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CHEMICAL COMPOSITION OF MEALWORM LARVAE (*TENEBRIO MOLITOR*) REARED IN SERBIA

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SUMMARY

The use of insects as "novel" and natural feed materials seems to be an attractive alternative protein source for poultry, pigs and fish since more than 80% of their chemical composition (dry weight basis) is crude protein and crude fat content. Therefore, the aim of this study was to determine some chemical composition parameters of powdered mealworm larvae, as a potential animal feed as well as human novel food. It was found that the crude protein (55.83%) and crude fat (25.19%) content was predominant, as well as that the content of nitrogen-free extract was low. Coincided with the high protein content, the levels of the most important amino acids were found to be considerable, especially lysine (3.18%) and threonine (1.34%). Crude fibre content was 7.15%, while ash was 4.84%. The samples were found to be rich in most nutritive elements, especially phosphorus (1.06%) and potassium (1.12%) in terms of macroelements, and zinc (138.2 mg/kg) in terms of microelements. Based on our research and other experimental results, it can be concluded that meals from the insects originating from the order Coleoptera, Tenebrionidae (mealworms) may be successfully used as feed material in diets of livestock animals, especially poultry.

Key words: *chemical composition, larvae, Serbia, Tenebrio molitor*

INTRODUCTION

The decrease of arable land suitable for agricultural production, along with the increasing demand for valuable protein sources for human population, has become a major global challenge (Jozefiak et al., 2016). Breeding insects is more environment-friendly and cheaper production, since it is more efficient than breeding large animals, it requires significantly less space and generates less waste (Durst and Shono, 2010). Additionally, the ability of insects to convert plant into animal biomass surpasses the ability of large animals (Premalatha et al., 2011). Insects are a constituent part of the traditional diet of more than 2 billion people, mostly in Asia, Africa, and South America (Van Huis et al., 2013). Moreover, insects are becoming recognized as an alternative source of high-quality protein along with other nutritionally valuable substances that are exploitable by the food industry (Schlüter et al., 2017).

A short reproduction cycle of insects and their high nutritious value, especially in protein and sulphur containing amino acids, are the most important prerequisites for ensuring cost-effective insect-based protein production. Furthermore, insects should be easy to rear in intensive production sites to guarantee a constant insect supply (Hossain and Blair, 2007).

The larval stage of the yellow mealworm beetle (*Tenebrio molitor* L., *Coleoptera*, *Tenebrionidae*) is considered to have a potential for industrial food and feed production (Van Huis, 2013). Insects are known to be resistant to temperature changes. The optimal rearing temperature for mealworms is 28 °C, although they easily survive 15 °C

for 48 h. On the other hand, they die very quickly under high humidity conditions (>70%). Among insects, the most widely reared for human food in Europe are yellow mealworm larvae (Caparros Megido et al., 2014).
Mealworm is considered to be one of the largest beetles (cca 15 mm long) infesting stored food products. It feeds on plant products and causes damage to their total mass and nutritive value. Besides, mealworm contaminates plant products with exudates, excrements, body parts and whole dead insects (Siemianowska et al., 2013).
Mealworms are common stored product pests, especially in mills, facilities for storing plant products, grocery shops and warehouses (Cosimi et al. 2009). They are easily reared and highly nutritious, and commonly used as feed for pets and many exotic animals to which they are usually served live, canned dried or lyophilized (Aguilar-Miranda et al., 2002; Veldkamp et al., 2012).
The nutritious value of insects depends on their life stage and rearing conditions, as well as the composition of the growth media (Makkar et al., 2014). Since the water content of live insects is rather high (average of 30%), this may cause problems in commercial feed milling operations. Therefore, it is imperative to standardize the processing conditions for meal production in order to obtain high-quality raw material (Jozefiak et al., 2016).
The first report on the proximate composition, amino acid profile, selected vitamins and minerals of mealworms was provided more than forty years ago. Barker et al. (1998) endeavored to deliver a more comprehensive summary of the nutritional composition of some whole invertebrates that can be used to evaluate the diets of animals in captive situations such as zoological parks. Their results of chemical composition showed 33% fat content of mealworm larvae.
Ravzanaadii et al. (2012) proposed the commercial use of mealworms as substitution of conventional protein sources. The authors also indicated that these insects contain approximately 46% protein and 33% fat (dry weight basis). Increasing popularity and production of mealworm larvae for human consumption in Europe is giving them a title of a novel source of food lipids, as well (Zhao et al., 2016). Siemianowska et al. (2013) found that the fresh larvae contained more total protein, total fat and ash compared to meats of farm animals, fish and eggs. Mealworms contain about 20% of fat (dry weight basis) which is even higher than soybean (Christensen, 2009). High amounts of crude protein (60%, dry weight basis) and fat (40%, dry weight basis) were reported by Finke (2015) and Barker et al. (1998), respectively. The content of essential amino acids meets the requirements for adults recommended by WHO (FAO/WHO/UNU, 1986). Finke (2002) pointed out that about 75% of the total fatty acids (FA) are unsaturated. The oleic acid (44%) was the most abundant FA. Fresh mealworm larvae contain 40% dry matter and 1–4.5% crude ash (Józefiak et al., 2016). Józefiak et al. (2016) concluded that the abovementioned features of mealworm larvae are motives for limiting their use in broiler diets. Insect species considered as animal feed have high levels of protein and suitable amino acid profiles to be used as feedstuffs (Makkar et al., 2014) and can be used as a natural nutrient source for poultry (Józefiak et al., 2016).
According to the investigation of Siemianowska et al. (2013), fresh and powdered mealworm larvae contain higher amounts of phosphorus, magnesium, zinc, iron, copper and manganese compared to eggs and meat obtained from farm animals in Poland. Particularly valuable were high concentrations of magnesium, zinc, iron, copper and manganese. The results of Ravzanaadii et al. (2012) show that total protein content of *T. molitor* larvae was 46.44%; resulted lower protein content compare to the previous study of Aguilar-Miranda et al. (2002), which was 58.4%. Mealworm, as well as other insects (Ravzanaadii et al., 2012), is a poor source of calcium (434.59 mg/kg for larvae), but on the other hand it contains a high level of phosphorus (Allen et al., 1996). A diet high in calcium fed to insects may increase their calcium content. Feeding alfalfa or commercially balanced diets to mealworms rather than bran or cereal grain substrates can significantly improve their nutritional quality (Frye, 1991).
To the best of the authors' knowledge, no investigation has been carried out to date to specifically correlate the chemical composition of mealworm larvae reared in Serbia. Moreover, no study has been carried out to determine their protein content, amino acid composition and mineral composition in our country. Therefore, the aim of the study was to determine some parameters of the chemical composition of the powdered mealworm larvae as potential animal feed as well as human novel food.

MATERIAL AND METHODS

Rearing of insects

The beetles were obtained from the Department of Plant and Environmental Protection, Faculty of Agriculture, University of Novi Sad, Serbia. Mealworm specimens were maintained in an incubator in controlled conditions (temperature: 27±1 °C, photoperiod: 0 h light - 24 h dark, relative humidity: 55%) in 12l plastic containers (20 cm x 40 cm x 15 cm). The insects were grown on a food mixture which contained 400 g of wheat bran, 250 g of dried barley germs, 200 g of dried oat germs, 50 g of barley flakes, 50 g oat flakes and 50g of powdered beer yeast. Pieces of apple were spread over the food mixture to provide additional moisture to the insects.

Preparing insects for drying and cooking

Insects were sieved through a sieve with large holes (the diameter of each hole was 2.5 mm) and the remaining insect parts were removed with weak wind flow produced by a hair dryer. The sieved larvae were moved to a sieve with smaller holes and with weaker wind flow the remains of the insect bodies were removed. Afterwards, the cleaned larvae were transferred into 2 l plastic container, and gently washed under water jet. The insects were then placed into a container with boiling water and cooked for 180 seconds. Thereafter, the entire content of the cooking pot was filtered through a sieve in order to remove water, and then the larvae were transferred to a filter paper in a thin layer to allow the excessive water to evaporate during 24 h. The dried insects were collected and placed on a new filter paper and left to dry for one more day (24 h).

Chemical analysis

Moisture content (MC) was determined as weight loss after drying (AOAC Official Method 934.01). Crude protein (CP) was analyzed according to standard Kjeldahl method (AOAC Official Method 2001.11), while crude fat content (EE) was determined as petroleum ether extract (AOAC Official Method 991.36). Crude fiber (CF) was determined according to AOCS Approved Procedure Ba 6a-05 (AOCS, 2005). Ash content was obtained by applying dry ashing at 600 °C during 2 hours (AOAC Official Method 942.05). Nitrogen-free extract (NFE) was calculated as follows: NFE (%) = 100 - MC (%) - CP (%) - EE (%) - CF (%) - Ash (%).

Mineral analysis was performed after dissolving the ash in diluted hydrochloric acid. Calcium, sodium and potassium were detected on flame photometer (Sherwood, UK), while phosphorus was determined on UV-Vis spectrophotometer (EU Instruments, China). Microelements and magnesium were analyzed on atomic absorption spectrometer (Perkin Elmer, Singapore).

Amino acids were determined on an Agilent Technologies 1260 series HPLC system (Agilent Technologies, USA) by applying previously established analytical conditions (Jajić et al., 2013).

RESULTS AND DISCUSSION

The chemical composition of mealworm larvae is presented in Table 1. The obtained results, regarding the basic parameters, showed predominant content of crude protein (55.83%) and crude fat (25.19%) and low content of nitrogen-free extract. Crude fibre content was 7.15%, while ash was 4.84%. The samples were found to be rich in most nutritive elements, especially phosphorus (1.06%) and potassium (1.12%) in terms of macroelements, and zinc (138.2 mg/kg) in terms of microelements. Since protein content was rather high, the essential amino acid portion was of great importance, especially lysine (3.18%) and threonine (1.34%).

Table 1. Chemical analysis of insects powder, mealworm larvae (n=5)

Parameter	Dry Matter (DM) basis		
	Mean value (Xsr)	Standard deviation (SD)	Coefficient of variation (CV), %
Basic parameters			
Crude protein, %	55.83	0.893	1.60
Crude fat, %	25.19	1.133	4.50
Crude fibre, %	7.15	0.192	2.69
Ash, %	4.84	0.331	6.84
Nitrogen-free extract (NFE), %	3.68	0.503	13.67
Macroelements			
Calcium (Ca), %	0.21	0.016	7.62
Phosphorus (P), %	1.06	0.105	9.91
Sodium (Na), %	0.21	0.011	5.24
Potassium (K), %	1.12	0.035	3.13
Magnesium (Mg), %	0.30	0.007	2.33
Microelements			
Ferrum (Fe), mg/kg	71.5	1.563	2.19
Zinc (Zn), mg/kg	138.2	7.389	5.35
Copper (Cu), mg/kg	19.4	1.520	7.86
Manganese (Mn), mg/kg	5.7	0.233	4.09

Amino acids, % *			
Threonine (THR)	1.338 (23.96)	0.093 (1.661)	6.95 (6.93)
Methionine (MET)	0.540 (9.67)	0.035 (0.620)	6.48 (6.41)
Lysine (LYS)	3.183 (57.02)	0.055 (0.977)	1.73 (1.71)

Legend: *values in brackets in mg/g protein

Our results were in agreement with the study performed by Ng et al. (2001) on mealworm meal. The authors reported 57.6% crude protein, 28.6% lipid, 4.5% ash and 6.9% crude fibre (results on a dry matter basis). Our results were similar regarding crude protein, crude fibre and ash content, but lower in fat content. Somewhat higher protein content of mealworm larvae (58.4%) was obtained by Aguilar-Miranda et al. (2002), while fat content was considerably higher (32.40%) in comparison with our results.

As opposed to aforementioned studies, the results in our study differed greatly from results reported by Siemianowska et al. (2013). The authors established considerably lower protein (44.72%) and ash (3.69%) content. On the other hand, fat content (42.48%) obtained by the same authors was noticeably high compared with our results. In the report by Ravzanaadii et al. (2012) larvae samples contained 46.44% protein and 32.87% fat. This was far lower in terms of protein content and considerably higher regarding fat content compared to our results.

Most species of insects contain a low level of calcium because insects as invertebrates do not have a mineralized skeleton. Calcium levels are typically lower than 0.3% dry matter (Barker et al., 1998; Finke; 2002). In our study, a level of 0.22% was found. In spite of low calcium levels in mealworm, we obtained rather high phosphorus content of 1.06%. This was in agreement with the results presented by Allen et al. (1996), who obtained 0.98% of this mineral.

WHO (2007) established amino acids requirements for human nutrition: threonine 23 mg/g protein; methionine 16.0 mg/g protein; lysine 45.0 mg/g protein. In the investigation by Finke (2002) on amino acids content in mealworm larvae, the authors found 41.8, 12.8 and 54.5 mg/g protein for threonine, methionine and lysine, respectively. In our study, threonine content was 1.34% (23.96 mg/g protein). This was lower compared to the results of previous studies by Aguilar-Miranda et al. (2002) and Ravzanaadi et al. (2012), in which it amounted to 2.70% and 1.81%, respectively. Methionine content in our study (0.54% or 9.67 mg/g protein) was also lower in comparison with the previous investigation (0.67%) by Ravzanaadi et al. (2012). On the other hand, lysine content obtained in our investigation (3.18% or 57.02 mg/g protein) was higher than the values presented by Aguilar-Miranda et al. (2002) and Ravzanaadi et al. (2012), which were 1.60% and 2.91%, respectively. The results obtained for threonine and lysine contents in our investigation were also compared with essential human requirements of amino acid (FAO/WHO/UNO, 1986), showing that the amino acid composition met the requirements for adult people amounting to 0.9% and 1.6%, respectively.

CONCLUSION

The use of insects as “novel” and natural feed materials seems to be an attractive alternative protein source for poultry, pigs and fish since more than 80% of the chemical composition (dry weight basis) is crude protein and crude fat content. Based on our research and other experimental results, it can be concluded that meals from insects belonging to the family *Tenebrionidae* (mealworms) have a potential to be successfully used as feed material in farm animal diets, especially poultry. These results show new ways of consuming mealworms preferably as a source of animal feed, which will find its place in the market of South-East Europe region. Further research is clearly required to ensure that mealworm proteins are safe for animal consumption, especially if the worms were reared on waste materials. The effect of mealworm-based diets on meat quality of farm animals will need further evaluation.

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