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ORIGINAL PAPER

EFFECT OF TEMPERATURE AND FEED ON THE MINERAL CONTENT AND THE CONTENT OF SELECTED HEAVY METALS IN MEALWORM*

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Abstract

Minerals are important components of thousands of enzymes and other compounds in the body. Although the content of minerals in the human body is small, their importance is immense. Edible insects can provide many of these minerals in abundance, and are therefore thought to be a good source of micronutrients. This study dealt with the effect of a rearing temperature and feed on the mineral content and on the content of selected heavy metals in mealworm (*Tenebrio molitor*). Mealworm larvae were kept at temperatures of 15, 20 and 25°C, and fed with wheat bran, lentil flour and a mixture of both. The types of feed for the experimental groups were chosen purposefully, considering the availability on site, price and especially the impact on the nutrition composition suitable for human. The concentrations of the most significant elements in terms of human nutrition concerns, that is zinc and copper, were determined with inductively coupled plasma mass spectrometry. The results showed that the normality condition was not achieved for Ca, Fe, P and Pb ($p<0.05$). Therefore, these data were compared using the Kruskal-Wallis method, while ANOVA test was performed for the other elements. There was a statistically significant dependence of the Cd concentration on feed at a constant rearing temperature of 15°C and 25°C over the entire feed change range. The results included a change in Mg over the whole range of monitored values with a change of the rearing temperature and constant feed of lentils-bran,and with a change of feed and constant rearing temperature of 15°C. In contrast, concentrations of Na, P, Ca and Cu over the whole observed range of feed or rearing temperature changes were independent from the variables. With an appro-

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priately chosen rearing temperature and feed, it is possible to obtain mealworm with specific nutritional properties for a target group of consumers.

Keywords: *Tenebrio molitor*, minerals, wheat bran, lentil flour, nutrition, rearing temperature, ICPMS.

INTRODUCTION

Interest in nutrition is growing constantly. Balanced intake of all nutrients is important for the health of consumers. With 1.5-3 million species, insects are is the most diverse class in the world (Zielińska et al. 2015). Edible insects were used in the past as traditional food, for example in China, where people ate silkworms pupae, which are still consumed in many areas of Southwest China (Feng et al. 2018).

The question of edible insects is an increasingly more popular topic for experts in nutrition as well as general public, also in countries where entomophagy is not common (Velíšek 2002, Horniaková et al. 2010, van Huis et al. 2013). The chance of using edible insects as a valuable source of protein, fats, vitamins and minerals is especially appreciated in areas where there are shortages of these nutrients (Velíšek 2002, Horniaková et al. 2010, Józefiak et al. 2016, Gere et al. 2019). Other advantages are higher feed conversion, low greenhouse gas emission, more optimized use of land and conversion of secondary organic materials into valuable products. This strategy can lead to financial savings and ecological advantages (Defoliart 1992, Cerritos 2011, Ramos Elorduy et al. 2011, van Broekhoven et al. 2015, van Huis 2016). Insects also have a great potential as feed, for example in aquacultures (van Huis 2016). The reason is their high protein content and the concentrations of sulphuric amino acids, which can be successfully used as feed for poultry (Józefiak et al. 2016). Payne et al. (2016) concluded that entomophagy does not have a worse effect on human health than eating conventional meat; on the contrary, it can lead to better health. It is presumed that with the right choice of a rearing temperature and feed (diet), it is possible to modify the nutritional value of insects. Hence, it is necessary to make nutritional analyses of insects depending on the applied feed and rearing temperature in order to acquire the requested nutritional values.

Edible insects could be a possible alternative source of minerals that are essential in human nutrition. They are important for biological processes taking place in the body, which is why they became the focus of our study. Mineral deficiencies can cause growth problems, immunological disorders and mental development disorders (Zielińska et al. 2015). Iron and zinc are especially important as these minerals are often deficient in developing countries (Rumpold Schlüter 2013, Manditsera et al. 2019). Unlike plant sources, some insects contain bioavailable iron, which is better dissolved and used by a living organism (Latunde-Dada et al. 2016). Latunde-Dada et al. (2016) reports that "*populations subsisting predominantly on plant food sources of iron in the diet have a high incidence of iron-deficiency anemia (IDA).*" The combination of copper and iron is very important for the formation of blood cells (Velíšek 2002, Horniaková et al. 2010). Finke (2002) measured the copper and iron content in mealworm. The copper content was 6.1 mg kg^{-1} and that of iron reached 20.6 mg kg^{-1} (Finke 2002). Zielińska et al. (2015) determined the copper content at 18.6 mg kg^{-1} and the iron content at 32.9 mg kg^{-1}. Zinc is the second most abundant metal in organisms, with 2-4 g found in the human body. It is a biologically essential trace element, critical to the cell growth, development and differentiation (John 2010). The amount of zinc was determined to be 52.0 mg kg^{-1} (Finke 2002) and Zielińska et al. (2015) reports a zinc content of 112 mg kg^{-1}.

The most common heavy metals include cadmium (Cd) and lead (Pb). Cadmium is a potent human carcinogen that primarily causes cancer of the prostate, lung, kidney and pancreas. Lead is toxic to humans and animals, and its persistence causes long-term occurrence in the environment (Jomova 2011). Dangerous concentrations of these metals have not been detected in common farmed insects.

MATERIAL AND METHODS

Mealworm larvae (*Tenebrio molitor*) were used for the analysis. Samples were bought from the company Radek Frýželka, Brno, while the rearing of larvae was carried out by Marie Borkovcová, Assoc. Prof. at Mendel University in Brno. The insects were divided into 3 experimental groups. The first group was fed only wheat bran, the second received only lentil flour, and the third group was fed a mixture of 50% wheat bran and 50% lentil flour. For each experimental group, the feed was weighed, and the weight of a ration was the same for all groups. Nutrition values of the feed were (per 100 g):

Wheat bran/crude: energy value 1 210 kJ/292 kcal, fat 5.3 g, of which saturated fatty acids 0.88 g, carbohydrates 24.9 g, of which sugars 2.2 g, fibre 40.2 g, protein 16.2 g, salt 0.1 g. Producer: Country Life, s r. o., Beroun 1.

Lentil flour: energy value 1250 kJ/298 kcal, protein 24.1 g, fats 2.0 g, of which saturated fatty acids 0.5 g, carbohydrates 49.6 g, of which sugars 2.2 g, fibre 11.4 g, salt 6.7 mg. Producer: Extrudo Bečice s r. o., Bečice 7, 375 01 Týn nad Vltavou.

Before analysis, the samples were prepared as follows: mealworm larvae in ultimate and penultimate instar development (with a full body length just before pupation) were taken from the rearing experiment. The next step was fasting the insects for 48 hours, sacrificing them in boiling water (100°C) and drying at 105°C. Samples thus prepared were homogenized and stored in a cooling box at 4 to 7°C until analysis. Samples for excrement analysis were taken at 20°C, homogenized and stored at 4 to 7°C until analysis.

After the experiment, the substrate with excrements was taken from the rearing boxes. These samples were homogenized and stored at 4 to 7°C until analysis.

Determination of mineral elements

Sample preparation

The volumetric equipment was soaked in 2% ANALPURE ultra HNO_3 overnight and thoroughly rinsed with Purelab Elga water before use. High purity 18.2 MΩcm water was obtained from a Purelab Classic Elga purification system for use in the preparation of all solutions. HNO_3 was delivered as concentrated 65% grade ANALPURE ultra.

Solid samples were decomposed with a mixture of 65% ANALPURE ultra HNO_3 and ANALPURE ultra H_2O_2 in a microwave system Milestone Ethos One. Each sample was weighed, divided into approximately 1g individual samples, and added 5 mL of 65% ANALPURE ultra HNO_3 and 1 mL of ANALPURE ultra H_2O_2. Afterwards, all vessels were closed and inserted into the segment to adjust the working pressure (performed with an adjustable torque wrench), and then inserted into a twisting rotor. The next step was the decomposition process according to a temperature programme suitable for cereal materials. Two sets of calibration standard series were prepared to match the expected concentration ranges in the samples of the 25 and 12 elements selected for this study - high standard series: Be, Zn, Cu, Ni, Al, Ga, Mg, Co, Li, Sc, Ag, Mn, Sr, Ba, TI, Bi, Ce, Cs, Ho, In, Rh, Ta, Tb, U and Y at 3–35 μg L^{-1} and low standard series: As, Ca, Cd, Cr, Fe, Hg, K, Na, Pb, Se, Sn and Ti at 0.5-1.0 μg L^{-1}.

Quality control (QC)

Standard solutions in 2% HNO_3 were prepared from 3-35 μg L^{-1} of the multi-element ICP standard (Analytika, CR) containing the elements: Be, Zn, Cu, Ni, Al, Ga, Mg, Co, Li, Sc, Ag, Mn, Sr, Ba, TI, Bi, Ce, Cs, Ho, In, Ta, Tb, U and Y in the original concentration (3-35) μg L^{-1} and half the concentration (1.5-17.5) μg L^{-1}. An internal standard (^{103}Rh) was used with 10 μg L^{-1} solution to improve the accuracy and truthfulness of the measurements.

The original multi-element ICP standard (Analytika,CR) with the concentration of 335 μg L^1 of individual elements was used as a continuing calibration verification (CCV) standard. For the concentration values, see Table 1.

Instrumentation

Analyses were carried out on a quadrupole-based Thermo Scientific iCAP Qc inductively coupled plasma-mass spectrometer (ICP-MS), equipped with nickel cones, a Peltier-cooled low-volume conical quartz spray chamber

Table 1

Concentration of individual elements in multi-element ICP standard (Analysis)

Element	STD25 (µg L^{-1})
Be	35 +/- 1.75
Zn	20 +/- 1.00
Cu, Ni	15 +/- 0.75
Al, Ga, Mg	10 +/- 0.50
Co, Li, Sc	8 +/- 0.40
Ag, Mn	6 +/- 0.30
Sr	5 +/- 0.25
Ba, TI	4 +/- 0.20
Bi, Ce, Cs, Ho, In, Ta, Tb, U, Y	3 +/- 0.15

to improve the instrument's performance, a concentric PFA nebulizer, a collision cell (QCell) with helium to remove undesirable molecule ions or to discriminate their kinetic energy (CCT and KED mode), a peristaltic sample delivery pump, and a Cetac 520 autosampler. The instrument's operating settings were optimized for the analysis of samples after MW decomposition with relatively simple matrices.

Mass calibration and detector cross-calibration were performed according to the instrument manufacturer's instructions, using the prescribed solutions obtained from Thermo. A sensitivity check using a 10 µg L^{-1} Ba, Be, Bi, Ce, Co, In, Li, Ni, Pb, U tuning solution preceded the onset of the analytical measurements. The resulting performance report included important sensitivity data, such as the extent of the formation of oxide ions ($156CeO^{+}/140Ce^{+}$) and doubly charged ions ($137Ba_2^{+}/137Ba^{+}$), in addition to count rates for the tuning solution elements, whereby the optimized status of the instrument could be assessed.

Elements that may distrub the quantification of elements due to interferences were screened. Although elements such as oxygen (O), argon (Ar) and chlorine (Cl) may also be present and complexed with the other elements, thereby generating interferences, they were eliminated using the CCT/KED mode for selected elements.

Furthermore, an internal standard (^{103}Rh) was used to improve the accuracy and truthfulness of the measurements. This element/isotope was used as a universal element the weight of which, resp. m/z of 103, lies in the middle of the range of masses of commonly determined elements (^{7}Li – ^{238}U).

The validation of the analytical method was ensured by conducting a simultaneous analysis of the certified reference material CRM 12-02-01 (Bovine Liver). Analytical data obtained for all determined elements were found within the confidence interval given by the manufacturer of the CRM for these elements (Kucera 1990).

Statistical evaluation

The data were analyzed in Excel 2013 (Microsoft Corporation, USA) and Statistica Cz version 12 (StatSoft, Inc., USA). The results were expressed as means ± standard deviation.

Statistical processing and data evaluation proceded through several steps. The Shapiro-Wilk test of normality was used first. The results showed that the normality condition was not observed for Ca, K, Fe and Pb (p<0.05). Subsequently, the Levene, and the Brown and Forsythe (HOV) tests of homogenity were performed. In both tests, the results showed that the homogenity condition was not fulfilled for P, Ca, K, Fe and Pb. Therefore, the data were compared using the Kruskal-Wallis method. An ANOVA test was performed for the remaining elements. Finally, the Tukey HSD tests were carreid out on the ANOVA data at a constant rearing temperature or feed.

RESULTS AND DISCUSSION

Insects can be a valuable source of a wide range of minerals. Among significant minerals and selected heavy metals, this research focused on Ca, P, K, Na, Zn, Cu, Fe, Cd and Pb, as it can be seen in Table 2. It might have been suspected that the KED-mode should have been more affected by the m/z differences between the internal standard and analyzed elements due to the effects of the initial kinetic energy and collision rate of ions and He-gas. The difference in the collision rate could lead to less efficient compensation by the internal standard with an increase in the m/z difference. While there were clear differences in the signal loss ratio between the KED- and standard-mode, depending on the size and m/z for each element, the ratios were consistent enough so that there was little or no effect indicating which internal standard would be preferable to use.

Calcium is one of the most essential elements in nutrition because it ensures the running of metabolic processes as well as enzymatic and hormonal reactions. In the mealworm samples analyzed, its content increased with the rearing temperature in treatments where the mixture of bran and lentils was used as a feed. For the combined protein and carbohydrate diet (lentils-bran), a higher calcium content was measured at 15°C than at 20°C and 25°C. There was a tendency towards higher Ca from 20°C. The highest content of calcium in the body of mealworm was determined at 25°C for lentil flour diet (0.453 mg kg^{-1}in DM). A similar calcium content was determined in mealworm fed bran and maintained at 25°C (0.450 mg kg^{-1} DM), and receiving the mixture of lentils and bran and kept at 15°C for (0.452 mg kg^{-1} DM). The difference in the calcium content between the two types of diet is minimal. The analysis showed that the amount of calcium intake in feed is very similar to the calcium content in excrements, sugges-

Table 2

Content of determined mineral elements and selected heavy metals in mealworm (*Tenebrio molitor*) depending on the rearing temperature and feed

Group	Type of feed	Ca (μg kg^{-1})		P (mg kg^{-1})		K (mg kg^{-1})		Na (mg kg^{-1})		Zn (mg kg^{-1})		Cu (mg kg^{-1})		Fe (mg kg^{-1})		Cd (μg kg^{-1})		Pb (μg kg^{-1})	
		M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
Feed	bran	375.5	2.3	419.7	19.2	293.1	1.8	2.6	0.3	33.9	3.4	11.7	0.6	0.6	0.6	50.6	2.9	64.8	5.2
Feed	lentils	378.5	0.7	115.3	2.5	295.8	0.3	4.7	0.3	11.1	0.2	8.2	0.2	5.4	2.7	9.9	0.6	41.8	18.3
15°C rearing	bran	437.5	1.4	395.4	14.8	346.1	5.0	71.0	7.6	52.7	2.2	21.9	1.1	12.1	0.9	52.2	3.1	49.9	5.0
20°C rearing	bran	445.5	7.5	383.9	18.4	352.2	4.3	71.2	7.1	51.6	2.4	21.9	1.1	14.4	0.5	52.1	4.6	56.5	5.8
25°C rearing	bran	450.8	1.0	439.5	14.9	352.4	0.9	93.7	4.2	56.2	2.3	27.1	0.9	16.7	0.3	62.8	3.2	51.6	3.9
15°C rearing	lentils - bran	452.8	8.8	361.0	20.4	358.9	3.0	69.7	3.5	46.8	3.5	22.0	1.2	15.5	0.3	57.6	2.8	50.4	3.2
20°C rearing	lentils - bran	441.3	12.6	380.8	40.8	356.3	1.4	75.0	3.8	46.5	3.7	23.3	1.3	14.1	0.3	53.8	3.7	57.8	0.9
25°C rearing	lentils - bran	449.6	5.9	379.0	15.1	358.0	1.0	79.3	4.9	47.0	1.9	23.9	1.0	15.8	0.5	52.0	2.7	59.5	2.0
15°C rearing	lentils	437.9	7.9	440.2	12.7	351.7	0.2	96.9	4.6	54.4	2.6	25.4	1.1	20.2	1.5	75.4	3.7	71.7	2.0
20°C rearing	lentils	439.5	6.8	432.0	9.7	352.4	0.7	95.1	5.1	49.5	1.7	25.1	1.2	18.5	0.6	66.7	3.4	51.4	4.9
25°C rearing	lentils	453.3	3.7	411.6	9.7	359.2	0.3	94.1	4.3	49.5	1.5	25.3	0.9	21.4	1.2	67.8	2.6	57.1	9.5
Excrements	bran	377.4	1.5	726.3	28.6	294.6	1.2	2.7	0.1	44.8	1.4	15.2	0.3	47.4	3.1	53.8	3.2	241.5	13.6
Excrements	lentils	376.7	1.3	188.5	4.7	294.1	1.0	2.0	0.1	17.1	0.5	9.3	0.4	17.9	0.5	14.7	1.2	32.2	0.9
Excrements	lentils - bran	415.9	40.3	301.4	5.9	324.7	31.5	2.5	0.2	21.1	0.7	10.1	0.4	23.7	0.5	22.9	1.2	85.8	3.2

ting that the calcium intake was sufficient for this rearing regime and did not accumulate in the larva's body.

Another macronutrient important in metabolism and energy transformation (ATP, AMP) that we analyzed was phosphorus. When mealworm larvae were reared at a temperature of 20°C or lower, there is a higher proportion of phosphorus in the groups fed with lentils. As the rearing temperature rose, the phosphorus content decreased slightly in these groups. The study shows that feeding with bran, which has about three-fold more phosphorus than lentils, led to a smaller amount of phosphorus being detected in the larval body. Determination of the phosphorus content relative to the feed supplied to mealworm (*Tenebrio molitor*) was studied by Oonincx et al. (2015), who described a change in the phosphorus content ranging from 7700 mg kg^{-1} DM to 9700 mg kg^{-1} DM. Significant changes in a carbohydrate and protein diet did not significantly affect the phosphorus content. However, the results of the cited study do not correspond to our data.

Potassium and sodium are important for osmotic pressure and acid-base balance. Both feeds are comparable sources of potassium, and this was manifested in the bodies of mealworm larvae, with the highest potassium content identified in the group reared at 25°C. According to the nutritional recommendation, the sodium to potassium ratio should be from 1:1 to 1:4. *Tenebrio molitor* fulfills this requirement. The highest measured values of sodium were in the group fed with lentils, the type of feed comparable to bran but with a higher proportion of this macronutrient.

A significant benefit of edible insects as food or feed is the content of zinc and copper, the two metals which play several functions in the body of an animal (nutrient metabolism, reproduction, bone formation and hematopoiesis). The average zinc content in all monitored groups ranged from 46 to 56 mg kg^{-1} DM, while the average copper content ranged from 21 to 27 mg kg^{-1} DM. In terms of nutritional recommendations (Zn 10 mg kg^{-1} and Cu 1 mg kg^{-1}), the *Tenebrio molitor* species can be a significant source of these minerals. The Zn and Cu levels in a carbohydrate feed are higher, which was evidenced by the zinc content in the experimental groups of insects at 20°C, but did not correspond to the determined copper content.

The combination of copper and iron, two metals of which edible insects can be a good source of, is crucial for the formation of blood cells. In the samples submitted to our research, an average iron value of 12 mg kg^{-1} to 21 mg kg^{-1} DM was determined. This is the same or lower than results provided by other authors. Finke (2004) reports up to 54 mg kg^{-1}. Therefore, the iron content is generally equal or higher then the iron content in beef or pork. Menezes et al. (2010) determined 49 mg kg^{-1} in raw beef and 21 mg kg^{-1} in raw pork, Velíšek (2002) reports 22-30 mg kg^{-1} for beef and 10-20 mg kg^{-1} for pork, and Gerber et al. (2009) determined 16.1 mg kg^{-1} (rib-eye) and 14.2 mg kg^{-1} (brisket) in raw beef and 9.8 mg kg^{-1} (neck steak) and 8.3 mg kg^{-1} (belly) mg kg^{-1} in raw pork. At 25°C, the iron content of the mealworm larvae fed lentils is higher than in those fed bran, but in the excrements analyzed, the iron content was found in the groups receving bran (Velíšek 2002, Horniaková et al. 2010). When analyzing excrements from the larvae of the species analyzed, the higher content of minerals was found in excrements than in the feed. The reason for this may be the binding of minerals to indigestible fiber in the diet.

In this study, we also looked at the safety of edible insect material in terms of its content of heavy metals such as cadmium and lead, which is must be monitored due to accumulation from feed in the body of insects. Heavy metals are a risk posed by edible insect consumption. Other threats are microbiological, physical and allergenic ones (Cappelli et al. 2020). The measured cadmium content ranged from 0.052 to 0.075 mg kg^{-1} DM in the experimental groups. These values do not exceed the limit of 0.5 mg kg^{-1} in the fresh state (FW) set for crustaceans, into which edible insects are currently classified because of a lack of specific legislation. Similarly, the lead content in the analyzed samples ranged from 0.049 to 0.072 mg kg^{-1} DM. The limit for this metal is the same as for cadmium, i.e. 0.5 mg kg^{-1} in FW (EC/1881/2006). Concerning lead and cadmium, the food appears safe.

The same issue of the mineral content in *Tenebrio molitor* was dealt with by other authors. According to Nowak et al. (2016), the main mineral substances contained in mealworm were iron in the amount of 3.29 mg 100 g^{-1} DM, copper 1.86 mg 100 g^{-1} DM, zinc 11.2 mg 100 g^{-1} dry matter, potassium 835 mg 100 g^{-1} dry matter, magnesium 304 mg 100 g^{-1} DM, sodium 57 mg 100 g^{-1} DM and calcium 41 mg 100 g^{-1} DM (Nowak et al. 2016). Finke et al. (2015) determined minerals at levels: calcium 156 mg kg^{-1}, phosphorus 2640 mg kg^{-1}, magnesium 620 mg kg^{-1}, sodium 225 mg kg^{-1}, potassium 3350 mg kg^{-1}, chlorine 1760 mg kg^{-1}kg, iron 20.7 mg kg^{-1}, zinc 49.5 mg kg^{-1}, copper 8.3 mg kg^{-1}, manganese 3.2 mg $kg^{-1}$and selenium 0.123 mg kg^{-1}. Baek et al. (2019) determined minerals in freeze drying material of mealworms with moisture at levels: calcium 341.9 mg kg^{-1}, phosphorus 5931.7 mg kg^{-1}, sodium 1082.3 mg kg^{-1}, potassium 8651.5 mg kg^{-1} and iron 20.7 mg kg^{-1}. Ravzanaadii et al. (2012) determined the mineral content for larvae and adults of *Tenebrio molitor*. The most abundant elements were potassium, phosphorus, sodium, magnesium and calcium, while the smallest quantities were determined for zinc, iron and copper (Ravzanaadii et al. 2012). These studies differ significantly in results, possibly due to differences in feeds and rearing conditions, which the authors did not specify in detail. For this reason, some results of our study may differ from the literature data.

The results of statistical processing show that there is a difference between all the measured elements (p<0.050) except from potassium. The results of the Tukey HSD tests and the graphical representation of the results in the form of mean and standard deviation show a statistically significant dependence of the Cd concentration on the feed at a constant rearing temperature of 15°C and 25°C over the entire feed change range. There was a change in Na in many range parts of monitored values. On the contrary, independence in many parts of the observed feed or rearing temperature range was noted for Ca relative to the rearing temperature change and the lentil feed, for P for the rearing temperature change and constant lentils-bran feed, for Ca for the feed change and constant temperatures of 20°C and 25°C, for Cu for the rearing temperature change and constant feed of lentils, for Zn for the rearing temperature change and constant lentils-bran feed. The non-parametric Kruskal-Wallis method was used for Ca and P, although it is also possible to use a graph of values. Regarding the other determinations, a statistically significant difference was detected in only a certain portion of the range, or else the difference or relationship could not be determined.

CONCLUSIONS

Changing the feed composition for edible insect can significantly affect its nutritional value. Mineral substances as an unmistakable component of organic substances were detected in *Tenebrio molitor* samples in a wide range. The most significant content of the monitored elements in terms of human nutrition was determined for zinc and copper. Zinc could be used to prevent parakeratosis. These findings could in the future allow the use of edible insects as a tool for the targeted nutritional supplementation of a specific animal species and a targeted enrichment of a diet with certain nutritional values for a particular group of consumers. It is believed that a suitably selected concentration of certain nutrients in insect feed could provide a specific diet to improve consumer health.

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