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Investigation of fly larvae *Lucilia Caesar* application in pet feed composition

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Abstract. The biomass of insect larvae is world-widely used as a valuable raw material for the pharmaceutical, microbiological, cosmetic industry and feeding production, also in the food industry. There is certain complex technology for processing biomass of insect larvae, which affords to isolate many physiologically active substances - chitin, antimicrobial peptides, fatty acids mixture, organic forms of mineral substances, hormones, etc.
The company New Biotechnology (Lipetsk, Russia) has developed a technical process for producing of the protein-lipid preparation (commercial name is Zooprotein) based on the fly larvae of the species *Lucilia Caesar*. The utilization of food waste as a substrate, unpretentiousness to cultivating environment and high protein content are capable of considering insects of the species *Lucilia Caesar* as a promising object of cultivation and a reliable, cheap, replenishable source of nutrients for resource-saving process of the feed production.
On the bases of ITMO University, an investigation is being conducted on the qualitative composition of the Zooprotein and the possibility of pet feed application. Cats are the most demanding animals to the quantitative and qualitative composition of protein fractions of feed. In present research an evidence-based calculation of the balance of the Zooprotein composition is presented as a feed component for cats during growth. Accordingly, the unique chemical composition of the development product based on fly larvae *Lucilia Caesar* makes possible to maintain that it is a promising functional ingredient in feeding rations for various animal species.

Key words: fly larvae, *Lucilia Caesar*, feed production, protein sources, raw materials; nutrients, fatty acids, amino acids, protein-lipid preparation, energy, pets.

INTRODUCTION

At the present time, the production of prepared animal feed is one of the leading sectors of the agro-industrial complex that includes manufacturing of animal feed, feed additives, drugs, etc., and is based on modern achievements of microbiological, pharmaceutical, food, chemical and other manufacturing sectors (Afanasyev, 2012).

About 4 billion tons of animal feed is produced annually in the world, whereof only 600 million tons are creep feed according to the International Federation of the Feed Industry (FAO, 2018).

Nowadays, secondary raw ingredients of animal and vegetable origin are widely used in feed production: whey, buttermilk, meat, bone, blood and fish meals, vegetable oil cake, etc. (Sencic et al., 2011). The main components of plant origin, which are contain high level of carbohydrates for animal feed are: grain varieties (wheat, barley, oats, millet, triticale, corn) – up to 85%; oilseed meal (flax, soybean, sunflower) – up to 15–25%; legumes with a high protein content (soybeans, beans, peas, chickpeas, lupins) – up to 45%; oilseeds (rapeseed, sunflower, cotton, camelina, colza) – up to 15%; hay, straw, other high-fiber roughage; grain and food industry waste; amino acids; mineral mixtures; vitamin supplements; antibiotics and biostimulants (Yasir et al., 2017). However, the use of secondary food raw materials for the production of feed is currently in competition with their involvement in the composition of food products. The use of biotechnology makes it possible to obtain valuable food ingredients and food products from secondary raw materials of animal and vegetable origin (Kuznetsova et al., 2014; Suchkova et al., 2014). Thus, the use of secondary food resources for the production of products with higher added value reduces the attractiveness of this raw material for feed production.

Increased world population, environmental effects, augmented land use resources and growth of demand for nutrients and non-renewable energy are predicted for coming decades. About 70% of all agricultural land uses is livestock production (Zanten et al., 2016) The global demand for livestock products is expected to almost double by 2050, thus innovative production solutions are required. Insect farming has been suggested as a good alternative to conventional livestock farming for future feed production (Van, 2013).

Growing insects in special farms under controlled conditions has several advantages. One of the advantages is the possibility of growing insects based on organic waste, which confirms the environmental and resource-saving aspects of this production. In addition, insects farming produces less greenhouse gases and ammonia, for instance, compared to cattle or pigs (Halloran et al., 2014). Cultivation of insects is also economically advantageous, since this type of production requires little resources in terms of land and water as contrasted with the cost of breeding cattle, etc.

However, at present, the cultivation of insects has not received a wide scale in the world. Most often, industrial enterprises of insect farming are a family business, and their main focuses are growing flour worms, crickets and grasshoppers for feeding animals in zoos and processing them for pet food (Belluco et al., 2013).

Aquaculture (fish farming, crustaceans and other aquatic animals) is one of the fastest extending industries. Nevertheless, the main obstacle to sustainable growth in the industry is the feed cost, in particular fish meal and fish oil (Van, 2013). Approximately 10% of fish products are processed into fish meal, and stocks of ocean fish are depleted due to overfishing as feed. The need for effective competition in this aspect indicates the feasibility of finding new sources of raw materials for feed production, for instance, various types of insects (Raubenheimer & Rothman, 2011).

The urgency of finding alternative feed of animal origin in regard to fish meal and soy meal has led to the recognition of insect protein in the market (Van et al., 2015). Insect-based feed products can provide an alternative to fish meal and soy meal, which are the most popular components used in feed mixtures for aquaculture and animal husbandry (Nadtochii et al., 2017) That fact creates prerequisites for investigating the possibility of using insects, which are widely distributed in nature, as a renewable source of protein in reference to feed industry, that has become more cost-effective. Studies on livestock have shown that insect meal is poised to replace fish meal in feed, which reduce overfishing (Makkar et al., 2014).

One of the advantages of using insects as an alternative source of protein in feed is that some insects recycle waste material into an extremely useful animal protein.

Many researchers have reported the insect larvae application as a renewable source of protein for pigs, birds, and fish feeding (Awoniyi et al., 2004; Charlton et al., 2015; Schiavone et al., 2017).

It is estimated that there are about 2,000 species of edible insects in the world; of these species, those that are considered most suitable for animal feed production include worms (*Tenenbrio Molitor, Zophobas Morio, Alphitobus Diaperinus),* and the larvae of the black soldier fly (*Hermetia Illucens*) or house flies (*Musca Domestica*) (Jongema, 2014).

Insects are a source of energy, protein, fat, minerals and vitamins, while the energy content is on par with other sources of fresh meat (based on fresh weight); the exception is pork due to its high fat content (Rumpold & Schluter, 2013a). The average estimates show that energy levels are around 400–500 kcal per 100 g of dry matter, which makes it comparable to other protein sources (Rumpold & Schluter, 2015). It has been reported that the inclusion of insects in broiler chicken feed will not reduce growth rates, and in some cases increase the growth rates of chickens (Rumpold & Schluter, 2013b). The best feed conversion was observed in chickens fed insects (Marono, 2017).

Many scientists have concluded in their research that fly larvae meal has a suitable nutritional composition and can serve as a substitute for fish meal, as well as other sources of protein commonly used in animal nutrition (Ramos et al., 2002; Magalhaes et al., 2017; Wang et al., 2017).

Table 1 is represented a comparison between fish meal, sunflower meal, soy meal and fly larvae meal. As can be seen from this table, the fly larvae meal surpasses some other traditional protein sources used in animal feeding, but, in some cases, is inferior (Aniebo et al., 2011; De Koning, 2005).

Table 1. Comparison of the nutritional value of fly larvae meal with indicators of other commonly used protein sources

Indicators	Fly larvae meal	Fish meal	Soy meal	Sunflower meal (after squeezing
Composition (dry matter), %				
Crude protein	50.86	68.84	49.44	35.56
Essential extract	27.32	5.66	0.45	1.22
Crude Fiber	8.10	1.07	7.87	26.67
Ash	6.75	20.38	7.64	–
Amino acid profile:				
Lysine	6.52	8.86	3.02	1.11
Histidine	3.34	2.88	1.31	0.61
Threonine	2.19	5.34	1.93	1.17
Arginine	6.26	7.04	3.53	2.56
Valin	3.90	6.83	2.33	1.78
Methionine	2.46	2.35	0.70	0.56
Isoleucine	3.30	5.55	2.20	1.11
Leucine	6.86	8.00	3.81	1.78
Phenylalanine	4.28	4.91	2.43	1.28
Tryptophan	–	1.07	0.83	0.50
Cystine	0.56	4.48	0.74	0.56
Tyrosine	3.14	4.70	2.15	–

In Table 1 is shown that the fly larvae meal has a high content of crude protein than soy and sunflower meal, but lower than fish meal. The fly larvae meal is represented by an excellent amino acid composition as well. Besides the fly larvae meal has higher concentrations of histidine and methionine compounds than fish meal.

Insects are able to thrive on a wide range of substrates, for instance, food waste consisting of animal and vegetable waste (Surendra et al., 2016). These food wastes can be used in order to breed insects, that are a cheap and even income-generating substrate. In addition, studies have shown that one of the insect species allowed for use as animal feed, larvae of black soldier flies (*Hermetia Illucens*) reduced organic waste by 60% in 10 days (Salomone et al., 2017). The fly larvae can be grown on a wide range of wastes thereby offering a solution to the growing problem of organic waste. In concordance with above mentioned, protein production for livestock feed may be derived from by-products and waste resulting from human consumption (Diener et al., 2009).

There are several key ways that agricultural insects could be a profitable alternative for agricultural protein production. Mass insect cultivation requires 50–90% less land than conventional agriculture per kilogram of protein, and can reduce greenhouse gas emissions from livestock about 50% by 2050 (Tilman & Clark, 2014).

The fly larvae of the species *Lucilia Caesar* are unpretentious to the conditions of cultivation, in particular, they are grown on food waste as a substrate. Despite this, the results of a previous study were obtained, which showed the antimicrobial activity of *L. caesar* larvae using indicator microorganisms, which are pathogenic for humans and birds (*Salmonella enteritidis, Staphylococcus aureus, Enterococcus faecium, Listeria monocytogenes, Clostridium perfringens, Escherichia coli 015, Pseudomonas aeruginosa*). Russian scientists have shown that growing *L. caesar* fly larvae on forcemeat, artificially contaminated, caused growth inhibition of pathogenic bacteria from the substrate. The obtained results on the antimicrobial activity of *L. caesar* larvae towards a number of bacterial infections in humans and birds allows us to consider the protein-lipid preparation from dried and crushed fly larvae of the species *Lucilia Caesar* as a promising feed production resource for pets admissible to the safety standards (Teymurazov et al., 2018).

However, the use of organic waste as a feed ingredient for insects is another legislative issue. In the short term, legislation in frame of European Union (EU) does not allow for the use of protein from insects as feed for animals. The Transmissible Spongiform Encephalopathies regulation at the European level also blocks the introduction of insects as an ingredient for feed (Regulation (EC) No 999/2001). Currently, in other regions the use of fly larvae protein is an acceptable procedure where this practice has long been conducted and large-scale production has been established. The largest producers of fly larvae protein as an additive in pet feed are regions of North and South America, Eastern Europe, Asia Pacific, Middle East and Africa (Kone et al., 2017; Kenis et al., 2018).

In this regard, the search for new forms of high-quality and safe raw materials are the overriding priority of productive feed production.

In present study, we consider the possibility of using the larvae of the *Lucilia caesar* flies as a raw ingredient in the production of high-quality feed for adult cats.

MATERIALS AND METHODS

Sampling

As objects of research was used following raw ingredients:

– fish meal in accordance with GOST (the State Standard of the Russian Federation) No 2116/2000 'Feed meal from fish, marine mammals, crustaceans and invertebrates'.

– meat and bone meal in accordance with GOST (the State Standard of the Russian Federation) No 17536/1982 'Feed meal of animal origin'.

– the protein-lipid preparation (commercial name – 'Zooprotein') was manufactured at the enterprise 'New Biotechnologies' LLC (Lipetsk) according to the technical specifications. This company specializes in the production of high-protein product from dried and crushed fly larvae of the species *Lucilia Caesar*.

The fly larvae cultivation

Growing fly larvae (species *Lucilia Caesar*) was carried out in a special chamber (cage) by placing eggs from adult flies on a nutrient medium. The nutrient medium served as crushed meat obtained from a forced killed and fallen bird, crushed meat was supplied by the poultry factory of the Lipetsk region (Russia). The nutrient medium was used for growing the fly larvae in the amount of a daily portion 1,000 ± 5% grams per control group (Teymurazov et al., 2018).

The fly larvae incubation was implemented without special lighting at 25 ± 2 °C with 70% relative humidity for 5 days. Throughout the process, constant air ventilation was maintained to prevent the accumulation of heat, to provide a certain level of oxygen and to remove excess CO^2. According to data (Liland et al., 2017), the larvae amount per cage was maintained at an average 830,000 larvae m^{-3}.

The manufacture of the protein-lipid preparation 'Zooprotein'

The fly larvae samples were subjected by heat treatment at 60–70 °C during the day until the mass fraction of moisture was no more than 4%. As a result, dried samples of the preparation Zooprotein were obtained, which were packed in sterile plastic containers and transported to the laboratory for further investigation.

Determination of amino acid composition of the protein-lipid preparation 'Zooprotein'

The tests were carried out at the laboratory of the organization Limited Liability Company Research and Testing Center 'CHERKIZOVO' on the amino acid analyzer HITACHI L–8900.

The total content determination of (free and bound forms) of individual amino acids was conducted according to GOST (the State Standard of the Russian Federation) No 32195/2013 'Feed, all-mash. Method for determination of amino acid content'.

Determination of fatty acid composition of the protein-lipid preparation 'Zooprotein'

Tests to determine the fatty acid composition of the preparation Zooprotein were carried out at the laboratory of the organization Limited Liability Company Research and Testing Center 'CHERKIZOVO' (Russia), according to the procedure described below.

A drop of fat (5 mg) was diluted with 1 ml of dichloromethane, 40 µl of BSTFA was added, kept for 4 hours and analyzed on a chromatography-mass spectrometry system including a Finnigan Trace GC Ultra gas chromatograph and a Finnigan PolarisQ mass spectrometer. The analysis was performed in the mode without dividing the flow with the start of purging after 0.1 min; the temperature of the injector (sampling device) was 260 °C; helium carrier gas flow rate was 1 mL min^{-1}. Column is DB–5 ms 30 m–0.25 mm–0.25 µm. The initial temperature of the chromatographic oven was 60 °C (2 min), then sample was heated to 310 °C at a rate of 6 °C min^{-1} and kept at this temperature for 12 min. The interface and the ion source temperatures of the mass spectrometer were 230 °C and 220 °C, respectively. The mass range is 40–550 atomic mass units (amu). Identification was performed by expert analysis using the NIST MS mass spectra collection. The assessment of the relative content was carried out according to the method of internal normalization.

Calculating BMR:

Basal metabolic rate (BMR) is the daily energy amount expended in the period of sleep in 12–18 hours after eating under normal temperature conditions. For cats and dogs weighing more than 2 kg, the amount of basal energy is determined by the following formula:

$$\text{BMR (kcal day}^{-1}) = 30 \times \text{body weight}^{*} + 70 \quad (1)$$

where *the body weight of an animal is 2.5 kg.

Calculating MER:

Maintenance energy requirements (MER) are determined by the amount of energy consumed by a moderately active animal under thermoneutral conditions. Maintenance energy for cats weighing more than 2 kg (kcal day^{-1}) is following:

$$\text{MER} = 1.4 \times \text{BMR} \quad (2)$$

Calculating ME:

The daily requirement for the animal's metabolizable energy (kcal day^{-1}) is calculated considering the increase ratio in the energy needs of the animal. The increase ratio in the energy needs of the cat during the growth period (3–6 months) is 1.6. Thus, the daily needs for metabolizable energy of a cat (3–6 months of age) is equal to:

$$\text{ME} = 1.6 \times \text{MER} \quad (3)$$

Calculating metabolizable energy (ME in feed):

For predictive equations of ME in prepared pet foods for cats and dogs (dry and wet) the following 4-step-calculation can be used (NRC 2006):

$$\text{Gross Energy (GE), kcal} = (5.7 \times \%\ \text{protein}) + (9.4 \times \%\ \text{fat}) + \\ +[4.1 \times (\%\ \text{NFE}^{**} + \%\ \text{crude fibre})] \quad (4)$$

$$\text{Energy Digestibility (ED), \%} = 87.9 - (0.88 \times \%\ \text{crude fibre in DM}) \quad (5)$$

$$\text{Digestible Energy (DE), kcal} = (\text{GE} \times \text{ED}) \div 100 \quad (6)$$

$$\text{Metabolizable Energy (ME in feed), kcal} = \text{DE} - (0.77 \times \%\ \text{protein}) \quad (7)$$

where **NFE – Nitrogen free extract is 0.4.

The satisfaction degree assessment of the daily need for macro- and micronutrients

$$\text{Units } 1{,}000 \text{ kcal}^{-1} = \frac{\text{Nutrient requirement per day (Units kg}^{-1}\text{metabolic BW)} \times 1{,}000}{\text{DER (kcal kg}^{-1}\text{metabolic BW)}} \quad (8)$$

Statistical analysis

Studies of the chemical properties of the samples were carried out in triplicate, the data were processed by the method of mathematical statistics with using MS Excel, finding the confidence interval and a probability of 0.95.

RESULTS AND DISCUSSION

Production waste can potentially be used to grow insects, in particular fly larvae (Diener et al., 2009). A comprehensive scheme for waste processing industry using fly larvae (species *Lucilia Caesar*) is represented in Fig. 1, which makes it possible to turn a large amount of organic waste into fertilizers for the agricultural industry, as well as protein-rich biomass, which is considered as an alternative raw material source for feed production. A comparative analysis of the chemical composition of traditional raw materials for feed production is represented in Table 2.

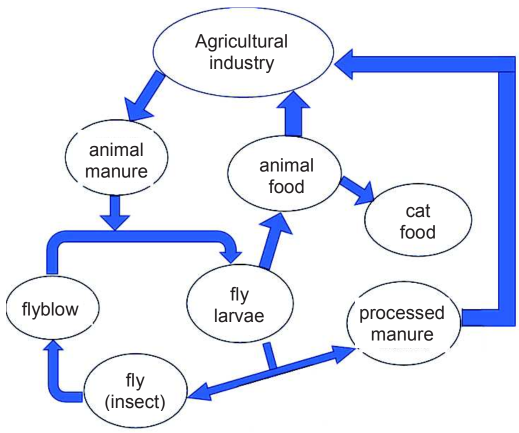

Figure 1. Resource-saving technologies based on fly larvae.

Based on the data in Table 2, it can be noted that Zooprotein is slightly inferior to fish meal and meat and bone meal in terms of crude protein (by 5.8%) and fat (by 2.4 and 1.4%, respectively), however is significantly superior in fiber content (by 8.1 and 6.1%).

Table 2. The chemical composition of raw materials for feed production

Indicators	Raw material resources, g 100 g^{-1} sample		
	Fish meal	Meat and bone meal	Zooprotein
Crude protein, no less than	50.0	50.0	44.2
Crude fat, no more than	14.0	13.0	11.6
Crude fiber, no more than	0.0	2.0	8.1
Moisture content, no more than	12.0	12.0	12.0

According to the analysis of the table data, Zooprotein can be considered as a raw ingredient in animal feed. In present research, this hypothesis was evaluated from thepoint of view to satisfy the needs of cats, taking into consideration that cats are carnivorous and most demanding on the protein-lipid composition of feeding rations. Specific differences in the cat nutrition compared with dogs are due to behavioral, anatomical, physiological and metabolic features (Hill et al., 2009). The diet of cats is distinguished by an increased need for protein as contrasted with the diets of dogs, in particular, cats require 50% more protein during their growth period and in adult status.

The studied samples are considered as dry feed for kittens during their intensive growth (at the age of 6 months), when there is an increased need for proteins, fats, etc. Based on the available data, the daily need for six months old kittens at body weight 2.5 kg is 100 g for female animal and 110 g for male animal of dry feed per day (Lewis et al., 1987). Table 3 is shown the calculation of animal's need satisfaction in the nutrients calculated as 100 g of dry matter due to the consumption of the test samples during the growth period.

Table 3. The cat's need satisfaction in nutrients, per day

Estimated factor	Cat needs for growth, pregnancy and lactation day^{-1}	Fish meal	Meat and bone meal	Zooprotein
Gross Energy, kcal g^{-1} of dry matter	≥ 4.5	4.7*	4.7*	4.5*
Protein, g 100 g^{-1} of dry matter	> 35.0	56.8*	56.8*	50.2*
Fat, g 100 g^{-1} of dry matter	≥ 17.0	15.9	14.8	13.2
Fiber, g 100 g^{-1} of dry matter	< 5.0	0.0	2.3*	9.2**

*completely satisfies the need in nutrient; **exceeds nutrient requirements.

The daily requirement for the animal's metabolizable energy (ME) was determined to 325 kcal day^{-1}, which is based on the calculation of the basal metabolic rate (BMR), equal to 145 kcal day^{-1}, and maintenance energy requirements (MER), equal to 203 kcal day^{-1}.

The metabolizable energy (ME in feed) was calculated separately for each test sample: fish meal – 372.29 kcal, meat and bone meal – 363.36 kcal and Zooprotein – 316.5 kcal per 100 g sample. The metabolizable energy (ME in feed) based on calculation of following indicators: gross energy (GE), equal for fish meal – 474.9, for meat and bone meal – 472.7, for Zooprotein – 445.1 kcal per 100 g; energy digestibility (ED), equal for fish meal – 87.9, for meat and bone meal – 85.9, for Zooprotein – 79.8; digestible energy (DE), equal for fish meal – 416.03, for meat and bone meal – 407.1, for Zooprotein – 355.2 kcal. Consequently, the daily requirement in feed for providing the animal with the necessary energy amounted to 87.3 for fish meal, for meat and bone meal – 89.4, for Zooprotein – 102.7, which corresponds to the need in feed for kittens aged 6 months, equal to 100–105 g.

Thus, Zooprotein is the balanced feed resource for cats and not inferior to other studied samples. However, it is important that the animal receives a balanced amount of energy and nutrients with the feed including essential feed components. If animals receive a diet that is unbalanced there may be a shortage of all nutrients. This deficiency is especially often observed during the period of growth and during lactation, in the case of feeding animals with low-calorie industrial feed or homemade ration. In addition, to satisfy the energy needs, the animal is forced to eat a large amount of feed. As a result, the kitten's belly hangs down, growth slows down, etc. (Baker, 1991).

Since cats are carnivores, it is especially significant to study their needs for amino acids by the consumption of various types of raw materials. It is important to evaluate the cats needs for certain amino acids, in particular for arginine, during their growth. In this regard, the usual diet of cats should contain many animal components with a fairly high protein content, including arginine. Table 4 is represented the protein component of Zooprotein, consisting of 10 amino acids, among which the arginine, methionine and tryptophan are indispensable in feeding kittens.

Table 4. Amino acid composition of Zooprotein

Amino Acids	The content of essential amino acids in Zooprotein	
	g 100 g^{-1} of protein	g 100 g^{-1} of product
Arginine	3.03	1.34
Histidine	1.97	0.87
Isoleucine	3.51	1.55
Leucine	5.63	2.49
Lysine	6.04	2.67
Methionine	1.92	0.85
Methionine + cystine	2.83	1.25
Phenylalanine	5.77	2.55
Threonine	3.55	1.57
Tryptophan	1.11	0.49
Valine	4.73	2.09

The lipid component of the feed. is not a primary factor in feeding cats However, investigations have been clearly shown that domestic cats cannot synthesize linoleic acid (LA) and have limited ability to synthesize arachidonic acid (AA) during metabolism (Bukkens et al., 1997; Bauer, 2004).

The most important essential fatty acid is linoleic acid (omega-6). It was found that it is an important dietary component necessary for the growth and prevention of skin lesions in animals. Linoleic acid (omega-6) can be converted into long chain polyunsaturated fatty acids that have additional functions as precursors of eicosanoids,which are powerful physiological mediators of cellular functions. Thus, essential fatty acids in the diet of cats are naturally polyunsaturated fatty acids. This group includes linoleic acid (LA), α-linolenic acid (ALA) and, under certain conditions, docosahexaenoic acid (DHA) and arachidonic acid (AA).

The Table 5 is shown the results of the lipid component of Zooprotein, which is confirmed by the data in Fig. 2. The research results confirm that Zooprotein is a source of necessary linoleic acid (omega-6) in the diets of feeding kittens, which is contained in the amount of 0.48 g per 100 g of Zooprotein.

Table 5. Fatty acid composition of Zooprotein

Lipid compounds	Content ratio, %
n-paraffins	2.20
Fatty acids:	
C3:0	0.88
H-C12:0	0.05
C14:1	0.03
H-C14:0	0.43
H-C15:0	0.06
C16:2	0.09
C16:1	4.04
н-C16:0	7.52
н-C18:0	4.57
C18:1	20.71
C18:2	0.48
C22:1	2.8
Other compounds	
Trimethylphosphate	1.70
Cholesterol	7.64
Cholest-5-en-3-ol	3.98
Cholesterol derivative	0.78
3a-Methoxy-9b-lanosta-7.24-dien-26.23-olide	1.74

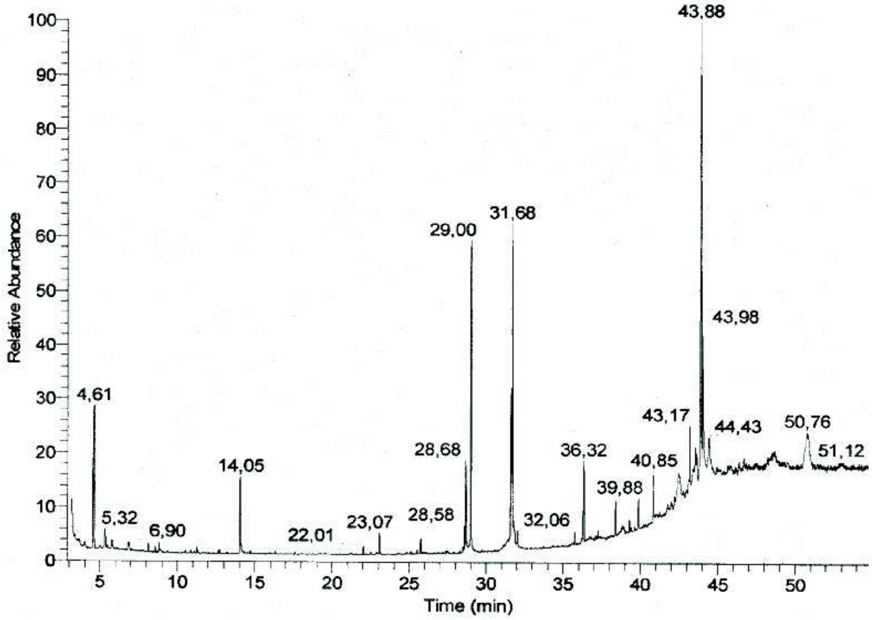

Figure 2. Mass chromatogram of Zooprotein lipid compounds on the full ionic current.

In present research the reference data were used for assessing the need satisfaction of kittens in nutrients during their growth period (FEDIAF, 2017). In Table 6 is represented data on the needs of kittens in the nutrients in terms of daily metabolizable energy, which was examined earlier and was equal to 325 kcal. The daily portion of Zooprotein is 102.7 g which provides the kitten's need for energy.

The nutrient levels for cats during growth are noted in a separate column: minimum recommended nutrient levels (on the left) and maximum recommended nutrient levels (on the right and indicated by *) and legal maximum nutrient levels for sodium (on the right and indicated by **). Legal maximum in EU legislation is expressed on 12% moisture content and there is not account for energy density.

Based on the data in Table 6, we can conclude that Zooprotein is an excellent source of amino acids, fatty acids and minerals. In particular, the daily portion of Zooprotein satisfies the minimum requirement for kittens in 11 amino acids by more than 100%. In addition, the daily dose of Zooprotein partially satisfies the animal's need for the maximum recommended amount of certain essential amino acids, in particular 48.59% arginine, 83.02% methionine and 36.96% tryptophan. The kitten's need of for essential linoleic fatty acid (ω-6) is satisfied by 111.11%, taking into account the daily intake of Zooprotein. The minimum need in minerals is satisfied over 100% at the level of potassium (138.78%) and sodium (353.85%) and partially at the level of calcium (65.43%) and phosphorus (88.24%), and the sodium levels in the daily portion of Zooprotein provide 37.71% of the kitten's need for this mineral.

It is obvious that Zooprotein outperforms other studied samples in a number of indicators (energy value, proteins (amino acids), fats (essential fat acid), fibers and minerals.

Table 6. The nutrient levels for need satisfaction of kittens with consideration to the daily portion of Zooprotein

Major nutrients	Minimum recommended nutrient levels for cats during growth (except * and **)		The content of nutrients in 102.7 g of Zooprotein (daily portion)	The level of satisfaction of the nutrients need, %
	g 1,000 kcal^{-1} of ME	g 325 kcal^{-1} of ME		
Amino Acids (Essential Amino Acids)				
Arginine	2.78 **(8.75*)**	0.90 **(2.84*)**	1.38	153.33 (**48.59**)
Histidine	0.83	0.27	0.89	329.63
Isoleucine	1.35	0.44	1.59	361.36
Leucine	3.2	1.04	2.56	246.15
Lysine	2.13	0.69	2.74	397.10
Methionine	1.10 **(3.25*)**	0.36 (**1.06***)	0.88	244.44 (**83.02**)
Methionine + cystine	2.20	0.72	1.29	179.17
Phenylalanine	1.25	0.41	2.62	639.02
Threonine	1.63	0.53	1.62	305.66
Tryptophan	0.40 **(4.25*)**	0.13 (**1.38***)	0.51	392.31 (**36.96**)
Valine	1.6	0.52	2.15	413.46
Taurine (dry pet food)	0.25	0.08	0.24	300.00
Essential Fat Acid				
Linoleic acid (ω-6)	1.38	0.45	0.50	**111.11**
Minerals				
Calcium	2.50	0.81	0.53	65.43
Phosphorus	2.10	0.68	0.60	88.24
Potassium	1.50	0.49	0.68	138.78
Sodium	0.40 **(3.75**)**	0.13 **(1.22**)**	0.46	353.85 **(37.71)**

* N – Recommended maximum, not harmful to the animal; ** – Scientific data show that sodium levels up to 3.75 g per 1,000, kcal ME are safe for healthy cats. Higher levels may still be safe, but no scientific data are available.

A comparative analysis of the prime cost of the studied samples was examined, in particular, the indicator for fish meal was 1,320–1,590 euro ton^{-1}, for meat and bone meal – 0,530–0,660 euro ton^{-1}, Zooprotein – 1,320 euro ton^{-1}. Thus, the competitive cost of Zooprotein proves the perspectivity of its use in combination with traditional feed raw materials, which can be widely used in food production.

CONCLUSIONS

This research confirms that Zooprotein is a high-quality feed resource in countries where using of fly larvae protein is an acceptable procedure. It has been established that the energy requirements for kittens at the age of 6 months, it is enough for them to consume 102.7 g of Zooprotein per day. The consuming of the daily portion of Zooprotein allows to satisfy the needs of kittens in most essential nutrients, such as essential amino acids (arginine, methionine, tryptophan, taurine etc.), essential fat acid (linoleic acid) and minerals (calcium, phosphorus, potassium, sodium). The competitive prime cost of Zooprotein also allows us to consider it as a promising raw material resource. In future research, it is advisable to find an optimal recipe for cat feed based on several raw ingredients using the method of multivariate modeling.

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