Featured Article

Protein quality of edible insects in the view of current assessment methods

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Implications

- Protein quality refers to the ability of dietary protein sources to supply indispensable amino acids (IAA) to meet the requirements of the target organism in either feed or food. Methods for quantifying protein quality are based on measures for either digestibility or growth.
- For insect protein quality, the evidence is growing though still a limited number of studies are conducted. Applying appropriate methods is critical to ensure the best future applications of insect protein for animal feed and direct human consumption.
- For evaluating the protein quality of insects for humans using DIAAS method, the generalized nitrogen-to-protein conversion factor of 6.25 is the recommended standard for quantifying crude protein. This factor results in an underestimation of protein quality due to the nature of the chitin non-protein nitrogen in insects. Thus, the evaluation should consider IAA instead of crude protein.
- *Tenebrio molitor* (Yellow mealworm) and *Hermetia illucens* (Black soldier fly) are the most studied species. Across studies, *T. molitor* has the highest crude protein digestibility, while the effect on animal growth performance is similar. Both insect species are suitable to be used in animal feed.
- The few studies investigating edible insect protein quality for humans indicate that among the species relevant for insect farming, cricket species may have slightly better protein quality than mealworm species, though inconclusive till more data is available.

Key words: amino acids, amino acid requirement, digestibility, insect protein, protein quality

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Introduction

Over the past decade, following the rise in global awareness of the potential of edible insects, their nutritional properties have been studied, though still in infancy compared to established feed and food sources. The nutritional composition among more than 2,000 insect species recorded to be utilized for animal feed or direct human consumption differs widely in nutritional content, though sharing that protein is a significant component (van Huis et al., 2021). Protein and amino acid (AA) content and composition differ between species, developmental stages (pupae, larva, and adult) (Jonas-Levi and Martinez, 2017); location and season of the collection; the processing of samples, etc (Churchward-Venne et al., 2017; Janssen et al., 2019).

Following the great diversity of edible insects, the AA profile of insects shows equal variability hence, it also challenges establishing a clear picture of the protein quality. Overall, insects are considered a valuable source of alternative protein for feed and food, with the potential for sustainable mass production. To fully release these potentials, insect protein needs to be characterized for quality to ensure the best future applications.

This review aims to provide an overview of suitable methods to evaluate protein quality and, secondly, provide a concise review of the available information on insect protein quality.

Protein quality

Protein quality refers to the ability of dietary protein sources to supply IAAs to meet the requirements of the target organism, in either feed or food (Fact box 1). Hence, commonly used principles of expressing protein quality are based on the ability of a protein source to supply sufficient IAA for a specific target group. Over a century, different methods have been developed to quantify and classify protein quality. The methods build on two principles, either as evaluation of the contents and digestibility of IAA or measured as the physiological impact, such as the growth of the target organism. For humans, IAA requirements are defined for different age groups. Except for breast milk for infants, no single dietary protein fully matches the requirements (FAO, 2013).

Assessment of the digestibility of the protein sources is used as an indicator to which extent the AA can be hydrolyzed by

gastrointestinal enzymes and be available for absorption in a target organism. (Stein et al., 2007). Evaluation of protein digestibility can be carried out either in-vivo or in-vitro. For the in-vivo digestibility methods, the point of sampling for digesta, i.e., either at the ileum or after the passage of the total gastrointestinal tract in the feces, has a significant impact on the protein quality measurements (Stein et al., 2007).

Total tract digestibility – also termed as "apparent total tract digestibility" (ATTD) – is the simplest and longest-standing method of measuring fecal nitrogen (N) recovery relative to intake, assuming AAs are absorbed throughout the digestive tract (Stein et al., 2007). However, in the large intestine of monogastric animals and humans, no significant AA absorption takes place, and N may be absorbed as ammonia and excreted in the urine (van der Wielen et al., 2017). Also, the microbiota in the large intestine catabolizes and synthesizes AA, making fecal digestibility not truly represent the protein source (Hendriks et al., 2012). To adjust for this, ATTD can be corrected for endogenous losses using an N-free diet but remain to account for N or AAs absorbed in the large intestine.

Ileal digestibility has been developed and standardized by the swine and poultry industries for feed formulation and adapted to improve human protein quality measures. The apparent ileal digestibility (AID) – overcomes the limitations of ATTD, but the measure is more complicated by requiring surgically fitted T-cannula to tap ileal digesta (Stein et al., 2007). Also, the ileal digesta is not exclusively of dietary origin, and basal and diet-specific endogenous AA losses add some noise to this method. The "true ileal digestibility" (TID) method overcomes these limitations by adding protein-free or well-defined AA diets, along with tracers or markers, to follow the transition in the tract. The TID is also called "standardized ileal digestibility" (SID) when no diet-specific endogenous AA losses exist. TID is considered the most accurate estimation of digestibility. However, it can also overestimate bioavailability for some diets, for example, due to the Maillard reaction (Jensen et al., 2019) between AAs and carbohydrates, not affecting the digestibility, but reducing the bioavailability of AAs.

Laboratory-based in-vitro digestibility methods that mimic digestive processes using proteolytic enzymes are widely used as a simple, less costly alternative to in-vivo digestibility (Egger et al., 2017). Although in-vitro digestion methods are being further developed into dynamic models of digestion (Egger et al., 2017), these methods can only partially mimic the actual gastrointestinal peristalsis conditions. Several insect species evaluated in in-vitro systems have been reviewed by Rodríguez-Rodríguez et al. (2022). This review concluded that there appeared to be great differences in in-vitro digestibility between insect species, but also found methodological issues calling for standardized in vitro protocols to secure comparable results between studies.

Protein quality based on in-vivo method

With few exceptions of human studies, in-vivo methods are carried out in animals assessing the protein quality in relation to animal nutritional requirements or used as an animal model for protein quality in relation to human requirements. The different in-vivo methods and their principles are based on either assessing growth or IAA digestibility or absorbed IAA (Osborne et al., 1919; FAO/WHO/UNU, 1991; WHO/FAO/UNU, 2007; FAO, 2013; Devi et al., 2018), as shown in Table 1.

Protein quality based on growth

Several methods are based on the assessment of the growth of animals, determined for feeding a diet containing a test protein and compared to a reference protein. The "protein efficiency ratio" (PER) and "NPR" (NPR) are widely used growth-based methods for protein quality evaluation. The PER in growing rats developed by Osborne et al. (1919) was the first method adopted for assessing the protein quality of human food. The PER method calculates the efficiency of dietary crude protein (CP) for body weight gain with casein as a reference protein. The PER ranks protein sources against a single reference, but this ranking is not proportional. The NPR is a modification of PER by adjusting for the weight loss of rats in the control group that is given a protein-free diet. The weight loss of the rat fed protein free diet is considered to be equivalent to the protein needed for maintenance. Both PER and NPR use rats and the need of Sulphur amino acids in rats is higher than in humans, and the outcomes thus can overestimate the protein quality for humans (Deglaire and Moughan, 2012).

Protein quality based on digestibility

The digestibility measures reflect a protein's enzymatic breakdown and adequacy to supply IAA by sampling either ileal digesta or feces from animals or humans. As outlined, ileal digesta is preferred over fecal digesta. For protein evaluation for food, humans are preferred, and as animal models, pigs are superior to rats because their digestibility and metabolism are more like humans (FAO, 2014). Only a few human studies have determined the ileal digestibility due to the technical, economic, and ethical barriers of invasive methods for ileal sampling. Consequently, most in-vivo data come from animal models.

The common methods for evaluating protein quality using animal models are: "biological value" (BV), "net protein utilization" (NPU), "protein digestibility-corrected AA score" (PDCAAS), and the "digestible indispensable AA score" (DIAAS) method. The BV, NPU, and PDCAAS use the fecal digestibility of CP in a rat model FAO/WHO/UNU (1991), whereas DIAAS uses ileal AA digestibility in a pig model (FAO, 2014).

The BV measures the proportion of absorbed N retained in the body and indicates how well the body utilizes CP, though not accounting for the digestion and the interaction with other foods. The NPU and BV are similar, except that NPU is calculated from ingested N and hence considers digestibility and is a better estimate for protein quality than BV (Hoffman and Falvo, 2004).

Table 1. In-vivo methods for determining protein quality of food

Method	Principle	Based on	Calculations	Remarks	Reference
Protein efficiency ratio (PER)	Assess the effectiveness of a protein on animal (rat) growth against casein as a reference protein.	Growth	Weight gain (g) TP/Amount of protein consumed (g)(TP)	• Not astrongcorrelationtothe growing needsofhumans because rats have a higher requirement of some amino acids than humans • Only accounts for growth and does not define protein used for maintenance purposes • The gain in body weight does not necessarily correspond to the gain in body protein • PER values vary with levels of protein intake	Osborne et al. (1919)
Net protein ratio (NPR)	Modification of PER method that uses protein-free diet in growing rats	Growth	[Weight gain TP (g)− an average weight loss of animals fed a non-protein diet (g)]/ Protein consumed (g)	• Same issues as for PER	FAO/WHO/UNU (1991)
Protein digestibility corrected amino acid score (PDCAAS)	Determines fecal amino acid and protein digestibility in a rat model using a protein-free diet	Digestibility	(mg of IAA_{lim} in 1 g TP/mg of same AA in 1 g reference protein) × fecal true protein digestibility (%)× 100	• Proteins with a higher score than 100% are truncated and do not acknowledge the extra dietary IAAs in high-quality protein that supplement and balance the amino acid composition of a mixed diet. • May overestimate protein digestibility due to microbial degradation of protein in the colon. • Uses values of total tract digestibility of CP based on the assumption that all AAs have the same digestibility as CP.	FAO/WHO/UNU (1991)
Digestible indispensable amino acid score (DIAAS)	Determines ileal digestible IAAs as the proportion of human requirement using a pig model. First, limiting IAA sets the score.	Digestibility	(mg of digestible IAA_{lim} in 1 g TP) /(mg of same aa in 1 g reference protein) × 100	• Recognized as the most suited animal model for protein quality evaluation in humans • Given that AA requirements in fast-growing animals like rats and pigs are predominantly directed toward tissue growth, whereas requirements in humans older than one year are used largely for maintenance, hence, sensible interspecies comparisons are difficult. • Total protein quantification critical for the score	FAO (2013)
Biological value (BV)	Measures percent of absorbed N retained in the body	Digestibility	[N intake of TP −(FecalN − Endogenous fecal N)−(Urinary N − Endogenous Urinary N)/N intake of TP −(Fecal N − Endogenous fecal N)] × 100	• Ignores factors that influence the digestion of protein and interaction of the protein with other food before absorption. • It only measures a protein's maximal potential quality and fails to measure estimates at requirement levels. • Relates N absorbed to N retained; thus, as an index, it excludes the digestibility factor. • BV results for the same food varies significantly depending on N intake.	FAO/WHO/UNU, (1991)
Net protein utilization (NPU)	Measures percent of dietary N retained in the body	Digestibility	[{N intake of TP −(FecalN − Endogenous fecal N)−(Urinary N − Endogenous Urinary N)}/N intake of TP] × 100 Or NPU = Digestibility × BV	• Measured when the protein content of the diet is below that of requirement and not appropriate when the diet is adequate	FAO/WHO/UNU, (1991); WHO/FAO/UNU, (2007)
Dual isotope tracer method	Compares bioavailability (digestion and absorption) of isotope-labeled IAA in a circular system from intrinsically labeled test protein consumed together with a reference protein with known digestibility labeled differently	Blood sampling	[{PlasmaAA (H labelled TP)− IAA}/ {mealAA (H labelled TP)−IAA}]/ [{plasma AA (C labelled RP)− IAA}/ {mealAA (C labelled RP)− IAA}] ×(100/1)× Digestibility of RP × Transamination correctionfactor	• Limited to measure the bioavailability of specific labeled IAA, and does not provide a complete measure for IAA digestibility and absorption from a food • Technically advanced and limited to experimental settings	Devi et al. (2018)

Abbreviations: TP = Test Protein; RP = Reference Protein; AA = amino acids; N = Nitrogen; IAA = indispensable amino acid; IAA_{lim} = first limiting indispensable amino acid.

The PDCAAS method was the first to be adopted that used an indirect measure of IAA bioavailability as apparent total tract CP digestibility (FAO/WHO/UNU, 1991) and was recommended as an alternative to the PER method. The PDCAAS is calculated by multiplying AA concentrations with ATTD of CP, providing the AA scores using the IAA requirements of a reference population. The lowest AA score is considered the first-limiting AA, defining the PDCAAS. Total tract CP digestibility can overestimate AA bioavailability of protein from ingredients with poor ileal digestibility and does not consider differences in digestibility among individual AA. Also, PDCAAS values higher than 100% are truncated to 100, implying that AAs supplied above requirements do not have additional physiological value. The truncation arbitrarily underestimates the protein quality of food sources with high protein values, such as meat and milk.

The DIAAS was developed to overcome the methodological limitations of PDCAAS. The DIAAS method is based on the cannulated sampling of ileal digesta and assesses digested AAs at the point of physiological absorption. The DIAAS relies on calculating a score for each IAA based on the AA content and the concentration of each digestible IAA and expresses the proportion of the reference patterns for the age groups for which the IAA has been set. The IAA with the lowest score relative to the reference pattern for the specific age is the first limiting IAA defining the DIAAS value (FAO, 2013). Compared with PDCAAS, there is no truncation for DIAAS values, and some protein ingredients can have values greater than 100. The FAO (2013) has recommended the DIAAS method, but the lack of ileal digestibility coefficients published in the literature has limited the widespread use of DIAAS. For the evaluation of insect protein quality using DIAAS, Malla et al. (2022) is currently the only published study. Though DIAAS is recommended for evaluating food's protein quality, PER, NPR, BV, NPU, and PDCAAS are still used until a sufficient database of ileal digestibility, and DIAAS values are available. Furthermore, DIAAS can also be determined by using dual stable isotope tracer method (FAO, 2014; Shivakumar et al., 2019).

Protein quality based on isotopic methods

More recently, the dual-isotope tracer method has been developed to determine true ileal IAA digestibility in humans (Devi et al., 2018). For this method, an intrinsically isotope-labeled test protein must be produced by growing the source, either plants or animals, on labeled substrates; the labeled protein is fed along with a different isotope-labeled "standard" protein of known digestibility. Then, the postprandial ratio of the differently labeled AA in the blood allows for determining the proper digestion and absorption of the test protein. This method measures AA bioavailability beyond intestinal digestibility. The method requires developing an intrinsic isotope labeled food, is expensive, and cannot be routinely conducted on all food ingredients.

Protein quality evaluation of insects

The different methods for assessing protein quality have been applied to insects in various studies, providing results that are to some extent comparable, but also represent different principles of evaluating protein quality (Longvah et al., 2011; Yang et al., 2014; Oibiokpa et al., 2018; Poelaert et al., 2018; Jensen et al., 2019; Hermans et al.,2021; Malla et al., 2022). In the following, we review the pool of published in-vivo studies. The overview of the studies reviewed on protein digestibility and quality measures by insect species across methods applied is shown in Table 2.

Protein quality of insects in pig and poultry diets

Growing research has been conducted on the effects of insect protein on the growth performance and nutrient digestibility of pigs and poultry (Table 3). For digestibility, we have reviewed in-vivo studies reporting CP, N, and AAs (Table 3), excluding in-vitro studies due to the limitations. Cho et al. (2020) reported that dietary supplementation of *Tenebrio molitor* (Yellow mealworm) larvae hydrolysate in diets of growing pigs had higher digestibility (AID and SID) of CP and some IAAs compared to fermented poultry by-product and hydrolyzed fish soluble. The AID tended to be higher in hydrolyzed than defatted *T. molitor* diet, likely explained by the low molecular peptides that are more easily absorbed. Another study also found that dried *T. molitor* larvae had better digestibility of CP and IAAs than fish, meat, or poultry meal (Yoo et al., 2019). The inclusion level of about 10% insect protein improved the digestibility of CP and AAs. Regardless of processing, *T. molitor* larvae protein's digestibility seems good.

Tan et al. (2020) compared two insects, *Hermetia illucens* (black soldier fly) and *Musca domestica* (housefly), and found lower SID of most IAAs in *H. illucens*, except for methionine and cysteine. In our study, Malla et al. (2022) also found the SID of CP of five species ranging from 61.6 for *H. illucens* to 77.8 for *Alphitobius diaperinus* (lesser mealworm). Both Tan et al. (2020) and Malla et al. (2022) indicated that the insects with higher CP and AAs content had higher digestibility of CP and AA. Different concentrations of chitin may also influence AA digestibility. Chitin contents were not analyzed in these studies, and the role of chitin in ileal digestibility remains understood but will likely reduce the SID of AA.

For growth performance as the measure of protein quality, studies in weaned piglets fed different insect species compared to different control diets as shown in Table 3 (Ji et al., 2016; Spranghers et al., 2018; Ao and Kim, 2019; Biasato et al., 2019; Cho et al., 2022). Some studies had low inclusion of insects in the feed. One study found an increasing *T. molitor* by up to 6% at the expense of soybean meal and soy oil, improved growth performance, explained by increasing feed intake due to improved flavor and palatability with increasing *T. molitor* level (Jin et al., 2016). The difference in growth performance between studies can be attributed to combinations of insect species, substituted ingredients, other feed protein sources, and

Table 2. Overview of the protein digestibility and quality measures by insect species across methods applied in the studies reviewed

						Reference protein pattern (years)															
		Number of studies				2–5						0.5–3				>3	Protein digestibility				
Insects	Common name	Total	Growth performance	Digestibility	Protein Quality	PER	NPR	BV	NPU	PDCAAS in-vivo	PDCAAS in-vitro	PER	BV	PDCAAS	DIAAS	DIAAS	AID	SID	ATTD	APD	TPD
Acheta domesticus	House cricket	2	–	3	2	–	–	–	–	–	–	**2.82**	–	83	76	89	–	75	–	78	83
Alphitobius diaperinus	Lesser mealworm	4	1	4	2	–	–	–	–	–	–	–	**63**	82	71	83	–	77	–	**87**	**93**
Cirina forda	Shea defoliator	1	–	1	1	–1.03	1.82	86	72	42	–	–	–	–	–	–	–	–	–	–	81
Gryllodes sigillatus	Banded cricket	1	–	1	1	–	–	–	–	–	–	–	–	–	**78**	**92**	–	64	–	–	–
Gryllus assimilis	Field cricket	1	–	1	1	**1.78**	**3.04**	**93**	75	73	–	–	–	–	–	–	–	–	–	–	80
Hermetia illucens	Black soldier fly	7	4	7	1	–	–	–	–	–	–	–	–	–	57	68	67–81	61	51–82	–	–
Holotrichia parallela	Chafer beetle	1	–	1	1	–	–	–	–	–	68	–	–	–	–	–	–	–	–	–	78
Macrotermis nigierensis	Termite	1	–	1	1	–2.25	1.32	85	70	42	–	–	–	–	–	–	–	–	–	–	90
Melanoplus foedus	Striped sand grasshopper	1	–	1	1	–1.25	2.4	84	**85**	46	–	–	–	–	–	–	–	–	–	–	84
Musca domestica	House fly	2	1	2	–	–	–	–	–	–	–	–	–	–	–	–	79–86	–	–	–	–
Ptecticus tenebrifer	–	1	1	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	78–60	–	–
Samia ricinii	Eri silkmoth	1	–	1	1	–	–	–	41	**99**	–	–	–	–	–	–	–	–	–	–	87
Tenebrio molitor	Yellow mealworm	12	6	12	3	–	–	–	–	–	–	2.20	56	76, **86**	54	64	80–**89**	71–**93**	60–**93**	85–86	90–91
Zophobas morio	Super worms	1	1		1	–	–	–	–	–	–	–	–	–	–	–	79–87	–	–	–	–

AID =”apparent ileal digestibility; ATTD”=”apparent total tract digestibility; BV =”biological value; DIAAS”=”digestible indispensable amino acid score; NPR”=”net protein ratio; NPU”=”net protein utilization; PDCAAS”=”protein digestibility corrected amino acid score; PER”=”protein ef!ciency ratio; SID”=”standardized ileal digestibility; TID”=”true ileal digestibility. The bold text represents highest value reported for each insect species within each method.

Table 3. Overview of studies on the effects of insect products on growth performance and/or protein digestibility

Animals	Age/life stage	Insect product	Replacement/ comparator	Inclusion (% of DM)	Effect on growth performance and nutrient digestibility	Remarks	References
Growing pigs	29 kg	*Tenebrio molitor* defatted, and *Tenebrio molitor* hydrolysate	Fermented poultry by-product andhydrolyzed fish soluble	10	**AID** of CP, lysine, methionine threonine (%) *Tenebrio molitor* defatted: 86.3, 79.6, 79.7, 79.3 *Tenebrio molitor* hydrolysate: 89.3, 79.9, 79.9, 79.9 Fermented poultry by-product:85.5, 78.8, 79.1, 78.9 Hydrolyzed fish soluble: 83.4, 78.7, 79.0, 78.8 **SID** of CP, lysine, methionine threonine (%) *Tenebrio molitor* defatted: 90.2, 83.5, 83.6, 83.2 *Tenebrio molitor* hydrolysate: 93.1, 83.7, 83.7, 83.7 Fermented poultry by-product: 89.3, 82.7, 83.0, 82.7 Hydrolyzed fish soluble: 86.4, 82.5, 82.9, 82.6	• Digestibility in CP, lysine, methionine, and threonine was higher for *Tenebrio molitor* hydrolysate diets than fermented poultry by-products and hydrolyzed fish soluble diets.	Cho et al. (2020)
Growing pigs	24 kg	*Tenebrio molitor*	Fish meal, meat meal, poultry meal	9.95	**AID** of CP, lysine, histidine, arginine, and cysteine (%) *Tenebrio molitor*: 89.5, 89.6, 89.6, 89.7, 89.5 Fish meal: 85.0, 86.1, 85.5, 87.3, 83.0 Meat meal: 86.7, 87.3, 86.6, 87.6, 86.0 Poultry meal: 87.8, 88.4, 88.2, 88.9, 87.6 **SID**: CP, arginine, and cysteine (%) *Tenebrio molitor*: 90.0, 89.9, 90.2 Fish meal: 85.5, 87.6, 83.6 Meat meal: 87.3, 87.9, 86.6 Poultry meal: 88.3, 89.1, 88.3	• The SID of arginine was higher in pigs fed *Tenebrio molitor* diet than in pigs fed fish or meat meal diets. • *Tenebrio molitor* diet had increased SID of cysteine than pigs fed fish meal diet. • Overall, *Tenebrio molitor* diet tended to show increased SID of CP, total AAs, and all AAs than meat meal, poultry meal, or fish meal diet.	Yoo et al. (2019)
Growing pigs	25.05 kg	*Musca domestica* and *Hermetia illucens*	N-free diet	Insects as the sole source of protein	**AID** of all IAA (%) *Musca domestica*: ranged from 84.4 to 92.8 *Hermetia illucens*: ranged from 73.5 to 82.1 **SID** of all IAA (%) *Musca domestica*:ranged from 87.8 to 98.8 *Hermetia illucens*: ranged from 76.7 to 191.8	• The *Musca domestica* has greater AID of all AA than *Hermetia illucens* • The *Musca domestica* has greater SID of all AA except methionine and cysteine than *Hermetia illucens*	Tan et al. (2020)
Growing pigs	35kg	*Alphitobius diaperinus, Tenebrio molitor, Acheta domesticus, Gryllodes sigillatus*and *Hermetia illucens*	N-free diet	Insect as sole source of protein	**SID** of CP (%) and IAA (%) range *Alphitobius diaperinus*: 77.8, 75.3 to 97.0 *Tenebrio molitor*: 71.7, 63.2 to 96.3 *Acheta domesticus*: 75.1, 78.7 to 96.7 *Gryllodes sigillatus*: 64.1, 63.2 to 95.0 *Hermetia illucens*: 61.6, 53.4 to 91.0	• SID of CP of Alphitobius diaperinus and Acheta domesticus comparable to Tenebrio molitor and Gryllodes sigillatus but higher than Hermetia illucens • SID of all IAAs are higher in *Alphitobius diaperinus* and *Acheta domesticus* than in *Gryllodes sigillatus* and *Hermetia illucens*	Malla et al. (2022)

Table 3. Contiuned

Animals	Age/life stage	Insect product	Replacement/ comparator	Inclusion (% of DM)	Effect on growth performance and nutrient digestibility	Remarks	References
Weaned piglets	24 days	*Tenebrio molitor* full-fatted and *Tenebrio molitor* hydrolysate powder	Spray-dried plasma protein	3	**ATTD** of N (%) Phase 1 (days 0 to 14) *Tenebrio molitor* full-fatted: 77.4 *Tenebrio molitor* hydrolysate:79.3 Spray-dried plasma protein: 78.2: Phase 2 (days 15 to 35) *Tenebrio molitor* full-fatted: 76.4 *Tenebrio molitor* hydrolysate: 79.7 Spray-dried plasma protein: 77.3	• No significant difference in growth performance between treatments • No significant difference in ATTD of N between treatments	Cho et al. (2022)
Weaned piglets	22 days	*Tenebrio molitor*	2% Fish meal	1, 2	**Average daily gain**(g) 1% *Tenebrio molitor*:404 2% *Tenebrio molitor*: 413 2% fish meal: 426 **Final body weight**(kg) 1% *Tenebrio molitor*: 20.0 2% *Tenebrio molitor*: 21.4 2% fish meal: 21.8 **ATTD** of N (%) 1% *Tenebrio molitor:* 79.5% 2% *Tenebrio molitor:* 81.2% 2% fish meal: 81.5%	• Feeding 1% *Tenebrio molitor* decreased average daily gain and final body weight but did not affect average daily feed intake or feed: gain ratio compared to2% fish meal • The growth performance of pigs fed 2% *Tenebrio molitor* was comparable to 2% fish meal • The ATTD of 1% *Tenebrio molitor* was lower than 2% fish meal • The ATTD of 2% *Tenebrio molitor* was comparable to 2% fish meal	Ao et al. (2020)
Weaned piglets	21 days	*Ptecticus tenebrifer* larvae	2% Fish meal	1, 2	**ATTD** of N (%) 1% *Ptecticus tenebrifer*: 78.57% 2% *Ptecticus tenebrifer*: 80.27% 2% fish meal: 81.61%.	• No difference in growth performance among treatment • The ATTD of 1% *Ptecticus tenebrifer* was lower than 2% fish meal • The ATTD of 2% *Ptecticus tenebrifer* was comparable to 2% fish meal	Ao and Kim (2019)
Weaned piglets	21 days	*Hermetia illucens* partially defatted	Soybean meal	5, 10	**ATTD** of CP (%) Phase 1 (days 1 to 23) 5% *Hermetia illucens*: 80.8 10% *Hermetia illucens*: 82.8 Soybean: 80.3 Phase 2 (days 24 to 61) 5% *Hermetia illucens*: 77.7 10% *Hermetia illucens*:79.5 Soybean: 76.6	• No difference in growth performanceamong treatment • *Hermetia illucens* meal at 5% and 10% inclusion did not influence the nutrient digestibility of the piglets in the present study	Biasato et al. (2019)

Table 3. Contiuned

Animals	Age/life stage	Insect product	Replacement/ comparator	Inclusion (% of DM)	Effect on growth performance and nutrient digestibility	Remarks	References
Weaned piglets	21 days	*Hermetia illucens* full-fat and defatted prepupae	Soybean(toasted)	Full fat: 4, 8 Defatted: 5.4	**AID and ATTD** of CP (%) 4% full-fat *Hermetia illucens*: 73.3–77.4 8% full fat *Hermetia illucens*: 67.4–77.6 Defatted *Hermetia illucens*: 73.3–78.3 Soybean meal (toasted): 69.7–76.8	• No differences in growth performance among treatments • The AID of CP was higher with 4% full-fat and defatted *Hermetia illucens* in the diet compared to the toasted Soybean • No difference in ATTD of CP among the different treatments	Spranghers et al. (2018)
Weaned pigs	28 days	*Tenebrio molitor, Musca domestica larvae*and*Zophobas morio*	5% plasma protein (powder)	5	**AID** of CP (%) and all AAs range (%) Phase 1 (days 1 to 28) *Tenebrio molitor:* 89.4, 69.5 to 94.0 *Musca domestica larvae:* 86.5, 77.6 to 94.6 *Zophobas morio:* 87.5, 67.3 to 94.0 5% plasma protein: 87.5, 70 to 94.6 Phase 2 (days 29 to 56) *Tenebrio molitor: Musca domestica larvae:* 79.1, 62.5 to 91.0 *Zophobas morio:* 79.1, 53.0 to 8.6 5% plasma protein: 79.6, 54.5 to 93.4	• No difference in growth performance among treatments • There is no significant difference in AID of CP and AA compared with the control group • AID of isoleucine for *Tenebrio molitor* (90 %)is higher *than for Zophobas morio* (75%) and comparable to *Musca domestica* (85.5%) and plasma protein (78.5) • AID of methionine is higher for *Tenebrio molitor* (97%) and *Musca domestica*(90.5%) compared to plasma protein (75.5%) and *Zophobas morio*	Ji et al. (2016)
Weaned piglets	28 days	*Tenebrio molitor* larvae	Soybean meal and soy oil	0, 1.5, 3, 4.5, 6	**Gain: feed ratio** 0%: 0.521 1.5%: 0.538 3%: 0.565 4.5%: 0.573 6%: 0.576 **Final body weight**(kg) 0%: 17.78 1.5%: 18.34 3%: 19.10 4.5%: 19.77 6%: 20.22 **ATTD** of CP (%) and N retention (g/d) 0%: 86.2, 2.20 1.5%: 90.2, 2.27 3%: 91.2, 2.33 4.5%: 92.1, 2.38 6%: 93.0, 2.42	• The increasing level of *Tenebrio molitor* up to 6% improved all the parameters of growth performance linearly • Linear improvements in CP digestibility and N retention by increasing levels of *Tenebrio molitor*	Jin et al. (2016)

Table 3. Contiuned

Animals	Age/life stage	Insect product	Replacement/ comparator	Inclusion (% of DM)	Effect on growth performance and nutrient digestibility	Remarks	References
Broiler	1 day	*Tenebrio molitor, Hermetia illucens, Alphitobius diaperinus*	Soybean and fish meal	10	**Feed conversion ratio** Control: 1.38 *Tenebrio molitor*: 1.35 *Hermetia illucens*: 1.38 *Alphitobius diaperinus*: 1.28 **N retention** *Tenebrio molitor*: 34.4 *Hermetia illucens*: 43.4 *Alphitobius diaperinus*: 35.6	• No effect on parameters of growth performance over the total experimental period • Higher N retention when feeding *Hermetia illucens* meal compared *Tenebrio molitor* and *Alphitobius diaperinus*	van der Heide et al. (2021)
Broiler	30 days	*Tenebrio molitor*	Soybean	30	**Feed conversion ratio**(g/d) *Tenebrio molitor*: 3.62 Soybean: 4.13 **AID** of CP (%) *Tenebrio molitor*: 80.2 Soybean: 87.3	• The feed conversion ratio was better for *Tenebrio molitor* group than the soybean group • No difference in other parameters (body weight gain and feed intake) of growth performance among treatments • Reduced AID for *Tenebrio molitor* group than soybean group	Bovera, et al. (2016)
Broiler	16 weeks	*Tenebrio molitor* *Hermetia illucens*	Basal diet	25	**ATTD** of CP (%), *Tenebrio molitor*: 60 *Hermetia illucens*: 51 **AID** of AA (isoleucine, lysine, methionine, phenylalanine, valine, alanine, aspartic acid, glycine, glutamic acid, and tyrosine)(%) *Tenebrio molitor*: 82, 85, 80, 91, 82, 93, 89, 89, 88, 83 *Hermetia illucens*: 45, 46,42, 63, 62, 86, 61, 67, 74, 43	• AID levels of isoleucine, lysine, methionine, phenylalanine, valine, alanine, aspartic acid, glycine, glutamic acid, and tyrosine in *Tenebrio molitor* were significantly higher than in *Hermetia illucens*	de Marco et al. (2015)
Laying hen	1 day	*Hermetia illucens* defatted	Soybean	25, 50	**Weight gain** 25% *Hermetia illucens:* 250.3 50% *Hermetia illucens:* 350.1 Soybean: 305.7 **AID** of CP (%) 25% *Hermetia illucens*: 81.1 50% *Hermetia illucens*: 76.0 Soybean: 86.1	• 50% *Hermetia illucens* group showed comparable weight gain to the soybean group and higher weight gain than the 25% *Hermetia illucens* group • Of all treatments, Soybean had better AID of CP followed by 25% *Hermetia illucens* and followed by 50% *Hermetia illucens* • Reduced AID of CP with increased inclusion level of *Hermetia illucens*	Bovera et al. (2018)
Laying hen	24 weeks	*Hermetia illucens* defatted	Soybean	17	**AID** of CP (%) *Hermetia illucens*: 67.6 *Soybean*: 78.2	• Laying hen fed with *a Hermetia illucens* based diet did not significantly differ in AID of CP to hen fed on a soybean-based diet.	Cutrignelli et al. (2018)

AA = amino acids; AID = apparent ileal digestibility; ATTD = apparent total tract digestibility; CP = crude protein; IAA = indispensable amino acid; N = Nitrogen; SID = standardized ileal digestibility.

Table 4. Insect protein quality*

Species/type	Test species	Method/outcome measure	Age group	Results	References
Acheta domesticus	Rats	PER and PDCAAS	6 months–3 years	PER: 2.82 PDCAAS: 83.9 (Leucine)	Poelaert et al. (2018)
Acheta domesticus	Growing pigs	DIAAS	birth–6 months 6 months–3 years >3years	45 (Tryptophan) 76 (SAA) 89 (SAA)	Malla et al. (2022)
Alphitobius diaperinus	Rats	BV and PDCAAS	6 months–3 years	BV: 63 PDCAAS: 82 (SAA)	Jensen et al. (2019)
Alphitobius diaperinus	Growing pigs	DIAAS	birth–6 months 6 months–3 years >3years	57 (Tryptophan) 71 (SAA) 83 (SAA)	Malla et al. (2022)
Alphitobius diaperinus	Humans	Isotope labeling		Muscle protein synthesis rates increased after both *Alphitobius diaperinus* and milk protein concentrate ingestion	Hermans et al. (2021)
Cirina forda	Rats	PER, NPR, NPU, BV, and PDCAAS	2–5 years	PER: −1.03 NPR: 1.82 NPU: 72.3 BV: 86.9 PDCAAS: 42 (SAA)	Oibiokpa et al. (2018)
Gryllodes sigillatus	Growing pigs	DIAAS	birth–6 months 6 months–3 years >3years	47 (Tryptophan) 78 (SAA) 92 (SAA)	Malla et al. (2022)
Gryllus assimilis	Rats	PER, NPR, NPU, BV, and PDCAAS	2–5 years	PER: 1.78 NPR: 3.04 NPU: 75.20 BV: 93.0 PDCAAS: 73 (Threonine)	Oibiokpa et al. (2018)
Hermetia illucens	Growing pigs	DIAAS	birth–6 months 6 months–3 years >3years	47 (Lysine) 57 (Lysine) 68 (Lysine)	Malla et al. (2022)
Holotrichia parallela	In-vitro	PDCAAS	2–5 years	PDCAAS: 68.2 (Threonine)	Yang et al. (2014)
Macrotermis nigierensis	Rats	PER, NPR, NPU, BV, and PDCAAS	2–5 years	PER: −2.25 NPR: 1.32 NPU: 70.4 BV: 85.4 PDCAAS: 42 (SAA)	Oibiokpa et al. (2018)
Melanoplus foedus	Rats	PER, NPR, NPU, BV, and PDCAAS	2–5 years	PER: −1.25 NPR: 2.40 NPU: 85.09 BV: 87.4 PDCAAS: 46 (Isolucine)	Oibiokpa et al. (2018)
Samia ricinii pre-pupae	Rats	NPU and PDCAAS	2–5 years	NPU: 41 PDCAAS: 99 (Leucine)	Longvah et al. (2011)
Samia ricinii pupae	Rats	NPU and PDCAAS	2–5 years	NPU: 41 PDCAAS: 100 (Leucine)	Longvah et al. (2011)
Tenebrio molitor	Rats	BV and PDCAAS	6 months–3 years	BV: 56.6 PDCAAS: 76 (SAA)	Jensen et al. (2019)

Table 4 Continued

Species/type	Test species	Method/outcome measure	Age group	Results	References
Tenebrio molitor	Rats	PER and PDCAAS	birth–6 months 6 months–3 years >3years	PER: 2.20 PDCAAS: 86.4 (SAA)	Poelaert et al. (2018)
Tenebrio molitor	Growing pigs	DIAAS	birth–6 months 6 months–3 years >3years	45 (SAA) 54 (SAA) 64 (SAA)	Malla et al. (2022)

*First-limiting amino acid is in parentheses. PER = protein efficiency ratio, NPR = net protein ratio; NPU = net protein utilization; BV = biological value; PDCAAS = protein digestibility corrected amino acid score; DIAAS = digestible indispensable amino acid score; SAA = Sulphur amino acids (methionine + cysteine). Poelaert et al. (2018); Jensen et al. (2019) and Malla et al. (2022), calculated protein quality of insects based on the FAO (2013) suggested pattern of amino acid requirements for age group, birth to 6 months (infants), 6 months to 3 years (young children), and >3 years (older children, adolescents, and adults). Longvah et al. (2011); Yang et al. (2014) and Oibiokpa et al. (2018) calculated protein quality of insects based on FAO/WHO/UNU (1985) suggested pattern of amino acid requirements for age group 2–5 years (pre-school children).

insects' inclusion levels. Standardized studies are needed to understand the value of insects in animal feed fully.

Different insect species assessed in weaned piglets show heterogeneous results in protein and AA digestibility across the studies (Table 3). As for growth performance, the difference in species, in replacement of other protein sources, and different insect inclusion levels limit the comparison among studies. Moreover, the precise characterization of the tested insect sample remains to be standardized, such as for the same growth stage, chitin content, etc., which may affect the digestibility. Also, some animals – as well as some human populations – may degrade chitin, as indicated by the identification of gene expression of chitinase in young pigs (Kawasaki et al., 2021).

Both broilers and laying hens fed with insect-based diets had reduced CP digestibility compared to soybeans (Bovera et al., 2016, 2018; Cutrignelli et al., 2018). Bovera et al. (2018) reported that CP digestibility reduced linearly with increasing levels of insect meal in laying hens. Cutrignelli et al. (2018) also observed that laying hens fed 17% defatted *H. illucens* had lower AID of CP than hens fed diets containing soybeans. Similar to pig studies, in broilers, the *H. illucens* had lower ATTD than the *T. molitor*. However, van der Heide et al. (2021) reported higher N retention for *H. illucens* compared to mealworms owing to their high content of Sulphur amino acids and lysine and high intake of N.

Protein quality of insects in human diets

The quality of insect protein in human diets must be considered in view of the method on which the evaluation is based. The studies available on determining the protein quality of insects for humans are outlined in Table 4. Oibiokpa et al. (2018) used a rat model for determining the protein quality of four species (*Macrotermes nigeriensis*, *Gryllus assimilis*, *Melanoplus foedus*, *Cirina forda*) reporting PER, NPR, NPU, BV, and PDCAAS. *Gryllus assimilis* (field cricket) had the highest protein quality among the four species, as reflected in the various protein quality indices. The protein quality of two species, *T. molitor* and *Acheta domesticus* (house cricket) was determined by Poelaert et al. (2018), and Jensen et al. (2019) assessed *T. molitor* and *A. diaperinus* also using the rat model. The PDCAAS values reported by Poelaert et al. (2018) and Jensen et al. (2019) are higher than the DIAAS values reported by Malla et al. (2022) for *T. molitor*, *A. diaperinus*, *A. domesticus*, conforming that the PDCAAS values likely to overestimate the protein quality.

Additionally, PDCAAS of pre-pupae and pupae of *Holotrichia parallela* (Chafer beetle) (Yang et al., 2014) was reported based on in-vitro digestibility of these insects. For the calculation of PDCAAS, static in-vitro measures were used as a surrogate for in-vivo total tract digestibility, which, however, lacks validation for generated digestibility coefficients for PDCAAS. True fecal total tract N digestibility for ileal digestibility of protein and free AA is currently the validated in-vitro method used for PDCAAS. The recent in-vitro method has been validated (Egger et al., 2017). However, this method is yet to be used to determine insects' protein quality based on DIAAS.

For studies in humans, Hermans et al. (2021) compare the impacts of ingesting *A. diaperinus* and milk-derived protein on protein and aa metabolism and muscle protein synthesis rates in young men. For this, they used intrinsically ^{13}C-labelled AA (phenylalanine and leucine) in *A. diaperinus* or milk-derived protein. During the five h postprandial period, there were no differences in the amount of *A. diaperinus* and milk protein–derived AA released into the circulation and muscle protein synthesis between groups receiving *A. diaperinus* and milk protein-derived phenylalanine. Further studies are needed to confirm this indication that insects can provide protein of similar quality to milk for human consumption.

Protein quality of insects in relation to nitrogen-protein (NP) conversion factor

The standard measure for CP content in feed and food is based on total N and the NP conversion factor of 6.25. This standardized measure was recommended for calculating DIAAS as described in the FAO protocol (FAO, 2013). While it is well described that the true NP conversion factor varies with the variation in the AA composition, for insects, the conversion is influenced by the chitin exoskeleton, a polymer of N-acetyl-glucosamine. This non-protein N contributed from chitin will overestimate the protein content when applying the standard NP conversion factor. Several studies have suggested modified NP conversion for insects, adjusting for the chitin-N (Janssen et al., 2017; Boulos et al., 2020). Boulos et al. (2020) established insect-specific NPs in three insect species and suggested 5.33 as a standard NP for insects. For the protein quality methods for which the quantification of AAs is already a demand, such as the DIAAS method, total AAs is considered to be a more relevant measure for total protein to overcome the overestimation of the protein content, hence underestimation of protein quality using NP of 6.25 (Malla et al., 2022). When using an insect-specific NP of 5.33 instead of 6.25, the DIAAS listed in Table 4 proportionally increased by 17%; for example, *T. molitor* scored 75 compared to 64, *A. domesticus* would score to be 103 rather than 89, and *Gryllodes sigillatus* scored 107 from 92. The intention of applying standardized assessment methods of protein quality, including a standardized NP factor, is to establish comparable measures across protein sources. However, for insects, the nature of the non-protein N contributed from the chitin exoskeleton should be considered in the application of methods for true quality evaluation.

Conclusion

To establish insects in the food systems as a protein source for animal feed and for direct human consumption, systematic, and comparable assessment of the protein quality is necessary. There are several methods for determining protein digestibility and quality to meet the requirements of animals and humans. These methods have advantages and limitations. From a scientific perspective, ileal digestibility measures expressed as TID and SID for animals, and DIAAS for humans, are considered the most accurate representation of protein quality. However, other methods could be sufficient to rank insects against other sources, such as growth studies for the quality of animal feed. Hence, the context under which the method will be utilized and available resources determine the choice.

The protein quality of insects must be considered in view of the applied methods. Also, the generalized NP of 6.25 for calculating CP needs reconsideration, accounting for non-protein N of, especially chitin. Across animal studies, *T. molitor* has higher protein digestibility and supports well animal growth performance and hence, is suitable to be used in animal feed. Across studies evaluating the protein quality of insects for human consumption, different species of cricket (*A. domesticus*, *G. assimilis*, and *G. sigillatus*) tend to have slightly better protein quality compared to mealworm species (*T. molitor* and *A. diaperinus*), though needs more data to conclude, and these species are all candidates as good alternative protein sources for human consumption.

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Fact box 1. Protein Quality for feed and food.

While animals and humans share the physiological need to rely on a dietary supply of indispensable AA (IAA), the protein quality of a protein source is more commonly used to describe the quality of human food than the quality of animal feed. In evaluating the nutritional adequacy of feed for monogastric livestock, the IAA concentrations, the IAA ileal digestibility, and the endogenous IAA losses of the protein sources are taken into account when formulating composite diets fulfilling the minimum nutritional requirements (Stein et al., 2007). In contrast, for humans, it is, in most cases, impossible and not desirable for healthy individuals to control dietary habits. Therefore, the concept of protein quality in single foods is used to facilitate guidance on protein sources in diverse diets based on consumers' preferences. Because of the wider diversity of foods that humans consume, the Food and Agriculture Organization (FAO) has facilitated the recommendation of specific standards based on scientific criteria for evaluating comparable measures for protein quality.

Literature Cited

Ao, X., and I.H. Kim. 2019. Effects of dietary dried mealworm (*Ptecticus tenebrifer*) larvae on growth performance and nutrient digestibility in weaning pigs. Livest. Sci. 230. doi:10.1016/j.livsci.2019.09.031.

Ao, X., J.S. Yoo, Z.L. Wu, and I.H. Kim. 2020. Can dried mealworm (*Tenebrio molitor*) larvae replace fish meal in weaned pigs? Livest. Sci. 239. doi:10.1016/j.livsci.2020.104103.

Biasato, I., M. Renna, F. Gai, S. Dabbou, M. Meneguz, G. Perona, S. Martinez, A.C.B. Lajusticia, S. Bergagna, L. Sardi, et al. 2019. Partially defatted black soldier fly larva meal inclusion in piglet diets: effects on the

growth performance, nutrient digestibility, blood profile, gut morphology and histological features. J. Anim. Sci. Biotechnol. 10:1–11. doi:10.1186/s40104-019-0325-x.

Boulos, S., A. Tännler, and L. Nyström. 2020. Nitrogen-to-protein conversion factors for edible insects on the Swiss market: *T. molitor*, *A. domesticus*, and *L. migratoria*. Front. Nutr. 7:1–12. doi:10.3389/fnut.2020.00089.

Bovera, F., R. Loponte, S. Marono, G. Piccolo, G. Parisi, V. Iaconisi, L. Gasco, and A. Nizza. 2016. Use of *Tenebrio molitor* larvae meal as protein source in broiler diet: Effect on growth performance, nutrient digestibility, and carcass and meat traits. J. Anim. Sci. 94(2):639–647. doi:10.2527/jas.2015-9201.

Bovera, F., R. Loponte, M.E. Pero, M.I. Cutrignelli, S. Calabrò, N. Musco, G. Vassalotti, V. Panettieri, P. Lombardi, G. Piccolo, et al. 2018. Laying performance, blood profiles, nutrient digestibility and inner organs traits of hens fed an insect meal from *Hermetia illucens* larvae. Res. Vet. Sci. 120:86–93. doi:10.1016/j.rvsc.2018.09.006.

Cho, K.H., S.W. Kang, J.S. Yoo, D.K. Song, Y.H. Chung, G.T. Kwon, and Y.Y. Kim. 2020. Effects of mealworm (*Tenebrio molitor*) larvae hydrolysate on nutrient ileal digestibility in growing pigs compared to those of defatted mealworm larvae meal, fermented poultry by-product, and hydrolyzed fish soluble. Asian-Australas. J. Anim. Sci. 33(3):490–500. doi:10.5713/ajas.19.0793.

Cho, K.H., V. Sampath, A.J. Kim, J.S. Yoo, and I.H. Kim. 2022. Evaluation of full-fatted and hydrolysate mealworm (*Tenebrio molitor*) larvae as a substitute for spray-dried plasma protein diet in weaning pigs. J. Anim. Physiol. Anim. Nutr. (Berl). 107(2):589–597. doi:10.1111/jpn.13763.

Churchward-Venne, T.A., P.J.M.M. Pinckaers, J.J.A.A. van Loon, and L.J.C.C. van Loon. 2017. Consideration of insects as a source of dietary protein for human consumption. Nutr. Rev. 75(12):1035–1045. doi:10.1093/nutrit/nux057.

Cutrignelli, M.I., M. Messina, F. Tulli, B. Randazzi, I. Olivotto, L. Gasco, R. Loponte, and F. Bovera. 2018. Evaluation of an insect meal of the black soldier fly (*Hermetia illucens*) as soybean substitute: intestinal morphometry, enzymatic and microbial activity in laying hens. Res. Vet. Sci. 117:209–215. doi:10.1016/j.rvsc.2017.12.020.

Deglaire, A., and P.J. Moughan. 2012. Animal models for determining amino acid digestibility in humans - a review. Br. J. Nutr. 108(2):S273–S281. doi:10.1017/S0007114512002346.

Devi, S., A. Varkey, M.S. Sheshshayee, T. Preston, and A.V. Kurpad. 2018. Measurement of protein digestibility in humans by a dual-tracer method. Am. J. Clin. Nutr. 107(6):984–991. doi:10.1093/ajcn/nqy062.

de Marco, M., Martínez, S., Hernandez, F., Madrid, J., Gai, F., Rotolo, L., Belforti, M., Bergero, D., Katz, H., Dabbou, S., Kovitvadhi, A., Zoccarato, I., Gasco, L., & Schiavone, A., 2015. 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 metabolizable energy. Anim Feed Sci Technol. 209:211–218. doi:10.1016/j.anifeedsci.2015.08.006.

Egger, L., P. Schlegel, C. Baumann, H. Stoffers, D. Guggisberg, C. Brügger, D. Dürr, P. Stoll, G. Vergères, and R. Portmann. 2017. Physiological comparability of the harmonized INFOGEST in vitro digestion method to in vivo pig digestion. Food Res. Int. 102:567–574. doi:10.1016/j.foodres.2017.09.047.

FAO/WHO/UNU. 1985. Energy and protein requirements. Report of a joint FAO/WHO/UNU Expert Consultation. WHO Tech Rep Ser. 724. Rome: Food and Agriculture Organization of the United Nations.

FAO/WHO/UNU. 1991. Protein quality evaluation. Report of a Joint FAO/WHO Expert Consultation. FAO food and nutrition paper 51. Rome: Food and Agriculture Organization of the United Nations.

Food and Agriculture Organization of the United Nations (FAO). 2013. Dietary protein quality evaluation in human nutrition. Report of an FAO expert group. FAO food and nutrition paper 92. Rome: Food and Agriculture Organization of the United Nations.

Food and Agriculture Organization of the United Nations (FAO). 2014. Research approaches and methods for evaluating the protein quality of human foods. Report of an FAO Expert Working Group. Rome: Food and Agriculture Organization of the United Nations.

Hendriks, W.H., J. van Baal, and G. Bosch. 2012. Ileal and faecal protein digestibility measurement in humans and other non-ruminants - A comparative species view. Br. J. Nutr. 108(2):S247–S257. doi:10.1017/S0007114512002395.

Hermans, W.J.H., J.M. Senden, T.A. Churchward-Venne, K.J.M. Paulussen, C.J. Fuchs, J.S.J. Smeets, J.J.A. van Loon, L.B. Verdijk, and L.J.C. van Loon. 2021. Insects are a viable protein source for human consumption: from insect protein digestion to postprandial muscle protein synthesis in vivo in humans: a double-blind randomized trial. Am. J. Clin. Nutr. 114(3):934–944. doi:10.1093/ajcn/nqab115.

Hoffman, J.R., and M.J. Falvo. 2004. Protein - Which is best? J. Sports Sci. Med. 3(3):118–130.

Janssen, R.H., J.P. Vincken, N.J.G. Arts, V. Fogliano, and C.M.M. Lakemond. 2019. Effect of endogenous phenoloxidase on protein solubility and digestibility after processing of Tenebrio molitor, Alphitobius diaperinus and Hermetia illucens. Food Res. Int. 121:684–690. doi:10.1016/j.foodres.2018.12.038.

Jensen, L.D., R. Miklos, T.K. Dalsgaard, L.H. Heckmann, and J. Nørgaard. 2019. Nutritional evaluation of common (Tenebrio molitor) and lesser (Alphitobius diaperinus) mealworms in rats and processing effect on the lesser mealworm. J. Insects Food Feed. 5(4):257–266. doi:10.3920/JIFF2018.0048.

Ji, Y.J., H.N. Liu, X.F. Kong, F. Blachier, M.M. Geng, Y.Y. Liu, and Y.L. Yin. 2016. Use of insect powder as a source of dietary protein in early-weaned piglets. J. Anim. Sci. 94:111–116. doi:10.2527/jas2015-9555.

Jin, X.H., P.S. Heo, J.S. Hong, N.J. Kim, and Y.Y. Kim. 2016. Supplementation of dried mealworm (*Tenebrio molitor* larva) on growth performance and nutrient digestibility and blood profiles in weaning pigs. Asian-Australas. J. Anim. Sci. 29(7):979–986. doi:10.5713/ajas.15.0535.

Jonas-Levi, A., and J.J.I. Martinez. 2017. The high level of protein content reported in insects for food and feed is overestimated. J. Food Compos. Anal. 62:184–188. doi:10.1016/j.jfca.2017.06.004.

Kawasaki, K., T. Osafune, S. Tamehira, and K. Yano. 2021. Piglets can secrete acidic mammalian chitinase from the pre weaning stage. Sci. Rep. 11:1–6. doi:10.1038/s41598-020-80368-0.

Longvah, T., K. Mangthya, and P. Ramulu. 2011. Nutrient composition and protein quality evaluation of eri silkworm (Samia ricinii) prepupae and pupae. Food Chem. 128(2):400–403. doi:10.1016/j.foodchem.2011.03.041.

Malla, N., J. V. Nørgaard, H. N. Lærke, L. H. L. Heckmann, and N. Roos. 2022. Some insect species are good-quality protein sources for children and adults: digestible indispensable amino acid score (DIAAS) determined in growing pigs. J Nutr. 152(4):1042–1051. doi:10.1093/jn/nxac019.

Oibiokpa, F.I., H.O. Akanya, A.A. Jigam, A.N. Saidu, and E.C. Egwim. 2018. Protein quality of four indigenous edible insect species in Nigeria. Food Sci. Hum. Wellness. 7(2):175–183. doi:10.1016/j.fshw.2018.05.003.

Osborne, T.B., L.B. Mendel, and E.L. Ferry. 1919. A method of expressing numerically the growth-promoting value of proteins. J. Biol. Chem. 37(2):223–229. doi:10.1016/s0021-9258(18)86353-0.

Poelaert, C., F. Francis, T. Alabi, R. Caparros Megido, B. Crahay, J. Bindelle, and Y. Beckers. 2018. Protein value of two insects, subjected to various heat treatments, using growing rats and the protein digestibility-corrected amino acid score. J. Insects Food Feed. 4(2):77–87. doi:10.3920/JIFF2017.0003.

Rodríguez-Rodríguez, M., F.G. Barroso, D. Fabrikov, and M.J. Sánchez-Muros. 2022. In vitro crude protein digestibility of insects: a review. Insects. 13(8):6821–6819. doi:10.3390/insects13080682.

Shivakumar, N., S. Kashyap, S. Kishore, T. Thomas, A. Varkey, S. Devi, T. Preston, F. Jahoor, M.S. Sheshshayee, and A.V. Kurpad. 2019. Protein-quality evaluation of complementary foods in Indian children. Am. J. Clin. Nutr. 109(5):1319–1327. doi:10.1093/ajcn/nqy265.

Spranghers, T., J. Michiels, J. Vrancx, A. Ovyn, M. Eeckhout, P. de Clercq, and S. de Smet. 2018. Gut antimicrobial effects and nutritional value of black soldier fly (*Hermetia illucens* L.) prepupae for weaned piglets. Anim. Feed Sci. Technol. 235:33–42. doi:10.1016/j.anifeedsci.2017.08.012.

Stein, H.H., B. Sève, M.F. Fuller, P.J. Moughan, and C.F.M. de Lange; Committee on Terminology to Report AA Bioavailability and Digestibility. 2007. Invited review: amino acid bioavailability and digestibility in pig feed ingredients: terminology and application. J. Anim. Sci. 85:172–180. doi:10.2527/jas.2005-742.

Tan, X., H.S. Yang, M. Wang, Z.F. Yi, F.J. Ji, J.Z. Li, and Y.L. Yin. 2020. Amino acid digestibility in housefly and black soldier fly prepupae by growing pigs. Anim. Feed Sci. Technol. 263:1–7. doi:10.1016/j.anifeedsci.2020.114446.

van der Heide, M., J.V. Nørgaard, and R.M. Engberg. 2021. Performance, nutrient digestibility and selected gut health parameters of broilers fed with black soldier fly, lesser mealworm and yellow mealworm. J. Insects Food Feed. 7(6):1011–1022. doi:10.3920/JIFF2020.0150.

van der Wielen, N., P.J. Moughan, and M. Mensink. 2017. Amino acid absorption in the large intestine of humans and porcine models. J. Nutr. 147(8):1493–1498. doi:10.3945/jn.117.248187.

van Huis, A., B. Rumpold, C. Maya, and N. Roos. 2021. Nutritional qualities and enhancement of edible insects. Annu. Rev. Nutr. 41:551–576. doi:10.1146/annurev-nutr-041520-010856.

WHO/FAO/UNU. 2007. Protein and amino acid requirements in human nutrition. Report of a joint FAO/WHO/UNU expert consultation. WHO technical reports series no. 935. Geneva (Switzerland): WHO.

Yang, Q., S. Liu, J. Sun, L. Yu, C. Zhang, J. Bi, and Z. Yang. 2014. Nutritional composition and protein quality of the edible beetle Holotrichia parallela. J. Insect Sci. 14(1):1–4. doi:10.1093/jisesa/ieu001.

Yoo, J.S., K.H. Cho, J.S. Hong, H.S. Jang, Y.H. Chung, G.T. Kwon, D.G. Shin, and Y.Y. Kim. 2019. Nutrient ileal digestibility evaluation of dried mealworm (Tenebrio molitor) larvae compared to three animal protein by-products in growing pigs. Asian-Australas. J. Anim. Sci. 32(3):387–394. doi:10.5713/ajas.18.0647.

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