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Black Soldier Meal in Feed Could Adversely Affect Organic Broiler Meat Quality When Used for the Total or Half Replacement of Diet Proteins

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Abstract: Organic poultry need high-quality proteins in their diet. The EU's organic regulation forbids synthetic amino acids; therefore, soybean, with its balanced essential amino acid content, has become the most used protein source, though much of it is imported from non-EU countries. Soybean, however, has some important problems related to sustainability and crop competition; therefore, it must be substituted with a high-protein-value alternative such as insect meal. In this study, 900 Aviagen Savanna broilers were reared using three different organic diets. The diets used the following as protein feed material: soybean only (S100), 50% black soldier fly larvae meal (BSL) and 50% soybean (BSL50), and 100% BSL only (BSL100). Broiler performance, welfare, and fatty acids (FA) in the thigh and breast were analyzed. BSL50 and BSL100 negatively affected growth performance, while BSL100 worsened all of the market-related measures of performance. Meat quality was worse in BSL50 and BSL100, with a significant increase in saturated FA (SFA) and a corresponding decrease in polyunsaturated FA (PUFA). Among SFA, lauric acid (C12:0) more notably increased. The increase in SFA represents a negative aspect of BSL meal inclusion, namely the production of lauric acid; however, this trend could have a beneficial effect, which will be more deeply explored with regard to its impact on animal and human health.

Keywords: organic broiler; protein feed; insect meal; lauric acid; meat quality; nutritional indices

1. Introduction

Soybean is the protein material most commonly used in animal feeding, especially in poultry production, thanks to its high amino acid profile [1]. However, increasing demand for soybean production brings up some important issues linked to economic, environmental, and social challenges. Fluctuating prices, deforestation, excessive water use, increased carbon dioxide (CO_2) emissions for long transport distances, and competition with human crops make the current level of soybean production unsustainable [2,3]. In organic poultry production, where synthetic amino acids are not allowed, soybean is the most widely used protein source, and among amino acids, its contents of lysine and methionine, two limiting amino acids for birds, have special weights [1]. Among EU countries, organic poultry production shows higher annual growth rates (11%) than other types of animal production and, at the same time, the feed industry's demand for high-quality proteins is increasing (A Decade of Organic Growth Organic Farming in the EU N°20 Agricultural Market Brief, 2023). Consequently, European soybean production cannot cover animal needs, meaning that organic poultry production depends on imports from non-EU countries, leading to it being afflicted by issues related to its sustainability. Therefore, alternative proteins are necessary to replace soybean in poultry feeds. The most promising protein alternative is insect meal, which has the same or an even better amino acid profile than soybean [4]. Insects can transform huge biomass into high-value protein, fatty acids, mineral compounds, and vitamins well suited for feeding poultry, fish and pigs, and they could help to reduce the EU's protein deficit [5]. Besides their nutritional value, insects contain bioactive molecules that contribute to improving the health of livestock animals [6]. Insects are authorized by EU for use as feed for poultry and pigs in Regulations EC 2021/1372, amending Annex IV of Regulation EC 2001/999. So far, insects have not been included in organic certification even if, according to Regulation EU 2018/848 art. 20 and 21, insects and insect meal can legally be used in organic farming, albeit only if reared and produced according to the rules of organic production. Among insects used for poultry feeding, Black Soldier fly (BS) is considered to be the most used and available insect species [5,7–9]. Compared to other insect species, BS needs the lowest rearing space, water, and energy resources and has the lowest substrate requirements; thus, it can be used on a large scale and in continuous industrial production [10,11]. Nogales-Mérida et al. [12] acknowledged that BS Larvae (BSL) meal has a desirable nutritional composition that includes not only favorable amino acid levels but also good antimicrobial properties. The nutrient composition of BSL varies depending on the breeding substrate [13]; in any case, BSL meal generally contains high levels of protein, ranging from 49 to 59% of dry matter (DM), with lysine, cysteine, methionine, threonine, and other nutrients, such as calcium and phosphorous, that are important for animal nutrition [14,15]. However, despite its good amino acid profile, many studies have shown that BSL has limited soybean substitution power due to its high saturated fatty acid content [11,16–20]. Generally, defatted insect meal is preferred for maintaining nutrient balance and ensuring uniform feed formulation. However, even if defatted, BSL meal is rich in medium chain saturated fatty acids, such as lauric acid (C12:0), produced by insects from unsaturated fatty acids (UFAs) [13,21–24]. During its lifetime, BSL bioaccumulates oleic acid (C18:1 n-9), linoleic acid (C18:2 n-6) (LA), and α-linolenic acid or ALA (C18:3 n-3), and it mainly metabolizes them to create lauric acid via a system of regulatory enzymes [25,26]. However, lauric acid can have beneficial effects, such as improving the intestinal microbiota of poultry [27,28]; however, from a food quality point of view, lauric acid can substantially affect meat quality in terms of fatty acid profile at the expense of polyunsaturated fatty acids (PUFA), the levels of which are significantly reduced [18,29–31].

Keeping in mind the positive and negative effects of using BSL meal in a broiler diet, it is important to substitute as much as organic soybean as possible to nullify the unsustainability of imported soybean.

Our work explored and analyzed the potential beneficial/unfavorable effects associated with the high replacement of organic soybean with defatted BSL meal (50%, and 100%) on performance, animal welfare, meat quality, nutritional indices, and potential side effects.

2. Materials and Methods

2.1. Animals, Management, and Feed

The broiler rearing cycle was carried out at a commercial poultry farm managed by the Council for Agricultural Research and Analysis of Agricultural Economics (CREA) in Monterotondo (RM), Italy. The poultry house had a 153 m^2 (18 m × 8.5 m) area equipped with automatic feeding and watering systems. Environmental conditions and animal behavior were detected using a robot equipped with cameras and sensors.

An aisle situated on the long side of the poultry house was used to monitor the animals without directly interfering with them.

In total, 900 Aviagen® Ranger SAVANNA female chicks were housed, in compliance with organic Regulation EU 848/2018, at one day of age on a 5 cm thick shaved wood litter, and they were vaccinated against coccidia, Marek's disease, pseudo-plague, infectious bronchitis, and Gumboro's disease. The house was divided into 3 boxes (7 × 6 m each), with each box containing 300 animals. The poultry house was heated with non-dazzle medium-wave heating infrared lamps (Syner Progetti, Mantova, Italy) 48 h before the animals' arrival, ultimately reaching a temperature of 30 °C. The room temperature was decreased to 20 °C until day 27. The birds had access to a 4 m^2 per bird outdoor area, divided into 3 sectors and covered by grass and trees, from the 28th day of age onward.

The animals were all fed *ad libitum* for their entire lifetimes. Until the 30th day of their lives (1° period), the chickens were fed with the same organic feed, purchased via the market, which had a protein content of 21.6 g/100 g. The protein source was only soybean, representing the 33.80% of feed composition (Tables 1 and 2).

Starting from the 31st days of life (2° period), the 3 groups were fed 3 different diets. In Group 1, also known as the control group, animals were fed with an organic diet, with only soybean making up the high protein matter (S100); group 2 was fed a diet of 50% soybean replaced with BSL defatted meals (BSL50), while group 3 was fed 100% BSL defatted meal as a protein source (BSL100) (Table 1). The feed was formulated in collaboration with an Italian organic feed mill. Table 2 shows the chemical composition of each diet. BSL, in dehydrated and defatted form, was purchased from Hexafly (Ireland), and its protein content was 53.10% (Table 3).

During rearing, only the normal routine activities of a commercial farm were performed, and no interventional operation was performed on the animals.

Table 1. 1° and 2° period's feed ingredients.

Raw Material	1° Feed Period	2° Feed Period S100 (%)	2° Feed Period BSL50 (%)	2° Feed Period BSL100 (%)
Soybean cake	33.80	27.00	13.99	0.00
Maize	30.00	28.60	38.01	33.30
Wheat	15.00	20.50	13.81	15.99
Bran	7.00	0.00	0.00	0.00
Maize gluten	4.20	3.00	4.69	4.79
Barley	4.00	14.30	11.96	15.99
Soybean vegetable oil	2.38	2.62	2.12	1.69
Calcium carbonate	1.10	1.22	0.88	0.00
Monocalcium phosphate	0.87	0.00	1.00	0.90
Sodium chloride	0.35	0.34	0.36	0.29
Dicalcium phosphate	0.30	1.50	0.00	0.00
Lysine	0.10	0.12	0.39	0.66
Choline chloride	0.10	0.10	0.09	0.09
Betaine anhydrous	0.20	0.20	0.14	0.14
Oligo-vitamin supplement	0.60	0.50	0.50	0.49
Insect meal	0.00	0.00	12.00	25.60

1° feed period = from day 1 to 31. 2° feed period = from day 32 to 75. S100 = 100% soybean feed, BSL50 = 50% soybean and 50% black soldier fly larvae meal feed. BSL100 = 100% black soldier fly larvae meal feed.

Table 2. 1° and 2° period's feed composition.

Analytical Component	1° Feed Period	2° Feed Period S100	2° Feed Period BSL50	2° Feed Period BSL100
Crude protein (%)	21.60	19.00	19.50	19.50
Crude fiber (%)	3.87	3.30	4.60	3.70
Ashes (%)	6.00	5.80	5.70	5.90
Fats (%)	6.50	6.20	6.40	6.50
Lysine (mg/kg)	1.25	1.00	1.02	1.00
Methionine (mg/kg)	0.50	0.45	0.53	0.47
Calcium (mg/kg)	0.90	1.00	1.00	1.00
Sodium (mg/kg)	0.17	0.17	0.19	0.18
Phosphorus (mg/kg)	0.70	0.65	0.66	0.64

1° feed period = from day 1 to 31. 2° feed period = from day 32 to 75. S100 = 100% soybean feed, BSL50 = 50% soybean and 50% black soldier fly larvae meal feed. BSL100 = 100% black soldier fly larvae meal feed.

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Table 3. BSL meal analytical component.

Analytical Component	BSL Meal
Crude protein (g/100 g)	53.10
Crude fiber (g/100 g)	11.50
Moisture (g/100 g)	6.50
Ashes (g/100 g)	6.30
Fats (g/100 g)	11.90
Lysine (g/100 g)	2.94
Methionine (g/100 g)	0.47
Cysteine (g/100 g)	0.41
Threonine (g/100 g)	3.24
Calcium (g/kg)	7.77
Sodium (g/kg)	4.39

BSL = black soldier fly larvae 2.2 Sample collection and analysis.

At 75 days old, the broilers were slaughtered in an EC-authorized, commercial slaughterhouse in the presence of a veterinarian. The animals were slaughtered before the 81st day of life to ensure compliance with Regulation 848/2018. The live weights at slaughter are shown in Table 4.

Table 4. Broiler performances.

	S100	BSL50	BSL100	RMSE	*p*-Value
Live weight (g)	3887.53 ^{a}	3672.67 ^{b}	3656.87 ^{b}	82.08	0.001
Carcass weight (g)	3127.30 ^{a}	3177.67 ^{a}	2959.70 ^{b}	295.77	0.014
Thigh (g)	215.43 ^{a}	189.73 ^{b}	200.08 ^{b}	23.21	0.001
Breast (g)	795.50 ^{a}	776.56 ^{a}	720.14 ^{b}	53.74	0.022

S100 = organic feed with 100% soybean. BSL50 = 50% soybean and 50% black soldier fly larvae meal feed. BSL100 = 100% black soldier fly larvae meal feed. Different letters in the same row indicate significant differences per $p < 0.05$.

Some welfare parameters, such as the cleanliness of plumage, pododermatitis, hock burning, breast reddening, and breast lesions, which were extrapolated from the "Welfare Quality® assessment protocol for poultry", were measured at the slaughterhouse. Litter quality and lameness were assessed in the poultry house once per week during the last three weeks. The scores for all indicators were 0 for the best condition and 3 for the worst condition.

After slaughtering, at the slaughterhouse, 15 carcasses were randomly selected from each diet group, out of a total of 90 animals, before being weighed and dissected in CREA laboratories in Monterotondo (RM). Thigh and breast muscles were separated from the carcasses, before being homogenized and stored at −80 °C until the fatty acid analysis was performed.

Total fat were extracted according to the method of Folch et al. [32]. In total, 100 mg of fat extract were methylated via the addition of methanolic potassium hydroxide (2N), based on the IUPAC procedure (1992), to obtain fatty acids methyl ester (FAME). The C19:0 fatty acid was used as the internal standard. Methyl esters were injected into a GC-FID gas chromatograph (GC 6890 N, Agilent, Inc., CA) using a CP-Sil88 100 m 0.25 (0.2) column (Agilent Technologies) under the operating conditions described by Failla et al. [33]. Fatty acid methyl ester standards, such as Supelco 37 Component FAME Mix, C22:4 n-6, C22:5 n-3 DPA, and C19:0 (Sigma Aldrich (Oakville, ON, Canada), were used to identify fatty acids. Fatty acids were expressed as the percentage of total FAME. Furthermore, we calculated the principal fatty acid classes as the sum of the saturated fatty acids (ΣSFA), the sum of the monounsaturated fatty acids (ΣMUFA), the sum of the polyunsaturated fatty acids (ΣPUFA), the sum of the n-6 fatty acids (Σn-6 PUFA), the sum of the n-3 fatty acids (Σn-3 PUFA), the n-6-to-n-3 ratio (n-6/n-3), and the PUFA-to-SFA ratio (PUFA/SFA).

Some nutritional indices were calculated besides the PUFA-to-SFA ratio (PUFA/SFA), which is normally used as the atherogenicity indicator of food.

The index of atherogenicity (IA) only takes into consideration the following three hypercholesterolaemic SFA [32]:

$$IA = [C12{:}0 + (4 \times C14{:}0) + C16{:}0]/\Sigma UFA. \quad (1)$$

The index of thrombogenicity (IT), as developed by Ulbritcht and Southgate, in 1991 [32]:

$$IT = (C14{:}0 + C16{:}0 + C18{:}0)/[(0.5 \times \Sigma MUFA) + (0.5 \times \Sigma n\text{-}6\ PUFA) + (3 \times \Sigma n\text{-}3\ PUFA) + (n\text{-}3/n\text{-}6)]. \quad (2)$$

The hypocholesterolemic/Hypercholesterolemic ratio (HH), a nutritional index proposed for the first time by Santos-Silva et al. [33], with a formula optimized by Mierlita [34], adding C12:0 in hypercholesterolemic fatty acid:

$$hH = (cis\text{-}C18{:}1 + \Sigma PUFA)/(C12{:}0 + C14{:}0 + C16{:}0). \quad (3)$$

The health-promoting index (HPI) is the inverse of the atherogenic index proposed by Chen at al. [35]:

$$HPI = \Sigma UFA/[C12{:}0 + (4 \times C14{:}0) + C16{:}0] \quad (4)$$

The following four nutritional indices assess the unsaturated fatty acid content. UI indicates the degree of unsaturation in lipids, and it is calculated as the sum of the percentage of each of the unsaturated fatty acids multiplied by the number of double bonds within that FA [36]:

$$UI = 1 \times (\%\ monoenoics) + 2 \times (\%\ dienoics) + 3 \times (\%\ trienoics) + 4 \times (\%\ tetraenoics) + 5 \times (\%\ pentaenoics) + 6 \times (\%\ hexaenoics) \quad (5)$$

FLQ, which was used originally for fish lipid quality [37], calculates the sum of the EPA and DHA as a percentage of the total fatty acids:

$$FLQ = 100 \times (C22{:}\ 6\ n \times 3 + C20{:}\ 5\ n \times 3)/\Sigma FA \quad (6)$$

The LA/ALA ratio is one of the traditional indices used to evaluate food quality with two of the most representative n-6 and n-3 fatty acids.

2.2. Statistical Analysis

All data were analyzed via ANOVA (SAS Inst. Inc., Cary, NC, USA) using a mono-factorial model, and significant differences were evaluated using Tukey's test, with $p < 0.05$ being the significance limit.

3. Results

3.1. Animal Performance

Performances are described in Table 4. The live weight was significantly lower in animals fed with BSL50 and BSL100 ($p = 0.001$), which were 3672.67 and 3656.87 g, respectively, compared to the control weight of 3887.53 g.

The carcass weight showed no significant change between the control and BSL50, as well as a significant decrease between BSL50 and BSL100 ($p = 0.014$).

The thigh weight follows the same trend: the weight decreased in animals fed with BSL50 and BSL100 ($p = 0.001$).

The breast weight of BSL100 is significantly different to those of the control (S100) and BSL50 ($p = 0.022$), and there were no significant differences between the control (S100) and BSL50.

3.2. Animal Welfare

Animal-based measures (ABMs) assessed in the slaughterhouse and the poultry house (lameness) showed a high degree of welfare. The percentage of animals scoring 0 for all ABMs was

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100% for every ABM indicators. The litter scored 0 for all the groups, 1 in the second week, and 1 in the third week.

3.3. Fatty Acid Composition of BSL Meal

The fatty acid composition is shown in Table 5. Among SFA (69.31% of total FAs), lauric acid accounted for 44.70%, followed, in decreasing order, by palmitic and myristic acids, with compositions of 13.72% and 8.77%, respectively. Oleic acid was not detected (amount less than 0.01). Lower amounts were shown for MUFAs and PUFAs, i.e., 13.66 and 15.51%, respectively. Among PUFAs, the only one linoleic acid (C18:2 n-6) was detectable, accounting for 14,27% of the total, while the n-3 class (1.24%) was, in practice, exclusively represented by ALA (α-linolenic acid, C18:3 n-3).

Table 5. BSL meal fatty acid profile of FAME.

Fatty Acid Profile (%)	BSL Meal
C12:0	44.70
C14:0	8.77
C16:0	13.72
C18:0	2.12
[2] ΣSFA	69.31
C14:1	0.25
C16:1 n-7	2.46
C18:1 n-7	10.95
[2] ΣMUFA	13.66
C18:2 n-6	14.27
C18:3 n-3	1.24
[2] ΣPUFA	15.51
n-6	14.27
n-3	1.24
n-6/n-3	11.51

[1] FAME = fatty acid methyl esters. [2] SFA: saturated fatty acids; MUFA: monounsaturated fatty acids. PUFA: polyunsaturated fatty acids. Lipid composition analysis have also included some components (C22:0, C18:1 n-9, C18:3 n-6, C20:2 n-6, C20:3 n-6, C20:4 n-6, C20:5 n-3, C22:5 n-3, C22:6 n-3) that were not reported in the table due to their limited quantities (<0.01%).

3.3.1. Fatty Acids in Thigh

An insect diet showed significant increased levels of SFA (Table 6), namely 28.17%, 31.83%, and 34.64% for S100, BSL50, and BSL100, respectively ($p < 0.001$). Within SFA, palmitic (C16:0), lauric (C12:0), and myristic (C14:0) acids increased with the inclusion of BSL in feed, with the highest value being found in BSF100 ($p < 0.0001$). In particular, lauric acid increased from 0.30 (S100) to 4.32% in BSL100. Stearic fatty acid (C18:0) BSL50 was significantly different from S100 and BSL100, while the last two were not significantly different.

Unlike SFA, MUFAs have not been significantly affected by BSL inclusion. The most representative fatty acid among MUFAs is oleic acid (C18:1 n9), while the control and BSL50 were not different from each other, though a significant lower amount was found in BSL100 ($p < 0.001$).

PUFA, total n-6, and total n-3 significantly decreased in proportion to the level of the inclusion insect meals in feed ($p < 0.001$). PUFAs decreased from 33.53% in S100 to 27.55% in BSL100 ($p < 0.001$), total n-6 decreased from 30.34% in S100 to 24.91% in BSL100, ($p < 0.001$), total n-3 decreased from 3.18% in S100 to 2.64% in BSL100 ($p < 0.001$). As among PUFA, linoleic acid (C18:2 n-6) is the prevailing acid present, its trend is the same as that of PUFA, decreasing from 27.58% in S100 to 22.26% in BSL100 ($p < 0.001$).

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ALA followed the same decreasing trend ($p < 0.001$). EPA did not appear to be influenced by BSL feed, while the DPA and DHA contents were lower ($p < 0.001$) for the diet with the inclusion of BSL (both for 50% and 100% substitution).

Table 6. Fatty acid profile in thigh (percentage of total FAME [1]).

THIGH	S100	BSL50	BSL100	RMSE	*p*-Value
C12:0	0.30 [c]	2.67 [b]	4.32 [a]	0.392	<0.0001
C14:0	0.63 [c]	1.51 [b]	1.75 [a]	0.236	<0.0001
C16:0	19.54 [c]	20.39 [b]	21.08 [a]	0.790	<0.0001
C18:0	7.45 [a]	7.03 [b]	7.28 [a]	0.452	0.0023
C22:0	0.25 [a]	0.23 [a]	0.20 [b]	0.042	0.0007
[2] ΣSFA	28.17 [c]	31.83 [b]	34.64 [a]	1.138	<0.0001
C14:1	0.07 [b]	0.16 [a]	0.19 [a]	0.062	<0.0001
C16:1 n7	2.38 [b]	2.91 [a]	3.07 [a]	0.410	<0.0001
C18:1 n9	30.79 [a]	30.53 [a]	29.64 [b]	0.841	<0.0001
C18:1 n7	1.79	1.79	1.85	0.128	0.128
[2] ΣMUFA	35.03	35.39	34.75	1.014	0.053
C18:2 n6	27.58 [a]	24.71 [b]	22.26 [c]	1.172	<0.0001
C18:3 n6	0.22 [b]	0.25 [a]	0.23 [b]	0.032	0.021
C18:3 n3	2.30 [a]	2.12 [b]	1.95 [c]	0.241	<0.0001
C20:2 n6	0.26 [a]	0.22 [b]	0.19 [c]	0.022	<0.0001
C20:3 n6	0.04	0.04	0.04	0.020	0.750
C20:4 n6	2.24 [a]	1.72 [b]	2.19 [a]	0.352	<0.0001
C20:5 n3	0.07	0.08	0.07	0.013	0.128
C22:5 n3	0.43 [a]	0.32 [b]	0.33 [b]	0.068	<0.0001
C22:6 n3	0.37 [a]	0.27 [b]	0.28 [b]	0.065	<0.0001
[2] ΣPUFA	33.53 [a]	29.72 [b]	27.55 [c]	1.436	<0.0001
[2] Σn6	30.34 [a]	26.93 [b]	24.91 [c]	1.282	<0.0001
[2] Σn3	3.18 [a]	2.79 [b]	2.64 [c]	0.284	<0.0001
[2] Σn6/[2] Σn3	9.59	9.70	9.60	0.904	0.876
[3] Other	3.27	3.05	3.06	0.467	0.118

S100 = 100% soybean feed. BSL50 = 50% soybean and 50% black soldier fly larvae meal. BSL100 = 100% black soldier fly larvae meal. [a–c] Different letters in the same row indicate significant differences. [1] FAME = fatty acid methyl esters. [2] ΣSFA = Σ(C12:0, C14:0, C16:0, C18:0); ΣMUFA = Σ(C14:1, C16:1 n7, C18:1 n9, C18:1 n7). ΣPUFA = Σ(C18:2 n-6, C18:3 n-6, C18:3 n-3, C20:2 n-6, C20:3 n-6, C20:4 n-6, C20:5 n-3, C22:5 n-3, C22:6 n-3). Σn-6 = Σ(C18:2 n-6, C18:3 n-6, C20:2 n-6, C20:3 n-6, C20:4 n-6). Σn-3 = Σ(C18:3 n-3, C20:5 n-3, C22:5 n-3, C22:6 n-3). [3] Other: non-detected, complement to 100.

3.3.2. Fatty Acids in Breast

Also, in breast, total SFA significantly increased with the administration of a BSL-based diet ($p < 0.001$), even though no differences were noted between BSL50 and BSL100 (Table 7). As in thigh, breast showed significant proportional increases in lauric and myristic acids, ranging from S100 (0.35 and 0.62%) to maximum replacement (BSL100) (3.09 and 1.53%) ($p < 0.001$), while palmitic acid only revealed a significant rise in BSL50. Having a different trend to that of thigh, stearic fatty acid in BSL100 was significantly different from S100 and BSL50, while the last two were not significantly different from each other.

In BSL100, MUFAs were significantly different to S100 and BSL50 ($p < 0.001$). The increasing percentage of MUFAs was due to the content of oleic acid, and the fatty acid was most abundant among MUFAs, the trends of which remained unchanged up to BSL50 and significantly increased in BSL100 ($p < 0.001$).

PUFA, total n-6, and total n-3 showed the same trend and were negatively correlated with the inclusion of insect meals in feed, decreasing from 34.60% in S100 to 29.44% in BSL100.

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Also, breast linoleic acid is the prevailing acid content among PUFAs, with a significant decrease recorded as BSL increased in feed from S100 (27.37%) to BSL100 (24.85%). ALA was not influenced by the feed up to BSL50, decreasing only in BSL100 ($p < 0.001$).

EPA showed a dissimilar behavior in breast because BSL100 was not significantly different to S100, but it was different to BSL50. DPA and DHA were adversely affected by inclusions at any level ($p < 0.001$).

Unlike in thigh, where no differences were detected, n-6/n-3 only significantly increased from BSL50 to BSL100 ($p < 0.001$).

Table 7. Fatty acid profile in breast (percentage on total FAME [1]).

BREAST	S100	BSL50	BSL100	RMSE	*p*-Value
C12:0	0.34 [c]	1.81 [b]	3.09 [a]	0.284	<0.0001
C14:0	0.62 [c]	1.14 [b]	1.53 [a]	0.145	<0.0001
C16:0	19.33 [b]	20.32 [a]	19.81 [ab]	1.510	0.0433
C18:0	7.63 [a]	7.40 [a]	6.47 [b]	0.624	<0.0001
C22:0	0.25 [a]	0.13 [c]	0.22 [b]	0.036	<0.0001
[2] ΣSFA	28.18 [b]	30.81 [a]	30.98 [a]	1.781	<0.0001
C14:1	0.08 [c]	0.15 [b]	0.17 [a]	0.017	<0.0001
C16:1 n-7	2.54 [b]	2.55 [b]	3.12 [a]	0.363	<0.0001
C18:1 n-9	29.90 [b]	29.93 [b]	31.27 [a]	1.217	<0.0001
C18:1 n-7	1.74 [b]	1.90 [a]	1.84 [a]	0.166	0.0010
[2] ΣMUFA	34.26 [b]	34.53 [b]	36.41 [a]	1.163	<0.0001
C18:2 n-6	27.37 [a]	25.71 [b]	24.85 [c]	1.291	<0.0001
C18:3 n-6	0.23 [b]	0.27 [a]	0.23 [b]	0.024	<0.0001
C18:3 n-3	2.34 [a]	2.29 [a]	2.01 [b]	0.222	<0.0001
C20:2 n-6	0.41 [a]	0.27 [c]	0.32 [b]	0.035	<0.0001
C20:3 n-6	0.04 [b]	0.06 [a]	0.04 [b]	0.009	<0.0001
C20:4 n-6	3.37 [a]	2.11 [b]	1.57 [c]	0.290	<0.0001
C20:5 n-3	0.07 [b]	0.10 [a]	0.07 [b]	0.015	<0.0001
C22:5 n-3	0.41 [a]	0.33 [b]	0.20 [c]	0.052	<0.0001
C22:6 n-3	0.35 [a]	0.27 [b]	0.15 [c]	0.048	<0.0001
[2] ΣPUFA	34.60 [a]	31.41 [b]	29.44 [c]	1.465	<0.0001
[2] Σn-6	31.42 [a]	28.43 [b]	27.01 [c]	1.354	<0.0001
[2] Σn-3	3.10 [a]	2.89 [b]	2.36 [c]	0.224	<0.0001
[2] Σn-6/[2] Σn-3	10.17 [b]	9.89 [b]	11.54 [a]	0.771	<0.0001
[3] Other	2.96	3.24	3.02	0.607	0.1832

S100 = 100% soybean feed; BSL50 = 50% soybean and 50% black soldier fly larvae meal; BSL100 = 100% black soldier fly larvae meal. . [a–c] Different letters in the same row indicate significant differences. [1] FAME = fatty acid methyl esters. [2] ΣSFA = Σ (C12:0, C14:0, C16:0, C18:0). ΣMUFA = Σ(C14:1, C16:1 n-7, C18:1 n-9, C18:1 n-7). ΣPUFA = Σ(C18:2 n-6, C18:3 n-6, C18:3 n-3, C20:2 n-6, C20:3 n-6, C20:4 n-6, C20:5 n-3, C22:5 n-3, C22:6 n-3). Σn-6 = Σ(C18:2 n-6, C18:3 n-6, C20:2 n-6, C20:3 n-6, C20:4 n-6). Σn-3 = Σ(C18:3 n-3, C20:5 n-3, C22:5 n-3, C22:6 n-3). [3] Other: non-detected, complement to 100.

3.4. Nutritonal Indeces

3.4.1. Thigh

The nutritional indices in thigh are shown in Table 8. ΣPUFA/ΣSFA, HH, HPI, and UI indices reveal the same trend: a significant decrease from S100 to BSL100 ($p < 0.001$). Instead, FLQ indices only significantly decrease up to BSL50. In contrast, IA and IT revealed an opposite trend, as they significantly increase from S100 to BSL100 ($p < 0.001$).

Table 8. Nutritional indices in thigh (%).

THIGH	S100	BSL50	BSL100	RMSE	*p*-Value
ΣPUFA/ΣSFA	1.191 [a]	0.936 [b]	0.797 [c]	0.071	<0.0001
IA	0.326 [c]	0.447 [b]	0.521 [a]	0.030	<0.0001
IT	0.457 [c]	0.500 [b]	0.541 [a]	0.026	<0.0001
hH	3.147 [a]	2.457 [b]	2.111 [c]	0.155	<0.0001
HPI	3.073 [a]	2.243 [b]	1.928 [c]	0.165	<0.0001
UI	112.144 [a]	102.975 [b]	98.795 [c]	3.091	<0.0001
FLQ	0.341 [a]	0.280 [b]	0.285 [b]	0.057	<0.0001
LA/ALA	12.063	11.739	11.678	1.352	0.4989

S100 = 100% soybean feed. BSL50 = 50% soybean and 50% black soldier fly larvae meal. BSL100 = 100% black soldier fly larvae meal. ΣPUFA/ΣSFA = Σ(C18:2 n-6, C18:3 n-6, C18:3 n-3, C20:2 n-6, C20:3 n-6, C20:4 n-6, C20:5 n-3, C22:5 n-3, C22:6 n-3)/Σ(C12:0, C14:0, C16:0, C18:0). IA = index atherogenicity. IT = index of thrombogenicity. hH = hypocholesterolemic/hypercholesterolemic ratio. HPI = health-promoting index. UI = unsaturation index. FLQ = fish lipid quality/flesh lipid quality. LA/ALA = linoleic acid/α-linolenic acid ratio. . [a–c] Different letters in the same row indicate significant differences.

3.4.2. Breast

As for thigh, a significant decrease was found in breast for the PUFA/SFA, hH, HPI, and UI indices, starting from S100 to BSL100 ($p < 0.001$) (Table 9). Unlike thigh, the same decrease was also observed for FLQ indices, ranging from S100 to BSL100 ($p < 0.001$). IA significantly increased up to BSL100; conversely, in thigh, IT significantly increased up to BSL50.

Table 9. Nutritional indices in breast (%).

BREAST	S100	BSL50	BSL100	RMSE	*p*-Value
PUFA/SFA	1.233 [a]	1.026 [b]	0.955 [c]	0.111	<0.0001
IA	0.322 [c]	0.406 [b]	0.442 [a]	0.033	<0.0001
IT	0.461 [b]	0.499 [a]	0.484 [a]	0.042	0.0039
hH	3.194 [a]	2.655 [b]	2.500 [c]	0.285	<0.0001
HPI	3.117 [a]	2.479 [b]	2.278 [c]	0.237	<0.0001
UI	115.666 [a]	106.560 [b]	102.124 [c]	3.584	<0.0001
FLQ	0.322 [a]	0.287 [b]	0.176 [c]	0.048	<0.0001
LA/ALA	11.788 [b]	11.324 [b]	12.484 [a]	1.080	0.0003

S100 = 100% soybean feed. BSL50 = 50% soybean and 50% black soldier fly larvae meal. BSL100 = 100% black soldier fly larvae meal. ΣPUFA/ΣSFA = Σ(C18:2 n-6, C18:3 n-6, C18:3 n-3, C20:2 n-6, C20:3 n-6, C20:4 n-6, C20:5 n-3, C22:5 n-3, C22:6 n-3)/Σ(C12:0, C14:0, C16:0, C18:0). IA = index atherogenicity. IT = index of thrombogenicity. HH = hypocholesterolemic/hypercholesterolemic ratio. HPI = health-promoting index. UI = unsaturation index. FLQ = fish lipid quality/flesh lipid quality. LA/ALA = linoleic acid/α-linolenic acid ratio. . [a–c] Different letters in the same row indicate significant differences.

4. Discussion

4.1. Performances

Our study shows the negative impact of the large dietary substitution (50% and 100% of soybean) of BSL on broiler live weight (Table 5). This result confirmed the differences found by Facey et al. [19], using same BSL replacements for soybean meal (50 and 100%). However, they did not find significant differences in live weight with lower substitution rates (12.5 and 25% BSL meal). Also, Heuel et al. [18] demonstrated that the total replacement of soybean with defatted BSL meal in animals reared for 63 days caused a significant decrease in live weight. Schiavone et al. [20] and Dabbou et al. [7], with a high inclusion level of BSL (66% replacing soybean), also found significant decreases in live weight. In addition, live weight significantly decreased in a study in which broilers

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were fed with BSL meal substituted for soybean at proportions of between 29% and 58% [17]. In contrast, Altmann et al. [38] and Pieterse et al. [39], using 47.4% BSL meal and BSF pre-pupae 55% meal, respectively, as substitutes for soybean meal, did not find differences compared to the control. Similarly, Cullere et al. [40] reported no changes in live weight when feeding broiler quails with diet containing a less than 24.8% replacement of soybean meal with defatted BSL meal.

Kim et al. [17], who observed a decline in carcass characteristics with 50% BSL substitution, hypothesized that the decline might have been caused by anti-nutritional factors such as chitin content [14], which increase in BSLM diets, or other anti-nutritional factors that still need to be identified. To support this hypothesis, Dabbou et al. [7] observed negative gut development in terms of short villi, deep crypts, and a reduced villus height-to-crypt-depth ratio upon replacing soybeans with about 67% BSL with respect to other the lower replacement levels. In contrast, Islam et al. [41] suggested that chitin fragments had potential as probiotics for the improvement of the overall health and performance of chicken, while Tabata et al. [42] found that chickens had enzymes to digest chitin, which are expressed in the glandular tissues of the chicken stomach. It should also be noted that there are differences in the chemical and nutritional composition of BLS according to the rearing substrate [43,44] and biological phase (i.e., larva, pupa, and adult), as reported by Liu et al. [45], as well as larval development [44], and this might explain the variability in the results available in the literature.

Carcass weight was negatively influenced by BSL100 (p = 0.014). The same significant decrease in carcass weight was confirmed by Heuel et al. [18], who experimented with the total replacement of soybean with BSL meal (100%) compared to the control, and by Schiavone et al. [20], who performed 66% BSL replacement. Kim et al. [17] also found significant differences from the control with lower replacement (29% of BSL meal). Unlike those results, no significant differences in carcass weight were observed by Altmann et al. [38] with 47% BSL meal replacement; Pieterse et al. [39] with up to 55% BSL meal replacement; or Cullere et al. [40] with up to 25% BSL meal replacement.

Thigh weight decreased with BSL meal inclusion. These results are different to those of other studies using BSL in different percentages, which showed no significant differences in thigh weight compared to control [20,38,39].

Breast weight was only influenced by BSL100. This result was confirmed by Heuel et al. [18], who experimented with the complete replacement of soybean meal with BSL, noting a significant decrease in breast weight. As in our study, studies using the partial substitutions of BSL below 66% did not lead to significant reductions in breast weight [20,38–40]. The addition of BSL to control feed, on the other hand, led to significant losses in breast weight in the study of Seyedalmoosavi et al. [46]. As the major significative differences were only noted when the soybean was totally substituted with BSF meal, 50% substitution did not change commercial performances.

4.2. Fatty Acids in BSL Meal

Insects are known to be reservoirs of proteins suitable for poultry nutrition. However, the content of fatty acid, especially SFA, is not indifferent. The fatty acid profile is of great importance for the healthy value of food. Generally speaking, the composition of insect meal fatty acid depends on the feeding substrates, stage of growth, and insect physiology; however, in any case, BSL is always rich in SFA, particularly lauric acid [13,47]. A positive correlation was found between larval weight and the percentages of lauric acid and total SFA in larvae, suggesting that they increase in accumulation as the larvae gain weight [13]. In our study, SFA in BSL feed were 69.31% of the total FA, with lauric acid being more than half of the SFA (64,49%), which is similar to the value reported by other studies [13,18,48–51].

4.3. Fatty Acid and Meat Quality

The increasing level of SFA recorded in our study was confirmed by Popova et al. [31] using a feed with defatted BSL (22.5% defatted BSL replacing soybean). The same results were confirmed by Altmann et al. [38], who compared the leg meat intramuscular fat of two different broiler genotypes fed with BSL in diet (47% BSL replacing soybean). Different results were found by Kim et al. [17], and they did not demonstrate significant SFA differences in the thigh of animals fed through the

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replacement of 29% and 58% BSL meal. Among SFA, palmitic (C16:0), lauric (C12:0), and myristic (C14:0) acid contents increased with the inclusion of BSL in feed, confirming the results found by Popova et al. [30] and by Altmann et al. [38]. Lauric acid content was up to 14-fold higher in BSL100, ($p < 0.001$). Popova et al. [31] obtained a 79-fold increase with 22,5% soybean substitution, starting with a control feed equal to zero and conditions similar to those of Heuel et al. [18], noting a 80-fold increase.

In breast, SFA increased only significantly up to BSL50, and this result was confirmed by other studies, which tested BSL substitutions (from 16% to 25%) [18,28,30]. In contrast, Schiavone et al. [20] found no significant differences in breast SFA when substituting 16%, 41%, and 67% soybean with BSL.

Thigh and breast showed significant proportional increases in lauric and myristic acid contents. This result was confirmed via different BSL replacements [18,20,29,31], with no distinction made between the use of defatted and full-fat BSL meal.

The negative influences of the two increasing medium-chain FA, lauric, and myristic acids on human health are still widely debated SFA are considered to be a risk factor of cardiovascular disease. , , However, lauric acid could have some benefits for animal and human health. In broiler, lauric acid is known for its beneficial properties, as it is an antibacterial agent with the capacity to prevent necrotic enteritis lesions from inhibiting *C. perfringens* in broiler gut [52,53].

With regard to human health, it was observed that a long-term diet with high dose lauric triglycerides reduced cholesterol biosynthesis in obese rats [54]. It also can reduce blood pressure and heart rate in normotensive and hypertensive rats and reduce oxidative stress in the heart and kidneys [55]. Ekanayaka et al. [56] considered about the influence of lauric acid on cholesterol levels in human blood, suggesting its decrease in a diet with coconut fat with a high percentage of lauric acid. In addition, lauric acid does not have a metabolic inflammatory effect and can contribute to the preservation of skeletal muscle metabolic health [57].

In addition, a higher concentration of SFA brings a lower risk of meat oxidation [16,58], as well as a better shelf life, confirmed by Kim et al. [30] who, using BSL oil as a functional ingredient, found increased antioxidant ability in broiler meat.

Regarding BSL meal's impact on PUFA, in thigh and breast, the increases in SFA in our study occurred at the expense of PUFA. These results were confirmed by Popova et al. [31], even at lower percentages of BSL inclusion (22.5% BSL), who did not distinguish between defatted and full-fat BSL, as well as by Altman et al. [40]. Indeed, for PUFA, linoleic acid (C18:2 n-6) is the prevailing acid, and its trend influences PUFA behavior.

In addition, Popova et al. [59] hypothesized that if stearic acid decreased, as it did in our study in both substitutions, the stearoyl-CoA desaturase and elongase activities required to produce polyunsaturated fatty acids should be lower. Liland et al. [48] concluded that PUFA if the PUFA content depended on substrates. Also, Heuel et al. [18] found different proportions of SFA and PUFA on different substrates; therefore, to better balance the SFA/PUFA ratio in feed, it is important to find the right substrates and establish insect feeding protocols to produce more standardized products for the feeding industry. Lauric acid in broiler metabolism is often used for other metabolic pathways and energy utilization [18], however all the citied studies found large quantities of lauric acid in the meat, This assumptions would allow producers to better modulate the various ingredients required to rear substrates to balance SFA and PUFA.

Regarding DPA, the significant decrease recorded in our study was confirmed by other studies, with BSL replacement ranging from 16% to 67% [20,31]. For DHA, there were disagreements between studies: some found significant differences [20,29], whereas others did not find significant changes [18,31].

The n-6/n-3 ratio increased significantly in thigh and breast from BSL50 to BSL100 ($p < 0.001$). The same result was confirmed by Heuel et al. [18] and Schiavone et al. [20], who found no differences in substitution under 67%. On the contrary, Popova et al. [31] and Cullere et al. [29] found significant evidence even below the 25% BSL replacement level.

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The decrease in PUFA, combined with the consequent lower levels of omega 6 and omega 3, could be considered to be a negative nutritional aspect due to the PUFA' well-known cholesterol-lowering effects. In the human diet, PUFA reduce the risk of cardiovascular and chronic diseases and may regulate the antioxidant signaling pathway and modulate inflammatory processes [60,61]. Furthermore, deficiencies in essential fatty acids, such as linoleic, linolenic, and arachidonic acids, cause several negative effects, such as stunted growth, skin symptoms, malabsorption, and catabolic diseases [11]. One important characteristic of organically raised broilers is the increased amounts of ALA and the three long-chain fatty acids, namely EPA, DHA, and DPA, in meat compared to those solely reared inside the poultry house. ALA is associated with the grass eaten by outdoor animals, and it is the precursor of the three long-chain fatty acids, namely EPA, DHA, and DPA. [62,63]. This gained value is lost with the inclusion of BSL, which depreciates the qualitative profile of meat, thus decreasing PUFA.

Fatty acid evaluation, performed through nutritional indices used in recent years, allows us to better assess the meat quality profile [64]. All nutritional indices considered in the present work were negatively affected by the use of BSL in diet. There are very limited studies of the nutritional indices of broilers fed with insect meal. Popova et al. [31] found a significant IA increase, even with lower substitutions (22.5% BSL), but they found no difference for IT. Schiavone at al. [20] agreed with Popova et al. [31] about the significant increase in IA in broiler breast fed with 16, 41, and 67% BSL as a substitute for soybean, as well as no differences for IT. The PUFA/SFA ratio, AI, TI, hH, HPI were used to evaluate the impact of diet on cardiovascular health (CVH). SFA contribute to elevated serum cholesterol levels, while PUFA can depress low-density lipoprotein cholesterol [3]. Moreover, the role of fatty acids in human health is not limited based on the amount of PUFA; in fact, the n-6/n-3 fatty acids ratio should be as low as possible [65]. In our study, the impact of the total substitution negatively affects the breast n-6/n-3 ratio, and the PUFA/SFA ratio is considered to be an important index for broiler meat quality [66]. The AI and TI indices increased with the administration of a BSL diet. Higher AI values involve increasing levels of total cholesterol and LDL-C in human blood plasma, and higher TI values increased the risk of coronary heart disease [67,68]. There are no references regarding broiler meat for the HPI, UI and FLQ indices, which are generally evaluated for milk, fish, and vegetable products; these indices are affected by the presence of PUFA in low quantities.

5. Conclusions

In organic farming, the protein component of the diet plays a very important role in ensuring the sustainability of animal breeding. Since synthetic amino acids are prohibited in organic farming, organic soybean plays a crucial role due to its high amino-acid profile. However, concerns regarding the sustainability of soybean mean that it necessary to find nutritionally viable alternatives for organic broiler.

Insect meal could be an ideal substitute, even in high quantities, for drastically reducing the use of soybean. The replacement of 50% and 100% of the BSL meal of diet soybean influences performance, but from a commercial perspective, the differences between the carcass weights are not so relevant, and 50% replacement does not affect commercial performance.

However, the inclusion of BSL apparently worsened the quality profile of the breast and thigh of broilers fed with either 50% or 100% BSL replacement. The high increase in lauric and myristic acids and SFA, as well as the consequent pejorative nutritional indices, apparently posed an increased risk to consumers' health, with this risk mainly being related to heart disease.

The main component responsible for the increase in SFA is lauric acid; however, the antimicrobial effect of lauric acid could provide an important added value to the broiler health. In addition, lauric acid could have a beneficial effect on human health, contributing to reduced blood pressure and oxidative stress and preserved skeletal muscle metabolic heath.

Therefore, it is necessary to investigate the effects of lauric acid and its beneficial quantities for use in poultry and consequently in human nutrition.

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