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Protein composition and digestibility of black soldier fly larvae in broiler chickens revisited according to the recent nitrogen-protein conversion ratio

J. Nery[1], L. Gasco[2,3*], S. Dabbou[1] and A. Schiavone[1,3]

[1]Department of Veterinary Sciences, University of Turin, Largo Paolo Braccini 2, 10095 Grugliasco, Italy; [2]Department of Agricultural, Forest and Food Sciences, University of Turin, Largo Paolo Braccini 2, 10095 Grugliasco, Italy; [3]Institute of Science of Food Production, National Research Council, Largo Paolo Braccini 2, 10095 Grugliasco, Italy; laura.gasco@unito.it

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RESEARCH ARTICLE

Abstract

Specific nitrogen-to-protein (N:P) conversion factors have recently been defined for processed insect meal, because the presence of non-protein nitrogen in insects leads to an overestimation of the insect protein content. The aim of this paper was, on the basis of our previous study, to recalculate the digestibility of partially or totally defatted black soldier fly (BSF; *Hermetia illucens*) larva meals in broiler chickens. Sixty 26-days-old Ross 308 male chickens were randomly divided into fifteen cages (5 replicates/treatment). The chickens were fed a basal diet, and two BSF larva meal diets that differed according to their fat content. The digestibility trial lasted 10 days, and it included a 6-days adaptation period and 4-days of excreta collection. Four methods were used to recalculate the crude protein (CP) content of the diet and excreta, using either the conventional 6.25 N:P conversion ratio, the mean 5.60 N:P conversion ratio proposed in literature, or the weighted average of the specific N:P conversion ratio (5.62 for BSF meal, 5.68 for corn and 5.64 for soybean meal). The data were analysed using the *t*-test and differences were considered significant for $P<0.05$. Diet CP digestibility calculated using the conventional 6.25 N:P conversion factor was slightly lower than that calculated using the weighted average 5.66 and 5.65 N:P conversion factors ($P<0.005$). A significantly lower diet CP digestibility was found when using the conventional 6.25 N:P conversion factors for the basal diet and excreta and the weighted average 6.09 N:P conversion factors for the insect meal diets ($P<0.005$). As far as the latest developments pertaining the N:P conversion ratio in processed insect meal are concerned, the mean 5.60 N:P conversion ratio seems more accurate than the conventional 6.25 one to determine the CP content in poultry diets containing insect processed meal.

Keywords: insect meal, *Hermetia illucens*, poultry, crude protein conversion ratio, nitrogen

1. Introduction

Proteins are essential in animal nutrition for their correct growth, development and health, and their role was investigated in depth over the last century (Schaefer, 1946). Each animal species has its own protein requirements and different vegetable or animal protein sources can be used in feed formula to cover these requirements.

The protein content of raw materials can be measured in two ways: crude protein (CP), which is also sometimes referred to as total protein, and true protein. Historically, CP was estimated after measuring the total nitrogen content by means of the Kjeldahl method, while it has more recently been measured using Dumas analysis protocols and multiplying the obtained value by a conventional conversion factor (Chromy *et al.*, 2015; Sàez-Plaza *et al.*, 2013; Thompson *et al.*, 2002). Nevertheless, a fraction of the nitrogen contained in feedstuffs also comes from non-protein nitrogen (NPN) sources, such as ammonia, urea, nitrates, nitrites, amines, nucleic acids, phospholipids, chitin, etc. (Janssen *et al.*, 2017; Mariotti *et al.*, 2008; McDonald *et al.*, 2010; Sosulski and Imafidon, 1990). True protein is the sum of all the amino acids in the protein, and thus only reflects the amount of nitrogen associated with the amino acids (Salo-Vaananen and Koivistoinen, 1996; Sriperm *et al.*, 2011).

It is common practice to use CP as a reference for the protein content of feedstuffs or animal feeds, using a conventional nitrogen-to-protein (N:P) conversion factor value of 6.25 and assuming that a protein source contains 160 g of nitrogen per kg (Atwater and Bryant, 1906; Sosulski and Imafidon, 1990). Nevertheless, as reported in the last century (Jones, 1941), this N:P conversion factor is not related to any specific feedstuff, as each raw material has its own specific amino acid composition and thus its own specific nitrogen content. Jones (1941) investigated the nitrogen content of more than 121 different animal and protein sources and proposed special conversion factors. Jones concluded by stating that '…it is believed that they [these special conversion factors] will give the true protein content more accurately than the indiscriminate use of the conversion factor 6.25'.

The use of the conventional N:P conversion factor of 6.25 to express the protein content of feedstuffs in animal nutrition, can therefore lead to unreliable protein contents of feedstuff and consequently of the protein supply of diets (Janssen *et al.*, 2017; Mariotti *et al.*, 2008; Sriperm *et al*, 2010). It would be more accurate to use specific N:P conversion factors for each feed, as suggested by some authors (Farrel *et al.*, 2004; Mariotti *et al.*, 2008; Sriperm *et al.*, 2010; Yeoh and Wee, 1994). In order to better evaluate the quantity of protein in feedstuff, Mariotti *et al.* (2008) proposed a set of specific N:P conversion factors for different protein sources and suggested using an average value of 5.60 when no specific factor is indicated for a protein source and for mixed products made of several different protein sources. Similarly, Sriperm *et al.* (2010) proposed an average N:P factor of 5.68 for corn, 5.64 for soybean meal and 5.74 for corn distillers' dried grains with solubles.

In addition to correctly formulating diets, the use of the appropriate N:P conversion factors could also enable the economic value of a feedstuff to be better reflected on a protein basis.

Because of the world's growing demand for food and protein, the research efforts of recent years have been focused on the search for alternative sustainable protein sources for animal feeds (Van der Spiegel *et al.*, 2013).

Insect-derived products are considered to be one of the most promising sources in animal nutrition, as they can effectively substitute not only conventional proteins (Biasato *et al.*, 2016, 2017, 2018; Gasco *et al.*, 2016; Renna *et al.*, 2017; Roncarati *et al.*, 2015; Stadtlander *et al.*, 2017), but also lipids (Schiavone *et al.*, 2017a, in press). Moreover, insect-derived products can stimulate the immune system and thus sustain animal health (Bovera *et al.*, 2015; Henry *et al.*, 2018; Spranghers *et al.*, 2018; Vogel *et al.*, 2018).

Only a few studies have focused on the digestibility of insect meals (Allegretti *et al.*, 2018; Bosh *et al.*, 2014; De Marco *et al.*, 2015; Miech *et al.*, 2017; Panini *et al.*, 2017; Schiavone *et al.*, 2017b) and, as far as poultry trials are concerned, the standard 6.25 N:P conversion factor has been used to estimate the CP content of diets and the CP digestibility of insect meals. Janssen *et al.* (2017) have recently pointed out that NPN content of insects (namely chitin and excretion products like ammonia) leads to an overestimation of the insect protein content. Those authors determined the specific N:P conversion factor for *Tenebrio molitor*, *Alphitobius diaperinus*, and *Hermetia illucens* and proposed to setting the average N:P at 4.76 for whole larvae and 5.60 for insect protein extracts.

The aim of this paper was, on the basis of our previous study (Schiavone *et al.*, 2017b), to recalculate the digestibility of partially or totally defatted black soldier fly (BSF; *H. illucens*) larva meals in broiler chickens.

2. Material and methods

Animals, diets and digestibility trial

The research trial has been described in Schiavone *et al.* (2017b) and is briefly reported hereafter.

Sixty male 26-days-old broiler chickens (Ross 308) were randomly divided into fifteen cages and five replicates were allocated to each treatment. The chickens were fed a basal diet, and two partially or totally defatted BSF larva meal diets (BSFp and BSFh, respectively). The insect meal diets were formulated by replacing 250 g/kg (w/w) of the basal diet with insect larvae meal (either BSFp or BSFh). The ingredients of the basal diet and the nutritional composition of the diets are presented in Table 1.

Calculation of the crude protein

CP was calculated using the N:P conversion factors proposed by different authors and presented in Table 2. Four methods were used to recalculate the CP content of the diets and excreta using:

- [625]: the conventional 6.25 N:P conversion factor;
- [625_562]: the 6.25 N:P conversion ratio for the basal diet and excreta, and the weighted average of conventional 6.25 and the recent 5.62 N:P conversion factor proposed by Janssen *et al.* (2017) for the BSF meal diets;
- [560]: the mean 5.60 N:P conversion factor proposed by Mariotti *et al.* (2008) and Janssen *et al.* (2017) for excreta, basal and insect meal diets;
- [566_565]: the mean 5.60 N:P conversion factor proposed by Mariotti *et al.* (2008) for excreta, and the weighted

Table 1. Nutritional composition and ingredients of diets (Schiavone *et al.*, 2017b).

Ingredients and nutrients	Basal diet (g/kg as fed)	BSFp[1] (g/kg as fed)	BSFh[1] (g/kg as fed)
Maize meal	582.9		
Soybean meal	350.0		
Soybean oil	36.0		
Dicalcium phosphate	12.4		
Calcium carbonate	11.2		
Sodium chloride	2.0		
Sodium bicarbonate	1.5		
Trace mineral-vitamin premix[2]	4.0		
DM[3]	889	897	906
	(g/kg DM)	(g/kg DM)	(g/kg DM)
OM[3]	814	817	824
EE[3]	71	103	72
Chitin		17	21
	(MJ/kg DM)	(MJ/kg DM)	(MJ/kg DM)
Gross energy	18.7	20.0	19.4

[1] BSFp = black soldier fly larva meal diets, partially defatted; BSFh = black soldier fly larvae meal diets, totally defatted.
[2] Composition of the mineral-vitamin premix (Final B Prisma, IZA SRL): 2,500,000 IU of vitamin A; 1,000,000 IU of vitamin D3; 7,000 IU of vitamin E; 700 mg of vitamin K; 400 mg of vitamin B1; 800 mg of vitamin B2; 400 mg of vitamin B6; 4 mg of vitamin B12; 30 mg of biotin; 3,111 mg of Ca pantothenic acid; 100 mg of folic acid; 15,000 mg of vitamin C; 5,600 mg of vitamin B3; 10,500 mg of Zn, 10,920 mg of Fe; 9,960 mg of Mn; 3,850 mg of Cu; 137 mg of I; 70 mg of Se.
[3] DM = dry matter; EE = ether extract; OM = Organic matter.

Table 2. Nitrogen-to-protein (N:P) conversion factors used to calculate excreta and diet crude protein.

Method	Excreta	Basal diet	BSF meal diets
625	6.25	6.25	6.25
625_562			6.09[a]
560	5.60	5.60	
566_565		5.66[b]	5.65[c]

[a] Weighted average of the N:P conversion factors, considering 6.25 for the basal diet (75%) and 5.62 (Janssen *et al.*, 2017) for black soldier fly (BSF) larva meal (25%).
[b] Weighted average of the N:P conversion factors, considering 5.68 and 5.64 for corn and soybean meal, respectively (Sriperm *et al.*, 2011)
[c] Weighted average of the N:P conversion factors, considering 5.66 (Sriperm *et al.*, 2011) for the basal diet (75%) and 5.62 (Janssen *et al.*, 2017) for the BSF larva meal (25%).

average of the specific N:P conversion ratio of the feedstuffs (5.68, 5.64, and 5.62 for corn, soybean meal and BSF meal, respectively) proposed by Sriperm *et al.* (2011) and Janssen *et al.* (2017) for all diets.

Digestibility trials

As previously described by Schiavone *et al.* (2017b), the excreta collection lasted 4 days after 6 days of diet adaptation. Protein digestibility was calculated as follows (Schiavone *et al.*, 2017b):

$$\text{Total CP excreted} = \text{N:P} \times ([\text{excreta N}] - (1.2 \times [\text{excreta uric acid}]))$$

with N:P as defined in Table 2 and the excreta and uric acid concentrations as the % on a DM basis.

$$\text{Diet dCP} = \frac{(\text{total CP ingested} - \text{total CP excreted})}{\text{total CP ingested}}$$

Moreover, insect larva meal digestibility was calculated according to Nalle *et al.* (2012) as follows:

$$\text{Meal dCP} = \frac{(\text{dCP}_{\text{basal diet}} - (\text{dCP}_{\text{insect meal diets}} \times 0.75))}{0.25}$$

Statistical analysis

The data were analysed using IBM SPSS statistics (22.0; Armonk, NY, USA). The Shapiro-Wilk normality test was used to assess the data distribution. The statistical unit was the cage replicate (n=5 per dietary treatment) and the factors included the protein determination method (625, 625_562, 560 and 566_565). The *t*-test was used to assess the differences between methods in order to calculate the CP digestibility of the diets. In detail, the control, BSFp and BSFh treatments were selected individually and the differences between treatments were measured using the coupled t-test. The differences between the CP digestibility of the test diets and insect meal were assessed using one-way ANOVA followed by the LSD post-hoc test. Differences were considered significant for $P<0.05$.

3. Results

The CP content of the basal and insect meal diets, calculated using different N:P conversion factors, is presented in Table 3. The overestimation of the CP content using the conventional N:P conversion factor of 6.25, compared with mean conversion factor of 5.60 (Mariotti *et al.*, 2008), was 11.6 and 11.5% for BSFp and BSFh, respectively. A lower overestimation of the CP content was found when the conventional N:P conversion factor (7.6 and 7.8% for the BSFp and BSFh diets, respectively) was used than for the more accurate conversion factors (Janssen *et al.*, 2017; Sriperm *et al.*, 2011).

Diet and insect meal CP digestibility, as defined using the conventional N:P conversion factors of 6.25, varied significantly from the other methods when considering all the dietary treatments (Table 4). Diet CP digestibility, when calculated using the 625 method (which was equivalent to the 560 method), was lower than the diet CP digestibility obtained using the 566_565 method ($P=0.001$, and $P<0.001$, for the basal and insect meal diets respectively). The same was true for insect meal CP digestibility ($P<0.001$). Significantly lower diet CP digestibility was found when using the conventional 625 method for the basal diet and excreta and the 625_562 method for the insect meal diets ($P=0.001$, and $P<0.001$, for the basal and insect meal diets respectively). The same was observed for insect meal CP digestibility ($P<0.001$).

Table 3. Crude protein content of the diets (% as fed) calculated using different N:P conversion factors.

N:P calculation methods[1]	Basal diet	BSFp[2]	BSFh[2]
625	21.9	30.7	33.9
625_562	21.9	29.9	33.0
560	19.7	27.5	30.4
566_565	19.9	27.8	30.6

[1] 625 = the N:P conversion factor of 6.25; 625_562 = the N:P conversion factors of 6.25 for the basal diet and 6.09 (Janssen *et al.*, 2017) for the insect meal diets; 560 = the N:P conversion factor of 5.60 (Mariotti *et al.*, 2008); 566_565 = N:P conversion factors of 5.66 and 5.65 for the basal diet and insect meal diets, respectively (Janssen *et al.*, 2017; Sriperm *et al.*, 2011).
[2] BSFp = black soldier fly larva meal diets, partially defatted; BSFh = black soldier fly larvae meal diets, totally defatted.

Table 4. Excreta crude protein (CP) content, and diet and insect meal CP digestibility calculated using different N:P conversion factors.[1]

	N:P method[2]	Basal diet		BSFp		BSFh		*P*-value
		n	mean±SEM	n	mean±SEM	n	mean±SEM	
Diet dCP	625	5	$90.4\pm1.2^{a,A}$	5	$83.4\pm0.7^{a,B}$	5	$83.3\pm0.8^{a,B}$	<0.001
	625_562	5	$90.4\pm1.2^{a,A}$	5	$82.9\pm0.7^{b,B}$	5	$82.8\pm0.8^{b,B}$	<0.001
	566_565	5	$90.5\pm1.2^{b,A}$	5	$83.5\pm0.7^{c,B}$	5	$83.4\pm0.7^{c,B}$	<0.001
P-value			0.001		<0.001		<0.001	
Meal dCP	625			5	62.3 ± 2.8^{a}	5	61.9 ± 3.0^{a}	ns
	625_562			5	60.5 ± 2.9^{b}	5	60.1 ± 3.0^{b}	ns
	566_565			5	62.5 ± 2.8^{c}	5	62.2 ± 3.0^{c}	ns
P-value					<0.001		<0.001	

[1] a,b,c the means between the N:P method change for each dietary treatment; A,B the means between the dietary treatments change for each N:P method. BSFp = black soldier fly larva meal diets, partially defatted; BSFh = black soldier fly larvae meal diets, totally defatted.
[2] 625 = N:P conversion factor of 6.25; 625_562 = N:P conversion factors of 6.25 for the basal diet and 6.09 (Janssen *et al.*, 2017) for the insect meal diets; 560 = N:P conversion factor of 5.60 (Mariotti *et al.*, 2008); 566_565 = N:P conversion factors of 5.66 and 5.65 for the basal diet and insect meal diets, respectively (Janssen *et al.*, 2017; Sriperm *et al.*, 2011).

Overall, the differences found between the N:P methods did not lead to different results, as reported in our previous study (Schiavone *et al.*, 2017b). The CP digestibility of the BSF meal did not differ between the partially and totally defatted treatments.

4. Discussion

Over the last century several studies have pointed out the need to assess the CP content in several matrices as accurately as possible, and in particular in food and feeds. The generally accepted and widely used 6.25 N:P conversion ratio is a simple and practical way of estimating CP content of feeds (Chromy *et al.,* 2015; Sàez-Plaza *et al.,* 2013). Nevertheless, over the last decade, some studies have pointed out the need to revise this ratio (Mariotti *et al.,* 2008; Sriperm *et al.,* 2011). At the same time, studies on the nutritional composition of new feedstuffs, such as insect or seaweed meals, have focused, among other aspects, on specific N:P conversion ratios (Janssen *et al.*, 2017; Safi *et al.*, 2013; Shuuluka *et al.*, 2013). Janssen *et al.* (2017) highlighted that the NPN content of insect meals could lead to a significant overestimation of the CP content of this matrix, and proposed specific N:P conversion factors for different insect meals.

On the basis of our previous study (Schiavone *et al.*, 2017b), we have re-calculated the digestibility of partially or totally defatted BSF larva meals in broiler chickens using either the conventional 6.25 N:P conversion ratio, a combination of conventional and specific N:P conversion ratio, as proposed by Janssen *et al.* (2017), and a combined method as proposed by the authors Mariotti *et al.* (2008) and Sriperm *et al.* (2011).

The overestimation of CP content of the diet, when using the conventional 6.25 N:P conversion factor was used, was around 10%, compared with the more accurate mean N:P conversion ratios. In this study, the specific N:P conversion ratio used for soybean meal was 5.64, as recently proposed by Sriperm *et al.* (2011). According to the results presented by Mariotti *et al.* (2008), the overestimation of the CP content using the 6.25 N:P conversion factor could be even greater for diets containing soybean meal, because the conversion factors previously determined for this protein source varied between 5.44 and 5.64. The conventional 6.25 N:P conversion factor, which is used widely to determine the CP content of food and feeds, could lead to an even higher overestimation when considering other protein sources used in feeds, such as legumes (Mariotti *et al.*, 2008), and poultry by-product meal or meat and bone meal (Sriperm *et al.*, 2011).

The differences between the CP digestibility of the diet, calculated by means of the conventional 6.25 N:P conversion factor and the more accurate Mariotti *et al.* (2008) and Sriperm *et al.* (2011) methods, although statistically significant, do not seem to have any biological value. The numerical difference of the digestibility coefficients between these two methods, in fact, ranged between 0.1 and 0.3%. On the other hand, when the 6.25_562 method was used, lower diet digestibility was observed. The numerical differences between these two approaches led to lower diet digestibility coefficients of 0.5-0.6% and to digestibility differences of the insect larva meal of 1.8-2.1%. Furthermore when using specific weighted average N:P conversion ratio in diets containing new feedstuff, it would be reasonable to determine coherently the CP of both diet and excreta, as different conversion ratios could have a major impact not only on overestimation of CP but also on digestibility coefficients. Therefore, caution should be taken when using the N:P conversion factors of new nutritional approaches, while the conventional 6.25 N:P conversion ratio is generally acceptable for faeces and other diets and feedstuff. The overestimation of the protein content in insect has recently been commented by Jonas-Levi and Martinez (2017), who proposed quantifying the nutritious proteins form insects using the total N and the total non-digestible N multiplied by the appropriate conversion factor. This approach is time consuming but has been recommended to replace conventional methodologies. Nevertheless, determination of the protein content using accurate N:P conversion factors has not led to any different conclusions on the protein digestibility of BSF insect meal from our previous results (Schiavone *et al.*, 2017b).

5. Conclusions

Although it is undeniable that the 6.25 N:P conversion ratio is a practical and simple method, it is not sufficiently accurate to determine the CP content of feedstuff. If the latest developments pertaining to the N:P conversion ratio in processed insect meal (Janssen *et al.*, 2017) are considered, while maintaining a practical approach, the mean 5.60 N:P conversion ratio proposed by Mariotti *et al.* (2008) and Janssen *et al.* (2017) seems more accurate in determining the CP content of poultry diets containing processed insect meal. Otherwise the specific N:P conversion ratio should be used to accurately determine the CP content of all the diet components.

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