Italian Journal of Animal Science

ISSN: (Print) 1828-051X (Online) Journal homepage: http://www.tandfonline.com/loi/tjas20

In Vitro Crude Protein Digestibility of Tenebrio Molitor and Hermetia Illucens Insect Meals and its Correlation with Chemical Composition Traits

Stefania Marono, Giovanni Piccolo, Rosa Loponte, Carmelo Di Meo, Youssef A. Attia, Antonino Nizza & Fulvia Bovera

To cite this article: Stefania Marono, Giovanni Piccolo, Rosa Loponte, Carmelo Di Meo, Youssef A. Attia, Antonino Nizza & Fulvia Bovera (2015) In Vitro Crude Protein Digestibility of Tenebrio Molitor and Hermetia Illucens Insect Meals and its Correlation with Chemical Composition Traits, Italian Journal of Animal Science, 14:3, 3889

To link to this article: http://dx.doi.org/10.4081/ijas.2015.3889

Published online: 17 Feb 2016.

PAPER

In vitro crude protein digestibility of *Tenebrio molitor* and *Hermetia illucens* insect meals and its correlation with chemical composition traits

Stefania Marono,[1] Giovanni Piccolo,[1] Rosa Loponte,[1] Carmelo Di Meo,[1] Youssef A. Attia,[2] Antonino Nizza,[1] Fulvia Bovera[1]

[1]Dipartimento di Medicina Veterinaria e Produzioni Animali, University of Napoli Federico II, Italy

[2]Arid Land Agriculture Department, King Abdulaziz University, Jeddah, Saudi Arabia

Abstract

The aims of this study were to evaluate the correlation between *in vitro* crude protein digestibility coefficients of insect meals from *Tenebrio molitor* (TI) and *Hermetia illucens* (HI) and their chemical composition traits as well as to develop regression equations able to estimate the *in vitro* crude protein digestibility (CPd) from proximate analysis of insect meals. Twelve samples of insect meals (6 from TM larvae, TM 1-6 and 6 from HI larvae, HI 1-6) were obtained from different producers and analysed for chemical composition and *in vitro* crude protein digestibility by a two-step enzymatic method (digestion with pepsin and trypsin-enriched pancreatin). For both insect meal samples, CPd was negatively correlated to ADF and chitin contents, while just for HI there was a positive correlation ($P<0.01$) between CP percentage of the samples and CPd. For both insect meals the former variable chosen in the stepwise analysis was the chitin, explaining the 79.45% of CPd variability for *Tenebrio molitor* samples and the 98.30% for *Hermetia illucens*. In the second step, the amount of protein linked to ADF was added in the model for *T. molitor* and CP for *H. illucens* samples. The coefficients chitin is the main constituent of insect body able to affect the crude protein digestibility of *Tenebrio molitor* and *Hermetia illucens* larvae meals estimated by an *in vitro* enzymatic method.

Introduction

The demand of high value protein sources for feeds formulation, such as fishmeal and extracted soybean meal are growing as human population and food demand (FAO, 2013). As a consequence, prices for these raw protein sources have increased ever more in the recent years pushing new research into the development of alternative protein sources especially for aquaculture and poultry. Currently, insects are being considered as a new protein source for animal feed (Premalatha *et al.*, 2011). Insects provide food at low environmental cost, contributing positively to livelihoods, and play a fundamental role in nature (Van Huis *et al.*, 2013). They have a high feed conversion efficiency, the possibility to be reared on organic side-streams and to reduce environmental contamination, adding value to waste (Veldkamp *et al.*, 2012). Moreover, insects do not compete with humans and other farmed animals for nutrients and are particularly suitable for poultry and fish nutrition as a part of their natural diet.

The insect nutritive properties are not well known (Sánchez-Muros *et al.*, 2014) due to the only recent interest in the use of insects as an alternative protein source. Previous studies on the chemical composition of insects have focused on human nutrition (Banjo *et al.*, 2006), and most of them demonstrate a good composition (Ramos-Elorduy *et al.*, 1982). Nevertheless, the utilisation of insects in animal feeding has been less studied; insects exhibit great development potential for development as a standard ingredient in animal feeding (Sánchez-Muros *et al.*, 2014). Many species of insects have been considered for their possible use in feeds for livestock and some studies have been carried out on poultry (Ravindran and Blair, 1993; Wang *et al.*, 2005; Ojewola *et al.*, 2005; Oyegoke *et al.*, 2006), fish (Gasco et al., 2014a and b) and other species (St-Hilaire *et al.,* 2007; Ng *et al.,* 2001).

Among the different insect species, yellow mealworms (*Tenebrio molitor L.*) and black soldier flies (*Hermetia illucens*) seems to be very interesting (Schiavone *et al.,* 2014; Bovera *et al.*, 2015). *T. molitor* is a pest of flour, grain and food store and has a world-wide distribution (Ramos-Elorduy *et al.*, 2002). Larval and pupal stages of *T. molitor* are rich in protein and easy to breed and feed (Ghaly *et al.*, 2009). In addition, it grows well on organic waste (Khusro *et al.*, 2012). The black soldier fly is an extremely resistant species and can be reared on organic wastes by converting them into a protein-rich and fat-rich biomass suitable for various purposes, including animal feeding, biodiesel and chitin production (Diener *et al.*, 2011; Van Huis *et al.*, 2013).

The main objectives of this study were to evaluate the correlation between *in vitro* crude protein digestibility coefficients of insect meals from *Tenebrio molitor* and *Hermetia illucens* and their chemical composition characteristics as well as to develop regression equations able to estimate the *in vitro* crude protein digestibility from proximate analysis of insect meals.

Corresponding author: Prof. Antonino Nizza, Dipartimento di Medicina Veterinaria e Produzioni Animali, Università di Napoli Federico II, Via F. Delpino 1, 80137 Napoli, Italy.
Tel. +39.081.2536061; Fax: +39.081.292981.
E-mail: nizza@unina.it

Key words: *Tenebrio molitor; Hermetia illucens; In vitro* crude protein digestibility; Chemical composition; Regression analysis.

Received for publication: 5 February 2015.
Accepted for publication: 28 May 2015.

Italian Journal of Animal Science 2015; 14:3889
doi:10.4081/ijas.2015.3889

Materials and methods

Twelve samples of insect meals (six from *Tenebrio molitor* larvae, TM 1 6 and six from *Hermetia illucens* larvae, HI 1-6) were obtained from three different producers (Gaobeidian Shannon Biology CO., Ltd., Shannong, China: one sample of *T. molitor*; Kreca, The Netherlands: three samples of *T. molitor* from different batches; Enviroflight LCC, OH, USA: two samples of *T. molitor* and four of *H. illucens* from different batches; laboratory of Entomology, Wageningen University, The Netherlands: two samples of *H. illucens* from different batches). The samples were analysed according to AOAC (2004) using the following methods: dry matter (DM, method number 943.01), Ash (method number 924.05), crude protein (CP, method number 954.01), ether extract (EE, method number 920.39), neutral detergent fibre (NDF, method

number 2002.04) and acid detergent fibre (ADF, method number 973.18). The amount of protein linked to acid detergent fibre (ADIP) was also determined (AOAC, 2004). The amount of ADF was used to estimate the chitin content of insect meals. According to Bernard and Allen (1997), chitin can be estimated by determining the acid detergent fibre fraction corrected for ash content. However, Finke (2007) showed that amino acids represent from 14.2 to 68.8% of the ADF residue by weight, suggesting that the ADF represents both protein and chitin with protein. Starting from these assumption, we estimated the amount of chitin in insect meal as follows: chitin (%) = ash free ADF (%) - ADIP (%).

An *in vitro* assay was performed to simulate the digestion of insect meals protein through the stomach and the small intestine of a single-stomached animal. The *in vitro* assay was a two-steps method developed to maximize the hydrolysis of the animal protein peptide bonds with minimal enzyme usage (Qiao, 2001; Qiao *et al*., 2004). All the insect meal samples were ground to pass a 1-mm screen (Brabender Wiley mill, Brabender OHG Duisburg, Germany) and accurately weighed (about 0.5 g/sample, five replication per each sample) in 150 ml glass jars, without preliminary defatting. All the enzymes were from porcine origin and obtained from Sigma-Aldrich (S. Louis, MO, USA). In the first step, fresh pepsin (Enzyme Commission number 3.4.23.1, 250 U/mg solid) solution was prepared (10 mg/mL) in pH 4 citrate buffer solution (0.1µM) to avoid pepsin precipitation that occurs in pH 2 citrate buffer solution. An aliquot of the fresh pepsin solution was immediately transferred into each 150 mL jar to make desired concentrations of pepsin solution (0.25% of pepsin related to protein content of the sample). The final volume in each test jar (20 mL) was obtained using a pH 2 citrate buffer. Jars were incubated at 38°C for 24 h under continuous stirring.

In the step 2, fresh trypsin (Enzyme Commission number 3.4.21.4, 1000 BAAE units/mg solid) -enriched pancreatin (Enzyme Commission number 232.468.9, 8 x USP specifications) was prepared (trypsin 2 mg/mL, pancreatin 10 mg/mL, ratio 1:5) in pH 8.0 phosphate buffer solutions (0.1 mol/L). An amount of 30 ml of phosphate buffer solution was added to each jar and, after adjusting the pH at 7 by adding 0.1M NaOH, the trypsin + pancreatin solution was inoculated (7.5% enzyme protein relative to substrate protein, final substrate concentration 5 mg/mL). Then, the digestion was continued for 96 h more under continuous stirring. All buffers contained 0.06% (wt/v) sodium azide to prevent microbial growth.

The length of these incubation times correspond to the time needed to loose over 95% of the enzymes activity in order to maximize their efficacy, thus allowing for minimal enzyme usage. To correct the results for a possible amount of nitrogen in the reagent used in the trial, three tubes were incubated without substrates (blanks) and followed the same digestion process than the other samples. At the end of digestion, samples were filtered (Whatmann, 401) and the residual material were submitted to CP analysis according to AOAC (2004). The calculation of *in vitro* digestibility coefficients has been obtained from:

$$CPd = [CPs - (CPr - CPb)]/CPs \times 100$$

where:
CPd is crude protein digestibility;
CPs is the crude protein content of samples;
CPr is the crude protein content of residual material after digestion;
CPb is the average crude protein content of blanks.

The differences between the average values of chemical composition and crude protein digestibility of the *T. molitor* and *H. illucens* were analysed by *t*-test. The coefficients of correlation between the crude protein digestibility and the parameters of chemical composition were estimated using a PROC CORR procedure (SAS, 2000). Prediction equations of CPd from chemical analysis of insect meal samples were developed by a multiple stepwise regression analysis, using the REG procedure of SAS (2000). Only linear models were tested and it was assumed that there was no interaction among variables.

Results

Table 1 reports the chemical characteristics of the twelve insect meals tested, the *in vitro* coefficient of protein digestibility, as well as

Table 1. Chemical characteristics and *in vitro* crude protein digestibility of the 12 insect meal samples.

	DM	Ash	CP	EE	NDF	ADF	ADIP	Chitin	CPd
				%					
TM1	96.0	3.60	52.2	28.4	11.7	7.95	2.80	5.15	66.3
TM2	95.8	3.67	51.8	29.8	11.6	7.52	2.72	4.80	66.7
TM3	99.0	6.36	59.0	16.6	48.7	10.9	4.19	6.73	65.5
TM4	99.2	6.49	58.8	17.1	52.5	11.4	5.10	6.34	66.2
TM5	98.2	3.51	57.6	28.9	18.9	10.3	3.96	6.37	65.8
TM6	99.0	3.74	57.4	28.9	19.4	10.5	3.30	5.15	66.2
HI1	95.1	9.88	52.0	11.3	25.8	8.41	3.06	4.75	67.1
HI2	94.8	9.96	51.8	11.3	34.4	8.80	3.65	4.50	67.3
HI3	98.8	6.43	58.8	12.9	5.99	4.89	1.96	2.93	67.6
HI4	98.9	6.52	58.4	11.6	6.03	4.69	1.83	2.86	68.7
HI5	95.9	4.72	49.9	29.0	18.3	8.30	3.32	4.98	66.8
HI6	95.9	4.64	50.5	28.4	19.2	8.53	3.03	5.50	66.0
TM	97.9	4.56^{b}	56.1	24.9	27.1	9.77^{a}	3.68^{a}	5.75^{a}	66.1^{b}
HI	96.6	7.02^{a}	53.6	17.4	18.3	7.27^{b}	2.98^{b}	4.25^{b}	67.3^{a}
P value	0.106	0.028	0.254	0.119	0.339	0.035	0.036	0.012	0.022

TM 1-6: T*enebrio molitor* samples from 1 to 6; HI 1-6: *Hermetia illucens* samples from 1 to 6; DM, dry matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADIP, protein linked to ADF; CPd, crude protein digestibility. ^{a,b}P<0.05.

the average content for TM and HI meals for all the presented criteria. The dry matter content showed a low variability ranging from 94.8 to 99.2%. More variability had the CP content, as the percentage ranged from 49.9 of *Hermetia illucens* sample 5 to 59.0 of *Tenebrio molitor* sample 3. A wide variation showed the NDF percentage ranging from 5.99 and 52.55, respectively of HI3 and TM4. Also ether extract (from 11.3 HI1 to 29.8% of TM2), and ADF (from 4.69 of HI4 to 11.4 of TM4) showed variables values among the different samples. The average values of Ash were higher (P<0.05) in the samples of HI, while the ADF content was higher (P<0.05) in TM samples and the crude protein digestibility was the highest in the samples of *H. illucens*. Samples of *T. molitor* showed higher (P<0.05) values of ADIP and chitin than *H. illucens*.

Table 2 shows the correlation coefficients between the measured chemical characteristics and the *in vitro* protein digestibility coefficients of the insect meal samples from *Tenebrio molitor*. Crude protein digestibility was negatively correlated (P<0.05) to ADF and chitin contents. The percentage of Ash was correlated negatively (P<0.01) to ether extract and positively to NDF (P<0.01) and ADIP (P<0.05). The percentage of crude protein had a positive correlation to ADF (P<0.01) and ADIP (P<0.05), while the ether extract was negatively correlated to NDF (P<0.01) and ADIP (P<0.05). NDF was positively correlated to ADIP (P<0.01) and ADF had a positive correlation (P<0.05) to ADIP and chitin.

Table 3 reports the correlation coefficients between the chemical constituents and the in vitro protein digestibility of *H. illucens* meal samples. The CPd was correlated positively to CP percentage and negatively (P<0.01) to both ADF and chitin. The amount of protein was negatively correlated to ADF (P<0.01), ADIP (P<0.01) and chitin (P<0.01). NDF was positively correlated to ADF (P<0.05); ADF had a positive correlation (P<0.01) to ADIP and chitin and ADIP was positively (P<0.05) correlated to chitin.

The regression equations to predict CP digestibility from chemical characteristics of insect meals samples were presented in Tables 4 and 5, respectively for *T. molitor* and *H. illucens*. For both meals the former variable chosen in the stepwise analysis was the chitin, explaining the 79.45% of CP variability for *Tenebrio molitor* samples and the 98.30% for *Hermetia illucens*. In the second step, nADF was added in the model for *T. molitor* and this

Table 2. Correlation coefficients between crude protein digestibility and the different traits of chemical composition of *Tenebrio molitor* larvae meal samples.

CPd	-	-0.429	0.190	-0.459	-0.137	0.017	-0.747	0.036	-0.891
	(0.396)	(0.719)	(0.360)	(0.796)	(0.975)	(0.035)	(0.951)	(0.017)	
DM	-	-	0.617	0.582	-0.622	0.544	0.586	0.684	0.657
		(0.192)	(0.195)	(0.187)	(0.090)	(0.103)	(0.137)	(0.157)	
Ash	-	-	-	0.649	-0.993	0.981	0.687	0.599	-0.009
		(0.162)	(<0.001)	(0.005)	(0.137)	(0.214)	(0.099)		
CP	-	-	-	-	-0.667	0.777	0.989	0.859	0.633
			(0.148)	(0.069)	(<0.001)	(0.028)	(0.178)		
EE	-	-	-	-	-	-0.979	-0.687	-0.902	0.004
			(0.001)	(0.132)	(0.014)	(0.999)			
NDF	-	-	-	-	-	-	0.705	0.935	0.174
				(0.053)	(0.006)	(0.741)			
ADF	-	-	-	-	-	-	-	0.853	0.841
				(0.031)	(0.033)				
ADIP	-	-	-	-	-	-	-	-	-0.442
					(0.381)				

DM, dry matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADIP, protein linked to ADF; CPd, crude protein digestibility. P values are indicated in brackets.

Table 3. Correlation coefficients between crude protein digestibility and the different traits of chemical composition of *Hermetia illucens* larvae meal samples.

CPd	-	0.799	0.157	0.941	-0.699	-0.625	0.901	-0.791	-0.992
	(0.057)	(0.766)	(0.005)	(0.103)	(0.184)	(0.001)	(0.061)	(0.001)	
DM	-	-	-0.418	0.602	-0.221	-0.752	-0.677	-0.661	0.657
		(0.410)	(0.114)	(0.674)	(0.093)	(0.121)	(0.138)	(0.131)	
Ash	-	-	-	0.009	-0.793	0.604	0.220	0.280	-0.078
		(0.987)	(0.059)	(0.204)	(0.675)	(0.551)	(0.973)		
CP	-	-	-	-	-0.614	-0.756	-0.966	-0.922	-0.973
			(0.195)	(0.082)	(0.002)	(0.009)	(0.001)		
EE	-	-	-	-	-	-0.016	0.416	0.343	0.655
			(0.976)	(0.414)	(0.501)	(0.158)			
NDF	-	-	-	-	-	-	0.888	0.923	0.688
				(0.018)	(0.009)	(0.131)			
ADF	-	-	-	-	-	-	-	0.966	0.941
				(0.002)	(0.005)				
ADIP	-	-	-	-	-	-	-	-	0.846
					(0.034)				

DM, dry matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADIP, protein linked to ADF; CPd, crude protein digestibility. P values are indicated in brackets.

decreased the RSD by 47.8%. For *H. illucens* samples the second step of analysis included the CP in the model and the RSD was decreased up to 1.17. No further variables for both *T. molitor* and *H. illucens* were added in the model.

Discussion

The results of chemical composition analysis were in line with the data available in literature and recently reviewed by Sanchez-Muros *et al.* (2014). The dry matter content is higher than that reported in literature as the samples were provided to our laboratories as dehydrated meals. It is not easy to found data in literature on the amount of chitin in insects, however our results agree with Finke *et al.* (2012) who estimated an amount of chitin equal to 5.41% on dry matter basis in *H. illucens* larve. However, we have to consider that our calculated values could underestimate the true amount of chitin in the different insect meals as also chitin contains in their structure an amount of nitrogen. The differences in chemical constituents into the same insect species could be tied to the diet fed to insect along the production period. Unfortunately, we had not clear information from the producers about the details of insect production, however, it is known that the amount of chemical constituents of insect can change according to source of feeding (Makkar *et al*., 2014). Published studies showed that whole insects contain variable but significant amounts of fibre as measured by CF, ADF and NDF (Finke, 1984, 2002; Pennino *et al.*, 1991; Barker *et al.*, 1998). In plants, ADF is composed typically of cellulose and lignins whereas NDF is composed of cellulose, lignin, and hemicelluloses (Van Soest and Robertson, 1977). Although insects contain significant amounts of both ADF and NDF, the components that make up these fractions are unknown (Finke, 2007). Various authors have suggested that the fibre in insects is represented by chitin because chitin [(linear polymer of b-(1-4) N-acetyl-D-glucosamine units)] is structurally similar to cellulose [(linear polymer of b-(1-4)-D-glucopyranose units)] and because the ADF fraction has been shown to contain nitrogen (Finke, 1984, 2002; Barker *et al.*, 1998). As ADF is part of NDF, the amount of protein linked to ADF were included both in the CP and NDF: for this reason the sum of ash + CP + EE + NDF was in several cases higher than 130/100 g.

The values of *in vitro* protein digestibility coefficients found in our trial were in line with the findings of other authors. Sanchez-Muros *et al.* (2014) reported that the protein digestibility of proteins among insect species varies from 45.0 to 66.9% and this values, lower than that reported for the most of vegetable protein sources used in animal nutrition, have to be ascribed to chitin that interferes with the digestive use of proteins (Longvah *et al.*, 2011). This suggests that the estimation of chitin content is very important when insect meals were used in animal nutrition. In this regard, Finke (2007) reported that the fibre content of insects measured as ADF consists mainly of chitin with significant amount of associated cuticular proteins (Merzendorfer, 2014). Chitin is not degraded and absorbed in the small intestine (Vidanarachchi *et al.*, 2010) and thus can affect the protein digestibility (Schiavone *et al.*, 2014; Bovera *et al.*, 2015). This is in agreement with our results which indicate chitin as the main factor affecting the *in vitro* protein digestibility of both insect meals. In our trial, crude protein digestibility is not correlated to ADIP for both insect species. This aspect is not easy to explain and suggests a possible effect of other protein fractions in affecting crude protein digestibility of insect meals. However, further investigation need to clarify this point.

As showed in our trial NDF is not correlated to protein digestibility, suggesting that this analysis is not adequate to estimate the chitin content in insect meals. This is in accordance with Finke (2002 and 2007) who suggested that ADF is the better way to estimate chitin of insects. For *H. illucens* samples also a positive correlation between CP level an CPd was found, indicating that, as the amount of crude protein in the samples increases, also the crude protein digestibility increases and this can be tied to the negative correlation between crude protein and ADF or ADIP. From our results it seems that when CP level of HI samples increases, the amounts of ADF and of nitrogen linked to ADF decrease and this can affect the digestibility of crude protein. On the contrary, for TM samples a positive correlation was found between CP and ADF or ADIP: in this case the increase of CP level of body insect is tied to an increase of cuticular structures but this increase was unable to make a significant correlation between CP level and CPd. This differences can be attributed to insect species. As confirmation of our considerations, the chitin was the first independent variable included in the model estimated by the STEPWISE procedure to predict the in vitro crude protein digestibility from chemical composition of both insect meals, even if its efficacy on CPd estimation was stronger in *H. illucens* samples. However, for both insect meal samples the chitin is the most important criteria affecting body insect CP digestibility. Due to different relationship among CP, ADF and ADIP, the second variable included in the model was ADIP for *T. molitor* and CP percent-

Table 4. Regression of the crude protein digestibility on the variables of chemical characteristics variables of *Tenebrio molitor* larvae meal samples.

Variables		R^2	RSD	
Intercept	Chitin	ADIP		
68.195	-0.3325	-	0.7945	12.06
(0.4903)	(0.0845)			
72.425	-0.2672	0.0845	0.9213	6.29
(0.4959)	(0.0674)	(0.0385)		

ADIP, protein linked to ADF; RSD, residual standard deviation. The standard error values of the regression coefficients are indicated in brackets.

Table 5. Regression of the crude protein digestibility on the variables of chemical characteristics variables of *Hermetia illucens* larvae meal samples.

Variables		R^2	RSD	
Intercept	Chitin	CP		
71.57	-0.9705	-	0.9830	2.50
(0.2804)	(0.064)			
80.09	-1.412	-0.1243	0.9940	1.17
(3.62)	(0.191)	(0.0529)		

CP, crude protein; RSD, residual standard deviation. The standard error values of the regression coefficients are indicated in brackets.

age for *H. illucens* samples.

Conclusions

Our results indicated that the crude protein digestibility of *Tenebrio molitor* and *Hermetia illucens* larvae meals, estimated by a described *in vitro* enzymatic methods indicated was mainly affected by the chitin content. A negative and significant correlation was also detected with ADF, but in the final equation, next to the chitin, ADIP or CP were chosen, respectively for *T. molitor* and *H. illucens* samples to give an accurate estimation of crude protein *in vitro* digestibility from chemical composition on insect meals. Further investigation is needed to assess the estimation equations parameters tied to insect species.

References

AOAC, 2004. Official methods of analysis. 18th ed., Association of Official Analytical Chemists, Washington, DC, USA.

Banjo, A.D., Lawal, O.A., Songonuga, E.A., 2006. The nutritional value of fourteen species of edible insects in Southwestern Nigeria. Afr. J. Biotechnol. 5:298-301.

Barker, D., Fitzpatrick, M.P., Dierenfeld, E.S., 1998. Nutrient composition of selected whole invertebrates. Zoo Biol. 17:123-134.

Bernard, J.B., Allen, M.E., 1997. Feeding captive insectivorous animals: nutritional aspects on insects as food. PhD Diss., Michigan State University, East Lansing, MI, USA.

Bovera, F., Piccolo, G., Gasco, L., Marono, S., Loponte, R., Vassalotti, G., Mastellone, V., Lombardi, P., Attia, Y.A., Nizza A., 2015. Yellow mealworms larvae (Tenebrio molitor, L.) as protein source for broilers: effects on growth performance and blood profiles. Brit. Poultry Sci. (In press).

Diener, S., Zurbrügg, C., Roa Gutiérrez, F., Nguyen Dang Hong, M.A., Koottatep, T., Tockner, K., 2011. Black soldier fly larvae for organic waste treatment-prospects and constraints. pp 52-59 in Proc. 2nd Int. Conf. Solid Waste Management in the Developing Countries, Khulna, Bangladesh.

FAO, 2013. Edible insects: future prospects for food and feed security food and agriculture organization of the united nations. FAO Publ., Roma, Italy.

Finke, M.D., 1984. The use of nonlinear models to evaluate the nutritional quality of insect protein. Degree Diss., University of Wisconsin, Madison, WI, USA.

Finke, M.D., 2002. Complete nutrient composition of commercially raised invertebrates used as food for insectivores. Zoo Biol. 21:269-285.

Finke, M.D., 2007. Estimate of chitin in raw whole insects. Zoo Biol. 26:105-115.

Finke, M.D., 2012. Complete nutrient content of four species of feeder insects. Zoo Biol. 32:27-36.

Gasco, L., Belforti, M., Rotolo, L., Lussiana, C., Parisi, G., Terova, G., Roncarati, A., Gai, F., 2014a. Mealworm (Tenebrio molitor) as a potential ingredient in practical diets for rainbow trout (Oncorhynchus mykiss). Page 69 in Proc. 1st Int. Conf. Insects to Feed the World, Wageningen University, Wageningen, The Netherlands (Abstr.).

Gasco, L., Gai, F., Piccolo, G., Rotolo, L., Lussiana, C., Molla, P., Chatzifotis, S., 2014b. Substitution of fishmeal by Tenebrio molitor meal in the diet of Dicentrarchus labrax juveniles. Page 70 in Proc. 1st Int. Conf. Insects to Feed the World, Wageningen University, Wageningen, The Netherlands (Abstr.).

Ghaly, A.E., Alkoaik, F.N., 2009. The yellow mealworm as a novel source of protein. Am. J. Agric. Biol. Sci. 4:319-331.

Khusro, M., Andrew, N.R., Nicholas, A., 2012. Insects as poultry feed: a scoping study for poultry production systems in Australia. World. Poultry Sci. J. 68:435-446.

Longvah, T., Mangthya, K., Ramulu, P., 2011. Nutrient composition and protein quality evaluation of eri silkworm (Samia ricinii) prepupae and pupae. Food Chem. 128:400-403.

Makkar, H.P.S., Tran, G., Heuzé, V., Ankers, P., 2014. State-of-the-art on use of insects as animal feed. Anim. Feed Sci. Tech. 197:1-33.

Merzendorfer, H., 2014. The cellular basis of chitin synthesis in fungi and insects: common principles and differences. Eur. J. Cell Biol. 90:759-769.

Ng, W.K., Liew, F.L., Ang, L.P., Wong, K.W., 2001. Potential of mealworm (Tenebrio molitor) as an alternative protein source in practical diets for African catfish, Clarias gariepinus. Aquac. Res. 32:273-280.

Ojewola, G.S., Udom, S.F., 2005. Chemical evaluation of the nutrient composition of some unconventional animal protein sources. Int. J. Poultry Sci. 4:745-747.

Oyegoke, O.O., Akintola, A.J., Fasoranti, J.O., 2006. Dietary potentials of the edible larvae of Cirina forda (westwood) as a poultry feed. Afr. J. Biotechnol. 5:1799-1802.

Pennino, M., Dierenfeld, E.S., Behler, J.L., 1991. Retinol, alpha-tocopherol and proximate nutrient composition of invertebrates used as feed. Int. Zoo Yearbook 30:143-149.

Premalatha, M., Abbasi, T., Abbasi, T., Abbasi, S.A., 2011. Energy-efficient food production to reduce global warming and ecodegradation: the use of edible insects. Renew. Sust. Energ. Rev. 15:4357-4360.

Qiao, Y., 2001. Routine techniques for monitoring the nutritional value of animal meals. Degree Diss., North Carolina State University, Raleigh, NC, USA.

Qiao, Y., Lin, X., Odle, J., Whittaker, A., van Kempen, T.A.T.G., 2004. Refining in vitro digestibility assays: fractionation of digestible and indigestible peptides. J. Anim. Sci. 82:1669-1677.

Ramos-Elorduy, J., Avila Gonzalez, E., Rocha Hernandez, A., Pino, J.M., 2002. Use of Tenebrio molitor (Coleoptera: Tenebrionidae) to recycle organic wastes and as feed for broiler chickens. J. Econ. Entomol. 95:214-220.

Ramos-Elorduy, J., Bourges, H., Pino, J.M., 1982. Valor nutritivo y calidad de la proteína de algunos insectos comestibles de México. Folia Entomol. Mex. 53:111-118.

Ravindran, V., Blair, R., 1993. Feed resources for poultry production in Asia and the Pacific. World. Poultry Sci. J. 49:219-235.

Sánchez-Muros, M.J., Barroso, F.G., Manzano-Agugliaro, F., 2014. Insect meal as renewable source of food for animal feeding: a review. J. Clean. Prod. 65:16-27.

Schiavone, A., De Marco, M., Rotolo, L., Belforti, M., Martinez Mirò, S., Madrid Sanchez, J., Hernandez Ruiperez, F., Bianchi, C., Sterpone, L., Malfatto, V., Katz, H., Zoccarato, I., Gai, F., Gasco L., 2014. Nutrient digestibility of Hermetia illucens and Tenebrio molitor meal in broiler chickens. Page 73 in Proc. 1st Int. Conf. Insects to Feed the World, Wageningen University, Wageningen, The Netherlands (Abstr.).

SAS, 2002. SAS/STAT software, ver. 9. SAS Inst., Inc., Cary, NC, USA.

St-Hilaire, S., Sheppard, C., Tomberlin, J.K., Irving, S., Newton, L., McGuire, M.A., Mosley, E.E., Hardy, R.W., Sealey, W., 2007. Fly prepupae as a feedstuff for rainbow trout, Oncorhynchus mykiss. J. World Aquacult. Soc. 38:59-67.

Van Huis, A. 2013. Potential of insects as food and feed in assuring food security. Annu. Rev. Entomol. 58:121-130.

Van Soest, P.J., Robertson, J.B., 1977. What is

fibre and fibre in food. Nutr. Rev. 35:12-16.
Veldkamp, T., van Duinkerken, G., van Huis, A., Lakemond, C.M.M., Ottevanger, E., Bosch, G., van Boekel, M.A.J.S., 2012. Insects as a sustainable feed ingredient in pig and poultry diets - a feasibility study. Rapport 638 - Wageningen Livestock Research, Wageningen, The Netherlands.
Vidanarachchi, J.K., Kurukulasuriya, M.S., Kim, S.K., 2010. Chitin, Chitosan and their oligosaccharides in food industry. In: S.K. Kim (ed.), Chitin, chitosan, oligosaccharides and their derivatives: biological activities and applications. CRC Press, New York, USA, pp 543-560.
Wang, D., Zhai, S.W., Zhang, C.X., Bai, Y.Y., An, S.H., Xu, Y.N., 2005. Evaluation on nutritional value of field crickets as a poultry feedstuff. Asian Austral. J. Anim. 18:667-670.