**Effects of dietary *Tenebrio molitor* meal inclusion in free-range chickens**

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**Summary**

Insects are currently being considered as a novel protein source for animal feeds, because they contain a large amount of protein. The larvae of *Tenebrio molitor* (TM) have been shown to be an acceptable protein source for broiler chickens in terms of growth performance, but till now, no data on histological or intestinal morphometric features have been reported. This study has had the aim of evaluating the effects of dietary TM inclusion on the performance, welfare, intestinal morphology and histological features of free-range chickens. A total of 140 medium-growing hybrid female chickens were free-range reared and randomly allotted to two dietary treatments: (i) a control group and (ii) a TM group, in which TM meal was included at 75 g/kg. Each group consisted of five pens as replicates, with 14 chicks per pen. Growth performance, haematological and serum parameters and welfare indicators were evaluated, and the animals were slaughtered at the age of 97 days. Two birds per pen (10 birds/treatment) were submitted to histological (liver, spleen, thymus, bursa of Fabricius, kidney, heart, glandular stomach and gut) and morphometric (duodenum, spleen, thymus, bursa of Fabricius, kidney, heart, glandular stomach and gut) investigations. The inclusion of TM did not affect the growth performance, haematological or serum parameters. The morphometric and histological features were not significantly affected either, thus suggesting no influence on nutrient metabolism, performance or animal health. Glandular stomach alterations (chronic flogosis with epithelial squamous metaplasia) were considered paraphysiological in relation to free-range farming. The observed chronic intestinal flogosis, with concomitant activation of the lymphoid tissue, was probably due to previous parasitic infections, which are very frequently detected in free-range chickens. In conclusion, the findings of this study show that yellow mealworm inclusion does not affect the welfare, productive performances or morphological features of free-range chickens, thus confirming that TM can be used safely in poultry diets.

**Keywords** poultry, insect meal, yellow mealworm, histology, morphometry

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**Introduction**

Insects are currently considered a novel protein source for animal feeds (Sánchez-Muros et al., 2014). Insect meals exhibit a great potential for becoming a standard ingredient in animal feeding, because of the high quality and quantity of protein (Ramos-Elorduy, 1997). Furthermore, the low competitiveness with human food (Ballitoc and Sun, 2013) and the reduction in environmental impact in terms of energy cost, land area utilization and footprints (Oonincx and de Boer, 2012; Makkar et al., 2014; Sánchez-Muros et al., 2014) make insects promising in an ecological perspective. Invertebrates are included in the European Union Feed Material Register as a feed material, even though they are currently only authorized for pets. However, insect-derived feeds could also represent a possible ingredient for livestock animals, such as poultry, pigs and fish (Veldkamp et al., 2012; van Huis, 2013; Makkar et al., 2014; Henry et al., 2015).
In particular, the most promising insect species for industrial production are *Hermetia illucens* (black soldier fly), *Musca domestica* (common house fly), *Tenebrio molitor* (yellow mealworm), *Bombbyx mori* (silkworm) and several grasshoppers (van Huis, 2013).

Considering that insects are consumed naturally by wild birds and free-range poultry (Zuiddhof et al., 2003), some studies have evaluated the feasibility of using insects as an alternative feed source for poultry (Khatun et al., 2003; Wang et al., 2005; Oyegoke et al., 2006; Adenjii, 2007; Hwangbo et al., 2009; Ijaiya and Eko, 2009; Ballitoc and Sun, 2013). Some authors have observed no differences in growth performance (in terms of feed intake, body weight gain and feed conversion efficiency) in broilers fed a control diet and an insect-based diet (Wang et al., 2005; Oyegoke et al., 2006; Adenjii, 2007; Ijaiya and Eko, 2009). Other studies have reported that insect meal inclusion in chicken diets improved animal growth indexes (Khatun et al., 2003; Hwangbo et al., 2009; Ballitoc and Sun, 2013). The same studies also observed an improvement in carcass yield characteristics, such as dressing percentage, breast muscle, thigh muscle, slaughter, dressed carcass and eviscerated weights (Khatun et al., 2003; Hwangbo et al., 2009; Ballitoc and Sun, 2103).

The larvae of *Tenebrio molitor* (TM) are easily bred, because of their efficient growth on dried and cooked waste materials from fruit, vegetables and cereals in various combinations. For this reason, they are already industrially produced as feeds for pets and zoo animals, including birds, reptiles, small mammals, amphibians and fish (Makkar et al., 2014). On a dry matter basis, the meal derived from TM larvae contains a large amount of crude protein (440–690 g/kg) and fat (230–470 g/kg) (Veldkamp et al., 2012). In livestock, TM has been shown to be an acceptable protein source for broiler chickens (Ramos-Elorduy et al., 2006; Ballitoc and Sun, 2013) and fish (Belforti et al., 2015; Roncarati et al., 2015).

Intestinal morphology is the main indicator of gut health and functioning (Kuzmuk et al., 2005). Dietary protein level and digestibility have been reported to significantly affect the intestinal development and the mucosal architecture of the gastrointestinal tract of broilers (Laudadio et al., 2012; Qaisrani et al., 2014). Intestinal development can be assessed through morphometric measurements of the villus height (to determine the area available for digestion and absorption) and crypt depth (the region in which new intestinal cells are formed) (Franco et al., 2006). The villus height/crypt depth ratio can also be evaluated, because it generally gives an indication of the likely maturity and functional capacity of the enterocytes (Hampson, 1986).

The nutrient profile and structure of protein sources may also vary significantly, and thus have different effects on nutrient utilization and metabolism, and consequently on some blood constituents, such as serum cholesterol, triglycerides and uric acid (Wang et al., 2015). Some blood parameters have been used as physiological indicators of the stress response of chickens. The heterophils-to-lymphocytes (H/L) ratio is affected by stress factors and could be used as an indicator of stress in poultry (Salaman et al., 2010; De Marco et al., 2013). The H/L ratio is correlated to a bird’s health status and responds to stimuli associated with diet, chronic bacterial infections, stress, light and trauma, and it varies according to the change in the percentage of heterophils and lymphocytes in the blood (Gross and Siegel, 1983; Maxwell, 1993; Maxwell and Robertson, 1998). Finally, diet composition can also affect the litter quality (Lynn and Elson, 1990; Jones et al., 2005), which is directly related to the development of footpad dermatitis (FPD) (Bessei, 2006). FPD is a condition that involves inflammation and necrotic lesions on the plantar surface of the bird’s feet and is considered to be an important welfare indicator in broilers (Ekstrand et al., 1997; Meluzzi et al., 2008; Welfare Quality®, 2009).

Although insect meals are considered a suitable ingredient for poultry feeding (Veldkamp et al., 2012; van Huis, 2013; Makkar et al., 2014), there is currently a lack of data on their utilization. Apart from the evaluation of growth performance and carcass yield characteristics, no anatomopathological or morphometric investigations, blood traits analysis or welfare assessments have been carried out on chickens fed diets with insect meal inclusion. Therefore, the aim of this study was to evaluate the effects of TM dietary inclusion on the productive performance, intestinal morphology, histological features, haematochemical parameters and welfare of free-range chickens.

**Materials and methods**

The study was performed by the Department of Veterinary Sciences and the Department of Agricultural, Forest and Food Sciences of the University of Torino (Italy) in collaboration with an external farm called ‘Fattoria La Fornace’, located in Montechiaro d’Asti (At – 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 (Directive 2010/63/EU, put into force in Italy with D.L. 2014/26). The experiment was
carried out between November and December 2014, when the photoperiod was 9-10L:14-15D.

Birds and diets

In this experiment, a total of 140 female Label Hubbard hybrid chickens (female: JA 57 × male: S77CN), a medium-growing genotype, were used. All the birds were free-range reared, in identical environmental conditions, throughout the experimental trial. At the age of 43 days (average weight 715 g), the birds were randomly allotted to two dietary treatments, each consisting of five pens as replicates, with 14 chicks per pen. Each pen had an indoor area (2.5 × 3.5 m) and an outdoor paddock of the same dimension (2.5 × 3.5 m). The floor was covered, to a height of 10 cm, with wood shaving litter. The birds were only exposed to natural light. Two diets were formulated: a control diet (C), normally used by the breeder, and an experimental diet (TM), in which TM meal (Gaobeidian Biology, Gaobeidian, Hebei province – China) was included at 75 g/kg in substitution of corn gluten meal (Table 1). The diets were designed to meet or exceed the current poultry requirements (National Research Council (NRC), 1994). Both diets were isonitrogenous and isoenergetic and were formulated using the apparent metabolizable energy (AMEn) values for broiler chickens (De Marco et al., 2015). Feed and water were provided ad libitum.

The experiment lasted 54 days. The average chicken weight and feed intake were recorded at the beginning and at the end of the experiment on a pen basis. The final body weight was recorded on day 97, and the feed conversion ratio was calculated for the 43- to 97-day period.

The chicks were vaccinated at hatching against cocidiosis, Newcastle disease and infectious bronchitis. All the birds were individually identified with a shank ring.

Chemical analyses of the diets

The diets were subsequently ground to pass through a 0.5-mm sieve and stored in airtight plastic containers for DM, ash, CP, crude fibre (AOAC, 2005) and EE (Folch et al., 1957) determination. The fatty acid composition of the control and TM diets was assessed using the method described by Alves et al. (2008). Fatty acid methyl esters were separated, identified and quantified on the basis of the chromatographic conditions reported by Renna et al. (2014). Heptadecanoic acid (C17:0) was used as the internal standard. The results were expressed in absolute values as g/kg DM.

To perform the AA determination, samples of the diets were prepared using a 22-h hydrolysis step in 6 HCl at 112 °C under a nitrogen atmosphere. Performic acid oxidation occurred prior to acid hydrolysis for determination. The fatty acid composition (g/kg DM)

<table>
<thead>
<tr>
<th>Fatty acid composition (g/kg DM)</th>
<th>Control diet</th>
<th>TM diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>C16:0</td>
<td>3.13</td>
<td>6.24</td>
</tr>
<tr>
<td>C18:0</td>
<td>0.47</td>
<td>1.06</td>
</tr>
<tr>
<td>C18:1 n9</td>
<td>4.00</td>
<td>10.05</td>
</tr>
<tr>
<td>C18:2 n6</td>
<td>10.42</td>
<td>20.41</td>
</tr>
<tr>
<td>C18:3 n3</td>
<td>0.52</td>
<td>0.73</td>
</tr>
<tr>
<td>Other fatty acids‡</td>
<td>0.35</td>
<td>1.12</td>
</tr>
<tr>
<td>Total SFA†</td>
<td>3.66</td>
<td>7.75</td>
</tr>
<tr>
<td>Total MUFA‡</td>
<td>4.29</td>
<td>10.71</td>
</tr>
<tr>
<td>Total PUFA‡</td>
<td>10.94</td>
<td>21.15</td>
</tr>
</tbody>
</table>

‡ The mineral-vitamin premix (Trevioli Volatili 3.5 – Trei – Rio Saliceto (RE) Italy) given values are supplied per kg: 650.000 IU vitamin A; 65.000 IU vitamin D3; 650 IU vitamin E; 80 mg vitamin K; 80 mg vitamin B1; 150 mg vitamin B2; 770 mg vitamin B3; 80 mg vitamin B6; 0.5 mg vitamin B12; 240 mg pantothenic acid; 4700 mg betaine; 1750 mg iron (II) carbonate; 1835 mg Magnesium oxide; 1612 mg Zinc oxide; 178 mg Copper (II) oxide; 18.3 mg Potassium iodide; 6.6 mg Sodium selenite; 4100 mg DL-methionine; 5500 mg L-lysine; 120 g Calcium carbonate; 450 g Calcium phosphate; 11.5 g Sodium chloride. †SFA: saturated fatty acids. ‡MUFA: monounsaturated fatty acids. §PUFA: polyunsaturated fatty acids. ¶Other FAs (all <0.40 g/kg DM): C12:0, C14:0, C14:1 cis9, C16:1 cis9, C18:1 cis11, C20:0, C18:3 n6, C20:1 cis9, C20:1 cis11.
Haematological and serum parameters

At the end of the experiment (day 97), blood samples were collected at slaughtering from 4 birds per pen: 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 cell counts were determined in an improved Neubauer haemocytometer on blood samples previously treated with a 1:200 Natt–Herrick solution. One hundred leucocytes, 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 approximately two hours 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 total proteins were quantified by means of the ‘biuret method’ (Bio Group Medical System kit; Bio Group Medical System, Talamello, Italy); the electrophoretic pattern of the serum was obtained using a semi-automated agarose gel electrophoresis system (Sebia Hydrasys®, Norcross, GA, USA). The alanina-aminotransferase (ALT), aspartate aminotransferase (AST), 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., Florence, Italy).

Slaughtering procedures, footpad dermatitis lesion assessment, histological investigations and morphometric analysis

On day 97, all the chickens were individually marked, weighed and slaughtered 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 the breasts, thighs, deboned thighs, spleen, bursa of Fabricius, liver, gizzard and abdominal fat was recorded.

To evaluate the FPD lesions, the feet collected at the slaughterhouse were assessed macroscopically using the so-called Swedish footpad scoring system (Ekstrand et al., 1997). According to this system, 0 = no lesion, slight discoloration of the skin or healed lesion; 1 = mild lesion, superficial discoloration of the skin and hyperkeratosis; 2 = severe lesion, affected epidermis, blood scabs, haemorrhage and severe swelling of the skin.

After slaughtering, 2 birds per pen (10 birds/treatment) were sampled for anatopathological investigations. Intestinal segment samples (approximately 5 cm in length) of the duodenum, jejunum, ileum and caecum were excised and flushed with 0.9% saline to remove all the contents. The collected intestine segments were the loop of the duodenum, the tract before Meckel’s diverticulum (jejenum), the tract before the ileocolic junction (ileum) and the apex of the caeca (caecum). Samples of the liver, spleen, thymus, bursa of Fabricius, kidneys, heart and glandular stomach were also collected. The gut segments were fixed in both 10% buffered formalin (for the histological examination) and Carnoy’s solution (for the morphometric analysis), while the other organ samples were only fixed in 10% buffered formalin solution. The tissues were routinely embedded in paraffin wax blocks, sectioned at a thickness of 5 μm, mounted onto glass slides and stained with Haematoxylin & Eosin (H&E). Morphometric analyses (Image Pro-Plus software) (Fig. 1a) were performed on 10 well-oriented and intact villi and 10 crypts chosen from the duodenum, jejunum and ileum (Qaisrani et al., 2014). 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 villi to the submucosa) and their ratio (Vh/Cd) (Laudadio et al., 2012). Histological changes were scored using a semi-quantitative scoring system as follows: absent or minimal (score 0), mild (score 1) and severe (score 2). The semi-quantitative scoring system was assessed evaluating the defined parameters of each organ (Table 2).

Statistical analysis

The statistical analysis was performed using SPSS 17 for Windows (SPSS, Chicago, IL, USA; SPSS, 2008). The experimental unit was the pen. The influence of diet on the performance parameters, haematological and serum biochemical traits and intestinal morphometric measurements were analysed using Student’s t-tests for independent samples. Morphometric data were also analysed by means of one-way ANOVA (post hoc test: Duncan’s multiple range test) to evaluate the influence of the intestinal segment within each dietary treatment. Mann–Whitney U-tests were used to compare the histological scores between treatments for each considered organ (spleen, thymus, bursa of Fabricius, liver, glandular stomach, intestine, heart and kidney). Kruskal–Wallis tests were used to compare intestine alterations among the considered segments (duodenum, jejunum,
ILEUM and CAECUM), WITHIN EACH DIETARY TREATMENT. THE RESULTS WERE CONSIDERED STATISTICALLY SIGNIFICANT WHEN ASSOCIATED WITH A LOWER PROBABILITY THAN 5%, AND HIGHLY SIGNIFICANT IF THE PROBABILITY WAS LOWER THAN 1%. THE RESULTS WERE EXPRESSED AS MEAN AND POOLED STANDARD ERROR OF THE MEAN (SEM).

RESULTS

THE INCLUSION OF TM IN THE MEDIUM-GROWING DIET IN THE 43- TO 97-D PERIOD DID NOT AFFECT THE GROWTH AND SLAUGHTERING PERFORMANCES (TABLE 3), THE BLOOD AND SERUM TRAITS (TABLE 4) OR THE FPD LESION INCIDENCE. THE BLOOD PARAMETERS FELL WITHIN THE PHYSIOLOGICAL RANGE.

THE MORPHOMETRIC DATA ARE SUMMARIZED IN TABLE 5. THERE WAS NO SIGNIFICANT DIFFERENCE IN THE MORPHOLOGY OF THE SMALL INTESTINE BETWEEN THE TWO DIETARY TREATMENTS (P > 0.05). IN THE C GROUP, THE Vh WAS GREATER (P < 0.01) IN THE DUODENUM THAN IN THE OTHER GUT SEGMENTS, WHILE THE Vh/Cd RATIO WAS ONLY SIGNIFICANTLY DIFFERENT (P = 0.01) BETWEEN THE DUODENUM AND THE ILEUM. IN THE TM GROUP, Vh AND Cd WERE GREATER (P < 0.01 AND P = 0.03) IN THE DUODENUM THAN IN THE OTHER GUT SEGMENTS.

THE GLANDULAR STOMACH, INTESTINE, SPLEEN, THYMUS, BURSA OF FABRICIUS AND LIVER WERE THE MOST FREQUENTLY AFFECTED ORGANS, WHILE THE HEART AND KIDNEYS SHOWED NO SIGNIFICANT ALTERATIONS. CHRONIC INFLAMMATION, WITH LYMPHOID TISSUE ACTIVATION, WAS OBSERVED IN THE INTESTINAL SEGMENTS (FIG. 1b). THE GLANDULAR STOMACH SHOWED LYMPHOPLASMACYTIC FLOGOSIS, WITH FOCAL TO MULTIFOXLymphoid tissue activation and different degrees of severity of epithelial squamous metaplasia (FIG. 1c and d). THE JEJUNUM AND THE CAECUM SHOWED THE MOST SEVERE ALTERATIONS IN BOTH DIETARY TREATMENTS (P < 0.01). WHITE PULP HYPERPLASIA OR DEPLETION WAS IDENTIFIED IN THE SPLEEN, WHILE COR-TICAL DEPLETION WAS DETECTED IN THE THYMUS. FOLLICULAR DEPLETION, WITH OR WITHOUT INTRAFOXLymphoid cysts, WAS OBSERVED IN THE BURSA OF FABRICIUS (FIG. 2a AND b). FINALLY, THE LIVER SHOWED DIFFERENT DEGREES OF LYMPHOID TISSUE ACTIVATION.

**Table 2** Parameters evaluated for the histological semi-quantitative scoring system

<table>
<thead>
<tr>
<th>Organ</th>
<th>Alterations</th>
<th>Parameters evaluated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spleen</td>
<td>White pulp hyperplasia</td>
<td>Number and dimension of follicles</td>
</tr>
<tr>
<td></td>
<td>White pulp depletion</td>
<td>Number of apoptosis</td>
</tr>
<tr>
<td></td>
<td>Thymus</td>
<td>Cell types</td>
</tr>
<tr>
<td></td>
<td>Bursa of Fabricius</td>
<td>Cortico-medullar ratio</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>Number of apoptosis</td>
</tr>
<tr>
<td></td>
<td>Glandular stomach</td>
<td>Cell types</td>
</tr>
<tr>
<td></td>
<td>Intrafollicular cysts</td>
<td>Number of lymphoid aggregates</td>
</tr>
<tr>
<td></td>
<td>Intestine</td>
<td>Distribution of inflammatory infiltrates</td>
</tr>
<tr>
<td></td>
<td>Lymphoid tissue activation</td>
<td>Number and dimension of follicles</td>
</tr>
<tr>
<td></td>
<td>Lymphoidplasmacytic flogosis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lymphoid tissue activation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Intestinal squamous metaplasia</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lymphoidplasmacytic flogosis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lymphoid tissue activation</td>
<td></td>
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</tbody>
</table>

**Fig. 1** Histological and morphometric evaluation of the gastrointestinal tract of the free-range chickens. (a) TM group. Morphometric measurements of the villus height (Vh) and the crypt depth (Cd) in the jejunum segment. 2.5× H&E. (b) TM group. Severe and diffuse mucosal lymphoplasmacytic and macrophagic flogosis with activation of the lymphoid tissue (*) in the caecum. 5× H&E. (c) C group. A normal glandular stomach. 5× H&E. (d) C group. Severe and diffuse mucosal lymphoplasmacytic flogosis with epithelial squamous metaplasia (arrow) and activation of the lymphoid tissue (*) in the glandular stomach. 5× H&E.
Affect the performance, the blood and serum traits or free-range conditions. The inclusion of TM did not in the diet of medium-growing chickens reared in the trial was set up to study the effect of TM inclusion in the diet of Shaver brown broilers did not affect the final body weight, feed intake and feed efficiency, as well as the slaughter yield. Bovera et al. (2015) showed that the inclusion level of 10 g/kg TM had a great impact on the growth performance of the broilers and improved the final body weight, feed intake and feed efficiency. In another study, it was noted that TM could be successfully used to replace 4% soybean meal in laying hens diets (Wang et al., 2015). Footpad dermatitis is a condition that involves inflammation and necrotic lesions on the plantar surface of the bird’s feet. It is considered to be an important welfare indicator in broilers (Ekstrand et al., 1997; Meluzzi et al., 2008). It is histologically validated and is easy to use for the routine (Fig. 2c and d). The histological changes were not significantly different between the dietary treatments (p > 0.05).

### Discussion

The trial was set up to study the effect of TM inclusion in the diet of medium-growing chickens reared in free-range conditions. The inclusion of TM did not affect the performance, the blood and serum traits or the welfare parameters of the birds. These results confirm that TM can be used safely in poultry diets. Ramos-Elorduy et al. (2006) showed that up to 100 g/kg of dried yellow mealworms can be included in a broiler starter diet based on sorghum and soya bean meal, without negative effects on either performance or palatability. Ballitoc and Sun (2013), including different levels of TM meal (5, 10, 20 and 100 g/kg respectively) in a standard commercial broiler diet, pointed out that the inclusion level of 10 g/kg TM had a great impact on the growth performance of the broilers and improved the final body weight, feed intake and feed efficiency, as well as the slaughter yield. Bovera et al. (2015) showed that 30% inclusion of TM meal in the diet of Shaver brown broilers did not affect the final body weight or blood traits, except for uric acid, albumin/globulin ratio, AST and ALT. In another study, it was noted that TM could be successfully used to replace 4% soybean meal in laying hens diets (Wang et al., 2015).
assessment of broiler welfare in processing plants (Michel et al., 2012). A low prevalence and severity of FPD is highly desirable as far as the health of birds and product quality are concerned (Abd El-Wahab et al., 2012). Furthermore, the lesions can be a gateway for bacteria that may spread haematogenously and impair product quality. Birds with severe lesions may also show reduced weight gains, due to pain-induced decreases in feed intake (Martland, 1985). FDP can be caused by several factors, the most important being the condition of the litter in the broiler house (Bessei, 2006). Litter quality can be affected by diet composition, as well as by the number and design of the drinkers in the pen (Lynn and Elson, 1990; Jones et al., 2005). The score obtained in the present study was zero for all of the birds, thus showing that they were in a good welfare condition.

Intestinal morphology is the main indicator of gut health and functioning (Kuzmuk et al., 2005). In the present study, mealworm inclusion did not affect the morphology of the small intestine, thus suggesting no influence on nutrient metabolism or performance. The greater development of the duodenum, in relation to the other intestinal segments, is in agreement with the results of Uni et al. (1999), Kondo (2003) and Murakami et al. (2007). In fact, 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).

In the present study, mealworm inclusion did not induce histological changes, thus suggesting no negative influence on animal health. Glandular stomach alterations were considered paraphysiological because, in the authors’ opinion, they were probably related to the free-range farming system. Outdoor access in fact entails less control of animal feeding, with the possible ingestion of foreign bodies. However, the activation of the lymphoid tissue, in both dietary treatments, represents the most interesting result. It has been reported that keeping birds in free-range systems may favour the occurrence of gastrointestinal parasitic diseases, with a high prevalence of coccidiosis and helminthiasis (Magwisha et al., 2002; Tomza-Marciniak et al., 2014). Although all the birds were vaccinated against coccidiosis, the immunological variation between strains of the same species might not have conferred total protection (Chapman, 2014). Furthermore, the chronic flogosis observed in the intestinal segments could suggest previous parasitic infections, with subsequent activation of the lymphoid tissue. Tong et al. (2015) also evaluated the effects of outdoor access on the lymphoid organ index in a local chicken breed and found that the spleen was the only lymphoid organ that responded to outdoor access. The thymus, bursa of Fabricius and liver were not altered, but the possibility of an adaption of the birds to the environment, in terms of immune system modification, cannot be excluded.
In conclusion, the findings of this study suggest that the inclusion of yellow mealworm in free-range chicken diets does not affect the welfare, productive performance or morphological features of the birds. These results confirm previous data concerning the safety of TM meal use in poultry diets. A key point in future studies would be to establish the point of view of European consumers regarding the use of insects as feed for livestock. Legislative issues will also have to be addressed at a European level, in order to allow insect meal to be used as a possible alternative to soybean meal in broiler diets. British Poultry Science 56, 569–579.


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Tenebrio molitor meal in free-range chicken diets


