

## DIGESTIBILITY OF INSECT PROTEINS AND INSECT-BASED PRODUCTS

*This document is part of our literature review on nutritional quality, processing, functionality and shelf life and storage of insect-based products, and the associated analytical methods.*

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## 1. Digestibility and protein quality

Digestibility is an indication of the percentage of food absorbed by the gut into the body. It is the amount of food components (e.g. amino acids) absorbed and is generally calculated as the amount of a component consumed (IN) minus the amount of a component retained in the feces (OUT). In 1981, protein quality described by its amino acid score was linked to protein digestibility to reflect digestion and absorption differences between proteins (Blackburn and Southgate 1981). Digestibility had been then calculated by measuring nitrogen intake and fecal nitrogen output as:

Apparent Protein (N) Digestibility (%) =  $(I - F) / I \times 100$ , and **Equation 1**

True Protein (N) Digestibility (%) =  $(I - (F - F_k) / I) \times 100$ , Equation 2,

Where I = Nitrogen Intake, F = Fecal Nitrogen output on test diet, and F<sub>k</sub> = Fecal Nitrogen output on a non-protein diet.

As a result, the *Protein Digestibility–Corrected Amino Acid Score (PDCAAS)* had been adopted by the Food and Agricultural Organisation and World Health Organisation (FAO/WHO) in 1991 (FAO 2013) as the preferred method for the measurement of the protein value in human nutrition (Schaafsma 2000):

PDCAAS(%) = (mg of limiting amino acid in 1 g of test protein / mg of same amino acid in 1 g of reference protein) × fecal true digestibility (%) × 100, Equation 3

To address the limitations of this method, which are apparent after over 20 years of use, the FAO/WHO recommended replacing PDCAAS by the *Digestible Indispensable Amino Acid Score (DIAAS)* in 2013. Highlighting the main difference, the PDCAAS method estimates protein digestibility over the total gut (referred to as ‘faecal’ protein or digestibility), including microbial activities in the hindgut, whereas DIAAS method determines amino acid digestibility at the end of the small intestine (mouth to terminal ileum; referred to as ‘ileal’ digestibility). A good introduction showing results for the two methods applied to different protein sources has been contrasted elsewhere (Mathai, Liu et al. 2017). The PDCAAS method can be used on human subjects, but to estimate protein quality, which is meeting the requirements of essential amino acids, as well as the physiological needs, and digestibility for human consumption; rodent or porcine models are recommended. Due to the requirement of measuring amino acid concentrations at the end of the ileum, the DIAAS method is only performed in rats or preferably pigs with the latter having a permanent ileostomy to facilitate collection of *digesta* of individuals in repeated trials. This approach has also been used to measure protein digestibility in human adults and children, where possible, using the indicator amino acid oxidation (IAAO) method (Shivakumar, Jackson et al. 2020).

Although the *in vivo* DIAAS method is now considered the most reliable source for protein digestibility, the main disadvantages are the time and cost associated with conducting animal

based studies, as well as the associated ethical considerations. After two decades of using the PDCAAS method, this has also led to the development of *in vitro* methods simulating digestion processes include the oral, gastric, small intestinal phases, large intestinal fermentation as occasionally realized in artificial rumen, stomach, or gut. This allows the analysis of the products of all sequential processes, or investigations of different phases of digestion, rumen/gut microbial communities and their underlying processes.

The main *in vitro* method referred to in the literature is based on a multienzyme system consisting of trypsin, chymotrypsin and peptidase (Hsu, Vavak et al. 1977). One commercial test patented by Medaillon Labs and distributed by Megazyme is the Animal-Safe Accurate Protein Quality Score (ASAP-Quality Score Method) for determination of the PDCAAS. There is, however, no indication that this kit has been used in primary published literature (PubMed, Scholar) for investigating protein quality. There are various further developments of the widely used Hsu 3-enzyme and pH-drop method as summarized by Butts, Monro et al. (2012) or Qiao, Lin et al. 2004. Developing dynamic and static models of human digestion have led to the COST action INFOGEST an international consortium joined by more than 200 scientists from 32 countries working in the field of digestion (Dupont, Bordonni et al. 2011).

Despite international efforts to identify the best method for defining protein digestibility (*i.e. in vivo* DIAAS, *in-vitro* digestion models) plasma amino acid levels can yield insight into the digestibility of protein products after supplementation (Vangsoe et al., 2018). It could be shown that whey, soy, and insect protein isolate increased the blood concentration of essential amino acids, branched-chain amino acids, and leucine over 120 min period for all protein supplements with whey greater than insect and soy. However, blood amino acid concentrations were highest for insect protein supplementation at 120 min indicating a slower digestion. The same research group also intends to investigate in the inVALUABLE consortium insect proteins and their digestibility using the DIAAS method in pigs to obtain reliable data for correlating *in vitro* and *in vivo* measurements (Heckmann, Andersen et al. 2019).

## 2. Insect protein products and its digestibility

A partial Google Scholar literature search of 'digestibility of insects' combined with a 'digestibility AND insect' search (Title/Abstracts) in PubMed resulted in 152 articles related to the subject (see annex in this chapter). Based on the titles, 61 articles describe the effects of general and specific insect products in animal trials compared to usually used feeds evaluating the protein quality based on the results (1-61). A further 73 articles are related to either how insects respond to different feeds (rearing and growth performance of insects), processing of insects, and related product qualities (62-134). The remaining 18 articles explore general aspects or rearing and evaluating insect foods for human consumption (135-152). In the next table, is summarized some of the studies conducted to assess protein digestibility in several species. As it can be seen, the most of the species give values ranging from 66 to 95% of digestibility; however, the methods of determining digestibility are varied and need standardising. In addition to this, no one of the experiment was done in humans, and the results might be slightly different.

**Table 1**  
**Digestibility of several edible insect species. Based on Churchward-Venne, Pinckaers et al. (2017).**

Edible insects	Digestibility/Comments (%)	Reference
<b>Coleoptera (beetles, grubs)</b>		
<i>Holotrichia parallela</i>	78.4	Assessed in-vitro (Yang, Liu et al. 2014)
<i>Tenebrio molitor</i> (mixed meal containing 1.5% larvae)	90.3	Assessed in pigs; apparent digestibility (Jin et al. 2016)
<i>Tenebrio molitor</i> (mixed meal containing 3.0% larvae)	91.3	Assessed in pigs; apparent digestibility (Jin et al. 2016)
<i>Tenebrio molitor</i> (mixed meal containing 4.5% larvae)	92.2	Assessed in pigs; apparent digestibility (Jin et al. 2016)
<i>Tenebrio molitor</i> (mixed meal containing 6.0% larvae)	93.0	Assessed in pigs; apparent digestibility (Jin et al. 2016)
<i>Tenebrio molitor</i>	86.0	Assessed in chickens; apparent digestibility (De Marco, Martínez et al. 2015)
<i>Tenebrio molitor</i>	91.3	Assessed in-vitro (Bosch, Zhang et al. 2014)
<i>Tenebrio molitor</i>	66.3	Assessed in-vitro (Marono, Piccolo et al. 2015)
<i>Tenebrio molitor</i>	66.7	Assessed in-vitro (Marono, Piccolo et al. 2015)
<i>Tenebrio molitor</i>	65.5	Assessed in-vitro (Marono, Piccolo et al. 2015)
<i>Tenebrio molitor</i>	66.2	Assessed in-vitro (Marono, Piccolo et al. 2015)
<i>Tenebrio molitor</i>	65.8	Assessed in-vitro (Marono, Piccolo et al. 2015)
<i>Tenebrio molitor</i>	66.2	Assessed in-vitro (Marono, Piccolo et al. 2015)
<i>Zophobas morio</i>	92.0	Assessed in-vitro (Bosch, Zhang et al. 2014)
<b>Diptera (flies)</b>		
<i>Hermetia illucens</i>	67.1	Assessed in-vitro (Marono, Piccolo et al. 2015)
<i>Hermetia illucens</i>	68.0	Assessed in chickens; apparent digestibility (De Marco, Martínez et al. 2015)
<i>Hermetia illucens</i>	67.3	Assessed in-vitro (Marono, Piccolo et al. 2015)
<i>Hermetia illucens</i>	67.6	Assessed in-vitro (Marono, Piccolo et al. 2015)
<i>Hermetia illucens</i>	68.7	Assessed in-vitro (Marono, Piccolo et al. 2015)
<i>Hermetia illucens</i>	66.8	Assessed in-vitro (Marono, Piccolo et al. 2015)
<i>Hermetia illucens</i>	66.0	Assessed in-vitro (Marono, Piccolo et al. 2015)
<i>Hermetia illucens</i> (larvae)	89.7	Assessed in-vitro (Bosch, Zhang et al. 2014)
<i>Hermetia illucens</i> (pupae)	77.7	Assessed in-vitro (Bosch, Zhang et al. 2014)
<i>Musca domestica</i> (30% of mixed meal)	98.5	Assessed in chickens; apparent digestibility (Hwangbo et al. 2009)
<i>Musca domestica</i> (pupae)	84.3	Assessed in-vitro (Bosch, Zhang et al. 2014)
<b>Hemiptera (true bugs)</b>		
Ahuahutle	89.3	Assessed in-vitro (Ramos-Elorduy, Moreno et al. 1997)
Axayacatl	98.0	Assessed in-vitro (Ramos-Elorduy, Moreno et al. 1997)
<b>Hymenoptera (ants, bees)</b>		
<i>Apis mellifera</i> (18.8% of mixed meal; whole dried)	62.0	Assessed in rats; apparent digestibility, not corrected for N in chitin (OZIMEK, SAUER et al. 1985)
<i>Apis mellifera</i> (18.8% of mixed meal; whole dried)	68.5	Assessed in rats; apparent digestibility, corrected for N in chitin (OZIMEK, SAUER et al. 1985)
<i>Apis mellifera</i> (18.8% of mixed meal; whole dried)	71.5	Assessed in rats; true digestibility, not corrected for N in chitin (OZIMEK, SAUER et al. 1985)
<i>Apis mellifera</i> (18.8% of mixed meal; whole dried)	79.8	Assessed in rats; true digestibility, corrected for N in chitin (OZIMEK, SAUER et al. 1985)
<i>Apis mellifera</i> (16.6% of mixed meal; protein concentrate)	87.6	Assessed in rats; apparent digestibility (OZIMEK, SAUER et al. 1985)
<i>Apis mellifera</i> (16.6% of mixed meal; protein concentrate)	94.3	Assessed in rats; true digestibility (OZIMEK, SAUER et al. 1985)
<i>Atta mexicana</i> (ants)	87.6	Assessed in-vitro (Ramos-Elorduy, Moreno et al. 1997)
<i>Brachygastra mellifica</i>	85.2	Assessed in-vitro (Ramos-Elorduy, Moreno et al. 1997)
<i>Polybia parvulina</i>	86.4	Assessed in-vitro (Ramos-Elorduy, Moreno et al. 1997)
<i>Vespula squamosa</i>	76.6	Assessed in-vitro (Ramos-Elorduy, Moreno et al. 1997)
<b>Orthiptera (crickets, hoppers, termites)</b>		
<i>Cricket (Gryllodes sigillatus)</i>	76.2	Assessed in-vitro (Stone, Tanaka et al. 2019)
<i>Termite (Macrotermes subhylanus)</i>	90.1	Assessed in-vitro (Kinyuru et al. 2009)
<i>Green grasshopper (Ruspolia differens)</i>	79.6	Assessed in-vitro (Kinyuru et al. 2009)
<i>Locust (Anacridium melanorhodon)</i>	50	Assessed in-vitro (Nafisa et al. 2008)
<i>Brown grasshopper (Ruspolia differens)</i>	81.1	Assessed in-vitro (Kinyuru et al. 2009)

The need to find alternative feeds for animal farming to cope with an increasing global meat demand is a main driver to test insect proteins in poultry (mainly quail, chicken, potentially turkey), pigs and piglets for pork production, for dog and cat food, and even as a protein source in fish farms. Findings so far show that poultry, pork, and fish products after insect diets are at either equal or higher quality compared to those following standard feeds (32; 23; 36; 49).

There is additional research necessary to investigate the use and effects of waste streams on insect products (3). It was concluded that price of insect production and processing were not competitive (in 2015) to compete with conventional protein sources to sustain an increasing demand for protein.

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## 4. Annex: Literature research

“A partial Google Scholar literature search of ‘digestibility of insects’ combined with a ‘digestibility AND insect’ search (Title/Abstracts) in PubMed resulted in 152 articles related to the subject”

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