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Partial or total replacement of soybean oil by black soldier fly larvae (*Hermetia illucens L.*) fat in broiler diets: effect on growth performances, feed-choice, blood traits, carcass characteristics and meat quality

Achille Schiavone[a,b], Marco Cullere[c], Michele De Marco[a], Marco Meneguz[d], Ilaria Biasato[a], Stefania Bergagna[e], Daniela Dezzutto[e], Francesco Gai[f], Sihem Dabbou[a,d], Laura Gasco[d,f] and Antonella Dalle Zotte[c]

[a]Dipartimento di Scienze Veterinarie, University of Turin, Grugliasco, TO, Italy; [b]Istituto di Ricerche Multidisciplinari sulla Sostenibilità, University of Turin, TO, Italy; [c]Dipartimento di Medicina Animale, Produzioni e Salute, University of Padova, Legnaro, PD, Italy; [d]Dipartimento di Scienze Agrarie, Forestali e Alimentari, University of Turin, Grugliasco, TO, Italy; [e]Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, TO, Italy; [f]Istituto di Scienze delle Produzione Alimentari, National Research Council, Grugliasco, TO, Italy

ABSTRACT

The present research studied for the first time the potential application of the fat derived from the black soldier fly larvae fat (BSLF) in substitution to the soybean oil in the diet for broiler chickens: growth performances, feed-choice, blood traits, carcass characteristics and meat quality were considered in this study. A total of 150 male broiler chicks (Ross 308) at one-day of age were randomly allotted to 3 dietary treatments (5 replicates and 10 birds/pen): a basal control diet (C group), and the same diet in which the soybean oil was replaced by 50% (CH group) or 100% (H group) BSLF. Growth performances, feed-choice test, blood traits and slaughtering performances were not influenced by diets. Independently of BSLF inclusion, broiler chickens breast meat had also similar crude protein and ether extract contents and displayed similar thawing loss. Furthermore, pH, L*, a*, b* colour values, and drip loss were unaffected by dietary treatments both at 0 and 9 days of refrigerated storage. As expected, the fatty acid profile of broiler chickens breast was greatly affected by BSLF inclusion level. With increasing BSLF inclusion rate, the proportion of SFA increased (32.2, 37.8, 43.5% for C, CH and H breast meat, respectively, $p < .001$) to the detriment of the PUFA fraction (22.7, 23.0, 22.9% for C, CH and H breast meat, respectively, $p < .001$). On the contrary, MUFA fraction was unaffected. BSLF inclusion guaranteed satisfactory productive performances, carcass traits and overall meat quality, thus suggesting that BSLF could be a promising new feed ingredient for chickens.

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Hermetia illucens; dietary fat source; broiler; performance; meat quality

Introduction

The search for rapid and innovative feed solutions to improve the sustainability of the livestock sector as well as to provide enough food for the raising World population in a more sustainable way, is a global challenge of major importance for the near future (FAO 2014). Indeed, the 70% of the global freshwater withdrawals is already used by the agriculture, livestock production occupies the 70% of total land use, and the demand of meat should rise by 75% in 2050 compared to 2005–2006 (Ridoutt et al. 2012; van Huis et al. 2015).

In this perspective, insects have been addressed as a possible alternative feed for monogastric animals, because of their rich nutrient content and extremely low environmental impact (Henry et al. 2015; Biasato et al. 2016; Bovera et al. 2016). Indeed they generate low greenhouse gas and ammonia emissions, have a favourable feed conversion ratio as cold-blooded animals, and require few water and soil to grow (van Huis 2013; Makkar et al. 2014). Consumers seem to be willing to accept products obtained using these unconventional raw materials (Mancuso et al. 2016). Moreover, they can provide animal feed bio-converting food wastes thus ultimately not competing with humans for natural resources (Diener et al. 2011; Makkar et al. 2014).

Among insect species, the Diptera *Hermetia illucens* was indicated as one of the most promising candidate

CONTACT Prof. Laura Gasco laura.gasco@unito.it Dipartimento di Scienze Agrarie, Forestali e Alimentari, University of Turin, University of Turin, Largo Paolo Braccini 2, 10095 Grugliasco, TO, Italy

for industrial production in the Western world (Veldkamp & Bosch 2015). It is indigenous of the tropical, subtropical and warm temperate zones of America, but it's now distributed worldwide. Furthermore, it is not a pest and it can convert by-products and food wastes into valuable nutrients (Diener et al. 2011). Larvae are rich of nutrients, depending on the quantity of fat and protein that they can store in their body during the larval stage (Surendra et al. 2016). The typical fat content of black soldier fly prepupae was reported to be around 32%, with saturated fatty acids (SFA) being the main fatty acid group in which lauric acid (C12:0) is the predominant one accounting for the 67% of total SFA (Ramos-Bueno et al. 2016; Surendra et al. 2016). Despite this, insects' fat quantity and quality can be greatly affected by the rearing substrate (Makkar et al. 2014; St-Hilaire et al. 2007). Until now, literature data about the potential application of *Hermetia illucens* as poultry feed ingredient are scarce and evaluated exclusively its potential as an alternative protein source to soybean meal and fishmeal (Oluokun 2000; Widjastuti et al. 2014; Maurer et al. 2016) with promising results. Differently, the fat derived from the black soldier fly larvae has been recently and exclusively studied in the perspective of the biodiesel production (Surendra et al. 2016).

Therefore, the present research studied for the first time the potential application of the fat derived from the black soldier fly larvae in broiler chickens diets. The effects on productive performance (growth performance, feed-choice, carcass characteristics and meat quality) and animal welfare (evaluated by the blood traits) were considered in this study.

Materials and methods

Birds and husbandry

The present trial was performed through the collaboration of the Department of Veterinary Sciences of Turin (Italy), the Department of Agriculture, Forest, and Food Science of the University of Turin (Italy) and the Department of Animal Medicine, Production and Health of Padova (Italy). The experimental protocol was designed according to the guidelines of the current European and Italian laws on the care and use of experimental animals (European directive 86 609/EEC, put into law in Italy with D.L. 116/92). The experiment was carried out in the animal farm of the Department of Agriculture, Forest, and Food Science of the University of Turin (Italy). The poultry house was 7 m wide ×50 m long ×7 m high, equipped with waterproof floor and walls, covered completely by tiles and with an automatic ventilation system.

A total of 150 male broiler chicks (Ross 308) at one-day of age were randomly allotted to 3 dietary treatments, each consisting of 5 pens as replicates with 10 chicks per pen. The animals were reared until the slaughter age, set at 35 days. Each pen was 1.0 m wide ×1.5 m long and was equipped with a feeder, an automatic drinker and rice hulls as bedding. During the first three weeks animals were heated by infra-red lamps to maintain the suitable temperature according to standard breeding practices (Aviagen: Ross broiler management handbook, (Aviagen 2014)). Lighting schedule was 23L:1D until day 3, and 18L:6D until slaughter age. At hatching, chicks received vaccination against Newcastle disease, Gumboro disease, infectious bronchitis and coccidiosis. Birds which died during trial period were identified and the date, weight and pen were recorded.

Diets

The trial was carried out to evaluate the effects of a partial or total replacement of soybean oil with black soldier fly larvae fat (BSLF) on broiler chickens using two levels of inclusion. The BSLF was purchased from a leading European Company, specialised in *Hermetia illucens* as feed source. A basal diet based on corn meal, soybean meal and soybean oil (Table 1) was formulated and served as control group (C) and the 50 and 100% replacement of soybean oil with BSLF formed the two treatment groups (CH and H group, respectively). For each treatment, diets were split in two phases: a starter/grower diet (day 1 to day 21) and a finisher diet (day 21 to day 35). All diets were isonitrogenous to meet or exceed the NRC (1994) requirements and were adjusted according to Aviagen (2014) broiler nutrition specifications. Feed and water were provided *ad libitum* throughout the trial.

Free choice feeding

Fifteen male broiler chicks (Ross 308) were reared until 21 days of age in heated pen (1.0 m wide ×1.5 m long) and fed with the same standard control broiler-starter/grower diet. At day 21, chicks were randomly allocated to individual cages (0.5 m × 0.5 m) fitted with two feed troughs. The choice between C and H diets was offered to each bird. Feed was provided *ad libitum* for two consecutive weeks. The amount of feed consumed from each feeder was determined on the cage basis at the end of the experiment.

Table 1. Ingredients and chemical composition of the experimental diets.

	1–21 days			22–35 days		
Ingredients, g/kg	Control diet	50% BSLF	100% BSLF	Control diet	50% BSLF	100% BSLF
Corn meal	484.2	484.2	484.2	551.9	551.9	551.9
Soybean meal	335.0	335.0	335.0	294	294	294
Soybean oil	58.5	29.1	–	69.0	34.5	–
Black soldier fly larvae fat	–	29.1	58.5	–	34.5	69.0
Gluten meal	80.0	80.0	80.0	50.0	50.0	50.0
Dicalcium phosphate	7.0	7.0	7.0	4.1	4.1	4.1
Calcium carbonate	16.9	16.9	16.9	15.2	15.2	15.2
Sodium chloride	2.3	2.3	2.3	2.3	2.3	2.3
Sodium bicarbonate	1.2	1.2	1.2	1.3	1.3	1.3
DL-methionine	1.8	1.8	1.8	1.5	1.5	1.5
L-lysine	5.2	5.2	5.2	3.5	3.5	3.5
Threonine	1.6	1.6	1.6	1.0	1.0	1.0
Vitamin and mineral finisher premix	5.0	5.0	5.0	5.0	5.0	5.0
Choline chloride	0.2	0.2	0.2	0.2	0.2	0.2
3-phytase; (E-300; natuphos bio/G500)	1	1	1	1	1	1
Calculated composition, g/kg						
ME, MJ/kg,	12.9	12.9	12.9	13.4	13.4	13.4
Analysed composition, g/kg						
Dry matter	898.0	896.1	898.1	896.8	903.3	904.9
Crude protein	243.0	247.2	240.6	203.1	202.0	213.8
Crude fat	85.9	87.9	91.0	94.6	100.6	96.9
Crude fibre	67.4	59.7	67.5	61.1	62.8	50.2
Ash	61.9	64.6	68.2	55.9	53.1	55.2

Growth performances

Clinical signs and mortality were monitored daily during the whole experimental period. Final body weight (FBW) was recorded at day 35. Feed intake (FI), daily weight gain (DWG) and feed conversion ratio (FCR) were determined for the overall experimental period (1–35 days). All measurements were performed on the pen basis using a high precision electronic scale (Sartorius – Signum®).

Slaughtering procedure

At day 35, 15 birds (3 birds/pen) from each feeding group (chosen on the basis of pen average FBW) were individually identified with a shank ring and weighted. All selected chickens were slaughtered at a commercial abattoir where birds were slaughtered according standard procedures (electrical stunning and exsanguination). Head, neck, feet, abdominal fat and internal organs were removed in order to obtain chilled carcass (CC) (WPSA 1984). The weight of liver, spleen, heart and abdominal fat were immediately recorded and expressed in g. The weight of CC, breast and thighs were recorded after chilling 24 h at 4 °C. Breast and thighs weight were expressed as percentage of the CC. A total of 45 breasts (15 per dietary treatment, 3/pen) were then separated in their right and left side, individually vacuum sealed, and sent in refrigerated conditions (4 ± 1 °C) to the Department of Animal Medicine, Production and Health (University of Padova). For each bird the right breast was subjected to a retail display storage test, whereas the left breast was frozen at −40 °C until further meat chemical analysis.

Hematological and serum parameters

At the end of the experiment (day 35), blood samples were collected at slaughtering from 3 birds per pen: 2.5 mL was placed in an EDTA tube and 2.5 mL in a serum-separating tube. A blood smear was prepared, using one glass slide for each bird, from a drop of blood without anticoagulant. The smears were stained using May-Grünwald and Giemsa stains (Campbell 1995). The total red and white blood cell counts were determined in an improved Neubauer haemocytometer on blood samples previously treated with a 1:200 Natt-Herrick solution. One hundred leukocytes, including granular (heterophils, eosinophils and basophils) and non-granular (lymphocytes and monocytes) leukocytes, were counted on the slide and the H/L ratio was calculated. The tubes without anticoagulant were left to clot in a standing position at room temperature for approximately two hours to obtain serum. The serum was separated by means of centrifugation at 700 × *g* for 15 min and frozen at −80 °C until analysis. The total proteins were quantified by means of the 'biuret method' (Bio Group Medical System kit; Bio Group Medical System, Talamello, Italy); the electrophoretic pattern of the serum was obtained using a semi-automated agarose gel electrophoresis system (Sebia Hydrasys®, Norcross, GA, USA). The alanino-aminotransferase (ALT), aspartate-aminotransferase (AST), triglycerides, cholesterol, glucose, phosphorus, magnesium,

iron, uric acid and creatinine serum concentrations were measured by means of enzymatic methods in a clinical chemistry analyser (Screen Master Touch, Hospitex diagnostics Srl., Florence, Italy).

Meat rheological traits during retail display

Right breasts were freed from vacuum bags, dried and weighed. On the cranial and caudal ends of *Pectoralis major* muscles, pH was measured 48 h *post mortem* using a Mettler Toledo FE20 pH-meter. On the same portions, colour values of lightness (L*), redness (a*) and yellowness (b*) (CIE 1976) were subsequently measured using a RM200QC colorimeter (X-Rite, Co, Neu-Isenburg, Germany. Measuring Area: 8 mm; Measuring Geometrics: 45/0 Image Capture; Illuminant/Observer: D65/10). The colour measurements were performed with the instrument resting on samples kept in horizontal plane. The pH and colour values adopted are the average of two measurements for each sample.

Breasts were then individually placed in polystyrene trays, wrapped with PVC film and placed in refrigerated conditions (4 ± 1 °C), under fluorescent light illumination (L58 W/20, Osram, Germany) at 870 lux (MT 940, Major) for a 9-day retail display test. At day 9 of storage, breasts were dried and weighed in order to calculate drip loss. Subsequently, pH and colour measurements were carried out as described previously.

Fatty acid profile of diets, thawing loss, proximate composition and fatty acid profile of chicken breast meat

Frozen left chicken breasts were freed from vacuum bags and allowed thawing for 24 h at +4 °C. Afterwards they were dried and weighed again in order to calculate thawing loss. Subsequently, each breast was ground with a Retsch Grindomix GM 200 (10 s at 7000 *g*) and freeze-dried. On freeze-dried samples proximate composition was analysed on $n = 45$ *Pectoralis major* meat samples using AOAC methods (AOAC 1995), with protein content calculated by difference. Lipid extraction and Fatty Acid Methyl Esters (FAME) determination were performed on $n = 45$ *Pectoralis major* meat samples.

Lipid extraction of the meat was performed by Accelerated Solvent Extraction (M-ASE) using a binary solvent mixture chloroform:methanol (1:2). Samples were subsequently transmethylated using a methanolic solution of H_2SO_4 (4%) in order to determine fatty acid methyl esters (FAME). A biphasic separation was obtained by adding 0.5 ml of distilled water and 1.5 ml of N-Heptane to each sample. FAME were quantified by gas chromatography (Shimadzu GC17A, equipped with an Omegawax 250 column (30 m × 0.25 μm × 0.25 μm) and FID detector. Helium was used as carrier gas at a constant flow of 0.8 mL/min. Injector and detector temperatures were 260 °C. Peaks were identified based on commercially available FAME mixtures (37-Component FAME Mix, Supelco Inc., Bellefonte, PA), and data obtained were expressed as % of total detected FAME (Table 2).

Statistical analysis

The statistical analysis was performed using SPSS 17 software for Windows (SPSS, Inc., Chicago, IL). Data were tested by one-way ANOVA, followed by Duncan's post-hoc test. The experimental unit was the pen for growth performances, while the individual bird was used for slaughtering performances and meat quality parameters. For the free-choice trial, the Student's *t*-test was used. Significance was declared at $p < .05$. A statistical trend was considered for $p < .10$. Results are presented as the mean and standard error of the mean (SEM).

Results and discussion

At the beginning of the experiment, the body weight of chickens was the same in all groups (Table 3) and also their FBW did not differ among C, CH and H groups. Similarly, FCR, DWG and DFI were not influenced by the partial or total replacement of soybean oil with BSLF ($p > .05$); only DFI displayed a positive numerical trend from C to H groups (55.1, 61.2 and 65.4 g feed/day for C, CH and H chickens, respectively, $p = .084$). The chickens remained healthy (absence of clinical signs) throughout the study and no mortality occurred during the trial. The results of the free-choice test showed that birds did not express any preference for C or H diets; indeed, their 14-days feed intake was similar in the two groups (526.1 and 570.4 g of feed for C and H, respectively). The blood traits (Table 3) were not significantly affected by the dietary treatments and all the parameters evaluated in the present trial fell within the physiological ranges (Lumej 2008), thus suggesting that dietary BSLF inclusion does not affect health status of the animals.

Insects have recently started being studied as innovative feed ingredients for aquaculture (Belforti et al. 2015; Gasco et al. 2016) and poultry (Bovera et al. 2015; Biasato et al. 2016; Cullere et al. 2016) since the two main derived products (meal and fat)

Table 2. Fatty acid profile (% of total FAME) of the black soldier fly larvae fat (BSLF) and of the experimental diets.

		1–21 days			22–35 days		
Fatty acids	BSLF	Control diet	50% BSLF	100% BSLF	Control diet	50% BSLF	100% BSLF
C10:0	1.43	0.00	0.54	0.94	0.00	0.55	0.93
C12:0 (Lauric)	52.6	0.34	20.9	37.5	1.06	21.2	38.1
C14:0 (Myristic)	8.54	0.17	3.71	6.71	0.29	3.71	6.91
C16:0 (Palmitic)	10.9	12.4	12.8	12.9	12.5	12.5	13.0
C17:0	–	0.08	0.10	0.11	0.09	0.10	0.11
C18:0 (Stearic)	1.53	3.02	2.44	1.95	3.05	2.43	1.94
C20:0	–	0.22	0.16	0.06	0.25	0.16	0.12
C24:0	–	0.15	0.10	0.06	0.15	0.10	0.05
SFA	75.0	16.5	40.7	60.4	17.3	40.8	61.4
C14:1	0.17	0.00	0.07	0.13	0.00	0.07	0.12
C16:1	1.98	0.12	0.98	1.72	0.17	0.97	1.69
C17:1	–	0.11	0.00	0.06	0.00	0.06	0.06
C18:1 *n*-9 (Oleic)	6.16	22.0	16.3	11.2	22.3	16.4	11.5
C18:1 *n*-11	0.24	1.24	0.81	0.43	1.23	0.79	0.40
MUFA	8.55	23.5	18.2	13.7	23.7	18.3	13.7
C18:2 *n*-6 (LA)	11.6	52.4	36.1	21.8	51.5	33.9	20.9
C22:2	–	0.00	0.70	0.05	0.00	0.07	0.05
C18:3 *n*-3 (α-LA)	1.01	5.12	3.05	1.11	4.89	2.07	1.05
C20:4 *n*-6 (Arachidonic)	0.29	0.00	0.00	0.00	0.00	0.07	0.06
C20:5 *n*-3 (EPA)	0.00	0.31	0.17	0.06	0.30	0.17	0.05
PUFA	12.9	57.9	40.0	23.1	56.7	36.2	22.1
UFA/SFA	0.29	4.95	1.43	0.61	4.64	1.34	0.58
n-6	11.9	52.4	36.1	22.8	51.5	35.9	22.0
n-3	1.01	5.43	3.22	1.17	5.19	3.24	1.10
n-6/*n*-3	11.8	9.65	11.2	19.5	9.93	11.1	20.0
Identified FA, %	96.4	97.8	98.9	97.2	97.7	95.2	97.2

Table 3. Growth performance ($n = 5$ pens/dietary treatment), free-choice test ($n = 15$ chickens) and blood traits ($n = 15$) of broiler chickens fed with increasing levels of black soldier fly larvae fat (BSLF).

	Control (C)	50% BSLF (CH)	100% BSLF (H)	SEM	*p*-value
Growth performance					
Initial body weight, g (d 1; IBW)	45.20	45.10	45.20	0.14	.838
Final body weight, g (d 35; FBW)	1747	1763	1796	42.50	.901
Daily feed intake, g (DFI)	55.10	61.20	65.40	2.00	.084
Daily weight gain, g (DWG)	37.10	40.40	43.10	1.32	.157
Feed conversion ratio (FCR)	1.48	1.51	1.52	0.01	.479
Free-choice test, g/intake for 14 days	526	–	570	33.5	.524
Blood traits					
Erythrocyte, 10^6 cell/μl	4.26	4.14	4.15	0.47	.498
Leukocyte, 10^3 cell/μl	14.40	13.10	13.20	0.29	.103
H/L ratio	0.66	0.58	0.57	0.03	.360
Total protein, g/dl	3.77	3.38	3.42	0.09	.148
AST[a], UI/l	257.85	237.43	250.09	12.44	.800
ALT[b], UI/l	25.21	22.43	19.03	1.52	.305
GGT[c], UI/l	32.00	27.45	30.20	1.92	.622
Uric Acid, mg/dl	5.88	4.87	5.63	0.22	.125
Creatinine, mg/dl	0.34	0.36	0.34	0.01	.541
Triglycerides, mg/dl	48.16	56.16	49.45	2.00	.190
Cholesterol, mg/dl	76.80	69.70	71.78	3.55	.288
Phosphorous, mg/dl	4.98	4.57	4.68	0.75	.703
Magnesium, mEq/l	1.57	1.58	1.49	0.19	.890
Iron, μg/dl	57.81	53.41	59.11	5.88	.426

[a]AST: aspartate aminotransferase;
[b]ALT: alanine aminotransferase;
[c]GGT: gamma glutamyl transferase.

may be suitable for substituting/integrating the conventional protein and fat sources (soybean/fish meals, vegetable oils). The present experiment is the first one testing the black soldier fly larvae fat as a partial or total replacement of the soybean oil in the diet for growing chickens. The first study which assessed the nutritional value of *Hermetia illucens* larval meal for growing chickens reported that the black soldier fly meal can be considered a valuable source of energy and digestible amino acids, thus being a potential feed ingredient for chicken diets in the near future (De Marco et al. 2015). *Hermetia illucens* was also tested as an alternative protein source to soybean meal and revealed positive results in terms of productive performance (Cullere et al. 2016; Hale 1973; Oluokun 2000). The results of the present study corroborate these previous data.

Similarly to performance traits, carcass traits (Table 4) were not significantly affected by a partial/total replacement of soybean oil with BSLF: indeed, CC, breast, thighs and internal organs showed similar weights and incidences on the live weight in C, CH and H animals. Independently on the BSLF inclusion, broiler chickens breast meat had also similar crude protein (19.4, 19.2, 19.2% for C, CH and H dietary groups, respectively) and ether extract (4.05, 4.11, 3.91% for C, CH and H, dietary groups, respectively) contents and displayed similar thawing loss (1.83, 1.77 and 2.03% for C, CH, and H dietary groups, respectively) in the three experimental groups (Table 5).

As expected, the fatty acid profile of broiler chickens breast was greatly affected by the BSLF inclusion level (Table 6). With increasing the BSLF percentage,

Table 4. Slaughter performance and internal organs weight of broiler chickens ($n = 15$/dietary treatment) fed with increasing levels of black soldier fly larvae fat (BSLF).

	Control (C)	50% BSLF (CH)	100% BSLF (H)	SEM	*p*-value
Live weight, g (LW)	1776	1802	1781	30.7	.941
Carcass weight, g (CW)[a]	1197	1243	1206	24.8	.732
Breast[a], % CW	24.3	24.7	27.8	0.28	.912
Thighs[b], % CW	30.8	31.9	30.2	0.35	.147
Abdominal fat, % CW	1.3	1.1	1.5	0.14	.655
Liver, g	39.3	36.9	37.2	0.86	.488
Heart, g	12.0	12.1	11.8	0.35	.934
Spleen, g	1.6	2.0	1.7	0.07	.100

[a]Without bone and skin.
[b]With bone and skin.

Table 5. Thawing loss (%) and proximate composition (g/100 g meat) of breast meat (*Pectoralis major*) ($n = 15$/dietary treatment) derived from broiler chickens fed with increasing levels of black soldier fly larvae fat (BSLF).

	Control (C)	50% BSLF (CH)	100% BSLF (H)	SEM	*p*-value
Thawing loss	1.83	1.77	2.03	1.12	.3101
Water	75.30	76.90	75.60	0.66	.3252
Crude protein	19.40	19.20	19.20	0.63	.7165
Ether extract	4.05	4.11	3.91	0.58	.6108
Ash	1.28	1.28	1.27	0.03	.4231

Table 6. Fatty acid profile (% of total FAME) of breast meat (*Pectoralis major*) ($n = 15$/dietary treatment) derived from broiler chickens fed with increasing levels of black soldier larvae fat (BSLF).

	Control (C)	50% BSLF (CH)	100% BSLF (H)	SEM	*p*-value
C8:0	0.10	0.07	0.12	0.10	.3792
C10:0	0.03^{B}	0.18^{A}	0.28^{A}	0.09	<.0001
C12:0 (Lauric)	0.09^{C}	4.75^{B}	8.50^{A}	1.14	<.0001
C14:0 (Myristic)	0.32^{C}	2.20^{B}	3.59^{A}	0.45	<.0001
C15:0	0.02	0.03	0.11	0.10	.1005
C16:0 (Palmitic)	20.2	21.0	21.1	1.52	.2825
C17:0	0.11	0.05	0.05	0.09	.1588
C18:0	10.3	9.47	8.79	1.77	.1638
C20:0	0.09	0.10	0.09	0.10	.9672
C22:0	0.06	0.06	0.09	0.15	.8904
C24:0	0.97	0.87	0.71	0.18	.3776
SFA	32.2^{C}	37.8^{B}	43.5^{A}	2.44	<.0001
C14:1	0.00^{B}	0.13^{B}	0.31^{A}	0.12	<.0001
C16:1	1.42^{B}	2.27^{AB}	2.86^{A}	0.91	.0033
C18:1 *n*-9 (Oleic)	18.9	18.4	17.7	2.49	.5017
C18:1 *n*-11	2.41^{a}	2.18^{ab}	2.10^{b}	0.24	.0130
MUFA	22.7	23.0	22.9	3.31	.9804
C18:2 *n*-6 (LA)	27.4^{A}	22.4^{B}	18.1^{C}	2.54	<.0001
C18:3 *n*-6 (GLA)	0.13	0.17	0.19	0.10	.3738
CLA	0.20	0.25	0.22	0.01	.6778
C20:2	0.66	0.53	0.55	0.18	.2213
C20:3 *n*-6 (DGLA)	0.59	1.19	1.00	1.33	.5568
C20:4 *n*-6 (Arachidonic)	5.08	4.35	4.48	1.69	.7470
C18:3 *n*-3 (α-LA)	1.81^{A}	1.42^{AB}	0.94^{B}	0.37	<.0001
C20:5 *n*-3 (EPA)	0.23	0.29	0.29	0.31	.8528
C22:6 *n*-3 (DHA)	0.67	0.53	0.55	0.38	.6341
PUFA	36.8^{A}	31.1^{B}	26.4^{B}	3.66	<.0001
UFA/SFA	1.86^{A}	1.40^{B}	1.14^{C}	0.15	<.0001
n-6	33.4^{A}	28.3^{B}	24.1^{B}	3.33	<.0001
n-3	2.71^{A}	2.24^{AB}	1.79^{B}	0.51	.0008
n-6/*n*-3	12.7	13.2	13.8	2.62	.6253
Indentified FA, %	91.7	92.9	92.8		

[a-b]Rows with different letters differed significantly ($p < .05$);
[A-C]Rows with different letters differed significantly ($p < .01$).

Table 7. Drip loss, pH and L*a*b* colour values of breast meat (*Pectoralis major*) ($n = 15$/dietary treatment) derived from broiler chickens fed with increasing levels of black soldier fly larvae fat (BSLF), subjected to a 9 days retail display.

	Control (C)	50% BSLF (CH)	100% BSLF (H)	SEM	*p*-value
Day 0:					
pH	5.82	5.85	5.90	0.12	.1808
L*	52.0	52.10	51.30	2.55	.6520
a*	−0.69	−0.78	−0.87	1.02	.8780
b*	10.30	11.30	11.30	1.90	.2163
Day 9:					
pH	5.95	5.91	5.99	0.15	.6234
L*	50.40	50.50	50.50	2.98	.9972
a*	1.72	1.38	1.06	1.09	.4985
b*	9.93	11.0	11.10	1.90	.3881
Drip loss, %	4.05	5.23	4.45	0.81	.0675

the proportion of SFA increased (32.2, 37.8, 43.5% for C, CH and H breast meat, respectively, $p < .001$) to the detriment of the PUFA fraction (36.8, 31.1, 26.4% for C, CH and H breast meat, respectively, $p < .001$). On the contrary, the MUFA fraction was unaffected. The BSLF is a great source of SFA which accounted for the 75% of total FAME (Table 2) and were mainly represented by lauric (C12:0, 52.6%), myristic (C14:0, 8.54%) and palmitic (C16:0, 10.9%) FA. Medium chain fatty acids (6 to 12 carbon atoms, thus including lauric acid) are well known for their antimicrobial activity through cellular membrane disruption and can also be used as a nutritional supplements (Kim & Rhee 2016).

BSLF was found to be relatively scarce in PUFA which were almost exclusively represented by linoleic acid (C18:2 n-6, 89.9% of PUFA and 97.6% of n-6) and α-linoleic acid (C18:3 n-3, 7.8% of PUFA and 100% of n-3). These findings were in accordance with the fatty acid profile of BSLF recently presented by Surendra et al. (2016). Such composition lowered the content of the n-6 and the n-3 PUFA fractions as the dietary BSLF level increased (33.4, 28.3, 24.1% in C, CH and H breast meat for n-6, $p < .0001$; 2.71, 2.24, 1.79% in C, CH, and H breast meat for n-3, $p = .0008$). Despite such great changes in the proportions of breast meat FA, the n-6/n-3 ratio remained unaffected. In general, the fatty acid composition of BSLF worsened the lipid profile of the meat and this is certainly an aspect that requires further research efforts, since dietary tendencies of modern consumers are towards healthier meat and meat products (Olmedilla-Alonso et al. 2013). The FA composition of *Hermetia illucens* fat can change greatly according to the rearing substrate (Makkar et al. 2014; Leong et al. 2016), therefore research efforts should be carried out in order to assess how much the FA profile of *Hermetia illucens* fat can be improved through substrate composition.

Results of the 9-days retail display test (Table 7) showed that the pH (5.82, 5.85, 5.90 for C, CH and H dietary groups, respectively) and the L*, a*, b* colour values remained similar in all three experimental groups, both at 0 and 9 days of refrigerated storage. Similarly, also drip loss was the same in the three groups (4.05, 5.23, 4.45% for C, CH and H dietary groups, respectively). The physical traits greatly affect the visual appearance of the product, with subsequent huge impact on consumer's choice at purchase.

Conclusions

When BSLF substituted 50% or 100% of soybean oil in the diets for broiler chickens throughout their productive cycle, it guaranteed satisfactory productive performances, carcass traits and overall meat quality. Thus suggesting that BSLF could be considered a promising new feed ingredient for chickens. However, further research efforts are necessary to find strategies for improving the FA profile of the fat through substrate modulation, in order to provide a healthier meat, which is a key aspect for the modern consumer. Chickens also displayed the same preference for the basal diet and that with 100% black soldier fly larvae fat substitution level.

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ORCID

Achille Schiavone http://orcid.org/0000-0002-8011-6999
Marco Cullere http://orcid.org/0000-0001-9409-8202
Michele De Marco http://orcid.org/0000-0002-1715-487X
Marco Meneguz http://orcid.org/0000-0001-8007-2044
Ilaria Biasato http://orcid.org/0000-0002-8855-4248
Francesco Gai http://orcid.org/0000-0003-1037-9483
Sihem Dabbou http://orcid.org/0000-0002-3525-1614
Laura Gasco http://orcid.org/0000-0002-1829-7936
Antonella Dalle Zotte http://orcid.org/0000-0001-9214-9504

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