Productive performance and blood profiles of laying hens fed *Hermetia illucens* larvae meal as total replacement of soybean meal from 24 to 45 weeks of age

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ABSTRACT The aim of the research was to study the effects of an insect meal from *Hermetia illucens* larvae (HILM) as complete replacement of soybean meal (SBM) on productive performance and blood profiles of laying hens, from 24 to 45 wk of age. A total of 108 24-week-old Lohmann Brown Classic laying hens was equally divided into 2 groups (54 hens/group, 9 replicates of 6 hens/group). From 24 to 45 wk of age, the groups were fed 2 different isoproteic and isoenergetic diets: the control group (SBM) was fed a corn-soybean meal based diet, while in the HILM group the soybean meal was completely replaced by *Hermetia illucens* larvae meal. Feed intake, number of eggs produced, and egg weight were recorded weekly along the trial. At 45 wk of age, blood samples were collected from 2 hens per replicate. The use of HIML led to a more favorable ($P < 0.01$) feed conversion ratio in hens but lay percentage, feed intake, average egg weight, and egg mass were higher ($P < 0.01$) in hens fed the SBM diet. Hens fed insect meal produced a higher percentage of eggs from small (S), medium (M), and extra-large (XL) classes ($P < 0.01$) than SBM, while the SBM group had a higher percentage of eggs from the large (L) class ($P < 0.01$). The levels of globulin and albumin to globulin ratio were, respectively, higher and lower ($P < 0.05$) in HILM than the SBM group. Cholesterol and triglycerides were higher ($P < 0.05$ and $P < 0.01$, respectively) in hens from SBM than in the HILM group. Blood levels of Ca were higher ($P < 0.01$) in hens fed insect meal, while creatinine was higher ($P < 0.01$) in blood of hens fed SBM. *Hermetia illucens* larvae meal can be a suitable alternative protein source for laying hens even if the complete replacement of soybean meal needs further investigation to avoid the negative effects on feed intake.

Key words: *Hermetia illucens* larvae meal, laying hen, productive performance, blood profile

2017 Poultry Science 96:1783–1790
http://dx.doi.org/10.3382/ps/pew461

INTRODUCTION

The current food insecurity situation prevailing in many developing countries and future challenges of feeding over 9 billion people in 2050 (Makkar et al., 2014) led researchers from all over the world to find alternative protein sources for human and animal nutrition.

Currently, the main protein sources in animal feed are soybean and fish meal, but the global land availability for soy cultivation is limited and marine overexploitation has reduced the abundance of small pelagic forage fish from which fish meal and fish oil are derived (Veldkamp et al., 2012).

Insects seem to be a good candidate to meet the global challenge of finding new protein sources (Makkar et al., 2014), especially taking into account their nutritional value and low space requirement for raising. In addition, insects used for feed can be reared on organic wastes such as manure and fish offal, without evoking revulsion and ethical issues (Rumpold and Schlüter, 2013). These insects are able to produce antimicrobial peptides (Ratcliffe et al., 2014) to protect themselves from infections and also reducing the harmful bacteria in the manure (Erikson et al., 2004); in addition, during feed production, different systems of decontamination are used by producers (Rumpold and Schlüter, 2013). Compared to conventional livestock, insects have

Received May 13, 2016.
Accepted December 5, 2016.
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a higher feed conversion efficiency (Nakagaki and Defoliart, 1991; Veldkamp et al., 2012) and fecundity (Nakagaki and Defoliart, 1991). It has even been indicated that insects might contribute less greenhouse gases than pig and cattle (Oonincx et al., 2010).

Insects can be an interesting alternative protein source in particular for poultry, considering that they are part of the "natural" diet of chickens (Bovera et al., 2015).

Among insects, black soldier fly (*Hermetia illucens*, L), a *Diptera* of the Stratiomyidae family, native to the tropical, subtropical, and warm temperate zones of America, seems to be particularly suitable for poultry feeding. In fact, its larvae contain about 35 to 57% crude protein with an amino acid composition similar or superior to soybean (Veldkamp et al., 2012), in particular for lysine content that ranges from 6 to 8% of crude protein (Makkar et al., 2014), and are rich in calcium (5 to 8% DM) and phosphorus (0.6 to 1.5% DM) (Arango Gutierrez et al., 2004; St-Hilaire et al., 2007; Yu et al., 2009). Black soldier fly larvae meal contains also chitin, a polysaccharide present in arthropod's exoskeleton. Chitin is not digestible by monogastric animals (Sanchez-Muros et al., 2014) and can negatively affect the protein digestibility (Longvah et al., 2011) but may have a positive effect on the functioning of the immune system of poultry (Sanchez-Muros et al., 2014; Bovera et al., 2016).

Several studies are available in the literature on the use of insect meals in broiler diets (Finke et al., 1985; Ravindran and Blair, 1993; Wang et al., 2005; Oyegoke et al., 2006; Bovera et al., 2015) even if the findings on the use of *Hermetia illucens* are very few (Schiavone et al., 2014; De Marco et al., 2015). On the contrary, the use of insect meal in laying hens' feeding has been poorly investigated. Wang et al. (1996) observed that dried ground mealworms as replacement of fishmeal in laying hen diet resulted in 2.4% higher egg production. Agunbiade et al. (2007), in a study on 50-week-old laying hens, showed that maggot meal could replace 50% of fishmeal protein (5% in diet) without adverse effects on egg production and shell strength but 100% of replacement decreased egg production. A recent study (Maurer et al., 2016) showed that *Hermetia illucens* larvae meal included up to 100% on a protein basis in a laying hen diet had no negative effects on egg production, feed intake, or feed conversion efficiency.

The aim of this study was to evaluate the productive performance and blood profiles of laying hens fed *Hermetia illucens* larvae meal in total substitution of soybean meal from 24 to 45 wk of age.

MATERIALS AND METHODS

All the animals were humanely treated according to the principles stated by the EC Directive 86/609/EEC (Council Directive, 2008) regarding the protection of animals used for experimental and other scientific purposes.

Table 1. Chemical-nutritional characteristics of the *Hermetia illucens* larvae meal and soybean meal.

	Hermetia illucents larvae meal	Soybean meal
Chemical composition		
DM, %[1]	97.8	90.0
CP, % as fed[1]	61.3	43.4
Ether extract, % as fed[1]	4.61	1.10
ADF, % as fed[1]	12.1	5.90
ADF-linked protein, % as fed[1]	5.59	1.78
Ash, % as fed[1]	7.82	6.01
Ca, % as fed[2]	6.90	2.83
Total P, % as fed [2]	0.91	0.57
Na, % as fed[2]	0.12	0.16
Lysine, % as fed[2]	4.05	2.92
Methionine, % as fed[2]	1.30	0.61
Methionine+Cystine, % as fed[2]	1.42	1.33
Isoleucine, % as fed[2]	3.11	2.30
Tryptophan, % as fed[2]	0.30	0.73
Valine, % as fed[2]	5.02	2.11
Threonine, % as fed[2]	2.32	1.74

[1]Analyzed composition.
[2]Obtained by producers.

The trial was carried out on a private hen farm located in southern Italy for 21 wk, from September 2015 to February 2016. A total of 108 24-week-old Lohman Brown Classic laying hens (average live weight 1.78 kg ± 0.15 standard deviation) were equally divided into 2 groups (54 hens per group). The hens were housed in the same building in modified cages (800 cm^2/hen), under controlled temperature and humidity conditions. For each group, hens were distributed into 3 cages (18 hens/cage) and each cage was divided by 2 internal transects in 3 equal areas, to obtain 9 replicates of 6 hens per group. Feed and water were manually distributed and appropriate separations were placed along the trough and the line of egg collection to control feed intake and egg production per each replicate. The dark:light cycle was 9:15 hours.

The 2 groups were fed 2 isoproteic and isoenergetic diets, differing in the ingredients used as the main protein source. The soybean meal (**SBM**) group was fed a corn-soybean meal based diet, while in the *Hermetia illucens* larvae meal (**HILM**) group, the soybean was completely replaced by a defatted meal from *Hermetia illucens* larvae (Hermetia Deutschland GmbH & Co KG, Amtsgericht Potsdam, German). The diets were formulated to meet hens' requirements according to Lohmann Brown classic Management Guide (2011). Chemical characteristics of the 2 main protein sources (insect meal and soybean meal) and of the diets were determined according to AOAC (2004) and are presented, respectively, in Tables 1 and 2. Metabolizable energy of diets was calculated according to NRC (1994) procedure of estimation. Table 1 shows the chemical composition, amino acid profile, and some mineral content (Ca, P, and Na) of the 2 protein sources. Data on amino acids, minerals, and metabolizable energy from all the ingredients were supplied by the respective producers

Table 2. Chemical-nutritional characteristics of the diets used in the trial.

	Hermetia illucens larvae meal diet	Soybean meal diet
Ingredients, g/kg		
Maize grain	653.0	583.0
Soybean meal	-	235.0
Insect meal	170.0	-
$CaCO_3$ grains	80.0	80.0
Dehulled sunflower meal	50.0	50.0
Vegetable oil	10.0	15.0
MinVit[1]	30.0	30.0
Monocalcium phosphate	5.00	5.00
Salt	2.00	2.00
Chemical nutritional characteristics		
DM, %[2]	90.5	90.1
CP, % as fed[2]	17.9	18.1
Crude fibre, % as fed[2]	4.14	3.96
Ether extract, % as fed[2]	4.26	4.33
ADF, % as fed[2]	3.82	3.45
ADIP, % as fed[2]	2.88	1.52
Ash, % as fed[2]	14.2	14.2
NDF, % as fed[2]	15.2	14.0
Ca, % as fed[3]	4.96	4.26
Total P, % as fed[3]	0.67	0.69
Na, % as fed[3]	0.30	0.19
Lysine, % as fed[3]	0.91	0.90
Methionine, % as fed[3]	0.64	0.55
Methionine+Cystine, % as fed[3]	0.86	0.84
Isoleucine, % as fed[3]	0.79	0.77
Tryptophan, % as fed[3]	0.16	0.21
Valine, % as fed[3]	1.19	0.82
Threonine, % as fed[3]	0.64	0.63
ME, kcal/kg[3]	2,745	2,780

[1]Provided 20 g of celite and 10 g of mineral and vitamin supplements. Per kilogram: vitamin A (retinyl acetate) 20,000 IU, vitamin D3 (cholecalciferol) 6,000 IU, vitamin E (dl-α-tocopheryl acetate) 80 IU, vitamin B1(thiamine monophosphate) 3 mg, vitamin B2 (riboflavin) 12 mg, vitamin B6 (pyridoxine hydrochloride) 8 mg, vitamin B12 (cyanocobalamin) 0.04 mg, vitamin K3 (menadione) 4.8 mg; vitamin H (d biotin) 0.2 mg, vitamin PP (nicotinic acid) 48 mg, folic acid 2 mg, calcium pantothenate 20 mg, manganous oxide 200 mg, ferrous carbonate 80 mg, cupric sulphate pentahydrate 20 mg, zinc oxide 120 mg, basic carbonate monohydrate 0.4 mg, anhydrous calcium iodate 2 mg, sodium selenite 0.4 mg, choline cloride 800 mg, 4-6-phitase 1,800 FYT, D.L. methionine 2,600 mg, canthaxanthin 8 mg.

[2]Analyzed composition.

[3]Calculated composition.

ADIP: Protein linked to ADF.

ME: Metabolizable energy.

and used to calculate the correspondent contents in the diet. For the insect meal and the diets, the amount of protein linked to acid detergent fiber (**ADF**) was determined (AOAC, 2004) and, for only insect meal, it was used to estimate the amount of chitin, according to Marono et al. (2015): Chitin (%) = ash free ADF (%) – ADF-linked protein (%).

Mash diets and fresh water were administered ad libitum and feeds were manually distributed each day. After 2 wk of adaptation to the new diets (starting from 26 wk of age) the collection of data was started. Feed intake was measured weekly per replicate, weighing the amount of feed distributed and that of residual and scattered feed and was expressed as individual feed intake by day.

From 26 to 45 wk of age, the number of eggs produced and the individual egg weights were recorded per replicate every week. Weighed eggs were assigned to 4 weight classes as follows: up to 52 g, small (**S**) class; 53 to 63 g, medium (**M**) class; 64 to 73 g, large (**L**) class; and >73 g, extra-large (**XL**) class. Per each replicate of each group, egg mass was calculated by multiplying egg weight by egg production percentage, and feed conversion ratio (**FCR**) was calculated as gram of feed consumption per day divided by gram of egg weight per day.

At 45 wk of age, blood samples were collected from the wing vein of 2 birds per replicate (a total of 36 blood samples, 18 per group) in tubes with and without heparin. Serum was separated by centrifugation at 1,500 g for 15 min and stored at $-20\,^{\circ}$C until analysis. Whole heparinised blood was analyzed for haematocrit, haemoglobin, and blood cell count (white blood cells, **WBC** and red blood cells, **RBC**) using an automatic blood analyzer (ADVIA 120 Siemens, Munich, Germany); the differential count of WBC was performed on blood smears stained with May-Grunwald-Giemsa by counting 100 cells with an optic microscope. All biochemical traits of blood serum—total protein, albumin, globulin, glucose, cholesterol, triglycerides, aspartate aminotransferase (**AST**), alanine aminotransferase (**ALT**), gamma glutamyl-transferase (**GGT**), alkaline phosphatase (**ALP**), creatine kinase (**CK**), lactic dehydrogenase (**LDH**), lactate, blood urea nitrogen (**BUN**), creatinine (**Crea**), uric acid, calcium, phosphorus, magnesium, iron, and chloride—were determined using commercially available kits by Spinreact (La Vall d'en Bas, Girona, Spain) by enzymatic colorimetric or kinetic methods, according to the manufacturer's instructions. Spectrophotometric measurements were performed using an automatic biochemical analyzer AMS AUTOLAB (Rome, Italy). Globulin concentration was estimated as the difference between total protein and albumin. Thus, the albumin to globulin ratio was calculated.

Statistical Analysis

Data were processed by ANOVA using the PROC GLM of SAS (2000). Differences among groups regarding laying performance, body weight, weight gain, and blood profiles were analyzed by one-way ANOVA according to the following model: Yij = m + Di + eij, where Y is the single observation, m the general mean, D the effect of the diet (i = HILM or SBM), and e is the error. The data on laying performance were processed by a 2-way ANOVA according to the model: Yijk = m + Di + Wj + DWij + eijk, where Y is the single observation, m the general mean, D the effect of the diet (i = HILM or SBM), W the effect of the week of lay (j = from 26 to 45), and e is the error. Comparison among means was performed by Tukey's test (SAS, 2000).

Differences among weight classes of eggs for the entire period of the trial were tested by chi-square test.

Table 3. Changes in live weight of laying hens during the trial.

	Initial body weight, kg	Final body weight, kg	Weight gain, g
HILM	1.79	1.89[b]	102.2[b]
SBM	1.77	2.10[a]	328.9[a]
P-value	0.79	0.012	0.024
RMSE	0.162	0.154	181.81

HILM: *Hermetia illucens* larvae meal; SBM: soybean meal.
[a,b]$P < 0.05$; RMSE: Root mean square error; n = 18 (9/group).

Table 4. Effect of main protein source and wk of production on laying performance of hens.

	Lay, %	Feed intake, g/d/hen	Egg weight, g	Egg mass	FCR
HILM	91.9[b]	108.0[b]	59.9[b]	55.1[b]	1.97[b]
SBM	94.5[a]	125.1[a]	61.8[a]	58.3[a]	2.17[a]
P-values					
Group effect	<0.001	<0.001	<0.001	<0.001	<0.001
Week effect	<0.001	<0.001	0.087	0.001	0.423
Interaction effect	0.45	0.20	0.38	0.005	0.20
RMSE	6.580	9.192	2.781	5.921	0.252

HILM*:* *Hermetia illucens* larvae meal; SBM: soybean meal.
[a,b]$P < 0.01$; RMSE: Root mean square error; ; n = 360 (9 replicates x 20 weeks/group).

Table 5. Effect of dietary treatment on weight class of eggs during the entire period of the trial.

	S % (up to 52 g)	M % (53–63 g)	L % (64–73 g)	XL % (>73 g)
HILM	7.14[a]	69.1[a]	21.6[b]	2.17[a]
SBM	2.82[b]	66.2[b]	30.9[a]	0.00[b]
P value	<0.001	<0.001	<0.001	<0.001
RMSE	0.934	2.931	0.351	0.153

HILM: *Hermetia illucens* larvae meal; SBM: soybean meal.
[a,b]$P < 0.01$, n = 360 (9 replicates × 20 wk/group).

RESULTS

The average temperature and humidity during the trial were 21.3°C ± 0.5 and 60% ± 2.5 standard deviation, respectively. No mortality was recorded during the trial. The amount of ADF-linked protein in HILM was 5.55% as fed. As a consequence, the estimated chitin content resulted in 5.40% as fed (46.22% of ADF).

Table 3 shows the change in live weight of hens along the trial. The initial body weight was similar among groups, while at the end of the trial, the SBM group had an average live weight higher ($P < 0.05$) than the HILM group and the weight gain observed from 26 to 45 wk of age was 328.9 g for the SBM group and 102.2 for the HILM group ($P < 0.05$).

Table 4 shows the effect of main protein source, wk of age, and their interaction on laying performance. Lay percentage, feed intake, average egg weight, and egg mass resulted higher ($P < 0.01$) in hens fed SBM. FCR was more favorable ($P < 0.01$) in hens fed HILM.

Table 5 reports the percentage of the different weight classes of eggs of the groups along the entire experimental period. Hens fed insect meal produced a higher percentage of eggs from the S class than SBM but also higher percentages of eggs from M and XL classes ($P < 0.01$), while the SBM group had a higher percentage of eggs from the L class ($P < 0.01$).

Tables 6 and 7 show the effect of diet on blood profiles of laying hens. The level of globulin was higher ($P < 0.05$) in the HILM group and the opposite happened for the albumin to globulin ratio ($P < 0.05$). Cholesterol and triglycerides were higher ($P < 0.05$ and $P < 0.01$, respectively) in hens from the SBM group.

Table 6. Haematological traits, serum proteins, glucose, and lipids of laying hens fed insect and soybean meal from 24 to 45 wk of age.

	HILM	SBM	*P*-value	RMSE
Haematological traits				
Haematocrit, %	33.3	33.8	0.67	3.642
Haemoglobin, g/dl	11.1	10.1	0.071	1.451
RBC, $x10^6/mm^3$	3.65	3.61	0.85	0.654
WBC, $x10^3/mm^3$	21.1	20.9	0.86	2.982
Heterophils, %	37.1	37.3	0.35	0.841
Lymphocytes, %	47.3	48.9	0.34	0.173
Monocytes, %	2.94	2.69	0.97	0.471
Eosinophils, %	11.4	10.1	0.22	0.090
Basophils, %	1.31	1.00	0.12	0.472
H/L	0.79	0.77	0.11	0.534
Serum proteins, glucose and lipids				
Total protein, g/dl	5.18	5.31	0.58	0.629
Albumin, g/dl	2.72	2.58	0.44	0.501
Globulin, g/dl	2.74[a]	2.12[b]	0.030	0.763
Albumin/Globulin	1.01[b]	1.62[a]	0.033	0.771
Glucose, mg/dl	274	295	0.29	55.340
Cholesterol, mg/dl	108[b]	134[a]	0.010	26.582
Triglycerides, mg/dl	1296[B]	1942[A]	0.007	627.77

HILM: *Hermetia illucens* larvae meal; SBM: soybean meal; RBC: Red blood cells; WBC: White blood cells; H/L: Heterophils to lymphocytes ratio.
[A,B]$P < 0.01$; [a,b]$P < 0.05$; RMSE: Root mean square error; n = 36 (18/group).

Table 7. Electrolytes, liver, renal, and muscle function of laying hens fed insect and soybean meal from 24 to 45 wk of age.

	HILM	SBM	*P*-value	RMSE
Liver function				
AST, U/l	112	126	0.57	68.470
ALT, U/l	139	133	0.66	97.610
GGT, U/l	88.8	76.9	0.64	71.501
ALP, U/l	1191	1189	0.99	906.703
Electrolytes				
Ca, mg/dl	10.6[A]	9.46[B]	0.002	0.943
P, mg/dl	7.58	8.43	0.34	2.502
Mg, mg/dl	5.22	6.99	0.082	2.734
Fe, mcg/l	215	224	0.18	19.091
Cl, mmol/dl	133[b]	137[a]	0.038	7.682
Renal and muscle function				
BUN, mg/dl	0.79	0.89	0.38	0.304
Crea, mg/dl	0.29[B]	0.46[A]	0.002	0.143
Uric acid, mg/dl	4.15	5.02	0.24	2.060
CK, U/l	663	654	0.22	478.182
LDH, U/l	949	898	0.62	293.302
Lactate, mg/dl	105	121	0.11	27.942

HILM: *Hermetia illucens* larvae meal; SBM: soybean meal.
AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, GGT: Gamma glutamyl-transferase, ALP: Alkaline phosphatase, CK: Creatine kinase, LDH: Lactic dehydrogenase, BUN: Blood urea nitrogen, Crea: Creatinine,.
[a,b]$P < 0.05$.
[A,B]: $P < 0.01$; RMSE: Root mean square error; n = 36 (18/group).

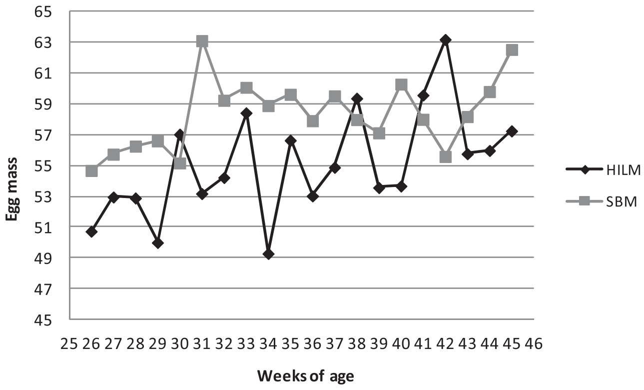

Figure 1. Egg mass of *Hermetia illucens* larvae meal (HILM) and soybean meal (SBM) groups during the wk of the trial. The values at wk 29, 31, 32, 34, 36, 37, 39, 40, 42, 44, and 45 are different at $P < 0.01$; wk 26, 28, 30, and 43 are different at $P < 0.05$; wk 27, 30, 33, 38, and 41 are not different.

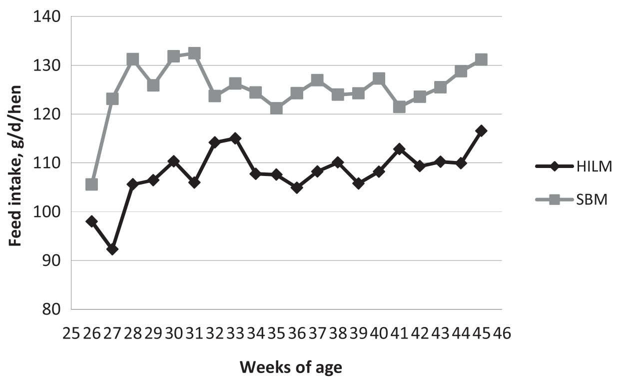

Figure 2. Feed intake of *Hermetia illucens* larvae meal (HILM) and soybean meal (SBM) groups during the wk of the trial. The values at weeks 27, 28, 29, 30, 31, 34, 36, 37, 39, 41, and 45 are different at $P < 0.01$; wk 33, 35, 38, 40, 42, and 44 are different at $P < 0.05$; weeks 26, 32, and 41 are not different.

Blood levels of Ca were higher ($P < 0.01$) in hens fed insect meal, while the opposite was for Cl ($P < 0.05$). Creatinine levels were higher ($P < 0.01$) in blood of hens fed SBM.

Figures 1 and 2 show, respectively, the trend of egg mass and feed intake for the wk of the trial for both dietary treatments. Egg mass showed a fluctuating trend and was, in general, higher in the SBM group; only at 4 wk (31, 38, 41, and 42) the egg mass values were higher in the HILM group. Feed intake of the SBM group was almost always significantly higher than in the HILM group and only at 26, 32, and 41 wk of age the differences were not significant.

DISCUSSION

Very few studies are available in literature on the use of insect meal as a possible alternative protein source for laying hens. The main protein sources of the 2 diets used in this study showed important differences not only in terms of macro-nutrients content, but also in mineral and essential amino acids, which are known to be important for poultry production. In particular, the levels of Ca, lysine, methionine, isoleucine, valine, and threonine in *Hermetia illucens* larvae meal were, respectively, 2.46, 1.40, 2.17, 1.35, 2.38, and 1.35 times higher than in soybean meal. The sum of methionine and cysteine was similar between the 2 protein sources, while SBM contained 2.33 times more tryptophan than insect meal. These reflected some slight differences between the diets, in particular for methionine content (+16 % than SBM diet). However, in both diets all the above-mentioned nutrients were of adequate levels to satisfy the requirements of Lohmann Brown laying hens from 26 to 45 wk of age, as reported by the Lohmann Brown Classic Management Guide (2011). In addition, Maurer et al. (2016) replaced soybean with *Hermetia illucens* in laying hen diet and also had a great difference in methionine level, higher than in our trial (+22 % in *Hermetia illucens* diet) without effects on productive performance.

Productive performance of laying hens fed *Hermetia illucens* defatted larvae meal as total replacement of soybean meal was lower than in that of the group fed SBM. This result seems to be in contrast with recent findings of Maurer et al. (2016) who found that egg production, feed intake, and FCR of Lohmann Leghorn laying hens fed experimental diets in which *Hermetia* meal replaced 100% of soybean cake were unaffected by dietary treatments. However, even if statistically not significant, in the trial of Maurer et al. (2016) feed intake and egg weight of the group in which *Hermetia* meal totally replaced soybean cake, were, respectively, −7.8 and −5.4% than the control. In our trial, feed intake of the HILM group was −13.6% than the control, but the differences between egg weights of the groups was 2.9%, lower than that found by Maurer et al. (2016). On the other hand, the trial of Maurer et al. (2016) was performed on a later laying period (starting from 64 wk of age) and for a shorter time (10 wk) with respect to ours. The lower productive performance of hens from the HILM group can be attributed to a lower feed intake. Since the 2 tested diets contained similar amounts of metabolizable energy, ADF, and protein, had a similar particle size, and, in addition, hens were submitted to the same environmental conditions, other factors affecting feed intake have to be considered. As observed in meat-type birds (Ferket and Gernat, 2006), in laying hens feed color also can affect their feed intake (Tabeekh, 2015). There are conflicting reports in feed color preference of poultry (Capretta, 1969; Cooper, 1971) and this was explained by Kennedy (1980) who concluded that all chicks showed a preference for diets whose color was the same as the diet fed after hatching. In addition, Tabeekh (2015) observed that there was a general tendency to decrease consumption of the colored diets. The diets used for laying hens are usually based on corn and soybean, and, therefore, the color of the control diet used in the present trial was not different from that of the diet fed to hens before the experimental period. On the contrary, *Hermetia illucens* meal had a brown color, darker than that of soybean. Also the different flavor of feed could affect the hens' intake, as poultry tend to avoid diets containing unusual ingredients in comparison with diets providing the basic nutrients from natural feed materials (Esmail, 2013). According to the Lohmann Brown Classic Management Guide (2011), the average feed intake from 26 to 45 wk of age was 117 g/d/hen, higher than that of the HILM group and lower than that of the SBM group. The higher feed intake of the SBM group also affected the weight gain and, as a consequence, the final body weight. At the end of the trial (45 wk of age) the average weight of hens from the HILM group fell in the range reported by the management breeder guide, while hens from the SBM group were slightly oversized. FCR was more favorable for the HILM group, thus indicating a more favorable use of feed nutrients. Such hypothesis is also confirmed by the average laying percentage of the HILM group (91.94%), which was not much lower than the standard one (92.36%) given by the breeder for Lohmann Brown classic in the same period; also the average egg weight of hens fed insect meal was 59.91 g (2.9% less than that of the other group) but it was in line with the average weight (59.57 g) indicated from the Lohmann standard along the same lay period; in addition, the egg size of hens fed insect meal showed a wide range of weight variability, including all 4 examined weight classes, while this did not happen for the SBM group in which the XL class of egg was not represented. It is known that protein intake is the main factor affecting the egg weight (Leeson et al., 2001): in the group fed insect meal the lowest feed intake affected, of course, protein intake even if the percentage of protein content in the insect diet (17.91%) was adequate to sustain the egg production of Lohmann Brown classic hens, with a daily feed consumption between 105 and 110 g in the period of 19 to 45 wk (Lohmann Brown Classic Management Guide, 2011).

The absence of mortality and clinical signs of trouble (such as diarrhea), as well as the absence of weight loss in both groups, indicated that the *Hermetia illucens* had no negative effects on laying hen health status. This is also confirmed by the results of blood analysis as most of the criteria were not different between the groups. Total protein and albumin are two criteria useful to evaluate body condition of poultry (Piotrowska et al., 2011) because plasma protein plays a key role in body homeostasis maintenance, and albumin serves as the most favorable source of amino acids for protein synthesis (Filipovic et al., 2007). Both criteria were not different between the 2 groups.

The blood level of globulin and the albumin to globulin ratio were, respectively, higher and lower in the HILM than in the SBM group. Griminger and Scanes (1986) stated that high globulin concentrations and low albumin/globulin ratios indicate a better disease resistance and immune response in birds. In a recent study on the use of *Tenebrio molitor* larvae meal to feed broilers from 30 to 62 d of age, Bovera et al. (2015) also found a lower albumin to globulin ratio in the group fed insect meal even if no differences were found in globulin content. The authors attributed the result to the properties of chitin contained in insect meal. The amount of chitin estimated in the HILM used in our trial was 46.2% of free ash ADF and 5.55% as fed, in line with the reports of Finke (2013) who estimated the chitin content of black soldier fly larvae as 5.4% of dry matter. Considering the average feed intake during the trial and the percentage of insect meal inclusion in the diet, hens from the HILM group ingested around 1.02 g/d of chitin.

According to Hossain and Blair (2007), the lower serum cholesterol and triglycerides observed in the HILM group also can be attributed to chitin. These authors included a commercial chitin in broiler diet from one to 21 d of age at zero, 25, 50, and 75 g/kg of diet and showed a cholesterol and triglycerides reduction at all levels, but more consistent at 50 g/kg of inclusion. The effect of chitin on lowering cholesterol and triglycerides could be ascribed to its positive charge able to attract negatively charged bile acids and free fatty acids (Prajapati and Patel, 2010).

In addition, recently, Ratcliffe et al. (2014) stated that insects such as black soldier fly and common housefly larvae may produce antimicrobial peptides to defend themselves from possible pathogens in manure and organic waste where they live and these peptides also can be useful for poultry (Veldkamp and Bosch, 2015).

Uric acid is the major poultry nitrogenous waste product (Harr, 2002) and its level in serum reflects protein catabolism. In our study, no differences were found between groups, indicating that the tested diets had no effects on protein metabolism, despite a different protein intake (19.35 vs. 22.60 g/d, respectively for HILM and SBM groups, according to the values calculated by multiplying the protein content of diet for its respective average intake). Creatinine, a byproduct of phosphocreatine breakdown in skeletal muscle, is another important indicator of protein metabolism (Piotrowska et al., 2011); its blood levels are related to muscle mass, age, physical activity, and diet (Wyss and Kaddurah-Daoukr, 2000; Rajman et al., 2006). In our trial the diet was the main factor affecting the creatinine level of hens, resulting lower in the HILM group. The lowest level of creatinine recorded in hens fed insect meal can be related to the lowest protein intake in birds of this group, as creatinine is also considered an index of protein metabolism and renal function. There are no evidences in literature on the effect of chitin on renal function, but some reports (Jing et al., 1997; Davis et al., 2003; Ahmed et al., 2014) indicated that chitosan, industrially produced by chitin deacetylation, had a positive effect on renal function of rats and dogs evidenced by decreasing serum creatinine levels.

Finally, it is not easy to explain the significant higher levels of Ca in blood of hens from the HIML group as, taking into account the average feed intake, the daily Ca intake was 5.36 and 5.33 g, respectively, for HILM and SBM groups.

CONCLUSIONS

Defatted *Hermetia illucens* larvae meal could be an interesting protein source for laying hens, able to sustain egg production without negative effects on animal health and enhancing immune status of birds. However, when used in total substitution of soybean meal, it negatively affected feed intake and thus production performance of hens, even if the feed conversion ratio of insect diet was more favorable than that of the soybean diet. This probably can be ascribed to the darker color of the insect meal as compared to that of soybean meal. A possible solution to this problem could be to use a lower percentage of *Hermetia illucens* larvae meal inclusion in hens' diet or to feed chicks with insect meal starting from the first d after hatching. This latter point deserves further investigation.

ACKNOWLEDGMENTS

The authors thank the owners and the staff of Casetta Rossa (Presenzano, Caserta, Italy) for making possible the trial and Raffaele Maglione from LABforVET (Napoli, Italy) for his technical assistance.

Authors contributions: SM: manuscript writing, performed the trial in the farm and the feed analysis; RL: performed the trial in the farm and the feed analysis; PL: performed blood analysis, contributed to manuscript writing and English check; GV: performed blood collection and analysis; MEP: performed blood analysis; FR: gave important support to make possible the trial by finding the farm and modifying cages; LG: supplied insect meal, contributed to manuscript revision; GParisi: contributed to manuscript revision; GPiccolo: contributed to manuscript revision; SN: contributed to manuscript revision; CDM: contributed to manuscript revision; YAA: contributed to manuscript revision: FB: planned and organized the trial, processed data, and wrote manuscript, tables, and graphs.

REFERENCES

Agunbiade, J. A., O. A. Adeyemi, O. M. Ashiru, H. A. Awojobi, A. A. Taiwo, D. B. Oke, and A. A. Adekunmisi. 2007. Replacement of fish meal with maggot meal in cassava-based layers' diets. J. Poult. Sci. 44:278–282.

Ahmed, F. A., M. S. Abdel-Lattife, A. S. Abd–El Azeem, A. M. Hegazy, H. Z. Hassoun, and M. A. Algalaly. 2014. The role of chitosan and wheat germ as antidiabetic substances in diabetic rats. Res. J. Pharmac., Biol. Chem. Sci. 5:457–469.

Arango Gutierrez, G. P., R. A. Vergara Ruiz, and H. Mejia Velez. 2004. Compositional, microbiological and protein digestibility analysis of larval meal of Hermetia illucens (Diptera:Stratiomyidae) at Angelopolis-Antioquia, Colombia. Rev. Facult. Nacl. Agron. Med. 57:2491–2499.

AOAC, Association of Official Analytical Chemists. 2004. Official Methods of Analysis. 18th edn. AOAC Int., Arlington, VA.

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

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

Capretta, P. J. 1969. The establishement of food preferences in chicks (Gallus gallus). An. Behav. 17:229–231.

Cooper, J. B. 1971. Colored feed for turkey poults. Poult. Sci. 50:1892–1893.

Council Directive, 2008. http://ec.europa.eu/food/fs/aw/aw_legislation/scientific/86-609-eec_en.pdf.

Davis, W., M. S. Lamson, V. Jonathan, and M. D. Wright. 2003. A case of early renal functional impairment resolved with nutrients and botanicals. Altern. Med. Rev. 8:55–58.

De Marco, M., S. Martínez, F. Hernandez, J. Madrid, F. Gai, L. Rotolo, M. Belforti, D. Bergero, H. Katz, S. Dabbou, A. Kovitvadhi, I. Zoccarato, L. Gasco, and A. Schiavone. 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. Anim. Feed Sci. Technol. 209:211–218.

Erickson, M. C., M. Islam, C. Sheppard, J. Liao, and M. P. Doyle. 2004. Reduction of Escherichia coli O157:H7 and Salmonella enterica serovar Enteritidis in chicken manure by larvae of the black soldier fly. J. Food Protection. 67:685–690.

Esmail, S. H. 2013. Factors affecting feed intake of chickens. World Poultry, http://www.worldpoultry.net/Nutrition/Articles/20131/3/Factors-affecting-feed-intake-of-chickens-1172230W/.

Ferket, P. R., and A. G. Gernat. 2006. Factors that affect feed intake of meat birds: A review. Int. J. Poult. Sci. 5:905–911.

Filipovic, N., Z. Stojeviae, S. Milinkoviae-Tur, B. B. Ljubiae, and M. Zdelar-Tuk. 2007. Changes in concentration and fractions of blood serum proteins of chickens during fattening. Vet. Arhiv. 77:319–326.

Finke, M. D., G. R. DeFoliart, and M. L. Sundae. 1985. An evaluation of the protein quality of mormon cricket (Anabrux simplex H.) when used as a high protein feedstuff for poultry. Poult. Sci. 64:708–712.

Finke, M. D. 2013. Complete nutrient content of four species of feeder insects. Zoo Biol. 32:27–36.

Griminger, P., and C. G. Scanes. 1986. Protein metabolism. Pages 326–345 in: Avian Physiology, 4th edn. P. D. Sturkie, Ed. Springer Verlag, New York.

Harr, K. E. 2002. Clinical chemistry of companion avian species: A review. Vet. Clin. Path. 31:140–151.

Hossain, S. M., and R. Blair. 2007. Chitin utilisation by broilers and its effect on body composition and blood metabolites. Br. Poult. Sci. 48:33–38.

Jing, S., L. Li, D. Ji, Y. Takiguchiand, and T. Yamaguchi. 1997. Effect of chitosan on renal function in patients with chronic renal failure. J. Pharm. Pharmacol. 49:721–723.

Kennedy, J. M. 1980. The development of dietary preferences in pigs and poultry. Pages 141–147 in: Palatability and flavour use in animal feeds. H. Bickel, Ed. Verlag Paul Parey Hamburg und Berlin.

Leeson, S., J. D. Summers, and L. J. Caston. 2001. Response of layers to low nutrient density diets. J. Appl. Poultry Res. 10: 46–52.

Lohmann Brown classic Management Guide. 2011. http://www.specialisedbreeders.com.au/wp-content/uploads/2016/04/LTZ-Management-Guide-LB-Classic-EN.pdf. Accessed 04/12/2016.

Longvah, T., K. Mangthya, and P. Ramulu. 2011. Nutrient composition and protein quality evaluation of eri silkworm (Samia ricinii) prepupae and pupae. Food Chem. 128:400–403.

Makkar, H. P. S., G. Tran, V. Heuze, and P. Ankers. 2014. State-of-the-art on use of insects as animal feed. Anim. Feed Sci. Technol. 197:1–33.

Marono, S., G. Piccolo, R. Loponte, C. Di Meo, Y. A. Attia, A. Nizza, and F. Bovera 2015. *In vitro* crude protein digestibility of Tenebrio molitor and Hermetia illucens insect meals and its correlation with chemical composition traits. It. J. Anim. Sci. 14:338–343.

Maurer, V., M. Holinger, Z. Amsler, B. Früh, J. Wohlfahrt, A. Stamer, and F. Leiber. 2016. Replacement of soybean cake by

Hermetia illucens meal in diets for layers. J. Insects Food and Feed. 2:89–90.

Nakagaki, B. J., and G. R. Defoliart. 1991. Comparison of diets for mass-rearing Acheta domesticus (Orthoptera, Gryllidae) as a novelty food, and comparison of food conversion efficiency with values reported for livestock. J. Econ. Entomol. 84:891–896.

National Research Council. 1994. Nutrient Requirements of Poultry, 9th Revised Edition. National Academy Press, Washington, DC

Oonincx, D. G. A. B., J. Van Itterbeeck, M. J. W. Heetkamp, H. Van den Brand, J. J. A. van Loon, and A. van Huis. 2010. An exploration on greenhouse gas and ammonia production by insect species suitable for animal or human consumption. PloS One. 5:12. doi: 10.1371/journal.pone.0014445.

Oyegoke, O. O., A. J. Akintola, and J. O. Fasoranti. 2006. Dietary potentials of the edible larvae of Cirina forda (westwood) as a poultry feed. Afr. J. Biotechnol. 5:1799–1802.

Piotrowska, A., K. Burlikowska, and R. Szymeczko. 2011. Changes in blood chemistry in broiler chickens during the fattening period. Folia Biol. (Kraków). 59:183–187.

Prajapati, B. G., and R. P. Patel. 2010. Nutrition, dietary supplements and herbal medicines: A safest approach for obesity. Res. J. Pharmac. Biol. Chem. Sci. 1:39–45.

Rajman, M., M. Jurani, D. Lamosova, M. Maejova, M. Sedlaekova, L. Kostal, D. Jeova, and P. Vyboh. 2006. The effects of feed restriction on plasma biochemistry in growing meat type chickens (Gallus gallus). Comp. Biochem. Physiol. A. 145:363–371.

Ratcliffe, N., P. Azambuja, and C. B. Mello. 2014. Recent advances in developing insect natural products as potential modern day medicines. Evid. Based Complement. Alternat. Med. 2014: Article ID 904958, 21 pages, doi:10.1155/2014/904958.

Ravindran, V., and R. Blair. 1993. Feed resources for poultry production in Asia and the Pacific. World's Poult. Sci. J. 49:219–235.

Rumpold, B. A., and O. K. Schlüter. 2013. Potential and challenges of insects as an innovative source for food and feed production. Innov. Food Sci. Emerg. Technol. 17:1–11.

Sánchez-Muros, M. J., F. G. Barroso, and F. Manzano-Agugliaro. 2014. Insect meal as renewable source of food for animal feeding: A review. J. Clean. Prod. 65:16–27.

SAS, Statistical Analyses System. 2000. SAS Institute Inc., SAS/STAT Software, Version 9, SAS Institute Inc., Cary, NC

Schiavone, A., M. De Marco, L. Rotolo, M. Belforti, S. Martinez Mirò, J. Madrid Sanchez, F. Hernandez Ruiperez, C. Bianchi, L. Sterpone, V. Malfatto, H. Katz, I. Zoccarato, F. Gai, and L. Gasco. 2014. Nutrient digestibility of Hermetia illucens and Tenebrio molitor meal in broiler chickens. Page 73 In: Proc. 1st Int. Conf. Insects to Feed the World, 14–17 May 2014, Wageningen, The Netherlands.

St-Hilaire, S., K. Cranfill, M. A. McGuire, E. E. Mosley, J. K. Tomberlin, L. Newton, W. Sealey, C. Sheppard, and S. Irving. 2007. Fish offal recycling by the blacksoldier fly produces a foodstuff high in omega-3 fatty acids. J. World Aquacult. Soc. 38:309–313.

Tabeekh, M. A. S. A. 2015. An investigation on preference of layers for different colored feed. Savant. J. Agric. Res. 1:1–5.

Veldkamp, T., and G. Bosch, 2015. Insects: A protein-rich feed ingredient in pig and poultry diets. Anim. Front. 5:45–50.

Veldkamp, T., G. van Duinkerken, A. van Huis, C. M. M. Lakemond, E. Ottevanger, G. Bosch, and M. A. J. S. van Boekel. 2012. Insects as a sustainable feed ingredient in pig and poultry diets – a feasibility study. In: Rapport 638. Wageningen Livestock Research, Available at: http://www.wageningenur.nl/uploadmm/2/8/0/f26765b9-98b2-49a7-ae43-5251c5b694f6234247%5B1%5D.

Wang, D., S. W. Zhai, C. X. Zhang, B. Y. Yu, A. S. Heng, and Y. N. Xu. 2005. Evaluation on nutritional value of field crickets as a poultry feedstuff. Asian-Aust. J. Anim. Sci. 18: 667–670.

Wang, Y. C., Y. T. Chen, X. R. Li, J. M. Xia, Q. S. Du, and C. Zhi. 1996. Study on rearing the larvae of Tenebrio molitor Linne and the effects of its processing and utilization. Acta Agric. Univ. Henanensis. 30:288–292.

Wyss, M., and W. Kaddurah-Daoukr. 2000. Creatine and creatinine metabolism. Phys. Rev. 80:1107–1213.

Yu, G. H., Y. H. Chen, Z. N. Yu, and P. Cheng. 2009. Research progress on the larvae and prepupae of black soldier fly Hermetia illucens used as animal feedstuff. Chinese Bull. Entomol. 46:41–45.