Yellow mealworm larvae (*Tenebrio molitor*) inclusion in diets for male broiler chickens: effects on growth performance, gut morphology, and histological findings

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ABSTRACT This study evaluated the effects of *Tenebrio molitor* (TM) larvae meal inclusion in diets for broilers. A total of 160 male broiler chicks (Ross 708) at one-day of age were randomly allotted to four dietary treatments: a control (C) group and three TM groups, in which TM meal was included at 50 (TM5), 100 (TM10), and 150 (TM15) g/kg, respectively. The experimental diets were isonitrogenous and isoenergetic. Each group consisted of five pens as replicates (8 chicks/pen). After the evaluation of growth performance and haematochemical parameters, the animals were slaughtered at 53 days and carcass traits were recorded. Morphometric investigations were performed on duodenum, jejunum, and ileum and histopathological alterations were assessed for liver, spleen, thymus, bursa of Fabricius, kidney, and heart. The live weight (LW) showed a linear (12 and 25 days, $P < 0.001$ and $P < 0.05$, maximum with TM15 and TM10) and quadratic (53 days, $P < 0.05$, maximum with TM5) response to dietary TM meal inclusion. A linear (1 to 12 and 12 to 25 days, $P < 0.001$, maximum with TM15) and quadratic (12 to 25 days, $P = 0.001$, maximum with TM15) effect was also observed for the daily feed intake (DFI). The feed conversion ratio (FCR) showed a linear response (25 to 53 and 1 to 53 days, $P = 0.001$ and $P < 0.05$, maximum with TM15). Haematological and serum biochemical traits, carcass traits and histopathological findings were not affected by dietary TM meal inclusion ($P > 0.05$). TM15 birds showed lower villus height ($P < 0.05$), higher crypt depth ($P < 0.05$), and lower villus height to crypt depth ratio ($P = 0.001$) compared with C and TM5. In conclusion, increasing levels of dietary TM meal inclusion in male broiler chickens may improve body weight and feed intake, but negatively affect feed efficiency and intestinal morphology, thus suggesting that low levels may be more suitable. However, no effect on haematochemical parameters, carcass traits, and histological findings were observed in relation to TM meal utilization.

Key words: broiler chickens, *Tenebrio molitor*, insect meal, growth performance, morphometry

2018 Poultry Science 97:540–548
http://dx.doi.org/10.3382/ps/pex308

INTRODUCTION

The market for poultry products (egg and meat) is growing worldwide owing also to the absence of cultural or religious obstacles. Protein sources represent the primary production costs in poultry diets (FAO, 2013). Soybean meal is the most used vegetable protein source in diet formulations for broilers and laying hens, because of the high quality and quantity of protein and the adequate amino acid profile (Veldkamp et al., 2012). However, due to its ever-increasing price, the sustainability of this production chain is becoming critical, in particular in some developing countries. Fishmeal has been widely used in poultry nutrition in the last decades, due to its excellent nutritive properties related to its very high nutrient density and digestibility. The protein in fishmeal has high biological value, because it is rich in essential amino acids, particularly lysine and sulphur-containing amino acids. In some developing countries, fishmeal is the most

© 2017 Poultry Science Association Inc.
Received August 22, 2016.
Accepted September 20, 2017.
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important conventional animal protein source, but its production availability and cost is a major issue (FAO, 2013). Severe environmental concerns must also be considered in relation to the massive use of soybean and fishmeal. On one hand, the increased soy cultivation causes deforestation, high water consumption and utilization of chemicals. On the other hand, fishmeal quantitatively and qualitatively depends on catch, which is destined to decrease in the coming years due to the risk of marine resources depletion (Veldkamp et al., 2012; Sánchez-Muros et al., 2014).

Some studies have recently shown the feasibility of insect-derived feeds as a useful alternative to soybean and fishmeal in animal nutrition (van Huis, 2013; Makkar et al., 2014; Belforti et al., 2015; Henry et al., 2015; Gasco et al., 2016). Certain insect species are efficient feed converters because they do not use energy to maintain a high body temperature (Sánchez-Muros et al., 2014). Furthermore, they can be packed into land much more intensively, are highly digestible and can even be fed waste products which would otherwise be environmental problems (FAO, 2013; Makkar et al., 2014; Sánchez-Muros et al., 2014). Furthermore, insects (adult, larval and pupal forms) are naturally consumed by wild birds and free-range poultry (Zuidhof et al., 2003; FAO, 2013). In the European Union, insects and insect meals are currently only authorized for pet animals and farm fish. The partial or total replacement of soybean or fish meal by black soldier flies (*Hermetia illucens* L.) (Maurer et al., 2015; Cullere et al., 2016), houseflies (*Musca domestica* L.) (Hwganbo et al., 2009), mealworms (*Tenebrio molitor* L.) (Ballitoc and Sun, 2013; Bovera et al., 2015; Biasato et al., 2016; Bovera et al., 2016), and silkworms (*Bombyx mori* L.) (Khatun et al., 2003; Ijaiya and Eko, 2009) has recently been investigated in poultry feeding. Some authors evaluated the effects of TM meal utilization on growth performance (Ballitoc and Sun, 2013; Bovera et al., 2015; Bovera et al., 2016), haematochemical profile (Bovera et al., 2015), and carcass traits (Ballitoc and Sun, 2013; Bovera et al., 2016) of broilers, while others focused their attention also on the assessment of intestinal morphology and histological features in free-range chickens fed diets including TM meal (Biasato et al., 2016). Despite dietary modifications being reported to widely affect gut morphology in poultry (Laudadio et al., 2012; Qaisrani et al., 2014), no studies in relation to insect meal utilization are currently available in broilers. The aim of the present study was to evaluate growth performance, haematochemical parameters, carcass traits, intestinal morphology, and histological features of male broiler chickens fed diets including TM meal.

MATERIALS AND METHODS

Birds and Husbandry

The present trial was performed in collaboration with a local poultry corporation named "O.R.A. Agricola S.r.l." sited in Cherasco (Cuneo, Italy). 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). Furthermore, the experimental protocol was approved by the Ethical Committee of the Department of Veterinary Sciences of the University of Turin (Italy). A poultry house of 14 m wide × 141 m long × 4.7 m high, equipped with waterproof floor and wall, completely covered with tiles and provided with automatic ventilation system was used.

A total of 160 male broiler chicks (Ross 708) at one-day of age were randomly allotted to 4 dietary treatments, each consisting of 5 pens as replicates with 8 chicks per pen. Each pen was 1.20 m wide × 1.20 m long and was equipped with a feeder occupying a surface of almost 1,800 cm^2, three nipple drinkers and rice hulls as litter. During the first 3 weeks, the animals were heated by infrared lamps to maintain the suitable temperature according to standard breeding practices (Aviagen, 2014). Lighting schedule was 23 h light:1 h darkness until day 3 and then 18 h light:6 hours darkness until slaughter age. At hatching, all chicks received vaccination against Newcastle disease, Gumboro disease, infectious bronchitis, and coccidiosis. Vaccine recalls were performed on day 9 for infectious bronchitis and on day 18 for Gumboro and Newcastle diseases.

Diets

A diet based on corn meal, corn gluten meal, and soybean meal was formulated and served as control (C), while 5, 10, and 15% full-fat TM larvae meal (Gaobeidian Shannong Biology CO., LTD, Gaobeidian, Hebei province, China) inclusion as a partial replacement of soybean meal, corn gluten meal and soybean oil constituted the 3 experimental treatment groups (TM5, TM10, and TM15) (Table 1). TM meal nutritive composition was previously analyzed as reported by De Marco et al. (2015) and summarized as follows: dry matter (**DM**), 948.0 (g/kg as fed); organic matter, 912.0 (g/kg as fed); crude protein (**CP**), 524.0 (g/kg as fed); ether extract (**EE**), 280.0 (g/kg as fed); neutral detergent fiber (**NDF**), 117.0 (g/kg as fed); acid detergent fibre (**ADF**), 79.5 (g/kg as fed); methionine, 5.9 (g/kg as fed); lysine, 14.7 (g/kg as fed); gross energy, 24.4 (MJ/kg DM); apparent metabolizable energy (**AMEn**), 16.02 (MJ/kg DM). The amount of chitin of the TM meal (46.2 g/kg as fed) was previously estimated by Bovera et al. (2016) as follows: chitin (%) = ash free ADF (%)—residual nitrogen in ADF × 6.25 (%) (Finke, 2007). For each dietary treatment, diets were divided into 3 phases: a starter diet (days 1 to 12), a grower diet (days 12 to 25), and a finisher diet (days 25 to 53). For each phase, the experimental diets were isonitrogenous and isoenergetic and were formulated using the AMEn values for TM calculated in vivo

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Table 1. Ingredients (g/kg as fed), apparent metabolizable energy (MJ/kg DM) and nutrient composition (%) of the experimental diets.[1]

Ingredients	First period (days 1 to 12)				Second period (days 12 to 25)				Third period (day 25 to slaughter)			
	Control	TM5	TM10	TM15	Control	TM5	TM10	TM15	Control	TM5	TM10	TM15
Corn meal	483.2	482.7	488.5	496.6	523.8	535.9	549.3	566.8	566.6	572.2	585.7	605.4
Soybean meal	345.0	333.8	304.0	262.0	317.0	294.0	254.1	203.9	275.5	259.0	219.0	164.0
TM larvae meal	0.0	50.0	100.0	150.0	0.0	50.0	100.0	150.0	0.0	50.0	100.0	150.0
Corn gluten meal	75.5	42.0	23.0	14.5	58.3	24.0	8.0	0.0	56.0	21.0	5.0	0.0
Soybean oil	54.0	50.3	43.5	34.8	64.9	59.9	51.7	41.6	68.9	64.9	56.7	45.8
Dicalcium phosphate	11.0	12.0	13.0	15.5	8.4	9.0	10.5	12.9	7.0	8.0	9.5	12.0
Calcium carbonate	17.5	16.5	16.0	15.0	15.0	15.0	14.5	13.0	14.5	14.0	13.5	12.2
Sodium chloride	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3
Sodium bicarbonate	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3
DL-methionine	0.8	0.9	0.9	0.8	0.8	0.9	0.9	0.8	0.4	0.6	0.6	0.5
L-lysine	3.1	1.9	1.2	0.9	2.0	1.3	0.9	0.8	1.3	0.4	0.0	0.0
Threonine	0.1	0.1	0.1	0.1	0.0	0.2	0.3	0.4	0.0	0.1	0.2	0.3
Trace mineral-vitamin premix[2]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Choline chloride	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
3-phytase	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Total	100	100	100	100	100	100	100	100	100	100	100	100
AMEn[3] (MJ/kg DM)	12.89	12.89	12.89	12.89	13.28	13.28	13.28	13.28	13.54	13.54	13.54	13.54
Nutrient composition (%)												
DM	86.6	86.6	86.7	86.6	86.7	86.8	86.6	86.8	86.8	86.7	86.7	86.8
CP	23.5	23.5	23.6	23.8	21.3	21.1	21.1	21.1	19.6	19.6	19.6	19.6
EE	7.9	8.3	9.0	9.6	9.0	9.2	9.8	10.3	9.5	9.7	10.4	10.8
NDF	9.4	9.8	10.0	10.1	9.4	9.8	10.0	10.1	9.4	9.7	9.9	10.1
ADF	3.8	4.1	4.3	4.4	3.7	3.9	4.1	4.1	3.5	3.8	3.9	4.0
Nutrient composition (%)[2]												
Calcium	1.1	1.1	1.1	1.1	0.9	0.9	0.9	0.9	0.8	0.8	0.8	0.8
Available phosphorus	0.6	0.6	0.5	0.6	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Digestible methionine	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.4	0.4	0.4	0.4
Digestible lysine	1.4	1.4	1.4	1.4	1.3	1.3	1.3	1.3	1.1	1.1	1.1	1.1
Digestible threonine	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.8	0.8	0.8	0.8

[1]Four dietary treatments: C = control; TM5 = 5% inclusion level of *Tenebrio molitor*; TM10 = 10% inclusion level of *Tenebrio molitor*; TM15 = 15% inclusion level of *Tenebrio molitor*.

[2]Mineral-vitamin 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 D3; 7,000 IU of vitamin E; 700 mg of vitamin K; 400 mg of vitamin B1; 800 mg of vitamin B2; 400 mg of vitamin B6; 4 mg of vitamin B12; 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 B3; 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.

[3]Calculated according to Sauvant et al. 2004 (ingredients AMEn, calcium, available phosphorus) and De Marco et al. (2015) (insect AMEn, digestible methionine, digestible lysine and digestible threonine).

TM, *Tenebrio molitor*; AMEn, apparent metabolizable energy; DM, dry matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fiber; ADF, acid detergent fiber.

for broiler chickens (De Marco et al., 2015). Diets met or exceeded (NRC 1994) requirements and were adjusted according to Aviagen (2014) broiler nutrition specifications. Feed and water were provided ad libitum.

The diets were ground to pass through a 0.5-mm sieve and stored in airtight plastic containers for DM (method number 943.01), ash (method number 924.05), CP (method number 954.01), EE (method number 920.39), NDF (method number 2002.04), and ADF (method number 973.18) determination (AOAC, 2004).

Growth Performance

The trial lasted 53 days. Health status and mortality were daily monitored during the whole experimental period. Live weight (**LW**), daily feed intake (**DFI**), and feed conversion ratio (**FCR**) were determined for each diet phase and for the overall experimental period. All measurements were made on pen basis using a high precision electronic scale (Sartorius—Signum).

Pre-slaughter Procedures

At day 53, all birds were individually weighed and 10 broilers/diet (2 birds/pen) were chosen on the basis of pen average LW and identified with a shank ring.

Haematological and Serum Parameters

At slaughter, blood samples of ten birds (two animals per pen) per feeding group were collected from the identified broilers: 2.5 mL was placed in an EDTA tube and 2.5 mL in a serum-separating tube. 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 (Campbell, 1995). The total red and white blood

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cell counts were determined in an improved Neubauer haemocytometer on blood samples previously treated with a 1:200 Natt-Herrick solution. One hundred leukocytes, including granular (heterophils, eosinophils, and basophils) and non-granular (lymphocytes and monocytes) leukocytes, were counted on the slide and the heterophiles to lymphocytes (**H/L**) ratio was calculated. The tubes without anticoagulant were left to clot in a standing position at room temperature for approximately two hours to obtain serum. The serum was separated by means of centrifugation at 700 × *g* for 15 minutes and frozen at −80°C until analysis. The total proteins were quantified by means of the "biuret method" (Bio Group Medical System kit; Bio Group Medical System, Talamello (RN), Italy); the electrophoretic pattern of the serum was obtained using a semi-automated agarose gel electrophoresis system (Sebia Hydrasys, Norcross, GA). The alanino-aminotransferase (**ALT**), aspartate-aminotransferase (**AST**), gamma glutamyl transferase (**GGT**), triglycerides, cholesterol, glucose, phosphorus, magnesium, iron, uric acid, and creatinine serum concentrations were measured by means of enzymatic methods in a clinical chemistry analyzer (Screen Master Touch, Hospitex diagnostics Srl., Firenze, Italy).

Carcass Traits

At the end of the trial, ten selected chickens (two birds per pen) per feeding group were euthanized by electrical stunning and bleeding in a commercial abattoir. The plucked and eviscerated carcasses were obtained and the head, neck, feet, and abdominal fat were removed to obtain carcass-for-grilling. The weight of liver, spleen, gizzard, and abdominal fat were immediately recorded. All slaughtered carcasses were stocked in a cooling chamber (0 to 4°C) for 24 h. Weights of carcass-for-grilling, breast and thighs were successively recorded. Carcass-for-grilling, breast, thighs, and organs weights were also expressed as percentage of LW.

Histomorphological Investigations

Ten birds per feeding group (two animals per pen) were submitted to anatomopathological investigations. Intestinal segment samples (approximately 5 cm in length) of duodenum, jejunum, and ileum were excised and flushed with 0.9% saline to remove all the content. 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 solution for morphometric analysis, while the other organ samples were fixed in 10% buffered formalin solution for histopathological examination. Tissues were routinely embedded in paraffin wax blocks, sectioned at 5 μm thickness, mounted on glass slides and stained with Haematoxylin & Eosin (**HE**). The evaluated morphometric indices were 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 villus height to crypt depth (**Vh/Cd**) ratio (Laudadio et al., 2012). Morphometric analyses were performed on 10 well-oriented and intact villi and 10 crypts chosen from duodenum, jejunum, and ileum (Qaisrani et al., 2014). The following histopathological alterations were evaluated: white pulp hyperplasia and depletion in spleen, cortical depletion in thymus, follicular depletion and intrafollicular cysts in bursa of Fabricius, and lymphoid tissue activation in liver (Biasato et al., 2016). Heart and kidney were assessed for inflammatory and degenerative diseases. The observed histopathological findings 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

IBM SPSS Statistics V20.0.0 software was used to perform statistical analysis. Shapiro-Wilk's test established normality or non-normality of distribution. All data from each pen were considered the experimental unit. Data collected for growth performance, blood parameters, and carcass traits were tested by one-way ANOVA, evaluating the effect of dietary TM inclusion by polynomial contrasts. χ^2 test was performed to evaluate the association between the mortality rate and the dietary treatments. Intestinal morphometric indices were analyzed by fitting a general linear model (**GLM**). The GLM allowed the morphometric indices (Vh, Cd, and Vh/Cd, separately) to depend on three fixed factors (diet, intestinal segment, and interaction between diet and intestinal segment). The interactions between the levels of the fixed factors were evaluated by pairwise comparisons. Statistical analysis was performed by procedure "General Linear Models > Univariate". Histopathological scores were analyzed by Kruskal-Wallis test (post hoc test: Dunn's Multiple Comparison test). P values <0.05 were considered statistically significant. Results were expressed as mean and pooled standard error of the mean (**SEM**).

RESULTS

Growth Performance

Birds remained healthy during the whole experimental period. The mortality rates of C (5%), TM5 (5%), TM10 (2.5%), and TM15 (2.5%) groups were not affected by dietary treatment ($P > 0.05$). Growth performance of the broiler chickens are summarized in Table 2. At 12 and 25 days of age, the LW increased linearly with increasing TM meal levels ($P < 0.001$ and $P < 0.05$, respectively) and the linear response increased to a maximum corresponding to the inclusion of

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Table 2. Effect of the dietary TM larvae meal inclusion on the growth performance of the male broiler chickens.[1]

Variable[3]	Age	Dietary treatments[2]				SEM	P[4]	
		C	TM5	TM10	TM15		Linear	Quadratic
LW (g)	12 d	287	308	350	358	10.12	<0.001	0.57
	25 d	1,105	1,129	1,272	1,253	43.80	0.018	0.67
	53 d	3,260	3,641	3,463	3,373	92.76	0.73	0.037
DFI (g)	1 to 12 d	23.8	30.0	30.5	35.6	0.75	<0.001	0.51
	12 to 25 d	81.4	111	104	113	2.55	<0.001	0.001
	25 to 53 d	184	220	209	225	11.65	0.06	0.43
FCR (g/g)	1 to 12 d	1.06	1.24	1.08	1.24	0.05	0.13	0.82
	12 to 25 d	1.33	1.78	1.47	1.73	0.10	0.07	0.38
	25 to 53 d	2.23	2.54	2.68	2.97	0.12	0.001	0.93
	1 to 53 d	1.92	2.26	2.18	2.46	0.11	0.010	0.80

[1]Each mean represents 5 replicates with 8 chicks/replicate (n = 40/treatment).
[2]Four dietary treatments: C = control; TM5 = 5% inclusion level of *Tenebrio molitor*; TM10 = 10% inclusion level of *Tenebrio molitor*; TM15 = 15% inclusion level of *Tenebrio molitor*.
[3]LW, live weight; DFI, daily feed intake; FCR, feed conversion ratio; n, number of pens.
[4]Statistical significance: $P < 0.05$.

Table 3. Effect of the dietary TM larvae meal inclusion on the haematological and serum parameters of the male broiler chickens.[1]

Variable[3]	Dietary treatments[2]				SEM	P[4]	
	C	TM5	TM10	TM15		Linear	Quadratic
Erythrocyte (10^6 cell/μL)	2.52	2.36	2.39	2.38	0.66	0.56	0.61
Leukocyte (10^3 cell/μL)	8.12	9.06	8.34	8.03	0.45	0.82	0.53
H/L ratio	0.82	0.83	0.89	0.83	0.03	0.73	0.62
Albumin (g/dL)	1.12	1.35	0.99	1.06	0.06	0.25	0.43
Total protein (g/dL)	3.40	3.71	3.43	3.13	0.20	0.57	0.48
GGT (UI/L)	23.04	20.64	21.07	19.48	0.89	0.23	0.83
AST (UI/L)	336.17	337.37	292.75	246.86	18.01	0.06	0.51
ALT (UI/L)	9.56	11.35	10.44	11.41	0.75	0.52	0.80
Uric acid (mg/dL)	5.85	4.12	4.85	4.32	0.33	0.20	0.36
Creatinine (mg/dL)	0.39	0.38	0.38	0.37	0.01	0.77	0.87
Triglycerides (mg/dL)	65.83	52.70	58.94	59.91	2.83	0.66	0.24
Cholesterol (mg/dL)	97.93	122.75	98.70	91.31	4.60	0.24	0.06
Glucose (mg/dL)	227.90	231.50	228.00	229.70	2.99	0.95	0.89
Phosphorus (mg/dL)	5.02	5.49	4.50	5.34	0.34	0.59	0.34
Magnesium (mEq/L)	1.49	1.15	1.02	1.32	0.10	0.30	0.80
Iron (μg/dL)	88.97	110.28	101.37	81.62	8.12	0.48	0.81

[1]Each mean represents 5 pens with 2 chicks/pen (n = 5/treatment).
[2]Four dietary treatments: C = control; TM5 = 5% inclusion level of *Tenebrio molitor*; TM10 = 10% inclusion level of *Tenebrio molitor*; TM15 = 15% inclusion level of *Tenebrio molitor*.
[3]H/L, heterophiles to lymphocytes ratio; GGT, gamma glutamyl transferase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; n, number of birds.
[4]Statistical significance: $P < 0.05$.

15 and 10% of TM meal, respectively. At 53 days of age, the LW showed quadratic response to increasing TM meal levels ($P < 0.05$), with a maximum corresponding to the inclusion of 5% of TM meal. In the period from 1 to 12 days of age, the response of DFI to the effect of TM meal inclusion was statistically significant ($P < 0.001$). In particular, the linear response increased to a maximum corresponding to the inclusion of 15% of TM meal. In the period from 12 to 25 days of age, the DFI increased linearly and quadratically with increasing TM meal levels ($P < 0.001$ and $P = 0.001$, respectively) and the linear and quadratic responses increased to a maximum corresponding to the inclusion of 15% of TM meal. On the contrary, the DFI showed no differences ($P > 0.05$) in the period from 25 to 53 days of age. In the periods from 1 to 12 and 12 to 25 days of age, the FCR was similar among the dietary treatments ($P > 0.05$). Differently, the FCR showed linear response to increasing TM meal levels in the periods from 25 to 53 ($P = 0.001$) and 1 to 53 days of age ($P < 0.05$), with a maximum corresponding to the inclusion of 15% of TM meal.

Haematological and Serum Parameters

Dietary TM meal inclusion did not influence ($P > 0.05$) the haematological and serum biochemical traits of the animals (Table 3).

Carcass Traits

Dietary TM meal inclusion did not affect ($P > 0.05$) the carcass traits of the broiler chickens (Table 4).

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Table 4. Effect of the dietary TM larvae meal inclusion on the carcass traits of the male broiler chickens.[1]

Variable	Dietary treatments[2]					P[3]	
	C	TM5	TM10	TM15	SEM	Linear	Quadratic
Live weight (LW) (g)	3,392	3,572	3,427	3,323	53.17	0.47	0.20
Carcass weight (g)	2,384	2,489	2,403	2,328	41.34	0.51	0.31
Carcass weight (% LW)	70.3	69.6	70.1	70.0	0.35	0.93	0.73
Breast (g)	622	656	664	613	16.20	0.91	0.22
Breast (% LW)	18.3	18.3	19.3	18.4	0.26	0.60	0.35
Thigh (g)	743	781	730	725	12.82	0.37	0.42
Thigh (% LW)	21.9	21.8	21.3	21.8	0.21	0.71	0.53
Spleen (g)	3.93	4.00	3.62	4.39	0.20	0.59	0.41
Spleen (% LW)	0.12	0.11	0.11	0.13	0.01	0.37	0.16
Liver (g)	59.4	66.1	57.8	54.0	1.93	0.15	0.16
Liver (% LW)	1.74	1.85	1.68	1.62	0.03	0.06	0.18
Gizzard (g)	68.6	77.6	68.8	65.9	2.02	0.34	0.14
Gizzard (% LW)	2.02	2.17	2.01	1.98	0.04	0.39	0.27
Abdominal fat (g)	40.5	43.3	33.8	43.5	2.25	0.99	0.46
Abdominal fat (% LW)	1.19	1.22	1.00	1.33	0.07	0.75	0.28

[1]Each mean represents 5 pens with 2 chicks/pen (n = 5/treatment).
[2]Four dietary treatments: C = control; TM5 = 5% inclusion level of *Tenebrio molitor*; TM10 = 10% inclusion level of *Tenebrio molitor*; TM15 = 15% inclusion level of *Tenebrio molitor*.
[3]Statistical significance: $P < 0.05$.

Table 5. Effects of diet, intestinal segment and interaction between diet and intestinal segment on the intestinal morphometric indices of the male broiler chickens.

Index	Fixed effect	d.f.[3]	F	P[4]
Vh (mm)	Diet[1]	3	2.194	0.09
	Intestinal segment[2]	2	48.132	<0.001
	Diet × Intestinal segment	6	1.195	0.32
Cd (mm)	Diet	3	2.875	0.040
	Intestinal segment	2	1.911	0.15
	Diet × Intestinal segment	6	0.124	0.99
Vh/Cd (mm/mm)	Diet	3	6.039	0.001
	Intestinal segment	2	15.813	<0.001
	Diet × Intestinal segment	6	0.671	0.67

[1]Four dietary treatments: C = control; TM5 = 5% inclusion level of *Tenebrio molitor*; TM10 = 10% inclusion level of *Tenebrio molitor*; TM15 = 15% inclusion level of *Tenebrio molitor*.
[2]Three intestinal segments: duodenum, jejunum and ileum.
[3]Degrees of freedom.
[4]Statistical significance: $P < 0.05$.
Vh, villus height; Cd, crypt depth; Vh/Cd, villus height to crypt depth ratio.

Table 6. Least square means of intestinal morphometric indices in male broiler chickens in relation to diet and intestinal segment.

Index	Fixed effect	Effect levels	Least square mean[1]	SEM
Vh (mm)	Diet[2]	C	2.05[a]	0.07
		TM5	2.06[a]	
		TM10	1.91[a,b]	
		TM15	1.87[b]	
	Intestinal segment[3]	DU	2.24[a]	0.06
		JE	2.16[a]	
		IL	1.52[b]	
Cd (mm)	Diet	C	0.17[a]	0.01
		TM5	0.17[a]	
		TM10	0.18[a,b]	
		TM15	0.19[b]	
	Intestinal segment	DU	0.18	0.01
		JE	0.18	
		IL	0.17	
Vh/Cd (mm/mm)	Diet	C	12.56[a]	0.54
		TM5	12.40[a]	
		TM10	10.66[b]	
		TM15	9.87[b]	
	Intestinal segment	DU	12.70[a]	0.47
		JE	12.19[a]	
		IL	9.23[b]	

[1]Means with different superscript letters (a, b) within the same column per fixed effect (i.e., diet, intestinal segment) differ significantly ($P < 0.05$).
[2]C = control; TM5 = 5% inclusion level of *Tenebrio molitor*; TM10 = 10% inclusion level of *Tenebrio molitor*; TM15 = 15% inclusion level of *Tenebrio molitor*
[3]DU = duodenum; JE = jejunum; IL = ileum.

Histomorphological Investigations

The effects of the diet, intestinal segment, and interaction between diet and intestinal segment on the intestinal morphometric indices are summarized in Tables 5 and 6. Vh depended on intestinal segment ($P < 0.001$) and showed a statistical trend for diet ($P = 0.093$). On the contrary, there was no influence of interaction between diet and intestinal segment ($P > 0.05$) on the morphometric index (Table 5). In particular, duodenum and jejunum showed higher Vh ($P < 0.001$) than ileum. Furthermore, lower Vh was found in TM15 animals ($P = 0.046$ and $P = 0.044$, respectively) compared with C and TM5 (Table 6). There was no effect of intestinal segment or interaction between diet and intestinal segment ($P > 0.05$) on Cd, while diet influenced ($P < 0.05$) the morphometric index (Table 5). In particular, TM15 birds showed higher Cd ($P < 0.01$ and $P < 0.05$, respectively) than C and TM5 (Table 6). Vh/Cd depended on diet and intestinal segment ($P < 0.01$ and $P < 0.001$, respectively), whereas there was no influence of interaction between diet and intestinal segment ($P > 0.05$) on the morphometric index (Table 5). In particular, TM10 and TM15 animals showed lower Vh/Cd compared with C ($P < 0.05$ and $P < 0.01$, respectively) and TM5 ($P < 0.05$ and $P < 0.01$, respectively). Furthermore, higher Vh/Cd was found in the duodenum and jejunum ($P < 0.001$) than the ileum (Table 6).

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Histopathological alterations varied from absent/minimal to severe in spleen, thymus, bursa of Fabricius, and liver for each dietary treatment, while heart and kidney showed no signs of inflammatory and/or degenerative diseases. Dietary TM inclusion did not affect ($P > 0.05$) the histopathological scores in any of the organs.

DISCUSSION

The present study evaluated the effects of dietary full-fat TM larvae meal inclusion on growth performance, haematochemical parameters, carcass traits, intestinal morphology, and histological findings of male broiler chickens.

Almost 50% of Italian male broiler chickens is generally slaughtered in the period from 50 to 60 days, in order to obtain heavy birds (3.4 to 3.6 kg of LW) for the production of cut-up and further processed products (Bianchi et al., 2007). The slaughter age of the present trial is also in agreement with previous studies (Sirri et al., 2016; Soglia et al., 2016).

Growth performance of the broiler chickens of the current study were consistent with the reference values recorded in the local poultry corporation in which the trial was conducted, but the final LW and overall FCR on average resulted −12.6% and +16.7% lower and higher than the Ross 708 broiler performance objectives (Aviagen, 2014). This could be related to the physical form of the feed, which was distributed in mash instead of the most frequently used crumble or pellet (Jahan et al., 2006). The body weight and feed intake of the birds in the present trial improved with increasing levels of TM meal inclusion, but the feed efficiency resulted impaired. Data regarding the TM meal utilization in poultry are limited and sometimes controversial. Ramos-Elorduy et al. (2002) showed no effects for the growth performance in fast-growing chickens fed sorghum-soybean meal-based diets in which the full-fat TM inclusion level ranged from 5 to 10% in partial substitution of soybean meal and vegetable oil. Similarly, Biasato et al. (2016) observed unaffected growth performance in intermediate-growing chickens fed corn-soybean-gluten meal-based diets in which the TM meal was included at 7.5% in complete substitution of corn gluten meal. On the contrary, Bovera et al. (2015 and 2016) found improved growth performance in fast-growing chickens fed on a corn-soybean meal-based diets in which the 29.6% of soybean meal was completely replaced by TM meal. In the current study, three different increasing levels of TM meal inclusion were tested in a corn-soybean-gluten meal-based diet replacing a portion of the dietary high-protein feedstuff sources (i.e., soybean and gluten meal) and soybean oil. In the present study, the body weight of the birds fed TM diets increased with increasing feed intake. Even if a feed-choice test was not performed, the increase of the average DFI in the birds may suggest an improvement of the diet palatability related to TM meal inclusion level. To the best of the authors' knowledge, no feed-choice trial has been performed to test the dietary inclusion of insect meal in broiler chickens. However, Cullere et al. (2016) performed a feed-choice test in broiler quails and observed that the birds tended to prefer the diets including *Hermetia illucens* meal. This may be related to the innate behavior of chickens, which naturally consume insects when reared in free-range systems (Zuidhof et al., 2003). However, the increase of the body weight and feed intake resulted in an overall impairment of the feed efficiency in the birds fed TM diets. Different hypotheses can be formulated to explain this negative effect. Firstly, the detrimental effect on FCR may be caused by the chitin content of TM diets, which has been reported to negatively influence the nutrient digestibility of crude protein (Razdan and Pettersson, 1994; Hossain and Blair, 2007) and organic matter (Razdan and Pettersson, 1994). Ravindran and Blair (1993) also pointed out that the chitin of insects is difficult to digest by domestic poultry. All these authors found no effects on growth performance of chickens fed diets supplemented with commercial sources of chitin instead of the whole insect of the current study (Razdan and Petterson, 1994; Hossain and Blair, 2007), but the chitin form may have influenced the results. Furthermore, the crude protein digestibility of TM meal used in the current trial was moderate (0.60), as reported by De Marco et al. (2015). Moreover, to the best of the authors' knowledge, this is the first study in which broiler chickens were fed diets with TM larvae meal from the hatch to the slaughter age. Indeed, the TM meal administration of previous studies started from 7 (Ramos-Elorduy et al., 2002; Ballitoc and Sun, 2013), 30 (Bovera et al., 2015), and 43 (Biasato et al., 2016) days of age, respectively, with an inclusion rate ranging from 0.5 (Ballitoc and Sun, 2013) to 29.6% (Bovera et al., 2015). Furthermore, in the present study modification in gut morphology has been observed, mainly in group TM15, and will be discussed successively.

The haematological and serum biochemical parameters give information about health status of the birds (Lumej, 2008; Salamano et al., 2010; De Marco et al., 2013). The results of the present trial suggest that dietary TM meal inclusion did not impair these blood parameters, thus confirming the safety of TM utilization in poultry diets, as previously observed by Bovera et al. (2015) and Biasato et al. (2016). Furthermore, all the blood parameters obtained in the present study fell within the physiological ranges (Lumej, 2008).

Dietary TM meal inclusion did not influence the carcass traits of the birds of the present trial. This is in agreement with Bovera et al. (2016) and Biasato et al. (2016), which did not find any differences for the same considered parameters. A similar figure was given by Hwangbo et al. (2009) and Cullere et al. (2016), who did not observe any effects after house-fly maggots and black soldier fly meal inclusion, respectively, in the diet of broiler chickens and quails. On the contrary, Ballitoc and Sun (2013) found improved slaughter, dressed carcass and eviscerated weights in broiler chickens fed TM diets.

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Dietary TM meal inclusion partially affected the intestinal morphology of the broiler chickens of the current study. In particular, shorter villi, deeper crypts, and reduced Vh/Cd were observed in the birds fed with 15% level of TM inclusion compared to the animals fed with TM5 and control diets, respectively. The small intestinal morphology, especially regarding crypts and villi of the absorptive epithelium, plays a key role in the final phase of nutrient digestion and assimilation (Wang and Pen, 2008). The physiological gut morphological development appears to be characterized by the identification of long villi and shallow crypts. Indeed, longer villi are generally associated with increased total luminal absorptive area and subsequent satisfactory digestive enzyme action and higher transport of nutrients (Laudadio et al., 2012). Simultaneously, shallower crypts reflect the prolonged survival of villi without the need for renewal (Oliveira et al., 2008), with reduced energy expenditure for this process and consequent enabled growth of other tissues (Miles et al., 2006). On the contrary, lower villus heights and greater crypt depths may lead to poor digestion and less absorption of nutrients and, as a consequence, poor performance of the animals (Qaisrani et al., 2014). Modifications of protein source and dietary structure has already been reported to negatively influence small intestinal morphology in broilers in terms of decreased Vh and increased Cd (Qaisrani et al., 2014). Therefore, the altered morphometric indices observed in male broilers fed with 15% level of TM meal inclusion could be related to the higher level of insects of this diet, thus also suggesting that lower levels may be preferable. This hypothesis seems to be supported by Biasato et al. (2016), which found no modifications of gut morphometric indices in free-range chickens fed diets including a 7.5% inclusion of TM meal. A similar consideration can also be applied for explaining the growth performance observed in the current study. Indeed, the overall FCR increased linearly with increased levels of TM meal, reaching its maximum with the inclusion of 15% of mealworms. The LW in the finisher period also increased quadratically till 5% level of TM meal inclusion, decreasing up to the inclusion of 15% of insects. Furthermore, the alterations of intestinal morphology can also justify the observed growth performance. Indeed, since it is well known that rapid growth of chickens directly depends on morphological and functional integrity of the digestive tract (Wang and Peng, 2008), the relationship between the altered gut morphometric indices and the worsening of the growth performance seems reasonable.

Apart from TM meal utilization, the current study confirms that morphometric indices followed a proximodistal decreasing gradient from the duodenum to the ileum, as previously reported (Iji et al., 2001; Forder et al., 2007; Murakami et al., 2007; Biasato et al., 2016). This is related to the intestinal absorption processes, which evolved differently depending on the considered segment. Indeed, the duodenum is the intestinal tract with the fastest cell renewal, and is also the first segment of the small intestine to receive physical, chemical and hormonal stimuli provoked by the presence of the diet in the lumen (Macari, 1998). Furthermore, the jejunum is an important site for nutrient digestion (Iji et al., 2001).

Dietary TM meal inclusion did not affect the histopathological features of the birds of the present trial, thus suggesting no negative influence on animal health. This is in agreement with Biasato et al. (2016), which did not find any differences in free-range chickens fed TM diets.

In conclusion, the present study suggests that increasing levels of dietary TM meal inclusion in male broiler chickens diets may improve body weight and feed intake, but negatively affect feed efficiency and intestinal morphology. However, no effect on haematochemical parameters, carcass traits, and histological findings are observed. These results advanced the hypothesis that low levels of TM meal inclusion (i.e., 5%) may be more suitable and overall confirm previous data concerning the safety of TM utilization in poultry feed.

ACKNOWLEDGMENTS

The authors are grateful to Dr. Oreste Massimino, Mr. Roberto Borgogno and Mr. Aldo Pozzo—"O.R.A. Agricola Srl" (Cherasco, CN—Italy)—for their technical support. The authors are also grateful to Dr. Paolo Montersino, Mr. Dario Sola and Mr. Mario Colombano for bird care and technical support. The research was supported by the University of Turin (2014-2015) and by the Regione Piemonte, Italy (PSR-PIAS no. 08000558869).

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