CZECH UNIVERSITY OF LIFE SCIENCE IN PRAGUE

Faculty of Agrobiology, Food and Natural Resources

Department of Microbiology, Nutrition and Dietetics

Evaluation of protein quality of Jamaican field crickets (*Gryllus assimilis*) reared on the dried rapeseed protein.

Master thesis

Thesis author**:** Bc. Yacouba Traore

Thesis supervisor**:** Ing. Martin Kulma, PhD.

Thesis consultant**:** Ing. Petra Škvorová

Declaration

I declare that I have prepared the master thesis "**Evaluation of protein quality of Jamaican field crickets (*Gryllus assimilis*) reared on the dried rapeseed protein**" independently under the guidance of a supervisor and using professional literature and other sources of information cited in the work are listed in the literature at the end of the thesis. As the author of the thesis, I further declare that I have not infringed the copyrights of third parties in connection with its creation.

Prague: 2022 --

ACKNOWLEDGEMENT

I am greatly indebted to my supervisor; Ing. Martin Kulma, PhD. for his guidance, support and advice from proposal inception to thesis write up.

I am also grateful to my colleagues who supported me in various ways during my study.

Finally, my appreciation to my family for their endless financial and moral support while pursued my studies.

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Summary

Food security is a serious concern particularly for developing countries. To overcome hunger and malnutrition, there is a need for increased research towards finding alternative and cheaper sources of nutrients. Insects have been reported to be good alternative source of proteins. This work therefore aimed to investigate the influence of addition of rapeseed protein into the Jamaican field cricket´s (*Gryllus assimilis*) diet on their amino acid profiles. The study included five dietary treatments of which four contained rapeseed oil by-products and the fifth was control diet. The chicken feed was used as feed in control group. All the tested feed substrates were based on the modified control diet, in which soybean meal was partially or completely replaced with rapeseed cakes as a food industry by-product. The amino acid profiles of samples were determined by acidic and oxidative hydrolysis of the samples, followed by evaluation using the Amino Acid Analyser with Na-citrate buffers and ninhydrin detection.

In comparison with the control group, the crickets fed by substrates containing 17.5 and 35.0 % (25 and 50 % of soybean meal was replaced) of rapeseed cakes had better amino acid composition expressed by essential amino acid index (EAAI), which ranged from 51.73% to 53.38%. The further increase of the rapeseed percentage up to 70 % (75 and 100 % of soybean meal was replaced) conversely caused the decrease of EAAI (from 48.66% to 48.24%). However, the statistic evaluation revealed that the differences in EAAI among the samples were not significant ($\alpha = 0.05$).

The most abundant amino acids in all groups were Glutamic acid followed by Aspartic acid, Alanine and Arginine. Although the levels of individual amino acids slightly varied among the samples, the significant differences were found between the contents of Cysteine, Proline, Tyrosine, Methionine and Threonine. Interestingly, the methionine, which was also determined to be limiting amino acid, content of crickets fed with rapeseed cakes was significantly higher than that of control group.

In conclusion, dietary rapeseed cakes, which are greatly available and cheap, could be an interesting alternative to conventional protein sources commonly used for crickets farming. This study revealed that the addition of rapeseed cakes had no significant influence on protein quality expressed by EAAI, even if the soya was completely replaced by rapeseed cakes.

Keywords: edible insects, novel food, amino acids, by-products, alternative.

Hodnocení kvality bílkovin u cvrčků banánových (*Gryllus assimilis*) krmených řepkovými výlisky

Abstrakt

Zabezpečení dostatečného množství kvalitních potravin budí vážné obavy zejména v rozvojových zemích. K překonání hladu a podvýživy je zapotřebí intenzivní výzkum zaměřený na hledání alternativních a levnějších zdrojů živin. Jedním z takovýchto alternativních zdrojů je hmyz.

Tato práce proto byla zaměřena na zkoumání vlivu přidání řepkových výlisků do krmné směsi cvrčka stepního (*Gryllus assimilis*) na profil aminokyselin. V této studii byli cvrčci krmeni pěti různými krmnými směsi, z nichž čtyři obsahovaly vedlejší produkty z výroby řepkového oleje. Jako pátá, kontrolní krmná směs, bylo použito krmivo pro brojlerová kuřata. Všechny testované krmné substráty byly založeny na modifikované kontrolní směsi, ve které byla sójová moučka částečně až úplně nahrazena řepkovým proteinem jakožto vedlejším produktem potravinářského průmyslu. Profil aminokyselin byl stanoven kyselou a oxidační hydrolýzou vzorků s následným vyhodnocením pomocí analyzátoru aminokyselin s Na-citrátovými pufry a ninhydrinovou detekcí.

V porovnání s kontrolní skupinou, kvalita proteinu cvrčků krmených krmnou směsí s obsahem řepkových výlisků 17,5 a 35,0 % (což odpovídá nahrazení 25 a 50 % sójového šrotu) vyjádřená indexem esenciálních aminokyselin (EAAI) byla vyšší než u skupiny kontrolní, přičemž se pohybovala od 51,73 do 53,38 procent. Naopak u cvrčků krmených směsmi s vyšším obsahem řepkových výlisků (nahrazeno 75 a 100 % sójového šrotu) byla hodnota EAAI nižší než v kontrolní skupině (48.66% a 48.24% respektive). Statistické vyhodnocení pokusu ovšem ukázalo, že výše uvedené rozdíly nejsou na hladině $\alpha = 0.05$ statisticky významné.

Nejhojněji zástupnými aminokyselinami ve všech analyzovaných vzorcích byl Glutamin, následován Asparaginem, Alaninem a Argininem. Přestože mezi hodnotami jednotlivých směsí byly určité rozdíly, statisticky významně se pokusné skupiny lišily pouze u Cystein, Prolin, Tyrosin, Methionin a Threonin. Zvláště zajímavé bylo zjištění, že směsi, které obsahovaly řepkový protein měly významně vyšší hodnoty Methioninu, který byl zároveň limitující aminokyselinou v měřených vzorcích.

Závěrem této práce je tedy zjištění, že řepkové výlisky, které jsou velmi dobře dostupné a levné, by se mohly stát zajímavou alternativou ke konvenčním zdrojům bílkovin v krmných směsích

pro hmyz. Tato studie prokázala, že i při úplném nahrazení sóji v krmné směsi za drcené řepkové výlisky nedojde ke zhoršení kvality proteinu sklizené biomasy.

Klíčová slova: jedlý hmyz, potraviny nového typu, aminokyseliny, vedlejší produkty, alternativa

Contents

1. Introduction

The daily consumption of animal protein is expected to increase by 22% in 2030 and by 25% in 2050 (FAO, 2017). Despite this expected increase, the levels are still not enough to feed the rapidly growing population (Boland et al., 2013). At the same time, the costs of increased meat production can therefore generate innumerable environmental impacts (Molden, 2013).

A reassessment of the food production system is needed to provide a secure source of food for the growing population. To overcome this problem, edible insects could be a good alternative for protein production. The Jamaican cricket (*Gryllus assimilis*), which is distributed in western India, southern parts of South America and Central America, belongs among most common edible insect species (Walker, 1962). Similarly, as house cricket (*Acheta domesticus*), it can be reared in a controlled environment in large colonies using similar rearing setup as house cricket (Rouge, 1990). Moreover, Jamaican field cricket is resistant to *A. domesticus* densovirus (AdDNV) which is a risk factor for the rearing of the house cricket (Szelei et al., 2011). Generally, crickets can convert organic by-products so that they have the potential to use a wide variety of plant materials, such as food waste or food industry by-products (van Huis & Oonincx, 2017). That offers new and interesting opportunities for their sustainable rearing and the circular economy. The use of by-products as feed for crickets could reduce the environmental impact with no negative influence on their performance, development, and quality from human nutrition point of view.

Currently, soybean meal is commonly used as protein source in feeds of insect industry (Cohen & Paarlberg, 2004). However, the use of soybeans has negative environmental impact mainly due to degradation of biologically valuable sites (Prudêncio da Silva et al., 2010). To improve sustainability of the insect rearing, it is necessary to find alternative way how to feed insects. These alternatives could come from by-products of the food industry that could eventually replace soybean meal as a more sustainable source of protein.

This study aims to evaluate the amino acid profiles of Jamaican field crickets (*Gryllus assimilis*) fed on substrate where soybean was replaced with different percentages of plant-based by-product, rapeseed protein. Due to high rapeseed oil production, the rapeseed meal, is available in large quantity in the Czech Republic, so could represent a good alternative. The scientific question of this study was: how will replacement of conventional protein sources by rapeseed protein into the feeding mixture influence life characteristics and protein quality of Jamaican field cricket (*Gryllus assimilis*)? Also, the most adequate ratio of substitution could be established.

2. Scientific hypothesis and objectives of work

The thesis aims to determine the amino acid profiles of Jamaican field crickets (*Gryllus assimilis*) reared on substrates containing rapeseed.

Scientific question: How will replacement of conventional protein sources by rapeseed protein into the feeding mixture influence protein of Jamaican field crickets (*Gryllus assimilis*)?

3. Literature review

3.1 Protein production and consumption

The demand for protein has increased over the past decade due to the population increase (FAO, 2017). The demand for animal proteins such as meat and milk are expected to increase by 58% and 70% respectively in 2050 compared to levels in 2010 (FAO, 2013a). Meat consumption is expected to increase by 5-6% per year over the next decade (Thornton, 2010). These increases in consumption will take place in a context of production under constraint for less emissions of greenhouse gas. FAO (2017) reports a 1% increase in meat and fish production in the world. Edible insects have been lacking in the global arena as an alternative source of protein but with the many benefits now attached to it, it may be the healthy, economic and ecological alternative.

3.2 Entomophagy

Entomophagy refers to the practice of eating insects and is a universal cultural phenomenon (Van Huis and Oonincx , 2017). Edible insects are a very important resource that contributes to livelihoods (Niaba et al., 2013). Entomophagy differs only in location, insect species and ethnic group involved (Jacob et al., 2013a).

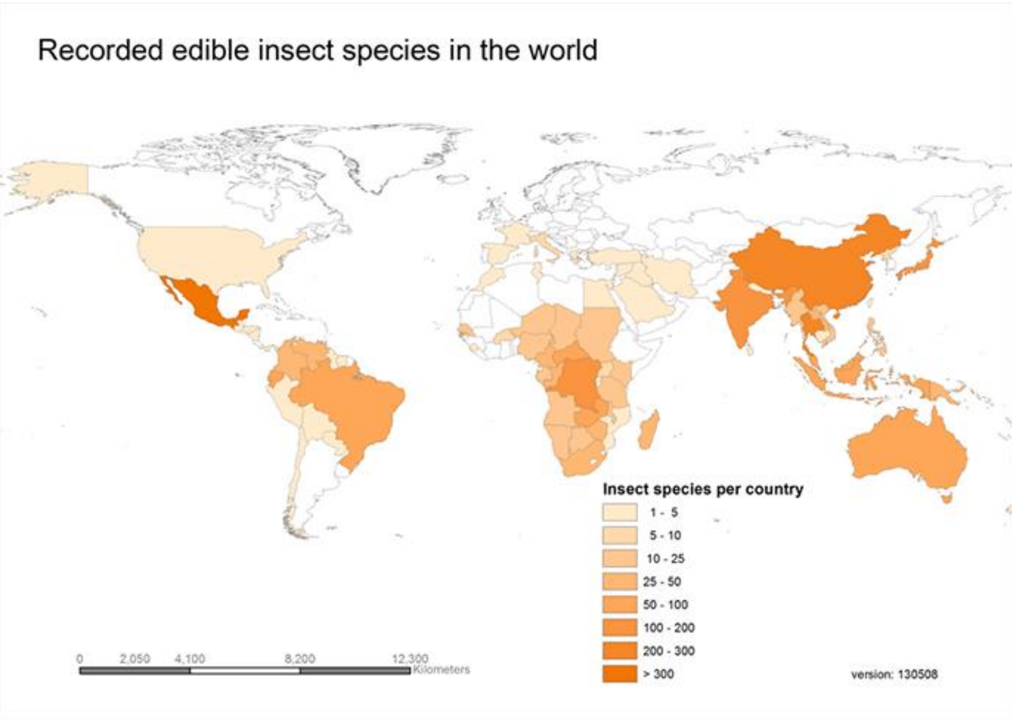


Figure 1: Number of edible insects species listed around the world (FAO, 2013b)

In Asia, Africa and South America, insects have long been used as a cheap and sustainable source of protein (Wang et al., 2005). As shown in Figure 1, about 1900 species are currently consumed worldwide and mainly in developing countries (Jacob et al., 2013a). The most common consumed insects in the world are crickets, grasshoppers, ants, beetle larvae and caterpillars (Braide & Nwaoguikpe, 2011). It has also been reported that some tribes in Venezuela, Colombia and South Africa prefer to eat insects rather than meat (Jacob et al., 2013a). The consumption of insects can take place at all stages of their development. Of course, majority of insects that are commonly eaten are those that can be easily collected from wild and eaten raw or cooked (Srivastava et al., 2009). Insect farming has remained an almost unknown practice until recently (Cadinu et al., 2020). Usually, insects were collected by wild harvest in Africa and Asia (Looy et al., 2014). The use of land for agriculture, desertification and urbanization have progressively induced a decline of insect wild catch (Looy et al., 2014). At the same time, the farming of edible insects has begun to rise as a valid, more sustainable alternative to wild harvesting (Raheem et al., 2019).

Excluding traditional entomophagous countries, insect cuisine is scarcely known also from other parts of world such as Asia and Europe (Jacob et al., 2013a). For example, cheese containing maggots is considered a popular delicacy in Italy (Alamu et al., 2013). Also, beetle larvae and grasshoppers were eaten by the Greeks and Romans (Pascucci et al., 2015).

3.3 Acceptance of edible insects

Insects have long been consumed as part of the diets of many African, Asian and South American cultures (Wilkinson et al., 2018). However, despite international agencies such as the Food and Agriculture Organization of the United Nations advocating the nutritional, environmental, and economic benefits of entomophagy, attitudinal barriers persist in Western societies (Wilkinson et al., 2018). For many consumers, insects are considered culturally not edible. The renewed interest of entomologists in insects as a food source is very recent (van Huis, 2013). Various approaches have so far been put forward in an attempt to make insects more attractive to consumers (Hartmann & Siegrist, 2017). People are unlikely more careful when it comes to trying new food. So it will be a great challenge to convince Western consumers of the advantage who place great importance on regular and abundant consumption of meat to find less attractive meat substitutes (Schösler et al., 2012). However, insects could be a promising option for consumer groups who value a sustainable food choice and seek for alternatives to the traditionally produced animal protein (Smetana et al., 2015).

3.4 Importance of insects as food

The history of civilization has made the consumption of insects a "primitive" act and aimed at the poor (Ayieko & Oriaro, 2008). However, certain shift in perception, leading to a renewed interest of populations in entomophagy has been recently detected (Bednárová et al., 2013). Few studies have recently shown that insects constitute quality food and feed, have high feed conversion rates and emit low levels of greenhouse gases (Ayieko et al., 2012; Pascucci et al, 2015). For example Gahukar (2011) reported the feed conversion efficiency of house crickets is twice than that of pigs and broilers, four times that of sheep, and six times that of beef when losses from trimming and dying dressing are taken into account.

3.4.1 Health and nutritional benefits of insects

Studies have shown that edible insects often contain more protein, fat, and carbohydrate than beef or fish, and a higher energy value than soybeans, corn, fish, and other beans (Gahukar 2011; Pascucci et al., 2015). By example the house cricket (*Acheta domesticus*) and the field cricket (*Gryllus testaceus*), have slightly higher protein than soybean (Pascucci et al., 2015; Wang et al., 2005). According to Gahukar (2011) and Srivastava et al. (2009) many insects contain low cholesterol levels compared to traditional sources of proteins.

Promotion of the use of lesser-known and cheaper sources of animal protein such as those from insects seems to be one of the best ways to tackle protein-energy malnutrition. Thus a 10% increase in the global supply of animal proteins through the mass production of insects for food can reduce or even completely eliminate malnutrition problems in the world (Jacob et al., 2013b).

So, insects are not only an important source of protein (see in Table 1), but also a very safe source to eat. It has been postulated by Roos & van Huis (2017) that eating insects can help manage conditions like hypertension, diabetes and obesity and improve immunity. Moreover, it has been proved (Figure 2), that edible insects are a source of biologically active substances and properties (Castro-López et al., 2020).

Table 1: Overview of nutrient content in insects and common foods (Ramos-Elorduy, 1998)

	Calories (kcal / 100 g)	Protein (g / 100 g)	Fats (g / 100 g)	Carbohydrates (g / 100 g)
Cockchafer	77.8	13.4	1.4	2.9
Ant	98.7	13.9	3.5	2.9
Cricket	112.9	12.8	5.7	2.6
Grasshopper	95.7	14.3	3,3	2.2
Bean	147.0	8.3	0.6	27.2
Ground beef	288.2	23.5	21.2	-
Egg	150.0	120.0	10.0	2.0

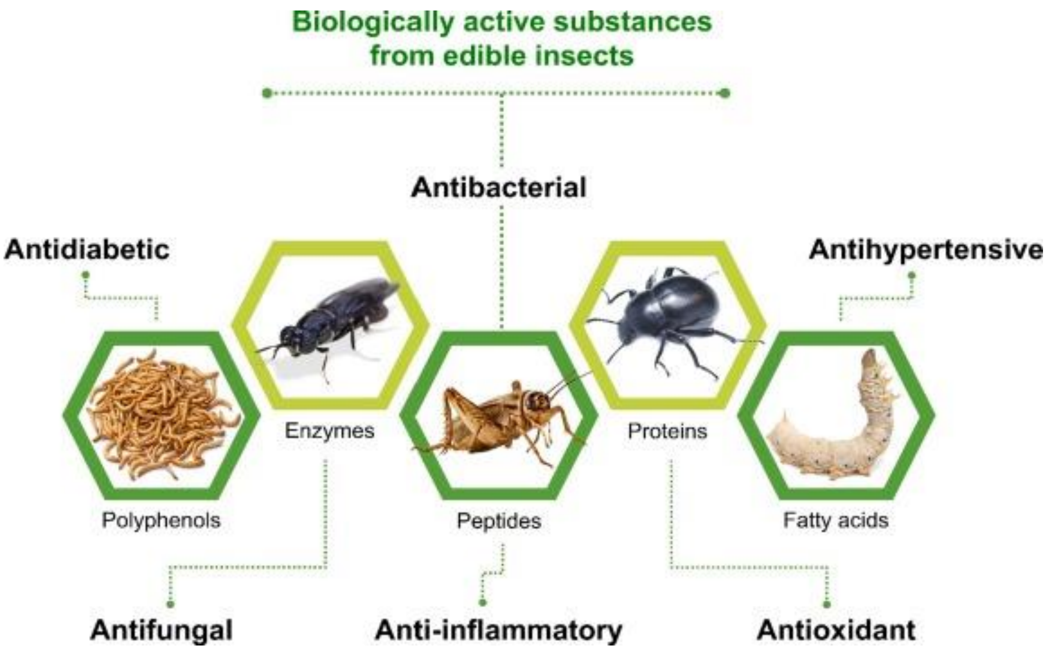


Figure 2: Edible insects as a source of biologically active substances and properties (Castro-López et al., 2020).

3.4.2 Socio-economic factors benefit of insects

Insects are more adaptable to climate change, and so, their rearing can help mitigate the effects of climate change and build farmers' resilience (Van Huis et al., 2013). Very often the insects consumed are collected from the wild, therefore they are generally free from pesticides and other chemical contaminants which thrive in places where there are conventional sources of protein (Patrick et al., 2010). Insects also process food much more efficiently than livestock (Van Huis and Oonincx, 2017). Some insect species can be reared on organic secondary streams, reducing environmental contamination, and turning waste into protein-rich foods that can replace increasingly expensive compound feed ingredients (Van Huis, 2013; Adegbola, et al., 2013; Van Huis and Oonincx, 2017). Usually when insects are consumed in society, they will not be pests but rather as a source of much needed protein.

Insect’s farming uses less land and energy than livestock (Premalatha et al., 2011). In addition, the insects grow very quickly and are prolific; this makes it a potentially reliable supplier of protein. Insect rearing is believed to reduce poverty levels through the creation of other employment and income opportunities (Van Huis et al., 2013). Studies have revealed that 29% of rural dwellers in South Africa generate their income from collecting and selling mopane caterpillars and in Thailand, so it is an important economic activity for the rural population (Halloran, 2017). In addition, consumption of these insects could reduce the rate of persistent malnutrition, especially in sub-Saharan Africa, thus improving the livelihoods of these populations.

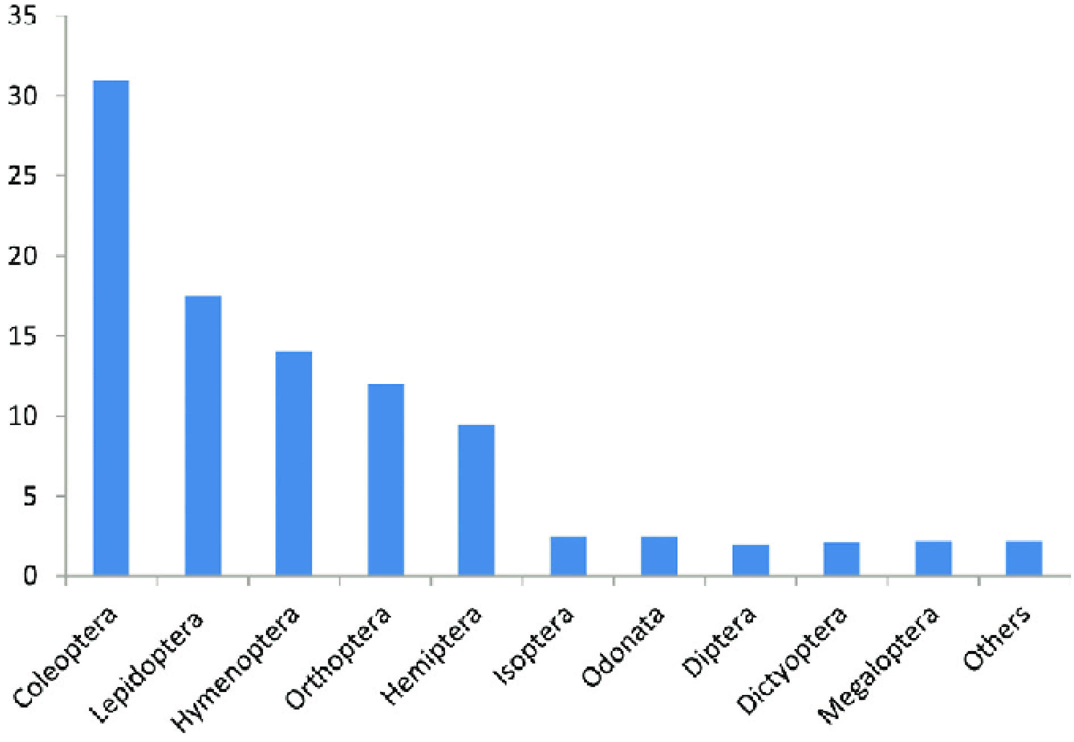
Interest in house crickets and field crickets is fuelled by several basic facts. These insect species lend themselves well to mass rearing under controlled conditions and can produce six to seven generations per year (Van Huis and Oonincx, 2017). Crickets adapt easily to domestic breeding and are rich source of nutrients (Van Huis et al., 2013).

Insects offer many opportunities to improve the living conditions of many people, as in developed as well as in developing countries. Although insect consumption is practiced by their own choice and not necessity, insects are an important source of animal protein for many communities around the world. Insect collection is a low-cost investment, so it is used especially in the poorer parts of the world, where people suffer from malnutrition and food insecurity. In such situations, insects can provide a cheap, effective, and highly nutritious resource food (van Huis, 2013).

3.5 Insect species consumed

Globally, the most common insects used for consumption are reported to be beetles, caterpillars, bees, wasps and ants. They are followed by grasshoppers, crickets, cicadas, termites, dragonflies, flies and other species – see Graph 1 (Jongema, 2015).

The European categorization of edible insects is provided by the European Food Safety Authority (EFSA), which aims to increase insect representatives for agricultural and food purposes. According to EFSA (2015) , the following species can be reared and consumed in Europe: mealworms (*Tenebrio molitor*), giant mealworms (*Zophobas morio*), lesser mealworms (*Alphitobius diaperinus*), house cricket (*Acheta domesticus*), Jamaican field crickets (*Gryllus assimilis)*, migratory locusts (*Locusta migratoria*), desert locusts (*Schistocerca gregaria*), honey bees (*Apis mellifera*) and wax moths (*Galleria mellonella*).



Graph 1: Number of insect species, by order, consumed in the world (Jongema, 2017)

3.6 Crickets

Crickets are nocturnal insects, which are specific by jumping hind legs with three tarsal segments (EL-Damanhouri, 2011). There are several species of crickets, but *Acheta domesticus* (house cricket), *Gryllus bimaculatus* (field cricket) and *Gryllus assimilis* (Jamaican field cricket) are the most popular among edible insects.

The male crickets only have two cerci at the end of their abdomen. They are omnivorous scavengers that feed on both animal and plant matter (Borror, 1989). They occasionally exhibit or display predatory behaviour upon the crippled or weak crickets or when food source is irregular (Borror, 1989). Crickets are also known to be cannibalistic when facing the lack of water.

3.6.1 Jamaican field cricket (*Gryllus assimilis*)

- **Description**

- Class: Insecta
- Order: Orthoptera
- Suborder: Ensifera
- Superfamily: Grylloidea
- Family: Gryllidae

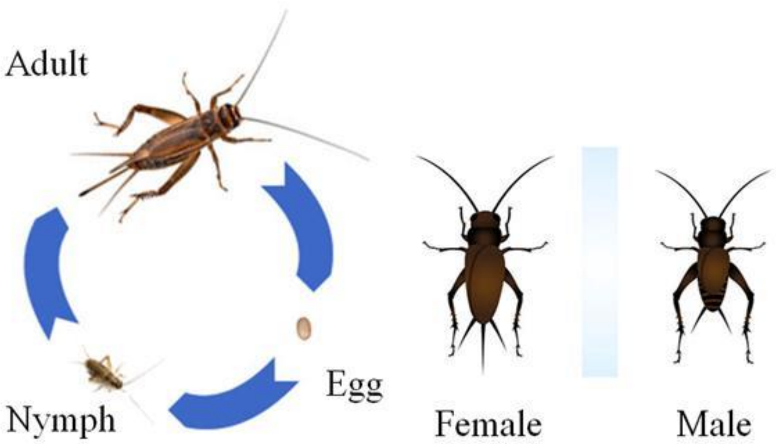
Figure 3 : A female adult of *Gryllus assimilis*

(https://www.biolib.cz/cz/person/id239/)

This thermophilic insect can reach in adulthood large 2-3 cm and often occurs in households (in warmer areas) replaces the role of cockroaches. Because it does not survive the cold winters, it withdraws to human dwellings fed on food leftovers and rubbish. They are not demanding on feed or living conditions but for successful reproduction and development, they require a higher rearing temperature in the range min. 25-30 °C and plenty of fluids. With a low temperature breeding completely loses its meaning because adults often die before reproducing and when the females already lay eggs, so they do not hatch for various reasons (Škrabalová, 2011).

- **Life cycle**

The life cycle depends on the species, and certain variations can occur even between different populations (Vahed, 2020). In the species of true crickets (Grylloidea) in Northern Europe (Figure 2), such as the field Cricket (*Gryllus assimilis)* , females deposit as many as 400 eggs via an ovipositor into damp soil. At suitably warm temperatures between 25 °C and 30 °C, eggs usually hatch in about eleven days (Vahed, 2020). Nymphal stages take place for another three six to seven weeks, at which point the insect reaches sexual maturity and the males begin calling for females. Eggs are often consumed cannibalistically by adults searching the soil for food.These reach adulthood by mid-summer and some of these adults may also survive a third winter (Brown, 1978). The adult life of crickets is relatively long, given that adults of either sex survive about 2 to 3 months, and even over 6 months in some extreme cases (Zefa et al., 2006).



(http://www.sugarglidertree.com/diet/diy-insect-breeding).

- **Nutritional value**

he insects presented variation in nutrient content during different biological cycles (Monter-Miranda et al., 2018). Insects are interesting in terms of the content of minerals such as iron, zinc, potassium, sodium, calcium, phosphorus, magnesium, manganese and copper (Kouřimská and Adámková, 2016). Among edible insects, *Gryllus assimillis* is a species with average nutritional composition (see tables 2 - 4) in all the categories with the exception of Palmitic

(16:0) fatty acid, of which it is quite a good source (Bednárová et al., 2013). According to Soares Araújo et al. (2019) *Gryllus assimillis* contain 65.52% of proteins, 21.80% lipids, 8.6% carbohydrates and 4.08% ash.

Table 2: Nutritional composition of *Gryllus assimilis* by (Oibiokpa et al., 2018)

Nutrients	**Proximate Composition (% in lyophilised samples)**
Protein	71.04
Fat	7.00
Energy (Kcal/100 G)	397.00
Fiber	8.28
Carbohydrate	12.46
Moisture	3.50
Ash	6.00

Table 3: Fatty acids profile of *Gryllus assimilis* (Mlček et al., 2018)

Fatty Acid Average Content (% of total fatty acids)

SFA				MUFA		PUFA	
C4 :0	0.01	C16:0	25.85	C14:1(cis-9)	0.061	C18:2(cis-9, 12)	26.13
C8:0	0.01	C18:0	14.07	C16:1(cis-9)	1.92	C18:3(cis-9, 12, 15)	1.60
C10:0	0.03	C20:0	0.56	C17:1(cis-10)	0.19	C20:2(cis-11, 14)	0.44
C12:0	0.12	C21:0	0.03	C18:1(cis-9)	25.03	C20:3(cis-11, 14, 17)	0.01
C13:0	0.02	C22:0	0.57	C20:1(cis-11)	0.24	C20:4(cis-5, 8, 11, 14)	0.21
C14:0	1.28	C23:0	0.22	C22:1(cis-13)	0.05	C22:2(cis-13, 16)	0.03
C15:0	0.37	C6:0	0.01	-	-	C20:5(cis-5, 8, 11, 14, 17)	0.38
C17 :0	0.57	-	-	-	-	-	-
TOTAL SFA	**43.72(%)**			Total MUFA 27.49		Total PUFA	28.80(%)
TOTAL N-3				**1.99**			
TOTAL N-6				**26.81**			
TOTAL N-9				**25.32**			

Table 4: Essential and non-essential amino acids of the *Gryllus assimilis* (Oibiokpa et al., 2018)

	Essential Amino Acids (g/100 g of protein)
Histidine	2.52
Isoleucine	3.36
Leucine	6.62
Lysine	5.29
Methionine	2.29
Phenylalanine	3.37
Threonine	3.09
Tryptophan	2.53
Valine	4.63
Total EAA	**33.70**
	Non-Essential Amino Acids (g/100 g of protein)
Alanine	6.23
Arginine	4.14
Cystine	1.14
Glutamic Acid	10.60
Glycine	4.03
Proline	5.09
Serine	3.80
Tyrosine	4.23
Aspartic Acid	8.25
Total NEAA	**47.51**

3.6.2 Crickets rearing

The use of crickets as food has been studied as an innovative source of high quality protein (Ayieko et al., 2012). Edible insects are generally harvested from the wild and the practice of rearing insects for human consumption is relatively new (FAO, 2013). Traditional collection is seasonal and less reliable, raising many concerns about sustainability (Ayieko et al., 2010). Thus, rearing procedures for large-scale production should be developed for crickets. Large-scale production systems have already been introduced in many countries (Riggi et al., 2013).

The growth and development of crickets is dependent on factors such as light, relative humidity and temperature (Ogah et al., 2012). Temperatures and relative humidity are known to be crucial effects influencing the behaviour, reproduction, survival, fertility and physiology of poikilotherms (Tochen et al., 2016).

3.6.3.Important factors in cricket rearing

a) Temperature

The development of insects generally occurs within a range of temperatures. Insects have a minimum and maximum development temperature which must be respected. Temperature as one of the abiotic factors, affects all metabolic and physiological processes. These include, but are not limited to, growth, survival, reproduction, and population size (Kuyucu & Çağlar, 2016). In fact, temperature affects the body's development more than growth (Forster & Hirst, 2012). Some of the mechanisms employed by insects include the induction of diapause or dormancy if the temperature rises above or exceeds the threshold for development (Hance et al., 2007). Crickets, as ectotherms, either respond to internal temperature changes behaviourally because they lack the neural mechanisms necessary to maintain a constant body temperature against changes in external temperature or adapt to environments suited to their conditions. physiological structures (Holmes, 2010). It has been reported that in crickets, temperatures have a great effect on the rate of chirping which is important for reproduction (Jang & Gerhardt, 2007), the rate of laying and the rate of hatching, diet (Adamo & Lovett, 2011) and growth rate (Mirth & Riddiford, 2007). Although crickets are known to adapt to varying temperature levels, a proper housing system should be able to maintain near optimal levels. They require a higher breeding temperature in the range min. 25-30 °C (Booth, 2020). Crickets raised at 25 °C grow slower (0.51 mg d1 , dry mass basis) and take longer to develop (119 d) but obtained a greater adult body mass (61 mg, dry mass) than crickets reared at 28 °C (0.99 mg d1 , 49 d, 48 mg) (Booth, 2020).

b) Light

Light intensity affects the growth and development of insects (Nakamura, 2002). Changes in the photoperiod have been found to influence insect behaviour (Bertram & Bellani, 2002). These changes affect the growth curve, development time, size of adults, and to some extent interfering with the life history and physiological traits of some organisms (Nakamura, 2002).

Very bright lighting has a negative impact on normal activities such as reproduction, oviposition, the stridulation period of males and feeding of insects (Bertram and Bellani, 2002). Crickets, as nocturnal insects, show negative photo-taxis, that is, show maximum activity at night. The photoperiod conditions recommended for crickets are a 16 h light:8 h dark cycle (Miki et al., 2020). The photoperiod 12:12 also can be used during the cricket rearing (Itoh & Sumi, 2000).

c) Relative humidity

Relative humidity has effects on various physiological processes in insects (Astuti et al., 2013). Low humidity can be the reason of desiccation of eggs and weight loss of young larvae and adults (Holmes, 2010). High humidity can also impact negatively hatching time and laying rate if it is not well controlled (Astuti et al., 2013), reproduction as well as the growth rate (Tochen et al., 2016). High humidity can even lead to high mortality due to infection with entomopathogens (Tochen et al., 2016). Miech et al. (2016) recommended a relative humidity of 50% for crickets rearing with a temperature range of 29° C to 35° C.

d) Cricket feed

Being omnivorous insects, crickets might be fed by substrates containing both animal and plant protein. In small scale rearing, dog or cat pellets are used for rearing purposes. On the other hand, many special substrates have been developed by the companies rearing crickets in large scale (Van Huis & Tomberlin 2017). Soybean meal is the main protein component of majority of such mixtures. Similarly, chicken feed with soybean protein is used as experimental diet. Nevertheless, the need for sustainable large-scale commercial production of insects has stimulated interest in the development of artificial diets including agro-byproducts (Nation, 2002). Van Huis (2013) sought to use organic side-streams in formulation of insect feed as a deliberate effort to help in waste management. Insects reared on various diets do not have similar growth rates and differ in developmental periods (Maklakov et al., 2008). The feed quality in terms physical attributes such as shape, colour, smell, hardness and allelochemical influences the insect capacity to consume and digest feed substrates (Lemoine & Shantz, 2016). Previous studies on the effects of feed on growth performance of insects and specifically crickets have yielded different results. El-Damanhouri (2011) observed that crickets fed on a high protein feed took a shorter period to reach adulthood as compared to the ones fed on a low protein feed. However, cricket feeding is an important component of the production process especially for the caged crickets and that insects reared on various diets do not grow at equal rates and differ in developmental periods (El-Damanhouri, 2011).

3.6.4 Effects of insect diet on insect nutrient composition

Diet can affect the nutritional value of an insect. The body composition of the insect can be altered to some extent through diet. Since the whole insect is normally eaten, the dietary effects may be partly due to the diet remaining in the gut as mentioned previously.

It seems that the fat content of insects is very variable and that the protein and ash content is subsequently affected. If an insect with a certain amount of protein increases its fat reserve, the percentage of protein decreases (Simpson & Raubenheimer, 2001). This could be the reason why the protein composition remains invariable in adulthood if a protein source is available and decreases if only a carbohydrate source is available (Nestel et al., 2005).

Unlike amino acid composition, it seems that the percentage of protein can be changed by diet. A study on the black soldier fly by Sealey et al., (2011) found no effect of diet on amino acid content. However, a study on yellow mealworms, reared on different diets, without causing significant differences in crude protein content but rather revealed small differences in amino acid profiles. As previously stated, the fat content of insects could be altered by diet. Studies carried out on fruit flies have revealed that fat composition is strongly influenced by diet (Bernard & Allen, 1997). For house crickets, several studies are available on their nutritional composition. The fat content is ranging from 17 to 37% dry matter (Finke, 2002 ;Oonincx et al., 2010).

3.6.5 Requirements for Specific Nutrients

a) Proteins

Proteins are important part of the insect diet as they provide amino acids that are assembled into structural tissues and enzymes (El-Damanhouri, 2011), enhances growth and longevity, reduces nymphal mortality, maturation of ovaries and eggs (Nation, 2002). The requirement of optimal protein in the diet, differs with age, sex and physiological stress.

b) Carbohydrates

Carbohydrates are a major source of energy, though insects do not require it in absolute for growth since it can be synthesized from amino acids and lipids (Nation, 2002). The carbohydrate composition of the haemolymph (blood sugar), trehalose, is greatly influenced by the diet (El-Damanhouri, 2011). It is stored in the fat body mainly in the form of glycogen, which can be rapidly hydrolysed into a readily useable form of energy, trehalose. Utilization of carbohydrates as source of energy is mostly during metamorphosis due to metabolic interconversions (El-Damanhouri, 2011), flight in *Hymenoptera, Diptera* and *Blattoidea*, egg maturation especially in cockroach, *Leucophaea maderae* (Nation, 2002) and male stridulation in crickets (Maklakov et al., 2008). Studies have also confirmed the close correlation between carbohydrate reserves and the efficiency of sexual reproduction (Vaillant-Gaveau et al., 2014). Therefore, the carbohydrates are necessary for reproduction.

c) Lipids

Lipids serve as sources of energy for insects (Nation, 2002; El-Damanhouri, 2011). The amount and composition of lipids in an insect vary between developmental stages and tissues (Tawes, 2014). They play an important role in the biochemistry of insects as structural components and hormones. Insects make efficient use of lipids for development, growth, and reproduction. The most critical lipid required in insect diets is sterol, which is useful for moulting and as a component of cell membranes (Nation, 2002). Although lipids are required to be present in insect diets, most lipids can be synthesized from carbohydrates and stored in the fatty tissue of the body. Dietary reference intakes suggest that adults consume 20% to 35% of their total calories from fat (Ryan-harshman & Aldoori, 2006).

3.6.6 Use of agro by-products as cricket feed

Industrial secondary products are wastes from industrial activities released into nature. Interest in waste disposal has triggered research into their alternative uses (Oonincx, 2015). Agro-industrial wastes are used for manufacturing of biofuels, enzymes, vitamins, antioxidants, animal feed, antibiotics, and other chemicals through solid state fermentation. Although it has been used for animal feed, their ability to feed insects so far has not been sufficiently studied.

a) Wheat bran

Wheat bran is a by-product of the manufacture of flour from the grains of common wheat (*Triticum aestivum* L.), a fine fraction consisting mainly of pod fragments and grain particles of which most of the albumen has been eliminated. It is a source of insoluble fiber. The nutritional value per 100g of wheat is 71g of carbohydrates, 12.2g of fiber, 12.6g of protein, 1.54g of fat, 13g of water (Saeid et al., 2015).

b) Rapeseed

Rapeseed belongs to the Brassicaceae family and contains more than 40% oil. The rest of biomass remains by-product used as fertilizer or as biomass to produce energy in many countries (Özçimen & Karaosmanoğlu, 2004). The use of rapeseed protein in the diet of crickets may be beneficial due to availability in small factories (Leming & Lember, 2005). The composition of essential amino acids is sufficient to support a very high biological value (Yang et al., 2014) making rapeseed a very interesting alternative to soy protein in the diet of insects. Comparison of these two meals is shown in Table 5. Several studies have evaluated the feasibility of using rapeseed proteins in animal feed.

Table 5: Composition and nutritional values of rapeseed and soybean meal

Relative to Gross Weight	Rapeseed Meal	Soybean Meal
Dry Matter (%)	88.7	87.8
Crude Protein (%)	33.7	45.3
Crude Fiber (%)	12.4	6
Cellulose: Adf-Adl (%)	10.1	6.6
Fat (%)	2.3	1.9
Total Soluble Sugars (%)	7.7	8.3
Crude Ash (%)	7	6.3
Calcium (g.kg-1)	8.3	3.4
Phosphorus (g.kg-1)	11.4	6.2
Sodium (g.kg-1)	0.7	0.1
Potassium (g.kg-1)	12.5	21
Magnesium (g.kg-1)	4.5	2.8
Vitamin B1 (mg.kg-1)	3	6
Vitamin B6 (mg.kg-1)	11	6
Vitamin E (mg.kg-1)	14	4
Vitamin B2 (mg.kg-1)	4	3
Gross Energy (kcal.kg-1)	4090	4130
Lysine (g.kg-1)	18 (Digestibility: 78%)	27.8 (Digestibility: 91%)
Methionine (g.kg-1)	6.9 (Digestibility: 87%)	6.4 (Digestibility: 91%)
Threonine (g.kg-1)	14.5 (Digestibility: 84%)	17.7 (Digestibility: 89%)

(Sauvant et al., 2004)

From a technical and economic point of view, the incorporation of rapeseed meal in the food ration becomes much interesting when its price is less than soybean s price. This co-product is therefore amply recoverable in the field of animal nutrition.

3.6.7 Feed intake and conversion

Understanding the efficiency of feed conversion is a very important aspect of insect production due to the increasing costs of livestock farming (Nation, 2002). It demonstrates the economic feasibility and the environmental impact of insect rearing (Van Huis and Oonincx, 2017). This is then useful for insect species selecting that maximize weight gain per unit of feed consumed (Spang, 2013). The quality of feed ingested by insects should be monitored (Kouřimská & Adámková, 2016), because although the digestibility of a particular food may be good, it may not be easily converted to body mass due to the nutritional imbalance.

The quantification of food conversion in insects can be an arithmetic or a geometric approach. The arithmetic approach calculates the average insect weight while geometric multiplies the initial and final weights, then calculates the square root of the product (Nation, 2002). Several measures and experimental procedures to calculate the efficiency of food conversion by insects have been postulated by the researchers. The most commonly used procedures are:

- The feed conversion rate which shows the efficiency with which the insect is able to transform the food consumed into body mass.
- And the approximate digestibility which directly measures the body mass gained relative to the amount of food ingested. It is the insect's ability to digest food and is expressed as a percentage as follows (Devi & Singh, 2002) .

$$\text{Approximate Digestibility } = \frac{\text{Dry weight of feed ingested} - \text{Dry weight of feaces}}{\text{Dry weight of feed ingested}} * 100$$

3.6.8 Growth measurement

Growth is the constant transformation of acquired resources and energy from the environment by insects into biomass. Crickets experience growth due to their significance as an exoskeleton, but the increase in biomass stops in adulthood (Maino & Kearney, 2015).

One method uses measurements the width of the head capsule or the length of any body. The other method is to measure growth as an increase in biomass. This can be done simply by weighing the insects individually or in groups over a specified time interval, then calculating the weight increase per time interval. Our study used the latter method to calculate the growth rates of crickets because it seems to give consistent results is and universally used.

4. Material and methods

4.1 Experimental design

The crickets (*Gryllus assimilis*) were reared in plastic boxes (56×39×28 cm) housed in the insectarium of the Czech University of Science with a temperature of 26±1˚C, relative humidity of 40-50%. The insectarium is lit by an automatic bulb, which lights up from 8 h to 20 h (photoperiod 12:12).

For experiments, the control group of crickets fed on soybean-based chicken diet (wheat + soybean meal 47,5 + rapeseed oil + L-lysin.HCL 98 + DL-methionin 99 + limestone + salt + MCP + sodium carbonate + BR supplement).

Initially, the 2 grams of freshly hatched crickets were weighed (KERN ABJ-NM/ABS-N, Czech Republic) and put into each box. In the trial, four experimental diets were provided to the crickets. In the experimental substrates, 25%, 50%, 75% and 100% of the soybean meal was replaced by rapeseed cakes. Each experimental substrate was provided into three parallel repetitions. Therefore,15 containers with insects were fed with five different substrates. The containers were covered with an anti-insect aluminium mesh to prevent the entry of predators and the escape of crickets at the same time to allow entry of air and light.

Water was supplied as a hydrogel (STOCKSORB © acrylic acid-homopolymer-potassium salt, pH 6.5–7.5) in petri dishes when needed. To prevent anxiety, egg trays were placed horizontally in the buckets to act as hide-outs.

In each container certain quantity of feed was provided in Petri dishes and recorded (KERN ABJ-NM/ABS-N, Czech Republic). All containers were checked one times per day, freshwater gel was supplied on the daily basis and feed when needed. The quantity of new substrate was gradually increased. Each time the substrate was replaced, the excrements, exuviae and feed residues were also collected, and their weight was taken. The experiment was terminated when crickets were 60 days old.

4.2 Experimental diets

The study included five dietary treatments of which four contained rapeseed by-products. The fifth control diet included feed for chickens. The by-product diets were based on the modified control diet, in which soybean meal was replaced with rapeseed protein as a by-product. Rapeseed protein was chosen based on its availability as a by-product of the Czech food industry. The nutrient content of each diet is shown in the following table.

Table 6: The nutrient content in the diets used for the experiment.

	Protein (%)	**Proportion of rapeseed in feed** (%)	**Experimental Boxes**
BK (control diet**)**	20,00	0	A1, A2, B2
RV17,5	21,45	17,5	B1, B2, B3
RV35	23,19	35	C1, C2, C3
RV50,5	24,41	50,5	D1, D2, D3
RV70	25,84	70	E1, E2, E3

4.3 Measurements

To monitor progress of crickets during the experiment, the weight of 20 random crickets from each box was measured individually on 20, 40 and 60th day of the experiment. Prior to harvest, the adult crickets were starved for 24 hours. The total biomass of crickets from each box was also weighed and then the insects were freeze killed in a -80 °C.

$$\text{Conversion efficiency} = \frac{\text{total weight gained (g)}}{\text{weight of feed provided (g)} - \text{feed residues (g)}} * 100$$

4.4. Chemical Analyses

4.4.1 Nitrogen content

The Kjeldahl method (ISO 1871: 2009) was used to determine the total nitrogen content of the test sample, which is then multiplied by a factor of 6.25. This method does not distinguish between protein and non-protein nitrogen. Under the conditions of this method, nitrogen cannot be determined in compounds with nitrogen oxide bonds (nitrates, nitrites, azo or hydrazo compounds). The nitrogen content is determined titrat ively, alkalimetrically or acidimetrically, after mineralization of the sample with hot sulfuric acid in the presence of a catalyst, whereby the nitrogen is converted into ammonium sulphate. The ammonia is then displaced from it with sodium hydroxide and distilled into sulfuric acid (alkalimetric titration) or boric acid (acidimetric titration). It is then titrated with a standard solution of sulfuric acid until the colour of the acid-base indicator used changes. The whole process of determining nitrogen content by the Kjeldahl method consists of phases: mineralization, distillation, and titration.

a) **Mineralization**

A dried sample of the Jamaican field cricket (*Gryllus assimilis*) was homogenized and then weighed into two glass cuvettes. Approximately 1 g of sample, two tablets of Selenic and 20 ml of sulfuric acid H_2SO_4 were weighed into each. At the same time, a 2x control blank was performed, in which distilled water was used instead of the sample. The sample thus prepared, and the blanks were placed in a mineralizer at 420 ° C and left for 105 minutes.

b) **Destillation**

Distillation was performed on Kjeltec 8400-protein analyzer unit (FOSS, Hilleroed, Denmark). The distillation time was 4 minutes. Chemicals were used for distillation: 160 ml of 40% sodium hydroxide NaOH, 70 ml of distilled water and 50 ml of boric acid.

c) **Titration**

After distillation, the sample was cooled, then titrated with a 0.2% solution of sulfuric acid H2 SO4 until purple. The total crude protein content was calculated by multiplying the nitrogen content by a factor of 6.25.

4.4.2 Amino Acid profile

The amino acid profile of samples was determined by acidic and oxidative hydrolysis of the samples, followed by evaluation using the Amino Acid Analyser 400 (INGOS, Czech Republic) with Na-citrate buffers and ninhydrin detection. To evaluate the protein quality, the essential amino acid index (EAAI) was calculated to determine the following essential amino acids: valine, leucine, isoleucine, threonine, phenylalanine, methionine, and lysine (Kulma et al., 2020).This index is based on the determined content of all the above-mentioned amino acids compared with those of a reference protein (Velíšek, 2012) which was whole egg protein in our study.

$$EAAI = \sqrt[7]{\frac{of\ lysine\ in\ 100\ g\ of\ analysed\ protein \times 100}{of\ lysine\ in\ 100\ g\ of\ reference\ protein}} \times \text{ (etc. for other EAA)}$$

4.5 Statistical analysis

The data were statistically evaluated with IBM SPSS Statistics 26.0 Armonk, New York: IBM Corporation software using a factorial analysis of variance (ANOVA), and Scheffe's post-hoc analyses with a significance level of α = 0.05. The results are expressed as arithmetical means ($\bar{x}$) ± standard deviations (SD). We observed how a different composition of the feed ration affected the content of amino acids. Using one-factor analysis, the statistical significance of the differences between the individual tested factors (feed, insects) was evaluated. Values higher than the selected α = 0.05 do not show a statistically significant difference between the pair of examined averages. Values lower than α = 0.05 indicate a statistically significant difference. After analysis of variance, if a statistically significant difference was found, we performed further analyses using Scheffe's post hoc test, a method of more detailed evaluation.

5. Results

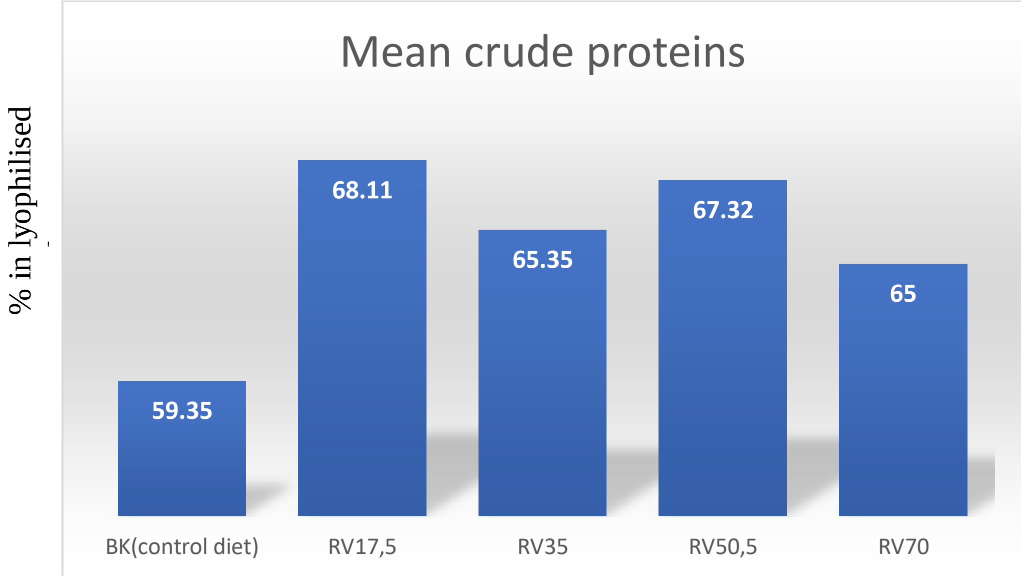
Mean values of the crude proteins and amino acids with standard deviations for all groups are provided in the table 7.

5.1 Total crude proteins content of *Gryllus assimilis*

The results of crude protein content for each group of GA (*Gryllus assimilis*) in dry matter are shown in Table 7. Measured data showed a statistically significant difference ($p < 0.05$) among the groups of GA reared on different diets. The significant difference ($p = 0.038$) was between the groups of GA reared on the control diet and the group reared on the experimental diet RV17.5 (59.35 vs. 68.11 % of protein). Between other groups, a statistically significant difference was not confirmed. In the table 7, we can see adult GA reared on the experimental diet RV17.5 and analysed in this study had the highest crude protein content. And the lowest protein content has the group of GA reared on the control diet (BK).

Table 7: Mean crude proteins of *Gryllus assimilis* reared on different diets

GROUPS (WHEAT+ % RAPESEED OIL)	EXPERIMENTAL BOXES	MEAN CRUDE PROTEINS (% IN LYOPHILISED SAMPLE)	DRY MATTER (G/KG OF LYOPHILISED SAMPLE)
BK (FEED: CONTROL DIET)	A1 A2 A3	59.36±1.78	979.69±0.73
RV 17,5 (FEED: WHEAT+17,5% RAPESEED OIL)	B1 B2 B3	68.11±1.66	978.09±1.70
RV 35 (FEED: WHEAT+% 35 RAPESEED OIL)	C1 C2 C3	65.36±7.40	978.94±1.72
RV 50,5 (FEED: WHEAT+ % 50,5 RAPESEED OIL)	D1 D2 D3	67.32±7.70	979.82±2.12
RV 70 (FEED: WHEAT+ %70 RAPESEED OIL)	E1 E2 E3	65.00±7.91	974.13±5.01



Graph 2: Mean crude proteins (percentage in lyophilised samples).

5.2 The amino acids content of *Gryllus assimilis*

Mean values of the amino acids with standard deviations for all groups are provided in the Table 8 and 9.

Tables 8 and 9 show data on the average of non-essential and essential amino acid contents measured for *Gryllus assimilis* reared on different diets combinations. The method used allowed an accurate measurement of 10 non-essential amino acids (NEAA) and of 7 essential amino acids (EAA).

Among the NEAAs in the groups of *Gryllus assimilis* (Table 8), the contents of cysteine, proline and tyrosine were significantly different between the groups of crickets. For other NAAs, a significant difference between the groups was not determined. As shown in the Table 8 the mean content of Proline in the experimental group RV35 was significantly (p=0.011) higher than that of the group RV70 (6.15 vs 4.43 g/100g protein). In the same table, we observed a significant difference ($p \leq 0.05$) in the cysteine level of samples between the control group and the experimental group RV17.5 (1.21 vs 1.66 g/100g protein). Cysteine average content in the control group BK was significantly (p=0.037) lower than that of the group RV50 (1.21 vs 1.43g/100g protein). The cysteine content in the group BK was also significantly (p=0.000) lower than that of the group RV70 (1.21 vs 1.59 g/100g protein).

Regarding the EAAs, we recorded a significant difference ($p \leq 0.05$) only in the content of Methionine, and Threonine among the groups. The addition of 17.5%, 35%, 50.5% and 70% of rapeseed protein in the diets did not change the rest of essential amino acid levels of the *Gryllus assimilis* when compared with the control group, even if slight changes in the amino acid's contents were observed. The average concentration of Methionine in the experimental group RV50.5 was significantly ($p=0.000$) higher than that of the control group BK (1.40 vs 0.97 g/100g protein). In the table 9, we can see significant difference ($p=0.000$) in the Methionine concentration between the control group BK and the experimental group RV70 (0.97 vs 1.38 g/100g protein). Moreover, the experiment showed some differences in the Methionine content between the control group and the experimental group RV17.5 (0.97 vs 1.34 g/100g protein).

The most abundant amino acids were Glutamic acid, followed by the Aspartic acid and Alanine. On the other hand, the Methionine was determined to be the limiting amino acids in all the sa mples. Most of the EAAs (essential amino acid) were found at higher concentrations in the RV35 experimental group (fed on diet with 35% of rapeseed meal), such as Lysine, Leucine, Threonine, Valine, Isoleusine and Phenilalanine compared to other groups. The highest concentration of Metionine was found in the experimental group RV50.5. Then, the highest EAAI (53.38%) in the experimental group, which was fed on a diet with 35% of rapeseed meal (RV35) was observed in the Table 8.

Except for Methionine, the general tendency observed in the Graph 3 is that the concentration of essential amino acids increases when we increased the percentage of rapeseed cakes in the diets of *Gryllus assimilis*. The increase in concentration reaches its maximum at 35%.

Table 8: Non-essential amino acid content in the Jamaican field cricket (*Gryllus assimilis*) analysed by Amino-Analyser

Diets / NEAA	**ALA**	**ARG**	**ASP**	**CYS**	**GLU**	**GLY**	**HIS**	**PRO**	**SER**	**TYR**
					g/100 g of protein					
BK (control diet)	6.55±1.42	4.83 ±0.23	5.81 ±0.53	1.21 ±0.10	8.12±0.55	3.85 ±0.53	2.04±0.61	5.07 ±1.18	3.02±0.24	4.37 ±0.46
RV17.5	6.01 ±1.00	5.31 ±0.69	6.28±1.20	1.66±0.10	7.82 ±0.99	3.61±0.35	2.07±0.85	5.26 ±1.01	3.65 ±0.85	3.53±3.52
RV35	6.68 ±1.33	5.42±0.50	6.26 ±0.71	1.43±0.17	7.82 ±1.00	3.86 ±0.50	2.52 ±0.85	6.15±0.96	3.37 ±0.23	3.85±0.50
RV50.5	5.53 ±0.31	5.03±0.19	5.57±0.20	1.66 ±0.03	7.48 ±0.29	3.47 ±0.08	1.59 ±0.06	4.57 ±0.31	3.11±0.12	3.40 ±0.13
RV70	5.75 ±0.21	4.82 ±0.31	5.91 ±0.71	1.59 ±0.08	7.68 ±0.39	3.47±0.15	1.64±0.08	4.43 ±0.17	2.98 ±0.19	3.63 ±0.30
P-Values	0.234	0.071	0.254	**0.000***	0.641	0.194	0.087	**0.012***	0.056	**0.004***

Results are presented as arithmetic means ± standard deviation (n = 6) and values with different superscripts are different at $p < 0.05$.

Asp = aspartic acid; Ser = serine; Glu = glutamic acid; Gly = glycine; Ala = alanine; Cys = cysteine; Tyr = tyrosine; His = histidine.

Arg = arginine; Pro = proline.

BK = control diet; RV17,5, RV35, RV50,5 and RV70 = experimental diets.

Table 9: Essential amino acid content in the Jamaican field cricket *(Gryllus assimilis)* analysed by Amino Acid Analyser

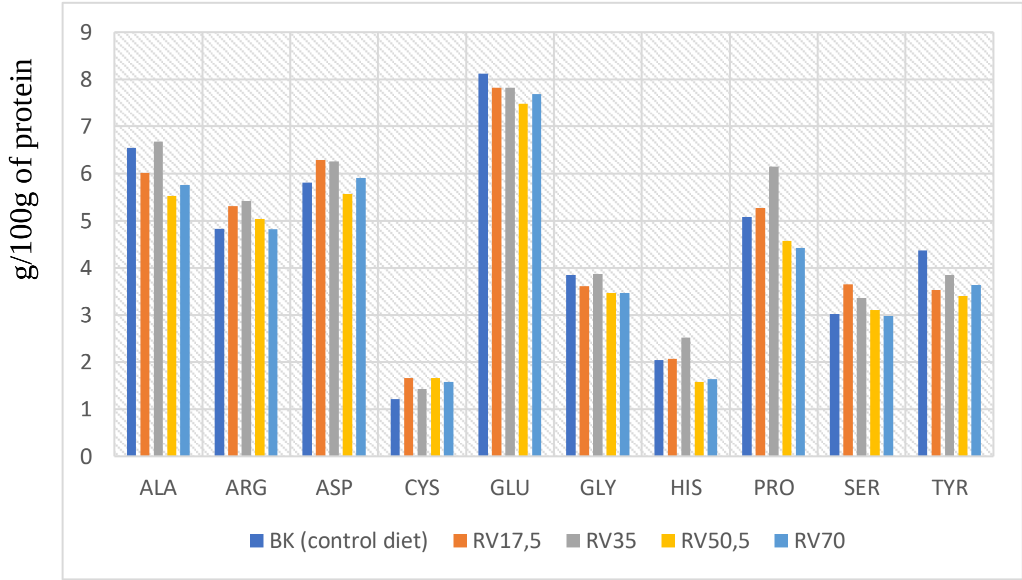
Diets / EAA	**ILE**	**LEU**	**LYS**	**MET**	**PHE**	**THR**	**VAL**	**EAAI%**
				g/100 g of protein				
BK (control diet**)**	2.96±0.27	5.02±0.55	4.23±0.67	0.97±0.06	2.50±0.20	2.83±0.28	4.38±0.54	49.67±4,49
RV17,5	3.01±0.39	5.01±0.78	4.34±0.92	1.34±0.04	2.47±0.41	2.87±0.39	4.10±0.57	51.73±6.10
RV35	3.12±0.28	5.18±0.81	4.67±0.84	1.17±0.18	2.62±0.27	3.07±0.24	4.43±0.63	53.38±6.27
RV50,5	2.82±0.06	4.69±0.17	3.82±0.09	1.40±0.08	2.30±0.06	2.62±1.91	0.87±0.11	48.66±0.84
RV70	2.68±0.09	4.72 ±0.26	3.90±0.24	1.38±0.12	2.26±0.15	2.58±0.17	3.88±0.18	48.24±2.35
P-Values	0.057	0.502	0.181	**0.000***	0.122	**0.030***	0.131	

EAAI – essential amino acid index.

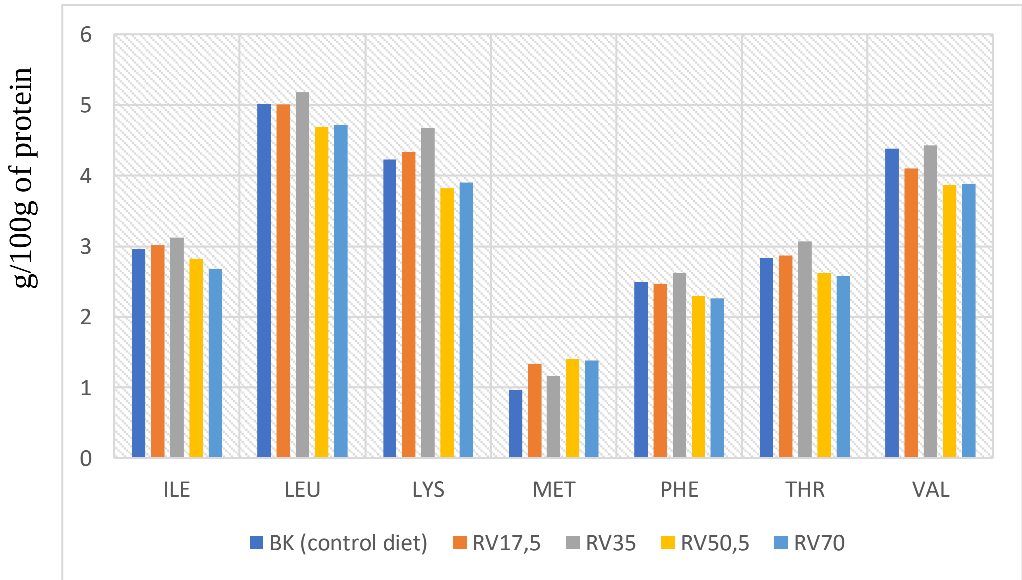
BK = control diets; RV17.5, RV35, RV50.5 and RV70 = experimental diets.

Results are presented as arithmetic means ± standard deviation (n=6) and values with different superscripts are different at $p < 0.05$.

Thr = threonine; Val = valine; Met = methionine; Ile = isoleucine; Leu = leucine; Phe = phenylalanine; Lys = lysine



Graph 3: Non-Essential amino acid content in the Jamaican field cricket (*Gryllus assimilis*) analysed by Amino Acid Analyser.



Graph 4: Essential amino acid content in the Jamaican field cricket (*Gryllus assimilis*) analysed by Amino Acid Analyser.

6. Discussion

6.2 Crude protein content of *Gryllus assimilis*

The average content of crude protein in insects vary from 15-81% of dry matter (Mlček et al., 2018). The values measured in this study varied from 59.36 to 68.11%. Regarding *Gryllus assimilis*, these values are comparable with the literature but slightly superior to previous results (55.6%) found by Mlček et al., (2018). Bednářová, (2013) also confirmed a lower value (56.49%). The difference between these values and our results may be caused by the fact that we used the rapeseed for feeding. Rapeseed is known to contain 20.7% of crude proteins (Burel & Kaushik, 2008). The protein content of the diet was then increased via the addition of rapeseed cake. As presented by Broekhoven et al. (2015), the nutritional composition of insects depends on the nutrition and temperature. In nature, insects feed on found and occasional food, while farm insects are supplied with all necessary nutrients like in our study for rapid growth.

6.3. The amino acids content of *Gryllus assimilis*

An essential amino acid, or indispensable amino acid, is an amino acid that cannot be synthesized by the organism to supply its demand and must therefore come from the diet. Thus, the most important factor in protein utilization is the EAA composition. Generally, the EAA profiles of insects are considered to be comparable to that of soya, superior to those of other vegetable proteins, and less than those of commercial livestock proteins (Yi et al., 2013). Moreover, compared to the protein content of the common meat sources, most of the edible crickets have a higher protein content than that of the roasted goat, broiler chicken, and pork (Magara et al., 2021).

In our study, all groups of *Gryllus assimilis* analysed were found to be good sources of several essential amino acids like Lysine, Threonine, Leucine, Isoleucine, Valine and Phenylalanine. It is interesting that the adult *Gryllus assimilis* reared on the experimental diet RV17.5 and analysed in this study had the highest crude protein content. But the highest quality of protein (EAAI=53.38%) was found in the group reared on the diet RV35. The lowest essential amino acid index (48.24 %) was then recorded in the groups of GA reared on the diet, which contained 70% of rapeseed protein. Generally, it has been proved that diets composed mainly of organic wastes or by-products may result in reduced growth performance and survival of crickets. This suggests that diets containing only by-products might be missing important and nutritionally necessary components for the development and growth of crickets. The by-products can be used as a source of protein for crickets when the diet is in balance with other nutritional components, such as carbohydrates and fats (Sorjonen et al., 2019).

The EAA content in *Gryllus assimilis* showed some significant differences ($p \leq 0.05$) in their quality across the groups analysed. These significant diet-related differences were detected in the Threonine and Methionine content. Compared to the values (72.3-77.1 %) found by Kulma et al., (2019) in the house cricket, *Acheta domestica*, the EAAI values in all the samples was quite low especially due to low amino acid scores of Methionine and Isoleucine. On the other hand, all tested crickets were quite rich in Lysine from this point of view. These differences could be explained by the diet used to feed experimental crickets and the species. Moreover, the EAAI was calculated for all the samples and based on this index it was obvious that the protein quality was feed dependent. Even though conditions in large scale commercial farming are expected to be more or less constant, specific changes in the rearing program such as temperature, age of harvested adults or diet probably caused these differences. Oonincx & Van Der Poel (2011) proved, that inclusion of wheat bran and carrot into the diet of crickets increased fat content and decreased protein quality.

Methionine was shown to be the limiting amino acid in all the groups of tested insects. Based on the analyses published by other authors, limiting amino acids vary according to species. Köhler et al. (2019) reported that tryptophan is the limiting amino acid in locusts and crickets, lysine in scarab beetles, and leucine in silkworms. In our study, tryptophan content was not measured because of hydrolysis during sample preparation, the possibility that tryptophan might be the limiting amino acid thus cannot be excluded. This finding agrees with the data previously reported in the literature, where lack of tryptophan analysis was obviously not unusual. Sánchez-Muros et al. (2014) considered sulfur-containing amino acids to be the limiting amino acids. Our results also do not match with Oibiokpa et al. (2018) study results where the limiting amino acid in the same cricket (*Gryllus assimilis*) was threonine. Oibiokpa et al. (2018) used corn starch as feed for the cricket. This phenomenon might be explained by the different feeding substrates provided to the insects, since rapeseed used in this study is known to contain low methionine level (Eklund et al., 2015). It was proved that the nutritional value of insects depends on their feed (Oonincx & Van der Poel, 2011). The very low content of methionine in our samples suggests some potential for the use of the insects in alternative diets for people with amino acid metabolism disorders (Harding & Blau, 2010). Methionine in the form S- adenosyl methionine is required for transmethylation reactions (Rubin et al., 2007).

Glutamine was then determined to be the major non-essential amino acid. These findings are in line with the results found by Kewuyemi et al. (2020). The study of Eklund et al. (2015) has shown that the major amino acid in the rapeseed is also the Glutamic acid (4.70 g/100g protein). It is therefore obvious that the protein profile was affected by the rapeseed in the diet of the groups of *Gryllus assimilis*. Non-essential amino acids hardly influenced by the diet composition (Kulma et al., 2019). Glutamic acid has been found to influence the taste of chicken meat. In addition to Glutamic acid , free aromatic amino acids such as Phenylalanine and Tyrosine, also play an important role in improving salty or umami taste at sub-threshold concentrations in the presence of salt and free acidic amino acid (Wattanachant et al., 2004).
Leucine was determined to be the major essential amino acid. Additionally, high lysine, valine, contents were also discovered (Table 9). These findings are in line with the published profiles of house cricket available in the literature (Finke, 2002). Eklund et al. (2015) has also recorded that the major essential amino acid in the rapeseed is Leucine. This study has demonstrated once again the effect of the rapeseed oil on *Gryllus assimilis* amino acid profile.
The experimental groups of *Gryllus assimilis* reared on the diets with the rapeseed oil possessed high values of lysine and threonine, which are lacking in some of the cereal proteins that are major parts of the daily diets of many households. Obviously, their consumption can help mitigate deficiencies in the required amino acids (Ghosh et al. 2017).

7. Conclusions

This work dealt with the possibility of protein quality manipulation in the Jamaican field cricket (*Gryllus assimilis*) by adjusting the diet. The insects were reared under laboratory conditions. In this study, five different diets were used to feed the cricket.

It is likely that the diet with rapeseed itself, present in the gut of the cricket, had an important effect on the proteins quality when we compare our result with previous studies. These differences may be due to different rearing methods the feed intake. The results also present some significant differences in the composition of crickets between the dietary groups, and thus confirmed our hypothesis that the replacement of conventional protein sources by rapeseed protein into the feeding mixture influences protein quality of Jamaican field cricket (*Gryllus* *assimilis*). This difference was higher ($p \leq 0.05$) between the diet which contains 35% of rapeseed proteins and the one with 70%. Crickets reared on the diet with 35% of rapeseed proteins have significantly the highest essential amino acids index (EAAI) than the other groups did. As we can see, the incorporation of rapeseed protein in the cricket's diet is problematical despite its interesting nutritional content. From a practical point of view, the use of rapeseed meals in the diet is suggested but the level of incorporation should not exceed to 35% in the diet. Because the proteins quality of crickets was reduced when more than 35% of rapeseed cake was incorporated into the diet in this study. Thus, even if this product seems to be much more promising as insect meal substitutes from a nutritive and economic point, we need to thank about the right percentage to be mixed.

This study, through the results, proved that crickets could be reared on rapeseed meal by-products without significant impact on the amino acid profile. Economically, rapeseed by-products can be a very attractive option because of the lower price. Research on this subject to find the compromise between good nutritional value and a reasonable price is therefore very necessary.

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9. List of abbreviations

AdDNV	Acheta domesticus DensoVirus,
Ala	Alanine
ANOVA	Analysis Of Variance
Arg	Arginine
Asp	Aspartic acid
Cys	Cysteine
EAA	Essential Amino Acid
EAAI	Essential Amino Acid Index
EFSA	European Food Safety and Authority
FAO	Food and Agriculture Organization
GA	*Gryllus Assimilis*
Glu	Glutamic acid
Gly	Glycine
H_2SO_4	Sulferic Acid
HCl	Clhloric acid
His	Histidine
Ile	Isoleucine
IBM	International Business Machines
ISO	International Organization of Standardization
Kcal	Kilocalorie
Kg	Kilogram
Leu	Leucine
Lys	Lysine
Met	Methionine
Mg	Miligram
MUFA	Polyunsaturated Fatty Acid
NaOH	Sodium Hydroxid
NEAA	Non-Essential Amino Acid
Phe	Phenylalanine
Pro	Proline

PUFA	Polyunsaturated Fatty Acid
Ser	Serine
SFA	Saturated Fatty Acid
Thr	Threonine
Tyr	Tyrosine
Val	Valine
WHO	World Health Organization

10. Enclosures

List of tables

List of images

List of graphs

- **Results of statistics for essential amino acid content of samples**

- **THR**

Tests of Between-Subjects Effects

Dependent Variable: VAR00003

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	,935[a]	4	,234	3,193	**,030**
Intercept	235,359	1	235,359	3214,469	,000
VAR00002	,935	4	,234	3,193	,030
Error	1,830	25	,073		
Total	238,124	30			
Corrected Total	2,766	29			

a. R Squared = ,338 (Adjusted R Squared = ,232)

- **VAL**

Tests of Between-Subjects Effects

Dependent Variable: VAR00004

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1,713[a]	4	,428	1,966	**,131**
Intercept	513,777	1	513,777	2359,308	,000
VAR00002	1,713	4	,428	1,966	,131
Error	5,444	25	,218		
Total	520,934	30			
Corrected Total	7,157	29			

a. R Squared = ,239 (Adjusted R Squared = ,118)

- **ILE**

Tests of Between-Subjects Effects

Dependent Variable: VAR00005

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	,694[a]	4	,173	2,645	**,057**
Intercept	255,999	1	255,999	3903,939	,000
VAR00002	,694	4	,173	2,645	,057
Error	1,639	25	,066		
Total	258,333	30			
Corrected Total	2,333	29			

a. R Squared = ,297 (Adjusted R Squared = ,185)

❖ LEU

Tests of Between-Subjects Effects

Dependent Variable: VAR00006

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1,154[a]	4	,288	,859	**,502**
Intercept	732,392	1	732,392	2179,946	,000
VAR00002	1,154	4	,288	,859	,502
Error	8,399	25	,336		
Total	741,945	30			
Corrected Total	9,553	29			

a. R Squared = ,121 (Adjusted R Squared = -,020)

❖ PHE

Tests of Between-Subjects Effects

Dependent Variable: VAR00007

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	,516[a]	4	,129	2,021	**,122**
Intercept	177,766	1	177,766	2784,942	,000
VAR00002	,516	4	,129	2,021	,122
Error	1,596	25	,064		
Total	179,877	30			
Corrected Total	2,112	29			

a. R Squared = ,244 (Adjusted R Squared = ,123)

❖ LYS

Tests of Between-Subjects Effects

Dependent Variable: VAR00008

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2,868[a]	4	,717	1,702	**,181**
Intercept	528,292	1	528,292	1254,054	,000
VAR00002	2,868	4	,717	1,702	,181
Error	10,532	25	,421		
Total	541,692	30			
Corrected Total	13,400	29			

a. R Squared = ,214 (Adjusted R Squared = ,088)

❖ **MET**

Tests of Between-Subjects Effects

Dependent Variable: VAR00009

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	,805[a]	4	,201	16,484	**,000**
Intercept	47,407	1	47,407	3885,037	,000
VAR00002	,805	4	,201	16,484	,000
Error	,305	25	,012		
Total	48,517	30			
Corrected Total	1,110	29			

a. R Squared = ,725 (Adjusted R Squared = ,681)