Yellow mealworm (*Tenebrio molitor* L.) larvae inclusion in diets for free-range chickens: effects on meat quality and fatty acid profile

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Abstract

This study evaluated the effects of a diet containing yellow mealworm (*Tenebrio molitor* L.; TM) larvae meal on quality parameters (pH_{24}, colour and drip losses), proximate composition and fatty acid (FA) profile of meat from free-range chickens.

A total of 140 medium-growing hybrid female chickens were free-range reared and randomly allotted to two dietary treatments: a control group and a TM group, in which TM meal was included at 75 g/kg as fed in substitution of corn gluten meal. Each group consisted of five pens as replicates, with 14 chicks per pen. At 97 days of age, ten birds (two birds/pen) from each feeding group were slaughtered at a commercial abattoir. Quality parameters and proximate composition of breast and thigh meat were not affected by treatment. The effects of dietary TM larvae meal on the FA profile of thigh meat were negligible. Breast meat from TM-fed chickens showed higher oleic and α-linolenic acids percentages as well as lower atherogenicity and thrombogenicity indexes.

In conclusion, this study demonstrated that TM inclusion in diets for free-range chickens did not prejudice meat quality traits. The obtained results confirm that TM can be considered a promising insect protein source for the poultry feed industry.

Key words: *Tenebrio molitor* larvae meal, free-range chickens, breast, thigh, fatty acids.

Introduction

In the poultry sector, chicken rearing in alternative systems, such as free-range or organic, is a profitable alternative, in a market with a great number of consumers willing to pay higher prices for the obtainable food products. Furthermore, the free-range system has a positive effect on meat quality traits (Castellini et al., 2002; Fanatico et al., 2005, 2007). Castellini et al. (2002) reported that the chicken meat quality enhancement in free-range birds is due to the higher total n-3 PUFA content in free-range birds, when compared to standard breeding. Regarding diet availability for free-range chickens, the birds roam freely through meadows and mimic their original foraging dietary habits, eating not only grass but also earthworms from the soil (Fanatico, 2006; Sossidou et al., 2011). The search for alternative feeds which can make the production of free-range birds viable is a way to adequately and economically replace the traditionally used feedstuffs. The use of insects as an alternative and attractive natural protein source in animal feeding is becoming globally more appealing, especially for its high sustainability (van Huis and Oonincx, 2017). Chickens with access to outdoor areas pick up insects at all life stages and eat them voluntarily, which indicates that they are evolutionarily adapted to eat insects as a natural part of their diet (Biasato et al., 2016). Current research has highlighted that insect-based protein meals could represent a valid alternative to conventional protein sources (fish or plant protein meals) or as a complementary feed source for poultry (Biasato et al., 2017, 2018; Schiavone et al., 2017a).

The use of insect meals in poultry feeds is not currently allowed in the European Community. Given, however, the potential ecological advantages and a good acceptance among producers consumers (Verbeke et al., 2015), it seems likely that the political legal frameworks may change in the near future, making the utilization of insect protein possible. This would imply a valuable potential also for organic systems (Leiber et al., 2017). Among insect species, yellow mealworm (*Tenebrio molitor* L.; TM), belonging to the Tenebrionidae family, is currently considered one of the most promising insect species to be used as innovative protein source for fishmeal and soybean meal (SBM) substitution in fish (Belforti et al., 2015; Gasco et al., 2016; Iaconisi et al., 2017; Piccolo et al., 2017) and poultry (Bovera et al., 2015, 2016; Biasato et al., 2016, 2017, 2018; Schiavone et al., 2017a) feeds.

Gasco et al. (2018) mentioned that TM larvae and adults contain a high amount of crude protein (44.1–60.3% dry basis) even if it has recently been reported that insect protein content is slightly overestimated due to the use of a wrong nitrogen to protein conversion factor (Janssen et al., 2017; Nery et al., 2018). Using the appropriate conversion factor, Janssen et al. (2017) reported that TM larvae contain about 45% of crude protein.

Bovera et al. (2015) compared the amino acids (AA) profile of TM larvae with SBM and reported that the two protein sources had a different composition in essential AAs, and this was particularly manifest for methionine and cystein. The authors concluded that only methionine and lysine contents limit the use of TM in poultry feeds.

In addition, TM contains fat (16.6–43.1% dry basis), minerals and vitamins (Gasco et al., 2018). It has been recently demonstrated that insect fat can successfully substitute conventional lipid sources in poultry diets, without affecting growth performance and gut histology (Schiavone et al., 2017b, 2018). Owing to the reasons mentioned above, the potential of insect protein and lipid in poultry diets has attracted much attention. In addition, in the only available study on free-range chickens, TM provided satisfactory results in terms of growth performance and gut morphology (Biasato et al., 2016). However, there is still lack of published data on the effects of dietary dried mealworm on meat quality of free-range chickens. Therefore, the aim of this study was to evaluate the effects of the inclusion of a full-fat TM larvae meal in a diet for free-range chickens on their meat quality and fatty acid (FA) profile.

Material and methods

Ethical approval

The study was performed by the Department of Veterinary Science (DVS) and the Department of Agricultural, Forest and Food Sciences of the University of Turin (Italy) in collaboration with a private farm called ‘Fattoria La Fornace’, located in Montechiaro d’Asti (Asti – Italy). The experimental protocol was designed according to the guidelines of the current European and Italian laws on the protection of animals used for scientific purposes (Directive 2010/63/EU, put into force in Italy with D.L. 2014/26). Furthermore, the experimental protocol was approved by the Ethical Committee of the DVS (protocol n. 1/2016).

Experimental design and feeds preparation

A detailed description of the experimental design is reported in Biasato et al. (2016). Briefly, at the age of 43 days, 140 female Label Hubbard hybrid chickens (female: JA 57 × male: S77CN; average initial live weight: 716.26±22.54 g), a medium-growing genotype, were randomly allotted to two groups (each consisting of five pens as replicates, with 14 birds per pen). Each pen had an indoor area (2.5 m × 3.5 m) and an outdoor paddock of the same dimension. The indoor floor was covered, to a height of 10 cm, with wood shaving litter. The birds were exposed to natural light only. A full-fat TM meal purchased from Gaobeidian Shannong Biology Co. Ltd (Hebei, China) was used. Two diets were formulated: a control diet, widely used in commercial farms, and an experimental diet with 75 g/kg of TM meal in substitution of corn gluten meal (Table 1). The diets were designed to meet or exceed National Research Council (1994) requirements and were formulated to be isonitrogenous and isoenergetic using the apparent metabolizable energy values for TM calculated for broiler chickens (De Marco et al., 2015).. Chickens had *ad libitum* free access to water and feed throughout the whole trial. As reported in Biasato et al. (2016), the average daily intake did not differ between groups (112.8 and 111.6 g for control and TM group, respectively). All the birds were individually identified with a shank ring.

Chemical composition and fatty acid profile of experimental diets

The diets were ground to pass through a 0.5-mm sieve. Samples were analysed for dry matter (DM, #934.01), and crude protein (CP, #984.13) according to AOAC International (2000);

ether extract (EE, #2003.05) and crude fiber (CF, #962.09) were determined following the procedures of AOAC International (2003) and AOAC International (2005), respectively. All chemical analyses were performed in duplicate.

A combined direct *trans*-esterification and solid-phase extraction (Alves et al., 2008) was used for the determination of the FA profile of the diets. Separation, identification and quantification of fatty acid methyl esters (FAME) were performed as reported by Renna et al. (2014). The results are expressed as g/100 g DM. The proximate and FA compositions of experimental diets are reported in Table 1.

Slaughtering procedures and muscle sampling

At 97 days of age, ten birds (two birds/pen) from each feeding group (chosen on the basis of pen average final live weight) were individually identified and weighed. The chickens were electrically stunned and then slaughtered at a commercial abattoir. The plucked and eviscerated carcasses were obtained, and the head, neck, feet and abdominal fat were removed to obtain the chilled carcass. The weight of the breasts, thighs, deboned thighs and abdominal fat were immediately recorded. The breast and thigh weights were expressed as percentage of live weight (LW). A total of ten breasts and ten thighs were collected in their right and left side, individually vacuum-sealed and refrigerated (4±1°C). Meat quality parameters (pH_{24}, color, and drip losses) were assessed on the *Pectoralis major* muscle on the right breast and on the *Biceps femoris* muscle on the right thigh, while the left breast and thigh meat were frozen at -20°C until further chemical analysis (proximate composition and FA profile).

Meat quality parameters

pH_{24}

The pH at 24 h postmortem was measured in duplicate using a Crison portable pH-meter (Crison Instruments, S.A., Alella, Spain) fitted with a spear-type electrode and an automatic temperature compensation probe.

Color

Meat color was measured at 24 h postmortem using a portable colorimeter Chroma Meter CR-400 Minolta (Minolta Sensing Inc., Osaka, Japan) with a 8 mm diameter measuring area, D65 illuminant and 2° standard observer. The results were expressed in terms of lightness (L*), redness (a*) and yellowness (b*) in the CIELAB color space (Commission Internationale de l'Éclairage, 1976). Chroma (C*) and Hue (H*) indexes were calculated using the following equations: $C^* = (a^{*2} + b^{*2})^{*0.5}$; $H^* = \tan^{-1} (b^*/a^*)$; $H^* = 180 + \tan^{-1} (b^*/a^*)$, when $a^*<0$. Chroma refers to the vividness or dullness of a color. Hue is the name of the color and is that quality by which we distinguish color families (red, green, blue, etc.). The color values were obtained considering the average of three readings per sample.

Drip losses

Twenty-four hours after slaughtering, breast and thigh were weighed and placed within a container on a supporting mesh and sealed. The samples were blotted for the excess surface fluids and reweighed. Drip losses were determined as percentage of weight lost by the samples during refrigerated storage period (Honikel, 1998).

Proximate composition and fatty acid profile

Breast and thigh samples were cut, homogenized and divided into two parts. A portion was used to determine moisture (#950.46) and ash (#920.153) contents according to AOAC International (2000) procedures. The remaining part was freeze-dried and afterwards analysed for protein and ether extract contents, and FA composition. The total N content was determined according to the Dumas method, using a macro-N Nitrogen analyzer (Foss Heraeus Analysensysteme, Hanau, Germany). The content of crude protein was calculated by multiplying the measured nitrogen quantity by the appropriate nitrogen-to-protein conversion factor (6.25). The ether extract content was determined by Soxhlet extraction with petroleum ether according to method #991.36 of AOAC International (2000). Proximate composition results were expressed as g/100g of fresh matter (FM).

The FA composition was assessed as detailed in Renna et al. (2019). Peaks were identified by injecting pure FAME standards as detailed by Renna et al. (2012). Quantification was assessed using tridecanoic acid (C13:0) as internal standard. The results were expressed as g/100 g of total detected FA.

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

$$AI = (C12{:}0 + 4 \times C14{:}0 + C16{:}0) / (\Sigma\ MUFA + \Sigma\ n\text{-}6 + \Sigma\ n\text{-}3)$$

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

where MUFA are monounsaturated FA.

Statistical analysis

The statistical analysis was performed using IBM SPSS Statistics v.21.0 for Windows (IBM SPSS Statistics, Armonk, NY, USA). The effect of the diet on the carcass characteristics, as well as on quality parameters, proximate composition and FA profile of meat were analysed using Student's *t*-tests for independent samples. The assumption of normality and homogeneity of variance were assessed using Shapiro-Wilk and Levene's tests, respectively. Results are reported as means and standard error of the mean (SEM). Significance was declared at $P<0.05$. A statistical trend was considered for $0.05 < P \leq 0.10$.

Results and discussion

The present study provides new insights into the use of TM larvae meal in the diet of medium-growing chickens reared in free-range conditions. All the experimental groups were kept on the same farm and were reared with the same free-range production system, allowing the birds to have access to outdoor paddocks. No mortality was recorded throughout the trial.

Carcass characteristics

The effect of TM larvae meal on carcass traits is reported in Table 2. Dietary TM inclusion did not affect the carcass characteristics of the birds. These results confirm the possibility of using insect meals in the diets of medium-growing hybrid chickens as an interchangeable ingredient compared to the conventional ingredients used in chicken nutrition. This promising result reinforces the potential of this innovative feed ingredient for poultry. To the best of our knowledge, no studies are currently available in the literature on the use of TM larvae meals in free-range chicken nutrition. For this reason, all the comparisons with literature data were done with chickens or other farmed birds. The results of this study are in agreement with those reported by Bovera et al. (2016) and Biasato et al. (2018) who did not find an influence of TM meal on slaughtering performance of broiler chickens. The results obtained in this trial do not always agree with those reported in other studies performed using TM. Indeed, Ballitoc and Sun (2013), using increasing levels of TM larvae meal (0.5, 1, 2 and 10% as fed), found improved slaughter yield, dressed carcass and eviscerated weights in broiler chickens fed TM diets with a 2% inclusion level. Biasato et al. (2017) evaluated the effects of a partial replacement of SBM, corn gluten meal and soybean oil with TM larvae meal on carcass characteristics of female broiler chickens, finding an increase of the carcass weight, abdominal fat weight and abdominal fat percentage with increasing levels of TM meal utilization. Hussain et al. (2017), including different levels of TM meal (1, 2 and 3 g/kg of diet) in a broiler diet, showed an improvement of carcass yield in all mealworm-supplemented broiler groups compared to a control group. Loponte et al. (2017), in a trial with barbary partridge (*Alectoris barbara*), formulated five diets substituting 25 and 50% of the SBM protein with TM larvae meal and a defatted *Hermetia illucens* (HI) larvae meal, respectively. The authors found improvements of carcass weights. In contrast, Cullere et al. (2016) in broiler quails and Schiavone et al. (2017b and 2018) in chickens fed diets with HI meal and fat, respectively, did not find significant effects on carcass traits.

Meat quality parameters of breast and thigh muscles

The pH_{24}, color and drip losses values of breast and thigh muscles of free-range chickens are reported in Table 3. All these meat quality traits were not affected by treatment. For both groups, breast pH_{24} fell in the range of standard poultry meat (5.77 and 5.73 for the control and TM group, respectively), as for values lower than 5.7 and higher than 6.2, breast broiler can be classified as PSE (pale, soft, and exudative) or DFD (dark, firm, and dry), respectively (Fletcher et al., 2000). Our results are in contrast with those reported by Bovera et al. (2016), who observed a higher pH value of breast muscle of broiler chickens fed TM compared to a control group. On the contrary, Cullere et al. (2016) in broiler quails fed diets with increasing levels of *Hermetia illucens* (HI) larvae meal reported a decrease in breast muscle pH and demonstrated that the differences could be ascribable to different muscle glycogen content. The observed differences could be related to species, genotype and rearing system (Mir et al., 2017).

Meat color is a very important quality parameter since it is directly perceived by the consumer. In our trial, the use of TM did not influence color parameters (*P*>0.05). To the best of our knowledge, no published data are currently available comparing muscle color of free-range chickens fed TM meals and other diets. In broiler chickens, Bovera et al. (2016) did not find a significant effect on raw and cooked color meat, as well as on skin, and they showed that meat from broilers fed TM meal could be easily accepted by consumers. A significant decrease for L* was reported by Pieterse et al. (2014) in breast muscle of broilers fed diets containing *Musca domestica* larvae meals compared to a fish meal based diet. Using HI larvae meal in broiler quails, Cullere et al. (2016) observed that redness index (a*) in the cranial and caudal part of the *Pectoralis major* muscle of broiler quails was significantly affected by the treatment and showed its highest (1.13) and lowest (0.46) values for HI groups, corresponding to 10% and 15% HI inclusion levels, comparing to a control group (0.81).

Proximate composition of breast and thigh meat

The proximate composition (water, ash, crude protein and ether extract) of breast and thigh meat was not affected by the dietary treatment (Table 4). The absence of differences in the proximate composition of meat from the two groups of chickens is an important finding for the positive evaluation of this new ingredient as a novel alternative feed in poultry nutrition. Our results are in agreement with those reported by Bovera et al. (2016). These authors did not find a significant effect in the proximate composition of meat obtained from the breast of broilers fed diets containing TM larvae meal during the growing period. The same results were found by Cullere et al. (2018) in broiler quails and Schiavone et al. (2017b) in chickens fed diets with HI meal and fat, respectively. Ballitoc and Sun (2013), including different levels of TM meal (0.5, 1, 2 and 10%) in a standard commercial broiler diet, reported that the inclusion level of 1% of TM showed the highest and lowest percentages of moisture in the thigh and breast portion, respectively, as compared to the other groups. These authors reported that, compared to a control group, the group fed 1% TM meal had a higher percentage of protein in the breast portion of the meat. The higher TM inclusion level (10%) in the trial performed by Ballitoc and Sun (2013) showed the highest percentage of fat for thigh and breast (6.30% and 1.25%, respectively); such values are comparable to those obtained in our experimental trial (5.64% and 0.50% of FM).

Fatty acid profile of breast and thigh meat

The FA composition of the full-fat TM larvae meal used in this trial was very similar to that recently reported for dietary TM oil by Kierończyk et al. (2018).

Table 5 shows the differences observed in terms of FA composition of breast and thigh meats between the control and TM groups. As expected, the predominant FA in the breast and thigh meat of the free-range chickens fed both the control and TM diets was C18:1 *c*9 (breast: 32.00 and 34.67 g/100g total FA, respectively; thigh: 38.21 and 39.41 g/100g total FA) followed by C16:0 (breast: 32.67 and 30.40 g/100g total FA; thigh: 26.67 and 26.44 g/100g total FA) and C18:2 n-6 (breast: 14.99 and 14.93 g/100g total FA; thigh: 15.89 and 15.43 g/100g total FA). The TM group showed significantly higher C18:1 *c*9 and C18:3 n-3 (P<0.05) percentages, a tendency (P<0.10) towards higher Σ MUFA rates, and contemporarily lower C16:0 and Σ

SFA rates in breast meat. Regarding thigh meat, only negligible differences were observed, with significantly higher rates of C14:0 and C20:0, in the TM group. Oleic acid is the predominant FA in TM larvae (Paul et al., 2017) and increased C18:1 *c*9 and Σ MUFA deposition in the breast muscle of chickens fed a diet containing TM oil when compared to a diet containing soybean oil was recently reported by Kierończyk et al. (2018). These authors also showed a significant reduction in Σ SFA in breast muscle when using dietary TM oil. Feeding broilers with a diet containing full fat TM meal resulted in an increased C12:0 and C14:0 percentages in breast meat (Loponte et al., 2018).

The Σ PUFA/Σ SFA ratio did not differ between groups and ranged in breast and thigh meat between 0.40 and 0.51. Turley and Thompson (2015) reported that, in human diets, this ratio should be maintained close to 1 and more generally in the range 0.34 to 2.99 to avoid promotion of tumor formation and atherogenicity.

Indexes (AI, TI) correlating the different amounts of some specific SFA, MUFA and PUFA of both the n-3 and n-6 series were proposed to indicate the contribution of these FA to the prevention or promotion of pathological phenomena in humans (Lands, 2014). Our results showed that the TM group had significantly lower AI and TI in the breast meat when compared to the control group. It has to be pointed out that, in both groups, the AI and TI values were low, and could be considered healthy for human consumers (Lazzaroni et al., 2009). Loponte et al. (2018) did not observe any difference between breast meat of broilers fed with TM larvae meal and those fed with SBM in term of quality indexes (PUFA n6/n3 ratio, AI and TI).

The FA profile of poultry meat usually mirrors that of the administered diet (Schiavone et al., 2007; 2010 and 2017b). In this study, the chickens were reared according to a free-range system, having access to outdoor paddocks. The observed little discrepancies in terms of meat FAs responses to dietary variations of FAs could be attributed to green grass and wild invertebrates consumption of the free-ranged chickens when accessing outdoor areas (Dal Bosco et al., 2014).

Conclusion

This study provided new data and knowledge on the potential use of a sustainable feedstuff for the nutrition of free-range chickens. The substitution of 75 g/kg of corn gluten meal with TM larvae meal in the diet did not affect the quality (pH_{24}, color and drip losses) of *Pectoralis major* and *Biceps femoris* muscles, and the proximate composition of breast and thigh meat. Regarding the FA profile of meat, only negligible differences in the thighs between the control and TM-fed chickens were observed. However, the dietary inclusion of TM increased the deposition of C18:1 *c*9 and reduced the atherogenicity and throbogenity indexes of breast meat. In conclusion, this study could help producers and farmers to make informed decisions on the use of TM meal in free range chicken diets.

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Conflict of interest

We declare that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

References

Alves SP, Cabrita ARJ, Fonseca AJM and Bessa RJB (2008). Improved method for fatty acid analysis in herbage based on direct transesterification followed by solid-phase extraction. *Journal of Chromatography A* **1209**, 212–219.

AOAC International (2000). Official methods of analysis (17th eds). Gaithersburg (MD, USA): Association of Official Analytical Chemists.

AOAC International (2003). Official methods of analysis (17th eds, 2nd revision). Gaithersburg (MD, USA): Association of Official Analytical Chemists.

AOAC International (2005). Official Methods of Analysis (18th eds). Washington (DC, USA): Association of Official Analytical Chemists.

Ballitoc DA and Sun S (2013). Ground yellow mealworms (*Tenebrio molitor* L.) feed supplementation improves growth performance and carcass yield characteristics in broilers. *Open Science Repository Agriculture* e23050425. http://doi.org/ 10.7392/openaccess.23050425.

Belforti M, Gai F, Lussiana C, Renna M, Malfatto V, Rotolo L, De Marco M, Dabbou S, Schiavone A, Zoccarato I and Gasco L (2015). *Tenebrio molitor* meal in rainbow trout (*Oncorhynchus mykiss*) diets: effects on animal performance, nutrient digestibility and chemical composition of fillets. *Italian Journal of Animal Science* **14**, 670–676.

Biasato I, De Marco M, Rotolo L, Renna M, Dabbou S, Capucchio MT, Biasibetti E, Tarantola M, Costa P, Gai F, Pozzo L, Dezzutto D, Bergagna S, Gasco L and Schiavone A

(2016). Effects of dietary Tenebrio molitor meal inclusion in free-range chickens. *Journal of Animal Physiology and Animal Nutrition* **100**, 1104–1112.

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

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

Bovera F, Piccolo G, Gasco L, Marono S, Loponte R, Vassalotti G, Mastellone V, Lombardi P, Attia YA and Nizza A (2015). Yellow mealworm larvae (*Tenebrio molitor*, L.) as a possible alternative to soybean meal in broiler diets. *British Poultry Science **56***, 569–575.

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

Castellini C, Mugnai C and Dal Bosco A (2002). Effect of organic production system on broiler carcass and meat quality. *Meat Science* **60**, 219–225.

Commission Internationale de l'Éclairage (1976). Recommendations on uniform colour spaces-colour difference equations, psychometric colour terms (Supplement no. 2 to CIE publication No. 15). Paris, France: Commission Internationale de l'Éclairage.

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

Cullere M, Tasoniero G, Giaccone V, Acuti G, Marangon A and Dalle Zotte A (2018). Black soldier fly as dietary protein source for broiler quails: meat proximate composition, fatty acid and amino acid profile, oxidative status and sensory traits. *Animal* **12(3)**, 640–647.

Dal Bosco A, Mugnai C, Rosati A, Paoletti A, Caporali S and Castellini C (2014). Effect of range enrichment on performance, behavior and forage intake of free-range chickens. *Journal of Applied Poultry Research* **23**, 137–145.

De Marco M, Martínez S, Hernandez F, Madrid J, Gai F, Rotolo L, Belforti M, Bergero D, Katz H, Dabbou S, Kovitvadhi A, Zoccarato I, Gasco L and Schiavone A (2015). Nutritional value of two insect meals (*Tenebrio molitor* and *Hermetia illucens*) for broiler chickens: apparent nutrient digestibility, apparent ileal amino acid digestibility and apparent metabolizable energy. *Animal Feed Science and Technology* **209**, 211–218.

Fanatico AC, Cavitt LC, Pillai PB, Emmert JL and Owens CM (2005). Evaluation of slow-growing broiler genotypes grown with and without outdoor access: Meat quality. *Poultry Science* **84**, 1785–1790.

Fanatico AC (2006). Alternative poultry production systems and outdoor access. NCAT Agriculture Specialist: ATTRA Publications (24p). <www.attra.ncat.org/attra-pub/poultry_access.pdf>.

Fanatico AC, Pillai PB, Emmert JL and Owens CM (2007). Meat quality of slow- and fast-growing chicken genotypes fed low-nutrient or standard diets and raised indoors or with outdoor access. *Poultry Science* **86**, 2245–2255.

Fletcher DL, Qiao M and Smith MP (2000). The relationship of raw broiler breast meat color and pH to cooked meat color and pH. *Poultry Science* **79**, 784–788.

Gasco L, Henry M, Piccolo G, Marono S, Gai F, Renna M, Lussiana C, Antonopoulou E, Mola P and Chatzifotis S (2016). *Tenebrio molitor* meal in diets for European sea bass (*Dicentrarchus labrax* L.) juveniles: growth performance, whole body composition and *in vivo* apparent digestibility. *Animal Feed Science and Technology* **220**, 34–45.

Gasco L, Gai F, Maricchiolo G, Genovese L, Ragonese S, Bottari T and Caruso G (2018). Fishmeal alternative protein sources for aquaculture feeds. In Gasco L, Gai F, Maricchiolo G, Genovese L, Ragonese S, Bottari T and Caruso G (eds), Feeds for the Aquaculture Sector – Current situation and alternative sources. *SpringerBriefs in Molecular Science*. Springer, Cham, pp. 1–20. https://link.springer.com/chapter/10.1007/978-3-319-77941-6_1

Honikel KO (1998). Reference methods for the assessment of physical characteristics of meat. *Meat Science* **49** 447–457.

Hussain I, Khan S, Sultan A, Chand N, Khan R, AlamW and Ahmad N (2017). Meal worm (*Tenebrio molitor*) as potential alternative source of protein supplementation in broiler. *International Journal of Bioscience* **10**, 255–262.

Iaconisi V, Marono S, Parisi G, Gasco L, Genovese L, Maricchiolo G, Bovera F and Piccolo G (2017). Dietary inclusion of *Tenebrio molitor* larvae meal: Effects on growth performance and final quality treats of blackspot sea bream (*Pagellus bogaraveo*). *Aquaculture* **476**, 49–58.

Janssen RH, Vincken JP, van den Broek L AM, Fogliano V, Lakemond CMM (2017). Nitrogen-to-Protein Conversion Factors for Three Edible Insects: *Tenebrio molitor*, Alphitobius diaperinus, and Hermetia illucens. *Journal of Agricultural and Food Chemistry* **65**, 2275−2278.

Kierończyk B, Rawski M, Józefiak A, Mazurkiewicz J, Świątkiewicz S, Siwek M, Bednarczyk M, Szumacher-Strabel M, Cieślak A, Benzertiha A and Józefiak D (2018).

Effects of replacing soybean oil with selected insect fats on broilers. *Animal Feed Science and Technology* **240**, 170–183.

Lands B (2014). Historical perspectives on the impact of n-3 and n-6 nutrients on health. *Progress in Lipid Research* **55**, 17–29.

Lazzaroni C, Biagini D and Lussiana C (2009). Fatty acid composition of meat and perirenal fat in rabbits from two different rearing systems. *Meat Science* **83**, 135–139.

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

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

Loponte R, Bovera F, Piccolo G, Gasco L, Secci G, Iaconisi V and Parisi G (2018). Fatty acid profile of lipids and caeca volatile fatty acid production of broilers fed a full fat meal from *Tenebrio molitor* larvae. *Italian Journal of Animal Science*. http://doi.org/10.1080/1828051X.2018.1502053.

Mir NA, Rafiq A, Kumar F, Singh V, Shukla V (2017). Determinants of broiler chicken meat quality and factors affecting them: a review. *Journal of Food Science and Technology* **54**, 2997-3009.

National Research Council (1994). Nutrient Requirements of Poultry. 9th revised eds. National Academy Press. Washington, DC, USA.

Nery J, Gasco L, Dabbou S, Schiavone A (2018). Protein composition and digestibility of black soldier fly larvae in broiler chickens revisited according to the recent nitrogen-protein conversion ratio. *Journal of Insects as Food and Feed* **4(3)**, 171–177.

Paul A, Frederich M, Caparros Megido R, Alabi T, Malik P, Uyttenbroeck R, Francis F, Blecker C, Haubruge E, Lognay G and Danthine S (2017). Insect fatty acids: A comparison of lipids from three Orthopterans and *Tenebrio molitor* L. larvae. *Journal of Asia-Pacific Entomology* **20**, 337–340.

Piccolo G, Iaconisi V, Marono S, Gasco L, Loponte R, Nizza S, Bovera F and Parisi G (2017). Effect of *Tenebrio molitor* larvae meal on growth performance, *in vivo* nutrients digestibility, somatic and marketable indexes of gilthead sea bream (*Sparus aurata*). *Animal Feed Science and Technology* **226**, 12–20.

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

Renna M, Cornale P, Lussiana C, Malfatto V, Fortina R, Mimosi A and Battaglini LM (2012). Use of *Pisum sativum* (L.) as alternative protein resource in diets for dairy sheep: Effects on milk yield, gross composition and fatty acid profile. *Small Ruminant Research* **102**, 142–150.

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

Renna M, Brugiapaglia A, Zanardi E, Destefanis G, Prandini A, Moschini M, Sigolo S and Lussiana C (2019). Fatty acid profile, meat quality and flavour acceptability of beef from double-muscled Piemontese young bulls fed ground flaxseed. *Italian Journal of Animal Science*, https://doi.org/10.1080/1828051X.2018.1530958.

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

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

Schiavone A, De Marco M, Martínez S, Dabbou S, Renna M, Madrid J, Hernandez F, Rotolo L, Costa P, Gai F and Gasco L (2017a). Nutritional value of a partially defatted and a highly defatted black soldier fly larvae (*Hermetia illucens* L.) meal for broiler chickens: apparent nutrient digestibility, apparent metabolizable energy and apparent ileal amino acid digestibility. *Journal of Animal Science and Biotechnology* **8**, 897–905.

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

Schiavone A, Dabbou S, De Marco M, Cullere M, Biasato I, Biasibetti E, Capucchio MT, Bergagna S, Dezzutto D, Meneguz M, Gai F, Dalle Zotte A and Gasco L (2018). Black soldier fly (*Hermetia illucens* L.) larva fat inclusion in finisher broiler chicken diet as an alternative fat source. *Animal* **12(10)**, 2032–2039.

Sossidou EN, Dal Bosco A, Elson HA and Fontes CMGA (2011). Pasture-based systems for poultry production: Implications and perspectives. *World's Poultry Science Journal* **67**, 47–57.

Turley J and Thompson J (2015). Nutrition: Your life Science. (2nd eds). Cengage learning, Boston. USA. pp. 97–168.

Ulbricht TL and Southgate DAT (1991). Coronary heart disease: seven dietary factors. *Lancet* **338**, 985–992.

van Huis A and Oonincx DGAB (2017). The environmental sustainability of insects as food and feed. A review. *Agronomy for Sustainable Development* **37(43)**. http://doi.org/10.1007/s13593-017-0452-8.

Verbeke W, Spranghers T, De Clercq P, De Smet S, Sas B and Eeckhout M (2015). Insects in animal feed: Acceptance and its determinants among farmers, agriculture sector stakeholders and citizens. *Animal Feed Science and Technology* **204**, 72–87.

Table 1.

Ingredients (g/kg as fed), chemical composition (g/kg DM, unless otherwise stated) and fatty acid profile (g/100g DM) of the experimental diets.

	Control diet	**TM diet**
Ingredients		
Corn meal	720.0	720.0
Soybean meal	170.0	170.0
Corn gluten meal	75.0	-
Tenebrio molitor meal	-	75.0
Vitamin-mineral premix*	35.0	35.0
Metabolizable energy (MJ/kg DM)	12.18	12.22
Chemical composition		
Dry matter	868	867
Crude protein	169	168
Ether extract	31	50
Crude fiber	23	22
Fatty acid composition		
C14:0	0.06	0.81
C16:0	16.56	15.76
C16:1 *c*9	0.19	0.61
C18:0	2.47	2.68
C18:1 *c*9 + C18:1 *c*11	22.25	26.15
C18:2 n-6	55.16	51.52
C18:3 n-3	2.74	1.84
C20:0	0.28	0.26
Other FA	0.29	0.38
ΣSFA	19.38	19.57
ΣMUFA	22.70	27.05
ΣPUFA	57.91	53.38
TFA	100.00	100.00

Abbreviations: *c*, *cis*; DM, dry matter; nd, not detected; Other FA = (C12:0 + C14:1 *c*9 + C18:3 n-6 + C20:1 *c*9 + C20:1 *c*11) - all < 45 g/100g DM in the diets; SFA, saturated fatty acids = (C12:0 + C14:0 + C16:0 + C18:0 + C20:0); MUFA, monounsaturated fatty acids = (C14:1 *c*9 + C16:1 *c*9 + C18:1 *c*9 + C18:1 *c*11 + C20:1 *c*9 + C20:1 *c*11); PUFA, polyunsaturated fatty acids = (C18:2 n-6+ C18:3 n-3+ C18:3 n-6); TFA, total fatty acids. All values are reported as mean of duplicate analyses.

*The vitam-mineral premix (Trevit Volatili 3.5 - Trei - Rio Saliceto (RE) Italy) given values are supplied per kg diet: 22750 IU of vitamin A; 2275 IU of vitamin D3; 22.75 IU of vitamin E; 2.80 mg of vitamin K; 2.80 mg of vitamin B1; 5.25 mg of vitamin B2; 26.95 mg of vitamin B3; 2.80 mg of vitamin B6; 0.02 mg of vitamin B12; 8.40 mg of pantothenic acid; 164.50 mg of betaine; 61.25 mg of Iron (II) carbonate; 64.22 mg of Magnesium oxide; 56.42 mg of Zinc oxide; 6.23 mg of Copper (II) oxide; 0.64 mg of Potassium iodide; 0.23 mg of Sodium selenite; 143.50 mg of DL-methionine; 192.50 mg of L-lysine; 4.20 g Calcium carbonate; 15.75 g Calcium phosphate; 0.40 g of Sodium chloride.

Table 2.

Effect of *Tenebrio molitor* (TM) larvae meal on the carcass traits of the free-range chickens.

	Control diet	**TM diet**	**SEM**	***P*-value**
Live weight at slaughter (d 97)* (g)	2220.80	2328.20	65.183	0.117
Chilled carcass weight* (g)	1459.30	1544.80	49.860	0.104
Breasts* (g)	347.09	370.82	20.890	0.271
Thighs* (g)	479.17	502.88	20.800	0.270
Thigh muscles* (g)	349.27	353.75	16.284	0.787
Thigh bone* (g)	83.80	89.28	3.920	0.180
Abdominal fat* (g)	40.62	45.14	13.696	0.745
Chilled carcass (% of live weight)	65.66	66.37	0.919	0.450
Breast (% of live weight)	15.60	15.92	0.720	0.662
Thigh (% of live weight)	21.55	19.45	2.216	0.350

Abbreviations: SEM, standard error of the mean.

*Source Biasato et al., (2016).

Table 3.

Effect of *Tenebrio molitor* (TM) larvae meal on the quality parameters of *Pectoralis major* (breast) and *Biceps femoris* (thigh) muscles of the free-range chickens.

	Control diet	**TM diet**	**SEM**	***P*-value**
Breast				
pH_{24}	5.77	5.73	0.046	0.353
Lightness (L*)	52.14	53.03	1.045	0.405
Redness (a*)	-0.88	-0.64	0.359	0.510
Yellowness (b*)	10.42	9.75	1.424	0.645
Chroma (C*)	10.48	9.82	1.423	0.647
Hue (H*)	94.95	93.64	2.267	0.569
Drip losses	7.40	7.99	0.817	0.478
Thigh				
pH_{24}	6.28	6.39	0.088	0.241
Lightness (L*)	54.31	54.53	1.747	0.898
Redness (a*)	0.98	0.80	0.482	0.710
Yellowness (b*)	7.03	6.37	1.457	0.660
Chroma (C*)	7.23	6.59	1.406	0.653
Hue (H*)	79.19	80.54	5.788	0.819
Drip losses	7.05	7.01	0.576	0.950

Abbreviations: SEM, standard error of the mean.

Table 4.

Effect of *Tenebrio molitor* (TM) larvae meal on the proximate composition (g/100g fresh matter) of breast and thigh meat of the free-range chickens.

	Control diet	**TM diet**	**SEM**	***P*-value**
Breast				
Water	74.09	74.00	0.231	0.714
Ash	1.26	1.22	0.034	0.256
Crude protein	24.49	24.36	0.259	0.619
Ether extract	0.44	0.53	0.060	0.137
Thigh				
Water	73.52	73.17	0.724	0.631
Ash	1.08	1.07	0.017	0.487
Crude protein	21.23	21.07	0.814	0.716
Ether extract	5.34	5.64	0.825	0.776

Abbreviations: SEM, standard error of the mean.

Table 5.

Effect of *Tenebrio molitor* (TM) larvae meal on the fatty acid composition (g/100 g of total FA) of breast and thigh meat of the free-range chickens.

	Breast				***Thigh***			
	Control diet	**TM diet**	**SEM**	***P*-value**	**Control diet**	**TM diet**	**SEM**	***P*-value**
C12:0	0.08	0.07	0.012	0.340	0.09	0.10	0.009	0.274
C14:0	0.75	0.84	0.286	0.073	0.67	0.85	0.045	0.001
C16:0	32.67	30.40	0.819	0.013	26.67	26.44	0.772	0.769
C16:1 *c*9	5.14	4.61	0.548	0.359	6.44	5.79	0.533	0.241
C18:0	8.72	8.56	0.469	0.731	7.28	7.39	0.455	0.824
C18:1 *c*9	32.00	34.67	0.976	0.013	38.21	39.41	1.028	0.259
C18:1 *c*11	3.17	2.96	0.134	0.130	2.68	2.59	0.113	0.418
C18:2 n-6	14.99	14.93	0.934	0.634	15.89	15.43	0.825	0.579
C18:3 n-3	0.19	0.28	0.026	0.004	0.39	0.40	0.027	0.583
C18:3 n-6	nd	nd	-	-	0.06	0.07	0.008	0.553
C20:0	0.44	0.38	0.072	0.029	0.03	0.04	0.004	0.013
C20:1 *c*11	nd	nd	-	-	0.27	0.29	0.025	0.387
C20:2 n-6	nd	nd	-	-	0.11	0.11	0.012	0.619
C20:3 n-6	nd	nd	-	-	0.13	0.13	0.012	0.935
C20:4 n-6	1.84	2.30	0.252	0.087	0.94	0.88	0.109	0.585
ΣSFA	42.68	40.24	0.879	0.013	34.88	34.94	0.930	0.946
ΣMUFA	40.31	42.25	1.112	0.099	47.60	48.07	1.136	0.681
ΣPUFA	17.01	17.51	0.731	0.506	17.52	17.02	0.858	0.565
ΣPUFA/SFA	0.40	0.43	0.020	0.102	0.51	0.49	0.031	0.568
Σn-3	0.19	0.28	0.026	0.004	0.39	0.40	0.027	0.583
Σn-6	16.82	17.23	0.717	0.577	17.13	16.62	0.843	0.548
AI	0.63	0.57	0.027	0.046	0.45	0.46	0.020	0.658
TI	1.45	1.30	0.052	0.010	1.03	1.03	0.043	1.000
TFA (mg/100g FM)	417.33	510.75	67.172	0.181	4014.94	4277.67	734.850	0.725

Abbreviations: nd, not detected; SEM, standard error of the mean; SFA, saturated fatty acids = (C12:0 + C14:0 + C16:0 + C18:0 + C20:0); MUFA, monounsaturated fatty acids = (C16:1 *c*9 + C18:1 *c*9 + C18:1 *c*11 + C20:1 *c*11); PUFA, polyunsaturated fatty acids = (C18:2 n-6 + C18:3 n-3 + C18:3 n-6 + C20:2 n-6 + C20:3 n-6 + C20:4 n-6); AI, atherogenicity index; TI, thrombogenicity index; TFA, total fatty acids; FM, fresh matter.