Nutritional value of *Tenebrio molitor* larvae meal for broiler chickens: metabolizable energy and standardized ileal amino acid digestibility

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Primary Audience: Nutritionists, Researchers, Feed Formulators

SUMMARY

Two experiments were conducted to determine the nitrogen-corrected apparent metabolizable energy (**AMEn**) (experiment 1) and the standardized ileal digestibility coefficients (**SIDC**) of amino acids (**AA**) (experiment 2) of *Tenebrio molitor* larvae meal (**TM**) for broilers. In experiment 1, 48 21-day-old male broilers were distributed in a completely randomized experimental design with 2 treatments (diets) and 6 replicates (metabolic cages)/treatment, with 4 birds each. Based on the total excreta collection method, cages were randomly assigned to 2 diets: a reference diet and a test diet prepared by substituting 300 g/kg of the reference diet with TM. Ferric oxide (1%) was added to the diets on the first and last day of excreta collection carried out from day 28 to 32, twice daily. In experiment 2, 120 one-day-old male broiler chicks were distributed in a completely randomized experimental design with 2 treatments (diets) and 6 replicates (pens)/treatment, with 10 birds each. At day 16, birds were assigned to 2 experimental diets: a semi-purified nitrogen free diet and a test diet with TM as the sole source of AA, formulated to be approximately 20% CP. Chromium oxide was used at 5 g/kg as an indigestible marker. On day 22, all birds were euthanized, and the contents of the distal ileum were collected. The determined AMEn for TM was 5,004 ± 121.2 kcal/kg DM, a value higher than that of common feed sources of animal and vegetable origin. The average SIDC of AA was 0.86, indicating a good availability of the nutrients for chickens. The values obtained for AMEn and SIDC of TM are comparable to those of other insect meals reported in the literature. Thus, AMEn and SIDC of AA of TM determined here might be applied as a guide to optimize poultry feed formulation using this ingredient.

Key words: digestibility, *Tenebrio molitor*, energy, amino acids, broiler chickens

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DESCRIPTION OF PROBLEM

The world population is growing over the years, and it is expected to increase by 2 billion people in 2050. As a result, the increased demand for animal protein will have to be met by a congruent production in accordance to sustainable and environmental concerns. This suggests that alternative feed ingredients should be introduced in the animal production chain (Henchion et al., 2017).

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Insects meal is one of the promising strategies proposed to be included among common ingredients for animal diets (Van Huis et al., 2013), being a rich source of nutrients (Van Huis, 2013; Sánchez-Muros et al., 2014; Glover and Sexton, 2015). The mealworm larvae (*Tenebrio molitor*) have a wide distribution in Brazil and is the most reared insect in Europe (Paul et al., 2017). Normally reared on dry co-products (e.g., from food and fuel processing) and animal and vegetable waste products (Ramos-Elorduy et al., 2002; Kim et al., 2016a, b; 2017), Tenebrio shows advantages to mass production because of its prolific population, short generation time, and easy handling (Van Huis et al., 2013). Likewise, this insect can provide high-value feedstuff, as good as plant-derived and animal-derived proteins, containing about 47 to 60% crude protein (**CP**) and 30% lipids on dry basis (Ravzanaadii et al., 2012; Makkar et al., 2014).

Chickens have the natural behavior of feeding themselves from a variety of insects during their entire lifecycle (Hwangbo et al., 2009; Khan, 2018), and entomophagy may represent a part of the bird ingested food (Van Huis et al., 2013). This statement is in agreement with our previous results in which broilers displayed an intense feed preference for insect meal offered simultaneously with other feed ingredients, for example soybean meal and corn (Nascimento Filho et al., 2020). Moreover, some studies have demonstrated that the use of insect meal, fully or partially substituting soybean meal in poultry diets, is extremely promising as it combines nutritional, health, and sustainable properties in an industrial sector that has never considered it before. Evaluating the feasibility of using an insect meal diet for growing chickens, Bovera et al. (2015) found that *T. molitor* larvae can be used to completely replace soybean meal in broiler diets, showing positive effects in feed conversion ratio and suggesting higher immune response. Likewise, increasing levels (up to 15%) of mealworm larvae meal in the diet (Biasato et al., 2017) resulted in increased weight gain and carcass yield of chickens as well as improved feed conversion. In the same context, the elevated nutritional value of Tenebrio meal, in terms of apparent metabolizable energy (**AME**) and apparent digestibility of amino acids (**AA**), was demonstrated by De Marco et al. (2015).

Given the increased interest in the use of insects in animal feeding, the need for additional knowledge on the nutritional value of insect meals of different origins for feed formulation, especially *Tenebrio molitor* larvae meal (**TM**), is evident. As current perspective, once insect protein becomes part of chicken diets, animals might benefit from an alternative feed ingredient of animal origin. Therefore, the objective of this study was to determine the values of nitrogen-corrected apparent metabolizable energy (**AMEn**) and standardized ileal digestibility coefficients (**SIDC**) of AA of TM for broiler chickens to be used in feed formulation.

MATERIAL AND METHODS

The experimental procedures were approved by the Institutional Animal Care and Use Committee, University of São Paulo, Piracicaba, SP, Brazil (protocol number: 2017.5.2568.11.5).

Experiment 1. Metabolizable Energy Assay

Animals, Diets, and Experimental Procedures. In experiment 1, 48 21-day-old male broiler chickens of a commercial strain (Ross AP95) were weighed and distributed to 12 metabolic cages (0.70 × 0.66 m) in an environmentally controlled room. The cages had wire mesh floors and metal trays covered with plastic on the bottom for excreta collection and were equipped with stainless steel feeders and waterers. The experiment was carried out in a completely randomized design, with 2 treatments and 6 replicates (cages) of 4 birds each. The treatments consisted of a grower reference diet, based on corn and soybean meal, formulated to meet birds' nutritional requirements for standard performance (Rostagno et al., 2017), and a test diet replacing 300 g/kg (w/w) of the reference diet with TM. The insect larvae meal was obtained from Vida Proteína Co. Ltda., Nerópolis, Goiás, Brazil. The adaptation period lasted 7 d, followed by a collection period of 5 d. Feed and water were available *ad libitum*.

For each cage and twice a day, total excreta output were collected from day 28 to 32 taking all the precautions to prevent contamination with foreign material and stored immediately at −20°C. Total feed intake and excreta production were quantified per cage at the end of the 5d trial. Ferric oxide was added at 1% to the diets on the first and last day of the collection period to identify precisely the excreta generated during the test period.

Measurements and Analytical Methods. Before performing the analyses, the 5d total excreta were thawed, homogenized, and pooled by cage. An aliquot of around 250 g of excreta from each cage was taken, weighed, dried in a forced-air oven at 65°C for 72 h, and finally ground to pass through a 1 mm sieve (ESALQ LAB, Piracicaba, Brazil). Similarly, samples of the diets and test ingredient were ground and stored in plastic bags. Analyses were carried out to determine the dry matter (**DM**), nitrogen (**N**), ether extract (**EE**), and gross energy (**GE**) of these samples. According to the standard procedure methods of AOAC (2005), diets and excreta samples were dried to a constant weight at 105°C to determine the DM content (procedure 930.15). Nitrogen was determined following the Dumas combustion method (LECO TruSpec N, St. Joseph, MI) using AOAC, 2016 (procedure 992.15) and CP value was computed (N × 6.25). The EE was determined by Soxhlet extraction method (AOAC, 2012; procedure 920.39) and GE by using an oxygen bomb calorimeter (Parr 6200; Parr Instrument Co., Moline, IL). Values of calcium, phosphorus, and fatty acids in TM were adopted from our previous study (Nascimento Filho et al., 2020) because TM source was the same. The apparent total tract digestibility coefficients (**ATTDC**) of nutrients were calculated based on the analytical results according to Schneider and Flat (1975): where:

$$\text{ATTDC } X_{diet} = [(\text{nutrient}_{consumed}(\text{g or kcal}) - \text{nutrient}_{excreted}(\text{g or kcal})) / \text{nutrient}_{consumed}(\text{g or kcal})]$$

$$\text{ATTDC } X_{test\ ingredient} = \left[\text{ATTDC } X_{test\ diet} - \text{ATTDC } X_{reference\ diet} \times 0.7\right] / 0.3$$

X = DM, N, or GE.

0.7 = inclusion ratio of the reference diet.

0.3 = inclusion ratio of the test ingredient.

Similarly, the values for AME and AMEn were calculated using the equations proposed by Matterson et al. (1965): where:

$$\text{AME}(\text{kcal/kg of DM}) = (\text{GE}_{consumed} - \text{GE}_{excreted}) / \text{DM}_{consumed}$$

$$\text{AME}(\text{kcal/kg of DM})_{test\ ingredient} = \text{AME}_{reference\ diet} + \left(\text{AME}_{test\ diet} - \text{AME}_{reference\ diet}\right) / 0.3$$

NB = nitrogen balance (Nconsumed - Nexcreted)

0.3 = inclusion ratio of the test ingredient.

The results were reported as average ± standard error of the mean, in which each value represents the mean of 6 replicates (4 birds per replicate).

$$\text{AMEn(kcal/kg of DM)}_{\text{diet}} = [(\text{GE}_{\text{consumed}} - \text{GE}_{\text{excreted}}) - 8.22 \times \text{NB}] / \text{DM}_{\text{consumed}}$$

$$\text{AMEn(kcal/kg of DM)}_{\text{test ingredient}} = \text{AMEn}_{\text{reference diet}} + \left(\text{AMEn}_{\text{test diet}} - \text{AMEn}_{\text{reference diet}}\right) \Big/ 0.3$$

Experiment 2. Amino Acid Digestibility Assay

Animals, Diets, and Experimental Procedures. In experiment 2, a total of 120 1-day old male broiler chicks of a commercial strain (Ross AP95) were weighed, placed in 12 floor pens (rice hulls as bedding) with 10 birds per pen and 6 replicates (pens) per diet in a completely randomized design. Each pen was 1.34 m wide × 2.00 m long and was equipped with a tube feeder and a nipple drinker line. All birds were fed a basal corn–soybean meal starter diet up to 15 d of age, formulated to meet their nutritional requirements for standard performance, according to Rostagno et al. (2017). On day 16, pens were randomly assigned to 2 experimental diets: a semi-purified nitrogen-free diet formulated to estimate the basal endogenous AA flow (adapted from Adeola et al., 2016) and a test diet with TM as the sole source of AA. The test diet was formulated to be approximately 200 g/kg CP, and the inclusion rate of TM was 378 g/kg. Chromic oxide was included in the diets at 5 g/kg as an indigestible marker. Feed and water were provided *ad libitum* throughout the experiment. The TM utilized in this experiment was the same as that of experiment 1.

On day 22, all birds were euthanized by cervical dislocation and contents from the distal third of the ileum (until approximately 2 cm above the ileocecal junction) were gently collected by flushing with distilled water. The ileal digesta samples from all birds, within each pen, were pooled and kept refrigerated during collection. The samples were stored at −20°C and then freeze-dried before analysis.

Measurements and Analytical Methods. Samples of the diets, TM, and ileal digesta were ground to pass through 1-mm sieve and stored in plastic bags. Analyses were carried out to determine the DM (AOAC, 2005; procedure 930.15), N (AOAC, 2016; procedure 992.15), AA, and chromium to estimate the amino acid digestibility coefficients. Except for tryptophan, the quantitative measurement of AA was determined using high-performance liquid chromatography (CBO Laboratorial Analyses, Campinas, Brazil) procedure with sample preparation by hydrolysis with hydrochloric acid method for most AA or by performic acid oxidation before the hydrolysis for methionine and cysteine analysis. Asparagine and glutamine are completely converted to aspartic acid and glutamic acid, respectively, and determined (AOAC, 1995, procedure 994.12) in conjunction with the latter 2 AA. The chromium concentration of the diets and ileal digesta samples was measured by the atomic absorption spectroscopy method (Agilent Technologies, Santa Clara, CA; AAS, model 240FS AA) (AOAC, 2016, procedure 968.08). The apparent and standardized ileal digestibility coefficients, as well as the basal endogenous losses of CP and AA, in TM were computed according to Moughan et al. (1992) using the following equations: where:

$$\text{AIDC} - \text{AA}(\%) = \left[1 - \left(\text{AA}_{\text{digesta}} \times \text{marker}_{\text{diet}}\right) / \left(\text{AA}_{\text{diet}} \times \text{marker}_{\text{digesta}}\right)\right] \times 100$$

$$\text{BEL} - \text{AA}(\text{g/kg DM}) = \text{AA}_{\text{digesta}} \times (\text{marker}_{\text{diet}} / \text{marker}_{\text{digesta}})$$

$$\text{SIDC} - \text{AA}(\%) = \text{AIDC} - \text{AA}_{\text{diet}}(\%) + [(\text{BEL} - \text{AA}) / (\text{AA}_{\text{diet}}) \times 100]$$

AIDC-AA = apparent ileal digestibility coefficient of amino acid.

$AA_{digesta}$ = amino acid content in g/kg DM of digesta.

AA_{diet} = amino acid content in g/kg DM of diet.

$marker_{diet}$ = content of the indicator in DM of diet.

$marker_{digesta}$ = indicator content in DM of digesta.

BEL-AA = basal endogenous loss of amino acid in g/kg DM intake.

SIDC-AA = standardized ileal digestibility coefficient of amino acid.

The results were reported as average ± standard error of the mean, in which each value represents the mean of 6 replicates (10 birds per replicate).

RESULTS

The composition and the analyzed nutrient values of the reference and assay diets in Experiment 1 are shown in Tables 1 and 2 and those of the nitrogen-free diet and test diet in Experiment 2 are shown in Tables 3 and 4, respectively. In addition, the analyzed chemical composition and AA contents of TM are shown in Tables 2 and 4, respectively.

The proximate nutritional composition of TM indicates that this ingredient is a rich source of CP (500.0 g/kg), ether extract (297.3 g/kg), and gross energy (6,366 kcal/kg), as fed basis (Table 2). The TM values of calcium and phosphorus (1.15 and 5.67 g/kg as fed basis, respectively) were those obtained from our previous study (Nascimento Filho et al., 2020).

The amino acid profile of TM (Table 4) indicates adequate balance compared with the main ingredients used in poultry feed. In general, values of histidine, valine, aspartic acid, glutamic acid, and tyrosine in TM were higher than those usually found in fish meal and soybean meal. The 3 main limiting AA in corn–soy diets for poultry, methionine plus cysteine, lysine, and threonine, are higher in TM (14.7, 32.6, and 20.9 g/kg, respectively) than in soybean meal (12.8, 28.1, and 18.0 g/kg, respectively) and similar to those in fish meal (14.9,

Table 1. Ingredient and nutritional composition of the reference diet, as fed basis (experiment 1).

Ingredients (g/kg, unless noted)	
Corn	554.0
Soybean meal	356.3
Soybean oil	55.9
Dicalcium phosphate	14.8
Limestone	6.5
Salt	4.9
DL-Methionine	2.8
L-Lysine HCl	1.5
L-Threonine	0.4
Vitamin premix[1]	1.5
Mineral premix[2]	0.6
Choline chloride 70%	0.8
Total	1,000
Nutritional composition (calculated)	
Metabolizable energy (kcal/kg)	3,150
Crude protein	208.3
Calcium	7.6
Available Phosphorus	3.7
Sodium	2.1
Digestible lysine	11.2
Digestible methionine + cysteine	8.3
Digestible threonine	7.4
Digestible tryptophan	2.4

[1]DSM Nutritional Products, Composition per kg of diet: Vit. A—13,500 UI; Vit. D_3—3,750 UI; Vit. E—30 UI; Vit. K_3—3.75 mg; Vit. B_1—3 mg; Vit. B_2—9 mg; Vit. B_6—4.5 mg; Vit. B_{12}—22.5 μg; Nicotinic acid—52.5 mg; Pantothenic acid—27 mg; Biotin—0.15 mg; Folic acid—2.25 mg; Selenium—0.375 mg.

[2]DSM Nutritional Products, Composition per kg of diet: Manganese—96 mg; Iron—60 mg; Zinc—60 mg; Copper—12 mg; Cobalt—1.2 mg; Iodine—1.2 mg.

Table 2. Analyzed chemical composition of the 2 assay diets (experiment 1) and of the *Tenebrio molitor* meal, as fed basis.

	Reference diet	Test diet[1]	*T. molitor* meal
Nutrient composition (g/kg, unless noted)			
Gross energy (kcal/kg)	4,235	4,878	6,366
Dry matter	893.0	909.9	970.2
Crude protein	218.4	298.4	500.0
Ether extract	24.1	94.0	297.3

Abbreviation: TM, *Tenebrio molitor* larvae meal.

[1]Test diet: substituting 300 g/kg (w/w) of the reference diet with TM.

29.5, and 18.6 g/kg, respectively), based on AminoDat 5.0 (Wiltafsky et al., 2016). Also, valine content in TM is at least 30% greater than in vegetable-based protein sources. Arginine is the only amino acid in TM with lower content compared to soybean meal or fish meal.

Table 3. Ingredient and nutritional composition of the experimental diets, as fed basis (experiment 2).

Ingredients (g/kg, unless noted)	NFD	NFD + TM
Dextrose	640.0	261.7
Tenebrio molitor meal	-	378.3
Corn starch	204.3	204.3
Cellulose	50.0	50.0
Soybean oil	50.0	50.0
Dicalcium phosphate	22.6	22.6
Limestone	8.6	8.6
Sodium bicarbonate	7.5	7.5
Chromium oxide	5.0	5.0
Potassium chloride	2.9	2.9
Potassium carbonate	2.6	2.6
Choline chloride 60%	2.5	2.5
Magnesium oxide	2.0	2.0
Vitamin premix[1]	1.5	1.5
Mineral premix[2]	0.5	0.5
Total	1,000	1,000
Nutritional composition (calculated)		
Metabolizable energy (kcal/kg)	3,332	3,884[3]
Crude protein	0	200.0
Ether extract	50.0	174.2
Calcium	8.8	9.2
Available phosphorus	4.2	6.3
Sodium	2.0	-
Chloride	1.4	-
Magnesium	1.1	-
Potassium	3.0	-

Abbreviations: NFD, nitrogen-free diet; NFD + TM, nitrogen free diet + *Tenebrio molitor* meal.

[1]DSM Nutritional Products, Composition per kg of diet: Vit. A—13,500 UI; Vit. D_3—3,750 UI; Vit. E—30 UI; Vit. K_3—3.75 mg; Vit. B_1—3 mg; Vit. B_2—9 mg; Vit. B_6—4.5 mg; Vit. B_{12}—22.5 µg; Nicotinic acid—52.5 mg; Pantothenic acid—27 mg; Biotin—0.15 mg; Folic acid—2.25 mg; Selenium—0.375 mg.

[2]DSM Nutritional Products, Composition per kg of diet: Manganese—80 mg; Iron—50 mg; Zinc—50 mg; Copper—10 mg; Cobalt—1 mg; Iodine—1 mg.

[3]Value based on the metabolizable energy of TM determined in experiment 1.

The determined values of ATTDC of nutrients and of AME and AMEn of the TM are shown in Tables 5 and 6, respectively. The TM had high digestibility coefficients for DM (0.70) and GE (0.80) as opposed to a low value for N (0.37). The mean AMEn value of TM was 5,004 kcal/kg, DM basis. Considering that the DM content of the TM used in this study was 970.2 g/kg, the AMEn of TM in natural matter was 4,855 kcal/kg.

The SIDC and the digestible content of AA of TM are presented in Table 7. The indispensable AA, arginine, and phenylalanine, as well as the dispensable amino acid tyrosine attained the highest values of SIDC ($\geq$0.90), whereas those of glycine and cysteine were the lowest (0.77 and 0.78, respectively). The remaining AA ranged in their SIDC from 0.80 to 0.89.

DISCUSSION

The nutritional composition of TM presented in our study (Table 2) was within the range reported in the literature (De Marco et al., 2015; Panini et al., 2017; Tran et al., 2019) for this novel ingredient. The protein content of TM is close to those of the commonly used protein sources in poultry feed industry such as soybean meal (440–480 g/kg) and fish meal (500–600 g/kg) (based on AMINODat 5.0), as described by Wiltafsky et al. (2016); however, TM has a higher ether extract (297 g/kg) and gross energy content (6,366 kcal/kg) values than those of soybean meal (216 g/kg and 4,180 kcal/kg, respectively) and fish meal (117 g/kg and 4,150 kcal/kg, respectively), according to the same reference. Regarding fatty acid content, TM stands out for being rich in

Table 4. Analyzed amino acid composition, crude protein, and dry matter content of the experimental diets (experiment 2) and of the *Tenebrio molitor* meal, g/kg as fed basis.

	NFD	NFD + TM	*T. molitor* meal
Indispensable amino acids			
Arginine	0.0	10.2	29.2
Histidine	0.0	6.1	18.2
Isoleucine	0.1	8.0	22.3
Leucine	0.1	13.4	37.4
Lysine	0.5	10.3	32.6
Methionine	0.1	2.8	7.8
Phenylalanine	0.2	8.0	23.6
Threonine	0.0	7.4	20.9
Valine	0.0	11.6	32.2
Dispensable amino acids			
Alanine	0.3	13.7	35.9
Aspartic acid	0.0	14.3	43.6
Cysteine	0.6	3.3	6.9
Glutamic acid	0.0	21.4	63.4
Glycine	0.0	10.3	29.0
Proline	0.0	11.4	33.0
Serine	0.0	7.6	21.5
Tyrosine	0.1	1.44	44.5
Crude protein	2.0	174.2	500.0
Dry matter	909.6	927.2	970.2

Abbreviations: NFD, nitrogen free diet; NFD + TM, nitrogen free diet + *Tenebrio molitor* meal.

unsaturated fatty acids (up to 73%), which are critical to perform physiological functions in metabolism (Ravzanaadii et al., 2012). Owing to the individuality of each insect species, it is not expected to find one unique fat or protein profile for insect ingredients, even within the same group. Differences in compositions may arise depending on factors such as the rearing substrate, stage of life, genetics, and processing of the raw material (Rumpold and Schlüter, 2013; Zielińska et al., 2015; Kouřimská and Adámková, 2016; Paul et al., 2017). Similarly, minerals have a variable composition in insects, and the data of our previous study (Nascimento Filho et al., 2020), using the same TM, are in agreement with reports in the literature (Ravzanaadii et al., 2012; Williams et al., 2016; Tran et al., 2019).

Table 5. Apparent total tract digestibility coefficient (ATTDC) of nutrients of *Tenebrio molitor* meal fed to broilers (experiment 1).[1]

	ATTDC[1]
Dry matter	0.70 ± 0.02
Nitrogen	0.37 ± 0.03
Gross energy	0.80 ± 0.02

[1]Each value represents the mean of 6 replicates (4 birds per replicate) ± standard error of the mean.

Based on the chemical composition, insect meals are considered rich sources of protein and fat, in which protein is the main feature. In fact, as previously pointed out, the data in Table 4 indicate that most indispensable AA in TM have levels similar to or greater than those found in high protein feeds, such as soybean meal and fish meal, except for arginine. It is possible to infer that this is a quality ingredient to be included in diet formulation to meet the amino acid requirements of chickens. The values from our study are in agreement with other researches on TM and various insects in the literature (Wang et al., 2007; Hwangbo et al., 2009; Zielińska et al., 2015, Józefiak et al., 2016, Tran et al., 2019).

The values for TM metabolizability have a close similarity to those of high protein animal-based ingredients such as meat and bone meal, fish meal and poultry by-product meal. However, the values found here are higher than those reported by De Marco et al. (2015), for digestibility coefficients of DM (0.70 vs. 0.60) and GE (0.80 vs. 0.64) in TM. Furthermore, Schiavone et al. (2017), evaluating partially and highly defatted black soldier fly larvae meal (*Hermetia illucens*), found moderate ATTDC of

Table 6. Determination of AME and AMEn of the experimental diets and of *Tenebrio molitor* meal fed to broilers. Values on dry matter basis (experiment 1).

Items	Reference diet	Test diet
Feed intake[1], kg	2.54	2.26
GE diet, kcal/kg	4,726	5,355
GE consumed, kcal	11,995	12,104
N diet, %	4.05	5.50
N consumed, g	102.8	124.3
Excreta production, kg	0.75	0.67
GE excreta, kcal/kg	4,084	4,332
GE excreted, kcal	3,048	2,889
N excreta, %	4.63	7.90
N excreted, g	34.59	52.57
NB (N consumed − N excreted), g	68.22	71.75
NB × 8.22, kcal	560.7	589.7
AME diet, kcal/kg	3,526 ± 22.5	4,075 ± 39.8
AMEn diet, kcal/kg	3,305 ± 21.4	3,815 ± 36.4
g DM test ingredient/g DM diet		0.30
AME test ingredient, kcal/kg		5,357 ± 132.8
AMEn test ingredient, kcal/kg		5,004 ± 121.2

Abbreviations: AME, apparent metabolizable energy; AMEn, nitrogen-corrected apparent metabolizable energy; DM, dry matter; GE, gross energy; N, nitrogen; NB, nitrogen balance.

[1]Each value represents the mean of 6 replicates (4 birds per replicate).

nutrients (0.63 and 0.59 for DM; 0.61 and 0.50 for GE, respectively). Both cited studies speculated that chitin might have negatively affected digestibility of nutrients because the insect's outer shell may be difficult to digest by birds. Moreover, Schiavone et al. (2017) indicated that the fat content in insect meal may also influence some nutrients metabolizability which is the case for highly vs. partially defatted insect meal. However, a lower value of ATTDC of N (0.37) was observed in the current study as compared with those (ranging from 0.50 to 0.60) reported in the literature (De Marco et al., 2015; Schiavone et al., 2017; Mwaniki and Kiarie, 2018). This reduced ATTDC of N in TM may be explained by the excess N content of the test diet obtained with the substitution method, whose calculated value was 343.8 g/kg of CP in DM, or 312.8 g/kg of CP on natural basis.

Regarding AMEn for TM (5,004 kcal/kg DM), the result indicates that this alternative ingredient has higher metabolizable energy value compared to common animal and vegetable origin feed sources as well as other insect larvae meals. Estimating AMEn of 2 insect meals fed to broiler chickens, De Marco et al. (2015) found values of 3,826 kcal/kg DM for *T. molitor* and 3,965 kcal/kg DM for *H. illucens*. In a precision feed assay, Wang et al. (2007) found nitrogen-corrected true metabolizable energy of 2,708 kcal/kg DM for Chinese grasshopper (*Acrida cinerea*). Furthermore, evaluating the effect of 10% inclusion of *H. illucens* larvae meal in the diet of quails, Woods et al. (2019) reported higher AME for the supplemented diet compared to the control diet. In all the above studies, as well as in ours, full-fat insect meals were used to estimate the metabolizable energy value.

In a recent review on feeding insects to animals, one of the most active research groups on the topic (Gasco et al., 2019) stated that manufacturers started to produce defatted larvae meal to facilitate and improve its use in the industry. They also indicated that the oil extracted in this process has potential as immunostimulant or modulator of the microbiota. Aiming at separately investigating the protein and fat components of insects, Schiavone et al. (2017) determined the AMEn of partially and highly defatted larvae of *H. illucens*, finding values of 3,552 and 2,357 kcal/kg DM, respectively. Also, Mwaniki and Kiarie (2018), when evaluating defatted larvae of *H. illucens*, reported an AMEn value of 2,902 kcal/kg DM. Both studies emphasized that defatted insect meal can provide an optimal high protein feed ingredient, although the resulting GE digestibility and AMEn were then considered moderate. Kierończyk et al. (2018) reported that the energy value of *T. molitor* oil is similar to that of soybean oil for chickens. Consequently, it is evident that the energy available in whole or defatted insect larvae meal or insect oil, as components of poultry diets, constitute excellent potential energy sources for feed formulation.

The data of SIDC of AA obtained here indicated that TM is a protein source as good as the major protein ingredients used in feed formulation for poultry (Ravindran et al., 2005). As for the main limiting AA for chickens, the values of standardized ileal digestible AA in

Table 7. Coefficients of apparent and standardized ileal digestibility of amino acids, total amino acid content, and standardized ileal digestible content of amino acids in *Tenebrio molitor* meal for chickens (experiment 2).[1]

	Coefficient of digestibility		Total AA	Digestible content
Amino acids	Apparent	Standardized	g/kg	g/kg
Indispensable AA				
Arginine	0.90 ± 0.01	0.92 ± 0.01	30.10	27.65
Histidine	0.79 ± 0.01	0.81 ± 0.01	18.76	15.22
Isoleucine	0.82 ± 0.02	0.86 ± 0.02	22.99	19.68
Leucine	0.85 ± 0.01	0.88 ± 0.01	38.55	34.07
Lysine	0.87 ± 0.02	0.89 ± 0.02	33.06	29.90
Methionine	0.85 ± 0.01	0.87 ± 0.01	8.04	7.04
Phenylalanine	0.87 ± 0.01	0.90 ± 0.01	24.33	21.90
Threonine	0.75 ± 0.01	0.82 ± 0.01	21.54	17.58
Valine	0.82 ± 0.01	0.86 ± 0.01	33.19	28.40
Dispensable AA				
Alanine	0.86 ± 0.01	0.88 ± 0.01	37.00	32.57
Aspartic acid	0.88 ± 0.01	0.89 ± 0.01	44.94	40.09
Cysteine	0.70 ± 0.03	0.78 ± 0.03	7.11	5.52
Glutamic acid	0.86 ± 0.01	0.88 ± 0.01	65.35	57.48
Glycine	0.74 ± 0.01	0.77 ± 0.01	29.89	23.15
Proline	0.82 ± 0.01	0.86 ± 0.01	34.01	29.38
Serine	0.74 ± 0.01	0.80 ± 0.01	22.16	17.73
Tyrosine	0.90 ± 0.01	0.92 ± 0.01	45.87	42.00
Overall mean	0.82 ± 0.01	0.86 ± 0.01	-	-

Abbreviations: AA, amino acid.

[1]Each value represents the mean of 6 replicates (10 birds per replicate) ± standard error of the mean.

TM for lysine, methionine, threonine, and valine (29.90, 7.04, 17.58, 28.40 g/kg DM, respectively) are comparable to those for soybean meal (28.35, 6.25, 17.06, 21.76 g/kg DM, respectively) and fish meal (31.52, 12.54, 20.28, 24.21 g/kg DM, respectively) as reported by Rostagno et al. (2017).

There are several reports on true and apparent ileal digestible AA of different insect meals in the literature. To the best of the authors' knowledge, except for a single report in which standardized ileal digestible AA were determined in defatted black soldier fly meal for chickens (Mwaniki and Kiarie, 2018), there is no determination of this parameter for other insect meals, including TM. The present study constitutes the first reference of SIDC of AA in TM. The results obtained here were, in general, close to those recorded in defatted black soldier fly meal by these researchers.

The average value of apparent ileal amino acid digestibility coefficients shown in Table 7 for TM (0.82) is slightly lower than that (0.86) determined by De Marco et al. (2015); the difference may be attributed to inherent aspects of the insect meal (source, processing) or to the experimental procedures (methodology, age of birds) In fact, these authors determined the digestibility coefficient by the indirect method using birds at 35 d of age, whereas we used the direct method and 22-day-old birds. Regarding black soldier fly meal, lower values of apparent ileal digestibility coefficient were observed in the full-fat meal (0.68) by De Marco et al. (2015) than in the partially and highly defatted meal (0.77 and 0.80, respectively) by Schiavone et al. (2017), although the same procedures were employed in both studies. It is worth noting that, according to these authors, insect fat may be a limiting factor for the ingredient digestibility.

A number of insects had the true ileal digestibility coefficient of AA determined, including adults of field crickets (Wang et al., 2005), Chinese grasshoppers (Wang et al., 2007), and house fly meal (Hall et al., 2018), with average values of 0.93, 0.94 and 0.89, respectively. Although recognizing that the data on metabolizable energy and digestibility of AA reported here refer to an ingredient whose composition may vary, the values determined in this study contribute to the nutritional characterization of TM with focus on its use in practical feed formulation for poultry.

CONCLUSIONS AND APPLICATIONS

1. *T. molitor* larvae meal is a rich source in protein (500.0 g/kg as fed basis) and fat (297.3 g/kg as fed basis), similar to common protein ingredients used in poultry rations.
2. The apparent metabolizable energy and the standardized ileal digestibility coefficients of AA of *T. molitor* larvae meal determined in the present study might be applied as a guide for broiler feed formulation using this ingredient.
3. The present report is the first study measuring the standardized ileal amino acid digestibility of *T. molitor* larvae meal in broilers, which allows precise ration formulation in practical diets for broilers.

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DISCLOSURES

The authors declare that there is no conflict of interest to disclose.

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