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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Running head: Black soldier fly meal in broiler chicken diets

Abstract
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) larvae meal (HI) 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
(2 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 color 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 a 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, 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 58-72% saturated fatty acids (SFA) and 19-40% mono (MUFA) and polyunsaturated 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) but data regarding the chemical safety of reared insects are very scarce. HI has recently been tested as a feed ingredient in conventional poultry diets as a fat source (Schiavone et al., 2017a and 2018) and in laying hens and broiler quails and chickens as protein source (Maurer et al., 2016; Cullere et al., 2016 and 2018),
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. HI 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, two hundred and fifty-six one-day-old male broiler chickens were reared from day 1 to day 35 and randomly allotted to four dietary treatments (8 pens/treatment
and 8 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 d of age, 16 animals (2 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 in two parts and frozen at -20°C for further meat analyses.

Meat quality parameters

The meat quality parameters (pH, color, 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 detailed 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 procedure (AOAC, 1990). Proteins were determined using the standard Kjeldahl copper catalyst method (AOAC, 1990). Total lipids were measured using a modification of the chloroform: methanol procedure described by Folch et al. (1957). FAs 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 4 breast meat pools (one pool per feeding group, each of them composed by 8 slaughtered animals [one bird per replicate pens]), as presented in Supplementary Material S1. The results were expressed as mg/kg 12% humidity.

**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). 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; 2 birds/pen). The carcass characteristics, meat quality parameters and FA profile data were tested by means of one-way analysis of variance (ANOVA), with experimental diet (HI0, HI5, HI10 and HI15) as fixed effect, and according to the following model:

\[ Y_{ij} = \mu + \alpha + \epsilon_{ij} \]

where \( Y_{ij} \) = the single observation; \( \mu \) = overall mean; \( \alpha \) = the effect of experimental diet (i= HI0 or HI inclusion level) and \( \epsilon \) = 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*) color 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 c9 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 dietary treatments were below the EU limits reported for chicken meat (EC, 2006).

Discussion

HI 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 unfavorable 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, 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, 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 color are controversial. Cullere et al. (2016) observed that meat 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 color. In regard to other insect meals, the use of MD larvae meal in broiler diets has also 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 color 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 color differences found in broiler chickens fed with HI larvae meal of the present study appear to be of little 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, slaughter weight) are also able to affect the FA 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. However, the total SFA remained constant, that is, at 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 c9) predominance (Klasing, 2000). An increasing percentage of MUFA, mainly due to the higher content of C18:1 c9, 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. HI 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 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). HI 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 larva meal was 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 metals 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.

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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.

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Table 1

Ingredients, apparent metabolizable energy and chemical composition of the experimental diets.

<table>
<thead>
<tr>
<th>Items</th>
<th>Starter period</th>
<th>Growing period</th>
<th>Finisher period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HI0</td>
<td>HI5</td>
<td>HI10</td>
</tr>
<tr>
<td>Ingredients (g/kg as fed)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maize meal</td>
<td>508.8</td>
<td>526.9</td>
<td>545.5</td>
</tr>
<tr>
<td>Soybean meal (48% CP)</td>
<td>345.3</td>
<td>299.0</td>
<td>248.0</td>
</tr>
<tr>
<td>HI larvae meal</td>
<td>0.0</td>
<td>50.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Corn gluten meal</td>
<td>54.0</td>
<td>32.0</td>
<td>15.0</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>46.3</td>
<td>45.1</td>
<td>43.0</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>6.5</td>
<td>8.5</td>
<td>10.5</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>17.3</td>
<td>16.4</td>
<td>15.6</td>
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<tr>
<td>Sodium chloride</td>
<td>2.3</td>
<td>2.3</td>
<td>2.3</td>
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<tr>
<td>Sodium bicarbonate</td>
<td>1.3</td>
<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
<td>DL-methionine</td>
<td>2</td>
<td>2.2</td>
<td>2.3</td>
</tr>
<tr>
<td>L-lysine</td>
<td>5.1</td>
<td>5.2</td>
<td>5.4</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
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<tr>
<td>--------------------------</td>
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</tr>
<tr>
<td>Threonine</td>
<td>1.9</td>
<td>1.9</td>
<td>1.9</td>
</tr>
<tr>
<td>Trace mineral-vitamin premix&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.0</td>
<td>8.0</td>
<td>8.0</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>3-phytase</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Total</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
</tr>
<tr>
<td>Nutrient composition&lt;sup&gt;c&lt;/sup&gt; (%)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>CP</td>
<td>23.01</td>
<td>22.98</td>
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<tr>
<td>EE</td>
<td>7.30</td>
<td>7.33</td>
<td>7.28</td>
</tr>
<tr>
<td>CF</td>
<td>3.25</td>
<td>2.99</td>
<td>2.70</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.96</td>
<td>0.96</td>
<td>0.96</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>0.48</td>
<td>0.48</td>
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</tr>
<tr>
<td>Methionine&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.56</td>
<td>0.56</td>
<td>0.56</td>
</tr>
<tr>
<td>Lysine&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.44</td>
<td>1.44</td>
<td>1.44</td>
</tr>
<tr>
<td>Threonine&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.97</td>
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</tr>
</tbody>
</table>
HI = *Hermetia illucens*; AME = apparent metabolizable energy; DM = dry matter; CP = crude protein; EE = ether extract; CF = crude fiber.

\(^a\)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 bisulfite), 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).

<table>
<thead>
<tr>
<th>FA Type</th>
<th>HI Larvae Meal</th>
<th>Starter Period</th>
<th>Growing Period</th>
<th>Finisher Period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HI0</td>
<td>HI5</td>
<td>HI10</td>
<td>HI15</td>
</tr>
<tr>
<td>C12:0</td>
<td>54.59</td>
<td>nd</td>
<td>2.73</td>
<td>5.46</td>
</tr>
<tr>
<td>C14:0</td>
<td>10.15</td>
<td>0.10</td>
<td>0.60</td>
<td>1.10</td>
</tr>
<tr>
<td>C16:0</td>
<td>12.03</td>
<td>10.37</td>
<td>10.43</td>
<td>10.48</td>
</tr>
<tr>
<td>C18:0</td>
<td>1.77</td>
<td>2.50</td>
<td>2.40</td>
<td>2.30</td>
</tr>
<tr>
<td>C18:1 c9</td>
<td>7.91</td>
<td>21.51</td>
<td>22.20</td>
<td>22.90</td>
</tr>
<tr>
<td>C18:2 n6</td>
<td>5.98</td>
<td>52.59</td>
<td>50.15</td>
<td>47.72</td>
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<tr>
<td>C18:3 n3</td>
<td>0.80</td>
<td>3.26</td>
<td>3.14</td>
<td>2.80</td>
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<tr>
<td>SFA</td>
<td>80.28</td>
<td>15.38</td>
<td>18.67</td>
<td>21.96</td>
</tr>
<tr>
<td>PUFA</td>
<td>6.85</td>
<td>58.50</td>
<td>55.82</td>
<td>53.15</td>
</tr>
<tr>
<td>Other FA</td>
<td>nd</td>
<td>9.60</td>
<td>8.34</td>
<td>7.23</td>
</tr>
</tbody>
</table>
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).

<table>
<thead>
<tr>
<th></th>
<th>Dietary treatments</th>
<th>SEM</th>
<th>P-value</th>
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<tbody>
<tr>
<td></td>
<td>HI0</td>
<td>HI5</td>
<td>HI10</td>
</tr>
<tr>
<td>Live weight (LW) (g)</td>
<td>2260.56</td>
<td>2259.44</td>
<td>2266.87</td>
</tr>
<tr>
<td>Carcass weight (g)</td>
<td>1594.84</td>
<td>1601.01</td>
<td>1607.84</td>
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<tr>
<td>Carcass weight (% LW)</td>
<td>70.55</td>
<td>70.86</td>
<td>70.92</td>
</tr>
<tr>
<td>Breast (% LW)</td>
<td>14.46</td>
<td>14.67</td>
<td>14.84</td>
</tr>
<tr>
<td>Thigh (% LW)</td>
<td>18.67</td>
<td>18.59</td>
<td>18.79</td>
</tr>
<tr>
<td>Spleen (% LW)</td>
<td>0.11</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Liver (% LW)</td>
<td>2.17</td>
<td>2.13</td>
<td>2.08</td>
</tr>
<tr>
<td>Heart (% LW)</td>
<td>0.64</td>
<td>0.60</td>
<td>0.56</td>
</tr>
<tr>
<td>Bursa of Fabricius (% LW)</td>
<td>0.29</td>
<td>0.26</td>
<td>0.26</td>
</tr>
<tr>
<td>Abdominal fat (% LW)</td>
<td>1.21</td>
<td>1.17</td>
<td>1.43</td>
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</table>

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

<table>
<thead>
<tr>
<th></th>
<th>pHu</th>
<th>Color</th>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>HI0</td>
<td>HI5</td>
<td>HI10</td>
<td>HI15</td>
<td>SEM</td>
<td>Linear</td>
<td>Quadratic</td>
<td></td>
</tr>
<tr>
<td>pHu</td>
<td>6.03</td>
<td>5.99</td>
<td>6.04</td>
<td>5.98</td>
<td>0.05</td>
<td>0.650</td>
<td>0.777</td>
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<tr>
<td>Color</td>
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<td></td>
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<td></td>
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<tr>
<td>Lightness (L*)</td>
<td>55.07</td>
<td>55.46</td>
<td>54.29</td>
<td>53.41</td>
<td>0.38</td>
<td>0.074</td>
<td>0.400</td>
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</tr>
<tr>
<td>Redness (a*)</td>
<td>2.72</td>
<td>3.18</td>
<td>2.87</td>
<td>3.71</td>
<td>0.14</td>
<td>0.030</td>
<td>0.469</td>
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<tr>
<td>Yellowness (b*)</td>
<td>11.80</td>
<td>9.68</td>
<td>8.19</td>
<td>7.57</td>
<td>0.39</td>
<td>&lt;0.001</td>
<td>0.174</td>
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</tr>
<tr>
<td>Drip loss (%)</td>
<td>1.24</td>
<td>1.36</td>
<td>1.26</td>
<td>1.39</td>
<td>0.06</td>
<td>0.211</td>
<td>0.928</td>
<td></td>
</tr>
<tr>
<td>Cooking loss (%)</td>
<td>20.11</td>
<td>20.49</td>
<td>19.31</td>
<td>19.71</td>
<td>0.36</td>
<td>0.480</td>
<td>0.990</td>
<td></td>
</tr>
<tr>
<td>Allo Kramer shear force (kg/g)</td>
<td>1.86</td>
<td>2.24</td>
<td>2.14</td>
<td>2.06</td>
<td>0.06</td>
<td>0.375</td>
<td>0.074</td>
<td></td>
</tr>
</tbody>
</table>

Proximate composition

<table>
<thead>
<tr>
<th></th>
<th>Moisture (%)</th>
<th>Protein (%)</th>
<th>Lipid (%)</th>
<th>Ash (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HI0</td>
<td>HI5</td>
<td>HI10</td>
<td>HI15</td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>76.14</td>
<td>75.51</td>
<td>75.50</td>
<td>75.24</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>22.37</td>
<td>22.28</td>
<td>22.42</td>
<td>23.09</td>
</tr>
<tr>
<td>Lipid (%)</td>
<td>1.56</td>
<td>1.76</td>
<td>1.85</td>
<td>1.75</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>1.23</td>
<td>1.33</td>
<td>1.24</td>
<td>1.31</td>
</tr>
</tbody>
</table>

HI= *Hermetia illucens.*
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).

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>HI0</th>
<th>HI5</th>
<th>HI10</th>
<th>HI15</th>
<th>SEM</th>
<th>Linear</th>
<th>Quadratic</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΣSFA</td>
<td>29.13</td>
<td>28.97</td>
<td>29.24</td>
<td>29.88</td>
<td>0.29</td>
<td>0.352</td>
<td>0.507</td>
</tr>
<tr>
<td>C12:0</td>
<td>nd</td>
<td>0.33</td>
<td>0.61</td>
<td>1.03</td>
<td>0.06</td>
<td>&lt;0.001</td>
<td>0.367</td>
</tr>
<tr>
<td>C14:0</td>
<td>0.23</td>
<td>0.43</td>
<td>0.54</td>
<td>0.74</td>
<td>0.03</td>
<td>&lt;0.001</td>
<td>0.965</td>
</tr>
<tr>
<td>C15:0</td>
<td>nd</td>
<td>0.02</td>
<td>0.05</td>
<td>0.04</td>
<td>0.01</td>
<td>&lt;0.001</td>
<td>0.041</td>
</tr>
<tr>
<td>C16:0</td>
<td>17.63</td>
<td>18.24</td>
<td>18.74</td>
<td>19.24</td>
<td>0.20</td>
<td>0.002</td>
<td>0.887</td>
</tr>
<tr>
<td>C17:0</td>
<td>0.15</td>
<td>0.12</td>
<td>0.13</td>
<td>0.14</td>
<td>0.01</td>
<td>0.705</td>
<td>0.315</td>
</tr>
<tr>
<td>C18:0</td>
<td>9.55</td>
<td>8.48</td>
<td>7.98</td>
<td>7.61</td>
<td>0.21</td>
<td>&lt;0.001</td>
<td>0.352</td>
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<tr>
<td>C20:0</td>
<td>0.19</td>
<td>0.24</td>
<td>0.23</td>
<td>0.23</td>
<td>0.01</td>
<td>0.152</td>
<td>0.245</td>
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<tr>
<td>C24:0</td>
<td>1.38</td>
<td>1.09</td>
<td>0.96</td>
<td>0.85</td>
<td>0.06</td>
<td>0.001</td>
<td>0.416</td>
</tr>
<tr>
<td>ΣMUFA</td>
<td>27.61</td>
<td>30.00</td>
<td>31.57</td>
<td>32.17</td>
<td>0.47</td>
<td>&lt;0.001</td>
<td>0.279</td>
</tr>
<tr>
<td>C14:1</td>
<td>nd</td>
<td>0.02</td>
<td>0.10</td>
<td>0.14</td>
<td>0.01</td>
<td>&lt;0.001</td>
<td>0.630</td>
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<tr>
<td>C16:1 n7</td>
<td>1.79</td>
<td>2.24</td>
<td>2.62</td>
<td>3.13</td>
<td>0.12</td>
<td>&lt;0.001</td>
<td>0.870</td>
</tr>
<tr>
<td>C17:1</td>
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<td>1.11</td>
<td>0.07</td>
<td>0.09</td>
<td>0.01</td>
<td>0.287</td>
<td>0.355</td>
</tr>
<tr>
<td>C18:1 c9</td>
<td>25.26</td>
<td>27.08</td>
<td>28.28</td>
<td>28.21</td>
<td>0.36</td>
<td>0.001</td>
<td>0.152</td>
</tr>
<tr>
<td>C20:1</td>
<td>0.20</td>
<td>0.26</td>
<td>0.28</td>
<td>0.34</td>
<td>0.01</td>
<td>&lt;0.001</td>
<td>0.330</td>
</tr>
<tr>
<td>FA</td>
<td>C24:1</td>
<td>C18:2 n6</td>
<td>C18:3 n6</td>
<td>C18:3 n3</td>
<td>C20:2 n6</td>
<td>C20:4 n6</td>
<td>C20:5 n3 (EPA)</td>
</tr>
<tr>
<td>-------------------</td>
<td>-------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
<td>----------------</td>
</tr>
<tr>
<td></td>
<td>0.31</td>
<td>0.28</td>
<td>0.22</td>
<td>0.26</td>
<td>0.01</td>
<td>0.091</td>
<td>0.182</td>
</tr>
<tr>
<td>C18:2 n6</td>
<td>31.44</td>
<td>30.62</td>
<td>29.61</td>
<td>28.32</td>
<td>0.41</td>
<td>0.004</td>
<td>0.763</td>
</tr>
<tr>
<td>C18:3 n6</td>
<td>0.20</td>
<td>0.24</td>
<td>0.22</td>
<td>0.25</td>
<td>0.01</td>
<td>0.355</td>
<td>0.974</td>
</tr>
<tr>
<td>C18:3 n3</td>
<td>2.31</td>
<td>2.40</td>
<td>2.47</td>
<td>2.20</td>
<td>0.06</td>
<td>0.641</td>
<td>0.150</td>
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<tr>
<td>C20:2 n6</td>
<td>0.56</td>
<td>0.57</td>
<td>0.57</td>
<td>0.47</td>
<td>0.03</td>
<td>0.319</td>
<td>0.345</td>
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<tr>
<td>C20:4 n6</td>
<td>0.13</td>
<td>0.17</td>
<td>0.17</td>
<td>0.17</td>
<td>0.01</td>
<td>0.207</td>
<td>0.375</td>
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<tr>
<td>C20:5 n3 (EPA)</td>
<td>1.09</td>
<td>0.81</td>
<td>0.62</td>
<td>0.72</td>
<td>0.05</td>
<td>0.002</td>
<td>0.039</td>
</tr>
<tr>
<td>C22:5 n3 (DPA)</td>
<td>0.76</td>
<td>0.49</td>
<td>0.34</td>
<td>0.46</td>
<td>0.04</td>
<td>0.001</td>
<td>0.004</td>
</tr>
<tr>
<td>C22:6 n3 (DHA)</td>
<td>0.90</td>
<td>0.97</td>
<td>1.29</td>
<td>1.30</td>
<td>0.08</td>
<td>0.032</td>
<td>0.814</td>
</tr>
<tr>
<td>Others FA</td>
<td>0.90</td>
<td>0.97</td>
<td>1.29</td>
<td>1.30</td>
<td>0.08</td>
<td>0.032</td>
<td>0.814</td>
</tr>
<tr>
<td>ΣPUFA/SFA</td>
<td>4.30</td>
<td>3.87</td>
<td>3.60</td>
<td>3.56</td>
<td>0.08</td>
<td>&lt;0.001</td>
<td>0.162</td>
</tr>
<tr>
<td>Σn3</td>
<td>38.06</td>
<td>36.19</td>
<td>34.29</td>
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<td>&lt;0.001</td>
<td>0.646</td>
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<tr>
<td>Σn6/n3</td>
<td>8.93</td>
<td>9.39</td>
<td>9.63</td>
<td>9.40</td>
<td>0.13</td>
<td>0.140</td>
<td>0.169</td>
</tr>
<tr>
<td>AI</td>
<td>0.26</td>
<td>0.29</td>
<td>0.31</td>
<td>0.34</td>
<td>0.01</td>
<td>&lt;0.001</td>
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<tr>
<td>TI</td>
<td>0.60</td>
<td>0.61</td>
<td>0.62</td>
<td>0.64</td>
<td>0.01</td>
<td>0.139</td>
<td>0.875</td>
</tr>
</tbody>
</table>

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.
### Table 6

Heavy metals in HI larvae meal.

<table>
<thead>
<tr>
<th></th>
<th>HI meal</th>
<th>MRL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(Directive 2002/32/EC)</td>
</tr>
<tr>
<td>As (mg/Kg 12% h)</td>
<td>&lt; 0.05</td>
<td>2</td>
</tr>
<tr>
<td>Cd (mg/Kg 12% h)</td>
<td>0.32</td>
<td>2</td>
</tr>
<tr>
<td>Pb (mg/Kg 12% h)</td>
<td>0.07</td>
<td>10</td>
</tr>
<tr>
<td>Hg (mg/Kg 12% h)</td>
<td>&lt; 0.02</td>
<td>0.1</td>
</tr>
<tr>
<td>Cr (mg/Kg)</td>
<td>0.23</td>
<td>Not legislated</td>
</tr>
<tr>
<td>Fe (mg/Kg)</td>
<td>189</td>
<td>Not legislated</td>
</tr>
<tr>
<td>Ni (mg/Kg)</td>
<td>0.18</td>
<td>Not legislated</td>
</tr>
<tr>
<td>Cu (mg/Kg)</td>
<td>10</td>
<td>Not legislated</td>
</tr>
<tr>
<td>Zn (mg/Kg)</td>
<td>157</td>
<td>Not legislated</td>
</tr>
<tr>
<td>Co (mg/Kg)</td>
<td>&lt; 0.05</td>
<td>Not legislated</td>
</tr>
<tr>
<td>Se (mg/Kg)</td>
<td>&lt; 0.02</td>
<td>Not legislated</td>
</tr>
</tbody>
</table>

HI = *Hermetia illucens*; MRL = Maximum Residue Limit; mg/kg 12% h = mg/kg 12% humidity.
Table 7

Heavy metals in breast meat of broiler chickens (one pool per feeding group, 8 birds/pool).

<table>
<thead>
<tr>
<th>Dietary treatments</th>
<th>MRL (Regulation 881/2006/EC)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HI0</td>
</tr>
<tr>
<td>As (mg/Kg 12% h)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Cd (mg/Kg 12% h)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Pb (mg/Kg 12% h)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Hg (mg/Kg 12% h)</td>
<td>&lt; 0.02</td>
</tr>
<tr>
<td>Cr (mg/Kg)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Fe (mg/Kg)</td>
<td>9.1</td>
</tr>
<tr>
<td>Ni (mg/Kg)</td>
<td>0.08</td>
</tr>
<tr>
<td>Cu (mg/Kg)</td>
<td>0.64</td>
</tr>
<tr>
<td>Zn (mg/Kg)</td>
<td>13</td>
</tr>
<tr>
<td>Co (mg/Kg)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Se (mg/Kg)</td>
<td>0.28</td>
</tr>
</tbody>
</table>

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

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

A. Schiavone, S. Dabbou, M. Petracchi, M. Zampiga, F. Sirri, I. Biasato, F. Gai, L. Gasco

Supplementary material S1

Material and methods

Meat quality parameters

pH
The ultimate pH (pH_u) measurement, at 48 h post mortem, was established using the dispersed phase method developed by Jeacocke (1977). Approximately 2.5 g of finely minced meat was homogenized for 30 seconds, by means of an Ultraturrax device, in 25 ml of sodium iodoacetate (5 mM) and 18 ml of potassium chloride (150 mM), previously equilibrated at pH 7 with the aid of a potassium hydroxide solution (0.1 N) and of hydrochloric acid (0.1 N). The pH of the homogenate sample, which was previously calibrated using buffer solutions at pH 4 and 7, was determined using a pH meter (Jenway 3519; Electrode 924001). The values of 2 replicates were considered, and their mean was subsequently utilized.

Color
The meat color was measured at room temperature (20°C) on the inner surface of the Pectoralis major muscle using a portable colorimeter Chroma Meter CR-400 Konica Minolta Sensing device (Minolta Sensing Inc., Osaka, Japan). The color measurements were reported in terms of lightness (L*), redness (a*) and yellowness (b*) in the CIELAB color space model (Commission Internationale de l’Éclairage, 1976). The values were recorded for CIE standard illuminant D65 and the CIE 2° standard observer. The color values were obtained considering the average of three readings per sample.

Drip loss
A sample weighing about 80 g (approximately 7 × 4 × 3 cm), obtained from the cranial portion of each fillet, was individually weighed and suspended in a plastic box at 2 to 4°C. After 48 h, the samples were blotted to remove the excess surface fluids, reweighed and the drip loss was determined as the percentage of weight lost by the sample during the refrigerated storage period (Petracci and Baéza, 2011).

Cooking loss
After determination of the drip loss, the meat samples were weighed individually, packaged in a plastic bag under vacuum, and cooked by immersion in a water bath (80°C) until their final internal temperature reached 80°C, according to the recommendations of Petracchi and Baéza, (2011). The cooked samples were cooled under running water for 30 min. The samples were then removed from the bags, blotted and weighed. The cooking losses were determined by calculating the difference in weight of the samples before and after cooking and they were expressed as percentages of the initial weight.

Allo Kramer shear force
Shear values were determined using a TAHDi Heavy Duty texture analyzer (Stable Micro Systems Ltd., Godalming, Surrey, the UK), equipped with an Allo-Kramer shear cell, using the procedure described by Sams et al. (1990). One meat sample (approximately 2 × 4 × 1 cm) from each sample used for the cook-loss determination was cut, parallel to the muscle-fiber direction, weighed, and sheared with the blades at a right angle to the fibers using a 250-kg load cell and a cross head speed of 500 mm/min. The shear values were reported as kilograms of shear per gram of sample.

**Fatty acid profile**

Fatty acids (FA) were determined as previously reported by Glass and Cristopherson (1969). In short, 250 µg of lipids and 500 µl of the methylating solution (KOH/methanol 2 N) were put into a vial containing 5 ml of hexane and 1 g of anhydrous sodium sulphate. The vial was mixed for 30 s and placed in a water bath at 40 °C for 15 min. The sample was then stirred and cooled on ice. The upper phase, containing fatty acid methyl esters (FAME), was collected and used for the separation of the FA using a Shimadzu GC17A gas chromatograph (Shimadzu Corporation, Tokyo, Japan) with a WP-4 Shimadzu integration system, equipped with a Varian CPSIL88 capillary column (100 m long, 0.25 mm i.d., 0.20 mm thick film) (Varian, Walnut Creek, CA, USA) and a flame ionization detector. The operating conditions of the gas chromatograph were as follows: the oven temperature was kept at 170°C for 15 min, increased to 190°C at a rate of 1 °C/min, then increased to 220°C at a rate of 5°C/min, and kept at this temperature for 17 min. The temperature of the injector and detector were 270°C and 300°C, respectively. Helium was used as the carrier gas at a constant flow rate of 1.7 mL/min. The identification of the individual FAs was carried out using PUFA-2 fatty acid methyl ester standards (Matreya, Pleasant Gap, PA, the USA), and FA quantification was obtained using methyl nonadecanoate 98% (C19:0) (Sigma, Saint Louis, the USA) as the internal standard, which was added prior to lipid extraction. The results were expressed as a percentage of each individual FAME per total FAME detected.

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

\[
AI = \frac{(C12:0 + 4 \times C14:0 + C16:0)}{[(\Sigma MUFA + \Sigma n-6) + \Sigma n-3]}
\]

\[
TI = \frac{(C14:0 + C16:0 + C18:0)}{[(0.5 \times \Sigma MUFA + 0.5 \times \Sigma n-6 + 3 \times \Sigma n-3) + (\Sigma n-3) / \Sigma n-6]}
\]

where MUFA are monounsaturated fatty acids.

**Heavy metals and arsenic**

The chromium, iron, cobalt, nickel, copper, zinc, selenium, arsenic, lead, cadmium and mercury concentrations in the HI larva meal and breast meat were determined by means of the accredited test MI 351 Rev. 1/2015 (UNI EN 13804:2002, UNI EN 15763:2010 and UNI EN 13805:2014). Aliquots of the samples (0.5 g) were weighed in allotted digestion vessels and a mixture of deionized water, nitric acid and hydrogen peroxide was added. The vessels were capped and the contents digested under high temperature and pressure using a single reaction chamber microwave digester system. The resulting solutions were transferred to pre-marked, acid-cleaned, plastic test tubes and diluted with deionized water. The metal content was determined by means of the ICP-MS technique (inductively coupled plasma spectrometry). Multi-element measurements were made using an Agilent 7700x ICP-MS (Agilent Technologies) with a collision cell.

**References**


