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Commentary

The high level of protein content reported in insects for food and feed is overestimated

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ABSTRACT

The potential of insects as a source of protein for future food and feed is widely admitted in the last couple years and is the object of numerous studies. The Kjeldahl method is widely used to quantify the crude protein content of insects which ranges from 8 to 70% of dry mass. This procedure evaluates the total concentration of Nitrogen (N), which is converted to protein by multiplying it by the nitrogen-to-protein conversion factor (N-factor) for meat (6.25). Giving that the insect cuticle contents large amounts of fibrous chitin, a polysaccharide rich in N, and proteins tightly embedded in its matrix, and is not digested by humans or domesticated animals, using the Kjeldahl method overestimates the digestible protein content of insects. We propose to evaluate digetible nitrogen by quantifying N in the cuticle and sustraiting it from the total nitrogen content, and to calculate a new N-conversion factor which should be similar for all the insects species and their development stages.

Insects are a promising, healthy and sustainable source of high-quality proteins (van Huis, 2013; Williams et al., 2016). They have been widely consumed throughout human history (McGrew, 2014). Entomophagy is still practised frequently in more than 90 developing countries (Defoliart, 1995), for a total of 1900 (van Huis, 2013) to 2163 (Jongema, 2012) edible insect species included in different orders, essentially beetles (Coleoptera), caterpillars (Lepidoptera) and bees/waps/ants (Hymenoptera), followed by grasshoppers and locusts (Orthoptera), termites (Isoptera) and other orders (van Huis et al., 2013). Following tradition and culture, and palatability of the species, people consumes young stages (larvae or nymphs), pupae or adults, or all stages of development of these insects. Concerned by the growing global human population and the expected increasing in protein demand for food and feed, the Food and Agriculture Organization of the United Nations (FAO) placed recently food production from insects on the global agenda (FAO, 2009). Insects have several advantages, including a higher feed conversion ratio (FCR) than common animal-based protein sources; considerably lower requirements for water, energy and land for production and lower ammonia emissions (Oonincx et al., 2010; van Huis et al., 2013), although more research is required to valid these advantages at an economically relevant production scale (Lundy and Parrella, 2015).

The rising interest in insects as a protein resource for humans and animals is reflected in the flourishing scientific literature. Protein levels as high as 13–77% of dry biomass have been reported in different insect types (Kupferschmidt, 2015; Sànchez-Muros et al., 2014; van Huis et al., 2013) or between 21 and 80% (Williams et al., 2016) (See Table 1 for examples). Clearly the accuracy of the quantification of protein in insects is central to defining their nutritional benefit.

The majority of research studies on insects as resource for human food or animal feed use the Kjeldahl standard protocol (FAO, 2003), for example Lundy and Parrella, (2015), Surendra et al. (2016) or Zielinska et al. (2015), while some others adopt the derived Dumas technique (e.g., Yi et al., 2013), following the protocols described in AOAC for analysis of protein in food (Latimer, 2016). The Kjeldahl method converts nitrogen (N) containing compounds (e.g., proteins, nucleic acids, amines, organic compounds) into ammonia, which is quantified carrying a three steps procedure: digestion of the sample in sulfuric acid, distillation with excess of base to convert ammonium sulfate to volatile ammonia which is steam-distilled into a solution of boric acid and then titration of the ammonium borate with chloric acid (Bruun Jensen et al., 2016). After quantification nitrogen N is converted to protein by multiplying it by a food-specific nitrogen-to-protein conversion factor (N-factor), 6.25 for meat for example (Merrill and Watt, 1973). In the Dumas method nitrogen is converted to N_2 by combustion, and the gas is detected by a thermal conductivity detector. Both methods were compared and discussed by Muller (2014). Both techniques have the advantage of being considered standard methods, allowing comparisons

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Table 1
Examples of studied insects for food and feed, and the methods used for protein and chitin quantifications. N-f-N to protein conversion factor; *N to protein conversion factor not stated; CP – crud protein, *N*-analysis method not declared; AA analysis – amino acid analysis by AA analyzer of by HPLC; - trp – tryptophan not tested in amino acid analyzer; ‡ fiber analysis method not declared, analyzed according to AOAC 1975; CF – crud fibers, double hot hydrolysis by 1.25% sulfuric acid following 1.25% sodium hydroxide, gravimetric weighing; Total carb. – total carbohydrates hydrolysis, HPLC; ADF – acid detergent fibers, hot hydrolysis by sulfuric acid or NIR reflectance spectroscopy; TDF – total dietary fiber calculated using enzymatic hydrolysis (EH): defat, amylase, protease, amiloglucosidase, alcohol precipitation, residual ash and N analysis. TDF = weight (residue of EH)-ash-*N* × 6.25; *N*-glucosamine – total chitin, 72% (w/w) H_2SO_4 1 h at 30 °C, subsequently 1M H_2SO_4 3 h at 100 °C and HPLC analysis; Δ – calculated from fresh weight.

Scientific name	Common name	Developmental stage	protein analysis method (N-f)	Protein, % (DW)	Dietary fiber analysis method	Dietary fiber, % (DW)	Reference citation
Acheta domestica	Cricket	adults	CP (6.25)	15.6			Payne et al. (2016)
Acheta domestica	Cricket	adults	Dumas (6.25)	46.8–68.5	aaaaaaaaaaaaaaaaaaa		Caparros et al. (2016)
Acheta domestica	Cricket	nymphs	CP (6.25)	17.79 Δ	TDF	1.18 Δ	Finke (2015)
Acheta domestica	Cricket	nymphs	CP (6.25)	17.79 Δ	ADF	1.92 Δ	Finke (2015)
Acheta domestica	Cricket	adults	Dumas (6.25)	73.6 Δ			Yi et al. (2013)
Allomyrina dichotoma	Japanese rhinoceros beetle	larvae	Kjeldahl (6.25)	54.18	TDF	4.03	Ghosh et al. (2017)
Alphitobius diaperinus	Lesser mealworm	larvae	Dumas (6.25)	58.0 Δ			Yi et al. (2013)
Alphitobius diaperinus	Lesser mealworm	larvae	AA analysis	49.58	*N*-glucosamine	4.4–9.1	Janssen et al. (2017)
Alphitobius diaperinus	Lesser mealworm	larvae	Dumas (4.86)	48.60	*N*-glucosamine	4.4–9.1	Janssen et al. (2017)
Alphitobius diaperinus	Lesser mealworm	larvae	Kjeldahl (6.25)	60.0			Adámková et al. (2016)
Analeptes trifasciata	Rhinoceros beetle	larvae	Kjeldahl (*)	22.3 Δ	CF	3.66 Δ	Banjo et al. (2006)
Analeptes trifasciata	Rhinoceros beetle	larvae	Kjeldahl (*)	30.28 Δ	CF	2.00 Δ	Banjo et al. (2006)
Anaphe infracta	African silkworm	larvae	Kjeldahl (*)	22.12 Δ	CF	2.66 Δ	Banjo et al. (2006)
Anaphe recticulata	African silkworm	larvae	Kjeldahl (*)	25.87 Δ	CF	3.47 Δ	Banjo et al. (2006)
Anaphe spp.	African silkworm	larvae	Kjeldahl (*)	20.42 Δ	CF	1.82 Δ	Banjo et al. (2006)
Anaphe venata	African silkworm	larvae	Kjeldahl (*)	28.40 Δ	CF	2.54 Δ	Banjo et al. (2006)
Apis mellifera	European honey bee	90% pupae, 10% larvae	CP (6.25)	23.4Δ	ADF	12.9 Δ	Finke (2005)
Apis mellifera	European honey bee	bee brood	CP (6.25)	15.2			Payne et al. (2016)
Apis mellifera	European honey bee	bee brood	Kjeldahl (*)	19.54 Δ	TDF	5.45 Δ	Adeyeye and Olaleye (2016)
Apis mellifera	European honey bee	bee brood	Kjeldahl (*)	23.0 Δ	CF	2.19 Δ	Banjo et al. (2006)
Blaptica dubia	Orange-spotted cockroach	adults	Dumas (6.25)	59.2 Δ			Yi et al. (2013)
Bombyx mori	Silkworm	larvae	Kjeldahl (*)	22.89 Δ	TDF	5.23 Δ	Adeyeye and Olaleye (2016)
Bombyx mori	Silkworm	pupae	CP (6.25)	17.9			Payne et al. (2016)
Bombyx mori	Silkworm	pupae	Kjeldahl (*)	21.65 Δ	TDF	5.85 Δ	Adeyeye and Olaleye (2016)
Brachytrupes orientalis	Mole cricket	adults	Kjeldahl (6.25)	65.7	CF	8.75	Chakravorty et al. (2014)
Brachytrypes spp.	Cricket	adults	Kjeldahl (*)	6.47Δ	CF	1.04 Δ	Banjo et al. (2006)
Chondacris rosea	Short-horned grasshopper	adults	Kjeldahl (6.25)	68.9	CF	12.38	Chakravorty et al. (2014)
Cirina forda	Pallid emperor Moth	larvae	Kjeldahl (*)	29.52 Δ	CF	2.63 Δ	Banjo et al. (2006)
Curculionidae	Snout beetle	larvae	Kjeldahl (*)	20.12 Δ	TDF	6.49 Δ	Adeyeye and Olaleye (2016)
Cytacanthacris aeruginosus unicolour	Short horned grasshopper	adults	Kjeldahl (*)	13.3 Δ	CF	1.65 Δ	Banjo et al.(2006)
Galleria mellonela	Waxworms	larvae	CP (6.25)	15.39 Δ	TDF	< 0.80 Δ	Finke (2015)
Galleria mellonela	Waxworms	larvae	CP (6.25)	15.39 Δ	ADF	1.62 Δ	Finke (2015)
Gonimbrasia belina	Mopane caterpillar	larvae	CP (6.25)	35.2			Payne et al. (2016)
Gryllodes sigillatus	Cricket	adult	Kjeldahl (6.25)	70.0	TDF	3.65	Zielinska et al. (2015)
Gryllus bimaculatus	Two-spotted cricket	adults	Kjeldahl (6.25)	58.32	TDF	9.53	Ghosh et al. (2017)
Hermetia illucens	Black soldier fly	larvae	AA analysis	36.00	*N*-glucosamine	4.4–9.1	Janssen et al. (2017)
Hermetia illucens	Black soldier fly	larvae	CP (6.25)	17.5			Payne et al. (2016)
Hermetia illucens	Black soldier fly	larvae	Dumas (4.67)	37.7	*N*-glucosamine	4.4–9.1	Janssen et al. (2017)
Hermetia illucens	Black soldier fly	prepupae	Dumas (6.25)	43.7	CF	10.1	Surendra et al. (2016)
Imbrasia belina	Emperor moth	larvae	Kjeldahl (*)	54-58			Dube et al. (2013)
M. falciger	Termite	alate	Kjeldahl (*)	21.2			Dube et al. (2013)
M. falciger	Termite	wingless	Kjeldahl (*)	41.8			Dube et al. (2013)
Macrotermes bellicosus	War-like Termite	alate, queen	Kjeldahl (*)	22.5 Δ	CF	3.0 Δ	Banjo et al. (2006)
Macrotermes natalensis	War-like Termite	alate, queen	Kjeldahl (*)	24.7Δ	CF	2.46 Δ	Banjo et al. (2006)
Macrotermes spp.	Termite	alate	CP (6.25)	24.5			Payne et al. (2016)
Macrotermes spp.	Termite	soldiers	Kjeldahl (*)	20.59 Δ	TDF	4.45 Δ	Adeyeye and Olaleye (2016)
Odontotermes sp.	Termite		Kjeldahl (6.25)	33.67	CF	6.30	Chakravorty et al. (2016)
Macrotermes subylanus	Termite	dewinged	Kjeldahl (6.25)	39.34	TDF	6.37	Kinyuru et al. (2013)
Pseudacanthotermes militaris	Sugarcane termite	dewinged	Kjeldahl (6.25)	33.51	TDF	6.59	Kinyuru et al. (2013)
Macrotermes bellicosus	War-like Termite	dewinged	Kjeldahl (6.25)	39.74	TDF	6.21	Kinyuru et al. (2013)
Pseudacanthotermes spiniger	Termite	dewinged	Kjeldahl (6.25)	37.54	TDF	7.21	Kinyuru et al. (2013)
Oecophylla smaragdina	Weaver ant		Kjeldahl (6.25)	55.28	CF	19.84	Chakravorty et al.

(continued on next page)

Table 1 *(continued)*

Scientific name	Common name	Developmental stage	protein analysis method (N-f)	Protein, % (DW)	Dietary fiber analysis method	Dietary fiber, % (DW)	Reference citation
							(2016)
Oecyphylla smaragdina	Weaver ant	adults	CP (6.25)	10.8			Payne et al. (2016)
Oryctes boas	Scarab beetles	larvae	Kjeldahl (*)	27.46 [Δ]	CF	3.59 [Δ]	Banjo et al. (2006)
P. sulcatus Smith	Wasp	larvae	AA analysis (-trp)	45.02			Ying et al. (2010)
P. sulcatus Smith	Wasp	larvae	Kjeldahl (*)	57.88			Ying et al. (2010)
Polistes sagittarius Saussure	Wasp	larvae	AA analysis (-trp)	36.11			Ying et al. (2010)
Polistes sagittarius Saussure	Wasp	larvae	Kjeldahl (*)	46.17			Ying et al. (2010)
Protaetia brevitarsis	White-spotted flower chafer beetle	larvae	Kjeldahl (6.25)	44.23	TDF	11.06	Ghosh et al. (2017)
Rhynchophorus phoenicis	Snout beetles	larvae	Kjeldahl (*)	31.61 [Δ]	CF	3.14 [Δ]	Banjo et al. (2006)
Rhynchophorus phoenicus	Palm weevil larvae	larvae	CP (6.25)	15.9			Payne et al. (2016)
Schistocerca gregaria	locusts	adult	Kjeldahl (6.25)	76.0	TDF	2.53	Zielinska et al. (2015)
Teleogryllus emma	Emma field Cricket	adults	Kjeldahl (6.25)	55.65	TDF	10.37	Ghosh et al. (2017)
Tenebrio molitor	Mealworm	larvae	Dumas (6.25)	52.3 [Δ]			Yi et al. (2013)
Tenebrio molitor	Mealworm	larvae	AA analysis	44.71	*N*-glucosamine	21	Janssen et al. (2017)
Tenebrio molitor	Mealworm	larvae	CP (6.25)	20.00 [Δ]	TDF	1.39 [Δ]	Finke (2015)
Tenebrio molitor	Mealworm	larvae	CP (6.25)	20.00 [Δ]	ADF	2.40 [Δ]	Finke (2015)
Tenebrio molitor	Mealworm	larvae	CP (6.25)	20.9			Payne et al. (2016)
Tenebrio molitor	Mealworm	larvae	Dumas (*)	68.6			Yi et al. (2016)
Tenebrio molitor	Mealworm	larvae	Dumas (4.75)	44.8	*N*-glucosamine	21	Janssen et al. (2017)
Tenebrio molitor	Mealworm	larvae	Kjeldahl (6.25)	52.35	TDF	1.97	Zielinska et al. (2015)
Tenebrio molitor	Mealworm	larvae	Kjeldahl (6.25)	53.22	TDF	6.26	Ghosh et al. (2017)
Tenebrio molitor	Mealworm	larvae	Kjeldahl (6.25)	63.0			Adámková et al. (2016)
V. basalis Smith	Wasp	larvae	AA analysis (-trp)	43.91			Ying et al. (2010)
V. basalis Smith	Wasp	larvae	Kjeldahl (*)	53.18			Ying et al. (2010)
V. mandarinia mandarinia Smith	Wasp	larvae	AA analysis (-trp)	52.20			Ying et al. (2010)
V. mandarinia mandarinia Smith	Wasp	larvae	Kjeldahl (*)	54.59			Ying et al. (2010)
Zonocerus variegatus	Variegated grasshopper	adults	Kjeldahl (*)	29.07 [Δ]	CF	2.60 [Δ]	Banjo et al. (2006)
Zophobas morio	Giant mealworm	larvae	Dumas (6.25)	51.6 [Δ]			Yi et al. (2013)
Zophobas morio	Giant mealworm	larvae	CP (6.25)	19.85 [Δ]	EH	1.54 [Δ]	Finke (2015)
Zophobas morio	Giant mealworm	larvae	CP (6.25)	19.85 [Δ]	ADF	2.50 [Δ]	Finke (2015)
Zophobas morio	Giant mealworm	larvae	Kjeldahl (6.25)	39.0			Adámková et al. (2016)

between studies. It is also universal, precise, reproducible, and inexpensive, and until 2013 more than 46,000 articles referred or used the Kjeldahl method in different research areas such industrial analysis of food, environment (water, waste water), agriculture or health (Chromy et al., 2015; Sàez-Plaza et al., 2013).

These procedures actually measure nitrogen, and have been validated for protein determination for meat, eggs or milk products and grains, using a specific conversion factor for different food assuming that all the nitrogen present is in the form of protein (Merrill and Watt, 1973). The N-factor for meat is 6.25, based on the idea that proteins content approximately 16% of Nitrogen (Merrill and Watt, 1973), and is also commonly and incorrectly used for insects (Kinyuru et al., 2013; Ramos-Elorduy Blasquez et al., 2012; Surendra et al., 2016; Zielinska et al., 2015). However, not all the nitrogen contained in insects and detected by these methods originate from proteins. The exoskeleton of arthropods (cuticle), is built primarily of chitin fibres, a polysaccharide of glucosamine and *N*-acetylglucosamine, both containing N atoms. Moreover not all the proteins in insects seems to be digestible for humans and animals. During the sclerotisation stage of development, numerous and diverse cuticular proteins harden the cuticle by linking the chitin fibres, through the reactions of quinones with the functional groups of these proteins (Andersen et al., 1995; Hopkins and Kramer, 1992). However these indigestible proteins are generally ignored or assumed to be available to animals (Bell, 1990), but Bosch et al. (2014) considered that some differences in protein digestibility from different insects resulted from different cuticular protein-sclerotisation Thus, protein content calculated using Kjeldahl analysis and conversion factors developed for other foods would be expected to overestimate the protein content of the whole insect, as it does not distinguish between easily-digested proteins, inaccessible proteins, chitin, and other N-containing molecules.

The logical conclusion would be that a different N conservation factor, specific to insects, is needed to allow the correct determination of proteins from a total nitrogen. In mushrooms, rich in chitin, the conservation factor is 4.39 (Wang et al., 2014). However the amount of chitin and non-digestible protein in insect cuticle is very variable: hard cuticles have a high protein contents between 70 and 85% (dry weight) and a low chitin content of 15–30%, while soft cuticles contains about 50% each of chitin and proteins (Chapman, pp. 483, 2013). As a consequence, protein quantity may vary enormously among different stages of the same animal life cycle (Hepburn, 1985). Nymphs and adults of insects with hemimetabolous (incomplete) metamorphosis (e.g. locusts and crickets) have a hard exoskeleton in contrast to larvae of insects with holometabolous (complete) metamorphosis (e.g. flies and beetles) that are often covered by a soft, thin cuticle. Thus, detailed studies would be needed to develop specific N conservation factors for each insect species and for each age/stage of each species. Janssen et al. (2017) began such a solid work with larvae of three insect species. However the wide variety of edible insects (as stated, at least 1900 species following van Huis, 2013) at their different development stages,

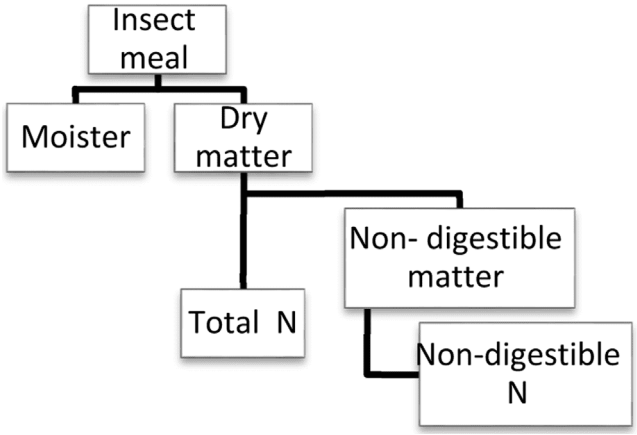

Fig. 1. The proposed process to quantify the nutritious proteins form insects: Digestible protein content = (Total N − non-digestible N) × conversion factor.

and heterogeneity in composition with respect to chitin and cuticular proteins would make it an impossible task.

We propose to evaluate the insect digestible protein contents. This can be done by subtracting the N quantity of the fibrous and other indigestible materials from the total N contents of the insect. The N-conversion factor for the digestible fraction should be similar in all insects. The non-digestible N content consisting of chitin and the proteins linked to the matrix of the cuticle, should be quantified by Kjeldahl method after an enzymatic procedure, based on the AOAC method (Prosky et al., 1988): briefly, after insect grinding, and lipid extraction, the insect meal is treated step by step, with alpha-amylase, protease, and amyloglucosidase, in proper solutions concerning pH and temperature, the fibres are then precipitated, filtered, dried and weighted. Cuticular non digestible Nitrogen is expected to be in the final sample and its amount can be known following the Kjeldahl method. But the different procedures which calculate dietary fibers, like acid detergent fibers or the suggested enzymatic method, contain another step of protein deduction from the weight material. These proteins are calculated as 6.25 multiply the measured N from the non-digested fraction. For insects this calculation is wrong, as it underestimates the true dietary fiber content due to the N from chitin. The right way for quantifying insects' chitin is through hydrolysis of the carbohydrates and analysis of *N*-glucosamine (Janssen et al., 2017). Thus, knowing the precise chitin amount dose not contribute for the nutritional protein quantification, as described before: we have to calculate the total N and the total non-digestible N multiplied by the conversion factor (Fig. 1).

This approach consumes time, but should replace the converted N-content evaluation methods for quantifying insect protein content that is nutritious for humans and animals.

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