



Nutritional value of three *Blattodea* species used as feed for animals

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ABSTRACT. Nutrient contents of subadult and adult *Blaptica dubia*, *Blaberus discoidalis* and *Blatta lateralis* roaches, commonly used as feed source for insectivorous animals in captivity, were determined. Contents of crude protein, fat, ash, chitin, carbohydrates, calcium and phosphorus were analysed. Adults of all species contained more crude protein ($602\text{--}678\text{ g} \cdot \text{kg}^{-1}\text{ DM} > 489\text{--}547\text{ g} \cdot \text{kg}^{-1}\text{ DM}$) and ash ($42\text{--}51\text{ g} \cdot \text{kg}^{-1}\text{ DM} > 36\text{--}40\text{ g} \cdot \text{kg}^{-1}\text{ DM}$) but less fat ($145\text{--}214\text{ g} \cdot \text{kg}^{-1}\text{ DM} < 236\text{--}363\text{ g} \cdot \text{kg}^{-1}\text{ DM}$) than subadults. Chitin content ranged $53\text{--}86\text{ g} \cdot \text{kg}^{-1}\text{ DM}$ and the calcium to phosphorus ratio was 1:1.9–23.5. Amino and fatty acid profiles were also determined. Very high levels of glycine ($66.4\text{--}166.2\text{ g} \cdot \text{kg}^{-1}\text{ DM}$) and alanine ($81.5\text{--}118.4\text{ g} \cdot \text{kg}^{-1}\text{ DM}$) were found in all examined samples. On the other hand, the lowest amino acid levels were determined in such sulphuric amino acids as cysteine ($0.5\text{--}2.1\text{ g} \cdot \text{kg}^{-1}\text{ DM}$) and methionine ($4.0\text{--}17.2\text{ g} \cdot \text{kg}^{-1}\text{ DM}$). Regarding essential amino acids, very high lysine ($48.6\text{--}94.0\text{ g} \cdot \text{kg}^{-1}\text{ DM}$) and valine ($53.3\text{--}84.0\text{ g} \cdot \text{kg}^{-1}\text{ DM}$) levels were determined. Essential amino acid index was found at the level of 0.4–0.9. In analysed cockroaches high oleic (38.0–44.2%), linoleic (8.5–15.3%) and palmitic (21.6–26.8%) fatty acids levels were determined. The obtained results indicate that quantity of protein and lipids varied between subadult and adult cockroaches within the species, while quality of these nutrients remained stable.

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Introduction

Unlike the past when insects were primarily used as feed for companion animals, today the usage of insects as an alternative source of nutrients in farm animal diets is also considered. The insects are generally known to be a sufficient source of protein and lipids. However, the nutritional requirements of animals are specified not only by nutrients quantity but also quality. Although the amino acid profile is the decisive characteristic of protein quality,

it's composition is known only for a few insect species. Yi et al. (2013) reported insect protein quality to be higher than soyabean and lower than casein proteins. Barroso et al. (2014) stated that amino acid profile of some *Diptera* is superior to soyabean meal.

From this point of view, the insect protein quality could also be compared with some conventional feed protein sources. On the other hand, Bosch et al. (2014) found that *in vitro* digestibility of insect protein is lower than that of commercially available protein feedstuffs. The quality of lipids depends on

their fatty acid profiles and therefore the determination of lipids composition in insects is also important. Fatty acid composition of insects was analysed by several authors, e.g., Finke (2002), Barroso et al. (2014) and Oonincx et al. (2015). It is also known, that fatty acid profile could vary significantly, because it reflects the fatty acid composition of animal diet (Sánchez-Muros et al., 2014).

Carbohydrates in insects are present basically in the form of chitin, usually indigestible for monogastric animals, in very low quantities (Finke, 2002). On the other hand, this long chain polymer of N-acetyl glucosamine may exert positive effect on the immune system (van Huis et al., 2013) and its antioxidative, hypocholesterolaemic and prebiotic effects are also known (Halder et al., 2013).

Insects are also a relatively good source of minerals, such as phosphorus, magnesium, sodium or chloride, and trace elements (Finke, 2002). Nevertheless, certain vital element levels are insufficient for the nutritional requirements of vertebrates. One of such elements is calcium (Barker et al., 1998; Finke, 2013).

There are many insects that are suitable for animal feeding. Sánchez-Muros et al. (2014) summarized that there are currently more than 150 commercially available species, whose nutrient content is species-dependent. Furthermore, not only various species, but also different developmental stages could be used as feed according to individual requirements. Despite the fact that nutritional value of more than 60 insect species is currently known (Sánchez-Muros et al., 2014), the information regarding the differences in insect chemical composition between several stages is only available for a limited number of species. *Acheta domestica* is one of such species – as an adult it contains more fat and protein but less ash than the nymphs (Barker et al., 1998; Finke, 2002). Regarding *Blattodea*, Oonincx and Dierenfeld (2012) found in *Eublaberus distanti* adults higher contents of ash and protein and lower levels of fat than their nymph counterparts, and in adults of *Gromphadorhina portentosa* approximately the same levels of fat and crude protein as the nymphs.

The investigated cockroaches varied in size, weight, sexual dimorphism and reproduction. In comparison to adults, subadult nymphs are smaller and their wings are not yet fully developed. Adult males of *Blatta lateralis* are 19–22 mm long and winged, whereas adult females are 22–25 mm long, oviparous and brachypterous. *Blaptica dubia* adults can reach 40 mm in length. Adult males of this species have long wings that cover the abdo-

men entirely, whereas ovoviviparous females have strongly reduced wings. Finally, *Blaberus discoidalis* are ovoviviparous and their winged adults can reach the length up to 55 mm. They all are considered as suitable feed for animals and are commercially available. Additionally, they are unable to fly or climb on smooth surfaces, which makes them significantly easier to manipulate and prevents from their accidental escape or contamination. Till this time, the information regarding nutritional values of above-mentioned species was either unavailable or concerned only adults, which made the comparison among different developmental stages impossible.

Due to the limited information, this study presents the nutritional value of subadults and adults of three cockroach species (*B. dubia*, *B. lateralis*, *B. discoidalis*) and compares the differences between their last two developmental stages from the perspective of nutritional composition. This knowledge will also allow to better understand the dietary habits of insectivorous animals or possibly to meet the requirements of animals, whose nutritional demands have already been standardized.

Material and methods

Sampling

All cockroaches were kept in plastic boxes (dimensions: 59 × 39 × 28 cm = 45 litres for *B. dubia* and 39 × 28 × 28 cm = 22 litres for *B. discoidalis* and *B. lateralis*) filled with flat egg trays. The boxes were covered by a lid equipped with aluminium insect mesh and no substrate was used. Heating foils were used to maintain a constant temperature of 27 °C, which was thermostatically controlled. All the cockroach colonies were fed dog granules (Brit Care Medium Adult Breed, Vafo, Chrášťany, Czech Republic) and old bread *ad libitum*. Slices of fresh vegetables and fruits (i.e. carrot, cucumber, turnip and apple) were provided daily. The vegetable and fruits scraps were constantly removed to prevent the occurrence of mildew. A water source was present in each of the breeding containers. Approximate populations of the colonies were as follows: *B. discoidalis* – 700 specimens, *B. dubia* – 1500 specimens and *B. lateralis* – 1700 specimens (all stages were kept together). Randomly selected subadults and adults (20–40 insects) of all investigated species were individually weighed to determine average weight per insect. Due to the difference in the weight of males and females, these values are reported separately ac-

ording to sex. Subsequently, samples (about 300 g) were removed from each colony and frozen alive (at -18°C). The samples were then lyophilized and homogenized.

Analysis of proximate composition

All investigated parameters were determined using the methods of European Commission Regulation (EC) No. 152/2009. The dry matter (DM) content was determined after 5 h of drying at 103°C . The total amount of nitrogen was evaluated by the Kjeldahl method (ISO 5983-1:2005) using the Kjeltec 2400 analyzer unit (Foss, Hilleroed, Denmark) and crude protein (CP) level was then calculated ($6.25 \times$ total nitrogen). Crude fat (petroleum ether extract; EE) content was determined gravimetrically after extraction with petroleum ether using Soxhlet apparatus SER 146 (Velp, Usmate, Italy). The samples were then mineralized in a muffle furnace at 550°C and the total amount of ash was measured. Additionally, chitin content was analysed according to Liu et al. (2012) using hydrolysis with 1M hydrochloric acid and 1M sodium hydroxide. Because chitin is a nitrogenous polysaccharide, the nitrogen content was also analysed in these hydrolysed samples to refine total crude protein content. Nitrogen-free extract (NFE) was calculated as:

$$\text{NFE} = 100 - (\text{moisture} + \text{CP} + \text{EE} + \text{ash} + \text{chitin}).$$

Finally, the energy content was evaluated using the standard calculation used by other authors such as Finke (2002):

$$\begin{aligned} \text{Energy content, kJ} \cdot \text{kg}^{-1} = \\ = (\text{CP} \times 17) + (\text{EE} \times 37) + (\text{NFE} \times 17). \end{aligned}$$

Analysis of amino acid composition

To describe the protein quality, the amino acid profile was analysed performing acid oxidation in Amino Acid Analyser 400 (INGOS, Prague, Czech Republic). Subsequently, protein quality was evaluated by the essential amino acid index (EAAI), which is based on the content of all essential amino acids except tryptophan in comparison to the reference (eggwhite) protein:

$$\begin{aligned} \text{EAAI} = 10 \sqrt{\frac{\text{mg of lysine in 1 g of test protein}}{\text{mg of lysine in 1 g of eggwhite protein}}} \times \\ \times (\text{etc. for other determined amino acids}). \end{aligned}$$

The determined amino acids were: valine, leucine, isoleucine, tyrosine, phenylalanine, histidine, lysine, arginine, cysteine and methionine.

Analysis of fatty acids

To determine the lipid quality, the fatty acid profile was analysed by gas chromatography-mass spectrometry technique (GC-MS). Prior to that, the Soxhlet extraction of lipids from samples was performed for 4 h, using petroleum ether. The base esterification method by 0.5M methanolic KOH and BF_3 -methanol was used for fatty acids derivatization (ISO 12966-2:2011). Methyl esters of fatty acids were analysed by GC-MS using Agilent 7890A GC (Agilent, Wilmington, DE, USA) equipped with a Restek Rt-2560 column (length – 100 m; ID – 0.25 mm; film – 0.25 μm ; Restek Corporation, Bellefonte, PA, USA) and coupled to Agilent 5975C single-quadrupole mass detector (Agilent, Wilmington, DE, USA). Hexane was used as a solvent and the sample volume of 1 μl was injected in split mode (ratio 50:1) into the injector heated to 225°C . The initial oven temperature was 70°C (hold 2 min), ramp1 was up to 225°C at $5^{\circ}\text{C} \cdot \text{min}^{-1}$ (hold 9 min) and ramp2 was up to 240°C at $5^{\circ}\text{C} \cdot \text{min}^{-1}$ (hold 25 min). Helium was used as a carrier gas with the flow rate of $1.2 \text{ ml} \cdot \text{min}^{-1}$. The mass spectrometry analysis was carried out in full scan mode, the electron ionization energy was set at 70 eV. Methylated fatty acids were identified using a Restek Food Industry FAME mix (cat no. 35077; Bellefonte, PA, USA) and by the comparison of their mass spectra with the National Institute of Standards and Technology Library (NIST, Gaithersburg, MD, USA). The proportions of fatty acids were calculated by area normalization method.

Results

As expected, the weight of roaches was proved to be species-specific. In all examined species the adult females weighed more than the subadult females, while weights of adult males were always lower than those of the subadults. The DM content amounted 307–422 $\text{g} \cdot \text{kg}^{-1}$ of the specimen fresh weight. In all adults it was found more crude protein but less crude fat and ash than in the subadults of respective species (Table 1). On the other hand, chitin levels showed some species-specific differences: whereas in adult roaches of *B. discoidalis* less chitin content was found than in their subadult counterparts, concentrations were higher in adults of *B. lateralis*. The difference between chitin levels in *B. dubia* adults and their subadult counterparts was less than $1 \text{ g} \cdot \text{kg}^{-1}$ DM. Concentration of calcium and phosphorus (Table 2) was lower than $50 \text{ g} \cdot \text{kg}^{-1}$ DM. In the investigated

Table 1. Nutrient content of three cockroaches (*Blattodea*) in adult and subadult stages

Species	Stage	Average weight		Nutrients content									Energy, MJ · kg ⁻¹ DM
		female	male	moisture	DM	EE	N × 6.25	chitin protein	CP	chitin	ash	NFE	
		mg per insect		g · kg ⁻¹			g · kg ⁻¹ DM						
<i>Blaptica dubia</i>	subadult	2034	1659	578	422	328	547	22	525	65	40	42	22
	adult	2213	1279	626	374	214	650	20	630	64	49	43	19
<i>Blaberus discoidalis</i>	subadult	3290	2612	659	341	236	536	33	503	86	36	139	20
	adult	3612	2212	657	343	203	678	26	652	66	42	37	19
<i>Blatta lateralis</i>	subadult	398	290	640	360	363	489	18	470	53	37	77	23
	adult	552	267	693	307	145	602	18	584	59	51	161	18

DM – dry matter; EE – petroleum ether extract of crude fat; N × 6.25 – total nitrogen amount; CP – crude protein; NFE – nitrogen-free extract

Table 2. Calcium and phosphorus content and their ratio of three cockroaches (*Blattodea*) in adult and subadult stages

Species	Stage	Content, g · kg ⁻¹ DM		Ca:P ratio
		Ca	P	
<i>Blaptica dubia</i>	subadult	2	43	1:21.5
	adult	2	47	1:23.5
<i>Blaberus discoidalis</i>	subadult	7	20	1:2.9
	adult	8	30	1:3.8
<i>Blatta lateralis</i>	subadult	7	12	1:1.8
	adult	9	25	1:1.9

Table 3. Non-essential amino acid content of three cockroaches (*Blattodea*) in adult and subadult stages

Amino acid	Non-essential amino acid content, g · kg ⁻¹ DM					
	<i>Blaptica dubia</i>		<i>Blaberus discoidalis</i>		<i>Blatta lateralis</i>	
	subadult	adult	subadult	adult	subadult	adult
Aspartic acid	26.3	28.0	33.0	25.3	22.4	39.3
Threonine	16.5	16.5	21.4	13.9	13.6	23.8
Serine	21.2	17.7	26.1	13.9	17.0	27.9
Glutamic acid	47.4	48.0	57.7	48.3	43.4	72.2
Proline	38.3	39.3	49.3	28.4	34.3	45.7
Glycine	98.4	166.2	116.1	69.2	66.4	128.6
Alanine	100.0	108.9	140.1	81.5	83.8	118.4

roaches more phosphorus than calcium was detected and the lowest Ca:P ratio was found in *B. dubia*.

In the examined cockroaches high levels of such non-essential amino acids as glycine and alanine were found (Table 3). In comparison to eggwhite, calculated EAAI values ranged from 0.4 to 0.9, and the highest EAAI was found in adult and subadult *B. discoidalis* roaches (Table 4).

Table 4. Essential amino acid content and essential amino acid index of three cockroaches (*Blattodea*) in adult and subadult stages

Amino acid	Essential amino acid content, g · kg ⁻¹ DM					
	<i>Blaptica dubia</i>		<i>Blaberus discoidalis</i>		<i>Blatta lateralis</i>	
	subadult	adult	subadult	adult	subadult	adult
Valine	58.8	66.3	84.0	54.4	53.3	81.3
Isoleucine	68.3	37.2	89.1	34.6	57.2	101.3
Leucine	36.0	64.6	26.6	61.5	18.9	9.7
Tyrosine	58.5	46.9	62.2	42.2	47.3	59.9
Phenylalanine	31.2	31.9	38.9	30.6	26.6	46.7
Histidine	34.8	36.6	50.7	30.8	29.6	47.7
Lysine	57.7	62.8	73.5	60.4	48.6	94.0
Arginine	53.8	56.1	71.3	57.2	44.9	89.8
Cysteine	2.1	1.7	1.4	2.0	0.5	0.9
Methionine	17.2	13.6	13.4	15.5	4.0	7.8
EAAI	0.4	0.7	0.9	0.9	0.7	0.6

EAAI – essential amino acid index

Conversely, contents of methionine and cysteine were the lowest.

In all cockroaches the oleic acid (C18:1*cis*-9) content was the highest among fatty acids, and ranged from 38.0 to 44.2% of total fatty acids (Table 5). Only in *B. lateralis* species more saturated (SFA) than monounsaturated (MUFA) fatty acids were determined. In the remaining two species there were more MUFA. The lowest polyunsaturated fatty acids (PUFA) levels (9.5% and 9.6% for adult and subadult stage, respectively) were found in *B. lateralis*. On the other hand, the highest PUFA amount (20.9%) was found in adults of *B. discoidalis*.

Table 5. Fatty acid profile of three cockroaches (*Blattodea*) in adult and subadult stages

Fatty acid	Fatty acid profile, % of total fatty acids					
	<i>Blaptica dubia</i>		<i>Blaberus discoidalis</i>		<i>Blatta lateralis</i>	
	subadult	adult	subadult	adult	subadult	adult
C4:0	ND	ND	ND	ND	ND	ND
C6:0	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
C8:0	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
C10:0	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
C12:0	0.3	0.3	0.1	0.1	0.2	0.1
C14:0	2.6	2.2	1.2	1.3	1.9	1.3
C14:1 <i>cis</i> -9	0.1	0.1	<0.1	<0.1	<0.1	<0.1
C15:0	0.2	0.2	0.2	0.3	0.1	0.2
C16:0	22.8	21.6	23.4	22.9	26.8	24.7
C16:1 <i>trans</i> -9	0.4	0.5	0.7	0.7	0.2	0.3
C16:1 <i>cis</i> -9	9.8	7.7	4.4	2.6	3.3	4.5
C17:0	0.5	0.6	0.3	0.6	0.4	0.5
C17:1 <i>cis</i> -10	0.3	0.5	0.2	0.3	0.1	0.2
C18:0	7.8	8.9	6.8	8.9	18.1	17.9
C18:1 <i>trans</i> -9	0.1	0.1	0.2	0.5	0.2	0.2
C18:1 <i>cis</i> -9	40.3	42.5	44.2	40.2	38.0	39.1
C19:0	0.1	0.1	ND	0.1	0.1	0.1
C18:2 <i>cis</i> -9,12	10.6	11.9	15.3	17.9	8.7	8.5
C20:0	0.2	0.3	0.3	0.3	0.6	0.9
C20:1 <i>cis</i> -11	0.3	0.3	0.3	0.3	0.2	0.3
C18:3 <i>cis</i> -9,12,15	3.2	1.8	1.7	1.9	0.7	0.7
C22:0	0.1	0.1	ND	ND	<0.1	0.1
C20:4 <i>cis</i> -5,8,11,14	0.2	0.3	0.5	1.0	0.2	0.4
C24:0	0.1	<0.1	<0.1	<0.1	<0.1	<0.1
SFA	34.7	34.3	32.4	34.6	48.3	45.8
MUFA	51.3	51.6	50.1	44.6	42.2	44.7
PUFA	14.0	14.1	17.5	20.9	9.6	9.5

ND – not detected; SFA – saturated fatty acids; MUFA – mono-unsaturated fatty acids; PUFA – polyunsaturated fatty acids

Discussion

Proximal composition. Information concerning the nutrient content of the investigated species is only available for the adults of *B. dubia* (Yi et al., 2013; Bosch et al., 2014) and young nymphs of *B. lateralis* (Oonincx and Dierenfeld, 2012). Therefore, the results should be compared mainly with other cockroaches or insect species. In all of the examined cockroaches high concentrations of crude protein (470–652 g · kg⁻¹ DM) as well as high levels of crude fat (145–363 g · kg⁻¹ DM) were determined. This means that both subadult and adult cockroaches used in our study are a similar source of protein and lipids like other species of cockroaches (Oonincx and Dierenfeld, 2012; Bosch et al., 2014),

house cricket (*Acheta domesticus*) (Barker et al., 1998; Finke, 2002) and other insects.

The ash content was 36–51 g · kg⁻¹ DM, which also corresponds with the findings of other authors (Barker et al., 1998; Finke, 2002; Oonincx and Dierenfeld, 2012). Additionally, we found rather low Ca:P ratios that are insufficient for the vertebrate diets (NRC, 1995, 2003; Finke, 2002); but these ratios are similar to those reported for most insect species (Barker et al., 1998; Finke, 2002; Oonincx and Dierenfeld, 2012). The above-mentioned results confirm the need for calcium supplements in the insectivore diets. In order to better serve the nutritional requirements of vertebrates, further research should be focused on possibilities of increasing Ca content in insects (such as gut loading, etc.) to be able to influence the unfavourable Ca:P ratio as well as the calcium accumulation in the body of insects.

The chitin content (53–86 g · kg⁻¹ DM) was similar to that of crickets (*Gryllus testaceus*) (Wang et al., 2005) and grasshoppers (*Acrida cinerea*) (Wang et al., 2007). Significantly lower chitin contents were reported by Finke (2013). However, these differences may result from different methodologies used. Additionally, crude protein content in chitin was 18–33 g · kg⁻¹ DM, which is clearly more than 4 g · kg⁻¹ DM reported by Bernard and Allen (1997).

Amino acid profile. In all tested samples contents of glycine (66.4–166.2 g · kg⁻¹ DM) and alanine (81.5–118.4 g · kg⁻¹ DM) were very high. It is more than in other studies focused on the protein quality in cockroaches, e.g., Yi et al. (2013) and Bosch et al. (2014). These differences were probably caused by feed residues in the cockroach digestive tract.

The examined cockroaches, especially *B. discoidalis*, also seem to be a good source of essential amino acids (EAA). The EEAI without tryptophan was 0.4–0.9, mainly because of high lysine level (48.6–94.0 g · kg⁻¹ DM). On the other hand, the lowest levels of sulphuric amino acids: cysteine (0.5–2.1 g · kg⁻¹ DM) and methionine (4–17.2 g · kg⁻¹ DM) were found. These findings are more or less in line with the results published by other authors. For adult females of the same species, Bosch et al. (2014) reported high contents of valine, leucine, histidine, lysine and arginine, which is consistent with the levels found in *B. dubia* in this study. Moreover, Józefiak et al. (2016) studied the EAA composition of *B. lateralis* subadult nymphs and found that tyrosine, arginine, valine and lysine were among the first five EAAs, which corresponds to the results obtained in this study. On the other hand,

the main EAA reported by Józefiak et al. (2016) was leucine, while in this paper the most abundant EAA is isoleucine ($101.3 \text{ g} \cdot \text{kg}^{-1} \text{ DM}$ for adult stage of *B. lateralis*).

Fatty acid profile. Finke (2002) and Oonincx et al. (2015) analysed the fatty acid profile of cockroaches and determined the same major fatty acids (mainly the oleic acid) in *B. lateralis* and *B. dubia*. Moreover, the presented fatty acids profiles were very similar to those obtained in our study. With a few exceptions, the dominant values of oleic, palmitic and linoleic fatty acids presented in this paper also correspond with the results of other authors (Finke, 2002; Barroso et al., 2014), who investigated fat composition of other terrestrial insect species. In line with these authors, no contents of omega-3 fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were detected. In this work, some differences in lipids composition were observed between the investigated species, but values within the one species varied only marginally. The results indicate that the fat of tested insects contained high proportion of SFA and MUFA. From this point of view, its nutritional quality is not so high and close to other animal fats. On the other hand, the proportion of PUFA is comparable to commonly used oils such as the olive, sunflower or rapeseed oil (Velišek, 2014). Moreover, it could even be optimized or improved during the rearing process by adding suitable supplements to the feeding mixtures for insects.

Comparison of developmental stages. Oonincx and Dierenfeld (2012) comparing two consecutive developmental stages of Turkestan roaches (*B. lateralis*) found that younger nymphs (2nd instar) contained more crude protein ($760.5 > 628.5 \text{ g} \cdot \text{kg}^{-1} \text{ DM}$), more ash ($78.8 > 68.9 \text{ g} \cdot \text{kg}^{-1} \text{ DM}$) and less crude fat ($144.5 < 265.0 \text{ g} \cdot \text{kg}^{-1} \text{ DM}$) than older nymphs (3rd instar). In our study less crude protein ($470 < 584 \text{ g} \cdot \text{kg}^{-1} \text{ DM}$), less ash ($37 < 51 \text{ g} \cdot \text{kg}^{-1} \text{ DM}$) and more crude fat ($363 > 145 \text{ g} \cdot \text{kg}^{-1} \text{ DM}$) were found in younger subadult nymphs than in adults. However, Oonincx and Dierenfeld (2012) did not determine these concentrations for adult roaches. Therefore, their presumption that earlier developmental stages contain more protein and less fat than older specimens of the same species seems dubious in the light of our findings. Nonetheless, further research is needed. We assume that the nutrient content might be significantly influenced by the various diets of the insects (e.g., total fat concentrations correlate with the energy value of the feed), ecdysis and the timing of the analysis.

In the dry matter of adults *B. dubia* roaches 630 g of crude protein and 214 g of crude fat per kg of DM were found, and these concentrations are similar to those reported by Yi et al. (2013) for this species (592 g of crude protein and 214 g of total fat per kg of DM) and Tzompa-Sosa et al. (2014) who reported 7.6 g of total fat from 100 g of fresh samples. The nutritional composition of roaches at the subadult developmental stage has been unknown until now.

This study was the first to determine the nutrient content of *B. discoidalis*. This content can be compared with that of *E. distanti*, which was selected mainly because it belongs to the same family (*Blaberidae*) and similar dimorphism (all adults are winged). The chemical composition of this species was reported by Oonincx et Dierenfeld (2012), whose findings partly correspond with our results. *E. distanti* adults contained more crude protein and ash but less fat than 4–5 cm long nymphs. However, most of the nutrient concentrations determined in this study (especially crude fat and crude protein) differed from those of *E. distanti*.

Conclusions

The obtained results showed differences in nutritional value among three studied species (*Blaptica dubia*, *Blaberus discoidalis* and *Blatta lateralis*). However, they were not as clear as the differences between adult and subadult cockroaches of the same respective species. All adults were better source of crude protein, ash and calcium, but contained less fat, than the subadults. On the other hand, it was determined that the quality (unlike the quantity) of lipids and proteins remained essentially the same.

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References

- Barker D., Fitzpatrick M.P., Dierenfeld E.S., 1998. Nutrient composition of selected whole invertebrates. *Zoo Biol.* 17, 123–134, [https://doi.org/10.1002/\(SICI\)1098-2361\(1998\)17:2<123::AID-ZOO7>3.0.CO;2-B](https://doi.org/10.1002/(SICI)1098-2361(1998)17:2<123::AID-ZOO7>3.0.CO;2-B)

- Barroso F.G., de Haro C., Sánchez-Muros M.-J., Venegas E., Martínez-Sánchez A., Pérez-Bañón C., 2014. The potential of various insect species for use as food for fish. *Aquaculture* 422–423, 193–201, <https://doi.org/10.1016/j.aquaculture.2013.12.024>
- Bernard J.B., Allen M.E., 1997. Feeding captive insectivorous animals: Nutritional aspects of insects as food. Nutrition Advisory Group Handbook, Fact Sheet 3, 1–7
- Bosch G., Zhang S., Oonincx D.G.A.B., Hendriks W.H., 2014. Protein quality of insects as potential ingredients for dog and cat foods. *J. Nutr. Sci.* 3, e29, <https://doi.org/10.1017/jns.2014.23>
- European Commission Regulation (EC), 2009, No. 152/2009. Official Journal of the European Union. Available from: <http://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32009R0152>
- Finke M.D., 2002. Complete nutrient composition of commercially raised invertebrates used as food for insectivores. *Zoo Biol.* 21, 269–285, <https://doi.org/10.1002/zoo.10031>
- Finke M.D., 2013. Complete nutrient content of four species of feeder insects. *Zoo Biol.* 32, 27–36, <https://doi.org/10.1002/zoo.21012>
- Halder S.K., Adak A., Maity C., Jana A., Das A., Paul T., Ghosh K., Das Mohapatra P.K., Pati B.R., Mondal K.C., 2013. Exploitation of fermented shrimp-shells hydrolysate as functional food: Assessment of antioxidant, hypocholesterolemic and prebiotic activities. *Indian J. Exp. Biol.* 51, 924–934
- ISO 5983-1:2005, 2005. Animal feeding stuffs – Determination of nitrogen content and calculation of crude protein content – Part 1: Kjeldahl method. International Organization for Standardization. Geneva (Switzerland)
- ISO 12966-2:2011, 2011. Animal and vegetable fats and oils – Gas chromatography of fatty acid methyl esters – Part 2: Preparation of methyl esters of fatty acids. International Organization for Standardization. Geneva (Switzerland)
- Józefiak D., Józefiak A., Kierończyk B., Rawski M., Świątkiewicz S., Długosz J., Engberg R.M., 2016. Insects – a natural nutrient source for poultry – a review. *Ann. Anim. Sci.* 16, 297–313, <https://doi.org/10.1515/aoas-2016-0010>
- Liu S., Sun J., Yu L., Zhang C., Bi J., Zhu F., Qu M., Jiang C., Yang Q., 2012. Extraction and characterization of chitin from the beetle *Holotrichia parallela* Motschulsky. *Molecules* 17, 4604–4611, <https://doi.org/10.3390/molecules17044604>
- NRC, 1995. Nutrient Requirements of Laboratory Animals. 4th Edition. National Academy Press. Washington, DC (USA)
- NRC, 2003. Nutrient Requirements for Nonhuman Primates. 2nd Edition. National Academic Press. Washington, DC (USA)
- Oonincx D.G.A.B., Dierenfeld E.S., 2012. An investigation into the chemical composition of alternative invertebrate prey. *Zoo Biol.* 31, 40–54, <https://doi.org/10.1002/zoo.20382>
- Oonincx D.G.A.B., van Broekhoven S., van Huis A., van Loon J.J.A., 2015. Feed conversion, survival and development, and composition of four insect species on diets composed of food by-products. *PLoS ONE* 10, e0144601, <https://doi.org/10.1371/journal.pone.0144601>
- Sánchez-Muros M.-J., Barroso F.G., Manzano-Agugliaro F., 2014. Insect meal as renewable source of food for animal feeding: a review. *J. Clean. Prod.* 65, 16–27, <https://doi.org/10.1016/j.jclepro.2013.11.068>
- Tzompa-Sosa D.A., Yi L., van Valenberg H.J.F., van Boekel M.A.J.S., Lakemond C.M.M., 2014. Insect lipid profile: aqueous versus organic solvent-based extraction methods. *Food Res. Int.* 62, 1087–1094, <http://dx.doi.org/10.1016/j.foodres.2014.05.052>
- van Huis A., Van Isterbeeck J., Klunder H., Mertens E., Halloran A., Muir G., Vantomme P., 2013. Edible insects: Future prospect for food and feed security. *FAO Forestry Paper* 171, pp. 201
- Velíšek J., 2014. *The Chemistry of Food*. Wiley-Blackwell. Chichester (UK)
- Wang D., Zhai S.W., Zhang C.X., Bai Y.Y., An S.H., Xu Y.N., 2005. Evaluation on nutritional value of field crickets as a poultry feedstuff. *Asian-Australas. J. Anim. Sci.* 18, 667–670, <https://doi.org/10.5713/ajas.2005.667>
- Wang D., Zhai S.-W., Zhang C.-X., Zhang Q., Chen H., 2007. Nutrition value of the Chinese grasshopper *Acrida cinerea* (Thunberg) for broilers. *Anim. Feed Sci. Technol.* 135, 66–74, <https://doi.org/10.1016/j.anifeedsci.2006.05.013>
- Yi L., Lakemond C.M.M., Sagis L.M.C., Eisner-Schadler V., van Huis A., van Boekel M.A.J.S., 2013. Extraction and characterisation of protein fractions from five insect species. *Food Chem.* 141, 3341–3348, <https://doi.org/10.1016/j.foodchem.2013.05.115>