Wageningen Academic Publishers

Hermetia illucens larvae reared on different substrates in broiler quail diets: effect on apparent digestibility, feed-choice and growth performance

M.J. Woods[1,2], M. Cullere[2], L. Van Emmenes[1,2], S. Vincenzi[3], E. Pieterse[1], L.C. Hoffman[1,4] and A. Dalle Zotte[2*]

[1]Department of Animal Sciences, Matieland 7602, Stellenbosch University, South Africa; [2]Department of Animal Medicine, Production and Health, University of Padova, Agripolis, Viale dell'Università 16, 35020 Legnaro, Padova, Italy; [3]Department of Agronomy, Food, Natural resources, Animals and Environment, University of Padova, Agripolis, Viale dell'Università 16, 35020 Legnaro, Padova, Italy; [4]Centre for Nutrition and Food Sciences, Queensland Alliance for Agriculture and Food Innovation (QAAFI), University of Queensland, Health and Food Sciences Precinct, 39 Kessels Rd, Coopers Plains 4108, Australia; antonella.dallezotte@unipd.it

Received: 7 June 2018 / Accepted: 17 December 2018

RESEARCH ARTICLE

Abstract

This research is aimed at improving the fatty acid profile of *Hermetia illucens* larvae and evaluating the effect of its inclusion on the apparent nutrient digestibility, feed choice, growth performance and slaughter traits of growing broiler quails (*Coturnix coturnix japonica*). *H. illucens* larvae (IM) were reared on two different substrates: layer mash (IM1) and 50:50 layer mash:fish offal (IM2). For the digestibility and feed choice trials, a total of sixty 16-day-old quails were assigned to three dietary groups: commercial diet (Control=C), a diet including 10% IM1 (IM1D), and a diet including 10% IM2 (IM2D). For the growth performance trial, a total of three hundred 10-day-old birds were allocated to the three dietary groups and fed the experimental diets until slaughter. Results of the digestibility trial showed a higher apparent metabolizable energy for larvae fed quail (14.0 and 13.9 MJ/kg DM vs 12.9 MJ/kg DM, (P<0.001). The IM2D quails also showed higher apparent digestibility for dry matter and organic matter. Feed choice results indicated that quails preferred the C diet compared to diets including *H. illucens* dried larvae. Productive performance, mortality and carcass traits were in line with commercial standards except for the IM2 quails which exhibited lower slaughter weight compared to C and IM1 fed quails. Based on the results of the present study, a 10% dietary inclusion of *H. illucens* larvae reared on a substrate rich in n-3 fatty acids did not negatively affect the apparent digestibility of nutrients, mortality, nor carcass yield. However, feed choice, growth rate and final carcass weight were negatively influenced by the IM2 diet. This result requires further investigations which should include the addition of an anti-oxidant.

Keywords: black soldier fly, substrate modulation, insect protein, larvae meal

1. Introduction

Soybean is one of the main feed ingredients for livestock and, for the poultry sector, it is used both as a protein and a fat source. However, soy requires substantial land and water to grow and therefore has a strong environmental impact (Charlton *et al.*, 2015). Furthermore, the soy currently used in European poultry farming is mainly imported from the United States, Argentina and Brazil (Boerema *et al.*, 2016) and, soy also being a food source for humans, the feed-food competition is predicted to increase soon; hence prices for this commodity will increase. This is caused by the growing world population and the consequent perspective of a growing global agricultural output to satisfy the increasing demand for food and livestock products (Van Huis, 2013). For all the above-mentioned reasons, the poultry sector is currently looking towards alternative feed ingredients.

In the context of an impellent search for new, sustainable and valuable feed ingredients for livestock and poultry, insects represent a great opportunity. Among insect species, the black soldier fly (*Hermetia illucens*) is considered one

of the most promising to be used for industrial production. *H. illucens* larvae meal showed to be an excellent source of apparent metabolizable energy and digestible amino acids for broilers (De Marco *et al.*, 2015; Schiavone *et al.*, 2017a). In fact, when different inclusion levels of defatted *H. illucens* larvae meals replaced fish meal in the diet for laying quails (Widjastuti *et al.*, 2014) and substituted soybean oil/meal in the diet for broiler quails (Cullere *et al.*, 2016, 2017) or Barbary partridge (Loponte *et al.*, 2017), no negative effects on the studied variables were observed.

Recent trials were also conducted to evaluate the potential of the fat extracted from *H. illucens* larvae to replace vegetable oils. Specifically, encouraging results were observed in broiler chickens fed with *H. illucens* prepupae fat in substitution to the soybean oil (Schiavone *et al.*, 2017b,c).

However, a drawback related to the use of *H. illucens* larvae or the derived products (meal and oil) in poultry diets was highlighted in all these studies when the fatty acid profile of the poultry meat was analysed. In fact, the fatty acid profile of *H. illucens* larvae is peculiar, being notably rich in saturated fatty acids and with a high n-6/n-3 ratio. Consequently, poultry species being monogastric animals, the fatty acid profile of meat derived when fed with diets containing *H. illucens* is sub-optimal in providing healthy meat for the modern consumer (Cullere *et al.*, 2017; Schiavone *et al.*, 2017c).

Overall, the lipid content of insects is largely dependent on their diet and stage of development. Moreover, the chemical composition of *H. illucens* larvae can theoretically be manipulated through diet as they are considered monogastric animals. Consequently, an appropriate growth substrate as well as the time of harvest could hypothetically improve their nutrients composition, which was partly demonstrated by some recent studies (Spranghers *et al.*, 2017; Tschirner and Simon, 2015). In addition, in the only two trials aiming to improve the fatty acids profile of *H. illucens* larvae by modulating the rearing substrate, it was shown that the larvae could successfully incorporate EPA and DHA n-3 fatty acids (Barroso *et al.*, 2017; St-Hilaire *et al.*, 2007). However, no data deriving from *in vivo* studies on poultry species fed with larvae enriched in n-3 fatty acids have been conducted until now.

Starting from these premises, the present research studied the effect of a dietary inclusion of *H. illucens* dried larvae reared either on a conventional substrate or on a substrate enriched in n-3 polyunsaturated fatty acids on broiler quails' apparent nutrient digestibility, feed choice, growth performance, slaughter traits, carcass and meat yields.

2. Material and methods

Insect farming

Black soldier fly (*H. illucens*; IM) larvae were harvested at Agriprotein (Pty) Ltd (Cape Town, South Africa). The larvae were reared on two different substrates: a commercial chicken layer mash (IM1), and a substrate made of 50% chicken layer mash:50% fish offal (IM2). Fish offal was provided by I&J commercial fishing company (Cape Town, South Africa) and analysed for heavy metals content on an ICP-MS as described by Bosch *et al.* (2016). After 18 days feeding, larvae were harvested, separated from the remaining residue by using a saturated salt solution in which the boxes were emptied, and collected (further details can be found in the Supplementary materials file). Subsequently, larvae were thoroughly washed with water to remove any residual salt and then dried in a commercial oven set at 65 °C for 16 hours. Dried larvae were then finely ground, vacuum-sealed and couriered to the Department of Animal Medicine, Production and Health (MAPS) of the University of Padova (Italy). The chemical composition, gross energy content, amino acids profile and main fatty acid classes of the larvae reared on IM1 and IM2 substrates are shown in Table 1.

Performance trial

The production trial was conducted at a private quail farm ('La Colombara' Società Agricola, Castelnovo di Isola Vicentina, Italy): for the experiment, a total of three hundred 10-day-old broiler quails (*Coturnix coturnix japonica*) of both sexes were weighed, marked and housed in batteries in an environmentally controlled room. The chicks (20 per cage) were allocated to 15 cages and received three dietary treatments (five replicates per treatment) until being slaughtered, at 29 days of age: a Control diet (C) which was formulated according to the common grower diet used on the farm, IM1 and IM2 diets in which conventional protein/fat sources were partly substituted with 10% IM reared on the two different substrates. All diets were formulated to meet the minimum requirements for Japanese quails (National Research Council, 1994). Ingredients, chemical composition and energy content of the diets are shown in Table 2 and 3, respectively. Mashed feeds and water were provided *ad libitum*. Mortality was monitored daily. Individual live weight (LW) was measured at the end of the experimental period whereas feed intake was measured within replicate throughout the trial. Body weight gain and feed conversion ratio were then calculated.

Digestibility trial

A total of sixty 16-day-old broiler quails were randomly selected from a large commercial flock and destined to an *in vivo* digestibility trial. These quails were different to

Table 1. Chemical composition, gross energy content, amino acid concentration (g/kg as fed) and main fatty acid classes (% of total fatty acids) of the two *Hermetia illucens* dried larvae (IM1, IM2) reared on different substrates.[1]

	IM1[1]	IM2[2]
Dry matter	934.9	940.1
Crude protein	336.6	343.1
Crude fat	342.0	366.9
Crude fibre	55.4	51.3
Chitin	24.8	24.2
Ash	135.2	99.2
Ca	43.4	29.1
P	5.91	5.75
Gross energy (MJ/kg)	22.63	23.36
Indispensable amino acids:		
Arginine	19.1	37.9
Histidine	13.4	21.2
Isoleucine	13.1	15.3
Leucine	20.4	21.8
Lysine	18.8	22.4
Methionine	5.00	6.30
Phenylalanine	13.9	21.4
Threonine	13.3	16.5
Valine	17.3	21.2
Dispensable amino acids:		
Alanine	20.6	15.3
Aspartic acid	30.3	6.80
Glycine	45.3	26.7
Glutamic acid	13.4	21.2
Proline	17.6	32.0
Serine	13.3	18.1
Tyrosine	16.0	17.6
Fatty acid classes:		
Saturated fatty acids	68.9	72.0
Monounsaturated fatty acids	17.7	18.7
Polyunsaturated fatty acids	12.6	6.99
n-6	12.1	5.42
n-3	0.53	1.57
n-6/n-3	22.9	3.45

[1] Heavy metals content of fish offal (mg/kg): Al = 39.34; Cr = 1.02; Mn = 169.6; Fe = 378.85; Cu = 10.31; Zn = 106.77; As = 4.89: Cd = 0.17; Hg = 0.14; Pb = 0.22.

[2] IM1 = dried and ground *H. illucens* larvae reared on layer mash.

[3] IM2 = dried and ground *H. illucens* larvae reared on 50:50 layer mash and fish offal.

Table 2. Ingredients of the experimental diets (g/kg as fed).[1]

Ingredients	Experimental diets[1]		
	Control	IM1D	IM2D
Ground corn	435.6	450.5	434.8
Soybean meal	460.4	377.0	373.5
Dried *Hermetia illucens* larvae (IM)	0.0	100	100
Whole wheat	23.5	42.5	61.7
Calcium carbonate	21.5	20.0	20.0
NaCl	2.70	2.70	2.70
L-Lysine	0.50	0.50	0.50
DL-Methionine	1.80	1.80	1.80
Vitamin-mineral premix[2]	5.00	5.00	5.00
Soybean oil	49.0	0.0	0.0

[1] IM1D = diet supplemented with 10% of dried *H. illucens* larvae reared on layer mash; IM2D = diet supplemented with 10% of dried *H. illucens* larvae reared on 50:50 layer mash and fish offal.

[2] Vitamin and mineral premix provided the following per kg of diet: vitamin A, 20,000 IU; vitamin D3, 6,000 IU; vitamin E (α-tocopherol acetate), 90 mg; vitamin K3, 7 mg; vitamin B1, 3.5 mg; vitamin B2, 16 mg; niacinamide, 100 mg; vitamin B6, 8 mg; vitamin B12, 0.04 mg; biotin, 0.4 mg; folic acid, 2.5 mg; Ca-panthotenate, 27.78 mg; Fe, 80 mg; Mn, 200 mg; Cu, 50 mg; Zn, 200 mg; Ca-iodate, 2 mg/kg; Se, 0.4 mg; E 1,604 Endo 14, 2,200 U; Endo 1, 3,000 FTU; Sepiolite, 175 mg.

those used for the performance trial. Digestibility cages were provided by the MAPS Department of Padova University (Italy). Quails were individually weighed and divided into three experimental feeding groups (C, IM1D and IM2D) with five replication each (four quails/replication). Each replication had similar mean live weight and standard deviation (592±16.2 g) and was balanced for gender. Quails were then subjected to a 10-day adaptation period to the experimental diets during which individual feed intake was determined. At the end of adaptation period and after 12 h fasting, quails were weighed again and then fed their corresponding experimental diet for 3 days followed by another 12 h of fasting, so that the feed intake and excreta could be accurately determined. The excreta samples were collected daily from each cage, carefully cleaned of feathers and feed, weighed, then promptly chilled. The total excreta, separated by cage, were freeze-dried, ground and stored at +4 °C until further analysis.

Feed choice test

After the digestibility trial, a total of fifteen 31-day-old male quails of similar LW were selected from the same 60 birds involved in the digestibility trial, individually caged, and simultaneously provided with three feeders containing C, IM1D and IM2D diets. After 10 days of adaptation to the new feeding condition, a 10-day feed choice trial was carried out. Feed and water were provided *ad libitum*. Feeders were placed in complete randomized order and their position within the cage was changed every 3 days. At the end of the experiment, the feed consumed from each feeder was

Table 3. Chemical composition, mineral and gross energy contents of the of the experimental diets (g/kg as fed).

	Experimental diets[1]		
	Control	IM1D	IM2D
Dry matter	901	900	901
Crude protein	243	239	240
Crude fat	59.8	56.9	55.9
Chitin	0.0	3.93	3.32
Ash	63.2	71.8	65.3
Starch	282	314	313
Ca	12.1	17.4	14.1
P	3.80	3.78	3.55
Na	1.46	1.81	2.01
Cl	2.24	4.06	4.00
K	12.2	10.2	9.40
Mg	2.45	2.27	2.13
Gross energy (MJ/kg)	17.43	17.85	17.81

[1] IM1D = diet supplemented with 10% of dried *Hermetia illucens* larvae reared on layer mash; IM2D = diet supplemented with 10% of dried *H. illucens* larvae reared on 50:50 layer mash and fish offal.

measured on the cage basis. Free choice was expressed as grams of dry matter (DM) per 100 g of LW. Birds used for the digestibility trial and free choice test were returned to the farmer after completion of the experiments.

Chemical analysis of the diets and the excreta

Analyses of IM1 and IM2 dried larvae, experimental diets and freeze-dried excreta were carried out in duplicate using AOAC (2012) methods to determine DM (method 934.01), crude protein (CP, method 2001.11), crude fibre (method 978.10), ash (method 967.05) and starch (amyloglucosidase-alpha-amylase, method 996.11) contents. Crude fat was determined after acid hydrolysis (EC, 1998). The methods to analyse the mineral content of the IM1, IM2 larvae and of the experimental diets were: method 968.08 (Ca and Mg), method 995.11 (P), method 956.01 (Na), method 975.03 (K), and method 943.01 (Cl) of the AOAC (2012). Gross energy of IM1, IM2 larvae, experimental diets and freeze-dried excreta was analysed with an adiabatic bomb calorimeter (ISO, 1998). The amino acid content of IM1 and IM2 larvae was analysed with a method developed by Grace Davison Discovery Sciences using high performance liquid chromatography. Lipid extraction and the subsequent fatty acid profile analysis of IM1 and IM2 larvae were performed following the method described by Cullere *et al.* (2016) for the defatted *H. illucens* larvae meal.

Crude protein content of excreta was corrected for uric acid content which was analysed according the procedure described by Fievez *et al.* (2001) with the modification reported in Cullere *et al.* (2016). Urinary nitrogen was estimated at 1.2 times uric acid content (Terpstra and De Hart, 1973). Experimental diets and freeze-dried excreta were analysed in triplicate for chitin content following the method by Zhang and Zhu (2006) with the modifications described in the Supplementary Material.

Slaughtering, carcass dissection and meat yields

At 29 days of age, after feed removal, quails of the performance trial were individually weighed (slaughter weight, SW) and transported to a commercial slaughterhouse (Quaja Veneta® Società Cooperativa Agricola, Malo, VI, Italy) located 8 km from the farm. After 6 hours fasting (from feed withdrawal until slaughtering), all birds were electrically stunned and processed under commercial conditions. Carcasses were bled, plucked, eviscerated and the head, neck, shanks and abdominal fat were removed. After one hour in the refrigeration tunnel (+2 °C), all carcasses were transported in chilled conditions to the MAPS Department of the University of Padova and stored at +2 °C.

The following day, carcasses were individually weighed (carcass weight; CW) and dressing percentage was calculated as a percentage of the SW. Breast muscle was excised and the yield as a percentage of CW was then determined.

Statistical analysis of results

Growth performance, carcass and breast meat yields, nutrients' apparent digestibility and nutritive values of the diets were subjected to a one-way analysis of variance (ANOVA) with experimental diet (Control, IM1D and IM2D) as fixed effect, following the general linear model (GLM) procedure of the SAS 9.1. statistical analysis software for Windows (SAS Institute, 2008). The experimental unit was the cage. A chi-squared test with the Marascuilo (1966) procedure was performed on mortality to detect differences among treatments. For nutrients' apparent digestibility and the nutritive value of the diets, the model considered the experimental diet as main factor. An one-way ANOVA of the GLM procedures of SAS was then performed that studied the effect of the experimental diet on the individual feed consumption. Differences were considered significant when $P \leq 0.05$.

3. Results and discussion

The results of the chemical composition of the two dried *H. illucens* larvae reared on different substrates partly confirmed the previous findings (Sprangers *et al.*, 2016;

Tschirner and Simon, 2015) crude fat and ash contents of the larvae were modified by the growing substrate, whereas their CP content remained constant. The CP contents of the IM1 and IM2 larvae were in line with values found by Tschirner and Simon (2015) but slightly lower compared to the range provided by Makkar *et al.* (2014) and Spranghers *et al.* (2017).

The amino acid profile of the two dried *H. illucens* larvae reared on different growing substrates differed markedly, and also differed from that reported by Spranghers *et al.* (2017) considering chicken feed, digestate, vegetable waste and restaurant waste as growing substrates. In fact, the addition of fish offal improved the amino acid content of *H. illucens* larvae (Treatment IM2), as it led to higher amounts of all the indispensable amino acids, when compared to the IM1 larvae, reared on a substrate made of 100% layer mash. As for the amino acid profile of the dried IM1 larvae, it is similar to values reported by De Marco *et al.* (2015) for a full-fat *H. illucens* larvae meal, for both indispensable and dispensable amino acids.

The average chitin content of both IM1 and IM2 larvae was only 24.5 g/kg as fed, lower than values (56-67 g/kg as fed) commonly found in literature (Spranghers *et al.*, 2017), which is important for better absorption of nutrients from the diet. This result can probably be ascribable to the fact that larvae were collected before the prepupae instar, thus having less chitin in the exoskeleton.

The addition of fish offal to the normal growing substrate influenced the main fatty acid classes of the larvae. The saturated fatty acids (SFA) proportion increased (72.0 vs 68.9% SFA for IM2 and IM1 larvae, respectively), whereas total polyunsaturated fatty acids (PUFA) decreased (6.99 vs 12.6% PUFA for IM2 and IM1 larvae, respectively). However, such reduction involved only the n-6 PUFA whereas the n-3 PUFA fraction showed a three-fold increase (1.57 vs 0.53% n-3 PUFA for IM2 and IM1 larvae, respectively), thus resulting in a nutritionally valuable improvement of the n-6/n-3 ratio (3.45 vs 22.9 for IM2 and IM1 larvae, respectively). Thus, the main objective of the substrate modulation has been successful. Such findings were coherent with those presented by St-Hilaire *et al.* (2007) who found that adding different proportions of fish offal from rainbow trout to cow manure, substantially enriched the larval content of n-3 fatty acids. Despite this, the enrichment of n-3 PUFA was much lower in the present study compared to the previous cited (0.23 vs 2.99% n-3 PUFA for larvae fed on 100% cow manure substrate vs larvae fed on 50% cow manure + 50% fish offal, respectively). Such difference could be explained by the different fish offal used in the two studies, with that of the present research being an undefined mixture coming from different marine fish species.

A further key aspect that should be considered, when using fish offal as growing substrate for insects, is its heavy metals content. In fact, Directive 2002/32/EG (EC, 2002) allows a maximum amount in feedstuffs of 2 mg/kg feed for Cd and 10 mg/kg feed for Pb. The bioaccumulation of some heavy metals by *H. illucens* larvae was previously studied by Diener *et al.* (2015) and it was observed that Cd readily accumulates into the insect body through the Ca^{2+} channel, as Cd^{2+} ions have very similar ionic radii. Conversely, Zn seemed to be actively regulated and Pb tended to be transported and immobilised in the exoskeleton. In the present experiment, the concentration of heavy metals in fish offal was 0.17, 106.8 and 0.22 mg/kg for Cd, Zn and Pb, respectively. These concentrations are far below the lower levels tested in the above cited experiment on bioaccumulation, and therefore they can be considered acceptable and safe.

Productive performances of growing broiler quails were overall consistent with commercial standards but slightly different compared to those presented by Cullere *et al.* (2016). At the end of the trial, quails fed the C and IM1D diets showed higher slaughter weight and body weight gain ($P<0.0001$) compared to quails fed the IM2D diet. However, no differences ($P>0.05$) for feed intake, feed conversion ratio, and for overall mortality, were observed among the three experimental groups (Table 4). The lower performance of quails fed the IM2 diet compared to the other dietary treatments was not expected. In fact, the chemical composition of the two diets with dried *H. illucens* larvae were very close to each other in terms of CP, crude fat, starch and overall gross energy contents. Results from the feed choice trial highlighted that the C diet was the most preferred by quails, but no difference was observed between IM1D and IM2D ($P<0.001$). Therefore, it was hypothesized that the marked fish aroma of the IM2 made the IM2D less palatable compared to the other two diets, and results of the performance trial seemed to corroborate this hypothesis, as IM2D quails exhibited a tendency ($P=0.0529$) towards a reduced feed intake. It was also observed that IM1D and IM2D had a darker colour compared to C which could have negatively affected feed choice. Since the IM2 larvae showed a three-fold increase in n-3 PUFA it could have been more prone to oxidative damages and therefore off-odours. The avian taste system is generally well developed but it can have a great variability among different species because of various feeding patterns and strategies. As reviewed by Roura *et al.* (2013), such differences reflect on a different number of taste buds and the size of the bitter taste receptors gene repertoire. Variability could also be observed within the same bird species, as different chicken breeds can display different sensitivities to the bitter taste in function of the total number of taste buds. Chickens have been reported to perceive also sweet, salty, sour, fat taste and calcium, with avoidance, preference and lowest-highest limits depending on several factors i.e. the age of the bird, physiological

Table 4. Effect of the dietary inclusion of dried *Hermetia illucens* larvae reared on two different substrates on the live performance and mortality of broiler quails.[1,2]

	Experimental groups			*P*-value	RSD
	Control	IM1D	IM2D		
No. (initial)	100	100	100		
LW (g)					
Initial weight (10 days)	77.8	77.7	77.7	0.9785	4.07
Slaughter weight (29 days)	240.6[a]	247.6[a]	228.7[b]	<0.0001	22.8
BWG (g/day)	9.0[a]	9.4[a]	8.4[b]	<0.0001	1.21
FI (g/day)[3]	29.1	29.9	27.5	0.0529	1.39
FCR[3]	3.4	3.4	3.5	0.8434	0.28
Mortality (%)	1	1	3	0.6388	1.63

[1] Means in a row with different superscript letters differ significantly.

[2] BWG = body weight gain; FCR = feed conversion ratio; FI = feed intake; LW = live weight; RSD = residual standard deviation.

[3] Five replicates per treatment.

status, etc. The experimental diets of the present trial also differed in starch content and some minerals such as Na, K and Cl thus probably affecting the palatability of feed. However, such hypothesis found no confirmation in previous literature, as data on taste patterns of Japanese quail are scarce. Only a preference for sucrose was reported in a previous study considering this avian species (Harriman and Milner, 1969), thus suggesting that sugars may play a key role in the feeding strategy of quails.

At the end of the digestibility trial, quails showed the same final LW, DM and water intake, however experimental groups differed in terms of excreta production (Table 5). In fact, IM1D and IM2D quails produced less excreta than control birds ($P<0.05$). Moreover, IM2D quails showed a higher apparent digestibility of DM ($P<0.05$) and organic matter ($P<0.05$) compared to C, with IM1D being intermediate. Starch was better digested in IM1D compared to C ($P<0.01$) but did not differ from IM2D. On the other hand, IM2D quails showed a lower ether extract (EE) apparent digestibility than C ($P<0.05$) but did not differ from IM1D. Quails belonging to the IM1D and IM2D groups displayed the highest gross energy apparent digestibility ($P<0.05$) with the same trend being observed for the metabolizable energy ($P<0.001$). Analysing these results, a further hypothesis to explain the lower productive performance of IM2D quails compared to the other groups is suggested. The IM2D quails exhibited a tendency ($P=0.0556$) towards a lower apparent digestibility of chitin compared to IM1D which probably had an effect in determining a lower apparent digestibility of the EE. Chitin is known to have a negative effect on the digestibility of protein and lipid fractions, which is particularly evident with increasing absolute chitin content or when animals have no chitinolytic activity (Kroeckel *et al.*, 2012). In fact, even though it was not significant ($P=0.3068$), the apparent digestibility of CP was ~10.5% lower in IM2D quails compared to IM1D ones. Another factor that could have contributed to the low digestibility of EE in the IM2D could be related to the different fatty acids composition of the larvae which had higher SFA and lower PUFA compared to those in the IM1D. For instance, it is known that the assimilation of dietary fats by chickens and turkeys is higher for unsaturated than for saturated (SFA) fatty acids. This phenomenon is due to unsaturated fatty acids spontaneously forming mixed micelles with monoglycerides and conjugated bile salts which are then easily transported to the mucosal surface and finally absorbed by the small intestine (Tancharoenrat *et al.*, 2014). On the other hand, SFA are non-polar, therefore requiring appropriate amount of bile salts for an efficient emulsification. Because of this, their incorporation into micelles is slower compared to unsaturated fatty acids (Zhang *et al.*, 2011).

Independently to the above cited results, the dietary inclusion of *H. illucens* larvae meal in both IM1D and IM2D groups did not negatively affect the overall apparent digestibility of nutrients. In fact, even if DM and organic matter apparent digestibility showed lower values than those previously reported by Sahin *et al.* (2006), the digestibility of CP, EE and starch for all treatments was consistent with values found on broiler quails by Cullere *et al.* (2016).

Interestingly, quails from the IM1D group performed better than C and IM2D groups, exhibiting the highest CW ($P<0.0001$) and breast meat weight ($P<0.01$), with more favourable carcass and breast yields ($P<0.01$) (Table 6). Even though IM2D birds showed the lightest carcasses, their

Table 5. Effect of the dietary inclusion of dried *Hermetia illucens* larvae reared on two different substrates on the quail nutrients' apparent digestibility and nutritive value of diets and feed choice.[1]

	Experimental groups[2]				
	Control	IM1D	IM2D	*P*-value	RSD[3]
No.	20	20	20		
Initial live weight (LW) (g)[4]	600	596	579	0.0904	14.3
Final LW (g)	703	698	682	0.3066	21.3
Average LW (g)	651	647	631	0.1866	17.6
Dry matter (DM) intake (g)	314.5	309.6	314.6	0.8651	16.7
DM intake (g/100 g LW)	48.3	47.9	49.9	0.3018	2.10
Total water intake (g)	957	865	817	0.1071	97.1
Excreta (g DM)	141.2^{a}	127.7^{b}	127.0^{b}	0.0184	7.52
Apparent digestibility (%):					
Dry matter	55.0^{b}	58.7^{ab}	59.6^{a}	0.0374	2.63
Organic matter	58.1^{b}	62.5^{ab}	62.9^{a}	0.0193	2.50
Crude protein	45.9	46.8	41.9	0.3068	5.02
Ether extract	93.0^{a}	91.9^{ab}	87.2^{b}	0.0274	3.11
Starch	90.3^{b}	95.5^{a}	93.2^{ab}	0.0018	1.76
Gross energy	66.6^{b}	70.6^{a}	70.4^{a}	0.0104	1.94
Chitin	–	55.3	45.3	0.0556	7.11
Nutritive value:					
Metabolisable energy (MJ/kg DM)	12.9^{b}	14.0^{a}	13.9^{a}	0.0008	0.38
Metabolizable protein (g/kg feed)	123.6	124.2	111.6	0.2812	13.4
Feed choice trial:					
Feed intake (g DM/100 g LW)	48.9^{a}	23.2^{b}	27.9^{b}	<0.0001	8.87

[1] Means in a row with different superscript letters differ significantly.

[2] IM1D = diet supplemented with 10% of dried *Hermetia illucens* larvae reared on layer mash; IM2D = diet supplemented with 10% of dried *H. illucens* larvae reared on 50:50 layer mash and fish offal.

[3] RSD = residual standard deviation.

[4] Four quails/replicated cage.

carcass and breast yields were comparable to those of the C group. Overall, considering results about performance, digestibility and carcass and meat traits, quails fed the IM1D diet performed the best. Moreover, despite the lower growth rate and EE digestibility, IM2D showed the same carcass yield, breast meat quantity and yield as the Control group.

The overall outcomes from the present research which was conducted under intensive conditions and using dried *H. illucens* larvae, when compared to recently published results testing a defatted *H. illucens* larvae meal on broiler chickens (Schiavone *et al.*, 2017a), Barbary partridge (Loponte *et al.*, 2017), broiler quails (Cullere *et al.*, 2016), and laying quails (Widjastuti *et al.*, 2014), as well as a further research testing *H. illucens* prepupae fat in the diet for broiler chickens (Schiavone *et al.*, 2017b,c), showed that productive performance, apparent digestibility of nutrients and carcass traits were always satisfactory. Such findings confirm once more the potential of the tested insect species as an alternative ingredient for poultry feeding, which could help in alleviating the competition between poultry species and humans for soya in the food-feed debate. Furthermore, results of the present research indicated that an appropriate substrate modulation is a key tool to improve the chemical composition of the *H. illucens*, thus helping to meet the nutrients requirements of different poultry species. Further research is necessary to investigate the exact reason why quails fed *H. illucens* larvae reared on a substrate enriched with fish offal exhibited the lowest growth rate. This research should include the evaluation of the level of lipid oxidation in the feed, and if required, the addition of an anti-oxidant. Moreover, the complete chemical composition and sensory traits of the derived meat needs further research.

Table 6. Effect of the dietary inclusion of dried *Hermetia illucens* larvae reared on two different substrates on the carcass and breast meat traits of broiler quails.[1]

	Experimental groups[2]			*P*-value	RSD[3]
	Control	IM1D	IM2D		
No.	99	99	97		
Carcass weight (CW) (g)	150.9[b]	159.2[a]	145.1[c]	<0.0001	15.4
Carcass yield (% CW)	62.7[b]	64.2[a]	63.1[b]	0.0039	3.01
Breast meat (g)	45.9[b]	49.8[a]	43.9[b]	<0.0001	6.50
Breast meat yield (% CW)	30.3[b]	31.3[a]	30.2[b]	0.0044	2.53

[1] Means in a row with different superscript letters differ significantly.

[2] IM1D = diet supplemented with 10% of dried *Hermetia illucens* larvae reared on layer mash; IM2D = diet supplemented with 10% of dried *H. illucens* larvae reared on 50:50 layer mash and fish offal.

[3] RSD = residual standard deviation.

Acknowledgements

Authors would like to thank Agriprotein (Pty, Ltd) (Cape Town, South Africa), I&J fisheries (Cape Town, South Africa), Rino Cailotto ('La Colombara' Società Agricola, Castelnovo di Isola Vicentina, VI, Italy), Quaja Veneta® Società Cooperativa Agricola (Malo, VI, Italy), and the feed company FANIN Srl (San Tomio di Malo, VI, Italy) for their crucial support for a successful research project. Authors acknowledge also Dr Giulia Tasoniero for the help in conducting the experiment and Dr Erik Claeys (Ghent University, Belgium) for the uric acid analysis. The research was supported by the University of Padova (Italy) funds (ex 60%, 2016 – DOR1603318), by a Senior Researcher Scholarship of Padova University (Prot. n. 1098, 2015) and by the Erasmus+ / KA107 mobility funds. This research was also supported by the South African Research Chairs Initiative (SARChI) and partly funded by the South African Department of Science and Technology. The financial assistance of the NRF towards this research is hereby acknowledged.

Declaration of interests

Authors declare the absence of conflicts of interest.

Ethics statement

The production trial was conducted after the approval by the veterinary authority and according to Article 2, DL 4 March 2014, No. 26 of the Official Journal of the Italian Republic (http://www.gazzettaufficiale.it/eli/id/2014/03/14/14G00036/sg), implementing the EU Directive 2010/63/EU on the protection of animals used for scientific purposes.

Software and data repository resources

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

Supplementary material

Supplementary material can be found online at https://doi.org/10.3920/JIFF2018.0027.

References

Association of Official Analytical Communities (AOAC), 2012. Official methods of analysis, 19th edition. Association of Official Analytical Chemists, Arlington, VA, USA.

Barroso, F.G., Sánchez-Muros, M.J., Segura, M., Morote, E., Torres, A., Ramos, R. and Guil, J.L., 2017. Insects as food: enrichment of larvae of *Hermetia illucens* with omega 3 fatty acids by means of dietary modifications. Journal of Food Composition and Analysis 62: 8-13.

Boerema, A., Peeters, A., Swolfs, S., Vandevenne, F., Jacobs, S., Staes, J. and Meire, P., 2016. Soybean trade: balancing environmental and socio-economic impacts of an intercontinental market. PLoS ONE 11: e0155222. https://doi.org/10.1371/journal.pone.0155222

Bosch, A.C., O'Neill, B., Sigge, G.O., Kerwatch, S.E. and Hoffman, L.C., 2016. Heavy metal accumulation and toxicity in smoothhound (*Mustelus mustelus*) shark from Langebaan Lagoon. South African Food Chemistry 190: 871-878.

Charlton, A.J., Dickinson, M., Wakefield, M.E., Fitches, E., Kenis, M., Han, R., Zhu, F., Kone, N., Grant, M., Devic, E., Bruggeman, G., Prior, R. and Smith, R., 2015. Exploring the chemical safety of fly larvae as a source of protein for animal feed. Journal of Insects as Food and Feed 1: 7-16.

Cullere, M., Tasoniero, G., Giaccone, V., Acuti, G., Marangon, A. and Dalle Zotte, A., 2017. 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. https://doi.org/10.1017/S1751731117001860

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.

De Marco, M., Martínez, S., Hernandez, F., Madrid, J., Gai, F., Rotolo, L. and Kovitvadhi, A., 2015. Nutritional value of two insect larval 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.

Diener, S., Zurbrüg, C. and Tockner, K., 2015. Bioaccumulation of heavy metals in the black soldier fly, *Hermetia illucens* and effects on its life cycle. Journal of Insects as Food and Feed 1: 261-270.

European Commission (EC), 1998. Commission Directive 98/64/EC of 3 September 1998 establishing community methods of analysis for the determination of amino acids, crude oils and fats, and olaquindox in feeding stuffs and amending Directive 71/393/EEC. Official Journal of the European Union L257: 14.

European Commission (EC), 2002. Directive 2002/32/EC of the European Parliament and the Council on undesirable substances in animal feed. Official Journal of the European Union L140: 1-29.

Fievez, V., De Fauw, K., Notteboom, K. and Demeyer, D., 2001. Effect of level and origin of rumen degradable nitrogen on rumen microbial growth and nitrogen utilization efficiency of animals fed maize silage at maintenance. Reproduction Nutrition Development 41: 349-364.

Harriman, A.E. and Milner, J.S., 1969. Preference for sucrose solutions by Japanese quail (*Coturnix coturnix japonica*) in two-bottle drinking test. American Midland Naturalist 81(98): 575-578.

International Organisation for Standardisation (ISO), 1998. Animal feeding stuffs, animal products and faeces or urine. Determination of gross calorific value – bomb calorimetric method. ISO 9831. ISO, Geneva, Switzerland.

Kroeckel, S., Harjes, A.G., Roth, I., Katz, H., Wuertz, S., Susenbeth, A. and Schulz, C., 2012. When a turbot catches a fly: evaluation of a pre-pupae meal of the black soldier fly (*Hermetia illucens*) as fish meal substitute – growth performance and chitin degradation in juvenile turbot (*Psetta maxima*). Aquaculture 364: 345-352.

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

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

Marascuilo, L.A., 1966. Large-sample multiple comparisons. Psychological Bulletin 65: 280-290.

National Research Council (NRC), subcommittee on Poultry Nutrition, 1994. Nutrient requirements of ring-necked pheasants, Japanese quail, and bobwhite quail. Nutrient requirements of poultry, 9th revised edition. National Academy of Sciences, Washington, DC, USA, pp. 44-45.

Roura, E., Baldwin, M.W. and Klasing, K.C., 2013. The avian taste system: potential implications in poultry nutrition. Animal Feed Science and Technology 180: 1-9.

Sahin, K., Onderci, M., Sahin, N., Gursu, M.F., Khachik, F. and Kucuk, O., 2006. Effects of lycopene supplementation on antioxidant status, oxidative stress, performance and carcass characteristics in heat-stressed Japanese quail. Journal of Thermal Biology 31: 307-312.

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., 2017c. 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, I., Capucchio, M.T., Bergagna, S., Dezzutto, D., Meneguz, M., Gai, F., Dalle Zotte, A. and Gasco, L., 2017b. Black soldier fly (*Hermetia illucens* L.) larva fat inclusion in finisher broiler chicken diet as an alternative fat source. Animal 12: 2032-2039. https://doi.org/10.1017/S1751731117003743

Schiavone, A., De Marco, M., Martínez, S., Dabbou 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 larva (*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: 51.

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

Statistical Analysis Software for Windows (SAS), 2008. Statistics version 9.1.3 ed. SAS Institute, Cary, NC, USA.

St-Hilaire, S., Cranfill, K., McGuire, M.A., Mosley, E.E., Tomberlin, J.K., Newton, L. and Irving, S., 2007. Fish offal recycling by the black soldier fly produces a foodstuff high in omega-3 fatty acids. Journal of the World Aquaculture Society 38: 309-313.

Tancharoenrat, P., Ravindran, V., Zaefarian, F. and Ravindran, G., 2014. Digestion of fat and fatty acids along the gastrointestinal tract of broiler chickens. Poultry Science 93: 371-379.

Terpstra, K. and De Hart, N., 1973. The estimation of urinary nitrogen and faecal nitrogen in poultry excreta. Journal of Animal Physiology and Animal Nutrition 32: 306-320.

Tschirner, M. and Simon, A., 2015. Influence of different growing substrates and processing on the nutrient composition of black soldier fly larvae destined for animal feed. Journal of Insects as Food and Feed 1: 249-259.

Van Huis, A., 2013. Potential of insects as food and feed in assuring food security. Annual Review of Entomology 58: 563-583.

Widjastuti, T., Wiradimadja, R. and Rusmana, D., 2014. The effect of substitution of fish meal by black soldier fly (*Hermetia illucens*) maggot meal in the diet on production performance of quail (*Coturnix coturnix japonica*). Animal Science Journal 57: 125-129.

Zhang, B., Haitao, L., Zhao, D., Guo, Y. and Barri, A., 2011. Effect of fat type and lysophosphatidylcholine addition to broiler diets on performance, apparent digestibility of fatty acids, and apparent metabolizable energy content. Animal Feed Science and Technology 163: 177-184.

Zhang, J. and Zhu, K.Y., 2006. Characterization of a chitin synthase cDNA and its increased mRNA level associated with decreased chitin synthesis in *Anopheles quadrimaculatus* exposed to diflubenzuron. Insect Biochemistry and Molecular Biology 36: 712-725.