

# Black soldier fly larva in Muscovy duck diets: effects on duck growth, carcass property, and meat quality

M. Gariglio,\* S. Dabbou,<sup>†,‡</sup> F. Gai,<sup>§</sup> A. Trocino,<sup>#</sup> G. Xiccato,<sup>||</sup> M. Holodova,<sup>¶</sup> L. Gresakova,<sup>¶</sup> J. Nery,\* S. Bellezza Oddon,\*\* I. Biasato,\*\* L. Gasco,<sup>§,\*\*,1</sup> and A. Schiavone\*,<sup>§</sup>

\*Department of Veterinary Sciences, University of Turin, Largo Paolo Braccini 2, 10095 Grugliasco, Turin, Italy; <sup>†</sup>Center Agriculture Food Environment (C3A), University of Trento, via E. Mach 1, 38010 San Michele all'Adige, Italy; <sup>‡</sup>Research and Innovation Centre, Fondazione Edmund Mach, 38010 San Michele all'Adige, Italy; <sup>§</sup>Institute of Science of Food Production, National Research Council, Largo Paolo Braccini 2, 10095 Grugliasco, Turin, Italy; <sup>#</sup>Department of Comparative Biomedicine and Food Science, University of Padua, Viale dell'Università 16, 35020 Legnaro, Padua, Italy; <sup>||</sup>Department of Agronomy, Food, Natural Resources, Animal, and Environment, University of Padua, Viale dell'Università 16, 35020 Legnaro, Padua, Italy; <sup>¶</sup>Institute of Animal Physiology, Center of Biosciences, Slovak Academy of Sciences, Soltessovej 4-6, 040 01 Kosice, Slovak Republic; and <sup>\*\*</sup>Department of Agricultural, Forest and Food Sciences, University of Turin, Largo Paolo Braccini 2, 10095 Grugliasco, Turin, Italy

**ABSTRACT** The aim of the study was to evaluate the effects of partially defatted black soldier fly (*Hermetia illucens*, **HI**) larva meal on the carcass characteristics and meat quality of Muscovy ducks (*Cairina moschata domestica*). A total of 192 female ducks aged 3 d were divided between 4 dietary treatments (6 pens/treatment; 8 birds/pen), characterized by increasing levels of substitution of corn gluten meal with HI meal (0%, 3%, 6%, and 9%; HI0, HI3, HI6, and HI9, respectively), and reared until 50 days of age. Twelve birds/treatment (2 birds/pen) were slaughtered on d 51 to evaluate the slaughter traits (i.e., carcass, breast, thigh, and organs weights), carcass yield and meat quality. The slaughter weight, hot and chilled carcass weights, and abdominal fat weight showed a quadratic response to HI meal (minimum for the HI6 group,  $P < 0.05$ ). Dietary HI meal inclusion did not influence the ultimate pH, the color, the proximate composition or the thiobarbituric acid

reactive substances (**TBARS**) values in either the breast or thigh meat. The mineral profile of the meat was slightly affected by the dietary treatment, with a linear increase in the Cu content of the thigh meat ( $P < 0.05$ ), whereas no differences were observed for Zn, Mn, or Fe. Dietary HI meal inclusion increased the saturated fatty acid rate in the thigh meat (maximum for the HI9 group,  $P < 0.05$ ), and the monounsaturated and polyunsaturated fatty acid content in the breast meat (maximum for the HI0 and HI9 groups, respectively,  $P < 0.05$ ). The  $\sum n-6/\sum n-3$  ratio decreased linearly in both the breast and thigh meat, with the HI9 group showing the lowest values ( $P < 0.05$ ). Finally, the heavy metal concentrations were below the EU limits for poultry meat. To conclude, the inclusion up to 9% of partially defatted HI larva meal in the diet of Muscovy ducks did not affect the slaughter traits or the meat quality, although it did affect the meat fatty acid profile.

**Key words:** Muscovy duck, *Hermetia illucens*, meat quality, fatty acid, heavy metal

2021 Poultry Science 100:101303

<https://doi.org/10.1016/j.psj.2021.101303>

## INTRODUCTION

The growing demand for poultry meat in the world has led to a considerable increase in duck meat production over the last 20 yr: from 2.5 million tons in 1997 to more than 4 million tons in 2017 (FAOSTAT, 2019). Asia is the main producer of duck meat, with more than

3 million tons produced in 2017 alone. However, the production and consumption of duck meat is much lower in other areas, such as Europe, due to the lack of promotion and a general scarcity of duck farmers (Aronal et al., 2012). France is the biggest duck meat producer in Europe, delivering more than 230,000 tons, followed by Hungary and Poland that produced more than 50,000 tons each in 2017 (FAOSTAT, 2019).

Duck meat is starting to receive more attention because it combines the characteristics of red meat, possessing more red muscle fibers than chicken, with the dietary characteristics of poultry meat, in particular its high polyunsaturated fatty acid (**PUFA**) content

© 2021 The Authors. Published by Elsevier Inc. on behalf of Poultry Science Association Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Received June 25, 2020.

Accepted June 1, 2021.

<sup>1</sup>Corresponding author: [laura.gasco@unito.it](mailto:laura.gasco@unito.it)

(constituting approximately 20–40% of total fatty acids; **FA**) (Chartrin et al., 2005; Ali et al., 2007; Aronal et al., 2012; Pingel et al., 2012). One consequence of the poultry industry's great expansion over recent decades has been the surge in the demand for vegetable protein sources as animal feeds. This has contributed to the overexploitation of lands and water resources, and augmented the environmental impact of the poultry industry.

From this standpoint, insects are now being recognized as a promising alternative protein source for poultry feeds, thanks to their nutritional composition and the low environmental impact of their production (Cullere et al., 2016; Loponte et al., 2017; Biasato et al., 2017, 2018; Dabbou et al., 2018, 2019; Gasco et al., 2019). Insect larvae can be successfully reared on a number of different food by-products (such as fruit and vegetable substrates), thereby transforming waste into nutrient-rich raw materials (Makkar et al., 2014; Meneguz et al., 2018). Depending on the species, insect-derived meals are characterized by their high quantity and quality of protein, with variable amounts of lipids and other nutrients, such as minerals (Makkar et al., 2014). The black soldier fly (*Hermetia illucens*, **HI**) is one of the most promising insect species for monogastric feeding. Depending on the type of rearing substrate, the crude protein (**CP**) content of HI larvae varies from 40 to 44% (on a dry matter, **DM**, basis) and the ether extract (**EE**) content varies from 15 to 49% (Makkar et al., 2014). The most abundant saturated fatty acid (**SFA**) in HI meal is lauric acid (C12:0, which varies from 21 to 43% of the total FAs), whereas oleic acid (C18:1 *n*9) is the prevalent monounsaturated fatty acid (**MUFA**), representing 12 to 32% of total FAs. However, the FA profile of HI can be modified by the type of diet used to feed the larvae (Makkar et al., 2014; Liland et al., 2017). In addition, the ash content is extremely variable, and the mineral profile of HI is closely linked to the mineral composition of the substrate. Spranghers et al. (2017) demonstrated a reduction in the major minerals (i.e., Fe and Zn) when the prepupae were fed on vegetable waste instead of a layer hen feed. In addition, these authors suggested that the mineral content in HI meal could be increased by defatting during meal processing. Finally, the ability of HI larvae to accumulate heavy metals and arsenic (**As**) should be considered. It has, in fact, been reported that HI larvae can accumulate Cd, Pb, Hg, and As (Biancarosa et al., 2017). Generally, the HI heavy metal content depends on the amount of these elements in the substrate, but different retention rates have been observed. Cadmium presents the highest retention rate (up to 93% in HI larvae reared on substrates enriched with seaweed), whereas As retention is much lower (up to 22%) (Biancarosa et al., 2017).

HI larva meal has been tested as a protein source for some poultry species, such as broiler chickens (Schiavone et al., 2019), broiler quails (*Coturnix coturnix japonica*) (Cullere et al., 2016, 2018), and Barbary partridges (*Alectoris barbara*) (Secci et al., 2018), by evaluating the quality traits and FA profile of the meat. The researchers obtained promising results in terms of carcass yield and meat proximate composition in broiler

quails and Barbary partridges (Cullere et al., 2016, 2018; Secci et al., 2018). On the other hand, conflicting results were reported in terms of meat FAs. In particular, increased SFA and MUFA content were observed in the meat of broiler quails fed a diet containing up to 15% HI larva meal (Cullere et al., 2018). Schiavone et al. (2019) found an increase in the MUFA content together with a decrease of the PUFA content, in breast meat of broiler chickens fed HI larva meal. However, no study has been conducted to evaluate the impact of partially defatted HI larva meal on the meat quality and safety of Muscovy ducks (*Cairina moschata domestica*).

Thus, the aim of the present study was to investigate the effects of partially defatted HI larva meal in the diet of female Muscovy ducks on the carcass characteristics and quality of meat.

## MATERIALS AND METHODS

### Birds and Diets

The study was carried out at the poultry facility of the University of Turin (Italy) and approved by the Bioethical Committee of the University of Turin (Italy) (Ref. 380576). A detailed description of the experimental design and duck farming conditions is reported in Gariglio et al. (2019a), which also reports data regarding the animals' growth performance and the nutrient digestibility of their feed.

In brief, 192 three-day-old female Muscovy ducklings (Canedins R71 L White, Grimaud Freres Selection, France) were randomly allotted to four groups (each consisting of 6 pens as replicates, with 8 birds per pen). A partially defatted HI larva meal, obtained by processing larvae reared on vegetable by-products and subsequently submitted to a defatting process (using a mechanical process, i.e., pressure) for a partial fat removal (Hermetia Deutschland GmbH & Co KG, Baruth/Mark, Germany), was used in this trial (DM, 92.4%; CP, 56.7% on DM; EE, 10.7% on DM; ash, 16.4% on DM; chitin, 6.43% on DM). Four diets (HI0, HI3, HI6, and HI9) were formulated, containing increasing levels of HI larva meal (0, 3, 6, and 9%, respectively) in substitution of corn gluten meal. Levels of HI larva meal were defined on the basis of performance results in broiler chickens (Dabbou et al., 2018). The diets were formulated to be isonitrogenous and isoenergetic (for three feeding periods) using the apparent metabolizable energy (**AMEn**) values for a partially defatted HI meal calculated for broiler chickens (Schiavone et al., 2017) and the INRA (2004) data relating to the other ingredients. The ducks had *ad libitum* access to water and feed throughout the whole trial. Detailed information about the diet ingredients and chemical composition are given in Table 1.

### Growth Performance, Slaughter Procedures, and Muscle Sampling

The live weight (**LW**) of each bird was assessed at 3 and 50 days of age. The average daily gain (**ADG**), the

**Table 1.** Ingredients (g/kg as fed) and chemical composition of the experimental diets.<sup>1</sup>

Ingredients	Starter period (d 3 to 17)				Grower period (d 18 to 38)				Finisher period (d 39 to 50)			
	HI0	HI3	HI6	HI9	HI0	HI3	HI6	HI9	HI0	HI3	HI6	HI9
Corn meal	600.0	600.0	600.0	600.0	638.0	638.0	638.0	638.0	670.0	670.0	670.0	670.0
Soybean meal	212.0	212.0	212.0	212.0	160.0	160.0	160.0	160.0	100.0	100.0	100.0	100.0
HI larva meal	0.0	30.0	60.0	90.0	0.0	30.0	60.0	90.0	0.0	30.0	60.0	90.0
Wheat bran meal	42.5	42.5	42.5	42.5	36.3	36.3	36.3	36.3	66.2	66.2	66.2	66.2
Corn gluten meal	90.0	60.0	30.0	0.0	90.0	60.0	30.0	0.0	90.0	60.0	30.0	0.0
Soybean oil	16.5	16.5	16.5	16.5	28.5	28.5	28.5	28.5	34.5	34.5	34.5	34.5
Dicalcium phosphate	10.0	10.0	10.0	10.0	13.0	13.0	13.0	13.0	4.0	4.0	4.0	4.0
Calcium carbonate	8.0	8.0	8.0	8.0	14.0	14.0	14.0	14.0	17.4	17.4	17.4	17.4
Sodium chloride	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Sodium bicarbonate	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
DL-methionine	2.5	2.5	2.6	2.8	1.7	1.8	1.9	2.2	0.3	0.4	0.5	0.8
L-lysine HCl	3.9	3.9	3.8	3.6	3.9	3.8	3.7	3.4	3.0	2.9	2.8	2.5
Mineral-vitamin premix <sup>2</sup>	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Choline chloride	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Optifos 250 bro <sup>3</sup>	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Avizyme 1500 x <sup>4</sup>	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Titanium dioxide	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Total	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000
AMEn (kcal/kg)	2897	2892	2888	2884	2994	2990	2986	2981	3052	3048	3044	3040
Nutrient composition (g/kg as fed)												
DM	893.0	898.0	901.0	897.0	888.0	892.0	891.0	891.0	887.0	890.0	893.0	889.0
CP	224.1	221.8	227.0	222.5	204.2	200.7	200.5	199.6	179.2	179.8	179.5	178.7
EE	42.0	43.1	44.1	45.7	55.1	55.3	57.0	58.8	64.8	65.9	67.9	68.5
NDF	113.4	110.5	114.4	111.2	114.6	116.9	112.3	113.2	112.6	116.6	113.4	114.7
ADF	30.4	31.4	33.3	29.6	31.1	31.2	33.0	31.2	30.2	31.2	31.3	30.2
Ash	50.0	53.9	50.5	52.0	69.3	66.9	66.8	71.3	57.7	57.0	61.6	59.6
Mineral composition												
Ca (%)	0.67	0.86	1.05	1.24	0.98	1.17	1.37	1.56	0.85	1.04	1.23	1.42
P (%)	0.57	0.57	0.55	0.58	0.55	0.55	0.58	0.56	0.43	0.46	0.44	0.45
nPP (%) <sup>a</sup>	0.15	0.14	0.14	0.14	0.13	0.13	0.14	0.13	0.10	0.11	0.10	0.10
Zn (mg/kg)	116.5	124.0	132.6	120.6	103.9	113.7	113.9	125.6	122.1	101.5	133.3	146.2
Fe (mg/kg)	191.4	185.4	193.2	180.5	154.6	160.3	169.4	186.0	146.6	113.5	140.1	147.2
Mn (mg/kg)	100.4	119.5	123.5	115.7	113.6	116.6	110.6	153.4	114.0	96.1	137.6	146.35
Cu (mg/kg)	15.6	18.0	18.0	14.9	13.9	17.4	14.7	17.0	14.7	11.0	16.6	14.9

Abbreviations: ADF, acid detergent fiber; AMEn, apparent metabolizable energy; CP, crude protein; DM, dry matter; EE, ether extract; HI, *Hermetia illucens*; NDF, neutral detergent fiber; nPP, non-phytate P.

<sup>a</sup>Calculated value (INRA, 2004).

<sup>1</sup>Diets HI0, HI3, HI6, and HI9 = dietary inclusion of HI larva meal at 0, 3, 6, and 9%, respectively. Table modified from Gariglio et al. (2019b).

<sup>2</sup>Mineral-vitamin premix per kg: vitamin A (retinyl acetate), 12,500 IU; vitamin D3 (cholecalciferol), 3,500 IU; vitamin E (DL- $\alpha$ -tocopheryl acetate), 40 mg; vitamin K (menadione sodium bisulfite), 2.0 mg; biotin, 0.20 mg; thiamine, 2.0 mg; riboflavin, 6.0 mg; pantothenate, 15.21 mg; niacin, 40.0 mg; choline, 750.0 mg; pyridoxine, 4.0 mg; folic acid, 0.75 mg; vitamin B12, 0.03 mg; Mn, 70 mg; Zn, 62.15 mg; Fe, 50.0 mg; Cu, 7.0 mg; I, 0.25 mg; Se, 0.25 mg.

<sup>3</sup>Optifos 250 bro: Phytase (EC 3.1.3.26) (250 OTU/kg diet), Huvepharma, Sofia, Bulgaria.

<sup>4</sup>Avizyme 1505X: Complex of Endo 1-4-Beta-Xylanase (EC 3.2.1.8) (256 U/kg), Subtilisin (Ec 3.4.21.62) (2560 U/kg diet) and Alpha-Amylase (EC3.2.1.1) (1472 U/kg diet), Danisco Animal Nutrition, Marlborough, Wiltshire, UK.

daily feed intake (**DFI**), and the feed conversion ratio (**FCR**) were calculated for the whole experimental period (3–50 days of age), as reported in detail in Gariglio et al. (2019a).

Forty-eight ducks (12 per diet, 2 birds/pen selected to be representative of the average final LW in each pen) were individually identified using a shank ring and weighed at 51 days of age. Following 12 h feed withdrawal, the animals were transported to a commercial abattoir and slaughtered according to the standard EU regulations.

Slaughtering weight (**SW**) was measured immediately before slaughter. Plucked and eviscerated carcasses were obtained after removing the head, neck, and feet. The hot carcass (**HC**) weight was then recorded. The spleen, liver, bursa of Fabricius, heart, and abdominal fat weights were immediately recorded and expressed as a percentage of the SW. The HC yield was calculated as a percentage of SW. The chilled carcass (**CC**) weight was registered following storage at +4°C for 24 h and their

CC yields were calculated as a percentage of SW. The breasts and thighs were then excised, and their weights expressed as a percentage of the CC weight. A total of 96 breasts and 96 thighs (right and left sides) were collected. The ultimate pH (**pHu<sub>24</sub>**) and color were assessed after 24 h of storage at +4°C on the *Pectoralis major* muscle on the right side of the breast and on the *Biceps femoris* muscle on the right thigh. In particular, the pHu<sub>24</sub> of the *Pectoralis major* and *Biceps femoris* muscles was measured in duplicate by means of a pH meter (Crison, Crison Instruments, SA, Alella, Spain) equipped with a specific electrode suitable for meat penetration. The lightness (**L\***), redness (**a\***), and yellowness (**b\***) color indexes (Commission International de l'Éclairage, 1976) were measured in the same muscles (taken at multiple locations and averaged) using a portable Chroma Meter CR-400 colorimeter (Konica Minolta Sensing Inc., Osaka, Japan).

The edible meat was then separated from the bones and cartilage of both whole thighs for use in subsequent

analyses. The meat from the right breast and right thigh was vacuum packaged and stored at  $-20^{\circ}\text{C}$  until further analysis. This meat was used to determine the content of heavy metals and thiobarbituric acid reactive substances (**TBARS**).

The meat from the left breast and left thigh was ground using a Grindomix GM 200 device (Retsch GmbH, Haan, Germany); one aliquot was used for FA analysis and the remaining amount was freeze-dried, reground, and analyzed to establish the proximate composition and mineral content.

### **Mineral Composition and Fatty Acid Profile of the Insect Meal and Experimental Diets**

The mineral analyses of the HI larva meal and experimental diets were performed at the Institute of Animal Physiology CBS, Slovak Academy of Sciences, the Slovak Republic. The Ca, total P, Zn, Fe, Mn, and Cu content of the feed were determined using a double-beam atomic absorption spectrophotometer (AA-7000 Series, Shimadzu Co., Kyoto, Japan), and certified reference materials LGC-7173 poultry feed (LGC Ltd., Teddington, UK) were used to verify instrument accuracy (Gresakova et al., 2016). The mineral profile of the HI larva meal was determined by inductively coupled plasma mass spectrometry or optical emission spectrometry (ICP-MS, Agilent 7900; ICP-OES, Agilent 5100, Agilent Technologies, Santa Clara, CA) (Gresakova et al., 2016). The mineral concentrations in all the samples were expressed as mg/kg.

The lipid extraction and FA profile of the HI meal and the experimental diets were carried out in the laboratory of the Department of Agronomy, Food, Natural Resources, Animal, and Environment, at the University of Padua, Italy. In brief, the total fat was extracted from fresh aliquots by means of accelerated solvent extraction (ASE, Dionex, Sunnyvale, CA, Application Note 334), using 2 extraction cycles with petroleum ether as solvent at  $125^{\circ}\text{C}$  and 10.3 MPa, a 6-min heating phase and a 2-min extraction phase. Then, 10 mL  $\text{NaSO}_4$  (0.47% in  $\text{H}_2\text{O}$ ) was added to the extracted lipids. The samples were kept at  $4^{\circ}\text{C}$  for 30 min and the supernatant (petroleum ether and lipids) was collected in another preweighed vial. Dry evaporation in  $\text{N}_2$  stream (Genevac EZ-2, SP Industries, Warminster, PA) was then performed; the residual samples (extracted lipids in vials) were weighed before adding 2 mL  $\text{H}_2\text{SO}_4$  (2% in methanol) (Christie, 1982). The vials were then incubated overnight at  $50^{\circ}\text{C}$ . Thereafter, the lipid rate was calculated. To this end, hexane (1 mL hexane/20 mg lipids) and potassium bicarbonate 2% (5 mL) were added. The obtained samples were centrifuged, stored at  $4^{\circ}\text{C}$  for 30 min, and the supernatant sampled for analysis using an Agilent 7890A GC System (Agilent Technologies, Santa Clara, CA). Supelco SP-2560 (Sigma-Aldrich, St. Louis, MO) (75  $\mu\text{m} \times 180 \mu\text{m}$  internal diameter, 0.14  $\mu\text{m}$  film

thickness, flow 0.25 mL/min) and Agilent J&W HP5ms (3.8 m  $\times$  250  $\mu\text{m}$  internal diameter, 0.25  $\mu\text{m}$  film thickness) were used with hydrogen as the carrier (split inlet, heater at  $270^{\circ}\text{C}$ , mode Pulse Split 25 psi until 0.30 min, split ratio 160:1, 40 mL/min). The temperature of the oven was set to  $40^{\circ}\text{C}$ , held for 2 min, raised to  $170^{\circ}\text{C}$  at a rate of  $50^{\circ}\text{C}/\text{min}$ , and held for 25 min, raised to  $250^{\circ}\text{C}$  at a rate of  $2^{\circ}\text{C}/\text{min}$ , and held for 14 min. GC IMAGE software, version 2.2b0 GC  $\times$  GC (Zoex Corporation, Houston TX), was used for data elaboration. The FAs were identified by comparing the retention time with that of a standard 52 FA methyl ester (**FAME**) mixture (GLC 463, NU-CHEK PREP Elysian, MN). Individual FAMES were expressed as the percentage of the total area of eluted FAMES.

### **Meat Quality Analyses**

Lipid oxidation was determined on 10 g samples of thawed breast and thigh meat by means of a TBARS assay, as described in Dabbou et al. (2014). The samples were analyzed in duplicate and the absorbance was read at 532 nm with a Helios spectrophotometer (Unicam Limited, Cambridge, UK). The results were expressed as  $\mu\text{g}$  malonaldehyde/g of meat.

The FA composition of the fresh meat from the breast and thigh was determined according to the method described above for HI meal and for the diets. Individual FAMES were expressed as the percentage of the total area of the eluted FAMES.

The atherogenicity (**AI**) and thrombogenicity (**TI**) indexes were calculated according to Ulbricht and Southgate (1991) as follows:

$$AI = [C12 : 0 + (4 \times C14 : 0) + C16 : 0] / (\sum MUFA + \sum n - 6 + \sum n - 3);$$

$$TI = (C14 : 0 + C16 : 0 + C18 : 0) / \left[ (0.5 \times \sum MUFA) + (0.5 \times \sum n - 6) + (3 \times \sum n - 3) \right] + \left( \sum n - 3 / \sum n - 6 \right).$$

Freeze-dried samples were used to determine the DM (934.01), ash (967.05), CP (2001.11), and EE (991.36) content (AOAC, 2000).

Moreover, the Zn, Fe, Mn, and Cu content of the freeze-dried samples were analyzed using a double-beam atomic absorption spectrophotometer (AA-7000 Series, Shimadzu Co., Kyoto, Japan), using pooled meat samples from each of the 6 replicate pens (Gresakova et al., 2016). The ERM-BB184 certified reference materials of bovine muscle (IRMM, Geel, Belgium) were included to verify the accuracy of the measurements. The mineral concentrations in all samples were expressed as mg/kg.

## Trace Elements, As, and Heavy Metal Determination

Trace element (Co, Cr, Ni, Se), As, and heavy metal (Hg, Cd, Pb) concentrations were determined for HI larva meal and on the 4 breast and thigh meat pools (1 pool per feeding group, each of which was composed of 6 slaughtered animals, 1 bird/pen) using the MI 351 Rev. 1/2015 accredited test (UNI EN 13804:2002, UNI EN 15763:2010 and UNI EN 13805:2014), as described by Schiavone et al. (2019). The results were expressed as mg/kg of sample with 12% humidity.

## Statistical Analyses

The statistical analyses were performed using the SPSS software package (version 21 for Windows, SPSS Inc., Chicago, IL). Shapiro-Wilk's test was used to establish the normality of distribution. The assumption of equal variances was assessed using Levene's homogeneity of variance test. The experimental unit was the pen. The collected data were tested by means of one-way ANOVA. Polynomial contrasts were used to test the linear and quadratic responses to increasing HI larva meal inclusion levels in the diet. Statistical significance was declared for  $P < 0.05$ . A statistical trend was considered for  $P < 0.10$ . The results are expressed as means and the standard error of the means (SEM).

## RESULTS

### Composition of Experimental Diets and Raw Materials

The trace element, As, and heavy metal concentrations in the partially defatted HI larva meal are reported in Table 2. The heavy metal concentrations were below the European Union (EU) limits reported for animal feeds (EC, 2002).

**Table 2.** Mineral, trace element, arsenic (As), and heavy metal concentrations in the *Hermetia illucens* larva meal.

Items	HI	MRL (Directive 2002/32/EC)
Minerals		
Zn (mg/kg)	172.7	
Fe (mg/kg)	218.7	
Mn (mg/kg)	255.2	
Cu (mg/kg)	14.9	
Trace elements, As, and heavy metal		
Co (mg/kg)	0.13	Not legislated
Cr (mg/kg)	0.57	Not legislated
Ni (mg/kg)	0.73	Not legislated
Se (mg/kg)	0.37	Not legislated
As (mg/kg 12% h)	0.08	2
Cd (mg/kg 12% h)	0.40	2
Hg (mg/kg 12% h)	0.02	0.1
Pb (mg/kg 12% h)	0.15	10

Abbreviations: MRL, maximum residue limit; HI, *Hermetia illucens*; mg/kg 12% h: mg/kg feed with 12% humidity.

As far as the FA profile is concerned, the partially defatted HI larva meal resulted rich in total SFAs (Table 3), with lauric acid (C12:0) being as the most represented FA (49.7% of the total FAs), followed by palmitic acid (C16:0; 13.3% of the total FAs), and myristic acid (C14:0; 10.1% of the total FAs). The HI larva meal contained a high amount of MUFAs (13.2% of the total FAs), predominantly represented by oleic acid (C18:1; 70.2% of MUFAs). The total quantity of lauric and myristic acids and total SFAs in the experimental diets increased as the percentage of insect meal inclusion increased and in line with the HI meal FA profile. Compared with the corn gluten meal, the HI meal contained a relatively low concentration of PUFAs (7.90% of the total FAs), which were almost entirely represented by linoleic (C18:2 *n-6*) and  $\alpha$ -linolenic (C18:3 *n-3*) acids. As a result, the *n-6* and the *n-3* PUFA content of the experimental diets decreased as the percentage of HI meal increased.

**Table 3.** Fatty acid composition of the *Hermetia illucens* meal and the experimental diets<sup>1</sup> (% of total FAs).

Fatty acids	HI meal	Starter period				Grower period				Finisher period			
		HI0	HI3	HI6	HI9	HI0	HI3	HI6	HI9	HI0	HI3	HI6	HI9
C12:0	49.70	0.07	2.73	5.49	8.11	0.07	2.57	4.82	7.67	0.07	2.07	4.82	6.35
C14:0	10.10	0.16	0.60	1.13	1.65	0.14	0.57	0.96	1.46	0.10	0.48	0.99	1.26
C16:0	13.29	14.99	13.91	13.71	13.62	13.99	13.69	13.36	13.06	13.78	13.53	13.01	12.71
C18:0	2.04	2.83	2.62	2.65	2.53	2.75	2.64	2.58	2.52	2.67	2.63	2.71	2.70
C16:1 <i>n-7</i>	3.09	0.15	0.29	0.44	0.57	0.15	0.29	0.39	0.53	0.14	0.26	0.42	0.50
C18:1 <i>n-9</i>	9.28	25.77	24.20	23.50	22.42	23.93	22.94	22.19	21.29	23.10	22.85	21.74	21.94
C18:1 <i>n-7</i>	0.44	1.10	1.03	1.00	1.03	1.21	1.15	1.12	1.08	1.22	1.16	1.10	1.07
C18:2 <i>n-6</i>	6.93	49.08	48.80	46.05	44.27	51.46	49.84	48.07	46.06	52.25	50.36	47.82	46.28
C18:3 <i>n-3</i>	0.86	3.27	3.40	3.35	3.29	4.02	4.13	4.05	4.06	4.48	4.43	5.28	4.98
$\sum$ SFA <sup>2</sup>	76.53	19.00	20.72	23.90	26.88	17.70	20.22	22.52	25.56	17.31	19.44	22.25	23.75
$\sum$ MUFA <sup>2</sup>	13.21	27.50	25.99	25.42	24.48	25.76	24.83	24.14	23.34	24.88	24.73	23.65	23.73
$\sum$ PUFA <sup>2</sup>	7.90	52.55	52.45	49.64	47.78	55.77	54.20	52.40	50.26	56.98	55.02	53.35	51.52
$\sum$ <i>n-3</i> <sup>2</sup>	0.89	3.31	3.44	3.38	3.32	4.08	4.16	4.091	4.10	4.52	4.46	5.30	5.00
$\sum$ <i>n-6</i> <sup>2</sup>	6.96	49.12	48.89	46.14	44.34	51.56	49.93	48.18	46.10	52.34	50.43	47.92	46.38
$\sum$ <i>n-6</i> / $\sum$ <i>n-3</i>	7.85	14.83	14.22	13.65	13.35	12.65	12.00	11.78	11.25	11.58	11.32	9.04	9.27
$\sum$ PUFA/ $\sum$ SFA	0.10	2.77	2.53	2.08	1.78	3.15	2.68	2.33	1.97	3.29	2.83	2.17	2.17

Abbreviations: FAs, fatty acids; HI, *Hermetia illucens*; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid;  $\sum$ *n-6*/ $\sum$ *n-3*:  $\sum$ PUFA *n-6*/ $\sum$ PUFA *n-3* ratio;  $\sum$ PUFA/ $\sum$ SFA: polyunsaturated fatty acid/saturated fatty acid ratio.

<sup>1</sup>Diets HI0, HI3, HI6, and HI9 = dietary inclusion of HI larva meal at 0, 3, 6, and 9%, respectively.

<sup>2</sup>Including minor FA.

**Table 4.** Growth and slaughtering performance of Muscovy ducks slaughtered at 51 days of age and fed increasing concentrations of partially defatted *Hermetia illucens* meal.

Items	Dietary treatments <sup>1</sup>				SEM	<i>P</i> -value	
	HI0	HI3	HI6	HI9		Linear	Quadratic
Growth performance (n = 6)							
Initial LW (3 d) (g)	71	70	73	72	0.60	0.405	0.733
Final LW (50 d) (g)	2541	2511	2456	2555	20.13	0.946	0.123
ADG (3–50 d) (g/d)	52.5	51.9	50.7	52.8	0.43	0.926	0.125
DFI (3–50 d) (g/d)	120.6	121.3	117.6	121.5	1.20	0.927	0.530
FCR (3–50 d) (g/g)	2.29	2.34	2.32	2.30	0.019	0.925	0.406
Slaughtering performance (n = 6)							
SW (g)	2492	2408	2374	2487	16.67	0.727	0.003
HC weight (g)	1606	1568	1551	1631	10.90	0.544	0.006
CC weight (g)	1577	1534	1521	1598	10.72	0.577	0.005
HC yield (% SW)	64.5	65.1	65.4	65.6	0.25	0.115	0.655
CC yield (% SW)	63.3	63.7	64.1	64.2	0.23	0.123	0.734
Breast yield (% CC weight)	20.0	20.5	20.0	20.6	0.24	0.575	0.977
Thigh yield (% CC weight)	29.4	29.6	30.2	29.5	0.21	0.657	0.403
Spleen (% SW)	0.08	0.07	0.08	0.09	0.00	0.831	0.157
Liver (% SW)	1.88	1.87	1.87	1.75	0.03	0.127	0.387
Bursa of Fabricius (% SW)	0.12	0.15	0.13	0.15	0.00	0.289	0.668
Heart (% SW)	0.61	0.60	0.58	0.58	0.01	0.247	0.623
Abdominal fat (% SW)	2.31	2.01	1.98	2.23	0.06	0.647	0.030

Abbreviations: ADG, average daily gain; CC, chilled carcass; DFI, daily feed intake; FCR, feed conversion ratio; HC, hot carcass; HI, *Hermetia illucens*; LW, live weight; SEM, standard error of the mean; SW, slaughter weight.

<sup>1</sup>Diets HI0, HI3, HI6, and HI9 = dietary inclusion of HI larva meal at 0, 3, 6%, and 9%, respectively.

## Slaughter Traits and Organ Weights

The growth performance of the Muscovy ducks (reported in detail in Gariglio et al., 2019a), is summarized in Table 4. LW, ADG, DFI, and FCR were not influenced by the dietary treatments throughout the entire trial.

Regarding the slaughter traits, the SW, and consequently the HC and CC weights, showed a quadratic response with the minimum corresponding to the HI6 group ( $P < 0.05$ ). Nevertheless, the HC and CC yields were not affected by the dietary treatments. The weights of the spleen, liver, bursa of Fabricius, and heart were similar among groups, whereas the abdominal fat showed a quadratic response, with the minimum corresponding to the HI6 group ( $P < 0.05$ ; Table 4).

## Meat Quality Traits

The pH<sub>u24</sub> and the color of the breast and thigh muscles and their chemical composition were not affected by the dietary treatment (Table 5). Only the Cu content increased linearly in the thigh meat, with a maximum for the HI9 group ( $P < 0.05$ ).

As for the FA profile of the meat (Table 6), the concentrations of lauric (C12:0) and myristic (C14:0) acids increased linearly as the HI meal inclusion in the diet increased, with the maximum concentration in the HI9 group ( $P < 0.05$ ) in relation to both breast (+85% and +35% for lauric and myristic acids, respectively) and thigh meat (+92% and +37% for lauric and myristic acids, respectively). Palmitic acid (C16:0) decreased linearly in the thigh meat and was about 4.8% lower in the HI9 group than in the HI0 group ( $P < 0.05$ ). Nevertheless, the SFA content of the breast meat was not

**Table 5.** pH<sub>u24</sub> and color, proximate composition (%), lipid oxidation (TBARS,  $\mu\text{g}$  malonaldehyde/g fresh meat) and mineral profile (mg/kg) of the breast meat (BM) and thigh meat (TM) of Muscovy ducks fed increasing concentration of partially defatted *Hermetia illucens* larva meal (n = 6, slaughtered at 51 days of age).

Items	Dietary treatments <sup>1</sup>				SEM	<i>P</i> -value		
	HI0	HI3	HI6	HI9		Linear	Quadratic	
pHu <sub>24</sub> and color								
pHu <sub>24</sub>	BM	5.88	5.86	5.84	5.83	0.02	0.401	0.860
	TM	5.92	5.89	5.95	5.90	0.02	0.976	0.778
L*	BM	45.6	46.1	46.9	45.9	0.42	0.683	0.374
	TM	44.4	46.1	46.3	45.1	0.45	0.528	0.116
a*	BM	13.1	12.5	12.6	13.7	0.34	0.566	0.232
	TM	11.4	11.7	10.3	11.2	0.33	0.549	0.682
b*	BM	11.3	11.1	11.3	10.8	0.27	0.549	0.719
	TM	10.7	11.3	10.1	10.3	0.20	0.190	0.542
Proximate composition								
Moisture	BM	77.2	77.1	77.2	77.2	0.06	0.629	0.808
	TM	74.4	74.8	74.6	74.8	0.09	0.250	0.697
Protein	BM	20.1	20.2	20.1	20.1	0.06	0.803	0.374
	TM	21.0	20.8	21.0	21.0	0.06	0.691	0.346
Ether extract	BM	0.88	0.91	0.85	0.87	0.02	0.412	0.823
	TM	2.57	2.48	2.74	2.48	0.09	0.991	0.638
Ash	BM	1.38	1.35	1.35	1.36	0.01	0.523	0.259
	TM	1.18	1.16	1.17	1.17	0.00	0.682	0.422
<i>TBARS</i>								
TBARS	BM	2.82	2.75	2.75	2.69	0.09	0.645	0.969
	TM	1.36	1.19	2.23	1.16	0.04	0.118	0.493
Mineral profile								
Zn	BM	10.6	10.5	10.3	10.3	0.09	0.281	0.675
	TM	20.5	21.2	21.6	21.2	0.26	0.308	0.337
Fe	BM	30.5	29.8	27.4	29.1	0.54	0.186	0.266
	TM	13.8	14.1	13.1	12.7	0.30	0.108	0.546
Mn	BM	0.16	0.13	0.13	0.14	0.00	0.103	0.055
	TM	0.11	0.10	0.11	0.11	0.00	0.276	0.391
Cu	BM	2.75	3.02	3.03	3.01	0.07	0.209	0.288
	TM	1.06	1.16	1.23	1.31	0.03	<0.001	0.814

Abbreviations: HI, *Hermetia illucens*; pH<sub>u</sub>, ultimate pH; SEM, standard error of the mean; L\*: lightness; a\*: redness; b\*: yellowness; TBARS, thiobarbituric acid-reactive substances.

<sup>1</sup>Diets HI0, HI3, HI6, and HI9 = dietary inclusion of HI larva meal at 0, 3, 6, and 9, respectively.

**Table 6.** Fatty acid composition (% of total FAME) of the breast meat (BM) and thigh meat (TM) of Muscovy ducks fed increasing concentration of partially defatted *Hermetia illucens* larva meal (n = 6, slaughtered at 51 days of age).

Fatty acids		Dietary treatments <sup>1</sup>				SEM	P-value	
		HI0	HI3	HI6	HI9		Linear	Quadratic
C12:0	BM	0.12	0.34	0.54	0.80	0.04	<0.001	0.610
	TM	0.13	0.60	1.19	1.66	0.09	<0.001	0.975
C14:0	BM	0.37	0.44	0.52	0.57	0.01	<0.001	0.634
	TM	0.49	0.57	0.74	0.78	0.02	<0.001	0.360
C16:0	BM	20.62	21.11	20.93	20.48	0.14	0.650	0.101
	TM	20.43	20.02	20.42	19.45	0.12	0.018	0.232
C18:0	BM	12.97	11.72	12.16	12.02	0.17	0.101	0.088
	TM	9.25	8.94	8.63	9.12	0.12	0.529	0.112
C16:1 n-7	BM	1.18	1.31	1.20	1.18	0.03	0.720	0.178
	TM	2.05	1.96	1.97	1.83	0.03	0.039	0.661
C18:1 n-9	BM	23.58	25.77	25.10	25.48	0.31	0.063	0.133
	TM	29.69	29.80	29.88	29.52	0.24	0.850	0.643
C18:1 n-7	BM	2.35	2.17	2.20	2.23	0.03	0.345	0.144
	TM	1.89	1.84	1.80	1.82	0.02	0.298	0.463
C18:2 n-6	BM	21.07	21.92	21.28	21.76	0.18	0.367	0.603
	TM	24.03	24.73	24.33	24.23	0.20	0.904	0.319
C18:3 n-3	BM	1.17	1.15	1.16	1.11	0.02	0.008	0.046
	TM	1.53	1.61	1.73	1.71	0.02	<0.001	0.158
C20:2 n-6	BM	0.51	0.43	0.47	0.46	0.01	0.257	0.180
	TM	0.22	0.22	0.21	0.22	0.00	0.787	0.780
C22:5 n-6	BM	1.99	0.73	0.82	0.77	0.03	0.026	0.080
	TM	0.73	0.64	0.61	0.67	0.02	0.359	0.133
C20:5 n-3 (EPA)	BM	0.16	0.14	0.16	0.17	0.00	0.449	0.154
	TM	0.12	0.11	0.10	0.12	0.00	0.933	0.218
C22:5 n-3 (DPA)	BM	0.72	0.57	0.64	0.60	0.02	0.112	0.172
	TM	0.50	0.46	0.44	0.46	0.01	0.263	0.270
C22:6 n-3 (DHA)	BM	0.67	0.52	0.58	0.58	0.02	0.217	0.041
	TM	0.50	0.45	0.41	0.47	0.02	0.385	0.110
$\Sigma$ SFA <sup>2</sup>	BM	34.59	34.10	34.68	34.42	0.11	0.958	0.600
	TM	30.54	30.38	31.24	31.29	0.11	0.001	0.601
$\Sigma$ MUFA <sup>2</sup>	BM	28.16	30.30	29.57	30.00	0.32	0.086	0.170
	TM	34.51	34.08	34.16	33.67	0.26	0.314	0.956
$\Sigma$ PUFA <sup>2</sup>	BM	36.35	34.77	34.87	34.69	0.32	0.092	0.277
	TM	34.15	34.38	33.46	33.87	0.26	0.457	0.860
$\Sigma$ n-3 <sup>2</sup>	BM	2.60	2.47	2.61	2.59	0.03	0.637	0.312
	TM	2.66	2.64	2.70	2.77	0.03	0.173	0.516
$\Sigma$ n-6 <sup>2</sup>	BM	33.54	32.10	32.05	31.87	0.30	0.059	0.287
	TM	31.28	31.53	30.54	30.90	0.23	0.319	0.918
$\Sigma$ n-6/ $\Sigma$ n-3	BM	12.93	13.04	12.30	12.31	0.09	<0.001	0.742
	TM	11.80	11.96	11.35	11.19	0.08	0.001	0.299
$\Sigma$ PUFA/ $\Sigma$ SFA	BM	1.05	1.02	1.01	1.01	0.10	0.137	0.469
	TM	1.12	1.13	1.07	1.08	0.01	0.051	0.948
AI	BM	0.34	0.36	0.38	0.36	0.00	0.020	0.281
	TM	0.33	0.33	0.36	0.36	0.00	<0.001	0.268
TI	BM	0.96	0.94	0.95	0.93	0.00	0.240	0.983
	TM	0.82	0.81	0.82	0.81	0.00	0.631	0.894

Abbreviations: AI, atherogenicity index; FA, fatty acids; HI, *Hermetia illucens*; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; SEM: standard error of the mean; TI, thrombogenicity index;  $\Sigma$ n6/ $\Sigma$ n3,  $\Sigma$ PUFA n6/ $\Sigma$ PUFA n3 ratio;  $\Sigma$ PUFA/ $\Sigma$ SFA, polyunsaturated fatty acid/saturated fatty acid ratio.

<sup>1</sup>Diets HI0, HI3, HI6, and HI9 = dietary inclusion of HI larva meal at 0, 3, 6, and 9%, respectively.

<sup>2</sup>Including minor FA.

influenced by the dietary inclusion level of HI meal, whereas a linear increase was observed in the thigh meat, with a maximum corresponding to the HI9 group ( $P < 0.05$ ). Among the MUFAs, palmitoleic acid (C16:1 n-7) in the thigh meat showed a linear decrease, with a minimum in the HI9 group, which contained 10% less palmitoleic acid than the HI0 group ( $P < 0.05$ ). The  $\alpha$ -linolenic acid (C18:3 n-3) decreased linearly and quadratically in the breast meat, with a minimum corresponding to the HI9 group (5.13% lower than the HI0 group;  $P < 0.05$ ). On the contrary,  $\alpha$ -linolenic acid increased linearly in the thigh meat according to the increasing dietary levels of HI meal, with a maximum in the HI9 group, which contained 10.5% more  $\alpha$ -linolenic

acid than the HI0 group ( $P < 0.05$ ). No significant difference was observed for eicosapentaenoic (EPA, C20:5 n-3) or docosapentaenoic (DPA, C22:5 n-3) acid in either the breast or the thigh meat samples. However, a quadratic response was observed for docosaheptaenoic acid (DHA, C22:6 n-3), being minimum in the HI3 group ( $P < 0.05$ ). A linear response was observed for n-6 docosapentaenoic acid (C22:5 n-6) in the breast meat, with a maximum corresponding to the control group ( $P < 0.05$ ). A linear trend was observed for the MUFA and PUFA content in the breast meat ( $P = 0.086$  and  $P = 0.092$ , respectively), whereas no significant difference was detected in the thigh meat. The  $\Sigma$ n-6/ $\Sigma$ n-3 ratio decreased linearly for both the breast and the thigh

**Table 7.** Heavy metal content in the breast meat (BM) and thigh meat (TM) of Muscovy ducks fed increasing concentrations of *Hermetia illucens* meal (one pool per feeding group, slaughtered at 51 days of age).

Items		Dietary treatment <sup>1</sup>				MRL (Regulation 881/2006/EC)
		HI0	HI3	HI6	HI9	
Trace elements						
Co (mg/kg)	BM	≤0.05	≤0.05	≤0.05	≤0.05	Not legislated
	TM	≤0.05	≤0.05	≤0.05	≤0.05	
Cr (mg/kg)	BM	0.09	0.33	0.52	0.27	Not legislated
	TM	0.05	0.07	≤0.05	0.06	
Ni (mg/kg)	BM	0.11	0.22	0.28	0.16	Not legislated
	TM	≤0.05	0.07	0.06	0.18	
Se (mg/kg)	BM	0.26	≤0.25	0.30	0.29	Not legislated
	TM	≤0.25	≤0.25	≤0.25	≤0.25	
As and heavy metals						
As (mg/kg 12% h)	BM	≤0.01	0.013	≤0.01	≤0.01	Not legislated
	TM	≤0.01	≤0.01	≤0.01	≤0.01	
Hg (mg/kg 12% h)	BM	≤0.01	≤0.01	≤0.01	≤0.01	Not legislated
	TM	≤0.01	≤0.01	≤0.01	≤0.01	
Cd (mg/kg 12% h)	BM	≤0.01	≤0.01	≤0.01	≤0.01	0.050 (EU n. 488/14)
	TM	≤0.01	≤0.01	≤0.01	≤0.01	
Pb (mg/kg 12% h)	BM	0.02	≤0.01	≤0.01	≤0.01	0.10 (EU n. 1005/15)
	TM	≤0.01	≤0.01	≤0.01	≤0.01	

Abbreviations: MRL, maximum residue limit; HI, *Hermetia illucens*; mg/kg 12% h: mg/kg 12% humidity.

<sup>1</sup>HI0, HI3, HI6, and HI9 = dietary inclusion of HI larva meal at 0%, 3%, 6%, and 9%, respectively.

meat, according to the increasing HI dietary levels, with the minimum observed in the HI6 and HI9 groups ( $P < 0.05$ ). A linear increasing trend was observed for the  $\sum$ PUFA/ $\sum$ SFA ratio in the thigh meat for increasing HI meal rates ( $P = 0.051$ ).

The lowest AI values for the breast and thigh meat were observed in the HI0 group ( $P < 0.05$ ), where linear responses were observed.

### Meat Trace Elements, As, and Heavy Metals

The concentrations of trace elements, As, and heavy metals in the breast and the thigh meat samples are summarized in Table 7. The As, Hg, Cd, and Pb concentrations were on average below 0.01 mg/kg at 12% humidity in both the breast and thigh meat, and below the EU limits reported for chicken meat (EC, 2006).

## DISCUSSION

HI larva meal is a promising high nutritional value ingredient to utilize in livestock feeds that could alleviate the pressure on conventional feed sources. The first 2 parts of our study (Gariglio et al., 2019a,b) described the effects, from the nutritional point of view, of the use of a partially defatted HI larva meal on the performance, digestive physiology, intestinal morphology, *in vivo* hematological parameters, and *post-mortem* organ traits of Muscovy ducks. In brief, these studies showed that increasing levels of HI larva meal inclusion did not affect the animals' growth performance, and that the digestive physiology, intestinal morphology, and hematological traits of the Muscovy ducks were unaffected or even improved. Indeed, a partially improved renal function and a significant reduction in serum cholesterol and triglycerides were

observed, together with an improved antioxidant status and a reduction in plasma oxidative metabolites (Gariglio et al., 2019b).

No study has so far evaluated the effects of the dietary inclusion of HI meal on the carcass characteristics, the meat quality traits or the safety of the meat derived from Muscovy ducks, despite the fact that the assessment of the meat quality plays a fundamental role in the application of feedstuffs. Overall, the results of the present study are encouraging; they demonstrate the meat from HI meal-fed ducks to have a comparable composition and quality to that of vegetable-fed animals.

Although the ducks that were slaughtered were selected on the basis of their average final LW within a pen, the SW, HC, and CC weights were lower in the HI6 group compared with the other feeding groups, most probably as a result of the lower amounts of abdominal fat in the slaughtered animals from this group. Moreover, the HC and CC yields were not affected by the dietary treatments and were consistent with what Schiavone et al. (2004) and Kowalczyk et al. (2012) observed for Muscovy and Pekin ducks, respectively. On the other hand, the SW, HC, and CC weights were on average higher than expected considering the rearing guide for this specific genotype at this age (Grimaud Freres, 2017).

As far as the use of HI larva meal in other poultry species is concerned, Schiavone et al. (2019) showed that up to 10% HI larva meal in the diet (in substitution of soybean meal) improved the slaughter and carcass weights of broiler chickens. Likewise, Altmann et al. (2018) detected heavier carcasses in chickens fed 50% HI larva meal in replacement of a soy-based protein compared with the control group. In a similar context, Loponte et al. (2017) reported higher carcass weights in Barbary partridges fed HI-based diets as a partial replacement of soybean meal (25 or 50%).



The pH<sub>u24</sub> values of the breast and thigh meat obtained in the present trial were in line with the values reported by Wawro et al. (2004) and Pingel et al. (2012). No significant difference in pH was observed compared with previous studies on Barbary partridges and broiler chickens fed HI meal (Pieterse et al., 2018; Secci et al., 2018; Schiavone et al., 2019). However, Cullere et al. (2016) reported a lower pH<sub>u</sub> in broiler quails fed diets containing 15% HI larva meal.

Color is one of the main qualitative aspects that can influence the acceptance of food products by consumers and it is frequently used as an indicator of the economic value of a foodstuff (Qiao et al., 2001). The color traits of the breast and the thigh muscles were not affected by the dietary treatments, as previously reported for broiler chickens fed HI meal (Altmann et al., 2018; Pieterse et al., 2018; Schiavone et al., 2019). Similarly, Secci et al. (2018) did not observe any significant differences, in terms of raw meat color in Barbary partridges fed diets in which HI meal replaced 25 and 50% of soybean meal.

The proximate composition of the breast meat measured in the present study is in line with the results of Wawro et al. (2004) for Muscovy ducks. The proximate composition was unaffected in both the breast and the thigh meat by the dietary inclusion of HI meal, and this result is consistent with previous studies on broiler quails, broiler chickens and Barbary partridges (Cullere et al., 2018; Pieterse et al. 2018; Secci et al., 2018). Schiavone et al. (2019), on the other hand, reported meat moisture to decrease and meat protein to increase in broiler chickens fed diets with increasing levels of HI meal.

According to Cullere et al. (2018), the oxidative status of both the breast and thigh meat in broiler quails fed HI meal (evaluated using TBARS levels) was unaffected by dietary HI meal inclusion, thus confirming a satisfactory shelf life of the product.

The overall mineral composition of the HI larva meal used in the present trial is similar to the mineral profile observed by Makkar et al. (2014) and Cullere et al. (2018) and its dietary inclusion did not affect the mineral content in either the breast or thigh meat, although the Cu content in the thigh meat was higher in the HI9 group (+19.1%) than in the control. This result is difficult to explain, but it could be related to a difference in Cu bioavailability between vegetable and insect protein sources. Aoyagi and Baker (1993) evaluated Cu bioavailability in vegetable protein meal and determined the values for soybean meal (40%) and corn gluten meal (50%). The low Cu bioavailability in vegetable protein meal could be related to the phytate Cu-binding effects (Leeson, 2009). On the other hand, Latunde-Dada et al. (2016) reported higher Cu solubility in some insect species (grasshoppers, crickets, and mealworms) than in vegetable meal (whole-wheat meal). This supports our findings.

The possibility of modifying the FA profile of meat has an important role in the production of high-quality products as it provides a means to increase the MUFA

and PUFA intake in order to satisfy human dietary recommendations. A reduction in SFA consumption in favor of an increased MUFA and PUFA consumption helps to reduce the risk of obesity, cardiovascular disease, and cancer (Jakobsen, 1999). According to the results obtained in the present trial, myristic (C16:0), stearic (C18:0), oleic (C18:1 n-9), and linoleic (C18:2 n-6) acids were the most representative in Muscovy duck breast and thigh meat, a result that was also found in a previous study (Aronal et al., 2012). On the other hand, several studies have highlighted the possibility of modifying the FA profile of duck meat by modifying the dietary FA profile (Schiavone et al., 2004; Schiavone et al., 2007; Schiavone et al., 2010). This was also observed in the present study. Indeed, a relationship between the FA profile of HI larva meal and duck meat was observed. In particular, increasing levels of lauric and myristic acids were observed in both the breast and the thigh meat with increasing HI larvae meal inclusion levels. Although the total SFA amount in the thigh meat was affected, this increase was only about +2.40% in the HI9 group, compared to the control group. Our results are partially in agreement with those of Cullere et al. (2018), who observed a significant increase in lauric, palmitic, and myristic acids in the breast meat of broiler quails, which consequently brought about an overall increase in the total SFA content.

The dietary  $\alpha$ -linolenic acid (C18:3 n-3) content was somewhat similar among treatments for each feeding period. However, in the finisher period, the amount of  $\alpha$ -linolenic acid was higher in the HI6 and HI9 groups (+15.2% and +10.0% compared with HI0, respectively) (Table 3). Although  $\alpha$ -linolenic acid decreased in the breast meat as the HI level is increased, this was only observed for the HI9 group and the magnitude of the decrease was not very large (-5.17% with respect to the HI0 group). On the other hand, the increase in  $\alpha$ -linolenic acid in the thigh meat was greater in the HI6 and HI9 groups (+11.6% and +10.5% compared with the HI0 group, respectively).

Unlike the results of previous studies with broiler quails and chickens (Cullere et al., 2018; Schiavone et al., 2019), the total MUFA content in the breast meat tended to decrease as HI increased, whereas PUFA increased. The  $\sum$ n-6/ $\sum$ n-3 ratios of the meat resulted higher (12.3 in the breast meat and 11.2 in the thigh meat for the HI9 group) than the recommended ratio of 4:1 for human nutrition (Gómez Candela et al., 2011). However, it has been reported that the lipid content and composition of HI may be modified by the substrate used to rear the larva. St-Hilaire et al. (2007) reported an enrichment in n-3 FA in HI prepupae fed a fish offal-based diet, indicating it to be an interesting feedstuff for fish and other monogastric animals.

The AI and TI represent the effect of dietary FA composition on the risk of developing coronary diseases in humans. The AI mean values observed in this study (0.36 and 0.34 for the breast and thigh, respectively) remained under the maximum recommended threshold of 0.55. The TI was not affected by the dietary HI larva

meal inclusion and the values were similar to or even lower than the maximum recommended threshold of 0.95 for poultry meat (Ulbricht and Southgate, 1991). From a dietetic point of view, the AI and TI indexes were under the values estimated for chicken meat by Ulbricht and Southgate (1991), that is AI 0.50 and TI 0.95. The AI and TI observed in our study revealed that duck meat of animals fed HI larva meal is a safe product concerning the risk for developing coronary heart disease.

In the same way, the evaluation of the potential risks related to heavy metal contamination in insect meal and therefore in the derived products is important for human health. Thus, one of the main issues for the insect production industries is to produce an insect meal that, not only provides a high nutritional value, but is also low in heavy metals in order to satisfy the need for a product with high dietetic and safety qualities. Several studies have evaluated the bioaccumulation of As and heavy metals in HI pupae and the results have indicated that Cd could be of concern in relation to insect production. Indeed, Gao et al. (2017) reported a relevant Cd accumulation in HI pupae, with a higher concentration in the body compared with the substrate. On the contrary, the concentration of Zn, Pb, and As in pupae were below the feed concentration limits for these heavy metals (Diener et al., 2015; Van der Fels-Klerx et al., 2016; Gao et al., 2017). The trace elements, As, and heavy metal concentrations remained below the EU limits defined for feedstuffs (EC, 2002) and foodstuffs (EC, 2006) in HI larva meal and in breast and thigh meat, respectively. These results are promising in terms of food safety. Similarly, Schiavone et al. (2019) investigated the As and heavy metal concentrations in broiler chickens fed diets with 5%, 10%, and 15% HI larva meal inclusion. The results showed the absence of any critical issues with regard to the heavy metal content in broiler chicken breasts, thus indicating the insects-rearing substrates to be safe (Schiavone et al., 2019).

## CONCLUSIONS

The results of the present study provide important and innovative information about the effects of partially defatted dietary HI larva meal in poultry. To the best of our knowledge, this study provides the first data on the evaluation of the meat quality from insects-fed Muscovy ducks. The inclusion of partially defatted HI larva meal in the diet of Muscovy ducks did not affect the slaughtering performance of the birds or the meat quality parameters. The comparable results, in terms of proximate composition, mineral profile (with the exception of Cu) and oxidative status, between the HI-fed ducks and the control group demonstrates the feasibility of introducing partially defatted HI larva meal into the diets of Muscovy ducks.

The reduction in  $\sum n-6/\sum n-3$  in the insect-fed group could represent a positive starting point for future investigations. Indeed, the use of HI meals obtained from n-3

rich substrates could positively affect the duck meat n-3 FA content.

Further research is necessary to guarantee safety and technological practices for the production of insects as an alternative source of poultry feed. In addition, investigations into sensory attributes will be important to obtain a better understanding of the effects of insect meal on duck meat quality and consumer sensory perception.

## ACKNOWLEDGMENTS

The authors are grateful to Heinrich Katz (Hermetia Baruth GmbH, Baruth/Mark; Germany), for providing the black soldier fly larva meal and A.I.A. Agricola Italiana Alimentare S.p.A (Fossano, CN, Italy) for providing the feed ingredients. The authors are also grateful to Dario Sola and Mario Colombano for the bird care and technical support. The research was supported by University of Turin (Italy) funding: [SCHA\\_RILO\\_16\\_02](#). This work is the result of the visit by Schiavone A. (21142/IV/19) funded by the Fundación Séneca-Agencia de Ciencia y Tecnología de la Región de Murcia in connection with the "Jiménez De La Espada" Regional Programme For Mobility, Collaboration And Knowledge Exchange.

## DISCLOSURES

The authors have no conflicts of interest.

## REFERENCES

- Ali, S., G. Kang, H. Yang, J. Jeong, Y. Hwang, G. Park, and S. Joo. 2007. A comparison of meat characteristics between duck and chicken breast. *Asian-Aust. J. Anim. Sci.* 20:1002–1006.
- Altmann, B. A., C. Neumann, S. Velten, F. Liebert, and D. Mörlein. 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* 7:34.
- AOAC International. 2000. *Official Methods of Analysis of AOAC International* (17th ed.). AOAC Int, Gaithersburg, MD.
- Aoyagi, S., and D. H. Baker. 1993. Estimates of copper bioavailability from liver of different animal species and from feed ingredients derived from plants and animals. *Poult. Sci.* 72:1746–1755.
- Aronal, A. P., N. Huda, and R. Ahmad. 2012. Amino acid and fatty acid profiles of peking and muscovy duck meat. *Int. J. Poult. Sci.* 11:229–236.
- Biancarosa, I., S. N. Liland, D. Biemans, P. Araujo, C. G. Bruckner, R. Waagbø, B. E. Torstensen, E. J. Lock, and H. Amlund. 2017. Uptake of heavy metals and arsenic in black soldier fly (*Hermetia illucens*) larvae grown on seaweed-enriched media. *J. Sci. Food Agric.* 98:2176–2183.
- Biasato, I., L. Gasco, M. De Marco, M. Renna, L. Rotolo, S. Dabbou, M. T. Capucchio, E. Biasibetti, M. Tarantola, C. Bianchi, L. Cavallarini, F. Gai, L. Pozzo, D. Dezzutto, S. Bergagna, and A. Schiavone. 2017. Effects of yellow mealworm larva (*Tenebrio molitor*) inclusion in diets for female broiler chickens: Implications for animal health and gut histology. *Anim. Feed Sci. Technol.* 234:253–263.
- Biasato, I., L. Gasco, M. De Marco, M. Renna, L. Rotolo, S. Dabbou, M. T. Capucchio, E. Biasibetti, M. Tarantola, L. Sterpone, L. Cavallarini, F. Gai, L. Pozzo, S. Bergagna, D. Dezzutto, I. Zoccarato, and A. Schiavone. 2018. Yellow mealworm larva (*Tenebrio molitor*) inclusion in diets for male broiler chickens:

- Effects on growth performance, gut morphology, and histological findings. *Poult. Sci.* 97:540–548.
- Chartrin, P., A. Schiavone, M. D. Bernadet, G. Guy, J. Mourot, M. J. Duclos, and E. Baéza. 2005. Effect of genotype and overfeeding on lipid deposition in myofibres and intramuscular adipocytes of breast and thigh muscles of ducks. *Reprod. Nutr. Dev.* 45:87–99.
- Christie, W. W. 1982. A simple procedure for rapid transmethylation of glycerolipids and cholesteryl esters. *J. Lipid Res.* 23:1072–1075.
- Commission Internationale de l'Éclairage. 1976. *Recommendations on Uniform Colour Spaces-Colour Difference Equations, Psychometric Colour Terms: 15* Supplement 2. Commission Internationale de l'Éclairage, Paris, France.
- Cullere, M., G. Tasoniero, V. Giaccone, R. Miotti-Scapin, E. Claeys, S. De Smet, and A. Dalle Zotte. 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.
- Cullere, M., G. Tasoniero, V. Giaccone, G. Acuti, A. Marangon, and A. Dalle Zotte. 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.
- Dabbou, S., L. Gasco, F. Gai, I. Zoccarato, L. Rotolo, S. Dabbou Fekih, A. Brugiapaglia, A. N. Helal, and P. G. Peiretti. 2014. Dried artichoke bracts in rabbits nutrition: effects on the carcass characteristics, meat quality and fatty acid composition. *Animal* 8:1547–1553.
- Dabbou, S., F. Gai, I. Biasato, M. T. Capucchio, E. Biasibetti, D. Dezzutto, M. Meneguz, I. Plachà, L. Gasco, and A. Schiavone. 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. *J. Anim. Sci. Biotechnol.* 9:49.
- Dabbou, S., L. Gasco, C. Lussiana, A. Brugiapaglia, I. Biasato, M. Renna, L. Cavallarini, F. Gai, and A. Schiavone. 2019. Yellow mealworm (*Tenebrio molitor* L.) larvae inclusion in diets for free-range chickens: effects on meat quality and fatty acid profile. *Renew. Agr. Food Syst* 1–8.
- Diener, S., C. Zurbrugg, and K. Tockner. 2015. Bioaccumulation of heavy metals in the black soldier fly, *Hermetia illucens* and effects on its life cycle. *J. Insects Food Feed.* 1:261–270.
- European Commission (EC). 2002. Council Directive (EC) 2002/32/EC of 7 May 2002 on undesirable substances in animal feed. *OJEC 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. *OJEC L364:5–24*.
- Gao, Q., X. Wang, W. Wang, C. Lei, and F. Zhu. 2017. Influences of chromium and cadmium on the development of black soldier fly larva. *Environ. Sci. Pollut. Res. Int.* 24:8637–8644.
- Gariglio, M., S. Dabbou, I. Biasato, M. T. Capucchio, E. Colombino, F. Hernández, J. Madrid, S. Martínez, F. Gai, C. Caimi, S. B. Oddon, M. Meneguz, A. Trocino, R. Vincenzi, L. Gasco, and A. Schiavone. 2019a. Nutritional effects of the dietary inclusion of partially defatted *Hermetia illucens* larva meal in Muscovy duck. *J. Anim. Sci. Biotechnol.* 10:37.
- Gariglio, M., S. Dabbou, M. Crispo, I. Biasato, F. Gai, L. Gasco, F. Piacente, P. Odetti, S. Bergagna, I. Plachà, E. Valle, E. Colombino, M. T. Capucchio, and A. Schiavone. 2019b. Effects of the dietary inclusion of partially defatted black soldier fly (*Hermetia illucens*) meal on the blood chemistry and tissue (spleen, liver, thymus, and Bursa of fabricius) histology of Muscovy ducks (*Cairina moschata domestica*). *Animals* 9:307.
- Gasco, L., I. Biasato, S. Dabbou, A. Schiavone, and F. Gai. 2019. Animals fed insect-based diets: State-of-the-art on digestibility, performance and product quality. *Animals* 9:1–32.
- Gómez Candela, C., L. M. Bermejo López, and V. Loria Kohen. 2011. Importance of a balanced omega 6/omega 3 ratio for the maintenance of health. Nutritional recommendations. *Nutr. Hosp.* 26:323–329.
- Gresakova, L., K. Venglovska, and K. Cobanova. 2016. Dietary manganese source does not affect Mn, Zn and Cu tissue deposition and the activity of manganese-containing enzymes in lambs. *J. Trace Elem. Med. Biol.* 38:138–143.
- Grimaud Frères. 2017. *Grimaud Frères Selection Rearing Guide. Roasting Canedins*. Grimaud Frères. France. Accessed Dec. 2019 <http://www.grimaudfreres.com/en/>.
- INRA. 2004. *Tables de Composition et de Valeur Nutritive des Matières Premières Destinées Aux Animaux d'élevage : Porcs, Volailles, Bovins, Ovins, Caprins, Lapins, Chevaux, Poissons*. INRA, Paris, France.
- Jakobsen, K. 1999. Dietary modifications of animal fats: status and future perspectives. *Fett/Lipid* 12:475–483.
- Kowalczyk, A., E. Łukaszewicz, M. Adamski, and J. Kuźniacka. 2012. Carcass composition and meat characteristics of Pekin ducks in relation to age at slaughter and level of maize distiller's dried grains with solubles in diets. *J. Anim. Feed Sci.* 21:157–167.
- Latunde-Dada, G. O., W. Yang, and M. Vera Aviles. 2016. In vitro iron availability from insects and sirloin beef. *J. Agric. Food Chem.* 64:8420–8424.
- Leeson, S. 2009. Copper metabolism and dietary needs. *World Poultry Sci. J.* 65:353–366.
- Liland, N. S., I. Biancarosa, P. Araujo, D. Biemans, C. G. Bruckner, R. Waagbø, B. E. Torstensen, and E. J. Lock. 2017. Modulation of nutrient composition of black soldier fly (*Hermetia illucens*) larvae by feeding seaweed-enriched media. *PLoS One* 12:e0183188.
- Loponte, R., S. Nizza, F. Bovera, N. De Riu, K. Fliegerova, P. Lombardi, G. Vassalotti, V. Mastellone, A. Nizza, and G. Moniello. 2017. Growth performance, blood profiles and carcass traits of Barbary partridge (*Alectoris barbara*) fed two different insect larva meals (*Tenebrio molitor* and *Hermetia illucens*). *Res. Vet. Sci.* 115:183–188.
- Makkar, H. P. S., G. Tran, V. Heuzé, and P. Ankers. 2014. State-of-the-art on use of insects as animal feed. *Anim. Feed Sci. Technol.* 197:1–33.
- Meneguz, M., A. Schiavone, F. Gai, A. Dama, C. Lussiana, M. Renna, and L. Gasco. 2018. Effect of rearing substrate on growth performance, waste reduction efficiency and chemical composition of black soldier fly (*Hermetia illucens*) larva. *J. Sci. Food Agric.* 98:5776–5784.
- Pieterse, E., S. W. Erasmus, T. Ushona, and L. C. Hoffman. 2018. 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. *J. Sci. Food Agric.* 30:893–903.
- Pingel, H., G. Guy, and E. Baéza. 2012. *Production de Canards (1st Ed.)*. Éditions Quæ, Versailles Cedex, France Quæ. France.
- Qiao, M., D. L. Fletcher, D. P. Smith, and J. K. Northcutt. 2001. The effect of broiler breast meat color on pH, moisture, water-holding capacity, and emulsification capacity. *Poult. Sci.* 80:676–680.
- Schiavone, A., I. Romboli, R. Chiarini, and M. Marzoni. 2004. Influence of dietary lipid source and strain on fatty acid composition of Muscovy duck meat. *J. Anim. Physiol. Anim. Nutr.* 88:88–93.
- Schiavone, A., M. Marzoni R.Chiarini, A. Castillo, S. Tassone, and I. Romboli. 2007. Breast meat traits of Muscovy ducks fed on a microalga (*Cryptocodinium cohnii*) meal supplemented diet. *Br. Poult. Sci.* 48:573–579.
- Schiavone, A., M. Marzoni, A. Castillo, J. Nery, and I. Romboli. 2010. Dietary lipid sources and vitamin E affect fatty acid composition or lipid stability of breast meat from Muscovy duck. *Can. J. Anim. Sci.* 90:371–378.
- Schiavone, A., M. De Marco, S. Martínez, S. Dabbou, M. Renna, J. Madrid, F. Hernandez, L. Rotolo, P. Costa, F. Gai, and L. Gasco. 2017. Nutritional value of a partially defatted and a highly defatted black soldier fly larva (*Hermetia illucens* L.) meal for broiler chickens: apparent nutrient digestibility, apparent metabolizable energy and apparent ileal amino acid digestibility. *J. Anim. Sci. Biotechnol.* 8:897–905.
- Schiavone, A., S. Dabbou, M. Petracchi, M. Zampiga, F. Sirri, I. Biasato, F. Gai, and L. Gasco. 2019. Black soldier fly defatted meal as a dietary protein source for broiler chickens: effects on carcass traits, breast meat quality and safety. *Animal* 13:2397–2405.
- Secci, G., G. Moniello, L. Gasco, F. Bovera, and G. Parisi. 2018. Barbary partridge meat quality as affected by *Hermetia illucens* and *Tenebrio molitor* larva meals in feeds. *Food Res. Int.* 112:291–298.
- Sprangers, T., M. Ottoboni, C. Klootwijk, A. Olyn, S. Deboosere, B. De Meulenaer, J. Michiels, M. Eeckhout, P. De Clercq, and

- S. De Smet. 2017. Nutritional composition of black soldier fly (*Hermetia illucens*) prepupae reared on different organic waste substrates. *J. Sci. Food Agric.* 97:2594–2600.
- St-Hilaire, S., K. Cranfill, M. A. McGuire, E. E. Mosley, J. K. Tomberlin, L. Newton, W. Sealey, C. Sheppard, and S. Irving. 2007. Fish offal recycling by the black soldier fly produces a foodstuff high in omega-3 fatty acids. *J. World Aquac. Soc.* 38:309–313.
- Ulbricht, T. L. V., and D. A. T. Southgate. 1991. Coronary heart disease seven dietary factors. *Lancet* 338:985–992.
- Van der Fels-Klerx, H. J., L. Camenzuli, M. K. van der Lee, and D. G. Oonincx. 2016. Uptake of Cadmium, Lead and Arsenic by *Tenebrio molitor* and *Hermetia illucens* from contaminated substrates. *PLoS One* 11:e0166186.
- Wawro, K., E. Wilkiewicz-Wawro, K. Kleczek, and W. Brzozowski. 2004. Slaughter value and meat quality of Muscovy ducks, Pekin ducks and their crossbreeds, and evaluation of the heterosis effect. *Arch. Anim. Breed.* 47:287–299.
- FAOSTAT. 2019. Accessed Dec. 2019. <http://www.fao.org/faostat/en/#data/QL>.