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(Article begins on next page)

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Nutritional value of two insect larval meals (*Tenebrio molitor* and *Hermetia illucens*) for broiler chickens: apparent nutrient digestibility, apparent ileal amino acid digestibility and apparent metabolisable energy.

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19 **Nutritional value of two insect larval meals (*Tenebrio***
20 ***molitor* and *Hermetia illucens*) for broiler chickens:**
21 **apparent nutrient digestibility, apparent ileal amino acid**
22 **digestibility and apparent metabolisable energy.**

23

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64 **Abstract**

65 The aim of this study was to determine the apparent
66 digestibility coefficients of the total tract (CTTAD) of nutrients
67 and the apparent metabolisable energy (AME and AMEn) of

68 two insect larval meals (*Tenebrio molitor* and *Hermetia*
69 *illucens*) for broiler chickens. The amino acid (AA) apparent
70 ileal digestibility coefficients (AIDC) was also determined. The
71 experimental diets were: a basal diet and two diets prepared by
72 substituting 250 g/kg (w/w) of the basal diet with *Tenebrio*
73 *molitor* meal (TM) or *Hermetia illucens* meal (HI). No
74 statistical difference was found between the two insect larval
75 meals for the CTTAD of the nutrients, except for the CTTAD
76 for ether extract ($P < 0.001$) where the HI meal proved to be
77 more digestible than the TM meal (0.99 and 0.88, respectively).
78 The CTTAD for DM was 0.60 and 0.53; 0.66 and 0.66 for OM;
79 0.60 and 0.51 for CP, whereas it was 0.64 and 0.69 for GE, for
80 TM and HI, respectively. No difference was observed between
81 TM and HI ($P > 0.05$) for AME or AMEn (AME = 16.86 and
82 17.38 MJ/kg DM, respectively; AMEn = 16.02 and 16.60
83 MJ/kg DM, respectively). The average AIDC of the 17
84 analyzed AAs was higher ($P < 0.001$) in TM than in HI (0.86
85 and 0.68, respectively) because the AIDC of isoleucine, lysine,
86 methionine, phenylalanine, valine, alanine, aspartic acid,
87 glycine, glutamic acid and tyrosine was higher ($P < 0.05$) in TM
88 than in HI. Overall, the present results have shown that TM and
89 HI meals are excellent sources of AME for broilers and a
90 valuable source of digestible AA, particularly as far as TM
91 meal is concerned.

92

93 **Keywords:** Insect larval meal; Amino acid; Metabolisable
94 energy; Apparent digestibility; Broiler chicken.

95

96 **1. Introduction**

97 Soybean meal is the most frequently used protein source
98 in diet formulations for broiler chickens. However, in recent
99 years, the increasing price of this raw material has become a
100 critical aspect for the economic sustainability of the poultry
101 meat industry, particularly in some developing countries
102 (Chadd, 2007). The evaluation of alternative ingredients that
103 are affordable and locally available as substitutes for
104 conventional protein meals is therefore required.

105 The use of insects as an alternative source of protein in
106 animal feeds is becoming more globally appealing.
107 Invertebrates constitute a raw material that is included in the
108 European Union Feed Material Register, and although they are
109 currently authorized only for fish and pets, insect-derived feeds
110 could also represent a suitable ingredient for feed
111 manufacturing for pigs and poultry in the near future. This
112 aspect could be a first step towards combating the severe
113 challenges of the global capacity to supply sufficient food. In
114 this context, insects have captured the interest as a
115 complementary source of protein, AA, fat, carbohydrates,
116 vitamins and trace elements (Chen et al., 2009). Number of
117 authors have reported interesting results about the suitability of

118 different types of insect meal as diet ingredients for livestock
119 animals (pigs, poultry, different fish species), (Veldkamp et al.,
120 2012; Van Huis, 2013; Makkar et al., 2014; Henry et al., 2015).

121 Among the different insect species, Black soldier fly
122 (*Hermetica illucens*, HI) and Yellow mealworm (*Tenebrio*
123 *molitor*, TM) show interesting characteristics, because they can
124 valorize organic waste producing proteins, fats and energy,
125 which are exploitable for feed (Zheng et al., 2013). These two
126 insects have the potential to recycle lost nutrients by
127 incorporating the residual AA and fatty acids of manure and
128 organic wastes into their biomass. This resulting biomass is
129 usually high in protein and fat, which makes it interesting for
130 incorporation into animal feeds (Makkar et al., 2014; Henry et
131 al., 2015). The meal derived from HI larvae is a high-value feed
132 source that is rich in protein and fat. It has been reported that
133 the crude protein content ranges between 350 and 570 g/kg
134 (Veldkamp et al., 2012). The amount of fat is extremely
135 variable and depends on the type of diet: values of 150-250
136 g/kg have been reported for larvae fed on poultry manure, 280
137 g/kg for those fed on swine manure, 350 g/kg for cattle manure
138 and 420–490 g/kg for oil-rich food waste (Makkar et al., 2014).
139 As a component of a complete diet, HI larvae have been found
140 to improve the growth rate of chickens (Hale, 1973; Oluokun,
141 2000), swine (Newton et al., 1977), and several commercial
142 fish species (Newton et al., 2005; St-Hilaire et al., 2007). The

143 larvae of TM are easy to breed, and they grow easily on dried
144 and cooked waste materials from fruit, vegetables and cereals
145 in various combinations. For this reason, they are already
146 produced industrially as feeds for pets and zoo animals,
147 including birds, reptiles, small mammals, amphibians and fish
148 (Makkar et al., 2014). The meal derived from TM larvae has a
149 high content of crude protein, which ranges between 440 and
150 690 g/kg, and a fat content that varies between 230 and 470
151 g/kg (Veldkamp et al., 2012). In livestock, TM has been shown
152 to be an acceptable protein source for African catfish (Ng et al.,
153 2001) and for broiler chickens (Ramos-Elorduy et al., 2002).

154 The potential of insects for use as livestock feeds may
155 also have a positive environmental impact: in fact, their
156 production involves less energy, land area utilization and
157 environmental footprints (Pimentel et al., 1975; Makkar et al.,
158 2014). All this evidence indicates that the use of insects in feed
159 formulations could be an opportunity to make the broiler
160 chicken supply-chain more sustainable than it currently is.
161 Moreover, it is also important to emphasize that insects are a
162 part of the natural diet of poultry. Nevertheless, at present,
163 information about insect digestibility in poultry is scarce, and
164 this limits the design of adequate insect-based diets for broilers.

165 For this reason, this study was undertaken to evaluate
166 the apparent nutrient digestibility, the apparent ileal AA

167 digestibility and the apparent metabolisable energy of HI and
168 TM meals fed to broiler chickens.

169

170 **2. Materials and methods**

171 The study was performed at the poultry facility of the
172 Department of Veterinary Sciences of the University of Turin
173 (Italy). The experimental protocol was designed according to
174 the guidelines of the current European and Italian laws on the
175 care and use of experimental animals (European directive 86
176 609/EEC, put into law in Italy with D.L. 116/92).

177

178 *2.1 Ingredients*

179 Two insect larval meals, namely, TM meal and HI meal,
180 were studied. The TM meal was obtained from Gaobeidian
181 Shannong Biology Co. Ltd., Gaobeidian, Hebei province
182 (China), while the HI meal was obtained from Hermetia
183 Futtermittel GbR, Baruth/Mark (Germany). The TM and HI are
184 omnivorous and were fed cereal by-products. The larvae weight
185 at collection ranged between 150 and 220 mg. The collected
186 larvae were dried for 20 h in an oven at low temperature (60
187 °C) and grinded to a meal. Both insect larval meals were full-
188 fat and produced from the larval stage of insects. Before the
189 digestibility trial, representative samples of the two insect
190 larval meals were analyzed, in triplicate, for dry matter (DM),

191 crude protein (CP), ether extract (EE), ash, gross energy (GE)
192 and AA composition.

193

194 *2.2 Pre-experimental period*

195 One-day-old male broiler chickens (Ross 708) were
196 raised in a floor pen till d 19 and fed a commercial broiler
197 starter diet (227 g/kg of CP; 13.4 MJ/kg metabolisable energy).
198 All the birds were vaccinated at hatching against Newcastle
199 disease, Marek disease, infectious bronchitis and coccidiosis.
200 At d 19, ninety birds of uniform body weight were chosen and
201 homogeneously distributed over thirty cages (3 birds *per* cage).
202 The cages (60 × 60 cm) were placed in an insulated room with
203 devices to control the temperature, light and humidity. Each
204 cage had a linear feeder at the front and a nipple drinker at the
205 back. Health status and mortality were monitored daily
206 throughout the whole experimental period. The birds were fed a
207 commercial finisher broiler diet (190 g/kg of CP; 13.6 MJ/kg
208 metabolisable energy) until the assay diets were introduced on
209 d 26. The feeds and water were provided *ad libitum*.

210

211 *2.3 Digestibility trial*

212 On day 26, the cages were randomly assigned to three
213 assay diets (10 replicates *per* diet). A basal diet, based on corn
214 and soybean meal, was formulated (Table 1), and two
215 experimental diets were subsequently formulated by

216 substituting 250 g/kg (w/w) of the basal diet with two insect
217 larval meals. Celite[®] (Celite Corp., Lompoc, CA, USA), was
218 added to each diet at 20 g/kg as an acid-insoluble ash (AIA)
219 digestibility marker in order to calculate the digestibility of the
220 AAs. The diet adaptation period lasted 6 d. Total tract
221 digestibility was evaluated *per* cage, through the total
222 collection of excreta method, from day 32 for four consecutive
223 days. Fresh feeds and water were available *ad libitum*. Feed
224 intake *per* cage was measured throughout the experiment and
225 the excreta was sampled daily during the test period. The total
226 fresh excreta *per* cage was weighed daily, frozen at -20°C and
227 lyophilized. 4 days excreta *per* cage was pooled for further
228 analysis.

229 On day 35, all the birds were euthanized by the
230 intravenous injection of sodium pentobarbital, and the content
231 of the lower half of the ileum was collected, according to the
232 procedures described by Ravindran et al. (2005). The ileum
233 was defined as that portion of small intestine extending from
234 Meckel's diverticulum to a point 40 mm proximal to the ileo-
235 cecal junction. The ileal content for each cage was pooled,
236 lyophilized, ground to pass through a 0.5-mm sieve, and stored
237 at -20°C in airtight containers until laboratory analyses were
238 conducted.

239

240 *2.4 Chemical analysis*

241 Both the dried excreta and diet samples were
242 subsequently ground to pass through a 0.5-mm sieve and stored
243 in airtight plastic containers for DM, ash, CP (AOAC, 2005;
244 procedure numbers of 930.15, 924.05, 984.13, respectively),
245 EE (Folch et al., 1957), GE (IKA C7000, Staufen, Germany)
246 and AIA (Vogtmann et al., 1975) analyses. The uric acid (UA)
247 content in the excreta samples was determined
248 spectrophotometrically according to the Terpstra and De Hart
249 (1974) method. The CP amount of excreta was calculated as
250 follows: $CP = (\text{total nitrogen} - \text{UA-nitrogen}) \times 6.25$.

251 The apparent digestibility trial was performed, using the
252 total excreta collection method, to determine the apparent
253 digestibility coefficients of the total tract (CTTAD) for DM,
254 organic matter (OM), CP, EE, GE, and the apparent
255 metabolisable energy (AME).

256 Ileal content samples from each cage were analyzed for
257 DM, AIA concentration and AA. In order to perform the AA
258 determination, samples of the diets, ileal digesta and insect
259 larval meals were prepared using a 22 h hydrolysis step in 6
260 HCl at 112°C under a nitrogen atmosphere. Performic acid
261 oxidation occurred prior to acid hydrolysis for methionine and
262 cystine. The AA in hydrolysate was determined by means of
263 HPLC after postcolumn derivatization, according to the
264 procedure described by Madrid et al. (2013). Tryptophan was
265 not determined.

266

267 *2.5 Calculations*

268 Two different methods were used for the TM meal and
269 the HI meal to calculate the CTTAD of the dietary nutrients,
270 AME and the apparent ileal digestibility coefficient (AIDC) of
271 the AAs (Ravindran et al., 2005; Nalle et al., 2012).

272 The CTTAD of the dietary nutrients of the insect larval
273 meals were calculated as follows:

$$274 \quad \text{CTTAD } X_{\text{diet}} = [(\text{total } X \text{ ingested} - \text{total } X \text{ excreted}) / \text{total } X \\ 275 \quad \quad \quad \text{ingested}]$$

$$276 \quad \text{CTTAD } X_{\text{insect larval meal}} = [\text{CTTAD } X \text{ of insect larval meal diet} \\ 277 \quad \quad \quad - (\text{CTTAD } X \text{ of basal diet} \times 0.75)] / 0.25$$

278 where X represents DM, OM, CP, EE and GE.

279 The AME values of the insect larval meals were
280 calculated using the following formula with appropriate
281 corrections made according to the differences in the DM
282 content:

$$283 \quad \text{AME}_{\text{diet}} (\text{MJ/kg}) = [(\text{feed intake} \times \text{GE diet}) - (\text{excreta output} \times \\ 284 \quad \quad \quad \text{GE excreta})] / \text{Feed intake}$$

$$285 \quad \text{AME}_{\text{insect larval meal}} (\text{MJ/kg}) = [\text{AME of insect larval meal diet} - \\ 286 \quad \quad \quad (\text{AME basal diet} \times 0.75)] / 0.25$$

287 Correction for zero nitrogen (N) retention was made using a
288 factor of 36.54 kJ *per* gram N retained in the body in order to

289 estimate the N-corrected apparent metabolisable energy
290 (AMEn) (Hill and Anderson, 1958). N retention was calculated
291 using the following formula:

$$292 \quad N_{\text{retention}} = [(\text{feed intake} \times N_{\text{diet}}) - (\text{excreta output} \times N_{\text{excreta}})] / \text{feed intake (kg)}$$

294 The AIDC of the AA of the insect larval meals was
295 calculated, using AIA as the indigestible marker, as follows:

$$296 \quad \text{AIDC of AAX}_{\text{diet}} = (\text{AAX/AIA})_{\text{d}} - (\text{AAX/AIA})_{\text{i}} /$$
$$297 \quad (\text{AAX/AIA})_{\text{d}}$$

298 The AIDC of AAX_{insect larval meal} = [(AIDC AAX of the insect
299 larval meal diet × AAX of the insect larval meal diet) – (AIDC
300 AAX of the basal diet × AAX of the basal diet × 0.75)] / (AAX
301 of the insect larval meal diet × 0.25).

302 where:

303 (AA/AIA)_d = ratio of the AA and AIA concentrations in the
304 diet;

305 (AA/AIA)_i = ratio of the AA and AIA concentrations in the
306 ileal digesta;

307 AAX : represents each AA evaluated.

308

309 *2.6 Statistical analyses*

310 The statistical analysis of the total tract digestibility
311 coefficients, apparent metabolisable energy and apparent ileal

312 digestibility coefficients was performed with SPSS 17 for
313 Windows (SPSS, Inc., Chicago, IL, USA). The experimental
314 unit was the cage. Data concerning total tract digestibility
315 coefficients, apparent metabolisable energy and apparent ileal
316 digestibility coefficients of the TM meal and HI meal were
317 analyzed using Student's t-test for independent samples. Before
318 testing for group differences, normality of the data distribution
319 and homogeneity of variances were assessed using the Shapiro-
320 Wilk test and the Levene test, respectively. Differences were
321 considered to be significant at $P \leq 0.05$.

322

323 **3. Results**

324 The proximate composition and GE of the three assay
325 diets and of the two insect larval meals are summarized in
326 Table 2. The TM meal resulted to have a higher CP content
327 than the HI meal (524 and 369 g/kg DM, respectively). On the
328 contrary, the EE content of the HI meal was higher than that of
329 the TM meal (343 and 280 g/kg DM, respectively). The GE
330 contents of the TM and HI meals were similar (24.4 and 23.8
331 MJ/kg DM, respectively).

332 The AA compositions of the three assay diets and of the
333 two insect larval meals are presented in Table 3. Lysine was the
334 most abundant indispensable AA in the TM meal, whereas
335 glutamic acid was the most abundant dispensable one. The
336 most represented indispensable AAs in the HI meal were

337 leucine and lysine. As in TM meal, glutamic acid was the most
338 abundant of the dispensable AAs. Both insect larval meals were
339 also good sources of methionine and threonine. The TM meal
340 showed higher lysine, methionine and threonine contents than
341 the HI meal.

342 The CTTAD of the nutrients, as well as the AME and
343 AMEn of the TM and HI meals are reported in Table 4. No
344 statistical differences were found between the tested insect
345 larval meals for any of the CTTAD of the nutrients, except for
346 EE ($P<0.001$), which was higher for the HI meal than the TM
347 meal (0.99 and 0.88, respectively). The CTTAD for DM was
348 0.60 and 0.53; 0.66 and 0.66 for OM; 0.60 and 0.51 for CP,
349 whereas it was 0.64 and 0.69 for GE, for TM and HI,
350 respectively.

351 No difference was observed between TM and HI
352 ($P>0.05$) for AME or AMEn. In particular, HI showed mean
353 AME and AMEn values of 17.38 and 16.60 MJ/kg DM,
354 respectively, while for TM, AME and AMEn they were 16.86
355 and 16.02 MJ/kg DM, respectively.

356 The determined values for the AIDC of the AAs are
357 presented in Table 5. The AIDC of the AAs in TM ranged from
358 0.80 to 0.93, while in HI it ranged from 0.42 to 0.89. Overall,
359 the AIDC of 17 AA was higher ($P<0.001$) in TM (0.86) than in
360 HI (0.68). This reflects the significantly higher ($P<0.05$) AIDC
361 levels of isoleucine, lysine, methionine, phenylalanine, valine,

362 alanine, aspartic acid, glycine, glutamic acid and tyrosine in
363 TM than in HI. Among the indispensable AAs, lysine and
364 methionine were the AAs that showed the greatest difference
365 between the two insect larval meals (AIDC for lysine: 0.85 and
366 0.46 in TM and HI, respectively, and AIDC for methionine:
367 0.80 and 0.42 in TM and HI, respectively).

368

369 **4. Discussion**

370 The compositional data have shown that the two insect
371 larval meals are good sources of protein and fat. In particular,
372 the TM meal has shown a higher CP content than soybean meal
373 which is close to that of meat meal, however it has a higher fat
374 content. This result indicates how this insect larval meal could
375 be used as both a protein and an energy ingredient for feeds
376 (Sauvant et al., 2004). The HI meal has shown a similar CP
377 content to some plant protein sources, such as sunflower meal,
378 lupins or faba beans, but also a higher fat content (Sauvant et
379 al., 2004). The CP and EE determined for the TM meal was
380 within the range reported by other researchers (Bernard et al.,
381 1997; Ramos-Elorduy et al., 2006; Barroso et al., 2014;
382 Sánchez-Muros et al., 2014). The fat content reported in the HI
383 meal was consistent with previous findings, while the protein
384 content was slightly lower (Newton et al., 1977; Sheppard et
385 al., 2007; Sánchez-Muros et al., 2014). This may be due to the
386 substrate where the larvae were raised, which can influence

387 variability in the amount of CP, EE and fatty acids composition
388 (Makkar et al., 2014).

389 The AA profiles of the TM and HI meals were within
390 the ranges reported by other authors (Ramos-Elorduy et al.,
391 2002; St-Hilaire et al., 2007; Barroso et al., 2014; Makkar et
392 al., 2014; Henry et al 2015). Both meals are a good source of
393 AA as they are both rich in methionine and lysine, which
394 content is higher than the common plant protein ingredients
395 used in poultry feeds (Ravindran et al., 1999; 2005; Nalle et al.,
396 2012; Barroso et al., 2014). The methionine and lysine contents
397 in the TM meal are slightly lower than those in fish meal, but
398 higher than those in meat meal (Ravindran et al., 1999; Sauvart
399 et al., 2004). The methionine and lysine contents in the HI meal
400 are in line with or slightly below those of meat meal
401 (Ravindran et al., 1999).

402 In this study, no differences have been found between
403 the TM meal and the HI meal in the CTTAD for DM, OM, CP
404 and GE. Nevertheless, differences have been found for CTTAD
405 of EE, where the HI meal has resulted more digestible than the
406 TM meal. Overall, the CTTAD of the nutrients were not very
407 high for either of the insect larval meals, except for EE. Little
408 information is available about the CTTAD of insects in
409 chickens, and to the best of the authors' knowledge, no studies
410 have dealt with CTTAD for TM meal or HI meal.
411 Consequently, a direct comparison between results is not

412 possible. Only two studies concerning insect digestibility have
413 been found, and both were carried out using dried housefly
414 meal. Hwangbo et al. (2009) fed 4-week old broilers a diet with
415 300 g/kg dried housefly larva meal or soybean meal for 7 days
416 and reported a very high AD coefficient of CP for housefly
417 larvae (0.98). Pretorius (2011) tested dried housefly larva meal
418 fed to 3-week old broiler chickens by substituting 500 g/kg
419 (w/w) of a maize meal-based diet with insect larval meal and
420 found a CP digestibility of 0.69. The CTTAD of nutrients
421 found in the present digestibility trial are lower than the two
422 above-mentioned studies, mainly with respect to those found by
423 Hwangbo et al. (2009). It can be speculated that the chitin
424 contained in the exoskeleton of the TM and HI larvae can
425 negatively affect CTTAD of nutrients. In this context,
426 Ravindran and Blair (1993) pointed out that the chitin
427 contained in the hard outer shell of insects is difficult to digest
428 by domestic poultry, although the high chitin content of insect
429 meals does not appear to have detrimental effects on poultry
430 performance.

431 The AME and AMEn values of the TM meal and HI
432 meal are comparable to such high-energy vegetable ingredients
433 as sunflower seed (Sauvant et al., 2004). The AME and AMEn
434 values found in the present study, as well as the CTTAD of the
435 nutrients, are not at the moment comparable with other insect
436 larval meals, because no similar studies have been found in

437 literature. However, the high CP and EE contents of the TM
438 meal and HI meal make these two ingredients have high
439 metabolisable energy values. In fact, with the exception of pure
440 fat ingredients, such as vegetable oils and animal fats, the
441 values of AME obtained in this study are higher than all the
442 ingredients normally used in poultry feeds (Sauvant et al.,
443 2004). This aspect could make these two insect larval meals
444 attractive and functional for poultry feed formulation. As
445 confirmation of this thesis, other studies reported how these
446 two meals can be used to feed poultry. Hale (1973) pointed out
447 that chickens fed a diet containing HI larva meal, as a substitute
448 of soybean meal, showed lower feed conversion ratio than the
449 control group.

450 Ramos-Elorduy et al. (2002) showed, with regards to
451 TM meal, how dried yellow mealworms included in quantities
452 of up to 100 g/kg in a broiler starter diet based on sorghum and
453 soybean meal could be used without any negative effects on
454 either the performances or palatability. In another study, it was
455 noted that TM could replace fishmeal in laying hen diets and a
456 2.4% higher egg-laying ratio than that obtained with good
457 quality feed could be obtained (Wang et al., 1996).

458 In the present study, differences in the AIDC of the AAs
459 have been found between the TM and HI meal. The AIDC of
460 17 AA in the TM meal was higher and showed fewer variations
461 than in the HI meal. Threonine (0.80) and methionine (0.80) for

462 TM, and methionine (0.42) and isoleucine (0.45) for HI were
463 the least digested indispensable AAs, while the most digestible
464 indispensable AAs were phenylalanine (0.91) and arginine
465 (0.90) in the TM meal, and arginine (0.83) and histidine (0.81)
466 in the HI meal. Moreover, it should be noted that the AIDC of
467 all the indispensable AAs in TM was greater than 0.80. It is
468 surprising low digestibility shown in HM for some
469 indispensable amino acids as methionine and isoleucine, which
470 may be inherent to the raw material or due to technical
471 processing for obtain this meal, which is unknown to us. To the
472 authors' knowledge, no studies on the AIDC of AAs in TM or
473 HI meal in broilers have been conducted. For this reason, it is
474 not possible to make a comparison of the values obtained in the
475 present study with published data. However, it has been
476 postulated that insect larval meals could be used in poultry
477 feeding to replace protein sources such as soybean meal
478 (Ramos-Elorduy et al., 2002; Veldkamp et al., 2012; Makkar et
479 al., 2014). In this sense, the average AIDC of the indispensable
480 AAs in TM coincides with the findings of Valencia et al.
481 (2009), Ravindran et al. (2005) and Huang et al. (2006) in 21,
482 42 and 49 day old broilers, respectively. It is worth noting that
483 both the concentration and the AIDC of lysine in the TM meal
484 were similar to that of the soybean meal analyzed in the above
485 studies (Ravindran et al., 2005; Huang et al., 2006, 2007),
486 although the AIDC for methionine was lower in the TM meal

487 than in the soybean meal. However, the concentration of
488 methionine in the TM meal was higher than that of soybean
489 meal, and TM has therefore resulted to be a good source of this
490 AA. Moreover, when the AIDC of the indispensable AAs in
491 TM was compared with other plant protein sources (pea protein
492 concentrate, full-fat soya bean and sunflower meal), it was
493 interesting to observe that the AIDCs were higher in the TM
494 meal than in the above-reported protein sources for most of the
495 AAs (Ravindran et al., 2005; Valencia et al., 2009). As far as
496 animal protein sources are concerned, it was noted that AIDC
497 was similar or slightly higher in the TM meal than in the fish
498 meal for most of the AAs (Ravindran et al., 2005), although the
499 AA content was lower in TM. The average AIDC of the
500 dispensable AAs calculated in TM was higher than in the
501 soybean meal and the other protein sources analyzed in the
502 above studies (Ravindran et al., 2005; Huang et al., 2006; 2007;
503 Valencia et al., 2009). In general, the AIDC of AAs results of
504 the TM meal can be considered interesting. Consequently, it is
505 reasonable to consider TM meal as an appealing protein source
506 for broiler feeds. As far as HI meal is concerned, the average
507 results of AIDC for the indispensable and dispensable AAs
508 were lower than those obtained for the soybean meal and other
509 protein sources examined by the previous authors (Ravindran et
510 al., 2005; Huang et al., 2006, 2007; Valencia et al., 2009).

511

512 **5. Conclusion**

513 Many authors have pointed out how there is a need for
514 the evaluation of the nutrient digestibility of processed insects
515 as a feed ingredient. Our study have shown that TM and HI
516 meals are valuable sources of AME and digestible AA. This
517 study has provided updated and never before determined
518 nutritional values of TM meal and HI meal, which could be two
519 potential future ingredients for use in the formulation of broiler
520 feeds. The acquired knowledge of AME and AMEn will be
521 useful for nutritionists and feed companies to obtain better
522 formulate innovative poultry feeds. Looking to the future, the
523 next foremost gamble will be to evaluate the point of view of
524 the European consumers in respect of the use of insects as a
525 livestock feed. Nowadays, little is known on the insects food
526 safety side and this can be of critical importance to meet
527 society's approval, especially if people are not accustomed to
528 eating insects, also indirectly. Legislative issues will also have
529 to be discussed and resolved.

530

531 **Conflict of interest statement**

532 The authors declare that there is no conflict of interest.

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694 **Table 1**

695 Composition (g/kg as fed) of the basal diet.

Ingredients	
Maize meal	580.0
Soybean meal	343.7
Soybean oil	45.0
Dicalcium phosphate	12.4
Calcium carbonate	11.2
Sodium chloride	2.2
Sodium bicarbonate	1.5
Trace mineral-vitamin premix ¹	4.0
Calculated analysis	
AME, MJ kg ⁻¹	12.2
Crude Protein	201
Methionine	4.0
Lysine	10.9
Methionine + Cysteine	6.1
Threonine	7.8
Calcium	8.7
Phosphorous	5.7

696 ¹Mineral-vitamin premix (Final B Prisma, IZA SRL), given
697 values are supplied per kg diet: 2.500.000 IU of vitamin A;
698 1.000.000 IU of vitamin D3; 7.000 IU of vitamin E; 700 mg of
699 vitamin K; 400 mg of vitamin B1; 800 mg of vitamin B2; 400
700 mg of vitamin B6; 4 mg of vitamin B12; 30 mg of biotin; 3.111
701 mg of Ca pantothenate acid; 100 mg of folic acid; 15.000 mg of
702 vitamin C; 5.600 mg of vitamin B3; 10.500 mg of Zn, 10.920
703 mg of Fe; 9.960 mg of Mn; 3.850 mg of Cu; 137 mg of I; 70
704 mg of Se.

705 **Table 2**

706 Analyzed chemical composition of the three experimental diets and of the two insect larval meals.

	Basal diet	<i>Tenebrio molitor</i> diet	<i>Hermetia illucens</i> diet	<i>Tenebrio molitor</i> (TM)	<i>Hermetia illucens</i> (HI)
Dry matter (g/kg diet)	903	914	917	948	957
Organic matter (g/kg DM)	830	850	833	912	827
Crude protein (g/kg DM)	198	270	235	524	369
Ether extract (g/kg DM)	65.7	107	121	280	343
Gross Energy (MJ/kg DM)	17.0	18.8	18.6	24.4	23.8

707

708 **Table 3**

709 Amino acid concentration (g/kg DM) of the three experimental

710 diets and of the two insect larval meals.

	Basal diet	<i>Tenebrio molitor</i> diet	<i>Hermetia illucens</i> diet	<i>Tenebrio molitor</i> (TM)	<i>Hermetia illucens</i> (HI)
Indispensable amino acids					
Arginine	16.6	19.3	18.2	28.0	19.4
Histidine	7.69	9.92	9.09	16.8	11.3
Isoleucine	9.74	12.4	10.4	22.1	17.2
Leucine	17.8	20.4	19.4	31.5	24.0
Lysine	9.84	15.5	11.2	35.9	22.3
Methionine	4.86	6.24	5.48	10.1	9.05
Phenylalanine	14.0	15.3	14.1	18.8	14.4
Threonine	9.54	11.6	11.3	18.5	15.2
Valine	9.80	13.7	12.0	28.2	22.0
Dispensable amino acids					
Alanine	7.01	14.8	13.0	38.9	30.3
Aspartic acid	17.2	23.2	19.7	43.7	32.2
Cysteine	4.56	6.83	6.98	12.5	13.8
Glycine	10.8	13.8	12.9	22.1	19.1
Glutamic acid	30.7	37.4	34.4	62.9	38.5
Proline	13.4	18.1	20.0	34.3	37.3
Serine	11.9	14.7	14.1	22.7	18.4
Tyrosine	9.71	15.0	10.8	32.8	21.6

711

712 **Table 4**

713 Apparent digestibility coefficients of the total tract (CTTAD) of
 714 the nutrients, AME and AMEn of insect larval meals for
 715 broilers¹.

	<i>Tenebrio molitor</i>		SEM	P
	(TM)	<i>Hermetia illucens</i> (HI)		
DM	0.60	0.53	0.02	0.20
OM	0.66	0.66	0.02	0.87
CP	0.60	0.51	0.03	0.23
EE	0.88	0.99	0.02	0.00
GE	0.64	0.69	0.02	0.23
AME (MJ/kg DM)	16.86	17.38	0.47	0.59
AMEn (MJ/kg DM)	16.02	16.60	0.46	0.54

716 DM = dry matter; OM = organic matter; CP = crude protein;

717 EE = ether extract; GE = gross energy; AME = apparent

718 metabolisable energy; AMEn = nitrogen-corrected apparent

719 metabolisable.

720 ¹Each value represents the mean of ten replicates (three birds

721 *per* replicate).

722 **Table 5**

723 Apparent ileal digestibility coefficients (AIDC) of amino acid
 724 of the two insect larval meals for broilers¹.

	<i>Tenebrio</i> <i>Hermetia</i>		SEM	P
	<i>molitor</i> (TM)	<i>illucens</i> (HI)		
Indispensable amino acids				
Arginine	0.90	0.83	0.03	0.23
Histidine	0.85	0.81	0.02	0.44
Isoleucine	0.82	0.45	0.05	0.00
Leucine	0.82	0.76	0.03	0.24
Lysine	0.85	0.46	0.05	0.00
Methionine	0.80	0.42	0.05	0.00
Phenylalanine	0.91	0.63	0.04	0.00
Threonine	0.80	0.75	0.03	0.46
Valine	0.82	0.62	0.03	0.00
Mean	0.84	0.64	0.03	0.00
Dispensable amino acids				
Alanine	0.93	0.86	0.02	0.04
Aspartic acid	0.89	0.61	0.04	0.00
Cysteine	0.84	0.82	0.02	0.52
Glycine	0.89	0.67	0.04	0.00
Glutamic acid	0.88	0.74	0.03	0.00
Proline	0.84	0.89	0.01	0.06
Serine	0.89	0.82	0.03	0.21
Tyrosine	0.83	0.43	0.05	0.00
Mean	0.87	0.73	0.02	0.00
Overall mean ²	0.86	0.68	0.03	0.00

725 ¹ Each value represents the mean of ten replicates (three birds

726 *per replicate*).

727 ² Average digestibility of 17 amino acids.