limited consumption have become a visible part of the fauna in Europe. Breeding programmes primarily focus on acquiring specimens with specific anatomical, morphological, and behavioural characteristics. These activities are aimed to obtain individuals with traits resembling those of wild birds capable of adapting to environmental pressures. Additionally, due to their valuable nutritional qualities, pheasants are also raised for both egg production and meat consumption (Ozbey et al. 2011; Geaumont et al. 2017; Uğurlu et al. 2017).

Pheasant meat is characterised by a fairly low fat content, a favourable fatty acid profile, particularly the proportion of n-3 to n-6 polyunsaturated fatty acids (Mieczkowska et al., 2015). However, pheasant eggs are mainly utilised for reproduction purposes due to their seasonal production, suboptimal hatchability (41–79%), and consequently low market availability.

The incorporation of a diverse diet in terms of the quantity and quality of protein, energy components and various feed additives, influences in turn the number of eggs, their quality and hatchability, and chemical composition (Gugała et al., 2019; Flis and Gugała, 2021; El-Saadany et al., 2022). Certain animal protein feedstuffs, in addition to plant feed materials, have been introduced in order to improve production results and administer diets closely resembling natural conditions of these birds (Marono et al., 2017; Secci et al., 2021; El-Sabrout et al., 2023). To address taste and odour concerns in eggs or meat, insect meal has been explored as a substitute for limited amounts of fish meal (Anankware et al., 2015). These meals contain significant quantities of crude protein (50–65%), crude fat (7.9–47%), minerals and vitamins (Benzertiha et al., 2020; Khalifah et al., 2023). Many studies have highlighted the suitability of insect meals for pigs and poultry (Kierończyk et al., 2022a; Kierończyk et al., 2023; Khalifah et al., 2023). The incorporation of insect meal has been found to influence the fatty acid profile in broiler meat (Kierończyk et al., 2023), layer eggs (Secci et al., 2018; Chatzidimitriou et al., 2022) and Japanese quail (*Coturnix japonica*) (Secci et al., 2021). Therefore, its addition may also affect the fatty acid composition and cholesterol content of pheasant egg yolks. Additionally, the inclusion of black soldier fly (BSF; *Hermetia illucens*) larval meal may influence not only the chemical composition of the final products, but also laying performance, and egg quality properties, such as egg weight, eggshell percentage, and thickness (Marono et al., 2017, Secci et al., 2020).

The authors postulated that the incorporation of *H. illucens* larval meal might affect selected hatchability parameters and the physicochemical properties of eggs due to the replacement of plant-derived feed material with insect biomass, which is more similar to the natural diet of birds. Therefore, the purpose of this study was to determine the effect of supplementing *H. illucens* larval meal in laying pheasant diets on egg production and morphometric parameters, selected hatchability indices, and egg yolk chemical composition, with particular emphasis on the fatty acid profile.

# Material and methods

The experimental procedures used in this study were approved by the Local Ethics Committee on Animal Experimentation of the University of Life Sciences in Lublin, Poland (approval No. 62/2022 of May 18, 2022).

## Birds and housing

The study was carried out on 63 female (21 per group, 7 per cage) and 9 male (1 per cage) pheasants (*Phasianus colchicus*) (12 weeks old, first reproductive season) from the breeding flock of the experimental station. The experiment was conducted on a pheasant aviary farm located in Poland (Wierzchowiny in the Radzyń district; latitude 51.611514411746676, longitude 22.69260406494141). The experiment lasted 17 weeks from March 2022 to September 2022. The average initial body weight of the birds was approximately 930 g for females and 1245 g for males. The birds were kept outdoors in cages with the following dimensions: 8.5 m × 5.0 m × 3.5 m (9 cages, 3 pens per group). Each cage was equipped with two nipple drinkers and an automatic feeder (40-cm-long, i.e., 4.0 cm of feeder edge per bird). A natural mating system was applied in each cage, at a sex ratio of one male to seven females. All pheasants had *ad libitum* access to feed and water. The composition of the experimental diets and nutritive values are presented in Tables 1 and 2. The diets were formulated to meet or exceed the nutritional requirements of the National Research Council (NRC, 1994). The experimental design was as follows: BSFL0 – basal diet without *H. illucens* larval meal; BSFL10 – experimental diet with 100 g/kg *H. illucens* larval meal; and BSFL20 – experimental diet with 200 g/kg *H. illucens* larval meal. Full-fat BSF larval meal was added as a partial replacement for soybean meal (BSFL10 group) or soybean meal, sunflower meal, and flax seeds (BSFL20).

## Insect meal

The insect biomass was supplied by HiProMine S.A. (Robakowo, Poland). *H. illucens* larvae were fed a mixture of plant byproducts following the protocol outlined by Kierończyk et al. (2022b). Full-fat insect meals were produced by freezing the insect biomass at −20 °C, air-dried at 50 °C for 24 h, and subsequently homogenised using a beater mill (with a diameter <0.1 mm). The raw material was hygienised by heating at 100 °C for 95 min in accordance with the guidelines of Annex IV of European Commission Regulation No. 141/2011

**Table 1.** Ingredients (g/kg, as-fed basis) of experimental pheasant diets

| Ingredients, g/kg | Treatment | | |
|---|---|---|---|
| | BSFL0 | BSFL10 | BSFL20 |
| Maize | 291.5 | 310.9 | 359.9 |
| Wheat | 222.0 | 222.0 | 222.0 |
| Soybean meal, 478 g CP/kg | 180.0 | 70.0 | 0.0 |
| Garden pea | 50.0 | 50.0 | 50.0 |
| Black soldier fly meal, 519 g CP/kg | 0.0 | 100.0 | 200.0 |
| Flax seed | 40.0 | 40.0 | 0.0 |
| Sunflower meal | 80.0 | 80.0 | 30.0 |
| Sorghum | 50.0 | 50.0 | 60.0 |
| Soybean oil | 10.0 | 0.0 | 0.0 |
| NaCl | 3.0 | 3.0 | 3.0 |
| Dicalcium phosphate | 10.0 | 10.0 | 10.0 |
| Limestone | 60.0 | 60.0 | 60.0 |
| Mineral-vitamin premix[1] | 2.5 | 2.5 | 2.5 |
| DL-methionine | 0.0 | 0.2 | 0.7 |
| L-lysine chloride | 1.0 | 1.4 | 1.9 |
| Total | 1000 | 1000 | 1000 |

BSFL0 – basal diet without black soldier fly larval meal application, BSFL10 – experimental diet with 100 g/kg black soldier fly larval meal, BSFL20 – experimental diet with 200 g/kg black soldier fly larval meal; [1] provided per kg of diet: mg: Mn 60, I 1, Fe 50, Zn 100, Cu 12, Se 0.2, vit. E 50, vit. $K_3$ 2, vit. $B_1$ 1.5, vit. $B_2$ 4.5, vit. $B_6$ 3, vit. $B_{12}$ 0.015, biotin 0.1, folic acid 0.8, nicotinic acid 20, pantothenic acid 12, choline 300; IU: vit. A 10000, vit. $D_3$ 2500

**Table 2.** Chemical composition of full-fat meal from *Hermetia illucens* larvae and experimental diets

| Nutrients | BSFL meal | Treatment | | |
|---|---|---|---|---|
| | | BSFL0 | BSFL10 | BSFL20 |
| Dry matter, g | 978.6 | 894.8 | 897.3 | 898.7 |
| Crude protein, g | 518.8 | 186.8 | 189.3 | 192.5 |
| Lysine, g | 24.6 | 9.52 | 9.47 | 9.49 |
| Methionine + cysteine, g | 11.2 | 6.45 | 6.41 | 6.38 |
| Crude fibre, g | 90.8 | 47.4 | 49.6 | 42.5 |
| Crude ash, g | 119.7 | 43.4 | 48.3 | 51.9 |
| Calcium, g | 9.4 | 25.7 | 26.1 | 26.5 |
| Total phosphorus, g | 8.5 | 5.96 | 6.04 | 6.12 |
| Ether extract, g | 110.6 | 51.7 | 49.6 | 42.5 |
| Lauric acid, C12:0, % | 37.96 | 0.01 | 3.81 | 7.61 |
| Myristic acid, C14:0, % | 8.37 | 0.12 | 0.94 | 1.77 |
| Palmitic acid, C16:0, % | 15.31 | 11.28 | 11.76 | 12.68 |
| Stearic acid, C18:0, % | 3.03 | 2.19 | 2.08 | 1.88 |
| Oleic acid, C18:1, n-9, % | 14.51 | 20.63 | 19.89 | 19.64 |
| Linoleic acid, C18:2, n-6, % | 12.99 | 48.89 | 44.94 | 41.85 |
| Linolenic acid, C18:3, n-3, % | 1.16 | 4.81 | 4.07 | 2.56 |
| $AME_N$, MJ/kg | 11.82 | 11.63 | 11.78 | 11.88 |

BSFL meal – black soldier fly larval meal, BSFL0 – basal diet without black soldier fly larval meal, BSFL10 – experimental diet with 100 g/kg black soldier fly larval meal, BSFL20 – experimental diets with 200 g/kg black soldier fly larval meal, $AME_N$ – apparent metabolisable energy corrected to zero nitrogen balance calculated according to Fisher and McNab (1987)

on the processing of animal byproducts. All feed materials were mixed and pelleted (0.5 mm diameter) at 60 °C.

## Data and sample collection

The birds were weighed at the beginning of the experiment and at the end of the egg production period using a laboratory scale (Balance XPR404S, Mettler-Toledo, LLC, Columbus, OH, USA). The diets were administered to the pheasants for 4 weeks before the laying period. Eggs obtained at the peak of the egg production period (from May 20 to June 20, 2022, i.e., 30 days) were used in the experiment (n = 1200). The collected pheasant eggs were placed in trays and kept at 18 °C for 7 days. The storage conditions applied were based on the recommendations of Demirel and Kirikci (2009), who have emphasised that these parameters do not negatively affect egg quality or incubation parameters. Subsequently, 150 eggs of average weight were chosen from each group and placed in an incubator. The weight of hatching eggs was determined using a Balance XPR404S electronic scale (Mettler-Toledo, LLC, Columbus, OH, USA) with an accuracy of ± 0.01 g. The length and width of the eggs were measured using an MN-85-100 electronic slide calliper (Rawlplug Ltd., Glasgow, United Kingdom) with a precision of ± 0.01 mm. The egg shape index was calculated as the ratio of the short axis to the long axis of the egg measured with an electronic calliper to an accuracy of ± 0.01 mm. Shell area was calculated using the following equation provided by Kokoszyński et al. (2011):

$$P_s = 4.835 \times W^{0.662},$$

where: $W$ – the egg weight.

Egg protein quality, expressed in Haugh units ($H_u$), was calculated as the natural logarithm of protein height (H) taking into account the egg weight ($W$):

$$H_u = I_n(H) + 7.57 - 1.7W^{0.37}.$$

During the incubation period, the fertilisation status of the eggs, the number of hatched chicks, and the count of embryos that died were recorded. Subsequently, the following percentage indices for the hatchlings were computed: fertility (%), hatchability from set eggs (%), hatchability from fertile eggs (%), and mortality at 0–25 days (% of set eggs).

Prior to placing the eggs in the incubator, 6 eggs from each weekly egg collection were selected from each group for analytical processing. The yolks were separated from the collected eggs (48 eggs from each group). Average pooled samples for analysis were obtained from 3 eggs (n = 16). Egg yolks were mixed and frozen at −20 °C until laboratory analyses.

## Chemical analysis

The nutritive value, including dry matter (DM; procedure 934.01), crude protein (CP; procedure 955.04), crude fibre (CF; procedure 962.09), ether extract (EE; procedure 920.39), crude ash (CA; procedure 942.05), and amino acid profile (procedure 994.12), of the basal diets was analysed according to the methods of AOAC (2000). The Ca content in the feed samples was determined using the AAS flame technique (AAS Unicam 939, Shimadzu Corp., Tokyo, Japan) after preheating to 550 °C, following the methods outlined by AOAC (2000) (procedure 927.02). The total P content (procedure 965.17) in the feed was identified colorimetrically using a Helios Alpha UV-VIS apparatus (Spectronic Unicam, Leeds, United Kingdom).

The total ether extract of the yolks for fatty acid profile analysis was determined using the chloroform/methanol technique, as described by Folch et al. (1957). The percentage of fatty acid methyl esters was estimated by gas chromatography using a Varian CP-3800 chromatograph (SpectraLab Scientific, Inc., Markham, Canada). The chromatographic operating conditions for fatty acid separation were described by Grela et al. (2020). Lipid quality indices, i.e., the atherogenicity index (AI) and thrombogenicity index (TI), were calculated based on the equations of Gao et al. (2020):

$$\text{atherogenicity index} = \left(\frac{C12:0 + 4 \times C14:0 + C16:0}{\sum MUFA + \sum(n\text{-}6) + \sum(n\text{-}3)}\right),$$

where: C12:0 – lauric acid, C14:0 – myristic acid, C16:0 – hexadecenoic acid, MUFA – monounsaturated fatty acid, n-6 – omega-6 fatty acid, and n-3 – omega-3 fatty acid;

$$\text{thrombogenicity index} = \left(\frac{C14:0 + C16:0 + C18:0}{0.5 \times \sum MUFA + 0.5 \times \sum(n\text{-}6) + 3 \times \sum(n\text{-}3) + \frac{\sum(n\text{-}3)}{\sum(n\text{-}6)}}\right),$$

where: C18:0 is stearic acid.

The hypocholesterolemic/hypercholesterolemic ratio (h/H) was calculated according to the formula described by Fernández et al. (2007):

$$h/H = C18:1\ n9 + C18:1\ n7 + C18:2\ n6 + C18:3\ n6 + C18:3\ n3 + C20:3\ n6 + C20:4\ n6 + C20:5\ n3 + + C22:4\ n6 + C22:5\ n3 + C22:6\ n3 / C14:0 + C16:0,$$

where: C18:1 n9 – oleic acid, C18:1 n7 – vaccenic acid, C18:2 n6 – linoleic acid, C18:3 n6 – gamma-linolenic acid, C18:3 n3 – alpha-linolenic acid, C20:3 n6 – dihomo-gamma linolenic acid, C20:4 n6 – arachidonic acid, C20:5 n3 – eicosapentaenoic acid, C22:4 n6 – adrenic acid, C22:5 n3 – docosapentaenoic acid, and C22:6 n3 – docosahexaenoic acid.

Cholesterol was determined by direct saponification of egg yolk followed by gas chromatography analysis (Varian CP-3800 chromatograph, SpectraLab Scientific, Inc., Markham, Canada), according to the method developed by Botsoglou et al. (1998). Aliquots (1 µl) of the saponified extracts were injected (split-less mode) into a fused silica capillary column (30 m × 0.53 mm i.d.) coated with a 1.0 µm thick SPB-1 film. The column temperature increase was programmed from 250 to 300 °C at a rate of 10 °C/min and held at 300 °C for 15 min. The injection port and flame ionisation detector temperatures were set at 300 °C. The hydrogen carrier gas flow rate was set at 3.4 ml/min. All injections were performed using a split-less mode. Cholesterol was identified by comparing sample retention times with those of an authenticated laboratory standard. Quantification was carried out against an external standard based on a curve plotted with cholesterol levels and peak area values. Cholesterol concentrations were expressed as mg/g yolk.

## Statistical analysis

For the selected egg morphometric parameters, each individual egg was defined as an experimental unit (total egg production excluding culled eggs, i.e., BSFL0, n = 316; BSFL10, n = 370; BSFL20, n = 452). Regarding hatchability parameters, 150 randomly selected eggs (n = 150/treatment) were used. The chemical composition and fatty acid profile of egg yolks were determined using 16 randomly selected eggs (n = 16/treatment). The obtained results were analysed statistically using STATISTICA ver. 13.3 (TIBCO Software, Inc., Palo Alto, CA, USA). All data were tested for normal distribution and homogeneity of variance using the Shapiro-Wilk test and Bartlett test, respectively. One-way ANOVA was performed to determine the effects of the experimental factors (diet with different levels of insect protein). The significance of differences between group means was estimated using Tukey's test at a confidence level of $P < 0.05$. A trend was considered significant at $P < 0.10$.

# Results

## Egg production and morphology

A significant difference in terms of egg production was observed (Table 3). The highest number of eggs was obtained in the BWF20 group, and the lowest in the BSFL0 group ($P = 0.001$). The BSFL10

**Table 3.** Effect of black soldier fly larval meal inclusion in pheasant diets on egg production and selected morphometric parameters

| Item | Treatment | | | SEM | *P*-value |
|---|---|---|---|---|---|
| | BSFL0 | BSFL10 | BSFL20 | | |
| Total number of eggs | 339$^{c}$ | 392$^{b}$ | 469$^{a}$ | 12.5 | 0.001 |
| Culled eggs, % | 6.80$^{a}$ | 5.60$^{ab}$ | 3.70$^{b}$ | 0.19 | 0.041 |
| Egg weight, g | 31.5 | 31.2 | 32.2 | 1.42 | 0.173 |
| Egg length, mm | 44.5 | 44.6 | 44.7 | 0.75 | 0.362 |
| Egg width, mm | 35.7 | 35.7 | 35.8 | 0.25 | 0.512 |
| Egg shape index, % | 80.2 | 80.0 | 80.1 | 0.21 | 0.473 |
| Eggshell area, cm$^2$ | 46.9 | 46.9 | 47.9 | 0.34 | 0.124 |
| Egg albumen height, mm | 3.97$^{b}$ | 4.17$^{ab}$ | 4.36$^{a}$ | 0.21 | 0.038 |
| Haugh index | 73.6$^{b}$ | 75.4$^{b}$ | 76.3$^{a}$ | 1.63 | 0.046 |

BSFL meal – black soldier fly larval meal, BSFL0 – basal diet without black soldier fly larval meal, BSFL10 – experimental diet with 100 g/kg black soldier fly larval meal, BSFL20 – experimental diets with 200 g/kg black soldier fly larval meal, SEM – standard error of the mean; means for selected morphometric parameters represent: BSFL0, n = 316; BSFL10, n = 370; and BSFL20, n = 452; $^{abc}$ – values within a row with different superscripts are significantly different at $P < 0.05$

treatment was characterised by an intermediate egg production. The percentage of culled eggs was lower ($P = 0.041$) in BSFL20 than in BSFL0, while BSFL10 exhibited no changes. Additionally, the lowest ($P = 0.038$) egg albumen height was determined in BSFL0, as opposed to BSFL20, where the highest value was recorded. No significant differences were observed between the experimental treatments. The Haugh index increased ($P = 0.046$) in response to the BSFL20 treatment. Neither BSFL0 nor BSFL10 diet had a notable effect on these parameters.

## Hatchability parameters

No significant differences ($P > 0.05$) were identified in the selected hatchability parameters, i.e., fertility, hatchability from set eggs and fertile eggs, as well as chick survivability between treatments (Table 4). However, it should be noted that there was a dose-dependent improvement trend for chick survival up to 7 days ($P = 0.068$) and 14 days ($P = 0.061$) after hatching.

**Table 4.** Effect of black soldier fly larval larval meal inclusion in pheasant diets on selected hatchability parameters

| Item | Treatment | | | SEM | *P*-value |
|---|---|---|---|---|---|
| | BSFL0 | BSFL10 | BSFL20 | | |
| Fertility, % | 92 | 88 | 93 | 2.30 | 0.348 |
| Hatchability from set eggs, % | 70 | 73 | 67 | 1.85 | 0.241 |
| Hatchability from fertile eggs, % | 76 | 83 | 72 | 2.25 | 0.117 |
| Survivability of chicks up to 7 days of age | 83 | 89 | 95 | 2.75 | 0.068 |
| Survivability of chicks up to 14 days of age | 73 | 81 | 88 | 1.84 | 0.061 |

BSFL0 – basal diet without black soldier fly larval meal, BSFL10 – experimental diet with 100 g/kg black soldier fly larval meal, BSFL20 – experimental diet with 200 g/kg black soldier fly larval meal, SEM – standard error of the mean; means represent 150 randomly selected eggs (n = 150/treatment); $P > 0.05$ – not significantly different

## Pheasant weight

No significant differences in body weight were recorded between treatments and after 3 months ($P = 0.525$) or in average daily gain (0–12 weeks; $P = 0.096$) (Table 5).

**Table 5.** Effect of black soldier fly larval meal inclusion in pheasant diets on body weight and average daily gain of female pheasants, g

| Item | Treatment | | | SEM | *P*-value |
|---|---|---|---|---|---|
| | BSFL0 | BSFL10 | BSFL20 | | |
| Initial body weight, g | 934 | 933 | 933 | 15.6 | 0.558 |
| Body weight after 3 months, g | 958 | 958 | 960 | 15.9 | 0.525 |
| ADG (0–12 weeks), g | 0.28 | 0.30 | 0.32 | 0.03 | 0.096 |

BSFL0 – basal diet without black soldier fly larval meal application, BSFL10 – experimental diet with 100 g/kg black soldier fly larval meal, BSFL20 – experimental diet with 200 g/kg black soldier fly larval meal, SEM – standard error of the mean, ADG – average daily gain; means represent 21 individual female birds (n = 21/treatment); $P > 0.05$ – not significantly different

## Egg chemical composition

Analysis of the chemical composition of pheasant egg yolk indicated an increased content of DM ($P = 0.045$) and CP ($P = 0.039$) in the BSFL20 group compared to the BSFL0 group (Table 6). Moreover, the inclusion of *H. illucens* larval meal in the pheasant diet tended to increase ($P = 0.075$) CA concentrations and reduce cholesterol levels ($P = 0.067$).

**Table 6.** Effect of black soldier fly larval meal inclusion in pheasant diets on the chemical composition (g/kg) of egg yolk

| Item | Treatment | | | SEM | *P*-value |
|---|---|---|---|---|---|
| | BSFL0 | BSFL10 | BSFL20 | | |
| Dry matter | 454$^{b}$ | 457$^{b}$ | 464$^{a}$ | 5.1 | 0.045 |
| Crude ash | 15.7 | 16.3 | 16.7 | 1.1 | 0.075 |
| Crude protein | 148$^{b}$ | 152$^{ab}$ | 158$^{a}$ | 8.9 | 0.039 |
| Ether extract | 258 | 258 | 258 | 12.1 | 0.205 |
| NfE | 32.1 | 31.5 | 31.1 | 1.3 | 0.102 |
| Cholesterol, mg/g | 10.5 | 9.93 | 9.87 | 0.34 | 0.067 |

BSFL0 – basal diet without black soldier fly larval meal, BSFL10 – experimental diet with 100 g/kg black soldier fly larval meal, BSFL20 – experimental diet with 200 g/kg black soldier fly larval meal, NfE – nitrogen-free extract, SEM – standard error of the mean; means represent randomly selected eggs, i.e., n = 16/treatment; $^{ab}$ – values within a row with different superscripts are significantly different at $P < 0.05$

The fatty acid profile and AI, TI, and h/H indices of pheasant egg yolks are summarised in Table 7. Notably, there was a significant increase in the proportion of saturated acids ($P = 0.035$), mainly

**Table 7.** Effect of black soldier fly larval meal inclusion in pheasant diets on the fatty acid profile in egg yolk

| Item | Treatment | | | SEM | *P*-value |
|---|---|---|---|---|---|
| | BSFL0 | BSFL10 | BSFL20 | | |
| Saturated | | | | | |
| lauric, C12:0 | 0.01$^{c}$ | 0.07$^{a}$ | 0.02$^{b}$ | 0.01 | 0.011 |
| myristic, C14:0 | 0.52$^{c}$ | 1.16$^{b}$ | 1.93$^{a}$ | 0.17 | 0.012 |
| palmitic, C16:0 | 26.2 | 26.8 | 26.9 | 0.38 | 0.103 |
| stearic, C18:0 | 8.88$^{a}$ | 8.80$^{a}$ | 8.07$^{b}$ | 0.21 | 0.042 |
| Unsaturated | | | | | |
| palmitoleic, C16:1, n-9 | 6.16$^{a}$ | 4.83$^{b}$ | 4.40$^{b}$ | 0.32 | 0.021 |
| oleic, C18:1, n-9 | 37.4 | 37.3 | 38.1 | 0.44 | 0.167 |
| vaccenic, C18:1, n-7 | 2.78$^{a}$ | 2.73$^{a}$ | 2.04$^{b}$ | 0.11 | 0.041 |
| linoleic, C18:2, n-6 | 13.9 | 13.8 | 13.8 | 0.34 | 0.226 |
| linolenic, C18:3, n-3 | 1.04 | 1.16 | 1.17 | 0.09 | 0.098 |
| gondoic, C20:1, n-9 | 0.28 | 0.30 | 0.27 | 0.02 | 0.185 |
| arachidonic, C20:4, n-6 | 0.82 | 0.85 | 0.79 | 0.09 | 0.152 |
| DHA, C22:6, n-3 | 0.50 | 0.51 | 0.47 | 0.04 | 0.169 |
| Summarized | | | | | |
| SFA | 35.8$^{b}$ | 37.1$^{a}$ | 37.3$^{a}$ | 1.01 | 0.035 |
| MUFA | 47.2$^{a}$ | 45.8$^{b}$ | 45.8$^{b}$ | 1.18 | 0.024 |
| PUFA | 16.6 | 16.7 | 16.6 | 0.49 | 0.171 |
| PUFA, n-3 | 1.75 | 1.89 | 1.88 | 0.11 | 0.095 |
| PUFA, n-6 | 14.9 | 14.8 | 14.7 | 0.32 | 0.224 |
| PUFA, n6:n3 | 8.50$^{a}$ | 7.82$^{b}$ | 7.83$^{b}$ | 0.14 | 0.037 |
| AI | 0.44$^{b}$ | 0.51$^{ab}$ | 0.56$^{a}$ | 0.05 | 0.038 |
| TI | 0.98 | 1.02 | 1.03 | 0.03 | 0.095 |
| h/H | 2.12 | 2.02 | 1.96 | 0.11 | 0.102 |

BSFL0 – basal diet without black soldier fly larval meal, BSFL10 – experimental diet with 100 g/kg black soldier fly larval meal, BSFL20 – experimental diet with 200 g/kg black soldier fly larval meal, SFA – saturated fatty acid, MUFA – monounsaturated fatty acid, PUFA – polyunsaturated fatty acid, AI – atherogenicity index, TI – thrombogenicity index, h/H – hypocholesterolemic/hypercholesterolemic ratio, SEM – standard error of the mean; means represent randomly selected eggs, i.e., n = 16/treatment; $^{abc}$ – values within a row with different superscripts are significantly different at $P < 0.05$

lauric acid (C 12:0) and myristic acid (C 14:0), in both experimental groups, and a decrease in stearic acid (C 18:0) in the group receiving 20% insect meal supplementation. A significant reduction ($P = 0.025$) in palmitoleic acid (C 16:1) was recorded in the experimental groups, which also had similar proportions of oleic acid (C 18:1, n-9); hence, the total proportion of monounsaturated fatty acids (MUFAs) was reduced. The percentage of n-6 polyunsaturated fatty acids (PUFAs) was similar in the control and experimental groups, while for n-3 PUFAs, there was a slight trend ($P = 0.095$) towards an increased proportion of n-6 polyunsaturated fatty acids (PUFAs) in egg yolks of pheasants fed a mixture with insect meal. Changes in the fatty acid profile contributed to an increase in the AI ($P = 0.038$) values in the group of birds administered feed with 20% insect meal addition and a favourable reduction in the n-6 to n-3 PUFA ratio ($P = 0.037$). No significant alterations were found in TI or hypo/hypercholesterolemic (h/H) acid ratio.

## Discussion

The diet of free-ranging pheasants comprises plant products, mainly cereal grains, as well as various insects and their larvae, with the feed composition influenced by the environmental conditions the birds inhabit (Anankware et al., 2015). Agricultural intensification has been shown to contribute to reduced reproductive success due to the limited proportion of invertebrates in pheasant diets (Geaumont et al., 2017). In poultry production, including pheasants, diets are largely composed of cereal grains, while by-products of the oil industry, such as soybean, rapeseed, and sunflower meals are used as protein feedstuffs (Kokoszyński et al., 2014; Gugała et al., 2019). In an attempt to mimic feed ingredients available in the natural habitats of pheasant, various high-protein components are being introduced, including those of animal origin, such as insect larvae or meal (Chatzidimitriou et al., 2022). Previous studies in other animal species have shown positive effects of such treatments (Kierończyk et al., 2022b) on growth performance (Kierończyk et al., 2022a) and egg quality (Liu et al., 2021).

The nutritive and hatching value of eggs is determined by their chemical composition, including amino acid and fatty acid profiles, as well as the content of biologically active substances (Sunwoo and Gujral, 2015). Recently, various feed materials have been evaluated for their impact on pheasant laying performance and egg chemical composition (Gugała et al., 2019; Flis and Gugała, 2021). However, there are no published reports concerning the application of insect meal in pheasant hen nutrition, its effects on production performance (laying and hatchability) or egg chemical composition. To date, a limited number of studies on pheasants have focused on the influence of the physical and chemical characteristics of eggs on the hatching and rearing of chicks. Ipek and ve Dikmen (2007) showed that egg weight significantly affected body weight of chicks, their rearing and later growth. Post-hatching body weight also plays a significant role in subsequent laying and the weight of eggs obtained (Ozbey et al., 2011). Similar results were reported by Ozbey et al. (2011) and Ashraf et al. (2016), highlighting a significant effect of egg weight on later body weight of chicks during hatching and rearing. In the present study,

no effect of insect biomass on egg weight or hatchability indices was observed. However, the administration of BSF larval meal in our study led to increased number of eggs laid, their size and weight, and the Haugh index, contributing significantly to the improved biological value of hatching eggs and embryo survival. This improvement can be attributed to optimal levels of crude protein and metabolisable energy (NRC, 1994), with the inclusion of insect meal yielding desired outcomes in reproduction. The protein content in the diet of pheasants is crucial, as indicated by Kokoszyński et al. (2011), who reported a deterioration in hatchability parameters at lower protein levels included in pheasant diets. Uğurlu et al. (2017) emphasised the necessity to appropriately optimise the level and quality of protein in birds' diets, as both deficiency and excess of protein in the feed can adversely affect certain aspects of birds' production. The available literature demonstrates that the utilisation of insect biomass in the nutrition of laying hens exerts various effects on egg production and morphometric indices. Nevertheless, as chicken eggs play a more important role in human nutrition, most related studies have focused on the chemical quality of eggs. However, the introduction of defatted *H. illucens* larval meal (170 g/kg) as a total replacement for soybean meal did not affect egg weight (Secci et al., 2018). On the other hand, Secci et al. (2020; 2021) reported positive effects of BSF and *Tenebrio molitor* larvae on egg weight increase; however, these studies assumed only a partial substitution of soybean meal in the birds' diets. Marono et al. (2017) emphasised that feeding hens with defatted *H. illucens* meal could reduce laying performance and egg weight. In the present study, a positive effect on the total number of eggs, as well as the percentage of culled eggs, was observed even when 200 g/kg full-fat BSF larval meal was applied. This finding is consistent with a natural behavioural nutritional model, where birds, particularly pheasants, are dependent on high-level crude protein diets containing components of animal-origin. Furthermore, the increase in egg albumen height and Haugh index associated with the highest dose of *H. illucens* larval meal administration might partly explain the tendency towards improved survivability of chicks up to 7 and 14 days of age. The Haugh index is a well-known indicator of internal egg quality. Moreover, an increase in albumen height positively influences albumen quality and thus the fertility and hatchability of chicks. However, comprehensive data and further nutritional experiments are essential to fully elucidate this phenomenon.

Diets containing BSF larval meal increased the dry matter and protein content of egg yolks and modified the fatty acid profile. These findings have positive implications for egg production and human nutrition, as elevated dry matter is primarily attributed to increased egg protein content, providing a rich source of exogenous amino acids. Crude protein concentrations exceeded values reported by Nowaczewski et al. (2013) and Song et al. (2000), i.e., 12.5%–13.4%. However, changes in the fatty acid profile, mainly in terms of SFA levels, mirrored the results of Chatzidimitriou et al. (2022), who reported that complete replacement of soybean meal with insect meal increased the percentage of saturated fatty acids (SFAs) and MUFAs, while decreasing PUFA levels in laying hen eggs. However, in the present study, an opposite trend was noted regarding the effect of insect biomass on the MUFA and PUFA contents in eggs. Furthermore, Secci et al. (2018) completely substituted soybean meal with insect meal in the diet of laying hens and did not observe significant differences in the SFA profile; however, the latter authors recorded decreased MUFA and increased PUFA concentrations compared to the control group. In contrast, Park et al. (2021) reported increased levels of MUFAs ($P < 0.01$) and decreased levels of PUFAs in eggs from laying hens fed diets containing insect meal. The divergent results across studies may stem from differences in insect developmental stages used during meal production, their nutritive value, and the bird species examined. The cholesterol-lowering trend of 5.9% in egg yolk ($P = 0.067$) was consistent with the findings of Secci et al. (2018) in Lohmann Brown Classic hens, where the use of *H. illucens* larval meal instead of soybean meal led to an up to 11% reduction in egg yolk cholesterol levels. It should be noted that cholesterol levels are primarily regulated endogenously and the compound is not absorbed from the GI tract, requiring further research to clarify the effect of diet on cholesterol concentrations. However, a commonly reported phenomenon in the available literature is the reduction of cholesterol levels in egg yolks by feeding *H. illucens* to laying hens (Secci et al., 2018). The aforementioned phenomenon may be explained by the presence of chitin and its ability to bind negatively charged fatty acids (Prajapati and Patel, 2010), but this mechanism requires further investigation. Surprisingly, the present study revealed a favourable reduction in the n-6/n-3 ratio in egg yolk, which is beneficial for the human diet (Attia et al., 2017). The available literature has frequently highlighted the effect of *H. illucens* products on the reduction of n-3 fatty acid levels

(Kierończyk et al., 2022a;b; 2023). In contrast, PUFA n-3 were found not to be affected in the present study. Moreover, the atherogenic index was increased by the BSFL20 treatment. Within the AI range proposed by Attia et al. (2017), i.e., from 0.434 to 0.533, the obtained results did not significantly exceed the standard. It should also be noted that *H. illucens* larval fat is characterised by high myristic and palmitic acid levels, common atherogenic agents. Therefore, it is crucial to modify the fatty acid profile of insect biomass during the rearing process through the chemical composition of the substrate (Kierończyk et al., 2023).

## Conclusions

The incorporation of black soldier fly larvae meal at 200 g/kg into pheasant diets yielded favourable results, particularly in terms of increased egg production and a tendency towards improved chick survival up to 7 and 14 days of age. Additionally, full-fat black soldier fly meal significantly modified the chemical composition of eggs by increasing the dry matter and crude protein content. Furthermore, the beneficial impact of black soldier fly larval meal was observed with respect to the yolk n-6:n-3 ratio and atherogenic index. Based on these findings, *Hermetia illucens* larval meal can be applied successfully in pheasant diets at a level of 200 g/kg.

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## Conflict of interest

The Authors declare that there is no conflict of interest.

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