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Evaluation of life cycle and nutrient content of Jamaican field crickets (*Gryllus assimilis*) reared on the dried rapeseed protein

Master´s thesis

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Declaration

I hereby declare that I have authored this master's thesis carrying the name „Evaluation of life cycle and nutrient content of Jamaican field crickets (*Gryllus assimilis*) reared on the dried rapeseed protein "independently under the guidance of my supervisor. Furthermore, I confirm that I have used only professional literature and other information sources that have been indicated in the thesis and listed in the bibliography at the end of the thesis. As the author of the master's thesis, I futher state that I have not infringed the copyrights of third parties in connection with its creation.

In Prague on 26th April 2021 _____

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Summary

Edible insect rearing could provide a good alternative for protein production in the future. Thus, the theoretical part of diploma thesis is focused on review of nutritional value and rearing technology of the crickets. Insect production has a smaller environmental impact comparing to the traditional livestock farming due to the insect's ability to convert organic side streams, and so, the work also deals with the possible use of waste and agricultural by-products as an alternative feed.

The practical part is devoted to the effect of addition of dried rapeseed cakes into the diet of *Gryllus assimilis* and its impact on their life characteristics and basic nutrient content. In this experiment, the soybean in the insect feed mixture was replaced by rapeseed cakes as the food industry by-product. The crickets were reared on the four different feed substrates containing different proportions of rapeseed cakes (17.5, 35, 52.5 and 70 %) and control group (chicken feed) in three repetitions. The whole life cycle was observed as well as the individual weights of the crickets were recorded. After cricket's harvest, laboratory analysis were performed to determine the essential nutrients.

Based on the statistical evaluation, the addition of rapeseed protein into the feed mixtures did not influence the content of basic nutrients and life characteristics of *Gryllus assimilis*. The nutritional value of the crickets, precisely the amount of dry matter, protein, fat, chitin, and ash content was in accordance with the cited literature, thus all feed mixtures enriched by rapeseed cakes were within the nutritional optimum.

In conclusion, the agricultural by-product – rapeseed cakes – could possibly be utilized as a part of the cricket feeds without any negative impact of its quality. Therefore, the obtained results may be used to improve the environmental sustainability of insect feed substrates, insect rearing technology and advance the goals of circular economy.

Keywords: edible insects, novel food, sustainability, food by-products, alternative

Content

1 Introduction

Given the ever-growing human population, which is expected to reach 10 billion in 2050, food insecurity is considered one of the most significant global problems today. This food problem represents a complex of economic, technological, social, demographic, and political aspects of food production, distribution, exchange, and consumption. Today's population is more separated than ever. The problem of food insecurity is significantly lesser in industrially advanced countries, so the people focus on two main factors - food safety and environmental sustainability in food production. For these reasons, it is necessary to find new ways to increase yields while maintaining the quality of food with respect for the environment. On the other hand, there exists about 1,2 billion people in developing countries suffering from constant hunger, which represents about one-fifth of the total population. The main world's challenge is therefore to find sustainable and high-quality food sources, including suitable protein.

From this point of view, insects are considered to be the alternative which can ease the world food shortage. The insects present a number of environmental benefits that are essential to human survival. Insects are important pollinators, contribute to the natural elimination of pests and improve soil fertility through bioconversion of waste. However, these invertebrates are also consumed in Africa and Asia for centuries. It is estimated that up to 1,900 insect species are part of the traditional diet of at least 2 billion people. There exist several reasons to include insects into the diet of both animals and humans. Insects have a good nutritional value. They contain high-quality proteins comparable to other animal or vegetable proteins, relatively high amount of fats with a higher proportion of polyunsaturated fatty acids and are a good source of vitamins and minerals. Insects also have a faster generation interval and feed conversion is more efficient compared to cattle, pigs, or poultry. Furthermore, rearing insects is undemanding and more eco-friendly due to the lesser emission of greenhouse gases and ammonia to the atmosphere and significantly lesser consumption of soil and water. These favourable attributes make insects an accessible opportunity to ensure food security within the world population.

The experimental organism in this diploma thesis is *Gryllus assimilis*, which is one of the most common reared and consumed insect species in the world. The theoretical part is devoted mainly to edible insects in general, its nutritional value and rearing technology. The work also focuses on the possible use of waste and agricultural by-products as an alternative feed. In the practical part, the work deals with the effect of addition of dried rapeseed cakes into the diet of *Gryllus assimilis* and its effect on their life characteristics and basic nutrient content. In this diploma thesis, the soybean in the insect feed mixture was replaced (from 25 – 100 %) by rapeseed cakes as the food industry by-product. Diet and feeding technology are one of the most important elements influencing the life cycle of insects. New findings of this work can be used in the future as a basis for the design of feed mixtures for crickets, and thus contribute to the optimization of insect rearing technology.

2 Scientific hypothesis and aims of the thesis

The thesis aims to determine content of basic nutrients and life characteristics of the Jamaican field crickets reared on substrate with rapeseed protein as the food industry by-product.

Scientific question:

How will replacement of the conventional proteins by rapeseed protein into the feeding substrate influence the development and content of nutrients in the Jamaican field crickets (*Gryllus assimilis*).

3 Literature research

3.1 Global search for alternative foods

Despite the rapid population growth, it is assumed that the population increases to at least 9,5 billion by 2050. Nowadays, the world population faces several challenges, where the food security is the major one. Overfishing of the oceans, global warming, land degradation, freshwater scarcity and waste of edible material pose a threat to decrease food supplies (Willet et al. 2019).

With an increasing wealth, a greater appetite for meat, eggs, and dairy products – simply the foods with high protein content - rises. The way we raise the cattle has a huge impact on the environment and is one of the biggest sources of greenhouse gas emissions. Eventhough the improved technologies have helped farmers to grow more, there might be still not enough food for everyone in the future (Ritchie & Roser 2020).

People make efforts to find various alternatives to produce more eco-friendly food in a way of growing plants with artificial light, growing mushrooms on logging residues or producing alternative foods from biomass (Baum et al. 2016). Among others, edible insects seem to be another promising alternative to achieve the food security in the upcoming global food crisis (Gravel & Doyen 2019).

3.2 Edible insects

The fact that humans have been consuming insects since ancient times is known for long time. Some of the first evidence is found, for example, in northern Spain on the painted walls of caves which dates from 9,000 to 30,000 BC (Mitsuhashi 2008). Other evidence has been discovered in Mexico and United States in fossil feces of humans and animals from the prehistoric period. These fossils mainly contained larvae, lice, ticks, and ants (Kouřimská & Adámková 2016).

Nowadays, edible insects represent a great natural food resource to many ethnic groups found, for example, in Africa, South America, Mexico, and Asia. It is estimated that at least two billion people worldwide consume insects. On the other hand, in most Western countries people evaluate insect consumption as something disgusting and primitive. This attitude has slowed down the entomophagy research and the interest in insect consumption has only recently started attracting people's attention (van Huis et al. 2013).

3.2.1 Most commonly consumed insects

It has been reported that it exists more than 1,900 insect species used as food (Huis et al. 2013). Yen (2015) found that approximately 88 % of edible insects occupy terrestrial ecosystems while 12 % live in or near water. Today, insects are consumed in 35 African countries, 29 Asian countries, 23 American countries, 14 countries in Oceania and 11 in European countries. Mexico, Thailand, India, and China are the leading countries in consuming insects (Jongema 2017).

The most commonly consumed insects worldwide (31 %) are beetles (Coleoptera) but the most favourite consumed ones are the crickets (Anderson 2018). Following caterpillars (18 %) (Lepidoptera) which are popular in sub-Saharan Africa. Then bees, wasps, and ants (14 %) (Hymenoptera). Next in a line grasshoppers, locusts, and crickets (13 %) (Orthoptera), cicadas, leafhoppers, planthoppers, scale insects and true bugs (10 %) (Hemiptera). Then termites, dragonflies, flies, and others (van Huis et al. 2013).

3.2.2 Insects as an alternative protein source

The human demands and needs for nutrition and food, particularly for animal protein, are still increasing, as the world population constantly grows (Kearney 2010). Lattre-Gasquet et al. (2018) declare that despite the pasture and arable lands expanded, the areas designated for agricultural production have been disappearing. Therefore, insects represent one of the good alternative protein sources and there are many reasons for that (Akhtar & Isman 2018).

Insects are known especially for their high protein content which generally ranges 40 – 70 % of dry matter. Besides other things, content of essential amino acids present in insects meets the World Health Organization's (WHO) requirements (Rumpold & Schluter 2013). The interesting fact is digestability of insect proteins. Comparing to plant-based proteins, such as lentils and peanuts with digestability of 52 %, insect proteins are 76 – 98 % more digestible. On the other hand, the animal-based proteins, such as egg or beef, are just a bit more digestible than insect proteins (United States Dairy Export Council 2004).

From another point of view, insect proteins are easier to gain thanks to the relatively easy, inexpesive and fast farming (van Huis 2013). For example, livestock rearing is much less eco-friendly comparing to insect rearing and produces 14 % of the global greenhouse gas emissions. It requires huge land area and consumes large amount of water (Gerber et al. 2013). To produce 1 kg of edible protein, mealworms require only 10 % of the land needed for beef production (Oonincx & de Boer 2012). Moreover, one of the advantages of rearing insects is its vertical farming (van Huis et al. 2013). It is expected that by 2025 two-thirds of the world will suffer water shortages (FAO Water 2013). According to Chapagain & Hoekstra (2003) 22,000 – 43,000 litres of water is needed to produce 1 kg of beef since water is used for feed production and forage. On the other hand, the amount of water used for insect production is much lower. Ramos-Elorduy et al. (2002) found that some insects such as the lesser mealworms and the yellow mealworms are drought resistant and can be reared on organic side streams.

3.3 Nutritional value of insects

Since the insects are the most diverse and largest group of organisms on Earth, their nutritional value is highly variable even within the same species group. This is dependent, for example, on the diet they take, on the habitat they occupy and also on the developmental stage of insects (Finke & Dennis 2014). The nutritional composition may be also influenced by the type of culinary preparation (frying, drying, boiling) and processing of harvested insects (Melgar-Lalanne et al. 2019).

Insect body contains proteins, fats, and carbohydrates. They have an interesting content of monounsaturated and polyunsaturated fatty acids, fibres, vitamins such as riboflavin,

pantothenic acid and biotin, and several minerals such as calcium, zinc, iron, copper, magnesium, manganese, phosphorous and selenium (Payne et al. 2016).

3.3.1 Dietary energy

It is assumed that dietary energy of insects is derived from fats, fibre, and protein (Wallig & Keenan 2013). Ramos-Elorduy et al. (1997) analysed 78 insect species from Oaxaca state, Mexico, and found that caloric content was 293 – 762 kilocalories per 100 g of dry matter. Table 1 shows energy values expressed in kilocalories per 100 g fresh weight of selected wild and farmed insects all around the world.

Table 1 Examples of energy content of different insect species (van Huis et al. 2013)

Common name	Scientific name	Location	Energy content (kcal/100g fresh weight)
Australian plague locust	*Chortoicetes terminifera*	Australia	499
Green (weaver) ant	*Oecophylla smaragdina*	Australia	1272
Red-legged grasshopper	*Melanoplus femurrubrum*	Canada	160
Yellow mealworm, larva	*Tenebrio molitor*	US, Illinois	206
Yellow mealworm, adult	*Tenebrio molitor*	US, Illinois	138
Termite, adult	*Macrotermes subhyalinus*	Yvory Coast	535
Leaf-cutter ant, adult	*Atta mexicana*	Mexico	404
Honey ant, adult	*Myrmecocystus melliger*	Mexico	116
Field cricket	*Gryllus bimaculatus*	Thailand	120
Giant water bug	*Lethocerus indicus*	Thailand	165
Rice grasshopper	*Oxya japonica*	Thailand	149
Grasshopper	*Cyrtacanthacris tatarica*	Thailand	89
Silkworm, pupa	*Bombyx mori*	Thailand	94
Migratory locust, adult	*Locusta migratoria*	Netherlands	179

3.3.2 Protein content

Generally, edible insects contain a high-quality protein due to the essential amino acids which are present within the recommended ratios (Belluco et al. 2013). The protein content of edible insect is shown in Table 2. Insect proteins vary between 35.34 % within termites (Isoptera) and 61.32 % within grasshoppers, locusts, and crickets (Orthoptera). It was discovered that the highest protein yields show the species from the order Ortophera such as *Sphenarium histrio* (71.15 – 77 %), *Melanoplus femurrubrum* (77 %), and *Melanoplus mexicanus* (58.90 – 77.13 %) (Ramos-Elorduy et al. 1998).

Table 2 Protein content [%] of edible insects (dry matter) (Rumpold & Schluter 2013)

Edible insects	Origin	Protein [%]
Blattodea (cockroaches)	Mexico, wild	57.30
Coleoptera (beetles, grubs)	Nigeria, Mexico	40.69
Tenebrio molitor (adult)	Mexico, reared	60.20
Tenebrio molitor (larvae)	Mexico, reared	47.70
Diptera (flies)	USA, Asia	49.48
Drosophila melanogaster	USA, reared	56.25
Musca domestica (larvae)	S. Korea, reared	63.99
Hemiptera (true bugs)	Mexico, wild	48.33
Hymenoptera (ants, bees)	Mexico, wild	46.47
Apis mellifera (larvae)	Mexico, wild	41.68
Atta mexicana (ants)	Mexico, wild	46.00
Oecophylla smaragdina (weaver ant)	Thailand	53.46
Vespula sp.	Mexico, wild	52.84
Isoptera (termites)	Nigeria, wild	35.34
Lepidoptera (butterflies, moths)	Mexico, Nigeria	45.38
Bombyx mori	Mexico, wild	58.00
Odonata (dragonflies, damselflies)	Mexico, USA	55.23
Orthoptera (crickets, grasshoppers, locusts)	Mexico, USA	61.32
Acheta domesticus (adults)	USA, reared	64.38
Acheta domesticus (nymphs)	USA, reared	67.25
Melanoplus mexicanus	Mexico, wild	77.13

It is also important to notice that insect proteins are highly digestible (77 - 98 %). Lower digestibility values are observable within insects whose exoskeleton containing higher levels of chitin, which reduces the digestibility (Belluco et al. 2013).

In general, the protein content of insects is comparable to the protein gained from other animal species. Table 3 shows the comparison of average protein content among insects, reptiles, fish, and mammals (van Huis et al. 2013).

Table 3 Comparison of average protein content of different animal species (van Huis et al.2013)

Animal group	Species and common name	Edible product	Protein content (g/100 g fresh weight)
Insects (raw)	Locusts and grasshopppers: *Locusta migratoria, Acridium melanorhodon, Ruspolia differens*	Larva	14-18
	Locusts and grasshopppers: *Locusta migratoria, Acridium melanorhodon, Ruspolia differens*	Adult	13–28
	Yellow mealworm (*Tenebrio molitor*)	Larva	14-25
	Silkworm (*Bombyx mori*)	Caterpillar	10-17
	Crickets	Adult	8-25
	Termites	Adult	13-28
Cattle		Beef (raw)	19-26
Fish (raw)	Finfish	Tilapia	16-19
		Mackerel	16-28
		Catfish	17-28
Crustaceans		Lobster	17-19
		Prawn	16-19
		Shrimp	13-27
Molluscs		Cuttlefish	15-18
Reptiles (cooked)	Turtles: *Chelodina rugosa, Chelonia depressa*	Flesh	25-27
		Intestine	18
		Heart	17-23

3.3.2.1 Amino acids

The amino acid content of edible insect varies within different species. Some of the amino acids are shown in Table 4. It has been discovered that insects are high in lysine, tryptophane and threonine, some insects are also rich in phenylalanine + tyrosine. Further studies show that all edible insects comply with amino acid requirements of adults for methionine and methionine + cysteine, just with one exception in Diptera order for cysteine (DeFoliart 1992). Besides that, all insect orders generally meet the requirements of the WHO for amino acids aside from the order Hemiptera which is low in lysine, valine, tyrosine, phenylalanine and isoleucine and the order Diptera being short of leucine and cysteine (WHO 2007).

Generally, edible insects especially the grasshoppers, crickets, and locusts (Orthoptera) are rich in high-quality protein thanks to their amino acid profile (Rumpold & Schluter 2013). Yi et al. (2013) found that the levels of essential amino acids in all insect species were lower for casein but comparable to soy protein. The nutrient quality of insect protein can be improved by the removal of the chitin (Rumpold & Schluter 2013).

Table 4 Amino acid content of edible insects [mg/g protein] (Rumpold & Schluter 2013)

Edible insects [mg/g protein]	Origin	Ile	Leu	Lys	Met	Cys	Met+Cys	Phe	Tyr	Phe+Tyr	Thr	Trp	Val
Blattodea (cockroaches)	Mexico, wild	29.9	56.4	48.0	29.8	11.6	41.4	30.6	62.3	92.9	34.6	6.0	53.8
Coleoptera (beetles, grubs)	Nigeria, Mexico	45.6	74.2	50.6	16.2	14.6	31.9	47.1	55.7	98.6	35.2	10.1	51.9
Tenebrio molitor (adult)	Mexico, reared	43.5	82.7	44.3	12.7	6.8	19.4	26.2	33.3	59.5	34.2	11.0	63.3
Tenebrio molitor (larvae)	Mexico, reared	50.3	106.4	54.5	12.8	8.6	21.4	35.3	74.5	109.8	41.8	8.0	58.8
Diptera (flies)	USA, Asia	32.6	57.4	62.9	27.2	5.3	36.6	50.6	56.7	107.3	38.8	28.3	46.9
Musca domesticus (larvae)	S. Korea, reared	22.8	45.3	81.6	36.6	6.6	43.1	55.8	71.1	126.9	35.5	49.5	45.6
Hemiptera (true bugs)	Mexico, wild	31.5	49.8	28.0	21.7	12.9	32.2	34.4	38.7	63.8	29.9	10.3	43.3
Hymenoptera (ants, bees)	Mexico, Thailand	47.8	78.4	53.8	23.8	12.9	30.5	47.5	55.3	104.3	41.7	10.3	60.5
Apis mellifera (honeybee)	Mexico, wild	53.0	93.0	56.0	17.0	0	0	39.0	37.0	76.0	43.0	0	52.0
Atta mexicana (ants)	Mexico, wild	53.0	80.0	49.0	19.0	15.0	34.0	41.0	47.0	88.0	43.0	6.0	64.0
Isoptera (termites)	Nigeria, wild	51.1	78.3	54.2	7.5	18.2	26.2	43.8	30.2	74.0	27.5	14.3	73.3
Lepidoptera (butterflies, moths)	Mexico, USA	40.4	62.7	57.7	22.1	12.2	34.7	46.3	49.1	95.8	40.0	11.2	54.1
Bombyx mori (larvae)	USA, reared	33.0	48.9	50.0	12.5	9.1	21.6	28.4	34.1	62.5	28.5	6.8	39.8
Orthoptera (crickets, grasshoppers, locusts)	Mexico, USA	39.6	74.8	53.9	19.3	12.8	29.8	46.6	61.5	100.3	35.8	8.1	50.3
Acheta domesticus (adults)	USA, reared	45.9	100.0	53.7	14.6	8.3	22.9	31.7	48.8	80.5	36.1	6.3	52.2
Acheta domesticus (nymphs)	USA, reared	42.9	95.5	53.9	13.0	8.4	21.4	27.9	55.2	83.1	35.7	5.2	49.4
Melanoplus femurrumbrum	Mexico, wild	26.4	58.2	61.7	29.8	11.6	41.4	22.5	56.4	78.9	37.0	6.4	40.9

3.3.3 Fat content

Fat is the second largest component of insect nutrients (Mlcek et al. 2014). The average fat content varies within different species and is 13.41 % in grasshoppers, locusts, and crickets (Orthoptera), 27.66 % in caterpillars (Lepidoptera), 29.90 % in cockroaches (Blattodea), 30.26 % in true bugs (Hemiptera), 32.74 % in termites (Isoptera) and 33.40 % in beetles and grubs (Coleoptera) (Rumpold & Schluter 2013). Mlcek et al. (2014) reported that adult insects contain less fat than larvae and pupae. Furthermore, they found that females are fatty than males. Interestingly, the fatty acid profile of insect seems to be dependent on diet and species (Schluter et al. 2017). Insects have generally less saturated fatty acids comparing to unsaturated fatty acids (de Castro et al. 2018). Orders such as Isoptera, Lepidoptera and Coleoptera show a significant source of the essential fatty acids such as α-linolenic and linoleic acid (Bukkens 1997). These essential fatty acids could possibly replace the potential deficient intake of omega-3 and omega-6 fatty acids which recently occurs in certain developing countries (Roos et al. 2010).

3.3.4 Fibre

Insects contain a significant amount of insoluble fibre derived from the exoskeleton, known as chitin (van Huis et al. 2013). Chitin is a derivate of glucose - a long-chain polymer of N-acetyl glucosamine and is similar to the cellulose which is commonly found in plants. It is believed that it is indigestible by humans, even though chitinase has been found in human gastric juices. Studies have shown that the presence of chitinase is associated with defense against parasitic infections and certain allergic conditions (Paoletti et al. 2007).

Bukkens (1997) found that insects with a hard exo-skeleton such as crickets, grasshoppers and termites contain something between 4.9 – 12.1 g/100 g dry weight of fibre. These data demonstrate that the fibre content of insects is surely higher comparing to other animal products and is similar to that of grains.

It has been discovered that insect chitin and chitosan show a large spectrum of biological activities, which could be useful for biomedicinal, food and industrial purposes. Their antibacterial and antioxidant effects with significant rheological properties could thus improve quality control, shelf-life, and food safety (Mohan et al. 2020). For example, Ma et al. (2015) found that chitosan extracted from mealworms, grasshopper and cicada slough species show a higher potential fat binding capacity (275 – 645 %) and water holding capacity (594 – 795 %) comparing to shrimp shell chitosan, so that this attribute could be a promising feature for food applications. Another study describes a better antifungal activity of chitin isolated from *Pterophylla beltrani* against the entomopathogenic fungi *M. anisoplia* (Torres-Castillo et al. 2015). Chung et al. (2004) found that chitosan isolated from shrimp and crab shell show a better action against Gram-negative bacteria comparing to Gram-positive microbes. This difference is caused mainly due to the hydrolysis of peptidoglycan where the positively charged chitosan molecules interact with negatively charged microbial cell membranes. This process leads to cell death due to the disintegration of cell membranes and consequent extinction of intracellular components (Chien et al. 2016).

3.3.5 Minerals and vitamins

Most edible insects such as crickets, mealworms, termites, and grasshoppers contain zinc, calcium, copper, magnesium, manganese, phosphorous and high amount of iron (de Castro et al. 2018). Comparing to beef with the iron content of 6 mg/100 g of dry weight, locusts contain 8 to 20 mg/100 g, caterpillar reaches the iron value of 31 to 77 mg/100 g (Oonincx et al. 2010).

Mlcek et al. (2014) found that edible insects contain vitamin K, D, E, C and B_1, B_2, B_6 which are responsible for stimulating metabolic processes and enhancing immune system functions. Orders such as Coleoptera and Orthoptera are rich in folic acid (Rumpold & Schluter 2013). Bukkens (1997) found that content of B_1 in edible insects ranges from 0.1 to 4 mg/100 g of dry matter and content of B_2 ranges from 0.11 to 8.9 mg/100 g, comparing to wholemeal bread which provides 0.16 to 0.19 mg/100 g of B_1 and B_2. Vitamin B_{12} is mostly presented in food of animal origin and its content is extremely limited within insect species (Finke 2002). Schmidt et al. (2018) found that vitamin B_{12} occurs in multiple different bioactive forms, known as vitamers, in Jamaican field crickets (*Gryllus assimilis*) with 2.88 µg/100 g, in mealworm larvae (*Tenebrio molitor*) with 1.08 µg/100 g, in cockroach (*Shelfordella lateralis*) with 13.2 µg/100 g and in grasshoppers (*Locusta migratoria*) with 0.84 µg/100 g dry weight. Oonincx & Dierenfeld (2012) described the content of vitamin E in fruit flies (*Drosophila melanogaster*) and false katydids (*Microcentrum rhombifolium*) which is about 110 mg/kg of dry matter. Palm weevil larvae (*Rhynchophorus phoenicis Fabricius*) contains 35 mg/100 g of α-tocopherol and 9 mg/100 g of β+γ tocopherol; the daily recommended intake is 15 mg (Bukkens 1997).

3.4 Rearing insects

The demand for foods and water is still increasing worldwide, thus the insects with its high nutritional value and eco-friendly impact on the environment become more and more popular to rear (Baiano 2020). Gerber et al. (2013) declare that between 2000 and 2050 the demand for livestock products will double due to the increasing request for meat products by the population of the developing countries.

There exists three ways of obtaining edible insects such as wild harvesting, semi-domestication, and farming (Yen 2015).

3.4.1 Wild harvesting

The most traditional way to gather insects is harvesting from the wild (waterways, forests, and agricultural fields) (Melgar-Lalanne et al. 2019). According to Yen (2015) about 92 % of edible insect is harvested from the wild, 6 % are semi-domesticated and only 2 % of insects are artificially raised.

Wild insect harvesting dominates in most of the Asian and Africain countries and serves as a seasonal product only (Raheem et al. 2018). A large variety of species can be collected depending on diverse life stages. Small-scale producers following the traditional and cultural practices have the necessary knowledge to recognize the proper conditions, timing, and host vegetation to produce specific edible species without damaging the environment (Durst & Hanboonsong 2015). Insect farms are hardly present, so the insects are mainly collected from

fields for home consumption. The method of collecting insects from the wild depends on insect behaviour. Some cricket species, for example, are located by the sound they make. Palm weevils are attracted to artificially created breeding sites, while some termites and grasshoppers can be lures into traps by light (Barretau 1999).

The growing demand for edible insect is still increasing but the lack of availability creates the accessibility issues and therefore, reduces opportunities for increasing trade (Shelomi 2015). It is then necessary to make the technological leap from wild harvesting to indoor farming with the help of indigenous people who have the knowledge associated with wild harvesting (Raheem et al. 2018).

3.4.2 Semi-domestication

Semi-domestication of insects is widely spread in Africa and Asia process, which involves some manipulation of insect's habitat to increase their production (Raheem et al. 2018). In total, three types of edible insects, whose production was enhanced by environmental manipulation, do exist. One of them is palm weevil which is semi-domesticated in Malaysia, Indonesia, Thailand, and Papua New Guinea and its production was enhanced by cutting down palm trees in order to provide new breeding sites for such beetle. Also, various caterpillars are reared in Africa. Finally, the eggs of aquatic Hemiptera, are semi-domesticated in Mexico, where the production was enhanced by better water management and provision of oviposition sites (Yen 2015).

The advantages of semi-domestication are more sustainable production, increased availability, and better control on the predictability of the target species while placing less exploitation stress on the environment. The techniques lay in the introduction of target insects on to food plants and in the planting of suitable food plants to increase insect populations. One of the examples could be also the use of alternative host plants. In Bas-Congo, the introduction of Australian plant *Acacia auriculiformis* helped five species of native edible caterpillars with the food intake; they were fed on its leaves (Latham 2003).

3.4.3 Farming

Insect farming can be accomplished in different ways such as simple single cage farming or large semi-automated factory. Small farm models have the potential to reduce loss of genetic diversity, to decrease a risk of insect disease destroying a whole colony and to keep a wider diversity of insects while factory scale production is focused on the production of large amounts of insects. Farming insects for feed and food purposes has, therefore, a great potential in business (Raheem et al. 2018).

An initial point when considering insect rearing is the factors related to the housing of insects and its substrates. These factors are life developmental stage, temperature, frequency, and timing of feeding, and nutritional composition of feed. All these factors vary within insect species (Raheem et al. 2018). According to Wook Jo et al. (2014) it takes a few months to raise crickets to maturity, while black soldier fly's life cycle duration is only about a few days. Additionally, it is known that temperature affects the growth of insects; the higher the temperature, the faster the development of insects (Booth & Kindell 2007). Oonincx et al. (2010) found that *ad libitum* feeding results in higher greenhouse gas production during the

growth. Also, the nutritional composition of feed is very important. For instance, Van Broekhoven et al. (2015) found that too much protein in the diet leads to the overproduction of uric acid, while a very small amount of protein is not good for insect development. Then, the availability of nutrients depends on airflow, moisture, pH, and particle size. It has been discovered that a smaller size in feed increases the availability of nutrients (Oonincx et al. 2010).

Currently, the house crickets and yellow mealworms are the major insects farmed as food. The reason for that is their commercial success; they are mostly used as pet feed in North America, Europe, and parts of Asia. Most farmed insects can be raised continuously and all year in small ventilated plastic containers in a short period due to their small size and short life cycle (house cricket: 30 - 45 days). The high ambient temperature (up to 30 °C) and relative humidity up to 70 % is required as well as the feed mostly composed of organic wastes and cereals (Guhakar 2016). Generally, a diet with high fat (around 9 %) and high protein content (around 20 %) is needed to obtain the best results in terms of feed conversion efficiency, nutritional composition and survival and development time. Fruits and vegetables in the diet are considered as a water source, however small quantity of water is added to prevent fungal contamination (van Broekhoven et al. 2015). Insects have high production densities, do not require sunlight during life stages and have low technical requirements (Hanboonsong et al. 2013).

3.4.3.1 Farming crickets

Currently, cricket farmers can maintain colonies of all sizes – from a single small container suitable for e.g., amphibian raising up to 10,000 square meters of rearing space across multiple buildings. The efficiency of the farm and of the yield per square meter increases at bigger scale. When rearing crickets, it is common to use multiple self-contained space which has its own population, food and water sources, and space maximizers (egg trays or cardboard stackers). To farm crickets, there exists multiple types of containers that each present their own advantages and challenges (see Figure 1). Some of the common options are as follows:

- Off-the-shelf plastic storage tub; 100 - 200 litres (Figure 1A)
- Larger cardboard, custom container, or fiberglass; 500 - 700 litres (Figure 1B)
- Concrete construction or a wood or metal frame draped in plastic sheeting; 3,000 - 8,000 litres (Figure 1C)
- Building containers for the crickets – all materials are placed on the floor of a large room, so that farmers can walk through and deliver water and food; 10,000 litres or more (Figure 1D)

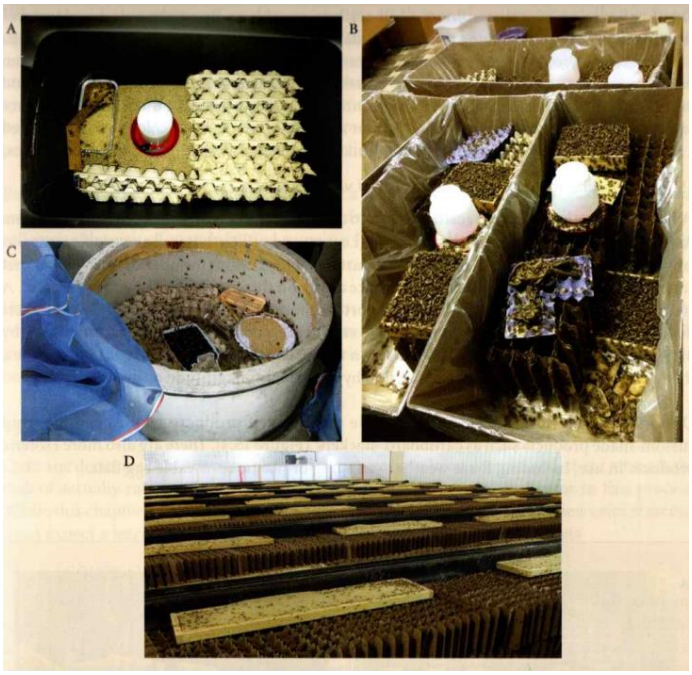

Figure 1 Suitable housing for crickets: (A) plastic storage tub (photo by Austin Miller); (B) plastic-lined cardboard boxes (photo by Gabe Mott); (C) trough at cricket farm in Chiang Mai, Thailand (photo by Arnold van Huis); (D) a room-sized farming area (photo by Entomo Farms) (van Huis & Tomberlin 2017)

As already mentioned, variety of less common containers exist. These are, for example, collapsible nets, custom stable stackable plastic boxes, modified two-litre soft drink bottles and many others (van Huis & Tomberlin 2017).

3.5 Using waste/by-products to feed the insects

Nowadays, one-third of all food produced annually ends up as waste. Large quantities of this organic waste come from the vegetable, fruit, fermentation, meat, dairy, or olive oil production. New measures and attitudes are being taken to create a more sustainable and healthier food production and consumption system which goal is to produce less waste. The European Commission launched the Food 2030 project which corresponds to the UN Sustainable Development Goals that is also relevant for the insect sector. It puts emphasis on the reduction of environmental impacts, it stresses on more sustainable general behavior and it encourages consumers to shift to more safe and nutritious diets. Insect farming and its feeding with by-products from the agri-food industries is one of the possible solutions of these societal challenges. Insects can be fed with co-products from the starch, grains, vegetable, and fruit supply chains, such as distiller grains, bran, unsold vegetable, and fruit, including peels (IPIFF 2019).

Insects can be raised on waste or side streams containing satisfactory nutrients or reared on land that is not suitable for other purposes (Dossey et al. 2016). According to van Broekhoven et al. (2015) balanced diet which is composed of organic by-products can be convenient for the great growth of mealworm species. Makkar et al. (2014) found that especially mealworms can convert low-quality, plant-derived waste into a high-quality feed rich in protein,

energy, and fat in relatively short time. It has been found that an organic food-based diet is crucial for colony maintenance, larval growth, and mass density (Morales-Ramos & Rojas 2015). In general, insects can convert feed to body weight much more efficiently comparing to conventional livestock. Furthermore, they can be reared on organic waste streams which are transformed into high-value food and feed (Makkar et al. 2014).

Several studies with promising results have been performed to test an artificial diet based on food wastes for mealworm and black soldier fly mass production. Three edible mealworm species, such as *Tenebrio molitor L*., *Zophobas atratus Fab*., and *Alphitobius diaperinus Panzer* were reared on mixed grains and following parametres, such as protein and fatty acid profile were observed. It has been found that larval protein content was not influenced by diet composition while larval fat composition was affected to a certain extent. Regarding the rearing of black soldier flies on various food waste, such as bread and biscuit remains, mixture of egg content, spent grains and beer yeast, potato steam peelings, waste plant tissues, catering waste, garden waste, municipal food waste, hatchery waste or wheat bran, the weight reduction of rested waste materials was determined. It thus showes a great ability of the black soldier fly to degrade food and plant organic waste. However, most studies provide no details about the impact of artificial diets on nutritional composition of edible insects, so more detailed studies are needed (Varelas 2019).

Recently, a great attention is focused on the wastes generated from the forest industry during the industrial processes comprise forest by-products such as stem wood, bark, wasted round wood, saw dust and foliage as well as wood processing industry by-products like shavings, bark, log-off cuts, product-off cuts, and sander dusts. This organic waste is full of lignin, starch, cellulose, hemicellulose, phenols, steroids, terpenes, fatty acids, and fatty alcohols, also resin acids, waxes, oils, phytosterold, flavonoids and tannins (Sjöström 1993). Thanks to the nutritionally rich substances that the waste contains, it could be used as an artificial diet for forest insects. Even though the forest waste has many uses in various areas of life (compost, biofuels), it has not yet been used as feed for insects, thus it attracts attention of the latest research (Varelas & Langton 2017).

3.6 Jamaican field cricket

3.6.1 Taxonomy

The Jamaican field cricket, previously known as *Acheta assimilis,* was first described by Danish zoologist Johan Christian Fabricius in the area of Jamaica and is now widespread in the West Indies. Its scientific name *Gryllus assimilis* was applied to all New World field crickets until 1957 because all species of field crickets looked pretty much alike. Finally, the biologists were able to separate the species based on the different calling songs that the crickets make. *Gryllus assimilis* belongs to the order of Orthoptera, family Gryllidae (Alexander & Walker 1962). Due to its convenient size, easy breeding and specific songs, field cricket becomes a favorite subject for studies of behavior, acoustic communication, and neurophysiology (Huber et al. 1989).

3.6.2 Distribution and habitat

Field crickets occur mainly in Mexico, southern United States specifically in Florida and Texas, West Indies, and parts of South America. Its characteristic habitat is lawns, roadsides, weedy fields, and other open areas. In the case of African field cricket in which all adults are long-winged, they were found more than 500 miles from shore flying at sea. It may be possible that Jamaican field cricket reached Florida from the West Indies by its own power (Huber et al. 1989).

3.6.3 Physical appearance and calling song

Generally, Jamaican field crickets are large (15 to 31 mm), all adults have long hind wings, that is why they are considered as macropterous and are probably the brownest of all American field crickets – the visible parts of abdomen and the front of the head are really black. The coloration is brownish red which is seen in the figure 2. The head is often narrower than pronotum and the dorsal pronotal surface is covered with short, brown, fine hairs which makes the cricket's look dull and more ruffled comparing to, for example, other Florida species. Most of the area around the eyes is light yellow-brown and the arms are well defined due to the Y-shaped, scientifically called ecdysial suture. Crickets have three pairs of limbs where the third pair consisted of long and strong shins and thighs is adapted to jumping. The abdomen has a cylindrical shape and contains copulating organs and ovipositor. The ovipositor of the female is always shorter than the body (Alexander & Walker 1962). The difference between the sexes is remarkable and consists mainly in the size of abdomen. The female is generally bigger, grows to lengths of 19 to 24 mm and 7 to 9 mm in diameter, does not have the rudimentary wings but has two small scales which cover the abdomen. The male's body is 18 to 23 mm in length and 5 to 7 mm in width (Friederich & Volland 2004).

Figure 2 *Gryllus assimilis*, female adult (available from: https://www.biolib.cz/en/taxonimage/id189682/?taxonid=285819)

As previously mentioned, it was the various calling songs of the crickets which finally led to their taxonomic classification (Alexander & Walker 1962). Characteristic attribute of calling song of Jamaican field cricket lies in a brief chirp which repeats about once per second. Each chirp has usually seven or more pulses where the initial ones are briefer and more rapid,

and the terminal pulses are rather longer and slower. The chirp of *Gryllus assimilis* sounds more like a continuous sound comparing to other chirping species thanks to the high pulse rate and the brief pulse intervals (Weissman et al. 2009).

3.6.4 Life cycle

Life cycle of *Gryllus assimilis* undergoes incomplete metamorphosis - egg, nymph, adult (Alexander & Walker 1962). At 25 ˚C, young crickets hatch from the eggs after 13 days. When the temperature rises to 30 to 33 ˚C, the process accelerates so it is possible to expect hatching after only 9 days. Nymphs mature in about 6 to 7 weeks. With increasing temperature, the time of maturing can be reduced to 5 weeks. The number of the offspring per female is 250 - 350. The adult females live about 12 weeks (Alexander & Walker 1962; Friederich & Volland 2004).

This species probably occurs in all stages at all time of the year which makes it suitable for mass production in colony and harvesting for use as a food and feed product (van Huis et al. 2013). According to Maciel-Vergara & Ros (2017), resistance to different diseases and pests is another advantage of rearing *Gryllus*. Comparing to *Acheta domestica*, *Gryllus assimilis* has larger body size and shows a similar or higher reproduction with the faster growth of the nymphs (Huber et al. 1989).

3.6.5 Rearing conditions

The size of the rearing container depends mainly on the size of the cricket's colony. The container should be at least 60 × 40 × 40 cm where the height is very important, so the crickets cannot easily escape. As illustrated in the figure 3, the whole container must be well sealed up and the lid of the container must be provided with a fine net due to the air which must flow naturally (Friederich & Volland 2004).

Figure 3 Rearing containers (Author of the thesis)

As the crickets are nocturnal insects, they accept a light/dark cycle which is characterised by 12 hours of light followed by 12 hours of dark. That is why the room equipped with an automatic lighting is needed. It is recommended to keep the humidity around 50 to 70 % and the temperature around 28 °C. However, research indicates that the higher temperature might generate faster growth. Moreover, several studies show that the higher humidity and temperature is, the higher risk of potential surface mould or mite invasion may appear. It is suggested to equip the container with egg trays which increase the living surface area of crickets, allow them to move and provide a suitable shelter to small individuals that can hide from the larger ones (see in the figure 4) or during ecdysis. In each container, there must be a suitable place where gravid females will lay the eggs. Various materials such as sand, perlite, potting soil, or peat moss are used. Eggs need to be kept moist during their development period. After about 7 days, young nymphs less than 3 mm large hatch from the oviposition sites. They shed their exoskeleton 11 times into adulthood and the whole development usually takes 6 weeks under temperature 28 - 30 °C. Nowadays, the most common diet for crickets is chicken feed composed mainly from grains and soy protein. Crickets can be also fed by grouts, old bread, oats, and dry dog/cat pellets. As a water source, fruit, vegetable, or green leaves may be used (van Huis & Tomberlin 2017).

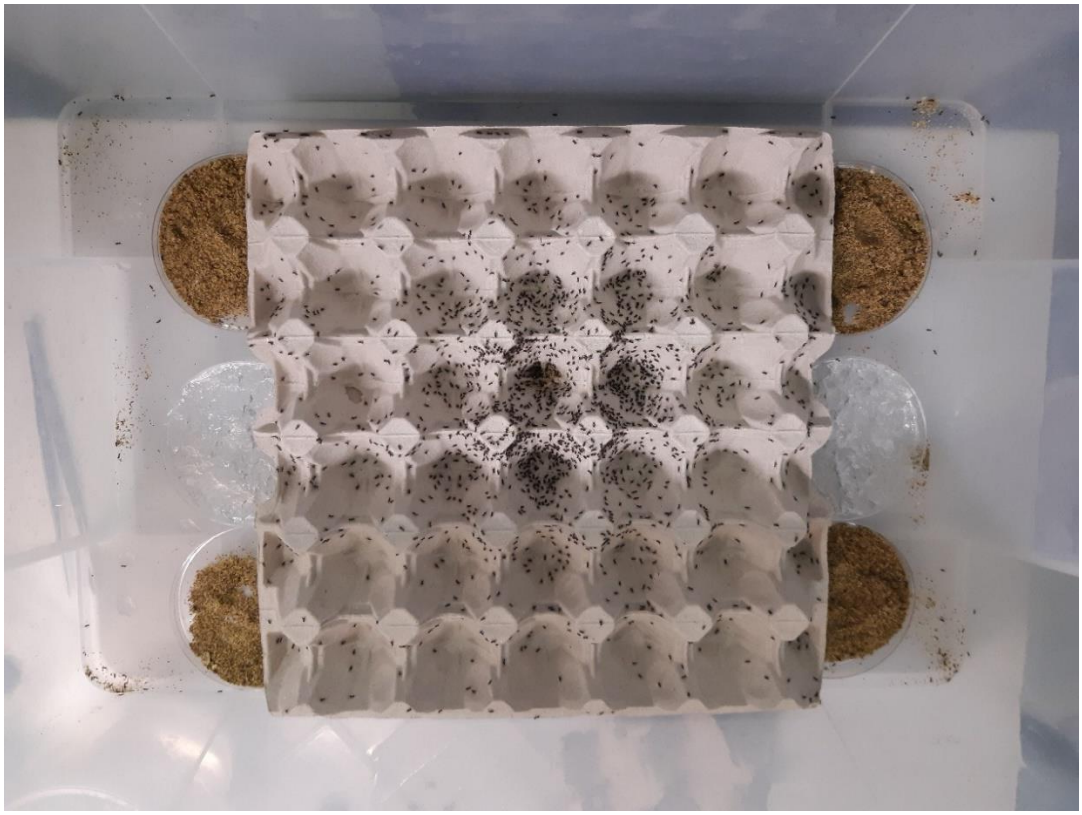

Figure 4 Rearing container equipped with egg trays (Author of the thesis)

3.7 Research relevance

Nowadays, insect rearing, and its consumption has become a big trend especially because of its excellent protein content and a good source of other substances necessary for nutrition. Unlike livestock farming, insects occupy much smaller areas and their breeding is generally

more environmentally friendly. The advantage is their low water consumption, incomparably lower greenhouse gas production, faster generation interval and more efficient feed conversion (van Huis 2013). Regarding the environment, animal health and welfare, it is necessary to continue exploring alternative sources of feed and food (Schluter et al 2017).

As previously mentioned, insects can be reared on the organic waste and by-products gained from the agriculture, food, and forest industries (Dossey et al. 2016). In the Czech Republic, rapeseed (*Brassica napus*) has become one of the most profitable crops and among European Union states represents the highest share in total crop area (USDA 2018). Therefore, it has been decided that in this experiment, the insects would be fed with mixtures containing rapeseed pomace. From an economic and ecological point of view, rapeseed could be a possible alternative which could, in the future, contribute to the optimization of breeding technology and therefore, subsequent reduction in insect production.

4 Methodology

4.1 Cricket rearing

Determination of the life cycle and nutritional value difference depending on the various feed mixtures was demonstrated on Jamaican field cricket - *Gryllus assimilis*.

4.1.1 Rearing conditions

All crickets were reared in the insectarium with an area of 10 m^2 at the Faculty of Agrobiology, Food and Natural Resources in the plastic containers of 56 × 39 × 28 cm and a volume of 45 liters. In total, 15 rearing containers were used. All experimental groups (B, C, D, E), including the control one (A), were placed into rearing containers in three replicates. The experimental groups differed in the food mixtures (RC 70, RC 52.5, RC 30, RC 17.5 – explained below) by which they were fed for a whole life cycle. The containers were sealed up with a plastic lid to prevent possible escape of crickets. The lid was provided with an aluminium fine net to allow the air to circulate. All containers were filled with several egg trays to provide a shelter to small crickets and to extend the space in the container. The whole insect laboratory was heated to 27 ± 1 ˚C with a photoperiod of 12: 12 and the air humidity of 30 - 40 %.

4.1.2 Feed mixtures and water source

As a feed, five types of feed mixtures were used: C which was given to control group A, RC 70 which was given to experimental group B, RC 52.5 given to group C, mixture RC 35 served to group D and feed of RC 17.5 given to experimental group E. The nutrient content of mentioned feed mixtures differed manly in the percentage of rapeseed pomace, wheat, and soya extract. This is shown in following tables 5 and 6.

Table 5 Composition of feed mixtures (provided by V. Plachý)

experimental group	**feed mixture**	**composition of feed mixture [%]**						
		wheat	soya extract	rapeseed pomace	rapeseed oil	calcite	salt	sodium carbonate
A	C	77.97	17.60	0.00	1.80	1.00	0.13	0.35
B	RC 70	21.57	0.00	70.00	5.30	1.00	0.13	0.35
C	RC 52.5	36.97	4.10	52.5	3.80	1.00	0.13	0.35
D	RC 35	50.37	8.60	35.00	3.40	1.00	0.13	0.35
E	RC 17.5	63.970	13.20	17.50	2.70	1.00	0.13	0.35

C Control group, no addition of rapeseed cakes; RC 70 containing 70 % of rapeseed cakes; RC 52.5 containing 52.5 % of rapeseed cakes; RC 35 containing 35 % of rapeseed cakes; RC 17.5 containing 17.5 % of rapeseed cakes.

Table 6 Nutrient content of 1 kg feed mixtures (provided by V. Plachý)

experimental group	feed mixture	nutrient content of 1 kg feed mixture [g]					
		NL	LYS	MET	MET+CYS	THR	linoleic acid
A	C	186.252	8.387	3.180	8.468	7.783	9.686
B	RC 70	186.806	9.065	4.576	8.275	8.278	39.714
C	RC 52.5	186.984	7.884	3.703	8.894	8.166	31.502
D	RC 35	186.387	8.040	3.523	8.734	8.023	24.550
E	RC 17.5	186.525	8.234	3.355	8.601	7.910	17.233

C Control group, no addition of rapeseed cakes; RC 70 containing 70 % of rapeseed cakes; RC 52.5 containing 52.5 % of rapeseed cakes; RC 35 containing 35 % of rapeseed cakes; RC 17.5 containing 17.5 % of rapeseed cakes.

The substrate was placed usually onto four Petri dishes and changed every time it ran out, so the crickets were fed *ad libitum*. The amount of used subtrate was always weighed and recorded to a laboratory notebook.

Aqua Crystal Gel (ACHETA farm, Mšené Lázně, Czech Republic) was used as a source of water. It was made of granulate which absorbed all the water into itself and thus increased its volume. Water gel was placed in the plastic Petri dishes and was exchanged for fresh every day.

4.1.3 Oviposition sites

For laying eggs, a small oviposition site of 11 × 8 × 5 cm was placed into a big rearing container to the crickets of generation 0. The small container was filled with a moist substrate and covered with a lid with a small metal net so that the crickets would not mess up the substrate and destroy the already laid eggs. The oviposition sites were moistened every day to keep the substrate damp most of the time. If the oviposition site had dry out, the eggs would not have hatched. On the other hand, if the oviposition site had been too wet, mold could have started forming on the egg substrate. Once the eggs were laid, the oviposition sites were transferred to a bigger container where, after a few days, the young nymphs started hatching. Meanwhile, a new oviposition site was given to the adult crickets and the whole process was repeated.

4.1.4 Life cycle and weighing

In the experiment, the crickets were harvested at the age of 60 days, when all the crickets were the same age (± 1 day). Everyday, precisely 2 g of fresh hatched nymphs (1 ± 1 days old) were weighed and transferred into each rearing container where they remained until the end of experiment. Each container was marked (see on the figure 5) with the name of the species (*Gryllus assimilis*), type of experimental group (e.g. C1), type of feed mixture (e.g. RC 52.5), day of hatching (e.g. 13.10.2021) and days of weighing (e.g. 20.: 2.11., 40.: 22.11.).

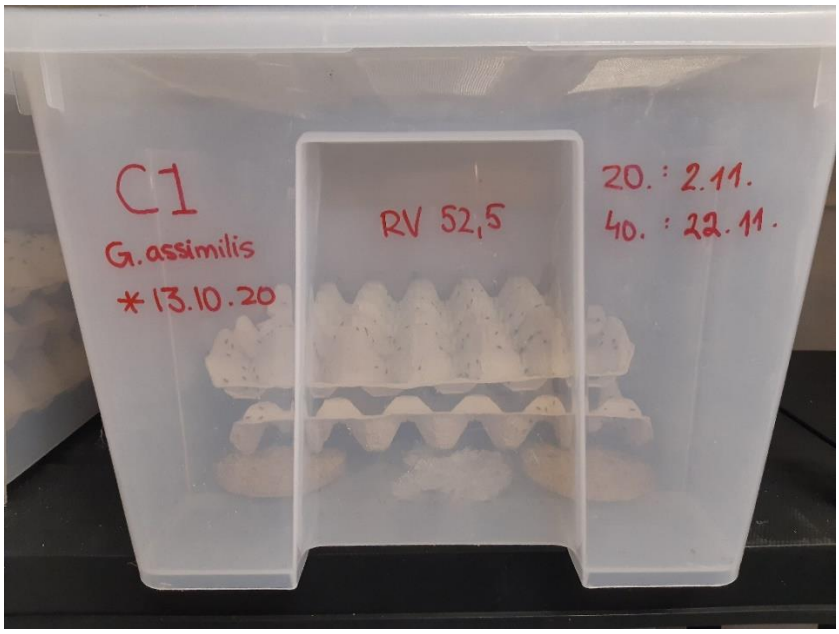

Figure 5 Marked rearing container (Author of the thesis)

Every 20th, 40th and 60th day, twenty crickets were weighed (see on the figure 6) and all the data were recorded into a laboratory notebook.

Figure 6 Weighing of adult female *Gryllus assimilis* (Author of the thesis)

Harvesting was done in all groups 60th day after hatching. Before harvesting, the feed residues were removed and weighed. Then, the crickets were starving for 24 hours to empty their digestive tract. This was necessary due to the contents remaining in their digestive tract that could possibly affect the results of the chemical analysis. After fasting, crickets were sieved from the faeces and all were transferred to a plastic box, weighed and freeze-killed in a freezer at the temperature of -70 °C.

4.1.5 Calculation of food conversion ratio

The feed conversion was calculated according to the formula below:

$$conversion = \frac{effective\ feed - feed\ leftovers}{total\ harvest}$$

4.2 Chemical analysis

4.2.1 Lyophilisation

Lyophilization was performed in a ScanSpeed MaxiVac apparatus (LaboGene, Lillerød Denmark). Samples which were frozen at -70 °C were placed into the device in closed containers and lyophilized for 72 hours, at a temperature of 28 °C, 200 rpm and at 1 - 5 millibars. Beforehand, the container, its lid and also the non-lyophilized insects contained in the container were weighed on the analytical balance. After lyophilization, the lid and the container together with the lyophilized insects were weighed again. The weight of the lyophilized crickets was calculated by subtracting the weight of the empty container and lid from the lyophilized insects in the container. In contrast to heat drying, a wide amount of substances is not destroyed during lyophilisation, but the maximum drying of the sample is not achieved.

4.2.2 Dry matter

The samples were dried at 103.5 °C in a Memmert UFB 500 oven (Memmert, Buechenbach, Germany). Firstly, an empty porcelain crucible was weighed and then 3 - 5 g of lyophilized sample was placed into it. Each sample had two replicates. These crucibles were left in the oven for 15 hours. The dried samples in the crucibles were placed in a desiccator where they remained for one hour until they cooled down. After cooling, the samples in the crucibles were reweighed. The amount of evaporated water from the lyophilized sample was calculated by subtracting the weight of the dried sample from the crucible from the weight of the lyophilized sample with the crucible. This was followed by conversion to the dry matter content of the fresh sample.

4.2.3 Ash

The ash was determined by burning the samples at 550 °C in a muffle furnace. Firstly, the weight of the porcelain crucible was weighed together with the insect sample and then the sample was incinerated. Each sample had two replicates. After incineration, the crucibles were transferred to a desiccator for one hour to cool down. Afterwards, the crucible with the ash was reweighed and the amount of ash in the samples was calculated.

4.2.4 Fat

The fat content was determined according to Soxhlet on the Soxtec SER 148 extraction apparatus (Velp, Usmate, Italy). To determine the fat content, it was necessary to prepare lyophilized and well-homogenized samples in two repetitions. Firstly, the glass containers had to be dried in an oven and then cooled down in a desiccator. Then, the paper cartridges were used to weight 4 - 5 grams of the sample and sealed with cotton wool. Next, the glass containers were filled up with 70 ml of petroleum ether. The samples in the cartridges were then placed in a Soxtec SER 148 extraction apparatus and immersed in the containers with petroleum ether. Firstly, the device was heated to 90 ˚C and then extracted the fat in three phases which lasted 110 minutes in total. After fat extraction, the glass containers with fat were transferred to an

oven where they were dried at 103.5 °C for 12 hours. After drying, the samples were cooled in a desiccator for one hour and then weighed on an analytical balance.

4.2.5 Crude protein

The crude protein content was determined by the Kjeldahl method on the Kjeltec 2400 apparatus (Foss, Hilleroed, Danmark). With the use of an analytical balance, sample of 0.2 g was weighed in three repetitions. This amount of the sample was placed in the glass tubes to which one copper tablet was added along with 10 ml of 96% sulfuric acid. Then, each glass tube was filled with 5 ml of hydrogen peroxide twice. The tubes were closed with lids and placed in a preheated (420 ˚C) heating mantle where they were left for 45 minutes to get the samples mineralized. After removal, the samples were green in color and 10 ml of distilled water was added to the samples while observing the colour change to blue. The tubes were then gradually placed in a Kjeltec™ 2400 apparatus which titrated the percentage of crude protein in the samples. This device multiplied the measured data by a conversion factor of 6.25.

4.2.6 Chitin

The chitin content was determined according to Woods et al. (2019) but the whole method was modified for the purpose of simplification and quicker evaluation of the measured results. The method was performed on the Velp Scientifica™ FIWE6 Raw Fiber Extractor (Velp, Usmate, Italy). This method consisted of three step process – first, removal of the fat, second, removal of the protein and mineral fractions with acid and alkali respectively, third, drying, weighing and incineration of the samples. Firstly, into a sintered glass filter 0.5 g of the sample was weighed in two replicates. Then, the sintered glass filters were placed to the Velp Scientifica™ FIWE6 COEX Cold Extractor (Velp, Usmate, Italy) to remove the fat. Each sintered glass filter was three times flushed with petroleum ether and then transferred to the Raw Fiber Extractor. Depending on the type of glass tubes, approximately 150 – 200 ml of 1 M hydrochloric acid was added to the glass tubes along with 5 drops of 1-octanol to prevent the excessive foaming. The demineralisation lasted one hour at 100 ˚C. After demineralisation was complete, the residue was washed three times with 250 ml of boiling distilled water to achieve a neutral pH. Deproteinisation was performed using an alkaline treatment with approximately 150 - 200 ml of 1 M sodium hydroxide solution and 5 drops of 1-octanol at 100 ˚C for two hours. After two hours, the solvent was filtered out under vacuum, the residue was washed three times again with 250 ml of boiling distilled water and the treatment was repeated. After deproteinization was ended, the sintered glass filters with the samples were placed to an oven where they were dried at 103.5 °C. The samples were then weighed, incinerated, and reweighed.

The chitin content was determined only in the samples with the largest addition (RC 70 groups) and no addition (C groups) of rapeseed cakes in the feed mixture. Due to the lack of time caused by epidemiological situation, the measurement was not carried out for other samples.

4.3 Statistical evaluation

The results are expressed as the arithmetical means and standard deviations. The data were statistically evaluated using the Statistica 13.2 software (StatSoft, Tulsa, USA) using one-way and two-way factorial analysis of variance (ANOVA) and Schéffe's post-hoc analyses with a significance level of $\alpha = 0.05$.

5 Results

5.1 Feed mixtures

Dry matter and the basic nutrients such as total fat, crude protein, and ash were determined in the different feed mixtures (see Table 7). It is apparent that the highest fat, protein, and ash content was in the feed mixture RC 70, while the lowest content of all nutrients shows the control (C) feed mixture.

Table 7 Contents of dry matter, total fats, crude protein, and ash in the experimental and control feed mixtures.

feed mixture	DM	TFC	CP	Ash
	g/kg feed		g/kg DM	
C	897.3	3.7	22.3	3.8
RC 70	921.5	15.7	28.0	5.4
RC 52.5	911.1	12.2	26.8	5.1
RC 35	911.5	11.7	25.7	4.4
RC 17.5	908.9	8.3	23.6	5.0

C Control group, no addition of rapeseed cakes; RC 70 containing 70 % of rapeseed cakes; RC 52.5 containing 52.5 % of rapeseed cakes; RC 35 containing 35 % of rapeseed cakes; RC 17.5 containing 17.5 % of rapeseed cakes.
DM = dry matter, TFC = total fat content, CP = crude protein

5.2 Life cycle and feed conversion ratio

The average initial weights and standard deviations of *Grylllus assimilis* characteristic for each experimental group are shown in the Table 8 as well as total harvest, effective feed, and feed conversion.

Table 8 The overall summary of cricket's initial weight, total harvest, effective feed, and efficiency of feed conversion ratio.

experimental group	initial weight	total harvest	effective feed	conversion
	[g]			
A	2.05 ± 0.05	130.8 ± 38.5	321.2 ± 55.4	2.6 ± 0.6
B	2.02 ± 0.02	114.8 ± 42.9	311.4 ± 21.5	3.2 ± 1.4
C	2.06 ± 0.07	142.6 ± 28.0	455.9 ± 8.7	3.3 ± 0.5
D	2.07 ± 0.03	157.5 ± 48.3	402.8 ± 84.3	2.6 ± 0.3
E	2.01 ± 0.01	324.0 ± 131.4	720.4 ± 219.1	2.4 ± 0.5

A = control group fed by C - no addition of rapeseed cakes; B = group fed by RC 70 containing 70 % of rapeseed cakes; C = group fed by RC 52.5 containing 52.5 % of rapeseed cakes; D = group fed by RC 35 containing 35 % of rapeseed cakes; E = group fed by RC 17.5 containing 17.5 % of rapeseed cakes.

It is obvious that the overall initial weights of the crickets within all experimental groups were almost the same, meaning that the accuracy while starting the experiment was high. As it is shown in the Table 8, the highest total harvest 324.0 ± 131.4 g was seen in the experimental

group E, while the lowest in B with 114.8 ± 42.9 g. These data are closely related to the amount of total effective feed which was the highest in E (720.4 ± 219.1 g) and the lowest in B (311.4 ± 21.5 g). The highest but statistically insignificant feed conversion (see Enclosure I and II) was determined in the experimental group E - 2.4 ± 0.5 g, while the lowest was in the group C 3.3 ± 0.5 g.

5.3 Weight at 60th day

Table 9 shows the average weights and standard deviations of twenty randomly selected and undamaged crickets represented by ten males and ten females at 60th day before freeze-killing. The weight of the male crickets at 60th day ranged from 0.662 to 0.812 mg and the weight of the female crickets at 60th day ranged from 0.979 to 1.051 mg. The average weight of the cricket at 60th day regardless of gender (column "total" in the Table 9) ranged from 0.834 to 0.932 mg. Enclosure III shows the differences in the experimental group E caused by higher weights of the male crickets which is evident from the graphic representation in Enclosure IV.

Table 9 Average weights of twenty randomly selected crickets (10 females and 10 males) at 60th day.

experimental group	**FW day 60 [g]**		
	female	male	total
A	0.979 ± 0.001	0.692 ± 0.017	0.836 ± 0.009^{Aa}
B	1.036 ± 0.014	0.677 ± 0.025	0.857 ± 0.019^{Bab}
C	1.002 ± 0.067	0.669 ± 0.010	0.834 ± 0.038^{Ca}
D	1.042 ± 0.061	0.662 ± 0.020	0.852 ± 0.040^{Dab}
E	1.051 ± 0.060	0.812 ± 0.052	0.932 ± 0.056^{Eb}

A = control group fed by C - no addition of rapeseed cakes; B = group fed by RC 70 containing 70 % of rapeseed cakes; C = group fed by RC 52.5 containing 52.5 % of rapeseed cakes; D = group fed by RC 35 containing 35 % of rapeseed cakes; E = group fed by RC 17.5 containing 17.5 % of rapeseed cakes.

FW = fresh weight

Values with different superscripts are different at $p < 0.05$. The values that are significantly different ($p < 0.05$) are marked with different letters.

5.4 Nutritional values

Table 10 shows basic nutrient content of *Gryllus assimilis*. All the values were statistically evaluated and displayedin tables and graphs attached in the enclosures (Enclosure V – Enclosure XIV).

Table 10 Basic nutrient contents of *Gryllus assimilis*.

experimental group	DM	TFC	CP	Chitin	Ash
	g/kg FW		g/100 g DM		
A	320.5 ± 12.0	33.4 ± 1.9	60.6 ± 1.7	5.6 ± 0.3	3.2 ± 0.1
B	312.9 ± 18.6	24.1 ± 1.6	69.6 ± 1.6	5.6 ± 0.1	4.3 ± 0.1
C	291.8 ± 5.0	27.3 ± 6.4	66.8 ± 6.9	***	4.5 ± 0.6
D	300.9 ± 18.9	26.1 ± 6.7	68.7 ± 7.1	***	4.3 ± 0.2
E	337.5 ± 6.8	26.2 ± 8.5	67.0 ± 7.7	***	4.2 ± 0.8

A = control group fed by C - no addition of rapeseed cakes; B = group fed by RC 70 containing 70 % of rapeseed cakes; C = group fed by RC 52.5 containing 52.5 % of rapeseed cakes; D = group fed by RC 35 containing 35 % of rapeseed cakes; E = group fed by RC 17.5 containing 17.5 % of rapeseed cakes.

DM = dry matter, FW = fresh weight, TFC = total fat content, CP = crude protein

*** Only the samples with the largest addition and no addition of rapeseed cake in the feed mixture were measured, therefore the measurement was not carried out for other samples.

The dry matter content of Jamaican field crickets which were fed by the different feed mixtures (RC 70, RC 52.5, RC 35, RC 17.5, and C) varied from 291.8 to 337.5 g/kg fresh weight. Showing in the enclosures, no statistically significant differences were found between the individual experimental groups fed by different substrates.

Crude protein content in dry matter in the samples ranged from 60.6 to 69.6 g/100 g. In comparison with control group A (CP = 60.6 ± 1.7), higher but statistically insignificant values were measured in experimental groups E – B (67.0 ± 7.7 - 69.6 ± 1.6). However, no statistically significant difference between the groups in the crude protein content was observed. Conversely, the highest fat content in dry matter was detected in the control group A (33.4 ± 1.9 g/100 g) and the lowest in the experimental group B 24.1 ± 1.6 g/100 g. Similarly, the ash content in experimental groups E - B was higher (4.2 ± 0.8 - 4.5 ± 0.6) than that of control group A, varied from 3.2 ± 0.1. Finally, the same chitin levels were analysed for experimental group B and control group A.

As is shown in Enclosures V - XIV, the statistical evaluation does not show any conclusive trend that would confirm that the change in rapeseed protein in the feed caused a significant change in the basic nutrient in the harvested biomass.

6 Discussion

In this diploma thesis, it has been assumed that the amount of added rapeseed protein into the feed mixtures will affect the life cycle of crickets including the total harvest, effective feed, feed conversion ratio, final weights, and nutritional value. The results and measurements may have been affected by various possible factors which could have happened in the beginning of the experiment. These factors could be the sudden deaths of the small nymphs caused by insufficient provision of water supply continuing in mutual cannibalism during the stripping.

6.1 Life cycle and weights

It has been shown that the diet RC 17.5 containing 17.5 % of rapeseed cakes is associated with the highest efficiency of feed conversion (2.4 ± 0.5 g). As it is shown in Table 5, the soya extract content in RC 17.5 feed mixture is the highest (13.2 %) comparing to other rapeseed feed mixtures (e.g. RC 52.5 with 4.1 %). Insects typically require nine to ten essential amino acids for successful development and growth (Oonincx & Dierenfeld 2011). The amino acid composition of rapeseed cakes is similar with soybean (see Table 6), so the feed containing lower levels of proteins and simpler amino acid profiles could modify the feeding behaviour of insects. Our data do not correspond to that of Sorjonen et al. (2019) who found that the high protein level in the by-product diets is associated with high efficiency of conversion of ingested food. The diet rich in high- and medium-protein broad bean shows the highest feed conversion within *Acheta domesticus* (10.1 ± 3.2 % and 10.0 ± 3.1 %) and *Gryllus bimaculatus* (29.5 ± 9.0 % and 22.7 ± 6.9 %). On the other hand, the lowest values of efficiency of feed conversion for *Acheta domesticus* were determined in chicken feed (3.8 ± 1.8 %) and low-protein barley feed diet (4.4 ± 1.3 %), whereas the lowest values of feed conversion ratio for *Gryllus bimaculatus* were observed in medium-protein barley feed diet (7.4 ± 2.3 %) and low-protein barley mash diet (9.5 ± 2.3 %). The highest feed conversion is closely related to the amount of effective feed which was the highest in the experimental group E (720.4 ± 219.1 g). Joern & Behmer (1997) explain that insects can compensate the poorer nutrient content of feed by increase of the consumption of lower quality feed to fulfil nutritional requirements.

The average individual weights at 60th day of randomly selected 10 females and 10 males from each rearing container ranged from 0.979 to 1.051 g for females and from 0.662 to 0.812 g for males. Statisticaly significant differences were observed only in the experimental group E fed by mixture containing 17.5 % of rapeseed cakes, which protein content was low in comparison with other experimental diets. This fact is connected to the higher weights of the male crickets. From this point of view, our data are not in accordance with the experiment of Bawa et al. (2020) which shows that the weight of adult crickets was influenced by the amount of protein in the feed. Crickets reared on a feed containing 16 % of protein weighed about 9 % less comparing to those which were fed by a substrate containing 22 % of protein. They also found that adding a pumpkin to the control feed could have a positive effect on the weight gain of the crickets. Sorjonen et al. (2019) tried to feed the crickets with various by-product protein sources such as barley mash, organic chicken feed, turnip rape, or broad bean-pea and found that the highest final weight was observed in *Gryllus bimaculatus* reared on medium (1.000 ±

0.061 g) and high-protein (0.986 ± 0.059 g) barley mash, and in *Acheta domesticus* on high-protein turnip rape (0.447 ± 0.039 g) and organic chicken feed (0.407 ± 0.039 g). For both species, low-protein turnip rape, barley mash and broad bean-pea produced particularly low-weight individuals. They also found that the fresh weight of female crickets was higher compared to the males. The mean weight of *G. bimaculatus* females and males was 0.912 ± 0.028 and 0.626 ± 0.028 g, which is a bit lower comparing to our data.

6.2 Basic nutrients

6.2.1 Dry matter

The dry matter content of the observed samples was determined between 29.2 and 33.7 % in the original samples. Bawa et al. (2020) declare that the dry matter content in the crickets ranged from 29.25 to 31.65 % and even the feed composition was changed. Study of Bednářová et al. (2013) shows higher average dry matter content (33.28 %), whereas the data of the dry matter content reported by Mlček et al. (2018) were lower (22.6 %). The difference in the measurement can be caused by different conditions of cricket rearing technology. According to the measurements, there was no change in the dry matter content after adding the rapeseed cakes into the feed, so the results of the work correspond to the cited literature.

6.2.2 Total fat content

In this work, the total fat content was determined in *Gryllus assimilis* as well as in different feed mixtures (C, RC 70, RC 52.5, RC 30, RC 17.5). Interestingly, the data show that the highest fat content (33.4 ± 1.9 %) was found in the control group A fed by feed mixture C (no addition of rapeseed cakes) having the lowest fat content of 3.7 g/kg. Similar data are published in the study of Bednářová et al. (2013) who found that the fat content in *Gryllus assimilis* in dry matter ranged around 34.3 %. Obtaining such high values of fat may correlate with the use of cricket nymphs, because according to Finke (2002) some insect species store more fat in their bodies in earlier developmental stages. On the contrary, crickets in our experiment with the lowest fat content (24.1 ± 1.6 %) were fed by the fatties feed mixture RC 70 (15.7 g/kg DM). Measured data of fat content do not correspond to that of Araújo et al. (2019) who determined the average total fat content of *Gryllus assimilis* at 21.8 ± 2.7 %. Also, our fat values do not match with the statement of Bawa et al. (2020), who declare that as the amount of fat decreases and the amount of protein in the feed increases, the fat content in the crickets decreases. According to Oonincx & Dierenfeld (2011), captive-bred insects have a higher percentage of body fat than wild-caught insects, which, according to Finke & Oonincx (2014), is probably affected by reduced physical activity and access to high-energy feeds. Sorjonen et al. (2019) claim that the diets consisting of barley mash and turnip rape showing the values of fat content of 5.4 – 6.5 % and 5.0 – 6.3 % have a positive effect on the overall development of *Acheta domesticus* and *Gryllus bimaculatus*. Oonincx et al. (2019) also confirmed that the fat content in house crickets can be increased by enriching the feed with linseed oil. Based on this diploma thesis, a different amount of fat was observed, but the trend of decreasing fat content in biomass while increasing amount of rapeseed cake protein in feed was not statistically confirmed.

6.2.3 Crude protein

Rumpold & Schlüter (2013) state that proteins are the dominant components of cricket's body and their content in dry matter ranges between 64.1 - 70.8 %. According to Araújo et al. (2019), the protein content of *Gryllus assimilis* in dry matter is 65.5 ± 1.4 % which corresponds to our measured values varying between 60.6 ± 1.7 and 69.6 ± 1.6 %. The highest protein content shows the experimental group B fed with the feed mixture with the highest protein content RC 70 containing 70 % of rapeseed cakes (28 g/kg DM). On the contrary, the lowest protein content is seen in the control group A fed by control feed mixture with no addition of rapeseed cakes with 22.3 g/kg DM of protein. Bawa et al. (2020) showed that the amount of protein content in dry matter of crickets increases with increasing protein content in feed. Crickets fed by high protein (22 %) and low carbohydrate feed contained 76 % of protein in dry matter. On the other hand, crickets fed by high proportion of both protein (18 %) and carbohydrates showed a reduction of protein content in the dry matter to 48.1 %. This is caused by excessive storage of carbohydrates in the form of fats, which leads to the reduction of protein content. Another study performed by Oloo et al. (2019) shows the effect of different diets on the protein profile of crickets. The crickets were fed by various agriculture by-products such as kales, sweet potato leaves, ugali, and banana peels with different protein values. It has been shown that the crickets fed by kale containing the highest protein content of 26.87 % showed the highest protein profile (82.4 %) comparing to other tested diet groups. Sorjonen et al. (2019) claimed that the diet rich in protein consisting mainly of turnip rape (30 %) and barley mash (22.5 %) seems to have a good balance of nutrients for growth of both studied cricket species such as *Acheta domesticus* and *Gryllus bimaculatus*. In this diploma thesis, the protein content of *Gryllus assimilis* was influenced by change of the rapeseed cakes in the diet, but the trend of increasing protein in biomass together with increasing protein content in feed was not statistically confirmed. Therefore, the results of the analysis are not corresponding to the results of the observations of Bawa et al. (2020) and Oloo et al. (2019).

6.2.4 Chitin

In this trial, the amount of chitin in dry matter was determined only in two experimental groups – control group A (5.6 ± 0.3 %) fed by feed mixture with no addition of rapeseed cakes and experimental group B (5.6 ± 0.1 %) fed by RC 70 containing 70 % of rapeseed cakes. Kulma et al. (2019) show that the chitin content in male house crickets vary between 6 to 6.2 % and in the female crickets between 5.4 to 5.5 %. Finke (2007) declares that the amount of chitin in *Acheta domestica* does not differ significantly and Ribeiro et al. (2019) claim that 5.1 % of chitin is found in the dry matter of the house crickets. In this diploma thesis, it has been shown that the amount of added rapeseed cakes in the feed did not increase or decrease the chitin levels in the crickets.

6.2.5 Ash

The ash content was determined between 3.2 to 4.5 %, which does not correspond with the results of Bednářová et al. (2013). The ash content in the dry matter measured by Rumpold & Schlüter (2013) varied between 3.6 to 5.1 %, whereas the content of ash determined by

Ribeiro et al. (2019) in the dry matter of *Acheta domestica* ranged around 4.9 % and of *Gryllodes sigillatus* around 4.2 %. In this diploma thesis, the ash content values were rather lower comparing to the cited literature. The results of performed analysis for this work showed that the amount of ash in the dry matter was not affected by addition of rapeseed cakes into the feed. The similar results are shown in the study of Bawa et al. (2020), where the increase or decrease in protein in the feed did not influence the ash content in the dry matter.

7 Conclusion

In this diploma thesis, the life characteristics, and basic nutrients of Jamaican field crickets (*Gryllus assimilis*) fed on diets with various levels of rapeseed cakes (side streams of rapeseed oil production) were determined. Normally, the chicken feed containing soybean meal as protein supply is used to feed the crickets. In the experiment, the 25 – 100 % of soybean meal was replaced by rapeseed cakes.

From nutritional point of view, no statistically significant differences were found at the significance level of 0.05 for any of the nutrients within the experimental groups and control. It has been also found that the amount of rapeseed protein added into the feed mixtures did not influence the conversion, development, and the final individual weights of *Gryllus assimilis*.

Based on this experiment, it was confirmed that the Jamaican field cricket is able to complete its development on all tested feed mixtures containing up to 70 % of rapeseed cakes without significant effect on both nutritional value and life characteristics. The nutritional value of the crickets, precisely the amount of protein, was in accordance with the cited literature, thus all tested protein levels in the feed mixtures seemed to be within the optimum. Therefore, no need to increase the protein content in the feed mixtures was observed.

The positive contribution of this work is the fact that the crickets could be reared on the agricultural rapeseed by-product, which was used to replace the soybean in the feed mixture. It has been proved that the nutritional value of the crickets was not changed. The advantage of using rapeseed cakes in the feeding mixtures instead of soybeans is their local availability and relatively low price. Therefore, rapeseed could be a possible alternative which could, in the future, contribute to the optimization and design of feed mixtures for commercial rearing technology of the crickets. Lastly, the use of agricultural by-products could improve the environmental sustainability of insect feed mixtures, insect rearing industry, and advance the targets of circular economy.

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9 List of abbreviations

FAO - Food and Agriculture Organization
WHO – World Health Organization

10 List of figures

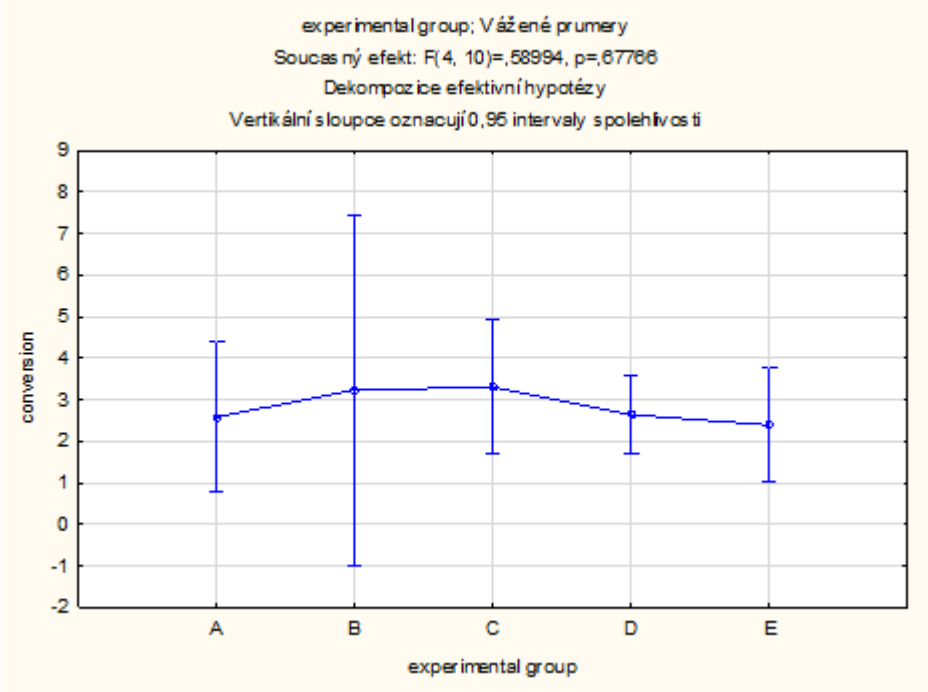
11 List of tables

12 List of enclosures

Enclosure I Scheffé test, conversion

C. bunky	Scheffeho test; promenná conversion (Gryllus_RC_výsledky final) Pravdepodobnosti pro post-hoc testy Chyba: meziskup. PC = ,85881, sv = 10,000					
	experimental group	1 2,5816	2 3,2311	3 3,3007	4 2,6374	5 2,3892
1	A		0,941315	0,917785	0,999996	0,999392
2	B	0,941315		0,999989	0,956858	0,865179
3	C	0,917785	0,999989		0,937040	0,829680
4	D	0,999996	0,956858	0,937040		0,998347
5	E	0,999392	0,865179	0,829680	0,998347	

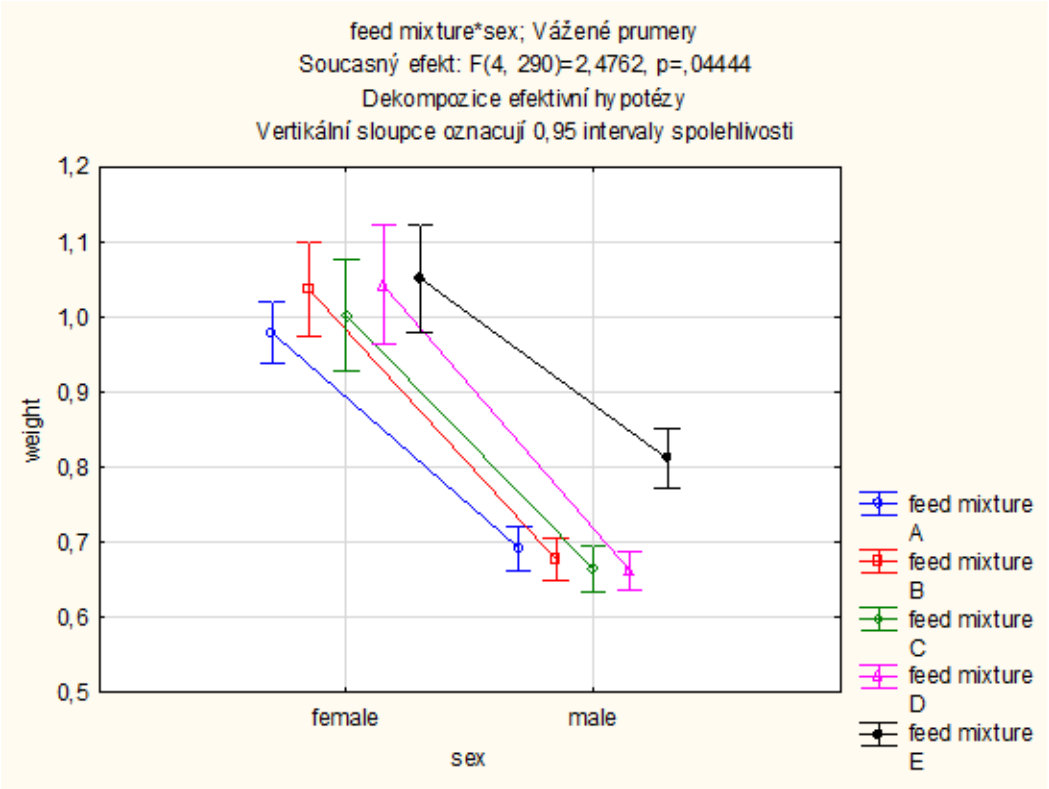
Enclosure II Scheffé post-hoc test, conversion



Enclosure III Scheffé test, average weights at 60th day

C. bunky	Scheffeho test; promenná weight (Gryllus_RC_analysis) Pravdepodobnosti pro post-hoc testy Chyba: meziskup. PC = ,02014, sv = 294,00					
	feed mixture	1 ,83572	2 ,85680	3 ,83366	4 ,85228	5 ,93169
1	A		0,955768	0,999995	0,981680	0,009252
2	B	0,955768		0,938577	0,999885	0,082280
3	C	0,999995	0,938577		0,971758	0,007216
4	D	0,981680	0,999885	0,971758		0,054507
5	E	0,009252	0,082280	0,007216	0,054507	

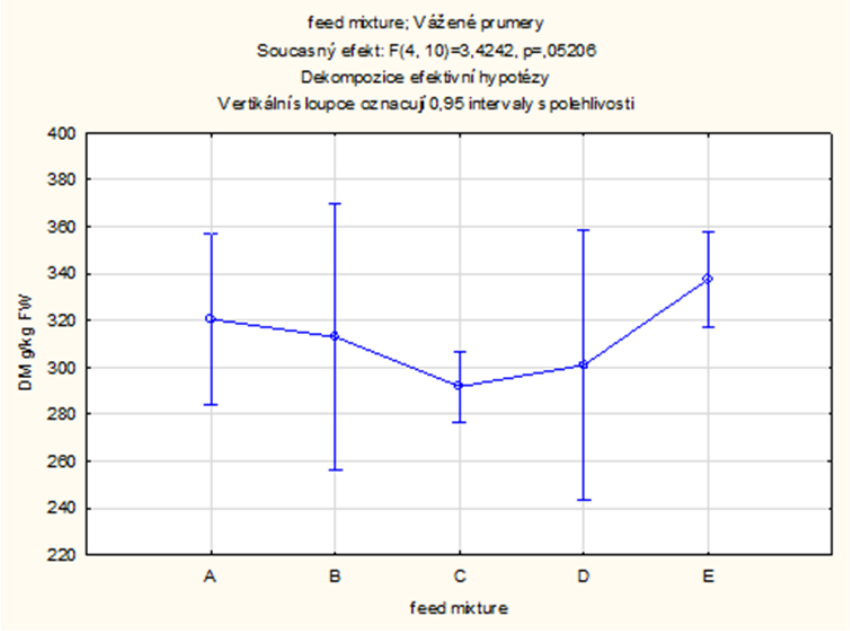
Enclosure IV Scheffé post-hoc test, average weights at 60th day



Enclosure V Scheffé test, dry matter

C. bunky	Scheffeho test; promenná DM g/kg FW (Gryllus_RC_výsledky final) Pravdepodobnosti pro post-hoc testy Chyba: meziskup. PC = 274,54, sv = 10,000					
	feed mixture	1 320,52	2 312,94	3 291,78	4 300,95	5 337,53
1	A		0,987166	0,396561	0,721231	0,807973
2	B	0,987166		0,663708	0,934698	0,538038
3	C	0,396561	0,663708		0,974239	0,081035
4	D	0,721231	0,934698	0,974239		0,200256
5	E	0,807973	0,538038	0,081035	0,200256	

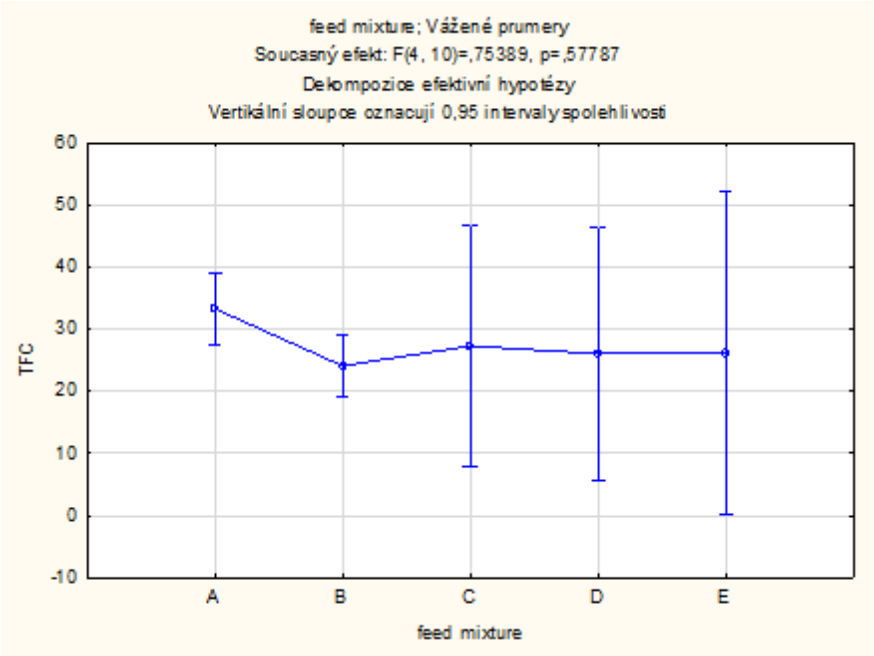
Enclosure VI Scheffé post-hoc test, dry matter



Enclosure VII Scheffé test, total fat content

C. bunky	Scheffeho test; promenná TFC (Gryllus_RC_výsledky final) Pravdepodobnosti pro post-hoc testy Chyba: meziskup. PC = 49,342, sv = 10,000					
	feed mixture	1 33,374	2 24,129	3 27,337	4 26,067	5 26,201
1	A		0,640074	0,886260	0,800592	0,810573
2	B	0,640074		0,987278	0,998145	0,997596
3	C	0,886260	0,987278		0,999648	0,999773
4	D	0,800592	0,998145	0,999648		1,000000
5	E	0,810573	0,997596	0,999773	1,000000	

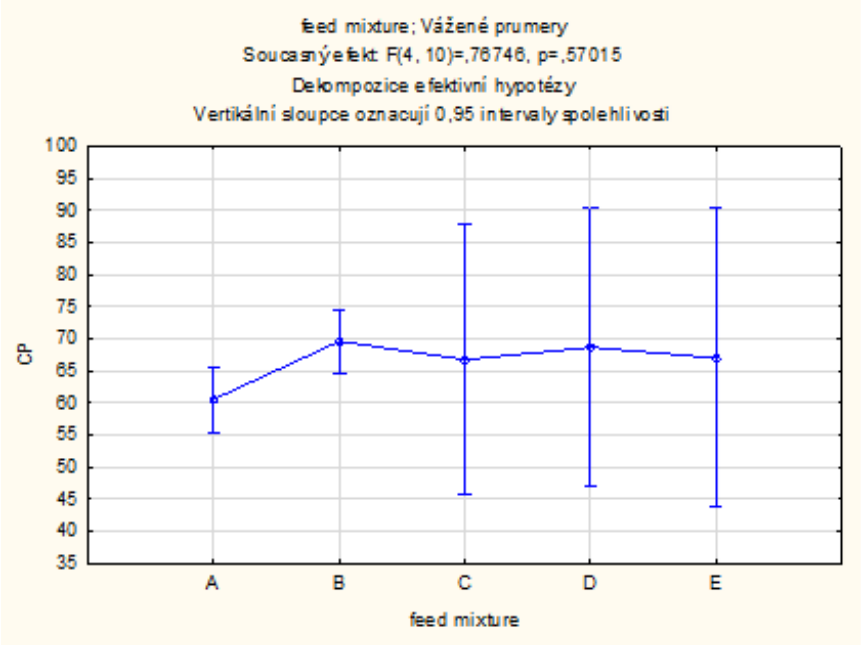
Enclosure VIII Scheffé post-hoc test, total fat content



Enclosure IX Scheffé test, crude protein

C. bunky	Scheffeho test; promenná CP (Gryllus_RC_výsledky final) Pravdepodobnosti pro post-hoc testy Chyba: meziskup. PC = 48,803, sv = 10,000					
	feed mixture	1 60,595	2 69,637	3 66,764	4 68,711	5 67,027
1	A		0,653312	0,876298	0,732630	0,859635
2	B	0,653312		0,991409	0,999897	0,994031
3	C	0,876298	0,991409		0,998070	0,999999
4	D	0,732630	0,999897	0,998070		0,998906
5	E	0,859635	0,994031	0,999999	0,998906	

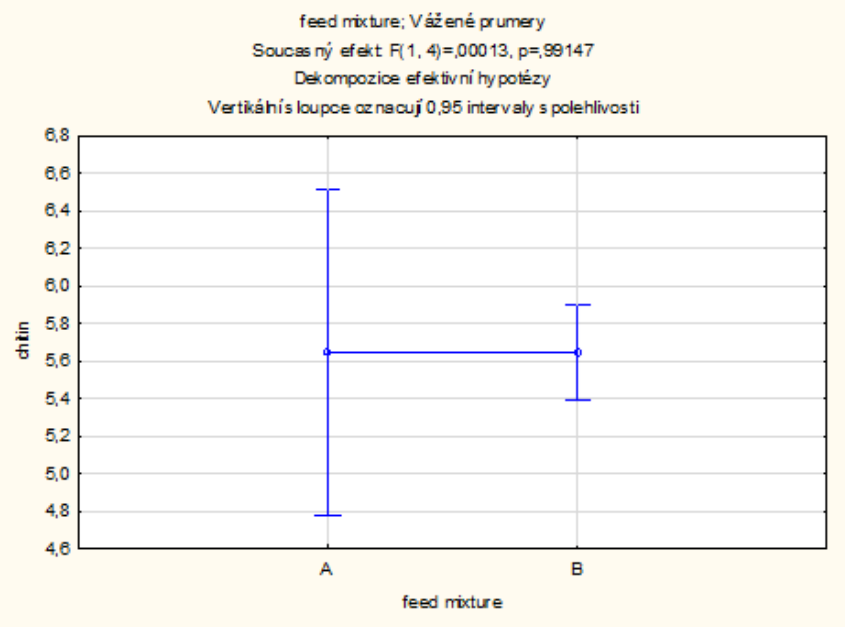
Enclosure X Scheffé post-hoc test, crude protein



Enclosure XI Scheffé test, chitin

C. bunky	Scheffeho test; promenná chitin (Gryllus_RC_výsledky final) Pravdepodobnosti pro post-hoc testy Chyba: meziskup. PC = ,06594, sv = 4,0000		
	feed mixture	1 5,6435	2 5,6459
1	A		0,991475
2	B	0,991475	

Enclosure XII Scheffé post-hoc test, chitin



Enclosure XIII Scheffé test, ash

C. bunky	Scheffeho test; promenná ash (Gryllus_RC_výsledky final) Pravdepodobnosti pro post-hoc testy Chyba: meziskup. PC = ,30042, sv = 10,000					
	feed mixture	1 3,2018	2 4,2986	3 4,5462	4 4,3031	5 4,1734
1	A		0,273746	0,135291	0,270425	0,377306
2	B	0,273746		0,987778	1,000000	0,999114
3	C	0,135291	0,987778		0,988595	0,946986
4	D	0,270425	1,000000	0,988595		0,998981
5	E	0,377306	0,999114	0,946986	0,998981	

Enclosure XIV Scheffé post-hoc test, ash