Animal (2018), **12:10**, pp 2032–2039 © The Animal Consortium 2018
doi:10.1017/S1751731117003743

animal

Black soldier fly larva fat inclusion in finisher broiler chicken diet as an alternative fat source

A. Schiavone[1,5], S. Dabbou[1], M. De Marco[1], M. Cullere[2], I. Biasato[1], E. Biasibetti[1], M. T. Capucchio[1], S. Bergagna[3], D. Dezzutto[3], M. Meneguz[4], F. Gai[5], A. Dalle Zotte[2†] and L. Gasco[4,5]

[1]Department of Veterinary Sciences, University of Turin, Largo Paolo Braccini 2, 10095 Grugliasco (TO), Italy; [2]Department of Animal Medicine, Production and Health, University of Padova, Agripolis, Viale dell'Università 16, 35020 Legnaro, Padova, Italy; [3]Veterinary Medical Research Institute for Piemonte, Liguria and Valle d'Aosta, Via Bologna 148, 10154 Turin, Italy; [4]Department of Agricultural, Forest and Food Sciences, University of Turin, Largo Paolo Braccini 2, 10095 Grugliasco (TO), Italy; [5]Institute of Science of Food Production, National Research Council, Largo Paolo Braccini 2, 10095 Grugliasco (TO), Italy

(Received 17 May 2017; Accepted 13 December 2017; First published online 18 January 2018)

*The objective of the present study was to evaluate the effects of partial or total replacement of finisher diet soybean oil with black soldier fly (*Hermethia illucens *L.; HI) larva fat on the growth performance, carcass traits, blood parameters, intestinal morphology and histological features of broiler chickens. At 21 days of age, a total of 120 male broiler chickens (Ross 308) were randomly allocated to three experimental groups (five replicates and eight birds/pen). To a basal control diet (C; 68.7 g/kg as fed of soybean oil), either 50% or 100% of the soybean oil was replaced with HI larva fat (HI50 and HI100 group, respectively). Growth performance was evaluated throughout the trial. At day 48, 15 birds (three birds/pen) per group were slaughtered at a commercial abattoir. Carcass yield and proportions of carcass elements were recorded. Blood samples were taken from each slaughtered chicken for haematochemical index determination. Morphometric analyses were performed on the duodenum, jejunum and ileum. Samples of liver, spleen, thymus, bursa of fabricius, kidney and heart were submitted to histological investigations. Growth performance, carcass traits, haematochemical parameters and gut morphometric indexes were not influenced by the dietary inclusion of HI larva fat. Histopathological alterations developed in the spleen, thymus, bursa of fabricius and liver and were identified in all of the experimental groups, but HI larva fat inclusion did not significantly affect (*$P > 0.05$*) the severity of the histopathological findings. The present study suggests that 50% or 100% replacement of soybean oil with HI larva fat in broiler chickens diets has no adverse effects on growth performance or blood parameters and had no beneficial effect on gut health.*

Keywords: *Hermetia illucens*, broiler chickens, dietary fat source, growth performance, gut morphometry

Implication

The black soldier fly (*Hermetia illucens* L.; HI) has been the subject of recent attention in poultry nutrition as an alternative ingredient. The lipid content of whole non-defatted black soldier fly larvae is notable (26% to 35%). The process of larva defatting results in two potential feed ingredients: fat and protein-concentrate meal. Recently, the fat derived from larvae of HI has been shown to be an acceptable lipid source for broiler chickens. This study demonstrated that fat from HI can totally and partly substitute for conventional lipid sources in finisher broiler diet without impairing performance, carcass characteristics, intestinal morphology or histological features.

† E-mail: antonella.dallezotte@unipd.it

Introduction

The majority of the lipid sources used in poultry diets consists mainly of rendered fat, coming from the part of the slaughtered animal which is not used for human consumption, and crude vegetable oils. Among vegetable oils, soybean oil is utilised as an ingredient in poultry diets due to its high energy value, digestibility and metabolizable energy content compared with other vegetable oils (Fascina *et al.*, 2009). However, currently, the limited supply of soybeans and their high price has caused increasing interest in the search for new alternative lipid sources for poultry feed.

Insects represent an opportunity for partial or total replacement of the conventional lipid feed sources. Furthermore, insects have a potential as a feed ingredient due to their appropriate nutritional quality (Veldkamp and Bosch, 2015)

and greater acceptance by poultry (Leiber *et al*., 2017). Indeed, insects (adult, larval and pupal form) are naturally consumed by wild birds and free-range poultry (Rumpold *et al*., 2016). In recent years, most of the attention on insects as a feed source has been focussed on their protein content (Belforti *et al*., 2015; Gasco *et al*., 2016; Renna *et al*., 2017). Certain insect species have a high fat content with higher levels in larval stages than in adults (Ramos-Elorduy, 1997; Barroso *et al*., 2014). In fact, lipids are also a main component of insects and are produced during protein isolation. Surendra *et al*. (2016) pointed out the importance of defatting insect meal and then using insect protein concentrate as an animal feed ingredient and the lipids for both animal nutrition and the production of biodiesel. Such authors showed that fat removal is necessary to improve the storability of the feed and to increase the protein digestibility of the insect-derived feed.

Black soldier fly (*Hermetia illucens* L.; HI) is a very promising species because of its amino acid profile, lipid content and calcium content (Cullere *et al*., 2016; Schiavone *et al*., 2017b). The larva of the black soldier fly contains up to 45% of lipids (Li *et al*., 2016). In the fatty acid (FA) profile of HI larvae, linoleic acid content is greater than α-linolenic acid content, similar to many plant oils (such as soybean oil and sunflower oil). The FA profile is also rich in medium-chain fatty acids (MCFAs) such as lauric acid (C12:0) and its esters, which represent 21.4% to 49.3% of the total profile (Tran *et al*., 2015; Li *et al*., 2016; Ushakova *et al*., 2016). The use of dietary fats rich in lauric acid and myristic acid (C14:0) in broilers could be advantageous in terms of gut health and growth performance. The underlying mechanism involves antimicrobial effects on gut bacteria and changes in gut morphology (Zeitz *et al*., 2015).

Chicken growth directly depends on the morphological and functional integrity of the digestive tract. Microscopic structure parameters such as villus height and crypt depth have been reported to be good indicators of intestinal development, health and functionality, influencing nutrient digestion and absorption (Wang and Peng, 2008). Despite the relationship between dietary modifications and gut morphology being widely investigated in poultry, limited data about the utilization of feed fats are currently available (Ozdogan *et al*., 2014; Zeitz *et al*., 2015).

Currently, knowledge about the suitability of HI larvae fat as a poultry feed ingredient is still scarce, and to the best of our knowledge, no studies have yet been carried out to determine the effects of soybean oil replacement by HI larva fat on gut morphology and histological features. For this reason, the objective of the present work was to evaluate the effects of finisher diet HI larva fat on the growth performance, carcass traits, blood parameters, intestinal morphology and histological features of broiler chickens.

Material and methods

Ethical approval

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 Sciences of the University of Turin (Italy).

Experimental design and feed preparation

The study was carried out at the experimental poultry farm of the Department of Agricultural, Forest and Food Sciences (DISAFA) of the University of Turin, located in Carmagnola (TO), Italy. The poultry house was 7 m wide $\times$ 50 m long $\times$ 7 m high, equipped with waterproof floor and walls, covered completely by tiles and with an automatic ventilation system. The impact of partial or total replacement of soybean oil with HI larva fat in broiler chickens' finisher diet (from 21 to 48 days of age) was evaluated by replacement in a basal control diet (C: 100% soybean oil; 68.7 g/kg as fed of soybean oil), either at 50% or 100% HI larva fat (HI50 and HI100 groups, respectively). All diets were formulated according to Aviagen (2014) broiler nutrition specifications (Table 1). Chickens had free access to water and feed throughout the trial.

Birds and husbandry

Day-old male broiler chickens (Ross 308) were farmed in a floor pen until 21 days of age and fed a commercial broiler starter diet (217 g/kg of CP; 12.9 MJ/kg metabolizable energy). At hatching, all the birds were vaccinated against Newcastle disease, Marek disease, infectious bronchitis and coccidiosis. At day 21, after equalizing for mean initial BW, 120 birds were chosen and homogeneously distributed over three dietary treatments, each one consisting of five pens as replicates with eight chicks per pen. The animals were reared to the slaughter age set at 48 days. Each pen was 1.0 m wide $\times$ 1.5 m long. Each pen was equipped with a feeder, an automatic drinker and rice hulls as litter. Up to 21 days of age infrared lamps were used to keep a suitable body temperature according to standard breeding practices (Aviagen, 2014). The lighting schedule was 18 h light : 6 h dark for the whole experimental period. Clinical signs of illness and mortality were monitored daily throughout the experimental period.

Chemical composition and fatty acid profile of the experimental diets

Chemical analyses were carried out on three replicates of each feed sample. Diet samples were ground through a 0.5-mm sieve and stored in airtight plastic containers. They were analysed for DM (#930.15), ash (#924.05), and CP (#984.13) according to the Association of Official Analytical Chemists (2005) methods. The gross energy content was determined using an adiabatic calorimetric bomb (IKA C7000; IKA, Staufen, Germany). The lipid extraction and FA profiling of the experimental diets were carried out at the laboratory of the Department of Animal Medicine, Production and Health, University of Padova, Legnaro, Italy. The lipid extraction was performed by Accelerated Solvent Extraction (M-ASE) using petroleum ether as a solvent. The FA profile was determined as described by Mattioli *et al*. (2016) and Schiavone *et al*. (2007). Samples were

transmethylated using a methanolic solution of H_2SO_4 (4%) in order to determine fatty acid methyl esters (FAME). A biphasic separation was obtained by adding 0.5 ml of distilled water and 1.5 ml of N-heptane to each sample. Fatty acid methyl esters were quantified by gas chromatography (Shimadzu GC17A), equipped with an Omegawax 250 column (30 m × 0.25 μm × 0.25 μm) and flame ionization detector. Helium was used as the carrier gas at a constant flow of 0.8 ml/min. The injector and detector temperatures were 260 °C. Peaks were identified based on commercially available FAME mixtures (37-Component FAME Mix; Supelco Inc., Bellefonte, PA, USA). The results are expressed as % of total detected FAME. The proximate and FA composition of the feeds are reported in Table 1.

Growth performance

During the whole experimental period (21 to 48 days), the BW and feed consumption (FC) of all birds were measured weekly to calculate daily feed intake, daily weight gain (DWG) and the feed conversion ratio (FCR). Final BW (FBW) was recorded on day 48. All measurements were carried out on the pen basis using a high precision electronic scale (Sartorius – Signum®).

Slaughtering procedures

At day 48, 15 birds (three birds/pen) from each feeding group (chosen on the basis of pen average FBW) were individually identified with a shank ring and weighed. The chickens were electrically stunned and 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. Then, the weights of the liver, heart, spleen, bursa of fabricius, abdominal fat, and breast and thighs were immediately recorded. The breast and thigh weights were expressed as percentage of live weight.

Haematological and serum parameters

At slaughtering (day 48), blood samples were collected from three birds per pen. A total of 2.5 ml was placed in an ethylenediaminetetraacetic acid tube and 2.5 ml in a serum-separating tube. Then, a blood smear was prepared, using one glass slide for each bird, from a drop of blood without anticoagulant. The smears were stained using May-Grünwald and Giemsa stains (Salamano *et al*., 2010). The total red (erythrocytes) and white (leucocytes) blood cell counts were determined in an improved Neubauer haemocytometer on blood samples previously treated with 1 : 200 Natt-Herrick solution. A total of 100 leukocytes, including granular (heterophils, eosinophils and basophils) and non-granular (lymphocytes and monocytes) leucocytes, were counted on the slide, and the H/L ratio was calculated. The tubes without anticoagulant were left to clot in a standing position at room temperature for ~2 h to obtain serum. The serum was separated by means of centrifugation at 700 × **g** for 15 min and frozen at −80°C until analysis. The electrophoretic pattern of the serum was obtained using a semi-automated agarose gel electrophoresis system (Sebia Hydrasys®, Norcross, GA, USA). The aspartate-aminotransferase (AST), alanine-aminotransferase (ALT), triglycerides, cholesterol, phosphorus, magnesium, iron, uric acid and creatinine serum concentrations were measured by means of enzymatic methods in a clinical chemistry analyser (Screen Master Touch; Hospitex diagnostics Srl., Florence, Italy).

Histomorphological investigations

The slaughtered animals were submitted to anatomopathological investigations. Intestinal segment samples (~5 cm in length) of duodenum, jejunum and ileum were excised and flushed with 0.9% saline to remove all the contents. The collected segments of intestine were the loop of the duodenum, the tract before Meckel's diverticulum (jejunum) and the tract before the ileocolic junction (ileum). Samples of liver, spleen, thymus, bursa of fabricius, kidney and heart were also collected. Gut segments were fixed in Carnoy's solutions for morphometric analysis, while the other organ samples were fixed in 10% buffered formalin solution for histological examination. Tissues were routinely embedded in paraffin wax blocks, sectioned at 5 μm thickness, mounted on glass slides and stained with haematoxylin and eosin (HE). The evaluated morphometric indexes were the villus height (Vh, from the tip of the villus to the crypt), crypt depth (Cd, from the base of the villus to the submucosa) and the Vh/Cd ratio (Laudadio *et al*., 2012). Morphometric analyses were performed on 10 well-oriented and intact villi and 10 crypts chosen from the three collected intestinal segments (Qaisrani *et al*., 2014). Histopathological alterations were evaluated using a semi-quantitative scoring system as previously assessed by Biasato *et al*. (2016): absent/minimal (score = 0), mild (score = 1) and severe (score = 2).

Statistical analysis

The statistical analysis was performed using the SPSS software package (version 17 for Windows; SPSS Inc., Chicago, IL, USA). The experimental unit was the pen for growth performance, while it was the individual bird for all of the other parameters. Data were tested by one-way ANOVA, followed by Tukey's *post hoc* test for growth performance, carcass traits and blood parameters. One-way ANOVA (Duncan's multiple range test) or the Kruskal–Wallis test (*post hoc* test: Dunn's Multiple Comparison test) was used to compare (1) the morphometric indexes among the dietary treatments within each intestinal segment and (2) the morphometric indexes among the intestinal segments within each dietary treatment. Histopathological scores were analysed by the Kruskal–Wallis test (*post hoc* test: Dunn's Multiple Comparison test). Significance was declared at $P < 0.05$. A statistical trend was considered for $P < 0.10$. The results are expressed as the mean and pooled standard error of the mean (SEM).

Results

Diet composition and fatty acid profile

The ingredients and the chemical and FA composition of the three experimental diets are summarized in Table 1.

Table 1 *Ingredients (g/kg as fed), chemical (g/kg DM) and fatty acid composition (% of total FAME) of the experimental diets*

	C	HI50	HI100
Ingredients			
Maize meal	547.4	547.4	547.4
Soybean meal	294	294	294
Soybean oil	68.7	34.3	–
Hermetia illucens larvae fat	–	34.3	68.7
Gluten meal	50	50	50
Dicalcium phosphate	12.4	12.4	12.4
Calcium carbonate	11.2	11.2	11.2
Sodium chloride	2.2	2.2	2.2
Sodium bicarbonate	1.5	1.5	1.5
DL-methionine	1.6	1.6	1.6
L-Lysine	3.6	3.6	3.6
Threonine	1	1	1
Mineral and vitamin finisher premix[1]	5	5	5
Choline chloride	0.4	0.4	0.4
3-phytase (E-300; natuphos bio/G500)	1	1	1
Analysed composition			
Gross energy (MJ/kg)	19.78	19.65	19.70
Chemical composition			
Dry matter (g/kg)	898.7	892.9	898.2
CP	219.5	210.6	210.4
Crude fat	90.3	85.7	90.1
Ash	57.5	55.3	57.0
Fatty acid composition (% of total FAME)			
SFA	16.0	36.2	56.9
C12:0	0.0	17.3	34.7
C14:0	0.0	3.4	6.5
MUFA	23.8	19.9	15.2
PUFA	59.8	43.3	27.9
UFA/SFA	5.22	1.75	0.76
∑n-6	54.9	40.1	26.2
∑n-3	4.88	3.22	1.66
∑n-6/∑n-3	11.2	12.5	15.8

C = control; HI50 = *Hermetia illucens* 50%; HI100 = *Hermetia illucens* 100%, DM = dry matter; AME = apparent metabolizable energy; FAME = fatty acids methyl esters; FA = fatty acids; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; UFA = unsaturated fatty acids.

[1]Mineral-vitamin finisher premix (Final B Prisma, IZA SRL, Forlì, Italy), given values are supplied per kg of diet: 2.500.000 IU of vitamin A; 1.000.000 IU of vitamin D_3; 7.000 IU of vitamin E; 700 mg of vitamin K; 400 mg of vitamin B_1; 800 mg of vitamin B_2; 400 mg of vitamin B_6; 4 mg of vitamin B_{12}; 30 mg of biotin; 3.111 mg of Ca pantothenate acid; 100 mg of folic acid; 15.000 mg of vitamin C; 5.600 mg of vitamin B_3; 10.500 mg of Zn, 10.920 mg of Fe; 9.960 mg of Mn; 3.850 mg of Cu; 137 mg of I; 70 mg of Se.

The three diets were isonitrogenous and had similar gross energy, total lipid, protein and ash contents but differed greatly in their FA composition. In the experimental diets, C12:0 and C14:0 FA and total saturated fatty acids (SFAs) increased with increasing inclusion of HI larva fat. Total monounsaturated FAs (MUFAs), polyunsaturated FAs (PUFAs), total n-3 PUFAs and total n-6 PUFAs decreased in the experimental diets as the HI larva fat inclusion level increased, which determined an increase of the ∑n-6/∑n-3 ratio in the experimental diets (from 11.2% to 15.8% of total FAME for HI0 and HI100, respectively).

Table 2 *Effects of dietary* Hermetia illucens *larvae fat (HI) inclusion on the growth performance of broiler chickens (n = 5 pens/treatment)*

	C	HI50	HI100	SEM	*P*-value
Initial BW (g; day 21)	823.1	823.1	817.8	3.12	0.762
Final BW (g; day 48)	3621.6	3576.9	3751.3	34.46	0.087
Daily weight gain (g)	107.6	105.9	112.8	1.36	0.084
Daily feed intake (g)	208.3	207.3	212.4	1.16	0.175
Feed conversion ratio	2.01	2.03	1.95	0.01	0.172

C = control; HI50 = *Hermetia illucens* 50%; HI100 = *Hermetia illucens* 100%.

Table 3 *Effects of dietary* Hermetia illucens *(HI) larvae fat inclusion on carcass traits and internal organs weight (n = 15/treatment)*

	C	HI50	HI100	SEM	*P*-value
LW (g)	3661	3681	3756	22.5	0.189
Chilled carcass (g)	2594	2624	2651	19.1	0.483
Chilled carcass (% of LW)	70.8	71.3	70. 6	0.25	0.494
Breast (g)	743.3	756.6	778.1	9.92	0.360
Breast (% of LW)	20.3	20.6	20.7	0.24	0.745
Thighs (g)	800	821	838	7.45	0.113
Thighs (% of LW)	21.9	22.3	22.3	0.15	0.373
Abdominal fat (g)	32.0	39.9	41.6	2.03	0.120
Liver (g)	69.1	66.9	64.0	1.29	0.289
Heart (g)	18.6	18.8	17.5	0.39	0.317
Spleen (g)	5.0	4.2	5.1	0.19	0.111
Bursa of fabricius (g)	8.1	7.8	7.6	0.39	0.846

C = control; HI50 = *Hermetia illucens* 50%; HI100 = *Hermetia illucens* 100%; LW = live weight.

Growth performance and slaughtering traits

During the whole experimental period, the birds remained healthy, no signs of illness were observed, and the mortality rate was zero in all groups. The growth performance of the broiler chickens is reported in Table 2. The initial BW of chicks did not differ ($P > 0.05$) among the three dietary treatments. Growth performance was not influenced by the partial or total replacement of soybean oil with HI larva fat ($P > 0.05$). However, a positive numerical trend was observed in the HI100 group for FBW ($P = 0.087$) and DWG ($P = 0.084$). No statistical differences were observed for FCR; nevertheless, the HI100 group displayed the lowest numerical value. Dietary HI larva fat inclusion did not significantly affect ($P > 0.05$) the slaughtering traits of the chickens (Table 3).

Haematological and serum parameters

The dietary HI larva fat inclusion level did not significantly influence ($P > 0.05$) the haematological or serum biochemical traits of the birds (Table 4).

Histomorphological investigations

The intestinal morphology of the broiler chickens is summarized in Table 5. Dietary HI larva fat inclusion did not significantly affect ($P > 0.05$) gut morphometric indexes. In all dietary treatments, the duodenum showed greater Vh

Table 4 *Effects of dietary* Hermetia illucens *(HI) larvae fat inclusion on blood parameters of broiler chickens (*n = *15/treatment)*

	C	HI50	HI100	SEM	*P*-value
Erythrocytes (10^6cell/μl)	4.19	3.90	3.88	0.08	0.208
Leucocytes (10^3 cell/μl)	12.3	12.5	12.4	0.17	0.861
H/L ratio	0.64	0.57	0.63	0.03	0.679
Total proteins	4.22	4.15	4.03	0.14	0.864
Uric acid (mg/dl)	3.47	3.85	3.61	0.23	0.808
Creatinine (mg/dl)	0.30	0.31	0.32	0.01	0.302
AST (UI/l)	279	285	294	9.31	0.817
ALT (UI/l)	22.7	25.7	23.7	0.88	0.363
GGT (UI/l)	29.8	25.3	26.4	1.46	0.407
Triglycerides (mg/dl)	32.3	32.7	33.0	1.30	0.980
Cholesterol (mg/dl)	59.8	66.7	65.8	1.58	0.160
Phosphorus (mg/dl)	6.51	6.84	5.72	0.24	0.151
Magnesium (mEq/l)	2.17	1.55	1.82	0.16	0.263
Iron (μg/dl)	72.95	73.86	73.27	3.47	0.994

AST = aspartate-aminotransferase; ALT = alanine-aminotransferase; GGT = gamma-glutamyl transferase; C = control; HI50 = *Hermetia illucens* 50%; HI100 = *Hermetia illucens* 100%.

Table 5 *Effects of dietary* Hermetia illucens *(HI) larvae fat inclusion on intestinal morphometric indexes of broiler chickens (*n = *15/treatment)*

	C	HI50	HI100	SEM	*P*-value
Duodenum					
Vh (mm)	2.53^{a}	2.52^{a}	2.38^{a}	0.10	0.579
Cd (mm)	0.15	0.17	0.15	0.01	0.415
Vh/Cd	17.62$^{\times}$	16.53	16.89$^{\times}$	1.42	0.859
Jejunum					
Vh (mm)	2.02^{b}	2.01^{b}	2.04^{b}	0.13	0.987
Cd (mm)	0.16	0.17	0.15	0.01	0.390
Vh/Cd	13.26^{y}	13.07	14.40^{xy}	1.06	0.469
Ileum					
Vh (mm)	1.74^{b}	1.71^{b}	1.56^{b}	0.09	0.352
Cd (mm)	0.16	0.15	0.13	0.01	0.159
Vh/Cd	11.71^{y}	11.60	11.98^{y}	0.78	0.942
P Vh	0.000	0.000	0.000	0.05	
P Vh/Cd	0.002	0.430	0.004	0.43	

Vh = villus height; Cd = crypt depth; Vh/Cd = villus height to crypt depth ratio; C = control; HI50 = *Hermetia illucens* 50%; HI100 = *Hermetia illucens* 100%. Different superscript letters in the same column mean significant differences ($P < 0.05$) among the intestinal segments (duodenum, jejunum and ileum) for Vh (a, b) and Vh/Cd (x, y) within each dietary treatment. *P*-values of the significant differences are indicated as *P* Vh and *P* Vh/Cd.

(C = 2.53 mm; HI50 = 2.52 mm; HI100 = 2.38 mm) ($P < 0.001$) than the jejunum (C = 2.02 mm; HI50 = 2.01 mm; HI100 = 2.04 mm) and ileum (C = 1.74 mm; HI50 = 1.71 mm; HI100 = 1.56 mm). In the C group, the Vh/Cd ratio was higher ($P < 0.01$) in the duodenum (17.62) compared with the jejunum (13.26) and ileum (11.71). In the HI100 dietary treatment, the Vh/Cd ratio was also higher ($P < 0.01$) in the duodenum (16.89) than the ileum (11.98). In contrast, no significant differences ($P > 0.05$) regarding Cd were observed within any intestinal segment in any of the groups.

Histopathological alterations developed for spleen, thymus, bursa of fabricius and liver and were observed in all of the dietary treatments. The histopathological scores of the broiler chickens are shown in Table 6. Dietary HI larva fat inclusion did not significantly affect ($P > 0.05$) the severity of the histopathological findings. Spleen showed mild (69% of the broilers in all the dietary treatments) to severe (C = 6%; HI50 = 19%; HI100 = 13%) white pulp depletion or hyperplasia. Of the animals, 25% (C), 13% (HI50) and 19% (HI100) had a normal spleen. In the thymus, mild cortical depletion was found in all the groups (C = 13% of the broilers; HI50 = 25%; HI100 = 13%). A normal thymus was observed in 88% (C), 75% (HI50) and 88% (HI100) of the animals. The bursa of fabricius showed mild (C = 69% of the broilers; HI50 = 63%; HI100 = 81%) to severe (C = 19%; HI50 = 13%; HI100 = 19%) follicular depletion. Of the animals, 13% (C), 25% (HI50) and 0% (HI100) had a normal bursa of fabricius. The liver showed mild (C = 25% of the broilers; HI50 = 50%; HI100 = 44%) to severe (C = 6%; HI50 = 0%; HI100 = 13%) perivascular lymphoid tissue activation. A normal liver was observed in 63% (C), 50% (HI50) and 44% (HI100) of the animals.

Table 6 *Effects of dietary* Hermetia illucens *(HI) larvae fat inclusion on histopathological scores of broiler chickens (*n = *15/treatment).*

	C	HI50	HI100	SEM	*P*-value
Spleen	0.84	1.06	0.94	0.14	0.488
Thymus	0.16	0.27	0.13	0.10	0.637
Bursa of fabricius	1.06	0.88	1.19	0.13	0.275
Liver	0.40	0.50	0.69	0.16	0.428
Heart	No alterations				
Kidney	No alterations				

C = control; HI50 = *Hermetia illucens* 50%; HI100 = *Hermetia illucens* 100%.

Discussion

Growth performance and slaughtering traits

The inclusion of HI larva fat substituting 50 or 100% of the soybean oil in finisher broiler chickens' diet did not lead to any adverse effects on growth performance or carcass traits. Feed intake was not affected by the partial or total replacement of soybean oil with HI larva fat, indicating that 50% and 100% HI inclusion were both acceptable to broiler chickens. This finding suggests that it is possible to replace up to 100% of the soybean oil with HI larva fat in diets for finisher chickens without any negative effects on feed utilization or productive performance. Comparing HI larva fat with other vegetable oils used in poultry diets, it seems to be similar to coconut oil rich in saturated oils (about 90%) with 60% of its total FA composition being MCFAs (Kappally *et al*., 2015). Furthermore, lauric acid content in coconut oil accounts for ~36.95 g/100 g FA (Wang *et al*., 2015). A study on male broiler finisher chickens (days 22 to 42) found that dietary replacement (25%, 50%, 75% and 100%) of soybean oil with coconut oil did not affect growth performance (Wang *et al*., 2015). The results of the present study agree with the findings of Schiavone *et al.* (2017a), which reported that the

partial or total replacement of soybean oil with HI larva fat in growing broiler diets did not affect the growth performance, the feed choice or the carcass traits. In the same context, Cullere *et al*. (2016) showed that defatted HI larva meal could be introduced into the diet for growing broiler quails at 10% and 15% inclusion levels, partially replacing conventional soybean meal and soybean oil, with no negative effects on productive performance, mortality or carcass traits. Regarding the use of HI larva fat in fish feed, Li *et al*. (2016) did not report any significant effects on growth performance in juvenile Jian carp (*Cyprinus carpio* var. Jian) fed with diets where soybean oil was substituted with 25%, 50%, 75% or 100% HI larva fat.

Haematological and serum parameters

The physiological and biochemical status of animals can be expressed through haematology and blood chemistry. All the blood parameters obtained in the present trial suggested that HI larva fat did not affect the health status of the animals. The H/L ratio has recently been used for measurement of distress conditions in chickens in which an increased H/L ratio may indicate that the animals suffered from infections, inflammation or stress (Salamano *et al*., 2010; De Marco *et al*., 2013; Pozzo *et al*., 2013). In the present study, the H/L ratio of chickens fed the experimental diets did not present any significant differences compared with the control group. Their leukocytes fell within the normal range, thus suggesting that the feeding treatments did not affect the immune system of the broiler chickens. It should be highlighted that the present study was performed in a standard environment without infections, stress or other factors that could have influenced the haematological parameters. The activities of serum AST and ALT are generally related to liver damage, acting as indicators of liver necrosis when they increase (Hyder *et al*., 2013). The identification of no influence on ALT or AST activity suggests that HI larva fat may not cause negative effects on the hepatopancreas or liver health. No significant differences ($P > 0.05$) were found in the mean values of creatinine, thus implying that HI larva fat has similar effects on the kidney functionality of the birds. The results of the present research agree with those of Schiavone *et al*. (2017a) and Li *et al*. (2016), who showed that the use of HI larva fat in substitution for soybean oil in broilers and juvenile Jian carp diets, respectively, had no negative effects on the blood traits of the animals and confirmed the nutritional adequacy of these diets.

Histomorphological investigations

An initial hypothesis was that lauric and myristic MCFA would exert a certain positive effect on chicken growth performance through two main mechanisms: an antimicrobial effect on gut pathogens that would improve gut health together with reducing the competition for nutrients, and modifications to the gut morphology, mainly increasing villi length and the villi : crypt ratio in the duodenum and jejunum, which could improve nutrient absorption (Zeitz *et al*., 2015). However, the intestinal morphology of broiler chickens was not affected by the dietary treatments of the present experiment in which important differences in the dietary contents of C12:0 and C14:0 FA were observed with increasing HI fat inclusion levels. Such finding could be due to the already optimal health status of the animals which was expressed by satisfactory productive performance and normal intestinal morphology. It is hypothesized that a sub-optimal farming condition and/or health status are necessary to test the real potential of dietary MCFA in exerting a possible positive effect on chicken gut health and growth performance. This confirmed the previous findings of Biasato *et al*. (2016), who observed no differences related to insect meal utilization in free-range chickens. According to previous studies (de Verdal *et al*., 2010; Biasato *et al*., 2016), the duodenum shows greater morphometric indexes among the intestinal segments. This finding represents physiological gut development because the duodenum is the segment with the fastest cell renewal and is also the first one to be stimulated by the presence of diet in the lumen (de Verdal *et al*., 2010).

Furthermore, the severity of the histopathological alterations in the broilers was not affected regardless of diet. However, the lymphoid system activation represented an interesting finding. Some stressful situations, in particular overcrowding, can frequently occur in modern poultry rearing operations. Measures of immunity that have been reported to be affected under stress conditions in poultry are lymphoid organ weight (Heckert *et al*., 2002), the H/L ratio (De Marco *et al*., 2013) and lymphocyte blastogenesis (Cunnick *et al*., 1994). Despite no histopathological studies being currently available in relation to stress evaluation in broilers, the lymphoid system activation detected in the present study may reflect this aspect. A potential role of HI in the development of these alterations seems unlikely as they were also found in the animals fed with the control diet, their severity was unaffected, and the percentage of their occurrence was heterogeneous and quite similar among the dietary treatments. The greater percentage of normal and moderately affected organs in all the groups may suggest the presence of tolerable stress levels. This hypothesis could also be supported by the identification of the overall positive health and welfare status of the broilers.

In conclusion, the important differences in the dietary contents of lauric and myristic FAs with increasing HI fat inclusion had no beneficial effect on chicken gut health. Despite this, the findings of this study suggest that HI larva fat could totally replace soybean oil in finisher chickens' diet without any adverse effects on growth performance, haematological parameters, serum biochemical indices, intestinal morphology or histological features. These findings suggest that HI larva fat can be a suitable ingredient for poultry diets. Further research efforts are necessary to deeply investigate the impact of HI larva fat on the meat quality traits and FA profile of broiler chickens.

Acknowledgements

The research was supported by a University of Turin (ex 60%) grant (Es. fin. 2014 and 2015), by University of Padova funds

(ex 60%, 2016 – DOR1603318) and by a University of Padova Senior Researcher Scholarship (Prot. n. 1098, 2015). The authors are grateful to Dr Paolo Montersino, Mr Dario Sola and Mr Mario Colombano for bird care and technical support.

Declaration of interest

The authors declare that they have no competing interests.

Ethics Statement

The experimental protocol was approved by the Ethical Committee of the Department of Veterinary Sciences of the University of Turin (Italy) (protocol number 1/2016).

Software and data repository resources

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

References

Association of Official Analytical Chemists 2005. Official methods of analysis, 18th edition. AOAC, Washington, DC, USA.

Aviagen 2014. Ross 708 broiler. Broiler performance objectives. Retrieved on 10 May 2017 from http://en.avi-agen.com/ross-708/.

Barroso FG, de Haro C, Sánchez-Muros MJ, Venegas E, Martínez-Sánchez A and Pérez-Bañón C 2014. The potential of various insect species for use as food for fish. Aquaculture 422, 193–201.

Belforti M, Gai F, Lussiana C, Renna M, Marfatto 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.

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, excreata microbial load, feed choice, performance, carcass and meat traits. Animal 10, 1923–1930.

Cunnick JE, Kojic LD and Hughes RA 1994. Stress induced changes in immune function are associated with increased production of an interleukin-1-like factor in young domestic fowl. Brain, Behavior and Immunity 8, 123–136.

De Marco M, Martinez S, Tarantola M, Bergagna S, Mellia E, Gennero MS and Schiavone A 2013. Effect of genotype and transport on tonic immobility and heterophil/lymphocyte ratio in two local Italian breeds and Isa Brown hens kept under free-range conditions. Italian Journal of Animal Science 12, 481–485.

de Verdal H, Mignon-Grasteau S, Jeulin C, Le Bihan-Duval E, Leconte M, Mallet S, Martin C and Narcy A 2010. Digestive tract measurements and histological adaptation in broiler lines divergently selected for digestive efficiency. Poultry Science 89, 1955–1961.

Fascina VB, Carrijo AS, Souza KMR, Garcia AML, Kiefer C and Sartori JR 2009. Soybean oil and beef tallow in starter broiler diets. Brazilian Journal of Poultry Science 11, 249–256.

Gasco L, Henry M, Piccolo G, Marono S, Gai F, Renna M, Lussiana C, Antonopoulou F, 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.

Heckert RA, Estevez I, Russek-Cohen E and Pettit-Riley R 2002. Effects of density and perch availability on the immune status of broilers. Poultry Science 81, 451–457.

Hyder MA, Hasan M and Mohieldein AH 2013. Comparative levels of ALT, AST, ALP and GGT in liver associated diseases. European Journal of Experimental Biology 3, 280–284.

Kappally S, Shirwaikar A and Shirwaikar A 2015. Coconut oil: a review of potential applications. Hygeia Journal of Drugs and Medicine 7, 34–41.

Laudadio V, Passantino L, Perillo A, Lopresti G, Passantino A, Khan RU and Tufarelli V 2012. Productive performance and histological features of intestinal mucosa of broiler chickens fed different dietary protein levels. Poultry Science 91, 265–270.

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.

Li L, Ji H, Zhang B, Tian J, Zhou J and Yu H 2016. Influence of black soldier fly (*Hermetia illucens*) larvae oil on growth performance, body composition, tissue fatty acid composition and lipid deposition in juvenile Jian carp (*Cyprinus carpio* var. Jian). Aquaculture 465, 43–52.

Mattioli S, Dal Bosco A, Szendrő ZS, Cullere M, Gerencsér ZS, Matics ZS, Castellini C and Dalle Zotte A 2016. The effect of dietary Digestarom® herbal supplementation on rabbit meat fatty acid profile, lipid oxidation and antioxidant content. Meat Science 121, 238–242.

Ozdogan M, Topal E, Paksuz EP and Kirkan S 2014. Effect of different levels of crude glycerol on the morphology and some pathogenic bacteria of the small intestine in male broilers. Animal 8, 36–42.

Pozzo L, Salamano G, Mellia E, Gennero MS, Doglione L, Cavallarin L, Tarantola M, Forneris G and Schiavone A 2013. Feeding a diet contaminated with ochratoxin A for broiler chickens at the maximum level recommended by the EU for poultry feeds (0.1 mg/kg). 1. Effects on growth and slaughter performance, haematological and serum traits. Journal of Animal Physiology and Animal Nutrition 97, 13–22.

Qaisrani SN, Moquet PC, van Krimpen MM, Kwakkel RP, Verstegen MW and Hendriks WH 2014. Protein source and dietary structure influence growth performance, gut morphology, and hind gut fermentation characteristics in broilers. Poultry Science 93, 3053–3064.

Ramos-Elorduy J 1997. Insects: a sustainable source of food? Ecology of Food and Nutrition 36, 247–276.

Renna M, Schiavone A, Gai F, Dabbou S, Lussiana C, Malfatto V, Prearo M, Capucchio MT, Biasato I, Biasibetti E, De Marco M, Brugiapaglia A, Zoccarato I and Gasco L 2017. Evaluation of the suitability of a partially defatted black soldier fly (*Hermetia illucens L.*) larvae meal as ingredient for rainbow trout (*Oncorhynchus mykiss Walbaum*) diets. Journal of Animal Science and Biotechnology 8, 57.

Rumpold BA, Klocke M and Schlüter O 2016. Insect biodiversity: underutilized bioresource for sustainable applications in life sciences. Regional Environmental Change 17, 1445-1454.

Salamano G, Mellia E, Tarantola M, Gennero MS, Doglione L and Schiavone A 2010. Acute phase proteins and heterophil:lymphocyte ratio in laying hens in different housing systems. Veterinary Record 167, 749–751.

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

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

Schiavone A, De Marco M, Martínez S, Dabbou S, Renna M, Madrid J, Hernandez F, Rotolo L, Costa P, Gai F and Gasco L 2017b. Nutritional value of a partially defatted and a highly defatted black soldier fly larvae (*Hermetia illucens* L.) meal for broiler chickens: apparent nutrient digestibility, apparent metabolizable energy and apparent ileal amino acid digestibility. Journal of Animal Science and Biotechnology 8, 51.

Surendra KC, Olivier R, Tomberlin JK, Jha R and Khanal SK 2016. Bioconversion of organic wastes into biodiesel and animal feed via insect farming. Renewable Energy 98, 197-202.

Tran G, Heuzé V and Makkar HPS 2015. Insects in fish diets. Animal Frontiers 5, 37–44.

Ushakova NA, Brodskii ES, Kovalenko AA, Bastrakov AI, Kozlova AA and Pavlov ADS 2016. Characteristics of lipid fractions of larvae of the black soldier fly *Hermetia illucens*. Doklady Biochemistry and Biophysics 468, 209–212.

Veldkamp T and Bosch G 2015. Insects: a protein-rich feed ingredient in pig and poultry diets. Animal Frontiers 5, 45–50.

Wang J, Wang X, Li J, Chen Y, Yang W and Zhang L 2015. Effects of dietary coconut oil as a medium-chain fatty acid source on performance, carcass composition and serum lipids in male broilers. Asian-Australasian Journal of Animal Sciences 28, 223–230.

Wang JX and Peng KM 2008. Developmental morphology of the small intestine of African ostrich chicks. Poultry Science 87, 2629–2635.

Zeitz J, Fennhoff OJ, Kluge H, Stangl GI and Eder K 2015. Effects of dietary fats rich in lauric and myristic acid on performance, intestinal morphology, gut microbes, and meat quality in broilers. Poultry Science 94, 2404–2413.