THESIS

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Effects of different nitrogen sources on growth performance of insects

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List of abbreviations

EU: European Union

IPIFF: International Platform of Insects for Food and Feed

FCR: Feed Conversion Ratio

FAO: Food and Agriculture Organization

PAPs: Processed animal proteins

CP: Crude protein

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1. Introduction

The consumption of animal products is expected to increase about 60-70% by the year 2050. This will demand a vast of resources, with the most difficult one being the feed of production animals due to the scarcity of natural options, the climate change, and the food-feed-fuel competition (Makkar et al., 2014; Tilman and Clark, 2014; Wang et al., 2010). The typical alternative feed supplies like soymeal and fishmeal require high production costs, and their future availability is also limited (Makkar et al., 2014). Therefore, there is an increased need for alternative protein sources as human food or animal feed (van Huis et al., 2013; Ribeiro et al., 2018).

Rearing of edible insects can be one of the solutions. Over 1900 insect species are considered edible, however only a few are bred in adequate quantities for mass production (van Huis et al., 2013). Examples of insect species that have been commercially mass-produced for animal feed or human consumption include crickets (house cricket: *Acheta domesticus*; tropical house cricket: *Gryllodes sigillatus*; Jamaican field cricket: *Gryllus assimilis*; two-spotted cricket: *Gryllus bimaculatus*), mealworms (yellow mealworm: *Tenebrio molitor*; Giant mealworm: *Zophobas atratus*, superworm: *Zophobas morio* and lesser mealworm: *Alphitobius diaperinus*), the housefly (*Musca doestica*) and the black soldier fly (*Hermetia illucens*) (Cortes Ortiz et al., 2016; Ribeiro et al., 2018).

The feedstuffs used in insect feeding follow the laws of European Union (EU). These include cereal-based materials, fruits and vegetables, commercial feed authorised for all animal species, vegetal origin unsold products from supermarkets and fat derived from the processing of slaughtered animal parts (International Platform of Insects for Food and Feed [PIFF], 2020). Restrictions on the feeds which may be given to 'farmed animals' are also applied to farmed insects. Waste materials of animal origin allowed for feeding to farmed animals are limited by Regulation 1069/2009 to Category 3 material (European Parliament and Council, 2009). Some insects such as crickets require protein rich feed with high quality proteins. Thus, it is important to find the best protein sources to achieve good performance and reduce costs.

2. Literature review

2.1. Insect production

Insects efficiently utilise water and feed than traditional livestock due to their physiology, consequently having a lower feed conversion ratio (FCR) and greater growth efficiency (Nakagaki and DeFoliart, 1991; Oonincx and de Boer, 2012). Being poikilothermic, insects

can use less energy to maintain homeostasis and prevent waste of protein for energy instead of growth, thus making them efficient feed convertors compared to conventional animals (Collavo et al., 2005). According to Oonincx and de Boer (2012) and Halloran et al.*(*2016) another advantage of insects being used as an alternative protein source, is the decreased impact on the environment compared to the traditional livestock. Rearing mealworms to be used as a human protein source, produces much less greenhouse gasses and requires much less land, but similar or higher energy use than traditional livestock (Oonincx and de Boer, 2012).

The Food and Agriculture Organization (FAO) estimated that insects can potentially replace fishmeal used in aquaculture and livestock and also be used in pet food (van Huis et al., 2013). According to Sealey et al. (2011), fishmeal replaced by black soldier fly in mixtures of 25% and 50% in feeding trials on trout diets, have shown results that were as good as the control diet that consisted of 100% fishmeal. Insect meal used as alternative protein source can be compared to fishmeal and soymeal, regarding the sustainability of the production and the protein nutritive properties (Barroso et al., 2014; Sánchez-Muros et al., 2014). Moreover, insects, such as mealworms, are able to utilise waste products with low-nutritive values and convert them into a diet of high protein content that can replace soymeal in animal feed (Cortes Ortiz et al., 2016). Overall, insects show promising results as a protein for animal feed, but their use as feed of food will only be considered as a sustainable alternative protein source only when their production costs will be comparable to production of more traditional protein sources like fishmeal or soybean (Ribeiro et al., 2018).

House cricket has been used in commercial for pet food and fishing bait for over 60 years in the USA (Cortes Ortiz et al., 2016). Additionally, it is considered a sustainable and nutritious food source of the future due to its nutritional value, especially its high protein content, and can potentially solve the global malnutrition (Bawa et al., 2020). Currently in the USA, several companies are processing cricket produced insect protein powders for human consumption and for manufacturing different food items including corn chips, cookies, energy bars, etc (Dossey et al., 2016). Finke (2002), calculated the nutrients contained by 100 g of crickets (protein: 46 g, energy: 447 kcal, omega-3 fatty acids: 0.25 g, iron: 5.0 mg) compared to 100 g beef (protein: 25.6 g, energy: 278 kcal, omega-3 fatty acids: 0.009 g, iron: 2.4 mg) and 100 g chicken (protein: 39 g, energy: 190 kcal, omega-3 fatty acids: 0.05 g, iron: 1.2 mg), which showed that crickets as food and feed contain higher nutritional values than conventional animals. In addition, cricket as food and feed can improve iron and B12 profile (Finke, 2002). However, the price of commercial cricket

products, like cricket powder, is much higher than the price of animal sources protein (Morales-Ramos et al., 2018).

2.2. Insect products

There is a wide range of possibilities of insect products for food or feed that can be commercialised. These depend on the product type, like whole insects or processed animal proteins (PAPs) and if the products will be used as food or feed. The EU restricts the use of some product types for some animal species. As an important milestone in September 2021 the European Commission (2021) authorised insect PAPs in the feed of farmed animals, including swine and chicken (Commission Regulation (EU) 2021/1372). Previously insect PAPs were only authorised in aquaculture (IPIFF, 2020). Besides that, insect PAPs are allowed to be used in pet food, fur animals and other non-food producing animals like reptiles and birds. Although insect PAPs can be used in some farm animals (except ruminants), the EU has restricted the insect PAPs source to seven species: black soldier fly (*Hermetia illucens*), house fly (*Musca domestica*), yellow mealworm (*Tenebrio molitor*), lesser mealworm (*Alphitobius diaperinus*), house cricket (*Acheta domesticus*), tropical house cricket (*Gryllodes sigillatus*) and Jamaican field cricket (*Gryllus assimilis*). However, these restriction does not apply to insect PAPs used in pet food, fur animals and other non-food producing animals (IPIFF, 2020).

Fats, oil, gelatine, and collagen extracted from insects can be used in feeds of non-ruminant livestock like poultry and pigs but also in pet food, fur animals and other non-food producing animals. Adversely, killed whole insects, treated (e.g., dry freeze) or untreated are prohibited to be used in feeds for food producing animals, but whole treated insects can be used in pet food and for technical uses (e.g., biofuels). Lastly, live insects as feed - if national authorities give the approval of commercialisation to the product and the processing method - and the hydrolysed insect proteins can be used in aquaculture feed, non-ruminant food producing animals (poultry and pigs) feed, pet food and in feeds of fur and other animals (IPIFF, 2020).

According to IPIFF (2021), the PAPs from insects contain amino acids that are highly digestible for animals and their profile corresponds to the nutritional needs of fish, poultry or swine. Insect meals used in animal feeds contain between 55% - 76% crude protein. Additionally, insects contain vitamins such as vitamin B12 (cobalamin) which is absent in vegetable origin products, and vitamin B2 (riboflavin), fatty acids, and chitin, which act as immunostimulant. These nutrients fit especially the young monogastric

animals. Animal feeds containing these also have shown increasing trends in growth rates and development as well as animal health and welfare. Therefore, including insects in such animal diets will refine the agri-food chain and improve the diet of livestock according to their nutritional needs (IPIFF, 2021).

2.3. Life cycle and rearing of insect species

Protein is the key macronutrient in the insect diet, especially omnivorous ones since diets with high protein content have shown higher yield and better growth performance. On the other hand, diets with lower protein content have shown delayed development time and individual biomass gain of crickets (Joern and Behmer, 1997; Patton, 1967). Soybean has been used as the main protein source in feeds in rearing insects, due to the high content and valuable amino acids (Cohen, 2015). The use of soybeans though, has a negative impact on the environment (da Silva et al., 2010). Therefore, it is important to find alternative protein sources for feeding insects to make the process more sustainable. But, in order to achieve a good nutritive profile of insects, we need to evaluate their given feed since their quality of nutrition is greatly dependent on what they eat (Oonincx et al., 2015; Oonincx and van Der Poel, 2011).

Cortes Ortiz et al. (2016), suggested that insects, like mealworms, can utilise waste product of low nutrient value and be able to provide a diet to other animals or humans with high protein content. However, Harsányi et al. (2020), found that nutrient-poor diets can be tolerable by mealworms in term of survival but they slow down the development and show lower weight. Also, nutrient-poor diets tend to result in lower protein content and higher fat content in the larvae on yellow mealworms, super worms, and house crickets.

2.3.1. House crickets and Jamaican field crickets

Acheta domesticus and *Gryllus assimilis* belong to the family *Gryllidae* of order *Orthoptera* and undergo a “hemimetabolism” or incomplete metamorphosis (Clifford et al., 1977). The females lay 340-1060 eggs using their ovipositor in the substrate (Murtaugh and Denlinger, 1985) and the eggs are incubated for 11-15 days from lay to hatch (Nowosielski and Patton, 1965). At 4-5th instar the females develop the ovipositor and in the last 2 instars, wings begin to develop, which adult males are using to produce the characteristic mating chirps (van Huis and Tomberlin, 2018).

Rearing conditions of house crickets and Jamaican field crickets are similar. House crickets have been studied throughout the years and minor differences were found in the

literature. Crickets tend to mature in 3-10 days. Natural mortality in the colony can happen depleting the population, therefore it is advised to harvest them when approximately 85% of the population have reached the adult stage (van Huis and Tomberlin, 2018). According to Patton (1978), the optimum temperature for rearing *A. domesticus* is at 32^{o}C with relative humidity kept between 70-75%. As long as other conditions are kept at optimum level the life cycle from egg to adult will take 6-7 weeks producing an adult cricket of 500 mg. However, this increased temperature can increase the stress and the risk of mortality or reduced fecundity (van Huis and Tomberlin, 2018). Morales-Ramos, Rojas and Dossey (2018) found that the optimal temperature and time for peak individual biomass gain of *A. domesticus* was 27^{o}C and by the end of 8th week of age. Therefore, knowing when to harvest them will aid in reducing the cricket production costs. According to van Huis and Tomberlin (2018), the relative humidity should be between 50-60%, to prevent mould and mite invasion when combined with high temperatures as well. The ventilation should also help in preventing these issues (van Huis and Tomberlin, 2018). Maximising the surface area using egg flats allow the increase of density, thus reducing the fixed costs (i.e., rent and utilities) and the variable costs (i.e., labour) per unit output (van Huis and Tomberlin, 2018).

Being nocturnal, crickets are suggested to be reared in a light/dark cycle of 12 hours of light and 12 hours of dark (van Huis and Tomberlin, 2018). Patton (1978) suggested that light/dark cycles can vary from 8-16, 16-8 and 24-0 hours. Crickets should be reared in groups rather than individually since the growth rate is higher in groups (McFarlane, 1965). Interestingly, group rearing influences how some nutrients affect the growth and development of the insects (McFarlane, 1965). An estimation of population density should not exceed 1 cricket per 2.5cm^{2} of space, even though it is unknown if crowding influences growth and development of *A. domesticus* since they are not territorial, and males do not fight. Despite this, cannibalism exists in both sexes (Patton, 1978).

2.3.2. Yellow mealworm

Tenebrio molitor belongs to the family *Tenebrionidae* of order *Coleoptera*. The yellow mealworm is needed in large quantities because of its importance in the diet of captive and wild birds, pets such as reptiles, larger predatorial insects like tarantulas, aquaculture and may even become an important protein source for human consumption (Morales-Ramos et al., 2011).

The females can lay 250-500 eggs one by one or in clusters in the substrate. The eggs hatch according to the incubation temperature: after 4 days at 26-30^{o}C, or up to 34 days at

15^{o}C (Ribeiro et al., 2018). Larval stages take an average of 112-203.3 days but can be as low as 57 days in controlled conditions. They moult several times with an average of 11-19 instars (Martin et al., 1976). Larvae close to pupation appear as a "C" shape. Pupal stage lasts 6-20 days. Initially, adults are white beetles with soft exoskeleton which eventually harden and become darker. Mating and oviposition start 3 days after they emerge. Adult stage lasts about 16-173 days (Ribeiro et al., 2018). The whole life cycle lasts 75-90 days under optimal conditions(Spencer and Spencer, 2006; Ribeiro et al., 2018).

The usual temperature for rearing mealworms is 25-28^{o}C (Kim et al., 2015; Koo et al., 2013; Ludwig, 1956; Punzo, 1975; Spencer and Spencer, 2006). Embryonic development is inhibited in temperatures below 17^{o}C (Koo et al., 2013) and mortality rate increases in temperatures above 30^{o}C (Koo et al., 2013; Ludwig, 1956). No significant differences were found in the temperature requirement for different stages of development in *T. molitor* species (Ribeiro et al., 2018). At 30^{o}C, the larval instars are more but shorter, leading to a longer total larval developmental phase at 30^{o}C than at 25^{o}C (Ludwig, 1956).

Optimum values for relative humidity have shown a variation from 60% to 75% (Punzo, 1975; Ribeiro et al., 2018). The number and duration of the instars, but also the water absorption capacity of the different stages are influenced by the temperature combined with relative humidity (Punzo and Mutchmor, 1980). Generally, the higher relative humidity the faster the growth rate. However, the high relative humidity, favours the contamination of the colony since microorganisms will thrive in such an environment (Fraenkel et al., 1950). Even though *T. molitor* can survive in dry conditions for a long time, larvae developmental time is faster in higher humid conditions (>70% relative humidity), but the likelihood of microorganisms development like fungi, bacteria or mites is a risk to consider (Fraenkel and Blewett, 1944). Higher growth rates were experienced where mealworms were reared on dry substrates with a water source (Oonincx et al., 2015). Water deprivation can cause *T. molitor* larvae to ingest less food and the FCR will decrease (Ribeiro et al., 2018). Adding a water source in the diet will increase the survival rates and reduce the development times (Oonincx et al., 2015).

Another parameter to consider in mass rearing of *T. molitor* is the population density because it affects the number and duration of the larval moults. The higher the population density the fewer the larval instars (Morales-Ramos et al., 2012; Morales-Ramos and Rojas, 2015; Weaver and McFarlane, 1990). Crowding, though, can lead to slower growth rate and incomplete transformation due to the reduced opportunity of feeding as a result of competition (Weaver and McFarlane, 1990). Crowding but also inhibits the pupation,

induces cannibalism (Tschinkel and Willson, 1971) and reduces the reproductive output (Morales-Ramos et al., 2012).

Being negatively phototropic and phototactic, adult and larger larvae of *T. molitor* place themselves below the substrate during the daylight and come out during darkness (Ribeiro et al., 2018). According to Kim et al. (2015), for optimal larval development, long-day conditions should be used in mass rearing with photoperiods of 14 hours of light and 10 hours of darkness.

2.3.3. Superworm

Zophobas morio is also a member of the family *Tenebrionidae* of order *Coleoptera* and it is a large neotropical beetle species (larval length can be up to 55 mm). Females can lay approximately 2000 eggs during their lifespan. The number of eggs is negatively correlated with female maternal age and positively correlated with adult density. Larvae hatch after 8 days at 25°C. For the pupation (which occurs after 16 or 17 moults) larvae should be kept isolated (Rumbos and Athanassiou, 2021). Larvae fail to pupate under crowded conditions and moulting continue to occur until death (Quennedey et al., 1995). This is an important difference between superworm and yellow mealworm larvae which significantly affect the industrialisation. The duration of the pupal stage is 13-15 days at 25°C. Adults have 38-57 mm body length and they can live up to 6-12 months.

The rearing conditions required by the superworm larvae are similar to that of the yellow mealworm. Superworm larvae are commonly fed with wheat bran which can be supplemented with cereal grains (Rumbos and Athanassiou, 2021). Ambient temperatures ranging between 25-28°C and relative humidity of 60-70% are adequate for superworms.

3. Aims

The aim of this study was to examine the effects of different nitrogen sources (micellar casein, urea, and defatted soybean meal) on the growth performance of four selected insect species such as Jamaican field cricket, house cricket, yellow mealworm and superworm. Urea is a non-protein nitrogen source which can substitute true protein sources thus reduce production costs. Micellar casein consists of 20% whey protein and 80% casein and considered to be a high-quality protein source. Soy is the most commonly used plant origin protein source in the feed industry.

4. Materials and Methods

4.1. Insect colonies

The insects were reared in the animal house of the Institute of Animal Breeding, Nutrition and Laboratory Animal Science, University of Veterinary Medicine, Budapest, Hungary.

House cricket and Jamaican field cricket colonies were housed in 790x570x420 mm transparent plastic boxes (Figure 1). Mealworm beetles and superworm beetles were housed in 570x390x280 mm transparent plastic boxes (Figure 2). The crickets and superworm beetles received *ad libitum* chicken grower feed and the mealworms received *ad libitum* wheat flour with wheat bran.

Figure 1. The house cricket colony

Figure 2. The mealworm colony

4.2. Experimental setup

The experiments were conducted in the animal house of the Institute of Animal Breeding, Nutrition and Laboratory Animal Science. The temperature and the relative air humidity were measured with a digital thermometer/hygrometer (TFA 30.5027.01, TFA Dostmann). The temperature and the relative air humidity of the room were 27±0.4°C and 50-60% respectively.

4.2.1. Crickets

The feeding experiments were carried out using 1-1.5 mm nymphs kept in plastic containers. The nymphs of each insect were randomly chosen and assigned in one of six experimental feed groups (Table 1). Small round plastic containers (66x20 mm) were used to put the feed

and the water for the cricket species. Paper towel was used as bedding material and paper egg holder as surface maximiser. Wood flakes were placed in the plastic container of water to provide standing area for the crickets to consume water and avoid drowning. For every diet treatment, four replicates of data were obtained.

The **Jamaican field crickets** were reared in a similar way, with *ad libitum* feeding and water in three categories. In experiment I, 15 crickets were housed in 206x156x83 mm containers (60 crickets for each feed group in total; Figure 3 and Figure 4). In experiment II, crickets were housed individually in 85x55 mm round plastic containers (4 crickets for each feed group in total).

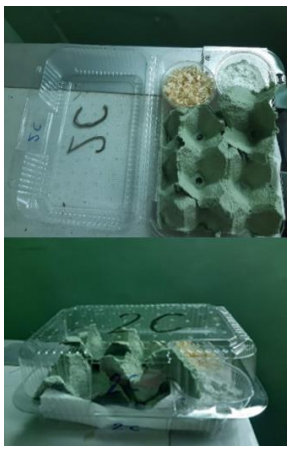

Figure 3. The containers for Jamaican field crickets

Figure 4. The housing of the crickets

The **house crickets** were reared in two different categories in plastic containers. In experiment I, 15 crickets were housed in 160x115x55 mm containers (60 crickets for each feed group in total). In experiment II, the crickets were housed individually (4 crickets for each group in total) in 85x55 mm round plastic containers (Figure 5 and Figure 6). Feed was offered in the paper egg holder and water was provided in plastic bottle cups with wood shaves as surface maximiser.

Figure 5. Container for individual housing

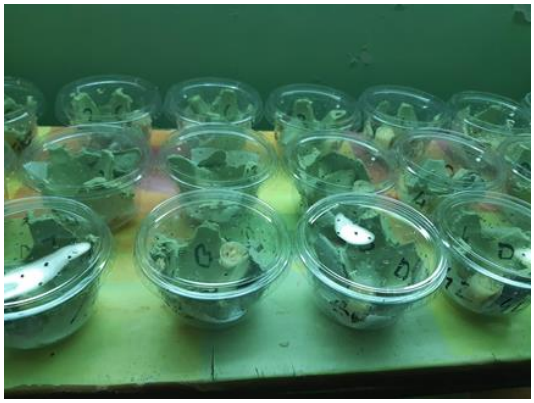

Figure 6. Group of individually housed crickets

4.2.2. Mealworms and superworms

Mealworm larvae (size: 10-10.3 mm) and superworm larvae (size: 10-10.5 mm) were reared in plastic containers with dimensions 140x100x75 mm, containing 50 g of feed (Figure 7 and Figure 8). Four containers for each feed group with 20 mealworms and 15 superworms were reared in each container (80 mealworms and 60 superworms per feed group in total). The feed was also offered *ad libitum* for each feed group, but no water was provided.

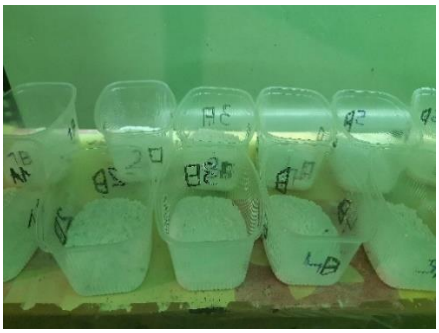

Figure 7. The containers of mealworm and superworm larvae

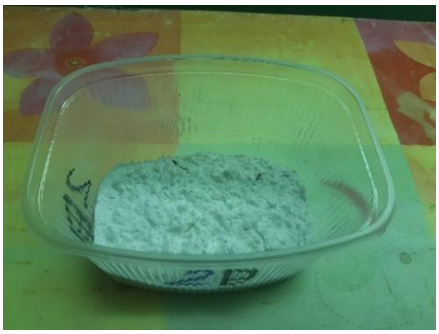

Figure 8. The container size of mealworm and superworm larvae

In the present study, six isonitrogenous feeds composed of 3.52% nitrogen and 22% crude protein were used to evaluate the growth performance of four different insect species, Jamaican field cricket, house cricket, yellow mealworm and superworm. Table 1 shows the composition of the 6 isonitrogenous feeds. Nitrogen contents of feed ingredients were measured with standard methods (AOAC, 1990). Table 2 shows the amino acid content of soybean meal and micellar casein.

Table 1. Ingredients of experimental isonitrogenous feed on dry matter basis (/100g)

Ingredients (g)	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Micellar casein	28.07	21.05	14.04	7.02	-	-
Urea	-	1.89	3.77	5.65	7.54	-
Defatted Soybean meal	-	-	-	-	-	42.2
Corn starch	71.93	77.06	82.19	87.33	92.46	57.8

Group 1: 100% micellar casein, Group 2: 75% micellar casein, 25% urea; Group 3: 50-50% micellar casein and urea, Group 4: 25% micellar casein, 75% urea, Group 5: 100% urea, Group 6: 100% defatted soybean meal

Table 2. Amino acid content of micellar casein and soybean meal (He et al., 2015)

Amino acids (/100 g protein)	Micellar casein	Soybean meal
Alanine	3.0	4.0
Arginine	3.7	7.0
Aspartic acid	7.0	11.3
Cystine	0.7	1.6
Glutamic acid	20.6	17.2
Glycine	1.7	4.0
Histidine	2.6	2.7
Isoleucine	5.2	4.9
Leucine	9.4	8.0
Lysine	8.3	6.4
Methionine	2.6	1.4
Phenylalanine	4.7	5.3
Proline	9.9	4.7
Serine	5.3	5.0
Threonine	4.1	4.2
Tryptophane	6.0	1.2
Tyrosine	4.6	3.9
Valine	6.0	5.3

4.3. Data collection and statistical analysis

The body weight and feed consumption were measured weekly with a digital scale (Tecator 6110). The insects were counted weekly. For each dataset, the mean, and the standard deviation (SD) were calculated. Analyses were performed using R 4.0.3. (R Core Team, 2020) software . P values lower than 0.5 were significant.

Normality of the data was tested with Quantile-Quantile Plot. Variances of the data were tested with Levene's test. One-Way ANOVA tests was performed to compare the normally distributed data. Tukey’s post-hoc analysis was performed if the result of One-Way AVOVA test was significant. Non-normally distributed data were analysed with Kruskal-Wallis rank sum test. Post-hoc Dunn Test was performed if the result of Kruskal-Wallis rank sum test was significant.

Survival rates and final mean individual body weight of crickets were tested on week 4. Development of crickets was followed for 13 weeks to see whether they reach sexual maturity. Survival rates and final mean individual body weight of yellow mealworm were compared on week 14. Data of superworm larvae were evaluated on week 5. FCR was calculated with the following formula FCR= amount of ingested food (g)/ weight gained (g).

5. Results

5.1. Crickets

The initial and final body weight of both cricket species were normally distributed and had equal variances ($p>0.05$). The initial body weight measurements of both cricket species were not significantly different. The survival rates of the house crickets were normally distributed and had equal variances. The survival rates of the Jamaican field crickets were non-normally distributed with equal variances. Due to frequent technical problems (moisture absorption), the feed intake of crickets could not be evaluated.

Table 3 and 4 show the results of the **house crickets**. There was a statistically significant difference in the final body weight ($p=0.014$) of group-housed crickets on week 4 between Group 5 and 1 ($p=0.0447$) and between Group 5 and 6 ($p=0.0213$). The mean individual body weight was the lowest in Group 5 and the highest in Group 6. The survival rate of the group-housed crickets was poor with the highest survival in Group 5 and the lowest in Group 3. The survival rates did not differ significantly ($p=0.854$).

Table 3. Results of group-housed house crickets on week 4

House cricket	**Number of live crickets**		**Mean individual body weight (g)**			**Survival rate**
	week 1	week 4	week 1	week 4	p value	
Group 1	60	13	0.0056±0.0007	0.0247±0.0048	g1-g5=0.0447 g6-g5=0.0213	21.1%
Group 2	60	15	0.0058±0.0007	0.0227±0.0052		25.0%
Group 3	60	11	0.0057±0.0006	0.0167±0.0051		18.3%
Group 4	60	16	0.0056±0.0008	0.0158±0.0049		26.7%
Group 5	60	17	0.0054±0.0007	0.0112±0.0046		28.3%
Group 6	60	12	0.0058±0.0008	0.0264±0.0054		20.0%

Group 1: 100% micellar casein, Group 2: 75% micellar casein, 25% urea; Group 3: 50-50% micellar casein and urea, Group 4: 25% micellar casein, 75% urea, Group 5: 100% urea, Group 6: 100% defatted soybean meal

Table 4 shows the final body weight of the individually housed crickets on week 4 which was significantly higher in Group 6 compared to those of the other groups. The mean individual body weight was the lowest in Group 5 and the highest in Group 6. Significant differences with p-values are shown in Table 4. The survival rate of the individually housed crickets was 100% on week 4.

Table 4. Results of individually housed house crickets on week 4

House crickets	**Number of live crickets**		**Mean individual body weight (g)**			**Survival rate**
	week 1	week 4	week 1	week 4	p value	
Group 1	4	4	0.0049±0.0049	0.0136±0.0023	g6-g1=0.03432 g6-g2=0.03933 g6-g3=0.01698 g6-g4=0.01354 g6-g5=0.00558	100%
Group 2	4	4	0.0050±0.0050	0.0133±0.0020		100%
Group 3	4	4	0.0052±0.0052	0.0126±0.0021		100%
Group 4	4	4	0.0052±0.0052	0.0122±0.0023		100%
Group 5	4	4	0.0049±0.0049	0.0092±0.0024		100%
Group 6	4	4	0.0050±0.0050	0.0235±0.0022		100%

Group 1: 100% micellar casein, Group 2: 75% micellar casein, 25% urea; Group 3: 50-50% micellar casein and urea, Group 4: 25% micellar casein, 75% urea, Group 5: 100% urea, Group 6: 100% defatted soybean meal

Table 5 and 6 show the results of the **Jamaican field crickets**. There were significant differences in the final body weight ($p<0.001$) of the group-housed crickets on week 4. The mean individual body weight was the lowest in Group 5 and the highest in Group 6. Significant differences with p-values are shown in Table 5. The survival rates of the group-housed Jamaican field crickets were generally low with the highest percentage in Group 2 and 4, and the lowest in Group 5, but the differences were not significant ($p=0.36$).

Table 5. Results of group-housed Jamaican field crickets on week 4

House crickets	Number of live crickets		Mean individual body weight (g)			Survival rate
	week 1	week 4	week 1	week 4	p value	
Group 1	60	12	0.0042±0.0004	0.0238±0.0034	g5-g2=0.0320 g2-g3=0.0254 g2-g4=0.0435 g2-g5=0.0064 g1-g6=0.0036 g2-g6=0.0165 g3-g6 < 0.001 g4-g6 < 0.001 g5-g6 < 0.001	20.0%
Group 2	60	17	0.0041±0.0003	0.0256±0.0038		28.3%
Group 3	60	15	0.0041±0.0004	0.0186±0.0037		25.0%
Group 4	60	17	0.0042±0.0004	0.0192±0.0035		28.3%
Group 5	60	10	0.0041±0.0003	0.0160±0.0037		16.7%
Group 6	60	12	0.0042±0.0003	0.0333±0.0035		20.0%

Group 1: 100% micellar casein, Group 2: 75% micellar casein, 25% urea; Group 3: 50-50% micellar casein and urea, Group 4: 25% micellar casein, 75% urea, Group 5: 100% urea, Group 6: 100% defatted soybean meal

Table 6 shows the final body weight of the individually housed Jamaican filed crickets with significant differences. The mean individual body weight was the lowest in Group 5 and the highest in Group 6. Significant differences with p-values are shown in Table 6. The survival rate of individually housed crickets was 100% on week 4.

Table 6. Results of individually housed Jamaican field crickets on week 4

Jamaican field crickets	Number of live crickets		Mean individual body weight (g)			Survival rate
	week 1	week 4	week 1	week 4	p value	
Group 1	4	4	0.0023±0.0007	0.0150±0.0028	g1-g5=0.0406 g6-g3=0.0386 g6-g4= 0.0160 g6-g5=<0.001	100%
Group 2	4	4	0.0024±0.0005	0.0141±0.0027		100%
Group 3	4	4	0.0024±0.0006	0.0120±0.0028		100%
Group 4	4	4	0.0025±0.0007	0.0112±0.0030		100%
Group 5	4	4	0.0025±0.0005	0.0079±0.0031		100%
Group 6	4	4	0.0023±0.0006	0.0188±0.0029		100%

Group 1: 100% micellar casein, Group 2: 75% micellar casein, 25% urea; Group 3: 50-50% micellar casein and urea, Group 4: 25% micellar casein, 75% urea, Group 5: 100% urea, Group 6: 100% defatted soybean meal

The tendencies of growth and survival rate of the house crickets and the Jamaican field crickets were similar. The final body weight was the highest in Group 6 (100% defatted

soybean meal) and the lowest in Group 5 (100% urea). The survival rates were equally poor between the two cricket species. However, the survival rate in Group 5 (100% urea) was the highest in the house crickets but the lowest in the Jamaican field crickets. In Group 6, both sexes of house cricket and Jamaican filed cricket reached maturity on week 11 and 12 respectively.

5.2. Yellow mealworm

The initial and final body weight of the mealworm larvae were normally distributed with equal variances ($p>0.05$). The initial body weight measurements of the mealworm larvae were not significantly different. Table 7 shows the results of the yellow mealworm larvae. There were significant differences in the final body weight ($p<0.001$) of the yellow mealworm larvae on week 14. The mean individual body weight was the lowest in Group 5 and the highest in Group 1. Significant differences with p-values are shown in Table 7. Only one individual reached pupation, in Group 1 on week 12.

Table 7. Results of yellow mealworm larvae on week 14

Yellow mealworm	**Number of live mealworms**		**Mean individual body weight (g)**			**Survival rate**
	week 1	week 14	week 1	week 14	p value	
Group 1	80	47	0.0104±0.0003	0.0405±0.0044	g1-g2=0.0012 g1-g3,4,5< 0.001 g2-g5=0.0172 g6-g3=0.0094 g6-g4=0.0067 g6-g5< 0.001	58.8%
Group 2	80	55	0.0105±0.0004	0.0250±0.0044		68.8%
Group 3	80	62	0.0105±0.0004	0.0188±0.0047		77.5%
Group 4	80	50	0.0104±0.0003	0.0183±0.0044		62.5%
Group 5	80	55	0.0105±0.0003	0.0134±0.0044		68.8%
Group 6	80	15	0.0105±0.0003	0.0313±0.0049		18.8%

Group 1: 100% micellar casein, Group 2: 75% micellar casein, 25% urea; Group 3: 50-50% micellar casein and urea, Group 4: 25% micellar casein, 75% urea, Group 5: 100% urea, Group 6: 100% defatted soybean meal

Table 7 and Figure 9 show the survival rates of the yellow mealworm larvae which were over 50%, with the exception of Group 6, which had a very poor survival rate (18.8%). The survival rates were normally distributed with equal variances. There were significant differences between Group 6 and Group 1, 2, 3, 4, 5 (<0.001).

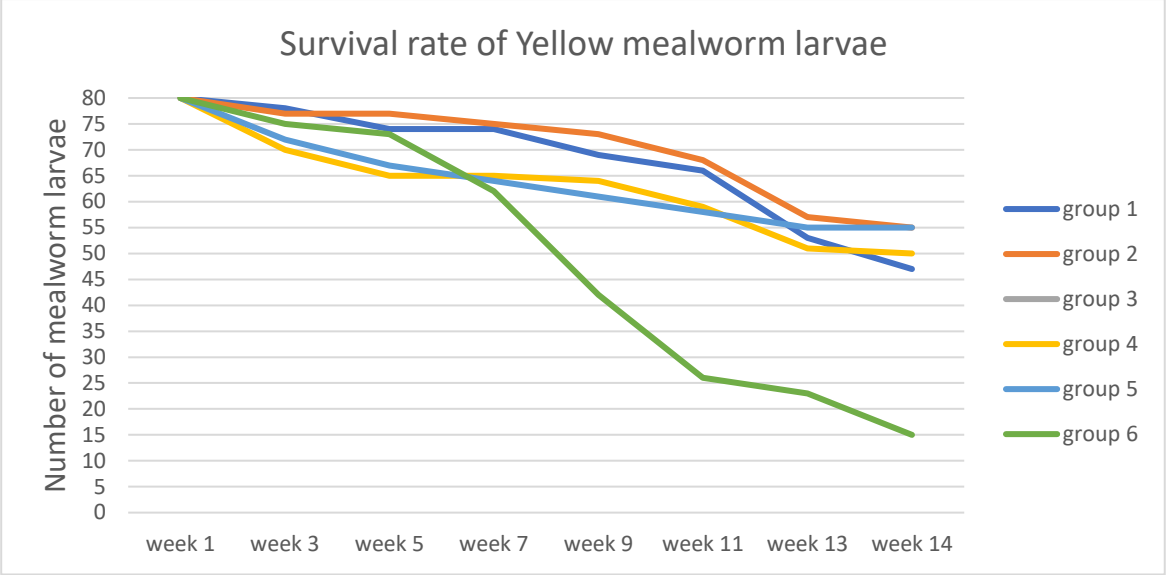

Figure 9. Survival rate of yellow mealworm larvae

Group 1: 100% micellar casein, Group 2: 75% micellar casein, 25% urea; Group 3: 50-50% micellar casein and urea, Group 4: 25% micellar casein, 75% urea, Group 5: 100% urea, Group 6: 100% defatted soybean meal

Table 8 shows the substrate reduction (feed consumption) of the mealworm larvae groups. The highest substrate reduction was noted in Group 5 and the lowest in Group 6. The substrate reduction differed significantly between Group 4 and 6 (p=0.0149) and between Group 5 and 6 (p=0.0035).

Table 8. Substrate reduction of yellow mealworm larvae

Yellow mealworm	**Substrate weight (g)**		
	week 1	week 14	Feed consumption
Group 1	50.17±0.2034	45.85±0.72	4.32
Group 2	50.18±0.2131	45.56±0.71	4.73
Group 3	50.20±0.2054	44.71±0.66	5.43
Group 4	50.18±0.2046	44.30±0.65	5.88
Group 5	50.20±0.2116	43.41±0.66	6.79
Group 6	50.17±0.2027	46.52±0.71	3.65

Group 1: 100% micellar casein, Group 2: 75% micellar casein, 25% urea; Group 3: 50-50% micellar casein and urea, Group 4: 25% micellar casein, 75% urea, Group 5: 100% urea, Group 6: 100% defatted soybean meal

Table 9 shows the FCR of the yellow mealworm. The FCR seemed to gradually worsen with the inclusion of urea level. The FCR was the best in Group 6 (100% defatted soybean meal) followed by Group 1 (100% casein) and the worst in Group 5 (100% urea).

Table 9. Feed conversion ratio of yellow mealworms

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
FCR	4.16±0.66	4.43±0.70	4.88±0.39	5.73±0.13	6.65±0.53	3.49±0.77

FCR: Feed Conversion Ratio, Group 1: 100% micellar casein, Group 2: 75% micellar casein, 25% urea; Group 3: 50-50% micellar casein and urea, Group 4: 25% micellar casein, 75% urea, Group 5: 100% urea, Group 6: 100% defatted soybean meal

5.3. Superworms

The initial body weight measurements of the superworm larvae were normally distributed with equal variances ($p>0.05$). Table 10 shows the results of the superworm larvae. The initial body weight measurements of the superworm larvae were not significantly different. The final body weight measurements were non-normally distributed with equal variances. The mean individual body weight was the lowest in Group 5 and the highest in Group 6. Significant differences with p-values are shown in Table 10.

The survival rates were normally distributed with equal variances. The survival rate decreased with the gradual increase of urea level. The highest was in Group 1 (100% casein) and the lowest in Group 6 (100% defatted soybean meal). The difference was significant between Group 1 and Group 2 ($p=0.0106$), 4, 5, 6 ($p<0.001$), Group 3 and Group 6 ($p=0.0185$).

Table 10. Results of superworm larvae on week 5

Super-worms	**Number of live superworms**		**Mean individual body weight (g)**			**Survival rate**
	week 1	week 5	week 1	week 5	p value	
Group 1	60	45	0.0281±0,0014	0.0449±0.0039	g1-g4=0.0488 g1-g5=0.0202 g2-g5=0.0233 g4-g6=0.0101 g5-g6=0.0029	75.0%
Group 2	60	29	0.0289±0.0014	0.0453±0.0033		48.3%
Group 3	60	34	0.0285±0.0012	0.0404±0.0035		56.7%
Group 4	60	22	0.0283±0.0011	0.0352±0.0034		36.7%
Group 5	60	22	0.0283±0.0014	0.0317±0.0033		36.7%
Group 6	60	20	0.0281±0.0014	0.0506±0.0038		33.3%

Group 1: 100% micellar casein, Group 2: 75% micellar casein, 25% urea; Group 3: 50-50% micellar casein and urea, Group 4: 25% micellar casein, 75% urea, Group 5: 100% urea, Group 6: 100% defatted soybean meal

Table 11 shows the substrate reduction (feed consumption) of the superworm larvae. The feed intake increased with increasing urea inclusion level. The substrate reduction did not differ significantly ($p = 0.2076$).

Table 11. Substrate reduction of superworm larvae

Superworm	**Substrate weight (g)**		
	week 1	week 5	feed difference
Group 1	50.24±0.20	49.58±0.3884	0.6567
Group 2	50.19±0.22	49.54±0.4220	0.6545
Group 3	50.23±0.20	49.56±0.4294	0.6697
Group 4	50.21±0.20	49.2971±0.41	0.9161
Group 5	50.23±0.21	49.0427±0.38	1.1907
Group 6	50.24±0.22	49.6283±0.38	0.6169

Group 1: 100% micellar casein, Group 2: 75% micellar casein, 25% urea; Group 3: 50-50% micellar casein and urea, Group 4: 25% micellar casein, 75% urea, Group 5: 100% urea, Group 6: 100% defatted soybean meal

The FCR of the superworms seemed to gradually worsen with the inclusion of urea level. The FCR was the best in Group 6 (100% defatted soybean meal) followed by Group 1 (100% casein) and the worst in Group 5 (100% urea). Overall, the FCRs were very poor over 15 kg/kg.

6. Discussion

There are 10 amino acids that are essential in arthropods' diet: leucine, isoleucine, valine, threonine, lysine, arginine, methionine, histidine, phenylalanine, and tryptophan. Tyrosine is a main component of sclerotin, and it is required in large amounts during moulting (Morales-Ramos et al., 2014). When there is a lack of nutrients in the diet of the yellow mealworm, the larvae tend to consume less and consequently gain less (Bordiean et al., 2020). As Table 2 shows, the micellar casein has a higher content of most of the essential amino acids (except arginine and phenylalanine) compared to the soymeal.

In order to decrease the production costs of products made from crickets agriculture by-products should be used in cricket feed formulations (Morales-Ramos et al., 2020a). By using the self-selection method of rearing crickets, it was discovered that by-products in feed formulations are more profitable. However, compared to a commercial diet, the rearing process was slower. Although there is evidence that most insects can self-select

macronutrient intake ratios, there is also evidence that insects can select vitamins to an optimal ratio as well (Schiff et al., 1988).

6.1. Crickets

The diet of the crickets is the most important factor in mass rearing since it is closely related to their nutritional profile (Oonincx et al., 2019). Crickets have a higher requirement in amino acids and protein than carbohydrates since they are omnivorous insects (Chapman, 2012). Interestingly, different diets, especially protein, carbohydrate and fat content can influence the survival rate, feed efficiency, and development time of the crickets (Oonincx et al., 2015). Generally the optimum protein content in the diet of crickets should be 20-30% (Orinda et al., 2017; Patton, 1967).

The FCR of crickets is quite bad (Finke, 2002; Nakagaki and DeFoliart, 1991). Being poikilothermic can prevent the protein consumption for energy instead of promoting growth (Collavo et al., 2005). Therefore, feeding crickets a high crude protein diet might be expensive, but less amount of feed is required, resulting in a higher yield output (Bawa et al., 2020). High protein diets can result in a high protein and low fat content in crickets (Bawa et al., 2020; Oonincx et al., 2015). Additionally, increasing the protein quality of the diet, the fat stores can be reduced in the crickets, since it increases the digestibility (Bawa et al., 2020). Another factor not only improving the survival rate but also the physiological functions of the crickets is the water supply (McCluney and Date, 2008).

The **level, source and quality of protein** influence the growth rate of crickets. Soy is a good and commonly used protein source for insects. This is also supported by this study as Group 6 (100% defatted soybean meal) had the highest final body weight measurements. As previously mentioned, crickets require protein rich diets. Crickets can be reared with lower levels of protein if their diet contains enough levels of the other nutrients (Bawa et al., 2020; Sorjonen et al., 2019). Food industry by-products with high protein content can replace the soybean that is usually used in rearing crickets. Harsányi et al. (2020), found that diets made of organic waste and of low-nutritive value (vegetable waste, garden waste, cattle/horse manure) cannot be used as a substrate to grow *A. domesticus*, *T. molitor* or *Z. morio* larvae, since these substrates decrease the protein concentration and increase the fat concentration in all three species.

Only a few studies tested milk powder or skim milk (Morales-Ramos et al., 2020a; Patton, 1978) thus it is difficult to compare the results of this study with that of others. Patton (1978) used a mixture feed as the optimum diet for rearing *A. domesticus* made from soybean

meal, wheat middling, powdered skim milk, corn meal, powdered brewer's yeast and powdered animal liver and calculated its contents as 30% protein, 3% carbohydrate, and 5% fat. The addition of pumpkin in the diet of crickets showed an improvement in the development time (Bawa et al., 2020; Cohen, 2015), but it resulted in crickets with lower protein and higher fat content because pumpkins are high in carbohydrates (Bawa et al., 2020).

A new formulation of diet was prepared by Morales-Ramos et al. (2020a), to study the self-selection of food ingredients and agricultural by-products of the house cricket. The skim milk was replaced by defatted dry and whole milk and the dry defatted pork liver with dry defatted beef liver. Vitamin C and sterols, which are abundant in whole milk, play an essential role in molting process of insects (Chapman, 2012; Cohen, 2015; Morales-Ramos et al., 2020a). The results showed high consumption of the whole dry milk (28.7 g) in a treatment compared to the classical defatted soybean meal (12.4 g). The liver powder in the mix offers a growth factor responsible for better performance. Morales-Ramos et al. (2020a), concluded that crickets fed on the former diets had faster development than the crickets fed on the new formulation. Lastly, although dry milk contains 40 ppb of vitamin B_{12}, Morales-Ramos et al. (2020a), found that it is not really required in the diet of the house cricket because it was absent in an earlier treatment and the crickets were still able to develop and reproduce. Nevertheless, it is known that adult crickets contain high amounts of vitamin B_{12} (Finke, 2002) which is believed to be synthetised by the symbiotic microflora of the house cricket (Ulrich et al., 1981).

The slight differences in the carbohydrate and fat content of the feeds have shown that apart from the proteins of the feed, the carbohydrates and the fats play a role in the performance of the crickets (Behmer and Elias, 1999; Cohen, 2015; Joern and Behmer, 1997). Crickets fed on diets with supplemented omega-3 fatty acids showed a higher content of omega-3 fatty acids in them (Oonincx et al., 2019).

Mean individual **body weight** of group-housed crickets of Group 1 (100% casein) and Group 6 (100% defatted soybean meal) had similar mean individual body weight with crickets kept on chicken feed in the study of Harsányi et al. (2020). The performance of other groups was lower. Cannibalism can explain the better performance of group-housed crickets in comparison to individually housed ones. Bawa et al. (2020) described higher individual body weight, but data were collected prior to harvesting on day 49, thus crickets were much larger. In the study of McFarlane (1965) the growth rate was also higher in groups than in the individually housed crickets.

Dobermann et al. (2019) conducted a four-week feeding trial with black crickets (*Gryllus bimaculatus*) to examine the effect of **survival** while feeding on bio-waste. The trial showed poor survival rate of the black cricket feeding on beer waste and cow manure, while feeding on the control feed, unprocessed vegetables and chicken feed showed medium levels of survival. On a large scale study, *A. domesticus* fed on municipal-scale food waste and on feeds made mainly from straw showed a mortality higher than 99% without reaching a harvestable size (Lundy and Parrella, 2015). Thus, a high quality feed is needed for crickets to reach a harvestable size with minimal mortality rate (Lundy and Parrella, 2015). High mortality was also noted in the study of Oonincx et al. (2015), with survival rates of 6-55% depending on the quality of the feed. The high protein (22.9%), low fat (1%) diet showed the lowest survival rate while being the best in the control group (17.2 CP and 4% fat). It is important to note that food by-product containing low quality protein sources might result in the poor survival rate in the high protein, low fat group (Oonincx et al., 2015). On the other hand Sorjonen et al. (2019) described 80% overall, 94% and 91% survival rate on medium- (22.5%) and high-protein (30.5%) barley mash respectively in house crickets. In the same study, the overall survival rate of *G. bimaculatus* was 44%, showing better results compared to the ones fed with high-protein (30%) turnip rape and chicken feed (15.2% protein). Sorjonen et al. (2019) concluded that, the best feeds for *A. domesticus* are the high- and medium-protein barley mas and for *G. bimaculatus* are the high-protein turnip rape. Despite the good quality of protein sources in our study (soy and casein) the survival rates of crickets were poor. This shows that beside diet, other factors also have a huge impact on the survival rate. As the inclusion level of urea did not significantly influence the survival rate, some other reasons led to this phenomenon. One reason of the high mortality can be the cannibalism, as the survival rates were much better in the individually housed crickets. Generally, these low survival rates can be a consequence of a densovirus (AdDNV), which is abundant in Europe and North-American house cricket facilities, interfering with the absorption of the nutrients, decreasing the growth rates and increasing the mortality (Liu et al., 2011; Oonincx et al., 2015; Pham et al., 2013; Szelei et al., 2011).

In a study investigating different plant-based by-product diets replacing soybean for *A. domesticus* and *G. bimaculatus* by Sorjonen et al. (2019), revealed that crickets fed on organic chicken feed showed the fastest **development** (34 and 24 days respectively) and crickets fed on low-protein barley showed the slowest development (45 and 28 days respectively). In the study of Oonincx et al. (2015) the development period was 48-167 days depending on the diet. The low protein (12.9%), high fat (14.6%) diet showed the slowest

development time while being the fastest in the control group (17.2 CP and 4% fat). In the current study it was difficult to evaluate the development time as only a few crickets reached maturity in Group 6 (100% defatted soybean meal). Therefore, the period of 77-84 days was much longer than the results of Sorjonen et al. (2019), but closer to the results of Oonincx et al. (2015).

6.2. Mealworms and superworms

The yellow mealworm can grow optimally in respect of development and population growth when it is reared using self-selection between wheat bran and dry potato flakes in different mixed diets (Morales-Ramos et al., 2011). Feed self-selection studies could help better understand the nutrition of *T. molitor* and figure out what are the optimal mixtures for mass rearing. This is also evidenced that *T. molitor* larvae have chemoreceptors in their antennae that may be used to recognise food ingredients (Morales-Ramos et al., 2011). The most common diet composition being used in the mealworm industry is bran, a water source like fresh vegetables (carrot, potato or apple) and/or a protein source like casein, beer yeast or soy protein (Ribeiro et al., 2018).

Nutritional requirements of mealworms are different than that of crickets as they require more carbohydrates. The optimal range of carbohydrates in the diet of *T. molitor* is 80-85% (Ribeiro et al., 2018). In diets with starch, sucrose, or lactose showed less growth performances than diets with glucose mixed with amino acids. Contrarily, the use of bacteriological dextrin as a carbohydrate source in diets enhanced weight gain almost twice as much as the diet with glucose (Davis, 1974). According to Rho and Lee (2016), the optimal protein to carbohydrate ratio for lifespan and reproductive success is 1:1, while Martin and Hare (1942), detected maximum growth in a diet with a minimum of 50% carbohydrate and a minimum of 15% protein. Mealworms tend to have higher body lipid content when fed low protein to carbohydrate ratios (Rho and Lee, 2016). Morales-Ramos et al. (2020b) examined the self-selection of food ingredients by *T. molitor*. The results on the best performing treatment, showed that the macronutrient intake ratios were 0.06±0.03 for lipid, 0.23±0.01 for protein, and 0.71±0.03 for carbohydrate. The food assimilation, food conversion, and biomass gain were impacted negatively by the intake of neutral detergent fibre, and positively by the intake of carbohydrate.

Similar to crickets, the **level, source and quality of protein** influence the growth rate of yellow mealworm and superworm larvae. Protein and amino acids in the diets positively affects positively the larval development time, survival and weight gain of *T.*

molitor (Ribeiro et al., 2018). The nutritional requirements of *T. molitor*, mainly protein and amino acids have been investigated in detail based on the mealworm larval tissues (Davis, 1975, 1974; John et al., 1979). The 10 essential amino acids of the larvae of *T. molitor* are the same as those required by rats, other vertebrates, and some protozoa. The non-essential amino acids that are not necessary for the growth of *T. molitor* include serine, tyrosine, glutamic acid and probably glycine. Alanine, cystine, proline and aspartic acid showed to be semi-essential (Davis, 1975). Threonine and tryptophan are to be considered as the limiting amino acids of *T. molitor* which are required to be in excess in the diet (John et al., 1979; Ribeiro et al., 2018). Adding protein to the feed of yellow mealworms, lowers significantly the development time, while adding fats has no significant effect. It is also shown that protein supplemented diet increases the pupal weight, fertility and number of laid eggs compared to a protein-free diet. The yellow mealworm is able to select the nutrients in the diet and showed that the conversion efficiency was the highest in mealworms fed on 80% dry potato, 10% dry egg (23.8%), followed by diet with 85% dry potato and 10% dry egg (23.7%) and by diet with 100% dry potato (20.6%). The diet consisting of only dry potato showed a significant improvement in food utilisation, development time, survival rate and fecundity compared to the diet consisting only of wheat bran (Morales-Ramos et al., 2013).

The supplementation of protein in their diet showed enhancement of growth rate. Supplementation of casein benefits the growth rate from 4.08 g to 6.16 g of larva by casein of 3% and 20%, respectively (Davis, 1970). Casein at concentrations of 2-32% and lactalbumin at lower concentrations also provide optimal effects (Davis and Leclercq, 1969). This shows that the amino acid composition of milk derived product is adequate for yellow mealworm. The current study also supports the adequacy of casein as the final body weight was the highest in Group 1 (100% casein).

Increased dietary protein does not lead to enhanced body protein content. In the study of van Broekhoven et al. (2015) the larval protein content was stable on diets that differed 2–3-fold in protein content. The best protein source was the yeast at concentrations of 5-10% (Martin and Hare, 1942). Diets with 10% yeast supplementation showed a weight gain per larva of 45.5-55.6 mg compared to a protein-free diet with weight gain of 2.3-2.9 mg (John et al., 1979). Even though soybean is high in protein, it contains a trypsin inhibitor that depresses the larval growth (Birk et al., 1962).

Mean individual **body weight** of yellow mealworms were lower than that of the mealworms and superworms kept on chicken feed in the study of Harsányi et al. (2020). Live larvae weight gain of yellow mealworms in this study was lower than weight gain in

the study of Morales-Ramos and Rojas (2015) where larvae were kept on wheat bran. In the study of Oonincx et al. (2015), the determining factor for **development** was the dietary protein content. Development was faster and survival of yellow mealworm was better on high protein diets (22-23%) than on low protein diets. In the study of van Broekhoven et al. (2015) the development time varried between 79-168 days. In the current study only one individual reached pupation in Group 1 on week 12 which is close to the 79 day of the van Broekhoven study (2015). On the other hand the rest of the mealwors did not reach pupation wich requires further investigation.

Yellow mealworm **survival rate** on high protein, low fat diet was 67% in the study of Oonincx et al. (2015). Supporting the same concept, van Broekhoven et al. (2015), resulted with the best survival rate (>80%) of yellow mealworm fed on high protein (24.1%) and high starch (28.4%), while the worst survival rate (0%) was observed when fed on low protein (20%) and low starch (19.4%). As reported by Dreassi et al. (2017), studying 6 different diets, the development of yellow mealworm fed 100% of either bread or oat flour experienced a mortality rate of >70% during the first 2 months. The best feed to reach 50% pupation rate (96.2±3.834 days) was the mixture of beer yeast (5%), wheat flour (47.5%), and oat flour (47.5%). Cannibalism occurs often in mealworm species which have a negative impact on the survival rate (Ichikawa and Kurauchi, 2009). In case of superworms the isolation prolongs larval development time in comparison to group-housed larvae (Quennedey et al., 1995). It is difficult to compare the results of this study with that of others as survival rates were recorded when the pupation rate reached a certain percentage. In the current study the survival rates were over 50%, but the pupation rate was almost zero.

The yellow mealworm FCRs of the current study were similar to that of Oonincx et al. (2015). It is clearly seen that the FCR of Group 1 (100% casein) and Group 6 (100% defatted soybean meal) was the best as these feeds were the highest in true protein. FCRs tended to be worse with the urea inclusion level which resulted in the worst FCR in Group 5 (100% urea). The superworm is not as well studied as the yellow mealworm, thus the evaluation of FCR results is difficult. Both weight gain and FCR were poor. These findings are in agreement with other studies where feed intake and consequently FCR was disadvantageous when larvae were fed with low quality feeds (Oonincx et al., 2015).

7. Conclusions

Both cricket species (house crickets and Jamaican field crickets) and the larvae of yellow mealworm and superworm species are able to utilise urea. The use of urea as the only

nitrogen source resulted in low final body weight. On nitrogen basis, urea can replace 25% of micellar casein without having negative effect on the growth performance and survival rate compared to 100% micellar casein group. However, in superworms the urea inclusion in the feed significantly decreased the survival rate. Further research is recommended to find out the reasons concerning the high mortality rate of crickets. Regarding the lack of pupation in the mealworms and superworms, more investigations should be done. Lastly, further study is needed to examine the effect of milk derived products on the performance and body composition of crickets, yellow mealworm and superworm larvae.

Abstract

The aim of this was study to examine the effects of different nitrogen sources (i.e.: micellar casein, urea, and defatted soybean meal) on growth performance of four selected insect species such as Jamaican field cricket ([JFC] *Gryllus assimilis*), house cricket ([HC] *Acheta domesticus*), yellow mealworm ([YM] *Tenebrio molitor*) and superworm ([SW] *Zophobas morio*).

Six isonitrogenous feeds composed of 3.52% nitrogen (22% crude protein) with 4 replicates/group were designed: Group 1 = 100% micellar casein, Group 2 = 75% micellar casein, 25% urea; Group 3 = 50-50% micellar casein and urea, Group 4 = 25% micellar casein, 75% urea, Group 5 = 100% urea, Group 6 = 100% defatted soybean meal). Beside the nitrogen sources corn starch was added as a carbohydrate source and feeds were provided *ad libitum*. In case of cricket species nymphs were either housed individually (n=4/group) or in groups (n=15/group). Larvae of YM (n=20/group) and SW (n=15/group) were group-housed. The survival rate, body weight and feed consumption were measured weekly. Data were evaluated on week 4, 5, 14 in crickets, JM and SW, respectively.

The final mean individual body weight of crickets was the lowest in Group 5 and highest in Group 6. In general group-housed crickets had better weight gain than individually housed crickets. Survival rate of individually housed crickets was 100% on week 4. Survival rates of group-housed crickets were low (<30%) in both cricket species without significant difference between the groups.

In the larvae, the highest mean individual body weight was recorded in Group 1 (YM) and Group 6 (SW), while it was the lowest in Group 5 (YM, SW). The survival rate of YM was the highest in Group 3 (77.5%) and the lowest in Group 6 (18.8%). The survival rate of SW decreased with the increased urea inclusion level. It was the highest in Group 1 (75%) and the lowest in Group 6 (33.3%). The feed conversion ratio of YM and SW increased stepwise with the urea inclusion level. The feed conversion ratio was the best in Group 6 followed by Group 1, and it was the worst in Group 5.

The selected insect species are able utilise urea. However, the urea as an only nitrogen source resulted in low final body weight. In HC, JFC and YM urea can replace 25% of micellar casein without having negative effect on the growth performance and survival rate in comparison to 100% micellar casein group. In SW 25% urea inclusion level did not have effect on the final body weight but significantly decreased the survival rate in comparison to 100% micellar casein group.

Összefoglaló: Különböző nitrogénforrások hatása a rovarok növekedési ütemére

A vizsgálat célja az volt, hogy különböző nitrogénforrások (micelláris kazein, karbamid, zsírtalanított szója) hatásait vizsgáljuk 4 rovarfaj, a banán tücsök (*Gryllus assimilis*), a házi tücsök (*Acheta domesticus*), a lisztbogár lárva (*Tenebrio molitor*) és a gyászbogár lárva (*Zophobas morio*) esetében.

Négy ismétlés/csoporttal hat *ad libitum* biztosított 3,52% nitrogéntartalmú (22% nyersfehérje) tápot alkalmaztunk: 1. Csoport = 100% micelláris kazein, 2. Csoport = 75% micelláris kazein, 25% karbamid, 3. Csoport = 50-50% micelláris kazein és karbamid, 4. Csoport = 25% micelláris kazein, 75% karbamid, 5. Csoport = 100% karbamid, 6. Csoport = 100% zsírtalanított szója. Szénhidrátforrásként kukoricakeményítőt használtunk. A növendék tücsköket egyesével (n=4/csoport), valamint csoportosan (n=15/csoport) helyeztük el. A lisztbogár (n=20/ csoport) és gyászbogár (n=15/ csoport) lárvák csak csoportokban voltak. A rovarok túlélési arányát, testsúlyát és a takarmányfelvételt hetente mértük. Az adatokat a 4. (tücskök), 5. (gyászbogár) és 14. (lisztbogár lárva) héten elemeztük.

A tücsköknél az átlagos egyedi zárósúly az 6. Csoportban volt a legnagyobb és az 5. Csoport a legkisebb. A csoportosan tartásban a súlygyarapodása jobb volt, mint az egyedinél. Az egyedileg elhelyezett tücskök túlélési aránya 100% volt a 4. héten. A csoportosan tartottak túlélési aránya nagyon kicsi (<30%) volt mindkét faj esetében és nem volt szignifikáns különbség a csoportok között.

A lárvák esetében az átlagos egyedi testsúly az 1. Csoportban (lisztbogár lárva) és a 6. Csoportban (gyászbogár lárva) volt a legnagyobb és az 5. Csoportban a legkisebb (lisztbogár és gyászbogár lárva). A lisztbogár lárvák túlélése a 3. Csoportban (77,5%) volt a lenagyobb és a 6. Csoportban (18,8%) a legkisebb. A gyászbogár lárvák esetében a növekvő karbamid részaránnyal csökkent a túlélési arány, ami az 1. Csoportban volt a legjobb (75%) és a 6. Csoportban a legrosszabb (33.3%). A takarmányhasznosítás mindkét lárva esetében lépcsőzetesen romlott az emelkedő karbamid részaránnyal. Értéke a 6. Csoportban volt a legjobb, amit az 1. Csoport követett és az 5. Csoportban volt a legrosszabb.

Az összes rovarfaj képes volt a karbamid hasznosítására, bár a 100%-os karbamid etetés alacsony zárósúlyt eredményezett. A tücsökfajok és a lisztbogár lárva esetében a karbamid helyettesítheti a micelláris kazein 25%-át anélkül, hogy negatívan hatna a túlélésre és a testsúlyra. A gyászbogár lárva esetében a karbamid helyettesítheti a micelláris kazein 25%-át anélkül, hogy negatívan hatna a testsúlyra, de a túlélési arányt jelentősen rontja.

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Thesis title: Effects of different nitrogen sources on growth performance of insects

Consultation – 1st semester

Timing				Topic / Remarks of the supervisor	Signature of the supervisor
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1.				TDK 2021	Dr. Hetényi Nikoletta

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The thesis meets the requirements of the Study and Examination Rules of the University and the Guide to Thesis Writing.I accept the thesis and found suitable to defence,

..... Dr. Hetényi Nikoletta.....
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Signature of the student: .....

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DECLARATION

I hereby declare that the thesis entitled **Effects of different nitrogen sources on growth performance of insects** is identical in terms of content and formal requirements to the TDK research paper submitted in 2021.

Date: Budapest, …08…. day…..…11……month…2022….year.

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We hereby confirm that we are familiar with the content of the thesis entitled **Effects of different nitrogen sources on growth performance of insects** written by **Yiannis Nikolaides** which we deem suitable for submission.

Date: Budapest, 2022.10.11.

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Dr. Hetényi Nikoletta

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Dr. Bersényi András

Institute for Animal Breeding, Nutrition and Laboratory Animal Science

Department of Animal Nutrition and Clinical Dietetics