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**Effects of yellow mealworm larvae ( *Tenebrio molitor* ) inclusion in diets for female broiler chickens: implications for animal health and gut histology**

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1           **Effects of yellow mealworm larvae (*Tenebrio molitor*)**  
2           **inclusion in diets for female broiler chickens: implications**  
3           **for animal health and gut histology**

4

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48

49 **Abstract**

50 The aim of the present study was to evaluate the animal  
51 performance, haematochemical parameters, intestinal  
52 morphology and histological features of broiler chickens fed  
53 diets including *Tenebrio molitor* (TM) larvae meal. A total of  
54 160 female broiler chicks (Ross 708) at one-day of age were  
55 randomly allotted to four dietary treatments: a control (C)  
56 group and three TM groups, in which TM meal was included at  
57 50, 100 and 150 g/kg, respectively. Each group consisted of  
58 five pens as replicates, with eight chicks per pen. After the  
59 evaluation of growth performance and haematochemical  
60 parameters, two birds per pen were slaughtered at 40 days and  
61 carcass traits were recorded. Morphometric investigations were  
62 performed on duodenum, jejunum and ileum and  
63 histopathological alterations were assessed for liver, spleen,  
64 thymus, bursa of Fabricius, kidney and heart. The live weight  
65 (LW) showed a linear (12 days,  $P < 0.05$ , maximum with  
66 TM15) and quadratic response (40 days,  $P < 0.05$ , maximum  
67 with TM5) to dietary TM meal inclusion. The average daily  
68 gain (ADG) showed a linear increase (1-12 days,  $P < 0.05$ ,  
69 maximum with TM15) in response to TM meal utilization. A  
70 linear effect (1-12 and 12-25 days,  $P < 0.01$  and  $P < 0.05$ ,  
71 maximum with TM15 and TM5) was observed for the daily  
72 feed intake (DFI). The feed conversion ratio (FCR) showed a  
73 linear response to TM utilization in the period 12-25 days ( $P <$

74 0.01, maximum with TM15). A quadratic effect ( $P < 0.05$ ,  
75 maximum with TM5) was observed for the carcass weight. The  
76 abdominal fat weight and percentage showed a linear response  
77 to dietary TM meal inclusion ( $P < 0.05$  and  $P < 0.01$ , maximum  
78 with TM15 and TM10). A quadratic increase ( $P < 0.05$ ,  
79 maximum with TM10) was observed for the erythrocytes,  
80 while the albumin and GGT showed a linear and quadratic  
81 decrease ( $P < 0.05$ , minimum with TM10) in relation to TM  
82 utilization. Gut morphology and histopathological findings  
83 were not significantly influenced ( $P > 0.05$ ) by dietary TM  
84 meal inclusion. The present study suggests that increasing  
85 levels of dietary TM meal inclusion in female broiler chickens  
86 diets may improve body weight and feed intake, but can  
87 partially worsen feed efficiency. However, positive effects on  
88 carcass traits and haematochemical parameters related to TM  
89 meal utilization are observed, along with no negative influence  
90 on gut morphology and histological findings.

91

92 **Keywords**

93 Poultry; *Tenebrio molitor*; insect meal; growth performance;  
94 histology; morphometry.

95

96 **Introduction**

97 World population is expected to increase by over a third,  
98 reaching over 9 billion people in 2050. This trend suggests that  
99 market demand for food will continue to grow. In particular,  
100 the demand for cereals and protein sources in both human food  
101 and animal feed is projected to have an exponential growth by  
102 2050 (FAO, 2013). Consequently, the world supply of some  
103 conventional feedstuffs like soybean and maize will  
104 increasingly compete between humans and livestock.  
105 Therefore, the foremost gamble will be the identification of  
106 alternative sources of protein, energy and other nutrients for  
107 livestock, in order to avoid such a competition.

108 The potential of insects for becoming a standard ingredient in  
109 animal feeds has already been emphasized by several studies  
110 (Veldkamp et al., 2012; Van Huis, 2013; Henry et al., 2015),  
111 because of the high quality and quantity of protein (Makkar et  
112 al., 2014), the low competitiveness with human food (Ballitoc  
113 and Sun, 2013) and the reduction of the environmental impact  
114 (Oonincx and de Boer, 2012; Makkar et al., 2014; Sánchez-  
115 Muros et al., 2014). Currently, the considered most valuable  
116 insect species to be used in livestock feeds are *Hermetia*  
117 *illucens* L. (black soldier fly), *Musca domestica* L. (common  
118 house fly), *Tenebrio molitor* L. (yellow mealworm), *Bombyx*  
119 *mori* L. (silkworm) and several grasshoppers (Van Huis, 2013).  
120 In particular, *Tenebrio molitor* (TM) and *Hermetia illucens*

121 have recently been used in poultry (Biasato et al., 2016; Bovera  
122 et al., 2016; Schiavone et al., 2017a) and fish (Belforti et al.,  
123 2015; Gasco et al., 2016; Renna et al., 2017) feeding. Yellow  
124 mealworms are already industrially produced as feed for pets  
125 and zoo animals, such as birds, reptiles, small mammals,  
126 amphibians and fish, and TM larvae are easily bred on dried  
127 waste materials, being able to recycle them into high-quality  
128 feed with less energy cost, land area utilization and footprints  
129 (Makkar et al., 2014). The influence of TM-based diets on  
130 growth performance (Bovera et al., 2015; Bovera et al., 2016;  
131 Biasato et al., 2016) haematochemical profile (Bovera et al.,  
132 2015; Biasato et al., 2016) and carcass traits (Ballitoc and Sun,  
133 2013; Bovera et al., 2016; Bisato et al., 2016), has recently  
134 been investigated. Gut morphology, which has been reported to  
135 be widely affected by dietary modifications in broilers  
136 (Laudadio et al., 2012; Gopinger et al., 2014; Qaisrani et al.,  
137 2014), has also been evaluated in free-range chickens fed diets  
138 with dietary TM larvae meal inclusion (Biasato et al., 2016).  
139 Despite insect meals being considered suitable ingredients for  
140 poultry feeding (Veldkamp et al., 2012; van Huis, 2013;  
141 Makkar et al., 2014), the implications of their utilization on  
142 poultry health and gut development are still very limited. The  
143 aim of the present study was to evaluate the growth  
144 performance, haematochemical parameters, carcass traits,

145 intestinal morphology and histological features of female  
146 broiler chickens fed diets including TM meal.

147

## 148 **Materials and Methods**

### 149 *Birds and Husbandry*

150 The present trial was performed in collaboration with a local  
151 poultry corporation named “O.R.A. Agricola S.r.l.” sited in  
152 Cherasco (Cuneo, Italy). The experimental protocol was  
153 designed according to the guidelines of the current European  
154 and Italian laws on the care and use of experimental animals  
155 (European Directive 86 609/EEC, put into law in Italy with  
156 D.L. 116/92). Furthermore, the experimental protocol was  
157 approved by the Ethical Committee of the Department of  
158 Veterinary Sciences of the University of Turin (Italy). A  
159 poultry house of 14 m wide × 141 m long × 4.7 m high,  
160 equipped with waterproof floor and wall, completely covered  
161 by tiles and provided with automatic ventilation system was  
162 used.

163 A total of 160 female broiler chicks (Ross 708) at one-day of  
164 age were randomly allotted to 4 dietary treatments, each  
165 consisting of 5 pens as replicates with 8 chicks per pen. Each  
166 pen was 1.20 m wide × 1.20 m long and was equipped with a  
167 feeder occupying a surface of almost 1800 cm<sup>2</sup>, three nipple  
168 drinkers and rice hulls as litter. During the first three weeks, the  
169 animals were heated by infrared lamps to maintain the suitable



170 temperature according to standard breeding practices (Aviagen,  
171 2014). Lighting schedule was 23 hours light : 1 hour darkness  
172 until day 3 and 18 h light : 6 hours darkness until slaughter age.  
173 At hatching, all chicks received vaccination against Newcastle  
174 disease, Gumboro disease, infectious bronchitis and  
175 coccidiosis. Vaccine recalls were performed on day 9 for  
176 infectious bronchitis and on day 18 for Gumboro and  
177 Newcastle diseases.

178

### 179 *Diets*

180 A basal diet based on corn meal, corn gluten meal and soybean  
181 meal was formulated and served as control (C) group, while 50,  
182 100 and 150 g/kg full-fat TM larvae meal (Gaobeidian  
183 Shannong Biology CO., LTD, Gaobeidian, Hebei province,  
184 China) inclusion as a partial replacement of soybean meal, corn  
185 gluten meal and soybean oil constituted the three experimental  
186 treatment groups (TM5, TM10 and TM15) (Table 1). TM meal  
187 nutritive composition and energy content were the following:  
188 dry matter, 939.0 (g/kg as fed); organic matter, 912.0 (g/kg as  
189 fed); crude protein (CP), 519.0 (g/kg as fed); ether extract (EE),  
190 236.0 (g/kg as fed); 117.0 (g/kg as fed); neutral detergent fibre  
191 (NDF); 79.5 (g/kg as fed); acid detergent fibre (ADF); DL-  
192 methionine, 10.1 (g/kg as fed); L-lysine, 35.9 (g/kg as fed);  
193 gross energy, 24.4; apparent metabolizable energy (AMEn),  
194 16.02 (MJ/kg DM). Three different diets were used per each

195 dietary treatments during the three phases of growth: a starter  
196 diet (days 1 to 12), a grower diet (days 12 to 25) and a finisher  
197 diet (day 25 to 40). For each phase, the experimental diets were  
198 isonitrogenous and isoenergetic and were formulated using the  
199 AMEn values for TM measured *in vivo* for broiler chickens (De  
200 Marco et al., 2015). Diets met or exceeded NRC (1994)  
201 requirements and were adjusted according to Aviagen (2014)  
202 broiler nutrition specifications. Feed and water were provided  
203 *ad libitum*.

204

#### 205 ***Chemical analysis***

206 The diets were ground to pass through a 0.5-mm sieve and  
207 stored in airtight plastic containers for DM (method number  
208 943.01), ash (method number 924.05), CP (method number  
209 954.01), EE (method number 920.39), NDF (method number  
210 2002.04) and ADF (method number 973.18) determination  
211 (AOAC, 2004).

212

#### 213 ***Growth Performances***

214 Health status and mortality were daily monitored during the  
215 whole experimental period. Live weight (LW) of the animals  
216 was recorded at an individual level at the beginning of the trial,  
217 at day 12, 25 and 40. Average daily gain (ADG) and average  
218 daily feed intake (DFI) were recorded at an individual and a  
219 pen level, respectively, at the end of each growth period. Feed

220 conversion ratio (FCR) was determined for each growth period  
221 and for the overall experimental period. All measurements were  
222 made on the pen basis using a high precision electronic scale  
223 (Sartorius – Signum®).

224

### 225 ***Slaughtering procedure and recordings***

226 At day 40, all birds were individually weighed and 10  
227 broilers/diet (2 birds/pen) were chosen on the basis of pen  
228 average LW and identified with a shank ring.

229 All selected animals were slaughtered in a commercial abattoir.

230 The plucked and eviscerated carcasses were obtained and the

231 head, neck, feet and abdominal fat were removed to obtain

232 carcass-for-grilling. The weights of liver, spleen, gizzard and

233 abdominal fat were immediately recorded. All slaughtered

234 carcasses were stocked in a cooling chamber (0-4 °C) for 24 h.

235 Weights of carcass-for-grilling, breast and thighs were

236 successively recorded. Carcass-for-grilling, breast, thigh and

237 organs weights were also expressed as percentage of LW.

238 Collected feet were examined macroscopically using the

239 Swedish FPD scoring system (Ekstrand et al., 1997). According

240 to this system, 0 = no lesion, slight discoloration of the skin or

241 healed lesion; 1= mild lesion, superficial discoloration of the

242 skin and hyperkeratosis; 2 = severe lesion, affected epidermis,

243 blood scabs, hemorrhage and severe swelling of the skin.

244

245 ***Haematological and serum parameters***

246 At slaughter, blood samples were collected from the identified  
247 broilers: 2.5 mL was placed in an EDTA tube and 2.5 mL in a  
248 serum-separating tube. A blood smear was prepared, using one  
249 glass slide for each bird, from a drop of blood without  
250 anticoagulant. The smears were stained using May-Grünwald  
251 and Giemsa stains (Campbell, 1995). The total red and white  
252 blood cell counts were determined in an improved Neubauer  
253 haemocytometer on blood samples previously treated with a  
254 1:200 Natt-Herrick solution. One hundred leukocytes, including  
255 granular (heterophils, eosinophils and basophils) and non-  
256 granular (lymphocytes and monocytes) leukocytes, were  
257 counted on the slide and the heterophils to lymphocytes (H/L)  
258 ratio was calculated. The tubes without anticoagulant were left  
259 to clot in a standing position at room temperature for  
260 approximately two hours to obtain serum. The serum was  
261 separated by means of centrifugation at  $700 \times g$  for 15 minutes  
262 and frozen at  $-80^{\circ}\text{C}$  until analysis. The total proteins were  
263 quantified by means of the “biuret method” (Bio Group  
264 Medical System kit; Bio Group Medical System, Talamello  
265 (RN), Italy); the electrophoretic pattern of the serum was  
266 obtained using a semi-automated agarose gel electrophoresis  
267 system (Sebia Hydrasys®, Norcross, GA, USA). The alanino-  
268 aminotransferase (ALT), aspartate-aminotransferase (AST),  
269 gamma glutamyl transferase (GGT), triglycerides, cholesterol,

270 glucose, phosphorus, magnesium, iron, uric acid and creatinine  
271 serum concentrations were measured by means of enzymatic  
272 methods in a clinical chemistry analyzer (Screen Master Touch,  
273 Hospitex diagnostics Srl., Firenze, Italy).

274

### 275 *Histomorphological investigations*

276 The slaughtered animals were submitted to  
277 anatomopathological investigations. Intestinal segment samples  
278 (approximately 5 cm in length) of duodenum, jejunum, ileum  
279 and caecum were excised from each bird and flushed with 0.9%  
280 saline to remove all the content. The collected segments of  
281 intestine were the loop of the duodenum, the tract before  
282 Meckel's diverticulum (jejunum), the tract before the ileocolic  
283 junction (ileum) and the apex of the caeca (caecum). Gut  
284 segments were fixed in Carnoy's solutions for morphometric  
285 analysis. Tissues were routinely embedded in paraffin wax  
286 blocks, sectioned at 5  $\mu$ m thickness, mounted on glass slides  
287 and stained with Haematoxylin & Eosin (HE). The evaluated  
288 morphometric indices were Vh (from the tip of the villus to the  
289 crypt), Cd (from the base of the villus to the submucosa) and  
290 the Vh/Cd ratio (Laudadio et al., 2012). Morphometric analyses  
291 were performed on 10 well-oriented and intact villi and 10  
292 crypts chosen from duodenum, jejunum and ileum (Qaisrani et  
293 al., 2014). Samples of liver, spleen, thymus, bursa of Fabricius,  
294 kidney and heart were also collected from each animal and

295 fixed in 10% buffered formalin solution for histopathological  
296 examination. Tissues were processed in the same way as gut  
297 and the following histopathological alterations were evaluated:  
298 white pulp hyperplasia and depletion in spleen, cortical  
299 depletion in thymus, follicular depletion and intrafollicular  
300 cysts in bursa of Fabricius and lymphoid tissue activation in  
301 liver (Biasato et al., 2016). Heart and kidney were assessed for  
302 inflammatory and degenerative diseases. The observed  
303 histopathological findings were evaluated using a  
304 semiquantitative scoring system as previously assessed by  
305 Biasato et al. (2016): absent/minimal (score = 0), mild (score =  
306 1) and severe (score = 2).

307

### 308 *Statistical Analysis*

309 IBM SPSS Statistics V20.0.0 software was used to perform  
310 statistical analysis. Shapiro-Wilk's test established normality or  
311 non-normality of distribution. The experimental unit was the  
312 pen for growth performance, haematochemical parameters and  
313 carcass traits and bird for histomorphological findings. Data  
314 collected for growth performance, blood parameters and  
315 carcass traits were tested by one-way ANOVA, evaluating the  
316 effect of dietary TM inclusion by polynomial contrasts.  $\chi^2$  test  
317 was performed to evaluate the association between the  
318 mortality rate and the dietary treatments. Intestinal  
319 morphometric indices were analyzed by fitting a general linear

320 model (GLM). The GLM allowed the morphometric indices  
321 (Vh, Cd and Vh/Cd, separately) to depend on three fixed  
322 factors (diet, intestinal segment and interaction between diet  
323 and intestinal segment). The interactions between the levels of  
324 the fixed factors were evaluated by pairwise comparisons.  
325 Statistical analysis was performed by procedure “General  
326 Linear Models > Univariate”. Histopathological and FPD  
327 scores were analyzed by Kruskal-Wallis test (post-hoc test:  
328 Dunn’s Multiple Comparison test). P values < 0.05 were  
329 considered statistically significant. The results were expressed  
330 as mean and pooled standard error of the mean (SEM).

331

## 332 **Results**

### 333 *Growth performance*

334 No clinical signs were observed and the birds remained healthy  
335 during the whole experimental period. The mortality rates of C  
336 (2.5%), TM5 (2.5%), TM10 (0%) and TM15 (2.5%) groups  
337 were not significantly different among treatments (P > 0.05).  
338 Growth performance of the broiler chickens are summarized in  
339 Table 2. At 12 days of age, the LW increased linearly with  
340 increasing TM meal levels (P < 0.05) and the linear response  
341 increased to a maximum corresponding to the inclusion of 150  
342 g/kg of TM meal. At 25 days of age, no significant effects  
343 related to TM meal utilization were observed. At 40 days of  
344 age, the LW showed quadratic response to increasing TM meal

345 levels ( $P < 0.05$ ), with a maximum corresponding to the  
346 inclusion of 50 g/kg of TM meal. In the period from 1-12 days  
347 of age, the ADG increased linearly with increasing TM meal  
348 levels ( $P < 0.05$ ) and the linear response increased to a  
349 maximum corresponding to the inclusion of 150 g/kg of TM  
350 meal. On the contrary, the ADG showed no differences ( $P >$   
351  $0.05$ ) in the periods from 12 to 25 and 25 to 40 days of age In  
352 the period from 1 to 12 days of age, the response of DFI to the  
353 effect of TM meal inclusion was statistically significant ( $P <$   
354  $0.01$ ). In particular, the linear response increased to a maximum  
355 corresponding to the inclusion of 150 g/kg of TM meal. In the  
356 period from 12 to 25 days of age, the DFI increased linearly  
357 with increasing TM meal levels ( $P < 0.05$ ) and the linear  
358 response increased to a maximum corresponding to the  
359 inclusion of 50 g/kg of TM meal. On the contrary, the DFI  
360 showed no differences ( $P > 0.05$ ) in the period from 25 to 40  
361 days of age. In the periods from 1 to 12, 25 to 40 and 1 to 40  
362 days of age, the FCR was similar among the dietary treatments  
363 ( $P > 0.05$ ). Differently, the FCR showed linear response to  
364 increasing TM meal levels in the period from 12 to 25 ( $P <$   
365  $0.01$ ), with a maximum corresponding to the inclusion of 150  
366 g/kg of TM meal.

367

368 *Slaughtering performance and footpad dermatitis (FPD)*

369 *score*



370 Table 3 summarizes the slaughtering performance of the broiler  
371 chickens. The carcass weight increased quadratically with  
372 increasing TM meal levels ( $P < 0.05$ ) and the quadratic  
373 response increased to a maximum corresponding to the  
374 inclusion of 50 g/kg of TM meal. The abdominal fat weight  
375 showed linear response to increasing TM meal levels ( $P <$   
376  $0.05$ ), with a maximum corresponding to the inclusion of 150  
377 g/kg of TM meal. Similarly, the abdominal fat percentage  
378 increased linearly with increasing TM meal levels ( $P < 0.01$ )  
379 and the linear response increased to a maximum corresponding  
380 to the inclusion of 100 g/kg of TM meal. On the contrary, no  
381 significant effects related to TM meal utilization were observed  
382 for the other carcass traits ( $P > 0.05$ ). FPD scores (C: 0.40;  
383 TM5: 0.20; TM10: 0.20; TM15: 0.00) were also not influenced  
384 by dietary TM meal inclusion ( $P > 0.05$ ).

385

### 386 *Haematological and serum parameters*

387 Haematological and serum biochemical traits of the broiler  
388 chickens are summarized in Table 4. The erythrocytes  
389 increased quadratically with increasing TM meal levels ( $P <$   
390  $0.05$ ) and the quadratic response increased to a maximum  
391 corresponding to the inclusion of 100 g/kg of TM meal. The  
392 albumin showed linear response to increasing TM meal levels  
393 ( $P < 0.05$ ), with a minimum corresponding to the inclusion of  
394 100 g/kg of TM meal. Similarly, the GGT decreased

395 quadratically with increasing TM meal levels ( $P < 0.05$ ) and the  
396 quadratic response decreased to a minimum corresponding to  
397 the inclusion of 100 g/kg of TM meal. On the contrary, no  
398 significant effects related to TM meal utilization were observed  
399 for the other blood and serum parameters ( $P > 0.05$ ).

400

#### 401 *Histomorphological investigations*

402 The effects of the diet, intestinal segment and interaction  
403 between diet and intestinal segment on gut morphometric  
404 indices of the broiler chickens are shown in Tables 5 and 6.  
405 There was no influence of diet or interaction between diet and  
406 intestinal segment ( $P > 0.05$ ) on the gut morphometric indices.  
407 On the contrary, Vh, Cd and Vh/Cd depended on intestinal  
408 segment ( $P < 0.001$ ,  $P = 0.001$  and  $P < 0.001$ , respectively)  
409 (Table 5). In particular, the duodenum showed higher Vh ( $P <$   
410  $0.001$ ) than the jejunum and ileum. Furthermore, higher Cd ( $P$   
411  $< 0.001$ ) was found in the duodenum and jejunum than the  
412 ileum. Similarly, the duodenum and jejunum showed higher  
413 Vh/Cd ( $P < 0.001$ ) than the ileum (Table 6).

414 Histopathological alterations were observed in all the dietary  
415 treatments and developed in spleen, thymus, bursa of Fabricius  
416 and liver, while heart and kidney showed no significant  
417 findings. Spleen ( $C = 1.50 \pm 0.22$ ; TM5 =  $1.20 \pm 0.25$ ; TM10 =  
418  $1.10 \pm 0.31$ ; TM15 =  $1.10 \pm 0.28$ ), thymus ( $C = 0.10 \pm 0.10$ ;  
419 TM5 =  $0.30 \pm 0.15$ ; TM10 and TM15 =  $0.10 \pm 0.10$ ), bursa of

420 Fabricius ( $C = 1.90 \pm 0.10$ ;  $TM5 = 1.50 \pm 0.27$ ;  $TM10 = 1.80 \pm$   
421  $0.20$ ;  $TM15 = 1.70 \pm 0.21$ ) and liver ( $C = 0.90 \pm 0.23$ ;  $TM5 =$   
422  $1.10 \pm 0.23$ ;  $TM10 = 0.90 \pm 0.23$ ;  $TM15 = 0.30 \pm 0.15$ ) scores  
423 were not influenced by dietary TM meal inclusion. Spleen  
424 showed moderate ( $C = 30\%$  of the broilers;  $TM5 = 40\%$ ;  $TM10$   
425  $= 10\%$ ;  $TM15 = 30\%$ ) to severe ( $C = 60\%$ ;  $TM5 = 40\%$ ;  $TM10$   
426  $= 50\%$ ;  $TM15 = 40\%$ ) white pulp depletion or hyperplasia. The  
427 10% I, 20% (TM5), 40% (TM10) and 30% (TM15) of the  
428 animals had normal spleen (Fig. 1A-B). In thymus, moderate  
429 cortical depletion was found in all the groups ( $C = 10\%$  of the  
430 broilers;  $TM5 = 30\%$ ;  $TM10$  and  $TM15 = 10\%$ ). A normal  
431 thymus was observed in 90% I, 70% (TM5) and 90% (TM10  
432 and TM15) of the animals. Bursa of Fabricius showed moderate  
433 ( $C$  and  $TM5 = 10\%$  of the broilers;  $TM10 = 0\%$ ;  $TM15 = 10\%$ )  
434 to severe ( $C = 80\%$ ;  $TM5 = 70\%$ ;  $TM10 = 90\%$ ;  $TM15 = 80\%$ )  
435 follicular depletion. The 0% I, 20% (TM5), 10% (TM10) and  
436 10% (TM15) of the animals had normal bursa of Fabricius (Fig.  
437 1C-D). In liver, moderate ( $C$ ,  $TM5$  and  $TM10 = 50\%$  of the  
438 broilers;  $TM15 = 30\%$ ) to severe ( $C = 20\%$ ;  $TM5 = 30\%$ ;  
439  $TM10 = 20\%$ ;  $TM15 = 0\%$ ) perivascular lymphoid tissue  
440 activation. A normal liver was observed in 30% I, 20% (TM5),  
441 30% (TM10) and 70% (TM15) of the animals.

442

## 443 **Discussion**

### 444 ***Growth performances***

445 Growth performances of the broiler chickens of the present  
446 study were consistent with the reference values recorded in the  
447 commercial farm in which the trial was conducted.

448 The body weight, weight gain and feed intake of the birds in  
449 the present trial improved with increasing levels of TM meal  
450 inclusion, but the feed efficiency resulted partially impaired.

451 Little information on the influence of dietary TM meal  
452 inclusion in broiler chickens is currently available. Ramos-  
453 Eldoury et al. (2002) and Biasato et al. (2016) did not show any  
454 effects for the growth performance in fast-growing and  
455 intermediate-growing chickens, respectively, fed diets in which  
456 the TM inclusion level ranged from 50 to 100 g/kg. Differently,  
457 Ballitoc and Sun (2013) and Bovera et al. (2015) observed  
458 improved growth performance in fast-growing chickens fed  
459 diets with low (from 5 to 100 g/kg) or high (296 g/kg) TM  
460 inclusion levels, respectively. Similar findings were obtained  
461 by Loponte et al. (2017) in partridges fed diets in which the TM  
462 inclusion level ranged from 250 to 500 g/kg. Recently, Islam  
463 and Yang (2017) also found a positive effect of a mealworm-  
464 based probiotic on broiler growth performance. Some authors  
465 also explored the possibility to use other insect meals in poultry  
466 feeding. Adeniji (2007) and Hwangbo et al. (2009) studied the  
467 effects of housefly-maggots as feed supplement in the diet of  
468 broiler chickens: the first found no differences for growth  
469 performance with inclusion levels ranging from 55 to 220 g/kg,

470 while the latter (inclusion rate: 50-200 g/kg) observed a linear  
471 increase in LW gain. Ijaiya and Eko (2009) evaluated the  
472 effects of replacing dietary fishmeal with silkworm meal  
473 (inclusion rate: 22-93 g/kg) on growth performance of broiler  
474 chickens, finding no differences related to insect meal  
475 utilization. Also Oyegoke et al. (2006) and Wang et al. (2005)  
476 observed no adverse effects on growth performance of broiler  
477 chickens fed diets with *Cirina forda* (inclusion rate: 20-40  
478 g/kg) and *Gryllus testaceus* (inclusion rate: 50-150 k/kg),  
479 respectively. Cullere et al. (2016) recently studied the influence  
480 of the inclusion (from 100 to 150 g/kg) of *Hermetia illucens*  
481 meal in quail diet, finding no differences for growth  
482 performances. Schiavone et al. (2017b) also evaluated the  
483 effects of replacing soybean oil with *Hermetia illucens* meal  
484 (inclusion rate: 500-1000 g/kg) on growth performance of  
485 broiler chickens, finding no differences related to insect meal  
486 utilization. The wide variability of the results obtained in the  
487 previous studies may be related to the nutritive value of the  
488 insect meal used, which can be influenced by the species, the  
489 insect life stage (adult, larva or pupa) and the insect rearing  
490 substrate (Sánchez-Muros et al., 2014).

491 The improvement of feed intake observed in the birds fed TM  
492 diets of the present trial was considered suggestive of increased  
493 feed palatability in relation to the addition of yellow  
494 mealworms, since insects are naturally consumed by wild birds

495 and free-range poultry (Zuidhof et al., 2003). In particular, the  
496 increased DFI observed in the starter period, which was  
497 accompanied by increased LW and ADG and unaffected FCR,  
498 was quite relevant. Indeed, starter period (from hatch to 10  
499 days) is considered the most important in broiler production,  
500 since growth and development take place at an incredible rate  
501 during it. In this period, the chicks' weight quadruples, thus  
502 influencing the following growth rate (Aviagen, 2014). On the  
503 contrary, the increased DFI observed in the growing period was  
504 accompanied by unaffected LW and ADG and subsequently  
505 impaired FCR, thus representing a negative effect related to  
506 TM meal utilization. De Marco et al. (2015) speculated that the  
507 chitin contained in the exoskeleton of the TM meal may  
508 negatively influence the apparent digestibility coefficient of the  
509 total tract of nutrients. Furthermore, Ravindran and Blair  
510 (1993) pointed out that the chitin of insects is difficult to digest  
511 by domestic poultry. As suggested by Rumpbold and Schlüter  
512 (2013), the partial chitin removal through high pressure  
513 processing could improve the use of insects as feeding  
514 ingredient thanks to disruption of the link between some chitin-  
515 bound proteins. However, the limited number of birds included  
516 in the current trial could have influenced the data interpretation.  
517 The results obtained need to be confirmed on a larger number  
518 of animals.  
519

520 *Slaughtering performance and footpad dermatitis (FPD)*

521 *score*

522 The majority of the carcass traits of the broilers in the present  
523 trial were not influenced by dietary TM meal inclusion, as  
524 previously observed by Bovera et al. (2016) and Biasato et al.  
525 (2016). Similar findings were obtained by Cullere et al. (2016)  
526 and Schiavone et al. (2017) in broiler quails and chickens fed  
527 diets with *Hermetia illucens* meal and fat, respectively.  
528 However, the carcass weight, abdominal fat weight and  
529 abdominal fat percentage increased with increasing levels of  
530 TM meal utilization. Loponte et al. (2017) also observed  
531 improved carcass weights when TM and *Hermetia illucens*  
532 meals were included in the diets of partridges. As already  
533 suggested by them, the differences in the eviscerated carcass  
534 weights can be partially explained by the increased final LW of  
535 the birds. Similar findings in terms of improved eviscerated  
536 carcass weights were obtained by Khatun et al. (2003),  
537 Hwangbo et al. (2009) and Ballitoc and Sun (2013), who also  
538 observed improved slaughter, dressed carcass, breast muscle  
539 and thigh muscle weights and dressing percentage in broilers  
540 fed diets with different insect meals inclusion. The differences  
541 in the abdominal fat weight and abdominal fat percentage  
542 observed in the birds of the present study are also in agreement  
543 with Ballitoc and Sun (2013) and suggests that yellow

544 mealworm utilization may improve fat mass in broiler chickens  
545 (USDA, 2011).

546 The majority of FPD scores obtained in the present trial was  
547 zero and no differences were found in relation to TM meal  
548 utilization. This is a positive result, since a low prevalence and  
549 severity of FPD is highly desirable as far as health of birds and  
550 product quality are concerned (Meluzzi et al., 2008).

551

### 552 *Haematological and serum parameters*

553 All the blood parameters obtained in the present trial fell within  
554 the physiological ranges (Lumej, 2008), thus suggesting that  
555 TM meal utilization does not affect health status of the animals.

556 In particular the H/L ratio, that is commonly used as indicator  
557 of stress in poultry (De Marco et al., 2013; Salamano et al.,  
558 2010), was not affected by dietary TM meal inclusion. As  
559 already observed by Bovera et al. (2015) and Biasato et al.  
560 (2016), the majority of the haematochemical and serum  
561 biochemical traits were not affected by yellow mealworm  
562 inclusion in the birds of the present study. Similar findings  
563 were obtained by Schiavone et al. (2017b) in broilers fed diets  
564 with *Hermetia illucens* fat. However, the erythrocytes increased  
565 and albumin and GGT decreased with increasing levels of TM  
566 meal utilization Loponte et al. (2017) also observed lower  
567 albumin when TM and *Hermetia illucens* meals were included  
568 in the diets of partridges. Interestingly, this finding was



569 accompanied by the increase of albumin/globulin ratio, which  
570 was also reported by Bovera et al. (2015) and ascribed to the  
571 properties of chitin contained in insect meal. High globulin  
572 concentrations and low albumin/globulin ratios generally  
573 indicate better disease resistance and immune response of birds  
574 (Griminger and Scanes, 1986). Another interesting finding is  
575 represented by the decrease of GGT serum levels in TM  
576 animals. Indeed, a high GGT concentration in birds is used as  
577 an indicator of liver disease and bile flow disorders (Ognik and  
578 Krauze, 2016). Therefore, GGT reduction can be considered a  
579 positive effect related to TM meal utilization.

580

#### 581 *Histomorphological investigations*

582 Dietary TM meal inclusion did not influence the gut  
583 morphology of the birds of the present study, as already  
584 observed by Biasato et al. (2016). Morphometric measurements  
585 of Vh and Cd are generally used to assess intestinal  
586 development (Franco et al., 2006), since represent useful  
587 indicators of gut proliferative and absorptive compartments  
588 (Lenhardt and Mozes, 2003). The Vh/Cd ratio is also evaluated,  
589 because it gives an indication of the likely maturity and  
590 functional capacity of the enterocytes (Hampson, 1986). As  
591 previously reported (Uni et al., 1999; Iji et al., 2001; Biasato et  
592 al., 2016), the present study confirms that both duodenum and  
593 jejunum show a greater morphological development compared

594 with the ileum. Indeed, the duodenum is the intestinal tract with  
595 the fastest cell renewal, and is also the first segment of the  
596 small intestine to receive physical, chemical and hormonal  
597 stimuli provoked by diet (Macari, 1998). Furthermore, the  
598 jejunum is an important site for nutrient digestion (Iji et al.,  
599 2001). Therefore, dietary TM inclusion preserves a  
600 proximodistal decreasing gradient of the morphometric indexes  
601 from the duodenum to the ileum, thus suggesting the  
602 maintenance of the physiological gut development.

603 The broiler chickens of the present trial showed different  
604 degrees of lymphoid system activation, with no differences  
605 related to dietary TM meal inclusion. This result could be  
606 related to the stress occurrence in modern poultry rearing  
607 operations. Stress can be caused by a variety factors,  
608 physiological (rapid growth rate) and social (overcrowding)  
609 ones included (Liles et al., 2015). However, a great deal of  
610 individual bird variability in some immunological measures  
611 (i.e., stimulation index, heterophils to lymphocyte ratio and  
612 lymphocyte blastogenesis) may also be considered (Talebi et  
613 al., 1995).

614

## 615 **Conclusion**

616 In conclusion, the present study suggests that increasing levels  
617 of dietary TM meal inclusion in female broiler chickens diets  
618 may improve body weight, weight gain and feed intake, but can

619 partially worsen feed efficiency. However, positive effects on  
620 carcass traits and haematochemical parameters are observed,  
621 along with no negative influence on gut morphology and  
622 histological findings. These results confirm previous data  
623 concerning the safety of TM utilization in poultry feed, even if  
624 legislative issues are still needed to allow insect meal to be  
625 used as transformed animal protein to feed monogastric farm  
626 animals.

627

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637

638

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

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 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 <sup>1</sup>	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Coline	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 <sup>2</sup> (MJ/kg)	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 (%) <sup>2</sup>												
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

862 <sup>1</sup>Mineral-vitamin premix (Final B Prisma, IZA SRL), given values are supplied per kg of diet: 2.500.000 IU of vitamin A;  
863 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  
864 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  
865 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  
866 mg of Se.

867 <sup>2</sup>Calculated according to INRA 2004 and De Marco et al. (2015).

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

870

871 **Table 2.** Effect of the dietary TM larvae meal inclusion on the growth performance of the female broiler chickens.

Variable <sup>2</sup>	Age	Dietary treatments <sup>1</sup>				SEM	P <sup>3</sup>	
		C	TM5	TM10	TM15		Linear	Quadratic
LW (g)	12 d	303.15	338.25	339.15	351.83	6.78	0.013	0.352
	25 d	1174.90	1234.77	1183.35	1179.79	14.14	0.775	0.280
	40 d	2078.46	2309.97	2115.91	2084.22	30.51	0.408	0.012
ADG (g)	1-12 d	23.61	26.80	26.89	28.04	0.61	0.012	0.350
	12-25 d	67.06	68.96	64.94	63.69	1.17	0.198	0.512
	25-40 d	60.24	71.68	62.17	60.29	1.91	0.552	0.071
DFI (g)	1-12 d	25.40	28.80	30.80	32.40	0.95	0.006	0.583
	12-25 d	95.00	117.00	109.40	116.60	2.95	0.014	0.129
	25-40 d	184.41	220.23	209.49	225.28	6.85	0.731	0.741
FCR (g/g)	1-12 d	1.08	1.07	1.14	1.15	0.03	0.336	0.925
	12-25 d	1.42	1.74	1.68	1.86	0.05	0.001	0.325
	25-40 d	2.40	2.16	2.13	2.31	0.11	0.775	0.404
	1-40 d	1.78	1.84	1.81	1.95	0.05	0.342	0.730

872 <sup>1</sup>Each mean represents 5 replicates with 8 chicks/replicate (n = 40/treatment).

873 <sup>1</sup>Four dietary treatments: C = control; TM5 = 5% inclusion level of *Tenebrio molitor*; TM10 = 10% inclusion level  
874 of *Tenebrio molitor*; TM15 = 15% inclusion level of *Tenebrio molitor*.

875 <sup>2</sup>LW, live weight; DFI, daily feed intake; ADG, average daily gain; FCR, feed conversion ratio; n, number of pens.



876 <sup>3</sup>Statistical significance:  $P < 0.05$ .

877

878 **Table 3.** Effect of the dietary TM larvae meal inclusion on the carcass traits of the female broiler chickens.<sup>1</sup>

Variable	Dietary treatments <sup>2</sup>				SEM	P <sup>3</sup>	
	C	TM5	TM10	TM15		Linear	Quadratic
Live weight (LW) (g)	1980	2149	2118	2018	33.68	0.771	0.054
Carcass weight (g)	1377	1501	1500	1276	38.80	0.348	0.025
Carcass weight (% LW)	69.46	69.75	70.77	62.86	1.78	0.256	0.266
Breast (g)	332	388	359	353	10.24	0.688	0.135
Breast (% LW)	16.64	17.92	16.94	17.43	0.24	0.512	0.406
Thigh (g)	411	433	434	402	6.00	0.646	0.026
Thigh (% LW)	20.80	20.20	20.51	20.00	0.16	0.163	0.883
Spleen (g)	2.62	2.84	2.83	2.80	0.13	0.672	0.677
Spleen (% LW)	0.13	0.13	0.13	0.14	0.01	0.742	0.768
Liver (g)	25.01	27.30	27.03	25.68	0.82	0.825	0.302
Liver (% LW)	1.26	1.26	1.27	1.26	0.02	0.921	0.941
Gizzard (g)	36.75	38.20	36.83	34.40	1.03	0.392	0.379
Gizzard (% LW)	1.83	1.77	1.74	1.69	0.04	0.168	0.914
Abdominal fat (g)	40.5	43.3	33.8	43.5	1.29	0.014	0.057
Abdominal fat (% LW)	0.66	0.86	1.08	0.98	0.23	0.005	0.074

879 <sup>1</sup>Each mean represents 5 pens with 2 chicks/pen (n = 5/treatment).

880 <sup>2</sup>Four dietary treatments: C = control; TM5 = 5% inclusion level of *Tenebrio molitor*; TM10 = 10% inclusion level

881 of *Tenebrio molitor*; TM15 = 15% inclusion level of *Tenebrio molitor*.

882 <sup>3</sup>Statistical significance:  $P < 0.05$ .

883

884 **Table 4.** Effect of the dietary TM larvae meal inclusion on the haematological and serum parameters of the female  
 885 broiler chickens.<sup>1</sup>

Variable <sup>3</sup>	Dietary treatments <sup>2</sup>				SEM	P <sup>4</sup>	
	C	TM5	TM10	TM15		Linear	Quadratic
Erythrocyte (10 <sup>6</sup> cell/ $\mu$ l)	2.27	2.41	2.47	2.39	0.28	0.073	0.040
Leukocyte (10 <sup>3</sup> cell/ $\mu$ l)	9.28	9.07	9.31	9.70	0.23	0.521	0.562
H/L ratio	0.83	0.68	0.75	0.77	0.03	0.632	0.095
Albumin (g/dl)	1.66	1.35	1.27	1.32	0.06	0.046	0.134
Total protein (g/dl)	3.31	3.80	3.85	4.10	0.14	0.068	0.663
GGT (UI/l)	26.86	22.13	21.61	25.56	1.05	0.629	0.046
AST (UI/l)	189.48	227.18	217.11	211.06	8.62	0.494	0.229
ALT (UI/l)	20.06	21.33	18.57	15.90	1.16	0.164	0.428
Uric Acid (mg/dl)	3.44	4.03	3.28	3.35	0.21	0.617	0.562
Creatinine (mg/dl)	0.36	0.38	0.36	0.38	0.00	0.410	0.957
Triglycerides (mg/dl)	42.44	44.48	52.80	36.03	3.05	0.686	0.133
Cholesterol (mg/dl)	60.33	71.12	70.87	77.80	3.71	0.138	0.799
Glucose (mg/dl)	222.60	221.10	219.70	227.00	1.62	0.431	0.196
Phosphorus (mg/dl)	3.67	4.19	3.93	5.56	0.33	0.067	0.376
Magnesium (mEq/l)	1.30	1.15	1.18	1.15	0.03	0.159	0.380
Iron ( $\mu$ g/dl)	102.44	81.20	81.26	103.89	11.64	0.985	0.392

886 <sup>1</sup>Each mean represents 5 pens with 2 chicks/pen (n = 5/treatment).

887 <sup>2</sup>Four dietary treatments: C = control; TM5 = 5% inclusion level of *Tenebrio molitor*; TM10 = 10% inclusion level

888 of *Tenebrio molitor*; TM15 = 15% inclusion level of *Tenebrio molitor*.

889 <sup>3</sup>H/L, heterophiles to lymphocytes ratio; GGT, gamma glutamyl transferase; AST, aspartate aminotransferase;

890 ALT, alanine aminotransferase; n, number of birds.

891 <sup>4</sup>Statistical significance:  $P < 0.05$ .

892

893

894 **Table 5.** Effects of diet, intestinal segment and interaction between diet and intestinal segment on the intestinal  
895 morphometric indices of the female broiler chickens.

<b>Index</b>	<b>Fixed effect</b>	<b>d.f.<sup>3</sup></b>	<b>F</b>	<b>P<sup>4</sup></b>
Vh (mm)	Diet <sup>1</sup>	3	1.210	0.310
	Intestinal segment <sup>2</sup>	2	68.115	< 0.001
	Diet × Intestinal segment	6	0.922	0.483
Cd (mm)	Diet	3	0.891	0.449
	Intestinal segment	2	7.275	0.001
	Diet × Intestinal segment	6	1.593	0.157
Vh/Cd (mm/mm)	Diet	3	0.705	0.551
	Intestinal segment	2	35.195	< 0.001
	Diet × Intestinal segment	6	0.277	0.947

896 <sup>1</sup>Four dietary treatments: C = control; TM5 = 5% inclusion level of *Tenebrio molitor*; TM10 = 10% inclusion level  
897 of *Tenebrio molitor*; TM15 = 15% inclusion level of *Tenebrio molitor*.

898 <sup>2</sup>Three intestinal segments: duodenum, jejunum and ileum.

899 <sup>3</sup>Degrees of freedom.

900 <sup>4</sup>Statistical significance: P < 0.05.

901 Vh, villus height; Cd, crypt depth; Vh/Cd, villus height to crypt depth ratio.

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

Index	Fixed effect	Effect levels	Least square mean <sup>1</sup>	SEM
Vh (mm)	Diet <sup>2</sup>	C	1.73	0.06
		TM5	1.67	
		TM10	1.57	
	Intestinal segment <sup>3</sup>	TM15	1.61	0.05
		DU	2.08 <sup>a</sup>	
		JE	1.65 <sup>b</sup>	
		I	1.20 <sup>c</sup>	
Cd (mm)	Diet	C	0.20	0.01
		TM5	0.20	
		TM10	0.20	
	Intestinal segment	TM15	0.21	0.00
		DU	0.21 <sup>a</sup>	
		JE	0.21 <sup>a</sup>	
		I	0.19 <sup>b</sup>	
Vh/Cd (mm/mm)	Diet	C	8.49	0.34
		TM5	8.51	
		TM10	8.00	
	Intestinal segment	TM15	8.00	0.47
		DU	10.06 <sup>a</sup>	
		JE	8.13 <sup>b</sup>	
		I	6.56 <sup>c</sup>	

904 <sup>1</sup>Means with different superscript letters (a, b) within the same column per fixed effect (i.e.  
 905 diet, intestinal segment) differ significantly (P < 0.05).



- 906 <sup>2</sup>C = control; TM5 = 5% inclusion level of *Tenebrio molitor*; TM10 = 10% inclusion level of
- 907 *Tenebrio molitor*; TM15 = 15% inclusion level of *Tenebrio molitor*
- 908 <sup>3</sup>DU = duodenum; JE = jejunum; I = ileum.

909 **Figure legends**

910

911 **Figure 1.** Histological findings of the female broiler chickens. **A)** TM5 group. A normal  
912 spleen. 5× Haematoxylin & Eosin stain. **B)** TM5 group. Spleen with severe and diffuse  
913 depletion of the white pulp. A high number of apoptotic cells (arrowheads) are observed. 20×  
914 Haematoxylin & Eosin stain. **C)** C group. A normal follicle in the bursa of Fabricius. 10×  
915 Haematoxylin & Eosin stain. **D)** C group. Bursa of Fabricius with mild and multifocal  
916 follicular depletion (arrow) associated with intrafollicular cyst (\*). 10× Haematoxylin &  
917 Eosin stain.