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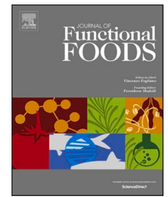

Investigation of *in vitro* and *in vivo* digestibility of black soldier fly (*Hermetia illucens L.*) larvae protein

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ABSTRACT

In this work, the *in vitro* and *in vivo* digestibility of the dried and low-fat black soldier fly (BSF, *Hermetia illucens L.*) larvae was carried out. The *in vitro* experiments demonstrated that the digestibility of the dried larvae protein was only 48%. Meanwhile, the protein digestibility of the defatted larvae biomass reached 75%. Based on these findings, the experimental feeds were composed: (a) control feed containing casein, (b) test feed containing defatted larvae biomass and (c) protein-free feed. For protein digestibility studies, experimental feeds were tested *in vitro* and *in vivo*. It was found that digestibility of larvae protein *in vivo* and *in vitro* reached 85% and 41% respectively. The DIAAS value (73%) for BSF larvae protein digestibility *in vivo* was determined for the first time. The obtained results suggest that larvae possess an easy digestible AA, therefore, they can be used as highly bio-available protein source for human nutrition.

1. Introduction

Given that the human population is growing exponentially and has a negative impact on the earth's ecological status, the alternatives for traditional protein sources are in high demand (Meybeck, Laval, Lévesque, & Parent, 2018). One of such alternative is the insects (Govorushko, 2019), having a high nutritional value and being consumed by one third of the world's total population. Despite their rich nutrient content, the insects possess other advantages. For example, their biomass can grow fast on the variety of organic waste materials and their cultivation does not require large areas. In addition, the maintenance of larvae is of low-cost; also, the insects might serve as the precursors for the chemical, pharmaceutical and other industries.

Black soldier fly (BSF) larvae are one of the most promising insect species for mass production (Wang & Shelomi, 2017). Being at the prepupae or the pupal stages, BSF can be a source of the nutrients such as protein (Al-Qazzaz & Ismail, 2016; Yu, Li, Chen, Rong, & Ma, 2019) and poly-unsaturated fatty acids (De Marco et al., 2015; Ravi et al., 2019; Spranghers et al., 2018). Therefore, the BSF larvae based products can replace the traditional ones and become a significant protein rich meal alternative in the future. Insect-based food is acceptable in the EU and regulated as novel food (EC 2015/2283). However, the consumption of the insect based food or food supplemented by the insect-ingredient is still rejected by the majority of Western consumers. It is usually determined by cultural reasons or environmental issues since mass-produced insects are raised in heated rooms, then freeze-dried, leading to a massive energy-consuming (Deroy, Reade, & Spence, 2015). Therefore, new arguments or strategies leading to the positive consumers' attitude towards the insects based food are needed (Gravel & Doyen, 2020). One of the possibilities to do so is to provide a scientifically proved argument

Abbreviations: AA, amino acids; BSF, black soldier fly; DIAAS, digestible indispensable amino acid score; FAO/WHO, Food and Agriculture Organization of the United Nations/World Health Organization; HPLC, high-performance liquid chromatography; IAA, indispensable amino acids; PD, protein digestibility; PDCAAS, protein digestibility corrected amino acid score; SSF, simulated salivary fluid; SGF, simulated gastric fluid; SIF, simulated intestinal fluid; TPD, true protein digestibility.

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that the food containing insects is rich in proteins that are easily accepted by the human digestive system. Moreover, to show that the insect protein are digested at a similar level as other animal protein and has a higher digestibility than the plant based protein. However, a nutrient content analysis alone does not provide information on the bio-accessibility of the particular nutrient. For this reason, a testing of the bio-accessibility is required in order to determine if the biologically active compounds and compounds with a nutritional value are released from the food matrix into a gastrointestinal tract making them accessible for an intestinal absorption. The bio-accessibility of nutrients in foods can be estimated using *in vivo* and/or *in vitro* methods (He et al., 2017; Peixoto, Devesa, Vélez, Cervera, & Cadore, 2016). A combination of these approaches provides complementary information leading to the facilitated interpretation and understanding of the nutrients' bio-accessibility by the human body. Among the fly larvae, the *in vitro* digestibility of termites and grasshoppers (Kinyuru, Kenji, Njoroge, & Ayieko, 2010) as well as cricket (da Rosa Machado & Thys, 2019) has been investigated. These studies demonstrate a different, but still significant uptake of the insects' protein. The limiting factor leading to the variation of the insects' protein digestibility is the amount of chitin, which is defined as insect fiber due to the structural similarity to cellulose. Since chitin is not decomposed and not absorbed in the small intestine, it may have a negative impact on the protein digestibility. Several research suggests that soft-bodied insects such as silkworm larvae contain less chitin. For this reason, they are more digestible compared to those larvae species having the chitin-based shells (Frye & Calvert, 1989; Finke, 2007). Despite the decreased protein digestibility, chitin has a number of benefits. For example, this structural polysaccharide is known to improve gastrointestinal health due to its prebiotic potential (Selenius, Korpela, Salminen, & Gallego, 2018). Moreover, it has been shown that the consumption of chitin improves glucose intolerance and reduce the level of LDL-cholesterol in the blood system (Lopez-Santamarina et al., 2020). Hence, in order to maintain a high nutritional value and all beneficial properties, chitin was not removed from the larvae used in this work.

Insect-based meals has been widely studied from a variety of scientific point of view: from the nutritional value to the digestibility of separate components. However, there is still little information on how *in vitro* and *in vivo* digestibility of the particular nutrients can correlate. To address this issue, the main objective of this work was to evaluate and compare the bio-accessibility of BSF protein through *in vitro* and *in vivo* experiments. Despite that Food and Agriculture Organization (FAO) proposed that true ileal digestibility (i.e. determined at the end of the small intestine) of each amino acid should be preferably determined in humans, the research with pigs or rats are still appropriate (FAO/WHO, 2013). To the best of our knowledge, the *in vivo* experiments regarding the true ileal digestibility of BSF larvae were not carried out before.

2. Materials and methods

2.1. Materials

BSF larvae were obtained from local farm in Lithuania. The larvae were reared for 20 days at 25 °C temperature, maintaining the ambient humidity of 50%. Larvae were fed with grains containing 70% of moisture. The average weight of the grown larvae was found to be 200 mg. The grown larvae were frozen at −20, then thawed and dried at 105 °C for 2 h in the conventional oven.

The low-fat BSF larvae material was obtained by mechanical fat extraction of the dried larvae using a press ("Maslyachok PShU-4" Electromotor, Ukraine).

For the preparation of the feed pellets containing dried low-fat larvae material the following materials were used: corn starch (Roquette, France), casein (Polsero Sp.z.o.o, Poland), cellulose (Harke GmbH, Germany), premix of vitamins AIN-93 VX and premix of minerals AIN-93 M (MP Biomedicals, US), titanium dioxide (Wild ADM, Germany). The rapeseed oil and sucrose powder was purchased from the local supermarket. The formulations of the experimental diets are provided in Table 1.

For the larvae protein digestibility *in vitro*, the following reagents were used: KCl, KH_2PO_4, $NaHCO_3$ (99%), NaCl, $MgCl_2 \cdot 6H_2O$, $(NH_4)_2CO_3$, NaOH, HCl (37%), $CaCl_2$, α-amylase from human saliva (Sigma Aldrich, A1031), pepsin (Sigma Aldrich, 3200–4500 U/mg), pancreatin (Sigma Aldrich, 8 × U), bile salts (Sigma Aldrich). All the materials used for the chemical analyses were of analytical grade and used without further purification.

2.2. Methods

For the evaluation of the digestibility of larvae protein, *in vitro* and *in vivo* methods were applied.

The chemical analysis of dried larvae, feed pellets, digesta and faeces of the rats was carried out. Content of crude protein was determined by Kjeldahl method using the conversion factor 5.6. Fat content of larvae was determined by ethyl ether extraction (Soxhlet technique). Dry matter content was determined gravimetrically after drying at 105 ± 0.5 °C to constant weight; ash content was determined by incineration in muffle at 500–600 °C. Chitin in both, dried larvae raw material and larvae containing reduced amount of fat was determined based on the protocol reported by Soetemans, Uyttebroek, and Bastiaens (2020). Briefly, the homogenized samples of larvae biomass were demineralized with HCl (1 M) at room temperature for 1 h. The ratio of the larvae biomass and HCl was 1:10 m/V. The demineralized samples were washed with distilled water until neutral pH and further treated with NaOH (1 M) at 80 °C for 8 h in order to remove proteins. The ratio of the larvae biomass and NaOH was 1:25 m/V. After deproteinization, the samples were washed with distilled water until neutral pH and dried at 105 °C to constant weight. The proximate chitin content was calculated based on dry matter, according to the following formula:

$$\text{Approximate chitin}, \% = \frac{m_1(dm)}{m_2(dm)} \times 100 \quad (1)$$

m_1 (dm) – dry matter of the sample after the demineralization and deproteinization (g)

m_2 (dm) – dry matter of the sample before demineralization and deproteinization (g)

2.2.1. Determination of amino acids content

For the determination of the total amino acid content (both, for *in vivo* and *in vitro* experiments), the dried sample (0.1 g) was placed into the vessel followed by the addition of 25 ml of 6 M HCl. The covered vessel was placed into the furnace for 24 h at 110 °C. Then, the sample was cooled to room temperature and transferred to the volumetric flask of its volume of 250 ml. The sample was diluted using citrate buffer solution. The pH value of the obtained sample was adjusted to 2.20 by adding 17 ml of NaOH solution (7.5 Mol/L). Then, the solution was diluted to the final volume of 250 ml using citrate buffer. Finally, the obtained sample was filtered through a 0,22 μm membrane filter. The obtained sample was analyzed by HPLC system in order to determine the qualitative and quantitative content of amino acids. For the quantitative evaluation of the amino acid profile, the calibration curve was built in

Table 1
The formulations of the experimental diets (g/kg).

Ingredients	Control diet	Test diet	Protein-free diet
Corn starch	644	666	762
Casein	118	–	–
Low-fat dried larvae	–	162	–
Sucrose	100	100	100
Vegetable oil	40	24	40
Fiber	50	–	50
Mineral AIN-93 M premix	35	35	35
Vitamin AIN-93 VX premix	10	10	10
Titanium dioxide	3	3	3
Total	1000	1000	1000

the range of 10 μMol/L – 200 μMol/L. The content of amino acids in the sample was calculated using the following equation:

$$\text{Content of Amino acid (g/100g)} = M_{AA}\frac{\frac{(V\times C_{AA})}{1000000}}{m_1}\times 100 \quad (2)$$

V – the volume of the prepared sample (L)
C_{AA} – concentration of amino acid (μMol/L)
M_{AA} – molar mass of amino acid (g/Mol)
m_1 – mass of the sample (g)

2.2.2. The digestibility trial in vitro (static)

The *in vitro* experiments were carried out according to the standardized INFOGEST protocol (Minekus et al., 2014). Briefly, the simulated salivary (SSF), gastric (SGF) and intestinal (SIF) fluids were prepared by mixing appropriate quantities of the electrolyte stock solutions. All the simulated fluids were diluted with deionized water to the final volumes of 500 ml.

The experiment of larvae protein digestibility *in vitro* included three main stages: oral, gastric and intestinal digestion. At the first stage (oral digestion), the homogenized sample containing the same quantity of the proteins was transferred to the vessel and 3.5 ml of the freshly prepared SSF solution was injected on the sample. Then, 25 μl of $CaCl_2$ (0.3 Mol/L) and 975 μl of deionized water were added into the vessel. Finally, human α-amylase solution (prepared in SSF) was added to the vessel to achieve 75 U/ml in the final mixture. The vessel was closed and placed into the thermal shaker for 2 min at 37 °C. At the second stage (gastric digestion), 7.5 ml of SGF solution, 1.6 ml of pepsin solution (25000 U/ml, prepared in SGF), 5 μl of $CaCl_2$ (0.3 Mol/L) solution, 0.2 ml of HCl (1 Mol/L) and 1 μl of deionized water were added to the sample. The pH value was adjusted to 3 by using HCl (6 Mol/L) solution. The vessel with prepared sample was covered and placed into the thermal shaker for 2 h at 37 °C. At the third stage (intestinal digestion), 11 ml of SIF solution, 5 ml of pancreatine solution (2000 U/ml, prepared in SIF), 2.5 ml of bile salts (160 mM, prepared in SIF), 40 μl of $CaCl_2$ (0.30 mol/L), 0.15 ml of NaOH (1 M) and 1.31 ml of deionized water were added to the sample. The pH value was adjusted to 7 by using NaOH (1 Mol/L) solution. The vessel was closed and placed into the thermal shaker for 2 h at 37 °C. The shaking speed in all the digestion stages was set to be 350 rpm.

After the third stage of the digestion, the sample was washed with deionized water and centrifuged at 4000 rpm (5 times). The supernatant was removed and the precipitate was placed into the drying furnace for 24 h at 55 °C in order to remove the excess of water.

2.2.3. The digestibility trial in vivo. Statement of human and animal rights

Ethics Committee of the State Food and Veterinary Service of the Republic of Lithuania approved all experimental procedures involving animals conformed to the European Community guiding principles (permission No. G2-89).

The digestibility of the proteins of *BSF* larvae was carried out according to FAO/WHO guidelines [18]. The rats used for this study were housed and cared in Lithuanian University of Health Sciences Biological Research Centre. During the study, animals were housed under conditions specified in the EU requirement.

Three diets were composed for the *in vivo* experiments as follows: test diet including dried low-fat *BSF* larvae raw material; control diet including casein (reference diet); protein-free diet (for the determination of the endogenic protein loss).

The diets were composed of 1% of vitamin premix, 3.5% of mineral premix, 4% of lipids, 5% of fibers, 10% of protein and 10% of sugar. Cornstarch was added in order to reach the final composition of 100%. In addition, 0.3% of titanium dioxide was used as an indigestible marker. The quantity of titanium dioxide in feed pellets and digesta was evaluated by spectrophotometric method as described by Short, Gorton, Wiseman, and Boorman (1996). The experimentally obtained absorption values were used for the calculation of the digestible indispensable amino acid score (DIAAS).

2.2.4. The preparation of feed pellets

All the ingredients were mixed and pellets were formed by using small capacity screw press Farmer-Duo P (Farmet, Czech Republic). The temperature of the pellets measured at the end of pressing process was 80 ± 5 °C.

During the study, 27 male albino Wistar rats (weighted of 195–230 g) were used. Each group consisted of 9 animals. Animals were adapted to the environment and feeding routine by feeding them with commercial diet for two days. On the third day, the commercial diet was replaced by the experimental diets: (a) test diet including dried low-fat *BSF* larvae raw material; (b) control diet including casein (reference diet); (c) protein-free diet. In total, the animals were fed with test diets for 9 days. The animals were fed 200 g/kg of feed daily at 9 am, water was available ad libitum. From fifth to tenth day animals were housed in individual cages to accurately measure the residue of the food and collect faeces. The residue of food and feaces were collected every day. Faeces were frozen at −20 ± 1 °C temperature before analysis. On the tenth day, 2 h after last meal, animals were asphyxiated with CO_2 and decapitation or cervical dislocation was performed. Abdomens were opened and dissection of the ileum section 20 cm anterior to ileal-caecal valve was done. Digesta were flushed using a 2 ml syringe with distilled deionized water. Collected digestas of each animal were frozen immediately at −20 ± 1 °C temperature. Due to the low amount of the digesta, the pooled sample of the digesta of 9 rats was prepared for further analyses.

Protein digestibility corrected amino acid score (PDCAAS) was calculated according to the following equation:

$$\text{PDCAAS (\%)} = \text{Limiting amino acid score} \times \text{True protein digestibility} \quad (3)$$

Limiting amino acid score is ratio of the first limiting amino acid in a gram of target food to that in a reference protein. The amino acid reference pattern for children aged 6 months to 3 years was used (FAO/WHO, 2013).

True protein digestibility (TPD) was calculated according to the following equation:

$$\text{TPD (\%)} = ((\text{N intake} - (\text{fecal N loss} - \text{metabolic N loss}))/\text{N intake}) \times 100 \quad (4)$$

The value of metabolic nitrogen loss was determined as the amount of fecal nitrogen produced by rats consuming a protein-free diet.

The report of the FAO Expert Consultation recommends a new, advanced method for assessing the quality of dietary proteins (FAO/WHO, 2013). According to the report, digestible indispensable amino acid score (DIAAS) replaces the PDCAAS.

DIAAS was calculated using the amino acid reference pattern for children aged 6 months to 3 years, which was used in conjunction with the following equation:

$$\text{DIAAS \%} = 100 \times [(\text{mg of digestible dietary indispensable amino acid in 1g of the dietary protein}) / (\text{mg of the same dietary indispensable amino acid in 1g of the reference protein}] \quad (5)$$

2.2.5. Statistical analysis

All the measurements were performed in triplicate. The data were analyzed by analysis of variance, in cases where significant interactions were determined, multiple comparisons were made. The differences were classified by a *Duncan* multiple comparison test ($P < 0.05$). IBM SPSS software, version 25.0 (Chicago, IL, USA,) was used for the statistical analysis of the data.

3. Results and discussion

3.1. Characterization of larvae raw material

The general plan of an experiment is presented in Fig. 1. At the first stage of the research, there was a need to select the larvae raw material for the production of the feed pellets. The selection was based on the level of the larvae protein digestibility *in vitro*. Therefore, two samples of larvae raw material were selected: dried and low-fat larvae. Before the *in vitro* digestibility experiment, the chemical analysis of the selected raw larvae materials was carried out and fat, moisture and ash content were determined. Since the biological value of proteins depends not only on their amount and digestibility but also on the content of amino acids, the analysis of amino acid profile was performed for both types of the larvae raw material. As seen in Table 2, the chemical analysis of the larvae raw material showed that the defatting process allowed to decrease total fat content of around 80%, leading to the reduced risk of lipid oxidation and the extended shelf-life of the larvae biomass. Moreover, the total protein content doubled after defatting of the larvae biomass was performed.

3.2. In vitro digestibility of larvae protein

The experiment of larvae protein digestibility *in vitro* involved two main stages: (a) the investigation of the protein digestibility of the dry larvae and low-fat larvae and (b) the investigation of the feed pellets produced using the larvae containing a reduced amount of fat. As seen in Table 3, the digestibility of the protein of dry larvae raw material was found to be only 48%. Meanwhile, the low-fat larvae raw material demonstrated higher digestibility of the proteins that was found to be 75%. Such difference in the digestibility efficiency can be explained by the presence of the high amount fat in the larvae raw material. Recently,

Table 2
The chemical composition of dried and dried low-fat larvae raw material and content of amino acids.

	Dried larvae raw material	Dried low-fat larvae raw material
Dry matter (%)	96.93 ± 0.15	94.75 ± 0.31
Protein (DM %)	27.54 ± 0.22	55.42 ± 0.90
Fat (DM %)	51.53 ± 0.56	9.85 ± 0.89
Ash (DM %)	6.59 ± 0.35	8.10 ± 0.73
Chitin (DM %)	3.87 ± 0.31	7.21 ± 0.24
Essential amino acids (DM %) Ile	1.35 ± 0.14	2.56 ± 0.26
Leu	1.70 ± 0.22	3.90 ± 0.35
Lys	1.16 ± 0.23	2.90 ± 0.38
His	1.27 ± 0.11	2.77 ± 0.19
Met	0.95 ± 0.25	2.08 ± 0.11
Cys	0.12 ± 0.08	0.32 ± 0.07
Phe	1.11 ± 0.19	2.30 ± 0.26
Tyr	1.45 ± 0.16	3.74 ± 0.41
Val	1.85 ± 0.23	3.81 ± 0.38
Thre	1.03 ± 0.15	2.26 ± 0.26
Trp	0.29 ± 0.08	0.37 ± 0.12
Non-Essential amino acids (DM %) Arg	1.25 ± 0.17	3.09 ± 0.33
Ala	1.63 ± 0.24	4.26 ± 0.33
Asp	2.43 ± 0.21	5.34 ± 0.41
Glu	3.10 ± 0.24	7.69 ± 0.76
Gly	1.29 ± 0.18	3.50 ± 0.35
Pro	1.60 ± 0.20	4.06 ± 0.37
Ser	1.00 ± 0.16	2.60 ± 0.29

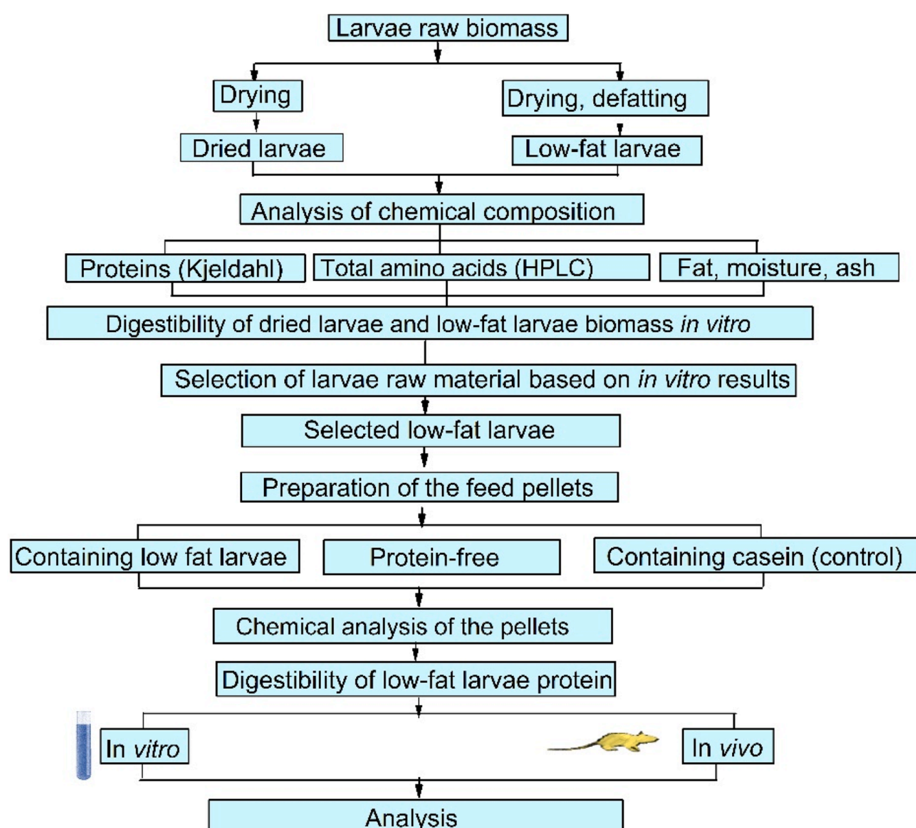

Fig. 1. The scheme of the experiment.

Table 3
The results of the protein digestibility *in vitro*.

Sample	The content of the protein in the sample before the digestion *in vitro*, %	The content of the protein in the sample after the digestion *in vitro*, %	Digestibility of the protein, %
Raw material			
Dried larvae raw material	27.54[a]	14.32[c]	48.00 [a]
Dried low-fat larvae raw material	55.42[b]	13.85[b]	75.00[b]
Casein	85.00[c]	0.00 [a]	100.00[c]
Feed			
The feed with low-fat larvae	8.96[a]	5.28[b]	41.00 [a]
The feed with casein	10.03[a]	0.00 [a]	100.00[b]

a, b – mean values within each column for separate material with different superscripts are different at $P < 0.05$

it was demonstrated that the digestibility of the proteins present in the complex systems (such as emulsions) could be significantly decreased. Such phenomenon occurs because the products of the lipid oxidation induce the aggregation of the larvae proteins, resulting in the limited access of the proteases to the protein complexes (Obando, Papastergiadis, Li, & De Meulenaer, 2015). For this reason, the digestibility of the proteins of dry larvae material is significantly lower comparing to those present in the low-fat larvae raw material. The similar results were obtained by Obando et al. (2015). They demonstrated that the digestibility of the casein in the presence of the oxidized fish oil amounted ~44%, whereas in the oil-free samples, the digestibility of casein reached 73%.

Based on the obtained results, the dried low-fat larvae raw material was selected for the experiments of protein digestibility *in vivo* and for the production of feed pellets. As the control sample, the pellets containing casein were produced. Both samples were digested parallel in order to maintain the same experimental conditions. As seen in Table 3 the digestibility of the pellets containing casein reached 100%. Meanwhile, the digestibility of the pellets containing low-fat raw larvae material was only 41%. Given that during the granulation process, the high temperature (90 °C) was applied for the production of the pellets, the proteins tend to aggregate and results in the restricted access of the enzymes to the protein aggregates. Possibly, for this reason, the efficiency of the digestibility was reduced. However, the casein was digested completely despite that the same parameters were used for the preparation of the pellets. According to Obando et al. (2015), the aggregation of the caseins can be induced by the interaction with lipid oxidation products. However, the impact of heat-induced aggregation remains restricted. Given that the low-fat larvae raw material consists of the different protein species, they are sensitive to the environmental factors (such as high temperature, pressure, pH, etc.). Therefore, the higher thermal stability of the casein comparing to the proteins present in the larvae raw material allows casein to be digested more efficiently. Chitin as a non-digestible fiber of the insects may also negatively affect the protein digestibility, since it is not degraded and absorbed in the small intestine (Marono et al., 2015).

Another factor that may determine the lower digestibility of the larvae protein comparing to casein is the processing of the larvae biomass. Given that the low-fat larvae material was obtained by mechanical fat extraction, it was affected by the temperature. As a result of temperature-induced protein denaturation, they may lose their solubility and digestibility. Therefore, it is likely that an alternative de-oiling approach, such as Soxhlet extraction, allow to avoid thermal denaturation of the protein leading to their increased digestibility.

3.3. *In vivo digestibility of the larvae proteins*

Calculated indispensable amino acid scores for low-fat larvae protein and casein are presented in Table 4. The limiting amino acid in low-fat larvae was found to be lysine with a score of 0.89. Some authors found that limiting amino acids of insects' proteins may vary depending on their species (Yang et al., 2014). Van Huis, Van Itterbeeck, Klunder, Mertens, Halloran, Muir, and Vantomme (2013) has also reported lysine to be the limiting amino acids in other edible insects.

True protein digestibility (TPD) determined using a rat assay for dried low-fat larvae and casein are presented in Table 5. The TPD value of 84.51% for low-fat larvae is in the range with the proteins digestibility values established by *in vivo* method for other insects. The TPD values for crickets (*Gryllus assimilis*), moth (*Cirina forda*), grasshopper (*Melanoplus foedus*) and termite (*Macrotermes nigeriensis*) were reported to be 80.82%, 81,71%, 84,98%, and 90.66% respectively (Oibiokpa, Akanya, Jigam, Saidu, & Egwim, 2018). The significantly lower TPD value for the low-fat larvae compared to casein may be attributed to the presence of chitin in larvae samples. Evaluation of the correlation between *in vitro* crude protein digestibility of insect meals from *Hermetia illucens* and chemical composition traits showed that chitin is the main constituent of the insect body able to affect the crude protein digestibility estimated by an *in vitro* enzymatic method (Marono et al., 2015).

PDCAAS values calculated using TPD and PD *in vitro* are presented in Table 4. As seen from the results, PDCAAS value is higher comparing to a value of PDCAAS determined *in vitro*. It was shown that *in vitro* methods used for PD determination may be more sensitive to different factors such as heat treatment or enzyme inhibitors in comparison to *in vivo* method using rats (Tavano, Neves, & da Silva Júnior, 2016). In our case, it can be assumed that the inhibitors (oxidized lipid derivatives) presence in the larvae raw material might have a different effect on protein digestibility. Therefore, the level of the protein digestibility *in vitro* and *in vivo* may differ.

The value of PDCAAS is usually determined using the value of the single faecal crude protein digestibility. Meanwhile, the determination of DIAAS is calculated based on the value of true ileal indispensable amino acid (IAA) digestibility what makes it more comprehensive. As

Table 5
Protein digestibility corrected amino acids scores of dried low-fat larvae and casein based on limiting amino acid score (AAS), and protein digestibility determined using *in vitro* and *in vivo* methods.

Protein source	Limiting AAS	True PD *in vivo*, %	PD *in vitro*, %	PDCAAS *in vivo*, %	PDCAAS *in vitro*, %
Casein	1.22 (Met + Cys)	96.69 ± 2.15	100.00 ± 0.00	100.00	100.00
Dried low-fat larvae	0.89 (Lys)	84.51 ± 1.87	41.00 ± 0.90	75.21	36.49

Table 4
Indispensable amino acids scores.*

Protein source	Thr	Ile	Leu	Lys	His	Met + Cys	Phe + Tyr	Trp	Val
Casein	1.29	1.47	1.39	1.35	1.45	1.22	1.54	1.53	1.49
Dried low-fat larvae	1.27	1.39	1.03	0.89	2.41	1.35	2.02	1.12	1.54

* Calculated based on FAO/WHO (2013) suggested pattern of amino acid requirements for the 0.5–3 years old child (g per kg protein).

Table 6
True ileal indispensable amino acids (IAA) digestibility, digestible IAA reference ratio and digestible indispensable amino acid score (DIAAS) of dried low-fat larvae and casein.

	True ileal IAA digestibility, %		Digestible IAA reference ratio	
	Dried low-fat larvae	Casein	Dried low-fat larvae	Casein
Thr	74.36 ± 3.10	88.58 ± 1.45	0.95	1.46
Ile	81.48 ± 2.12	95.34 ± 2.12	1.14	1.59
Leu	82.69 ± 1.95	95.68 ± 1.88	0.86	1.34
Lys	81.48 ± 2.45	96.44 ± 2.12	0.73	1.42
His	82.14 ± 1.54	93.58 ± 1.15	2.00	1.70
Met + Cys	82.10 ± 2.59	99.00 ± 0.80	1.11	1.24
Phe + Tyr	84.10 ± 1.86	94.23 ± 2.52	1.72	1.55
Trp	90.45 ± 2.05	98.14 ± 2.55	1.02	1.50
Val	83.92 ± 3.12	92.97 ± 2.16	1.31	1.51

DIAAS for casein – 124%.
DIAAS for dried low-fat larvae – 73%.

seen in Table 6, the digestibility of true ileal IAA as well as calculated digestible IAA reference ratio values based on FAO/WHO (2013) suggested a pattern of amino acid requirements for the 0.5–3 years old child (as in the case of PDCAAS) for dried low-fat larvae and casein. It was determined that the lowest IAA reference ratio for larvae raw material was common for lysine. Therefore, this ratio was used for the calculation of larvae DIAAS value that was found to be 73%. For casein, the lowest IAA reference ratio was common for Met + Cys. The calculated DIAAS value for casein was found to be 124%. Other authors report that the DIAAS value for the BSF larvae treated by different methods and digested *in vitro* reached up to 75% (Huang et al., 2019).

The dried low-fat larvae had higher PDCAAS values compared to those of DIAAS. The obtained results can be explained by the quality of the protein. High quality proteins such as casein are almost completely digested and absorbed in the small intestine (Chalupa-Krebzdak, Long, & Bohrer, 2018). Therefore, they show high ileal digestibility. However, the digestion and absorption of the lower quality protein may not be complete in the small intestine.

4. Conclusion

In this work, the *in vitro* and *in vivo* digestibility of the proteins of the oven dried Black soldier fly *Hermetia illucens* larvae was investigated. It was found that the *in vitro* protein digestibility of dry and low-fat larvae raw materials were 48% and 75% respectively. It was assumed that such difference is determined by the interaction of the proteins with lipid oxidation products. Such interaction restricts the access of enzymes, resulting in the low digestibility of the proteins. Further experiments have shown that the casein encapsulated in the food pellets was digested completely, while the pellets containing low-fat larvae raw material amounted only 41.00%. It was hypothesized that such difference was influenced by the technological parameters (mainly temperature) of the granulation process. It was also determined that the true digestibility of larvae protein and casein *in vivo* was 84.51% and 96.69% respectively. A comparison of the *in vitro* and *in vivo* digestibility of the proteins revealed that the *in vitro* method requires optimization. Therefore, further research must be carried out in order to determine the factors that may have influenced differences in digestibility values determined by different methods.

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CRediT authorship contribution statement

Lina Traksele: Conceptualization, Investigation, Writing - original draft, Writing - review & editing. **Vilma Speiciene:** Data curation, Formal analysis, Investigation, Writing - original draft. **Romualdas Smicius:** Methodology, Investigation, Formal analysis. **Gitana Alencikiene:** Project administration, Formal analysis. **Alvija Salaseviciene:** Resources, Funding acquisition, Data curation. **Galina Garmiene:** Supervision, Conceptualization, Writing - review & editing. **Vilma Zigmantaite:** Investigation, Methodology, Writing - original draft. **Ramune Grigaleviciute:** Investigation, Formal analysis. **Audrius Kucinskas:** Supervision, Resources, Funding acquisition, Writing - review & editing.

Ethics statement

All authors declare that they have read and adhere to the Publishing Ethics. All experimental procedures involving animals conformed to the European Community guiding principles and approved by the Ethics Committee of the State Food and Veterinary Service of the Republic of Lithuania (permission No. G2-89). Animals used for this study were housed and cared at the Lithuanian University of Health Sciences, Biological Research Centre. Animals were housed under conditions specified in the EU requirements, during the study.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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