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doi:10.1017/S1751731119000685

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Black soldier fly defatted meal as a dietary protein source for broiler chickens: effects on carcass traits, breast meat quality and safety

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(Received 8 October 2018; Accepted 5 March 2019)

*Finding insect meals as alternative sources of poultry feedstuffs is a recent research topic; therefore, the present study aimed to evaluate the effects of defatted black soldier fly (*Hermetia illucens L., **HI**) larvae meal in broiler chicken diets on the carcass characteristics and meat quality parameters, proximate composition, fatty acid profile and the heavy metal content of the breast meat. Four dietary treatments were designed: a control diet (HI0) and three experimental diets (HI5, HI10 and HI15), corresponding to 50, 100 and 150 g/kg HI inclusion levels, respectively. The inclusion of 50, 100 and 150 g HI meal per kg feed supply 16.56%, 33.01% and 49.63% of required crude protein. The broilers were slaughtered at day 35, the carcasses were weighed and the breast muscles were excised from 16 birds per each feeding group (two birds per replicate pens) and used for meat quality evaluation. Linear and quadratic responses were observed, for increasing HI meal levels, in the live and carcass weights (maximum for HI10). As far as the colour of the breast meat is concerned, redness (a*) showed a linear response, while yellowness (b*) linearly decreased with increasing HI meal levels (minimum for HI15). As the HI larvae meal increased in the diets, the moisture content linearly decreased and the protein content increased. The total saturated fatty acid and total monounsaturated fatty acid proportions rose to the detriment of the polyunsaturated fatty acid fraction. The HI larvae meal, used in the current study, represents a valuable protein source for broiler chickens when included by up to 100 g/kg in their diets, as an improved slaughtering performance was observed without any detrimental effects on meat quality parameters or heavy metal residues in the meat.*

Keywords: *Hermetia illucens*, broiler chickens, fatty acids, insect meal, heavy metals

Implication

Due to their nutritive value and low environmental impact, there is an increasing interest on black soldier fly larvae meal (*Hermetia illucens* L.) as potential feed source in poultry diets. This study showed that insect meal from *Hermetia illucens* larvae can substitute conventional ingredients in the diet for broiler chickens, without any detrimental effects on meat quality parameters or heavy metal residues in the meat.

Introduction

Nowadays, insects are considered a novel and promising alternative dietary protein source for monogastric animals (Makkar *et al.*, 2014). Insects contain high quality and quantity of protein and are characterized by high feed to protein conversion rate (Makkar *et al.*, 2014). Furthermore, they can easily be reared on different secondary raw materials, thus allowing to reduce their disposal costs and promote reutilization of by-products (Makkar *et al.*, 2014; Boccazzi *et al.*, 2017; Meneguz *et al.*, 2018; Ottoboni *et al.*, 2018). Among the different insect species, black soldier fly (*Hermetia illucens* L., **HI**) larvae meal is already being used in developed countries as a feed for pets and exotic animals, including birds and fish. In relation to the nutritive profile, HI larvae contain large amounts of lipids, which show an extreme quantitative and qualitative variability, depending on the chemical composition of the rearing substrate (Spranghers *et al.*, 2017; Meneguz *et al.*, 2018). In addition, HI contains 58% to 72% saturated fatty acids (**SFA**) and 19% to 40% mono (**MUFA**) and polyunsaturated

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fatty acids (**PUFA**) of the total fat content (Makkar *et al*., 2014). Moreover, the accumulation of toxic elements (i.e. heavy metals, mycotoxins and pesticides) is also one of the potential hazards associated with insect production (Belluco *et al*., 2013); however, data regarding the chemical safety of reared insects are very scarce. *Hermetia illucens* has recently been tested as a feed ingredient in conventional poultry diets as a fat source in broiler chickens (Schiavone *et al*., 2017a and 2018) and in laying hens and broiler quails as protein source (Cullere *et al*., 2016 and 2018; Maurer *et al*., 2016), providing satisfactory results in terms of animal performance and gut morphology. In male broiler chickens, Dabbou *et al*. (2018) demonstrated that increasing levels (50, 100 and 150 g/kg) of dietary HI meal inclusion may improve the live weight (**LW**) and dietary feed intake during the starter period. However, at the highest inclusion level, it may also negatively affect the feed conversion ratio and gut morphology. This suggests that low inclusion levels (50 or 100 g/kg) may be more suitable. However, no significant effects on the haematochemical and histological parameters were observed in relation to HI meal utilization. On the other hand, there is a lack of scientific information about the impact of the use of HI larvae meal on meat safety and quality traits. *Hermetia illucens* larvae meal utilization as protein source has recently been reported to not significantly affect meat quality parameters in broiler quails, with the only exception of a negative modulation of the fatty acid (**FA**) profile (Cullere *et al*., 2018). However, no studies are currently available on these aspects in broiler chickens.

Based on the above-mentioned paper and in order to provide reliable data on the potential use of insect meal in broiler chicken nutrition, the present research aims to evaluate the effects of dietary defatted HI larvae meal inclusion on the carcass traits and meat quality parameters, proximate composition, FA profile and heavy metals residues of broiler chicken breast meat.

Material and methods

Birds and diets

A detailed description of the experimental design is reported in Dabbou *et al*. (2018). Briefly, 256 one-day-old male broiler chickens were reared from day 1 to day 35 and randomly allotted to four dietary treatments (eight pens/treatment and eight birds/pen). Four experimental diets were formulated to be isonitrogenous and isoenergetic during the three phase-feedings. The diets were formulated according to Sauvant *et al*. (2004) for all feedstuffs, except for HI larvae meal for which chemical composition as described by Schiavone *et al*. (2017b) was used. The diets were prepared including, as a feed basis, increasing levels of HI larvae meal (0, 50, 100 and 150 g/kg; HI0, HI5, HI10 and HI15, respectively). The inclusion of 50, 100 and 150 g HI meal per kg feed supply 16.56%, 33.01% and 49.63% of required crude protein. A partially defatted HI meal derived from larvae that were fed with vegetable by-products (cereals). The defatting process was performed using high pressure and without solvents.

The ingredients and the chemical composition of the experimental diets are reported in Table 1.

Fatty acid profile of the insect meal and experimental diets

The FA composition of the HI larvae meal and experimental diets was assessed using the method described by Schiavone *et al*. (2007). The fatty acid methyl esters were separated, identified and quantified on the basis of the chromatographic conditions reported by Renna *et al*. (2014). The results were expressed as g/100g of total fatty acids (**TFA**) (Table 2).

Slaughtering procedures

At 35 days of age, 16 animals (two birds per replicate pens) from each feeding group (chosen based on the average final LW in each pen) were individually identified with a shank ring and weighed. The chickens were slaughtered at a commercial abattoir. Plucked and eviscerated carcasses were obtained, and the head, neck, feet and abdominal fat were removed to obtain the chilled carcass. Then, the liver, heart, spleen, bursa of Fabricius, abdominal fat, thigh and breast weights were immediately recorded. The breast and thigh weights were expressed as percentages of the LW. Twenty-four hours after slaughtering, breasts were separated into right and left sides, individually vacuum sealed and refrigerated (4±1°C). Breast meat was divided into two parts and frozen at −20°C for further meat analyses.

Meat quality parameters

The meat quality parameters (pH, colour, drip loss, cooking loss and shear force) were assessed on the *Pectoralis major* muscles of the right breast (from the 16 slaughtered animals per each feeding group) following the harmonized methodologies for the assessment of poultry meat quality features described in Supplementary Material S1.

Chemical composition and fatty acid profile

The left breast meat of the 16 slaughtered animals per each feeding group was used to perform chemical analysis. The moisture and ashes were determined according to the Association of Official Analytical Chemists (AOAC, 1990) procedure. Proteins were determined using the standard Kjeldahl copper catalyst method (AOAC, 1990).Total lipids were measured using modification of the chloroform: methanol procedure described by Folch *et al*. (1957). Fatty acids were then determined as reported in Supplementary Material S1. The results were expressed as g/100g of TFA. The health indexes were calculated as described in the Supplementary Material S1.

Heavy metals and arsenic

The analysis of heavy metals and arsenic were performed on HI larvae meal and four breast meat pools (one pool per feeding group, each of them composed by eight slaughtered animals (one bird per replicate pens)), as presented in Supplementary Material S1. The results were expressed as mg/kg 12% humidity.

Table 1 *Ingredients, apparent metabolizable energy and chemical composition of the experimental diets fed to broiler chickens*

	Starter period				Grower period				Finisher period			
Items	HI0	HI5	HI10	HI15	HI0	HI5	HI10	HI15	HI0	HI5	HI10	HI15
Ingredients (g/kg as fed)												
Maize meal	508.8	526.9	545.5	566.9	536.1	557.1	574.5	606.1	537.2	590.8	620.3	653.2
Soybean meal (48% CP)	345.3	299.0	248.0	193.0	332.0	277.4	230.0	153.0	300.0	253.0	178.0	100.0
HI larvae meal	0.0	50.0	100.0	150.0	0.0	50.0	100.0	150.0	0.0	50.0	100.0	150.0
Corn gluten meal	54.0	32.0	15.0	0.0	35.0	20.0	0.0	0.0	21.0	0.0	0.0	0.0
Soybean oil	46.3	45.1	43.0	40.0	59.3	56.4	54.9	48.0	71.2	70.0	63.2	56.0
Dicalcium phosphate	6.5	8.5	10.5	12.5	4.0	6.0	8.3	10.6	3.5	6.0	8.3	10.5
Calcium carbonate	17.3	16.4	15.6	14.8	16.7	15.8	14.8	14.0	15.1	14.0	13.2	12.4
Sodium chloride	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3
Sodium bicarbonate	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3
DL-methionine	2	2.2	2.3	2.3	1.8	1.9	2	2.0	1.8	1.9	1.9	1.8
L-lysine	5.1	5.2	5.4	5.8	3.8	4.1	4.2	5.0	3.3	3.4	4.1	5.0
Threonine	1.9	1.9	1.9	1.9	1.5	1.5	2.0	2.0	1.1	1.1	1.2	1.3
Trace mineral-vitamin premix[a]	8.0	8.0	8.0	8.0	5.0	5.0	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	0.2	0.2	0.2	0.2	0.2	0.2
3-phytase	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Total	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000
AME[b] (MJ/kg DM)	12.56	12.56	12.56	12.56	12.98	12.98	12.98	12.98	13.40	13.40	13.40	13.40
Nutrient composition[c] (%)												
CP	23.01	22.98	23.01	23.01	21.50	21.52	21.52	21.52	19.51	19.47	19.54	19.50
EE	7.30	7.33	7.28	7.14	8.63	8.50	8.50	8.01	9.86	9.89	9.41	8.88
CF	3.25	2.99	2.70	2.41	3.21	2.91	2.64	2.25	3.08	2.82	2.43	2.04
Calcium	0.96	0.96	0.96	0.96	0.87	0.87	0.87	0.87	0.79	0.79	0.79	0.79
Phosphorus	0.48	0.48	0.48	0.48	0.43	0.43	0.43	0.43	0.40	0.40	0.40	0.40
Methionine[d]	0.56	0.56	0.56	0.56	0.51	0.51	0.51	0.51	0.47	0.47	0.47	0.47
Lysine[d]	1.44	1.44	1.44	1.44	1.29	1.29	1.29	1.29	1.16	1.16	1.16	1.16
Threonine[d]	0.97	0.97	0.97	0.97	0.88	0.88	0.88	0.88	0.78	0.78	0.78	0.79

HI=*Hermetia illucens;* AME=apparent metabolizable energy; DM=dry matter; CP=crude protein; EE=ether extract; CF=crude fibre.
[a] Mineral-vitamin premix*:* vitamin A (retinyl acetate), 12500 IU; vitamin D3 (cholecalciferol), 3000 IU; vitamin E (DL-a-tocopheryl acetate), 60 IU; vitamin K (menadione sodium bisulphite), 1.02 mg; riboflavin, 2.0 mg; pantothenate, 8.0 mg; niacin, 6 mg; piridossin, 4 mg; folic acid, 0.5 mg; biotin, 0.10 mg; tiamin, 1.0 mg; vitamin B12, 20 mg; Mn, 120 mg; Zn, 80 mg; Fe, 52 mg; Cu, 15 mg; I, 1.5 mg; Se, 0.4 mg.
[b] Calculated according to Schiavone *et al.* (2017b) and Sauvant *et al.* (2004).
[c] Chemical analyses were carried out on three replicates of each feed sample.
[d] Digestible amino acid estimated according to Schiavone *et al.* (2017b) and Sauvant *et al.* (2004) for HI meal and other ingredients, respectively.

Table 2 *Fatty acid profile of HI larvae meal and experimental diets of broiler chickens (g/100 g of total fatty acids)*

		Starter period				Grower period				Finisher period			
	HI larvae meal	HI0	HI5	HI10	HI15	HI0	HI5	HI10	HI15	HI0	HI5	HI10	HI15
C12:0	54.59	nd	2.73	5.46	8.19	nd	2.73	5.46	8.19	nd	2.74	5.46	8.19
C14:0	10.15	0.10	0.60	1.10	1.60	0.10	0.60	1.10	1.61	0.09	0.60	1.10	1.61
C16:0	12.03	10.37	10.43	10.48	10.55	10.45	10.51	10.58	10.64	10.10	10.55	10.62	10.70
C18:0	1.77	2.50	2.40	2.30	2.17	2.51	2.41	2.29	2.13	2.42	2.39	2.21	2.03
C18:1 *c*9	7.91	21.51	22.20	22.90	23.65	21.87	22.43	23.17	23.85	22.15	22.73	23.31	23.107
C18:2 n6	5.98	52.59	50.15	47.72	45.30	53.05	50.63	48.20	45.81	51.26	50.83	48.45	46.09
C18:3 n3	0.80	3.26	3.14	2.80	2.41	3.27	3.09	2.77	2.22	3.12	3.03	2.48	2.13
SFA	80.28	15.38	18.67	21.96	25.25	15.24	18.52	21.80	25.05	15.83	18.43	21.70	24.93
MUFA	12.88	24.24	24.82	25.43	26.06	24.36	24.81	25.45	26.03	24.57	25.01	25.48	26.31
PUFA	6.85	58.50	55.82	53.15	50.44	58.68	56.00	53.32	50.52	57.78	56.03	53.23	50.43
Other FA	nd	9.60	8.34	7.23	6.12	8.74	7.58	6.43	5.55	10.86	7.13	6.36	6.18

HI=*Hermetia illucens*; nd=not detected; SFA=saturated fatty acid; MUFA=monounsaturated fatty acid; PUFA=polyunsaturated fatty acid; FA=fatty acids.

Table 3 *Effect of the dietary HI larvae meal inclusion level on the carcass traits of broiler chickens (n=16 animals/group)*

	Dietary treatments					*P-value*	
	HI0	HI5	HI10	HI15	SEM	Linear	Quadratic
Live weight (LW) (g)	2260.56	2259.44	2266.87	2070.12	15.69	<0.001	<0.001
Carcass weight (g)	1594.84	1601.01	1607.84	1469.65	11.73	<0.001	<0.001
Carcass weight (% LW)	70.55	70.86	70.92	71.00	0.20	0.453	0.785
Breast (% LW)	14.46	14.67	14.84	13.57	0.16	0.067	0.018
Thigh (% LW)	18.67	18.59	18.79	18.45	0.12	0.676	0.611
Spleen (% LW)	0.11	0.10	0.10	0.11	0.01	0.486	0.262
Liver (% LW)	2.17	2.13	2.08	2.25	0.05	0.707	0.368
Heart (% LW)	0.64	0.60	0.56	0.57	0.01	0.078	0.400
Bursa of Fabricius (% LW)	0.29	0.26	0.26	0.26	0.01	0.532	0.476
Abdominal fat (% LW)	1.21	1.17	1.43	1.44	0.05	0.042	0.813

HI=*Hermetia illucens*; LW=live weight.

Statistical analyses

The statistical analyses were performed using the IBM SPSS software package (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0; IBM Corp, Armonk, NY, USA). The Shapiro–Wilk test was used to test the normality of the data distribution. Individual bird was used as the experimental unit to analyse the carcass characteristics, meat quality parameters and FA profile ($n = 16$ per treatment; two birds/pen). The carcass characteristics, meat quality parameters and FA profile data were tested by means of one-way ANOVA, with experimental diet (HI0, HI5, HI10 and HI15) as fixed effect, and according to the following model:

$$Y_{ij} = \mu_i + \alpha + \varepsilon_{ij}$$

where Y_{ij} = the single observation; μ = overall mean; α = the effect of experimental diet (i = HI0 or HI inclusion level) and ε = residual error.

The effect of dietary HI meal inclusion was evaluated by means of polynomial contrasts (linear and quadratic responses). The results were expressed as the mean and standard error of the mean (**SEM**). Significance was considered at $P<0.05$.

Results

Carcass characteristics

The carcass characteristics of the broilers fed the experimental diets are presented in Table 3. Linear and quadratic responses were observed for increasing HI meal levels in the live and carcass weights ($P < 0.001$), with a maximum for the HI10 group. The breast yield increased quadratically for increasing HI meal levels ($P < 0.05$), and the quadratic response increased to a maximum for the inclusion of 100 g/kg of HI meal. The abdominal fat yield showed a linear response to increasing HI meal levels ($P < 0.05$), with a maximum for the inclusion of 150 g/kg of HI meal. On the other hand, no significant effects related to HI meal utilization were observed for the other carcass traits.

Meat quality parameters of the breast muscle

No significant differences were observed for the meat quality parameters, with the exception of the redness (a*) and yellowness (b*) colour traits (Table 4). The observed redness showed a linear response to increasing HI meal levels ($P < 0.05$), and the linear response increased to a maximum for the HI15 group. A linear decrease of yellowness was elicited for the HI meal as the level of HI increased with a minimum corresponding to the inclusion of 150 g/kg of HI meal.

Chemical composition and fatty acid profile of the breast meat

As far as the chemical composition is concerned, the moisture content decreased linearly with increasing HI meal levels ($P < 0.05$), and the linear response decreased to a minimum for the inclusion of 150 g/kg of HI meal. A linear response was observed for the protein content, with a maximum for the HI15 group. No significant effect, related to HI meal utilization, was observed for the lipid and ash contents (Table 4).

The effects of the experimental diets on the FA profile of breast meat are presented in Table 5. A linear increase was observed for C12, C14, C16, C14:1, C16:1 n7; C20:1 ΣMUFA and atherogenicity index, and the linear response increased to a maximum for the inclusion of 150 g/kg of HI meal. C15:0 and C18:1 *c*9 showed a linear response to increasing HI meal levels ($P < 0.05$), with a maximum for the inclusion of 100 g/kg of HI meal. A linear decrease was shown for C18:0, C24:0, C18:2 n6, C20:4 n6, ΣPUFA, Σn3, Σn6 and ΣPUFA/SFA. A linear and quadratic decrease was observed for C22:5 n3 and C22:6 n3 with a minimum for the HI10 group.

Heavy metal and arsenic determination

The concentrations of heavy metals and arsenic in the HI larvae meal and breast meat samples of the broiler chickens are summarized in Tables 6 and 7, respectively.

All the arsenic, cadmium, lead and mercury levels in the HI larvae meal were below the European Union (**EU**) limits reported for animal feeds (EC, 2002). The heavy metal and arsenic contents in the breast meat sample pools of the four

Table 4 *Effect of the dietary HI larvae meal inclusion on the meat quality and chemical composition in breast meat of broiler chickens (n=16 animals/group)*

	Dietary treatments					*P-value*	
	HI0	HI5	HI10	HI15	SEM	Linear	Quadratic
pH_u	6.03	5.99	6.04	5.98	0.05	0.650	0.777
Colour							
Lightness (L*)	55.07	55.46	54.29	53.41	0.38	0.074	0.400
Redness (a*)	2.72	3.18	2.87	3.71	0.14	0.030	0.469
Yellowness (b*)	11.80	9.68	8.19	7.57	0.39	<0.001	0.174
Drip loss (%)	1.24	1.36	1.26	1.39	0.06	0.211	0.928
Cooking loss (%)	20.11	20.49	19.31	19.71	0.36	0.480	0.990
Allo Kramer shear force (kg/g)	1.86	2.24	2.14	2.06	0.06	0.375	0.074
Proximate composition							
Moisture (%)	76.14	75.51	75.50	75.24	0.13	0.016	0.460
Protein (%)	22.37	22.28	22.42	23.09	0.12	0.035	0.118
Lipid (%)	1.56	1.76	1.85	1.75	0.05	0.134	0.110
Ash (%)	1.23	1.33	1.24	1.31	0.02	0.427	0.718

HI=*Hermetia illucens*.

dietary treatments were below the EU limits reported for chicken meat (EC, 2006).

Discussion

Hermetia illucens larvae meal has been recently proposed as emerging and innovative feed ingredient in poultry feeds (Cullere *et al*., 2016 and 2018; Loponte *et al*., 2017; Altmann *et al*., 2018; Pieterse *et al*., 2019) in order to improve the sustainability of poultry meat production. The defatting process results in insect meals with larger protein values and can reduce the risk of lipid oxidation, allowing for a longer shelf life of the product (Zheng *et al*., 2013). Our study investigated the effects of defatted HI larvae meal on carcass characteristics and meat quality of broiler chickens as well as the optimal level of inclusion.

The results of the relative carcass and organ yields showed a satisfactory effect of HI larvae meal inclusion on the carcass traits, a result that could be positive for commercial purposes. Positive linear and quadratic responses were observed up to 100 g/kg level of HI larvae meal for the parameters mentioned above, while HI15 group showed unfavourable results. The results observed in HI15 group are in agreement with our previous findings, which revealed a negative modulation of growth performance and gut morphology by insect meal utilization in HI15 birds (Dabbou *et al*., 2018). In fact, the authors observed the worst gut mucosal development (in terms of short villi, deep crypts and reduced villus height-to-crypt depth ratio) in HI15 group, which may explain the deterioration of the growth performance and consequently the carcass characteristics.

On the basis of the results of this research, the supplement of HI larvae meal up to 100 g/kg has been suggested to be suitable as a feed ingredient for broiler chicken diets.

The results obtained in this study are in agreement with those reported by Loponte *et al*. (2017). These authors reported greater carcass weights in Barbary partridges (*Alectoris barbara*) fed with HI and yellow mealworm (*Tenebrio molitor* L., **TM**) diets than control group as a partial replacement (25% or 50%) of soybean meal. However, our results are not consistent with the majority of studies about broiler chickens and quails, where all carcass traits were unaffected by dietary house-fly maggots (*Musca domestica* L., **MD**), TM and HI larvae meal inclusion (Bovera *et al*., 2016; Cullere *et al*., 2016; Biasato *et al*., 2017 and 2018; Pieterse *et al*., 2019).

In the current study, the redness (a*) index of breast meat was higher in the HI15 group, while the HI5 and HI10 groups showed intermediate values in comparison with HI0 group. These results may be due to a possible accumulation of insect meal pigments in the intramuscular fat. On the other hand, the yellowness value (b*) decreased in a linear fashion ($P < 0.001$), unlike the overall responses of redness to dietary HI larvae meal for an increased HI larvae meal content in the diet. This finding may be attributed to the decreased content of corn gluten meal in HI diets (Table 1). The effects of dietary insect meal on meat colour are controversial. Cullere *et al*. (2016) observed that redness (a*) in the breast meat of broiler quails was affected by increasing inclusion levels of HI larvae meal in diets, showing the highest (1.13) and lowest (0.46) values for 100 g/kg and 150 g/kg HI groups, respectively. On the contrary, Leiber *et al*. (2017), Altmann *et al*. (2018) and Pieterse *et al*. (2019) did not find any significant effect of dietary HI meal on broiler meat colour. In regard to other insect meals, the use of MD larvae meal in broiler diets has been associated with a significant decrease in breast muscle lightness (L*) (Pieterse *et al*., 2014). Differently, Bovera *et al*. (2016) did not find any significant effect on the colour of raw and cooked meat, or on the skin of broiler chickens, also showing that the meat from broilers fed with TM meal could be accepted by consumers. However, the meat colour differences found in broiler chickens fed with HI larvae meal of the present study appear to be of little

Table 5 *Effect of the dietary HI larvae meal inclusion on the fatty acid profile in breast meat of broiler chickens (g/100g of total fatty acids; n=16 animals/group)*

	Dietary treatment					*P*-value	
	HI0	HI5	HI10	HI15	SEM	Linear	Quadratic
ΣSFA	29.13	28.97	29.24	29.88	0.29	0.352	0.507
C12:0	nd	0.33	0.61	1.03	0.06	<0.001	0.367
C14:0	0.23	0.43	0.54	0.74	0.03	<0.001	0.965
C15:0	nd	0.02	0.05	0.04	0.01	<0.001	0.041
C16:0	17.63	18.24	18.74	19.24	0.20	0.002	0.887
C17:0	0.15	0.12	0.13	0.14	0.01	0.705	0.315
C18:0	9.55	8.48	7.98	7.61	0.21	<0.001	0.352
C20:0	0.19	0.24	0.23	0.23	0.01	0.152	0.245
C24:0	1.38	1.09	0.96	0.85	0.06	0.001	0.416
ΣMUFA	27.61	30.00	31.57	32.17	0.47	<0.001	0.279
C14:1	nd	0.02	0.10	0.14	0.01	<0.001	0.630
C16:1 n7	1.79	2.24	2.62	3.13	0.12	<0.001	0.870
C17:1	0.05	1.11	0.07	0.09	0.01	0.287	0.355
C18:1 *c*9	25.26	27.08	28.28	28.21	0.36	0.001	0.152
C20:1	0.20	0.26	0.28	0.34	0.01	<0.001	0.330
C24:1	0.31	0.28	0.22	0.26	0.01	0.091	0.182
ΣPUFA	42.36	40.06	37.89	36.65	0.51	<0.001	0.524
C18:2 n6	31.44	30.62	29.61	28.32	0.41	0.004	0.763
C18:3 n6	0.20	0.24	0.22	0.25	0.01	0.355	0.974
C18:3 n3	2.31	2.40	2.47	2.20	0.06	0.641	0.150
C20:2 n6	0.56	0.57	0.57	0.47	0.03	0.319	0.345
C20:4 n6	5.86	4.76	3.90	4.05	0.24	0.002	0.152
C20:5 n3 (EPA)	0.13	0.17	0.17	0.17	0.01	0.207	0.375
C22:5 n3 (DPA)	1.09	0.81	0.62	0.72	0.05	0.002	0.039
C22:6 n3 (DHA)	0.76	0.49	0.34	0.46	0.04	0.001	0.004
Others FA	0.90	0.97	1.29	1.30	0.08	0.032	0.814
ΣPUFA/SFA	1.46	1.40	1.30	1.23	0.03	<0.001	0.901
Σn3	4.30	3.87	3.60	3.56	0.08	<0.001	0.162
Σn6	38.06	36.19	34.29	33.08	0.45	<0.001	0.646
Σn6/n3	8.93	9.39	9.63	9.40	0.13	0.140	0.169
AI	0.26	0.29	0.31	0.34	0.01	<0.001	0.957
TI	0.60	0.61	0.62	0.64	0.01	0.139	0.875

HI=*Hermetia illucens*; nd=not detected; SFA=saturated fatty acids; MUFA=monounsaturated fatty acids; PUFA=polyunsaturated fatty acids; FA=fatty acids; AI=atherogenicity index; TI=thrombogenicity index.

practical relevance and potentially incapable of affecting the consumers' willingness to buy meat.

Orthogonal polynomial contrast test revealed that increasing the HI larvae meal of diets decreased the moisture content (linear effect, $P<0.05$), and increased protein, according to a notable linear trend, for increasing HI larvae meal inclusion levels. Even the effects of dietary insect meal on poultry meat proximate composition are conflicting. Cullere *et al.* (2018), Pieterse *et al.* (2019) and Schiavone *et al.* (2017a) reported no significant effects on meat chemical composition of broiler quails or chickens fed diets with HI meal and fat, respectively.

The FA composition of broiler meat basically depends on the dietary FA profile. Several factors (e.g. genotype, sex, age and slaughter weight) are also able to affect the FA

Table 6 *Heavy metals in HI larvae meal*

	HI meal	MRL (Directive 2002/32/EC)
As (mg/kg 12% h)	<0.05	2
Cd (mg/kg 12% h)	0.32	2
Pb (mg/kg12% h)	0.07	10
Hg (mg/kg 12% h)	<0.02	0.1
Cr (mg/kg)	0.23	Not legislated
Fe (mg/kg)	189	Not legislated
Ni (mg/kg)	0.18	Not legislated
Cu (mg/kg)	10	Not legislated
Zn (mg/kg)	157	Not legislated
Co (mg/kg)	<0.05	Not legislated
Se (mg/kg)	<0.02	Not legislated

HI=*Hermetia illucens;* MRL=Maximum Residue Limit; mg/kg 12% h=mg/kg 12% humidity.

composition of meat, the diet component being considered as a major effect in monogastric animals (Rymer and Givens, 2005; Schiavone *et al.*, 2007 and 2010). Consequently, as expected, the dietary inclusion of HI larvae meal influenced the FA profile of the broiler breast meat. The lauric and myristic acid contents increased with increasing levels of dietary inclusion of HI in the diet, and this increase was also noticed for C16:0. The total SFA remained constant, however C18:0 and C24:0 reduced linearly as the inclusion rate increased. These results are in agreement with those reported by Renna *et al.* (2017) and Cullere *et al.* (2018) for rainbow trout and broiler quail, respectively, fed defatted HI larvae meal. Avian FAs are typically monounsaturated due to an active hepatic delta-9 desaturase and an oleic (C18:1 *c*9) predominance (Klasing, 2000). An increasing percentage of MUFA, mainly due to the higher content of C18:1 *c*9, was observed as a result of increasing inclusion levels of HI meal in the diets. On the contrary, PUFA significantly decreased with greater decreases in C18:2 n6. *Hermetia illucens* larvae meal was found to be relatively scarce in PUFA, which were represented almost entirely by C18:2 n6 and C18:3 n3. Such a composition lowered the contents of the n-6 and the n-3 PUFA fractions as the dietary HI larvae meal inclusion level increased. Despite these remarkable changes in the breast meat FAs, the Σ n-6/ Σ n-3 ratio remained unaffected. Breast meat lipids are mainly composed of triacylglycerol and phospholipids, the latter being rich in very long chain n-3 PUFA (Eicosapentaenoic acid (**EPA**; C20:5 n3) and Docosahexaenoic acid (**DHA**; C22:6 n3)), which are well known for their high biological efficiency in the organism and their beneficial effects on human health (Rymer and Givens, 2005). A significant decreased content was observed in HI groups for Docosapentaenoic acid (**DPA**; C22:5 n3) (on average −34.25%) and DHA (on average −43.42%). These results are in agreement with those reported by Cullere *et al.* (2018), who found a significant reduction of DHA content in breast meat of Japanese quail fed increasing levels of HI meal. Renna *et al.* (2017) also observed lower contents of EPA and DHA in the fillet muscles of trout fed 400 g/kg of HI than other groups, while DPA was reduced with the lowest inclusion level of HI meal. The present study indicates that

Table 7 *Heavy metals in breast meat of broiler chickens (one pool per feeding group, eight birds/pool)*

	Dietary treatments				
	HI0	HI5	HI10	HI15	MRL (Regulation 881/2006/EC)
As (mg/kg 12% h)	<0.05	<0.05	<0.05	<0.05	Not legislated
Cd (mg/kg 12% h)	<0.05	<0.05	<0.05	<0.05	0.050 (EU n. 488/14)
Pb (mg/kg 12% h)	<0.05	<0.05	<0.05	<0.05	0.10 (EU n. 1005/15)
Hg (mg/kg 12% h)	<0.02	<0.02	<0.02	<0.02	Not legislated
Cr (mg/kg)	<0.05	<0.05	<0.05	<0.05	Not legislated
Fe (mg/kg)	9.1	6.5	6.5	6.7	Not legislated
Ni (mg/kg)	0.08	<0.05	<0.05	<0.05	Not legislated
Cu (mg/kg)	0.64	0.46	0.41	0.46	Not legislated
Zn (mg/kg)	13	13	13	13	Not legislated
Co (mg/kg)	<0.05	<0.05	<0.05	<0.05	Not legislated
Se (mg/kg)	0.28	<0.25	<0.25	<0.25	Not legislated

HI=*Hermetia illucens;* MRL=Maximum Residue Limit; mg/kg 12% h=mg/kg 12% humidity.

the inclusion of a defatted HI larvae meal in broiler chicken diets leads to significant modifications of the breast meat FA profile, with higher MUFAs and lower PUFAs in HI groups than control group. In order to balance and overcome these potential negative effects related to HI larvae meal utilization, a modulation of the larva rearing substrate should be recommended to obtain an improved insect larvae FA profile and provide healthier meat for the modern consumer. Little information on the influence of insect meals on meat sensory profile and consumer health and acceptance has been provided till now. The dietary inclusion of insect meal may affect the sensory profile of meat as reported by Pieterse *et al.* (2014), who found that the meat derived from chickens fed with a 10% MD larvae meal had a higher perception of metallic aroma and aftertaste but a higher sustained juiciness and a lower mealiness (dry sensation) in the mouth compared to the control group. Differently, Cullere *et al.* (2018) reported unaffected meat sensory profile of quails fed HI larvae meal. Further research should be focused on the evaluation of the consumer acceptance and health, and meat processing in order to successfully include HI meal in commercial poultry diets.

In the current research, the concentrations of heavy metals and arsenic in both the HI larvae meal and in the breast meat of the birds remained below the EU limits suggested for feed materials (EC, 2002) and foodstuffs (EC, 2006). *Hermetia illucens* larvae have been reported to accumulate cadmium (Van der Fels-Klerx *et al.*, 2016; Purschke *et al.*, 2017; Biancarosa *et al.*, 2018), lead (Van der Fels-Klerx *et al.*, 2016; Purschke *et al.*, 2017; Biancarosa *et al.*, 2018), mercury (Biancarosa *et al.*, 2018) and arsenic (Van der Fels-Klerx *et al.*, 2016; Biancarosa *et al.*, 2018) when reared on contaminated substrates. The results obtained in the present study suggest that the HI larvae were fed with a feeding media that did not contain heavy metals or arsenic levels that exceeded the maximum allowable limits. The cadmium and lead concentrations in the breast meat also remained below the EU limits, thus representing a relevant result in terms of food safety.

Conclusion

Overall, the present study has provided new data and knowledge on the potential use of a new sustainable feedstuff for broiler chickens. The main findings of the current research suggest that defatted HI larvae meal can be used to up to 100 g/kg level of inclusion in broiler chicken diets, without detrimental effects on carcass and meat quality parameters or heavy metal contents. Remarkable differences in the meat nutritional profile were found in relation to the FA composition, with an increase of MUFA content to the detriment of PUFA. Therefore, important efforts should be made to evaluate new substrates capable of improving the FA profile of larvae, thus potentially counteracting the negative effects on the nutritional value and perceived healthiness of the poultry meat. These substrates should also be evaluated from a safety point of view, as new evidence on their safety could be adopted to reduce the potential toxicity of the meal.

Acknowledgements

This research was supported by University of Torino (Italy) founding: SCHA_RILO_14_01 (2015 to 2017) and SCHA_RILO_16_02 (2016 to 2018).

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Declaration of interest

The authors declare that there is no conflict of interest.

Ethics statement

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) and approved by the Ethical Committee of the Department of Veterinary Science of the University of Turin (Italy).

Software and data repository resources

The data sets analysed in the current study are available from the corresponding author on reasonable request.

Supplementary material

To view the supplementary material for this article, please visit https://doi.org/10.1017/S1751731119000685

References

Altmann BA, Neumann C, Velten S, Liebert F and Mörlein D 2018. Meat quality derived from high inclusion of a micro-alga or insect meal as an alternative protein source in poultry diets: a pilot study. Foods 7, 1–15. doi: 10.3390/foods7030034.

Association of Official Analytical Chemists 1990. Official methods of analysis, 15th edition. AOAC, Arlington, VA, USA.

Belluco S, Losasso C, Maggioletti M, Alonzi CC, Paoletti MG and Ricci A 2013. Edible insects in a food safety and nutritional perspective: a critical review. Comprehensive Reviews in Food Science and Food Safety 12, 296–313.

Biancarosa I, Liland NS, Biemans D, Araujo P, Bruckner CG, Waagbø R, Torstensen BE, Lock EJ and Amlund H 2018. Uptake of heavy metals and arsenic in black soldier fly (*Hermetia illucens*) larvae grown on seaweed-enriched media. Journal of the Science of Food and Agricultural 98, 2176–2183.

Biasato I, Gasco L, De Marco M, Renna M, Rotolo L, Dabbou S, Capucchio MT, Biasibetti E, Tarantola M, Bianchi C, Cavallarin L, Gai F, Pozzo L, Dezzutto D, Bergagna S and Schiavone A 2017. Effects of yellow mealworm larvae (*Tenebrio molitor*) inclusion in diets for female broiler chickens: implications for animal health and gut histology. Animal Feed Science and Technology 234, 253–263.

Biasato I, Gasco L, De Marco M, Renna M, Rotolo L, Dabbou S, Capucchio MT, Biasibetti E, Tarantola M, Sterpone L, Cavallarin L, Gai F, Pozzo L, Bergagna S, Dezzutto D, Zoccarato I and Schiavone A 2018. Yellow mealworm larvae (*Tenebrio molitor*) inclusion in diets for male broiler chickens: effects on growth performance, gut morphology and histological findings. Poultry Science 97, 540–548.

Boccazzi IV, Ottoboni M, Martin E, Comandatore F, Vallone L, Spraghers T, Eechkout M, Mereghetti V, Pinotti L and Epis S 2017. A survey of the mycobiota associated with larvae of the black soldier fly (*Hermetia illucens*) reared for feed production. PLoS One 12, 1–15. doi: 10.1371/journal.pone.0182533.

Bovera F, Loponte R, Marono S, Piccolo G, Parisi G, Iaconisi V, Gasco L and Nizza A 2016. Use of *Tenebrio molitor* larvae meal as protein source in broiler diet: Effect on growth performance, nutrient digestibility, and carcass and meat traits. Journal of Animal Science 94, 639–647.

Cullere M, Tasoniero G, Giaccone V, Acuti G, Marangon A and Dalle Zotte A 2018. Black soldier fly as dietary protein source for broiler quails: meat proximate composition, fatty acid and amino acid profile, oxidative status and sensory traits. Animal 12, 640–647.

Cullere M, Tasoniero G, Giaccone V, Miotti-Scapin R, Claeys E, De Smet S and Dalle Zotte A 2016. Black soldier fly as dietary protein source for broiler quails: apparent digestibility, excreta microbial load, feed choice, performance, carcass and meat traits. Animal 10, 1923–1930.

Dabbou S, Gai F, Biasato I, Capucchio MT, Biasibetti E, Dezzutto D, Meneguz M, Plachà I, Gasco L and Schiavone A 2018. Black soldier fly defatted meal as a dietary protein source for broiler chickens: effects on growth performance, blood traits gut morphology and histological features. Journal of Animal Science and Biotechnology 9, 1–10. doi: 10.1186/s40104-018-0266-9.

European Commission (EC) 2002. Council Directive (EC) 2002/32/EC of 7 May 2002 on undesirable substances in animal feed. Official Journal of the European Communities L140, 10–21.

European Commission (EC) 2006. Council Directive (EC) 2006/1881/EC of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Communities L364, 5–24.

Folch J, Lees M and Sloane Stanley H 1957. A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry 226, 479–509.

Klasing KC 2000. Lipids. In Comparative avian nutrition. (ed. KC Klasing). pp. 171–173. CAB International, Oxon, UK.

Leiber F, Gelencsér T, Stamer A, Amsler Z, Wohlfahrt J, Früh B and Maurer V 2017. Insect and legume-based protein sources to replace soybean cake in an organic broiler diet: effects on growth performance and physical meat quality. Renewable Agricultural and Food Systems 32, 21–27.

Loponte R, Nizza S, Bovera F, De Riu N, Fliegerova K, Lombardi P, Vassalotti G, Mastellone V, Nizza A and Moniello G 2017. Growth performance, blood profiles and carcass traits of Barbary partridge (*Alectoris barbara*) fed two different insect larvae meals (*Tenebrio molitor* and *Hermetia illucens*). Research in Veterinary Science 115, 183–188.

Makkar HPS, Tran G, Heuzé V and Ankers P 2014. State of the art on use of insects as animal feed. Animal Feed Science and Technology 197, 1–33.

Maurer V, Holinger M, Amsler Z, Früh B, Wohlfahrt J, Stamer A and Leiber F 2016. Replacement of soybean cake by *Hermetia illucens* meal in diets for layers. Journal of Insects as Food and Feed 2, 89–90.

Meneguz M, Schiavone A, Gai F, Dama A, Lussiana C, Renna M and Gasco L 2018. Effect of rearing substrate on growth performance, waste reduction efficiency and chemical composition of black soldier fly (*Hermetia illucens*) larvae. Journal of the Science of Food and Agricultural 98, 5776–5784.

Ottoboni M, Spranghers T, Pinotti L, Baldi A, De Jaeghere W and Eeckhout M 2018. Inclusion of *Hermetia Illucens* larvae or prepupae in an experimental extruded feed: process optimisation and impact on in vitro digestibility. Italian Journal of Animal Science 17, 418–427.

Pieterse E, Erasmus SW, Uushona T and Hoffman LC 2019. Black soldier fly (*Hermetia illucens*) pre-pupae meal as a dietary protein source for broiler production ensures a tasty chicken with standard meat quality for every pot. Journal of the Science of Food and Agricultural 99, 893–903.

Pieterse E, Pretorius Q, Hoffman LC and Drew DW 2014. The carcass quality, meat quality and sensory characteristics of broilers raised on diets containing either *Musca domestica* larvae meal, fish meal or soya bean meal as the main protein source. Animal Production Science 54, 622–628.

Purschke B, Scheibelberger R, Axmann S, Adler A and Jäger H 2017. Impact of substrate contamination with mycotoxins, heavy metals and pesticides on the growth performance and composition of black soldier fly larvae (*Hermetia illucens*) for use in the feed and food value chain. Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment 34, 1410–1420.

Renna M, Gasmi-Boubaker A, Lussiana C, Battaglini LM, Belfayez K and Fortina R 2014. Fatty acid composition of the seed oils of selected *Vicia* L. taxa from Tunisia. Italian Journal of Animal Science 13, 308–316.

Renna M, Schiavone A, Gai F, Dabbou S, Lussiana C, Malfatto V, Prearo M, Capucchio MT, Biasato I, Biasibetti E, De Marco M, Brugiapaglia A, Zoccarato I and Gasco L 2017. Evaluation of the suitability of a partially defatted black soldier fly (*Hermetia illucens* L.) larvae meal as ingredient for rainbow trout (*Oncorhynchus mykiss* Walbaum) diets. Journal of Animal Science and Biotechnology 8, 957–969.

Rymer C and Givens DI 2005. n-3 Fatty acid enrichment of edible tissue of poultry: a review. Lipids 40, 121–130.

Sauvant D, Perez JM and Tran G 2004. Tables of composition and nutritional value of feed materials: pigs, poultry, cattle, sheep, goats, rabbits, horses and fish. Wageningen Academic Publishers INRA Editions, France.

Schiavone A, Chiarini R, Marzoni M, Castillo A, Tassone S and Romboli I 2007. Breast meat traits of Muscovy ducks fed on a microalga (*Crypthecodinium cohnii*) meal supplemented diet. British Poultry Science 48, 573–579.

Schiavone A, Cullere M, De Marco M, Meneguz M, Biasato I, Bergagna S, Dezzutto D, Gai F, Dabbou S, Gasco L and Dalle Zotte A 2017a. Partial or total replacement of soybean oil by black soldier larvae (*Hermetia illucens* L.) fat in broiler diets: effect on growth performances, feed-choice, blood traits, carcass characteristics and meat quality. Italian Journal of Animal Science 16, 93–100.

Schiavone A, Dabbou S, De Marco M, Cullere M, Biasato I, Biasibetti E, Capucchio MT, Bergagna S, Dezzutto D, Meneguz M, Gai F, Dalle Zotte A and Gasco L 2018. Black soldier fly (*Hermetia illucens L.*) larva fat inclusion in finisher broiler chicken diet as an alternative fat source. Animal 12, 2032–2039.

Schiavone A, De Marco M, Martínez S, Dabbou S, Renna M, Madrid J, Hernandez F, Rotolo L, Costa P, Gai F and Gasco L 2017b. Nutritional value of a partially

defatted and a highly defatted black soldier fly larvae (*Hermetia illucens* L.) meal for broiler chickens: apparent nutrient digestibility, apparent metabolizable energy and apparent ileal amino acid digestibility. Journal of Animal Science and Biotechnology 8, 897–905.

Schiavone A, Marzoni M, Castillo A, Nery J and Romboli I 2010. Dietary lipid sources and vitamin E affect fatty acid composition or lipid stability of breast meat from Muscovy duck. Canadian Journal of Animal Science 90, 371–378.

Spranghers T, Ottoboni M, Klootwijk C, Ovyn A, Deboosere S, De Meulenaer B, Michielis J, Eeckhout M, De Clercq P and De Smet S 2017. Nutritional composition of black soldier fly (*Hermetia illucens*) prepupae reared on different organic waste substrates. Journal of the Science of Food and Agriculture 97, 2594–2600.

Van der Fels-Klerx HJ, Camenzuli L, van der Lee MK and Oonincx DG 2016. Uptake of cadmium, lead and arsenic by *Tenebrio molitor* and *Hermetia illucens* from contaminated substrates. PLoS One 11, 1–13. doi: 10.1371/journal.pone.0166186.

Zheng L, Hou Y, Li W, Yang S, Li Q and Yu Z 2013. Exploring the potential of grease from yellow mealworm beetle (*Tenebrio molitor*) as a novel biodiesel feedstock. Applied Energy 101, 618–621.