UNIVERSITY OF WARMIA AND MAZURY

IN OLSZTYN

FACULTY OF AGRICULTURE AND FORESTRY

Anna Bordiean, M.Eng.

BIOMASS RESIDUE UTILSATION IN INSECT REARING

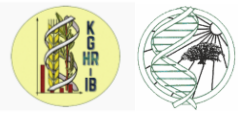
Interdisciplinary Doctoral Programme „Bioeconomy”
Financed by European Union under European Social Fund scheme (nr. POWR.03.03.00-00-M141/16)

The doctoral thesis carried out in the
Department of Genetics, Plant Breeding and Bioresource Engineering
under the supervision of
Dr. hab. Michał Zenon Krzyżaniak prof. UWM,
and
Dr. hab. Stanisław Czachorowski, prof. UWM,
Department of Ecology and Environmental Protection

Olsztyn 2022

UNIWERSYTET WARMIŃSKO-MAZURSKI W OLSZTYNIE

WYDZIAŁ ROLNICTWA I LEŚNICTWA



UNIWERSYTET WARMIŃSKO-MAZURSKI W OLSZTYNIE

WYDZIAŁ ROLNICTWA I LEŚNICTWA

mgr inż. Anna Bordiean

WYKORZYSTANIE POZOSTAŁOŚCI BIOMASOWYCH W HODOWLI OWADÓW

Interdyscyplinarny Program Doktorancki „Biogospodarka” Finansowany przez Unię Europejską w ramach programu Europejskiego Funduszu Społecznego (nr. POWR.03.03.00-00-M141/16)

Rozprawa doktorska wykonana w
Katedrze Genetyki, Hodowli Roślin i Inżynierii Biosurowców
pod kierunkiem
Dr hab. inż. Michała Zenona Krzyżaniaka, prof. UWM,
oraz
Dr hab. Stanisława Czachorowskiego, prof. UWM z Katedry Ekologii i Ochrony Środowiska

Olsztyn 2022

Contents

Summary 5

Streszczenie 7

Publications included in the doctoral thesis 9

1. INTRODUCTION 10

2. THE AIM OF THE STUDY 12

3. MATERIAL AND METHODS 13

3.1. Methodology of review study 13

3.2. Methodology of insect rearing experiments 13

3.2.1. Diet preparation 14

3.2.2. Feeding trial 15

3.2.3. Development measurements 16

3.3. Insect drying experiment 16

3.4. Statistical analysis 18

4. RESULTS AND DISCUSSION 20

4.1. Research on yellow mealworm in Europe (P1) 20

4.1.1. Mealworm as sustainable food of the future 20

4.1.2. Mealworm development on various feed materials 21

4.2. Results of the preliminary feeding trial (P2) 23

4.3. Results on long-term feeding trial (P3) 27

4.3.1. Survival and larval development 27

4.3.2. Individual fresh weight 27

4.3.3. Feed conversion efficiency 28

4.3.4. Efficiency of ingested feed 29

4.4. Influence of different drying methods on the yellow mealworm features (P4) 31

CONCLUSIONS 34

Acknowledgments 36

Funding 36

REFERENCES 37

Summary

Biomass of agricultural origin is an important source of nutrients. According to scientific sources and organizations such as FAO, about a third of this biomass is lost due to inefficient storage or technical limitations. The recovery of nutrients from it can be done with the help of its use as feed for the growth of insects. This theme is very current in Europe, because it allows an efficient bioconversion of nutrients by insects. The latter become the source of nutritional fodder for other animals, allowing to replace fish meal, currently used as a rich source of nutrients in animal breeding. The use of insects as a tool to recover valuable nutrients, but also as a feed source for animals, reduces the pressure on the environment in terms of the need in increasing the production of animal protein sources for growing global population. Recently in 2021, insects are an already accepted source of protein for human consumption. Yellow mealworm larvae (*Tenebrio molitor* L.) flour was the first that has been allowed on the European market. Yellow mealworm is a beetle from Coleoptera: Tenebrionidae. Research was conducted at the Department of Genetics, Plant Breeding and Bioresource Engineering, University of Warmia and Mazury in Olsztyn, Poland. The aim of the research was to analyse the progress and directions of research on yellow mealworm, as well to investigate the growth performance, survival, feed conversion ratio (FCR), efficiency of ingested feed (ECI), etc. on larvae grown on 13 different diets containing agro-industrial byproducts. In addition, influence of different drying methods on chemical and physical mealworm larvae nutritional features was evaluated.

Based on investigated literature from 2012-2020 (**P1**) it was concluded that yellow mealworm is widely bread insect species in Europe, being tested as feed for different livestock (poultry, fish, small birds, and pets). Western European researchers and research facilities are investigating the mealworm larvae on a small and large scale. Many studies are carried out on the influence of different feed and byproducts on the nutritional quality of the mealworm and its development.

Moreover, result on feeding experiment of mealworm larvae on various tested diets showed that the highest final fresh weight of larvae was on those reared on WB diets. Individual larval weight gain almost doubled weekly, excepting the larva fed on WS 100 diet (**P2**). The survival of mealworms on tested diets was high, between 92.2% and 97.7% for larvae grown on WB 100 and WS 25/CF 75, respectively (**P3**). However, the longest larval period (115 days) was recorded for mealworms fed on the WS 100 diet, compared to those fed on WB 25/FC 75 (73

days). The final results revealed the lowest FCR (1.53 to 1.59) were found on larvae fed on RM mixed with CF diets (**P3**). Adding CF into the diets significantly improved the ECI (**P2&P3**).

Mealworm larvae were also analysed for their physical and chemical composition after being subjected to a drying process (**P4**). Insects were blanched and dried with the use of two methods: convective drying and freeze-drying, at different drying temperatures and times. Dried insects were characterized by low moisture content in the range of 3.15% to 5.47%, and they differed considerably in water activity (0.06–0.55). Moisture content and water activity were substantially higher in larvae blanched for 60 s and freeze-dried than in larvae dried with the use of the remaining methods. It was shown that the shorter freeze-drying time resulted in a high value of water activity (a_w) (0.49–0.55), but still sufficient to inhibit the growth of microorganisms but insufficient to inhibit enzymatic reactions. In the case of extending the freeze-drying time during the main drying a_w values dropped significantly, especially in the larvae blanched for 60 s. The study of the proximate composition of larvae, however, did not show any significant changes in the crude content of protein, fiber or fat in insects after using various methods and drying parameters. However, it was noticed that the age of insects significantly influenced the features of the examined larvae, e.g. crude fat and protein content.

Keywords: *Tenebrio molitor*; edible insects; mealworms; small livestock; novel food; food and feed; nutritional value; larval development; feed conversion ratio; agricultural and industrial residues; bioconversion; proximate analysis; water activity.

Streszczenie

Biomasa pochodzenia rolniczego jest ważnym źródłem składników odżywczych. Według źródeł naukowych i organizacji, takich jak FAO, około jedna trzecia tej biomasy jest tracona z powodu nieefektywnego przechowywania lub ograniczeń technicznych. Odzyskiwanie z niej składników odżywczych można osiągnąć wykorzystując ją jako paszę dla owadów. Temat ten jest bardzo aktualny w Europie, ponieważ umożliwia wydajną biokonwersję składników odżywczych przez owady. Te ostatnie stają się źródłem paszy dla innych zwierząt, pozwalając zastąpić mączkę rybną, stosowaną obecnie jako bogate źródło składników odżywczych w hodowli zwierząt. Wykorzystanie owadów jako narzędzia do odzyskiwania cennych składników odżywczych, ale także jako źródła paszy dla zwierząt, zmniejsza presję na środowisko w zakresie konieczności zwiększenia produkcji źródeł białka zwierzęcego dla rosnącej światowej populacji. Od 2021 r., owady są akceptowanym źródłem białka do spożycia przez ludzi. Mączka z larw mącznika młynarka (*Tenebrio molitor* L.) była pierwszą dopuszczoną na rynek europejski. Mącznik młynarek to chrząszcz z rodziny Coleoptera: Tenebrionidae. Badania przeprowadzono w Katedrze Genetyki, Hodowli Roślin i Inżynierii Biosurowców Uniwersytetu Warmińsko-Mazurskiego w Olsztynie. Ich celem była analiza postępów i kierunków badań nad mącznikiem młynarkiem, a także ocena wydajności wzrostu, przeżywalności, współczynnika wykorzystania paszy (FCR), wydajności spożytej paszy (ECI) itp. na larwach hodowanych na 13 różnych mieszankach paszowych zawierających produkty uboczne z przemysłu rolno-spożywczego. Ponadto oceniono wpływ różnych metod suszenia na chemiczne i fizyczne cechy larw mącznika.

Na podstawie przeanalizowanej literatury z lat 2012-2020 (**P1**) stwierdzono, że mącznik młynarek jest szeroko rozpowszechnionym gatunkiem owada w Europie, testowanym jako pasza dla różnych zwierząt gospodarskich (drób, ryby, małe ptaki i zwierzęta domowe). Zachodnioeuropejscy naukowcy i ośrodki badawcze badają larwy mącznika na małą i dużą skalę. Prowadzonych jest wiele badań dotyczących wpływu różnych pasz i produktów ubocznych na jakość odżywczą mącznika i jego rozwój.

Wyniki doświadczenia z wykorzystaniem rożnych produktów ubocznych w żywieniu larw mącznika wykazały, że najwyższą końcową świeżą masę larw uzyskano u tych hodowanych na dietach WB. Przyrost masy poszczególnych larw prawie podwoił się tygodniowo, z wyjątkiem larw karmionych dietą WS 100 (**P2**). Przeżywalność mącznika młynarka na badanych dietach była wysoka, od 92,2% do 97,7% dla larw hodowanych odpowiednio na WB

100 i WS 25/CF 75 (**P3**). Jednak najdłuższe stadium larwalne (115 dni) odnotowano u mączników karmionych dietą WS 100 w porównaniu z tymi karmionymi dietą WB 25/FC 75 (73 dni). Ostateczne wyniki wykazały, że najniższy FCR (1,53 do 1,59) stwierdzono u larw karmionych dietą RM zmieszaną z dietą CF (**P3**). Dodanie paszy dla kurcząt do diety znacząco poprawiło wskaźnik ECI (**P2&P3**).

Larwy mącznika poddano również analizie ich składu fizycznego i chemicznego po uprzednim poddaniu ich procesowi suszenia (**P4**). Owady blanszowano i suszono dwiema metodami: suszeniem konwekcyjnym oraz liofilizacją, przy różnych temperaturach i czasach suszenia. Wysuszone owady charakteryzowały się niską zawartością wilgoci w zakresie od 3,15% do 5,47% i znacznie różniły się aktywnością wody (0,06–0,55). Wilgotność i aktywność wody były istotnie wyższe u larw blanszowanych przez 60 s i liofilizowanych niż u larw suszonych pozostałymi metodami. Wykazano, że krótszy czas liofilizacji skutkował wysoką wartością aktywności wody (a_w 0,49–0,55), wciąż wystarczającą do zahamowania wzrostu mikroorganizmów, lecz niewystarczającą do zahamowania reakcji enzymatycznych. W przypadku wydłużenia czasu liofilizacji podczas suszenia głównego wartości a_w znacznie spadły, zwłaszcza u larw blanszowanych przez 60 s. Badania składu podstawowego larw nie wykazały jednak istotnych zmian w zawartości białka, błonnika czy tłuszczu surowego u owadów po zastosowaniu różnych metod i parametrów suszenia. Zauważono jednak, że wiek owadów istotnie wpływał na cechy badanych larw, m.in. zawartość tłuszczu i białka surowego.

Słowa kluczowe: *Tenebrio molitor*; owady jadalne; larwy mącznika; małe zwierzęta gospodarskie; nowa żywność; żywności i pasza; wartość odżywcza; rozwój larw; współczynnik wykorzystania paszy; pozostałości rolnicze i przemysłowe; biokonwersja; analiza podstawowa; aktywność wody.

Publications included in the doctoral thesis

1. **Bordiean A.**, Krzyżaniak M., Stolarski M.J., Czachorowski S., Peni D. (2020). Will yellow mealworm become a source of safe proteins for Europe? Agriculture 10, 233; https://doi.org/10.3390/agriculture10060233 (IF=3.408) (**P1**)
2. **Bordiean A.**, Krzyżaniak M., Stolarski M.J., Peni, D. (2020). Growth potential of yellow mealworm reared on industrial residues. Agriculture 10, 599; https://doi.org/10.3390/agriculture10120599 (IF= 3.408) (**P2**)
3. **Bordiean A.**, Krzyżaniak M., Stolarski M.J. (2022). Bioconversion potential of agro-industrial byproducts by *Tenebrio molitor* - long-term results. Insects 13, 810. https://doi.org/10.3390/insects13090810 (IF=3.139) (**P3**)
4. Krzyżaniak M., Aljewicz M., **Bordiean A.**, Stolarski M.J. (2022). Yellow mealworm composition after convective and freeze drying - preliminary results. Agriculture 12, 149. https://doi.org/10.3390/agriculture12020149 (IF=3.408) (**P4**).

1. INTRODUCTION

Biomass is considered as a mass of living organisms [1]. The term of "biomass" is often used to define especially the feedstock/biomass for energy purposes such as heating, electricity generation and fuels. Biomass represents the organic material such as forestry (logging residues, wood processing byproducts, black liquor from the pulp and paper industry, etc.), agricultural (crop residues, animal waste, energy crops, etc.) and urban waste (municipal solid waste, sewage sludge, etc.) [2].

The biomass supply in EU27 in 2017, according to the FAO [3], was approximately 1 billion tons of dry mass. About 90% of it was produced in the EU27, 5% being imported, and the rest of the biomass origin was not identified. The highest rate of 69% of the total domestic biomass comes from agricultural sector, followed by forestry (31% of the dry matter content). The biomass supply from agriculture (713 million tonnes of dry vegetal biomass), 68% represents the crop production (~485 million tonnes), 13% grazing (~95 million tonnes) and 12% crop residues (~88 million tonnes) that are closer in weight but representing much smaller portions. About one-third of harvested residues (29 million tonnes) is used for feed, and the remaining two-thirds are used for other purposes (biomaterials or energy), lost or discarded. Approximately half of the produced crops in the EU27 were cereals (48%), fodder crops (31%) and root crops (8%). Due to the growth of the world's population [4] and other factors, according to the latest Crop Prospects and Food Situation Report emitted by FAO [5] the demand/utilization for cereals increased in 2021/2022 by 1.2%. With these increases, it is essential to mention that about a third of all food produced globally becomes waste during different stages (from production to processing and consumption) [6], including crops. Therefore, the increase in cereal production and production/demand for agricultural crops, inevitably leads to an increase in agricultural waste and residue volume. The food crisis appears in the context where the demand for some cereals and prices are high in some regions due to the COVID-19 pandemic effects. Another aspect represents the forecast of the supply disruption caused by the Russian aggression war against Ukraine. The cereal and food supply, and food insecurity become more difficult to avoid [5]. Moreover, all this is added to the about 900 million people who suffer from hunger and food insecurity every day [7].

The problem of waste of agricultural origin contradicts food security. These residues, as well food and agricultural waste, are not fully recovered in terms of nutritional elements. It is essential to mention that for the production of crops to produce feed and food, many natural

resources are used: soil, water, fuels, labour/workforce, logistical resources, etc. that become harder to access, especially in a climate-changing world. There is a growing demand for poultry and livestock farming for other types of animal-origin proteins. Almost 80% of the global agricultural land is used for growing feed for livestock. However, livestock provides less than 20% of the calories used in human diets worldwide [8]. Nowadays, there is a series of new protein sources available to combat the insufficiency and shortage of food, reducing the negative impact on the environment. Insects are known for their efficiency to convert low quality biomass into rich protein and nutrient sources [9]. They are rich in nutrients such as proteins, fats, and microelements, that cover requirements as livestock feed. In the growing population world, the entomophagy could be also a solution [10]. It is estimated that globally entomophagy is practiced by over 2 billion people [11], including over 2000 insect species [12]. Following the publication from 2015 listing 15 insects by EFSA [13], in Europe the subject of implementing insects use on a large scale, both as feed and as food, intensified. The use of whole insects as animal feed is currently not allowed, with a few exceptions (for pets or amphibians). Alternatively, the use of components (fat) from insects is accepted in aquaculture of few species (the house cricket, banded cricket, field cricket, yellow mealworm, lesser mealworm, black soldier fly, and the common house fly), although with some limitations, taking into account the regulations [14]. The use of powdered and dried yellow mealworm as human food was recently approved in Europe [15]. Until the end of 2021, the list of edible insects approved has been extended with frozen, ground, and dried of house cricket (*Acheta domesticus*) and migratory locusts (*Locusta migratoria*).

One of the most extensively investigated insects in Europe is the *Tenebrio molitor* beetle, known as the yellow mealworm, and its larvae. The species is often associated with stored grain [16]. On the other hand, the mealworm's gut is capable of digesting a manifold of substrates or by-products, such as waste from bakeries (bread remains, cookie remains), brewery yeasts, potato peelings, spent grains, beet molasses [17, 18], spirit distillers' grains, highly denatured soybean meal, mushroom spent corn stover [19, 20], agricultural by-products from the seed cleaning process of cereals (triticale, barley, durum wheat and oat) and legumes (vetch, pea, lupin, lentil, lucerne and broad bean) [21], as well as industrial by-products (chicken feed, wheat and rye brans, rapeseed meal and cake, flax cake, and *Silybum marianum* cake) [22]. Therefore, mealworm larvae contribute to the efficient bioconversion of valuable nutrients from by-products and waste, thus becoming a nutritious feed for other livestock (i.e., chicken, fish) [23, 24].

2. THE AIM OF THE STUDY

The aim of the PhD dissertation was:

- Review of the existing literature on the current state of yellow mealworm rearing for food and feed, especially in Europe. (Publication 1).
- Evaluation of progress and direction of research on yellow mealworm larvae (*Tenebrio molitor*) in Europe (Publication 1).
- Evaluation of growth performance, feed conversion ratio, efficiency of ingested feed by mealworm larvae reared on different types of agricultural and industrial residues in one trial (Publication 2).
- Investigation of growth performance, survival, larval development period, feed conversion ratio, and efficiency of ingested feed of yellow mealworm grown on different agro-industrial byproducts – long term results (Publication 3).
- Determination of effects of different drying methods on physical and chemical characteristics of yellow mealworm larvae (Publication 4).

3. MATERIAL AND METHODS

3.1. Methodology of review study

The reviewed articles were original research studies, reviews and observational studies published in English. The review focused mostly on European articles. Scopus and Science Direct data bases were searched to select articles published between 2012 and 2018 based on the following key words: yellow mealworm, Tenebrio, *Tenebrio molitor*, mealworm and mealworms. These keywords were used to identify original papers and review articles for the current review. The methodology was described in Publication 1 (**P1**).

The reviewed papers were divided into several categories based on different types of mealworm utilization as well as fields of research. The exclusion criteria for the selected articles included duplication of data, low relevance and focus on other mealworm species such as lesser/buffalo mealworm (*Alphitobius diaperinus*) or giant mealworm (*Zophobas morio*). The articles were collected and sorted according to the country of origin, the number of articles per country, the year of publication, and the most important keywords.

3.2. Methodology of insect rearing experiments

The mealworm larvae were obtained from a commercial supplier (CRICKETSFARM) specializing in growing insects in Poland (**P2&P3**). The insects were maintained under laboratory conditions (relative humidity: 55-60%, air temperature: 28°C, photoperiod: 12 hours). The purchased mealworm larvae were reared to obtain a colony of matured individuals (**Figure 1**). The mealworms were fed *ad libitum* with chicken feed milled at 3 mm and supplied with fresh carrots three times a week. The insects were kept in plastic containers (35×23×13 cm) with 3 mm aeration holes in the sides, closed with a lid. Hygrometers were installed to enable the verification of the parameters in the boxes. Three weeks later, the first pupas appeared. 6-7 days later after first pupation a new adult generation were obtained. The adults were placed in the "adult box" that was built with the mosquito mesh on the bottom that allowed the eggs to fall into a collection box. The pupas/beetles were collected until it was sufficient to obtain a high number of eggs in a short collecting period (3-4 days). Hatching was estimated to last 7-10 days after egg collection. Newly hatched larvae were fed *ad libitum* on the chicken feed, supplied with fresh carrot slices for four weeks. This procedure allowed the larvae to grow to a size enabling their easy and safe collection for the main experiment (feed conversion).



Figure 1. Adult mealworm beetle feeding.

3.2.1. Diet preparation

Before the beginning of the experiment, some of the cheapest types of agricultural/industrial residues were selected (**P2&P3**). Diets were obtained from commercial companies from Poland. The only exception was for willowleaf sunflower (*Helianthus salicifolius*) biomass, which were obtained from another experiment conducted in the Department of Genetics, Plant Breeding and Bioresource Engineering of the University Warmia and Mazury in Olsztyn, Poland. The experimental diet mixes were composed of (1) chicken feed used as a control feed (CF), (2) rapeseed meal (RM), (3) wheat bran (WB) and (4) pelleted willowleaf sunflower (WS) biomass originating from residues after supercritical CO_2 extraction of active compounds.

All feeds were comminuted to obtain 3 mm particles. The obtained meal was sieved through a 300 μm sieve to remove the smallest particles with sizes similar to the larva faeces. Subsequently, the nutritional value of all the feeds was determined, including dry matter, ash, nitrogen/protein content and crude fat (**Table 1**). Proximate analysis of experimental diets included ash and fiber content, crude fat, protein content and nitrogen-free extract (NFE). Ash content was measured by using an automatic ELTRA TGA-THERMOSTEP analyser (Neuss, Germany) according to the PN-EN ISO 16948:2015-07 and PN-EN ISO 16994:2016-10, and fiber was analysed according to the PN-EN ISO 13906:2009, by using ANKOM A200 (Macedon, NY, USA) respectively. Protein content was measured using the Kjeldahl method, and crude fat was determined using Soxhlet extraction with petroleum ether as a solvent.

The NFE estimates nonfibrous carbohydrates, such as soluble mono- and oligosaccharides and starches. The NFE was calculated according to Equation (1):

NFE (% dry mass) = 100% - {P (% d.m.) + CF (% d.m.) + CFb (% d.m.) +Ash (% d.m.)}, **(1)**

where P is crude protein content, CF crude fat, and CFb is the crude fiber content in the diets. Experimental feeds were mixed in different weight proportions with chicken feed: only residual feed (0%), 25% CF, 50% CF, and 75% CF. In this way, 13 types of feed mixes were obtained. The diets were stored at −20°C until the start of the experiment.

Table 1. Main component of chicken feed (control) and industrial residues used for different diets

Substrate	***Ash (% d.m.)***	***Fibre content (% d.m.)***	***Crude fat (% d.m.)***	***Protein (% d.m.)***	***NFE (% d.m.)***
Chicken feed (CF)	12.89	3.23	2.38	20.11	61.39
Rapeseed meal (RM)	7.39	11.12	1.78	35.52	44.19
Wheat bran (WB)	5.82	8.68	2.9	18.63	63.97
Willowleaf sunflower (WS)	9.49	22.64	0.76	10.56	56.55

3.2.2. Feeding trial

Fifty 3rd–4th instar was placed in a plastic container ("diet box") (22×13×5 cm) with aeration holes on the sides, covered by a lid (**P2&P3**). For reliable data collection, the full experiment included three separate trials, each conducted in 3 replicates (n=9). Therefore, 150 larvae were simultaneously tested on each diet per trial (50 per box) and 450 in total for three trials (**P3**). Each larvae box was provided with 3.1 g of experimental diet at the beginning of each experimental week and 2 g of carrots (twice a week). Subsequently, the feed and carrot amount were adjusted following the larvae's growth. Weekly larval development parameters were monitored by counting the larval survival rate and mealworm weight. The feed and carrot amounts were measured, and the non-consumed feed and carrot remains were dried to calculate the amount of consumed feed and carrots. The first trial (**P2**) was terminated when the first pupation occurred, regardless of a diet. The pupation time per diet was recorded in the second and third trials (**P3**). The pupation time in each box was recorded, and the larvae development time (larval stage) was considered to have been completed when at least one pupa was detected per diet.

3.2.3. Development measurements

The larvae weight gain (LWT), individual larvae weight (LW), and feed consumption were calculated. The larvae were screened for frass every week. Subsequently, the weight of 20 randomly chosen larvae from each box was registered. Therefore, following this measurement, the individual weight (LW) of larvae and larvae weight gain (LWT) (mg fresh matter (f.m.)) were calculated. All larvae were returned to the same box for further measurements (**P2&P3**). The final fresh and dry weights of larvae grown on WS 100 in this study are based on the second and third trials, as the harvesting on the first trial was terminated after the first pupation in the whole trial and larvae fed on WS 100 were still far from the pre-pupa stage (**P3**).

The feed consumption in the dry and wet forms was assessed by subtracting the remaining feed mass from the feed provided at the start of each experimental week. The same was done for consumed carrots.

Based on these parameters, the feed conversion ratio (FCR) and efficiency of conversion of ingested feed (ECI) were calculated. The FCR was calculated by dividing the total mean of individual larvae consumption by the total mean of individual weight gain (as described by Miech et al. [25] and calculated on a wet basis:

$$\text{FCR} = \text{weight of ingested food} / \text{weight gained} \quad (2)$$

The ECI values were recorded at the end of the experiment. The ECI was calculated based on Waldbauer [26] by dividing the final weight of larvae by the weight of ingested feed, and the results was expressed on the dry matter basis of feed and insects:

$$\text{ECI} = (\text{final weight} / \text{weight of ingested feed}) \times 100\ (\%) \quad (3)$$

3.3. Insect drying experiment

For the testing of drying methods experiment (**P4**) larvae purchased from CRICKETSFARM company were used. The purchased larvae were grown on chicken feed. At prepupal stage, the larvae were tested for different drying methods. In the first test, insects were fasted for 24 h, frass was filtered with a sieve, insects were frozen at a temperature of around −30 °C and stored until further analysis. The prepared insects were dried in a convection oven with a forced air circulation system, at a temperature of 70°C, 60°C and 50°C for 12 h. However, the results were not satisfactory due to the considerable browning of thermally processed larvae (**Figure 2**). Light-colored larvae (whole insects or meal) are more appealing to consumers. Therefore,

the experiment was modified as follows: larvae were immobilized by short-term chilling, and they were blanched (B), dried in a convection oven (T), or freeze-dried (FD).



Figure 2. Larvae from the first drying test at 12 h drying time. Drying temperature from the left to right: 70, 60, and 50 °C. Considerably browning of larvae can be observed.

Insects were fasted for 24 h; frass was filtered out on a 1 mm mesh sieve, and 10 larvae were weighed in triplicate to determine the fresh weight of individual larvae (**Table 2**). Insect specimens of 100 mL each were immobilized at 4°C for 2 h before drying. Immobilized insects were blanched in boiling water (10 parts water to one-part insects) for 60 and 180 s, strained with a sieve, and dried on paper towels. Insect specimens for convective drying were placed on steel trays lined with aluminum foil (to prevent insects from sticking to the metal surface). Insects were dried in two convection ovens (Binder FD53E3, Binder GmbH, Tuttlingen, Germany and Memmert UF750, Memmert GmbH, Schwabach, Germany) with a forced-air circulation system. This procedure allowed us to carry out the experiment at the same time for two different drying times. Parameters for convection drying were temperature: 60 and 80°C and time: 12 and 16 h. Larvae for the freeze-drying experiment were frozen at a temperature of −20°C for 4 h and lyophilized (Alpha 1-2 LDplus, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) at −30°C for 16 h or 24 h (main drying), followed by −40°C for 2 h (final drying). Insects were analyzed in four separate batches. The applied drying methods are presented in **Figure 3** and **Table 2**.

Table 2. Methods applied to dry mealworm larvae and fresh weight of single larvae per batch.

Code	*Batch No.*	*Drying Method*	*Blanching Time (s)*	*Temperature (°C)*	*Drying Time (h)*	*Fresh Weight of Single Larvae (g)*
B1T1	1	convective	60	60	12	0.101
B1T2	1				16	0.101
B1T3	3			80	12	0.133
B1T4	3				16	0.133
B2T1	2	convective	180	60	12	0.122
B2T2	2				16	0.122
B2T3	4			80	12	0.126
B2T4	4				16	0.126
B1FD1	1	freeze drying	60	−30/ (−40)	16/ (2)	0.101
B1FD2	3				24/ (2)	0.133
B2FD1	2	freeze drying	180	−30/ (−40)	16/ (2)	0.122
B2FD2	4				24/ (2)	0.126

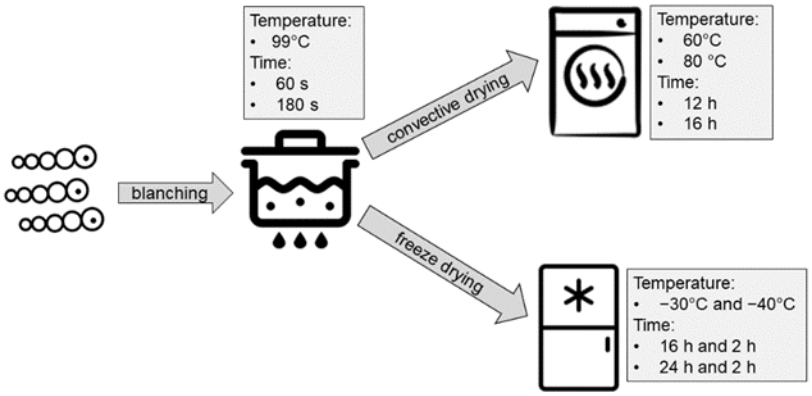


Figure 3. Diagram of methods applied to dry mealworm larvae.

Proximate analysis of larvae included moisture, water activity, protein, crude fat, crude fiber and ash. The detailed description of methods is explained in **P4**.

The purpose of this section was only to provide the reader with general information that was used in this doctoral dissertation. All detailed information on the measurements, analyzed features and experimental techniques used to perform the experiments have been described in the relevant publications on which this doctoral thesis is based.

3.4. Statistical analysis

A statistical analysis was performed with Statistica 13 software (Tibco, Palo Alto, CA, USA) (for **P2**, **P3** and **P4**). Experimental data, such as individual fresh weight, individual dry weight,

survival, FCR and ECI, were subjected to a one-way ANOVA analysis. Homogeneous groups for all features were determined using Tukey's (HSD) multiple tests at $\alpha = 0.05$. The results from the larval stage were not included in the ANOVA statistics because the data (being the results of two repetitions/trials) were considered insufficient to be processed statistically.

The applied drying methods (**P4**) were analyzed by agglomerative hierarchical clustering with the use of Ward's method, where Euclidean distance was the distance measure. Clusters were identified with the use of the grouping criteria proposed by Sneath. The results of the cluster analysis were used to develop a dendrogram of similarities between larvae dried by different methods. A heat map was also generated for all analyzed parameters of mealworm larvae. In the heat map, original data are subtracted from the sample mean, and the result is divided by the standard deviation of the sample.

4. RESULTS AND DISCUSSION

4.1.Research on yellow mealworm in Europe (P1)

A total of 291 articles by European researchers were chosen from the analysed databases. After a thorough analysis, 201 articles were included in the review (**Figure 4**). The selected papers were classified based on the year of publication. The analysis demonstrated that the number of research studies into mealworms had increased considerably since 2015, which could be attributed to the growing interest in mealworms in the scientific community. Other contributing factors include the opinion of the European Food Safety Authority (EFSA) of 8 October 2015 on the risk profile related to the production and consumption of insects as food and feed.

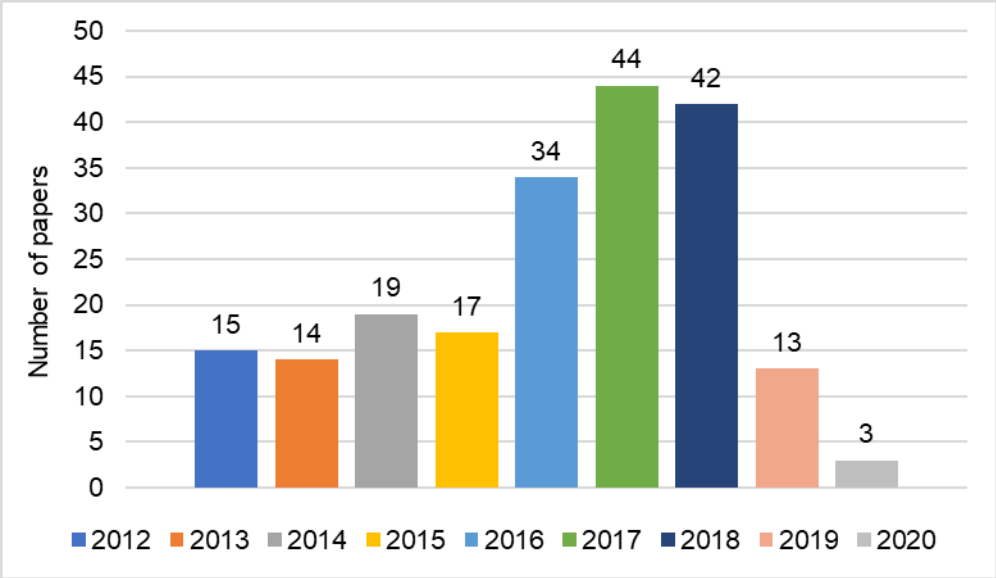


Figure 4. Number of research papers on mealworms published between 2012 and 2020 in Europe.

4.1.1. Mealworm as sustainable food of the future

The nutrient content of *Tenebrio molitor* larvae determines the extent to which mealworms can be regarded as a novel food for combating global malnutrition. Edible insects are a valuable source of protein, and their lipid, fat, mineral and vitamin content varies across growth stages, and is influenced by processing [27]. Fresh mealworm larvae are abundant in protein, and they can easily compete with raw livestock meat (**P1**). The protein content of mealworms varies subject to feed and rearing conditions. According to some studies [28] protein content was highest (51-59%) in commercial mealworm samples, and differences were observed between processing methods. In a study by Kulma et al. [29], the crude protein content was 63% d.m., and it was higher than that reported by other authors [30, 31]. Mealworm larvae have high fat

content. They can be recommended for the contemporary human diet due to low levels of SFAs, high PUFA content, and a low n-6/n-3 PUFA ratio [30, 32].

The elemental composition of mealworms differs in various stages of growth. Third-instar larvae (6.8±1.9 mm) are characterised by the highest content of Ca (736 mg kg^{-1}), Fe (250 mg kg^{-1}), K (14.6 g kg^{-1}), Mn (18.9 mg kg^{-1}), Na (2.1 mg kg^{-1}), S (9.1 g kg^{-1}), Sr (8.8 mg kg^{-1}) and Zn (171 mg kg^{-1}) [33]. The same authors found that the highest P level (65 g kg^{-1}) was reported in eight-instar larvae (7.8±1.3 mm), whereas last-instar larvae (20.4±1.2 mm) were most abundant in Mg (2.0 g kg^{-1}). The concentration of Cu (14.4 mg kg^{-1}) was higher in first instar and much higher in pupae or adults. Therefore, the decision to add mealworms to feed should be made based on the desired outcomes of supplementation. Fresh and powdered larvae also differ in mineral content. Powdered mealworms are more abundant in minerals than fresh larvae [34].

4.1.2. Mealworm development on various feed materials

Yellow mealworms can convert low-energy feeds, substrates and biomass into products with high nutrient content [16, 17]. Research has shown that mealworms are able to transform organic by-products from brewing, baking, bioethanol production and potato processing into nutritious feed for livestock and other components [18].

The optimal rearing conditions for *T. molitor* larvae that guarantee successful mass production have been described by numerous studies. Mealworms thrive at a temperature of 25–28 °C and relative humidity (RH) of ≥70% (the optimal RH range is 60–75%). Optimal growth is achieved when mealworm diets are abundant in yeasts (5–10%), carbohydrates (80–85%) and B-complex vitamins [35]. The composition of mealworm diets is crucial for obtaining high-quality larvae characterised by a high feed conversion ratio (FCR) and high body weight. Various diets have been analysed to determine their impact on the growth and nutritional value of larvae. The protein and starch content of some of the tested diets affected the growth, survival and chemical composition of mealworms. Van Broekhoven et al. [18] observed that high protein and high starch (HPHS) diets considerably accelerated larval development (79 days). High protein low starch (HPLS) diets were also found to improve larval survival rates. The feed conversion ratio ranged from 2.62 to 6.05 on a fresh weight basis. The crude protein content of mealworms was highly similar in all tested diets (between 45.1–48.6% dry matter (DM)). The crude fat content of mealworms ranged from 18.9% DM in insects fed LPLS (low

protein, low starch) diets to 27.6% DM in insects fed HPLS (high protein, low starch) diets [18].

Mealworm larvae are able to synthesise fat even if they are fed low-fat diets. Therefore, the final fat content of larvae fed low-fat diets is highly similar to that of larvae whose diets contain more fat. In a study where mealworms were fed diets with a fat content of 0.46–9.34% DM, the crude fat content of mealworm larvae was determined at 39.75–48.31% DM in the first generation and 34.42–48.17% DM in the second generation [30]. Yellow mealworms are less heat-adapted insects [36], and rearing temperature influences their fat content. The fat content of mealworms reached 47.4% at 31 °C, and it decreased to 30.2% at 37 °C and to 16% at 39 °C [36]. Adámková et al. [37] reported that fat content was maximised at a temperature of 23 °C (24.56%). An analysis of the effects of rearing temperature on the protein content of mealworms revealed that this parameter increases with a rise in temperature [36]. The complete data dedicated to the mealworm use in Europe investigation can be found in the reference publication (**P1**).

4.2. Results of the preliminary feeding trial (P2)

A nutritive control feed (CF) was chosen for the first weeks after the hatching of mealworms, which provided them with enough elements to grow until week 4. This feed was also safely used and verified before the experiment. In other experiments, however, wheat bran was used as the control feed [38, 39]. In the present study (**P2**), at the beginning of the experiment, the mean weight of mealworms was 0.88 mg f.m. (**Figure 5**). At the end of week 1 of the experiment, mealworm weight almost doubled for mealworms reared on wheat bran (2.05 mg f.m.). The lowest weight was found for larvae grown on willowleaf sunflower (WS 100) (1.27 mg f.m.). During the experimental period, mealworms doubled their mass weekly, except for those fed with WS 100. Overall, the mealworms fed diets mixes with wheat brans had the highest body weight during the rearing experiment compared with the mealworms fed other diets. Thus, at the last measurement (week 9) mealworms weight was 134.4., 125.9, 124.6 and 114.2 mg f.m. for WB 100, WB 75, WB 50, WB 25, respectively. A slightly lower fresh weight (114.7 mg per larvae) was found in another study for mealworms fed with wheat bran and cabbage leaves as a water source [40].

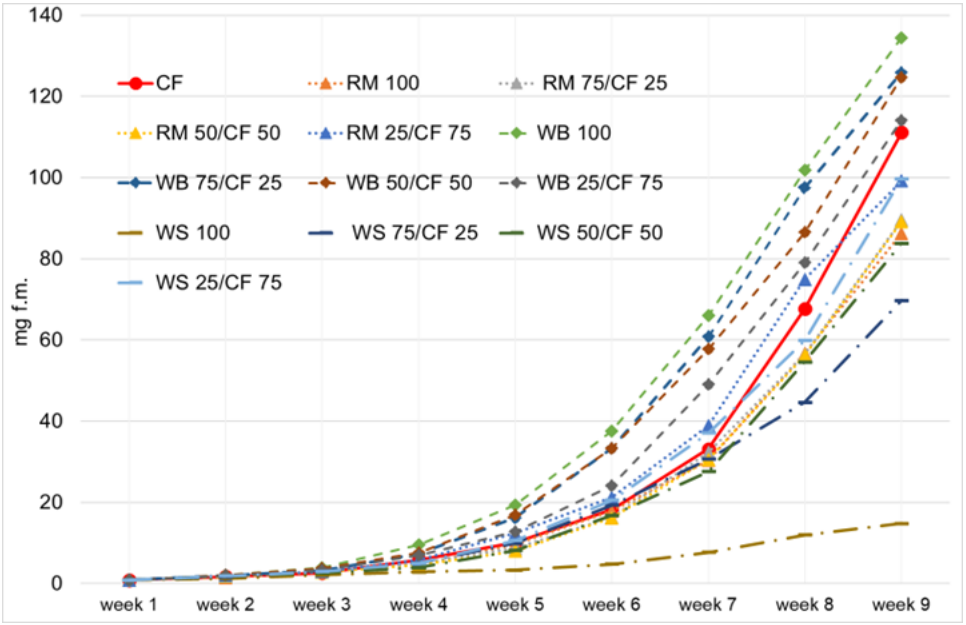


Figure 5. Individual larvae weight depending on the type of diet and week of rearing; CF (chicken feed), RM (rapeseed meal), WB (wheat bran) and WS (willowleaf sunflower).

The mean dry individual larval weight for all diets used in this experiment was 31.44 mg d.m. (**Figure 6**). The significantly highest dry weight was noted for mealworms reared on WB 100 and WB 75 diets and was 47.9 and 44.0 mg d.m., respectively. Other mixes of WB and CF produced very similar results. Mealworm reared on CF (control diet) were classified in the homogeneous group b together with diets RM 25/CF 75 and WS 25/CF 75 and the dry weights

ranged from 32.3 to 34.5 mg d.m. It was noted that a high CF share in WS diets increased the dry larval weight. Mealworms fed RM diets (except RM 25/RM 75) and the WS 50/CF 50 diet demonstrated the same dry individual larval weight (25.9–28.1 mg d.m.). In the present study, mealworms grown on WB diets displayed the highest dry weight. This is contrary to the observations made by Li et al. [40], who found that the dry weight of larvae fed wheat bran was approximately 35.5 and 16.6 for larvae grown on plant waste diet (fermented straw and old cabbage leaves). In our study, the significantly lowest dry individual larval weight was reported for diets based solely on WS, which was only 3.9 mg d.m. after week 9 of the experiment. It is possible that for other WS/CF mixes, mealworms fed more on CF and only accidentally on WS, based on self-selection of feed. The self-selection of feed was described by Morales-Ramos et al. [39], who noted that larvae selected the best components of mixed diets [41] to optimise their nutrition and growth. Willowleaf sunflower residues may be difficult to digest, possibly because of the high fibre content and low amount of non-fibrous carbohydrates (**Table 1**).

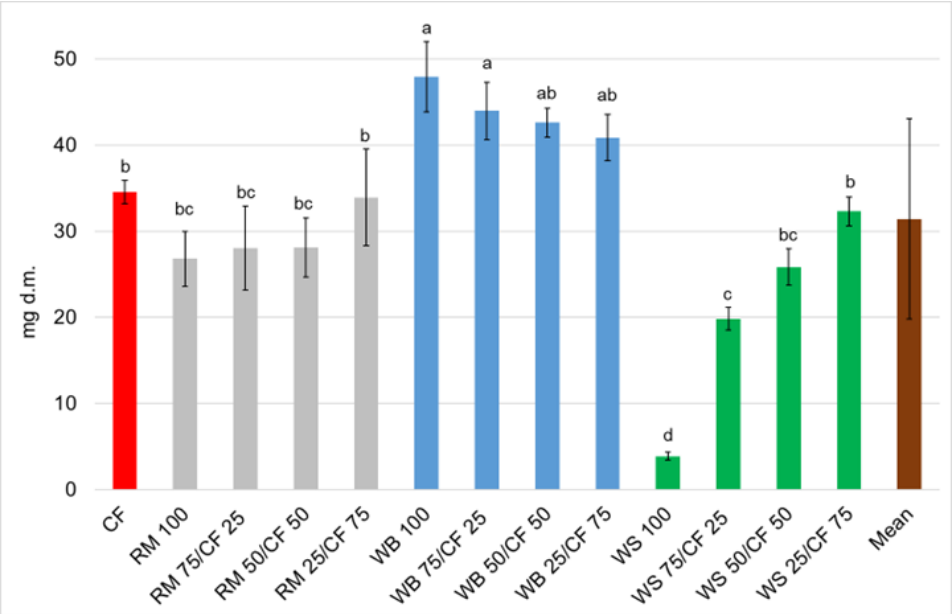


Figure 6. Final dry individual larva weight depending on the type of diet; CF (chicken feed), RM (rapeseed meal), WB (wheat bran) and WS (willowleaf sunflower); a, b, c…- letters mean that values are statistically different (Tukey's test at $p < 0.05$).

In the **P2** study, the FCR for all diets except WS 100 (**Figure 7**) was statistically the same and ranged from 1.57 to 2.08 for CF and WB 100, respectively. It was observed that mealworm fed diets containing WB showed a slightly higher FCR than other diets. The significantly highest FCR (4.42) was found for mealworm reared on the WS 100 diet. The experimental insects are cold-blooded mini-livestock and they do not need to maintain constant body temperature.

In a study by Smil [42], the FCR for chicken was 2.5, for swine was 5.0 and it doubled for cattle (when FCR is expressed in kg of feed per kg of live weight). These values for livestock are much higher if expressed in kg of feed per kg of edible weight and double or even higher for cattle (4.5, 9,4 and 25, for chicken, swine, cattle, respectively), while the mealworm larvae can be consumed entirely without any subsequent waste. Thus, insects have a better feed conversion ratio compared with other livestock [43].

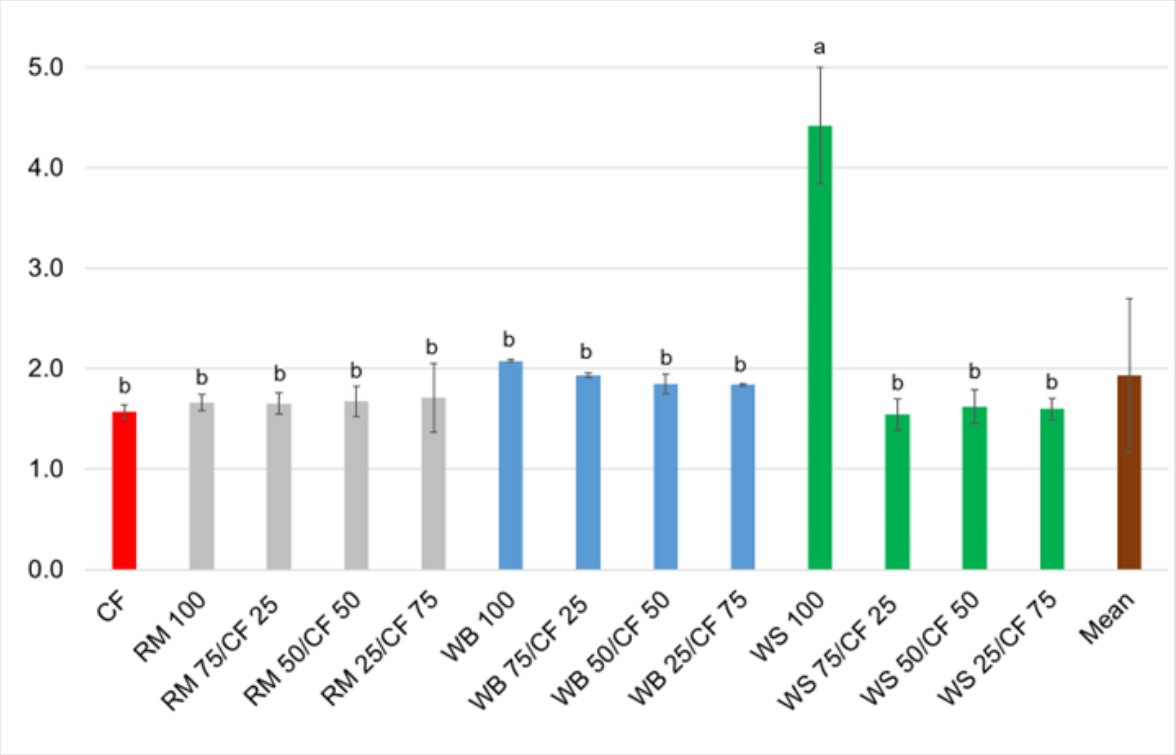


Figure 7. Mealworm feed conversion ratio (FCR); CF (chicken feed), RM (rapeseed meal), WB (wheat bran) and WS (willowleaf sunflower); a, b, c… - letters indicate that values are statistically different (Tukey's test at $p < 0.05$).

The results obtained on single trial on ECI of yellow mealworm larvae are significantly different for various feed types (**Figure 8**). The mean value for mealworms fed in this experiment was 20.1%. The highest ECI (23.9%) was noted for mealworms fed the RM 25/CF 75 diet. A very similar value (23.3%) was reported for mealworms fed the WS 25/CF 75 diet. A slightly (but statistically significant) lower ECI value was calculated for mealworms fed the RM 25/CF 50 diet. As for FCR, the ECI value for mealworms fed the WS 100 diet was significantly lower and was only 5.9%.

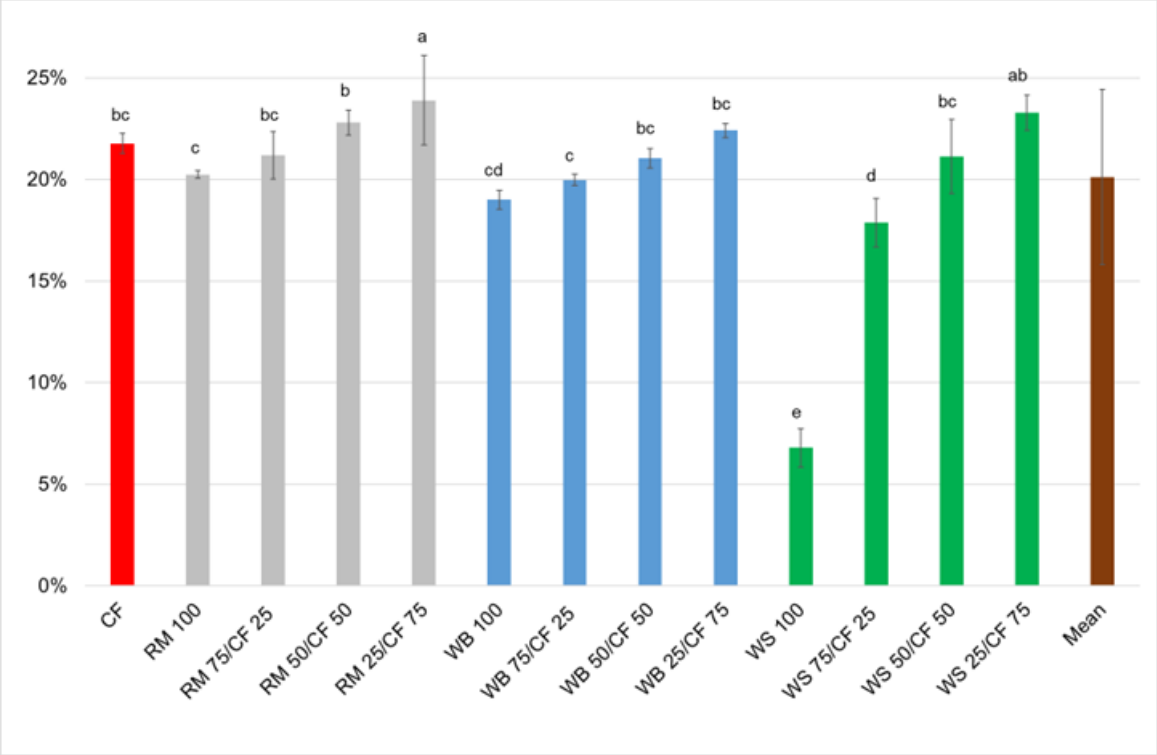


Figure 8. Mealworm efficiency of ingested feed (ECI) conversion; CF (chicken feed), RM (rapeseed meal), WB (wheat bran) and WS (willowleaf sunflower) a, b, c … - letters indicate that values are statistically different (Tukey's test at $p < 0.05$).

4.3. Results on long-term feeding trial (P3)

4.3.1. Survival and larval development

The survival of mealworms on the tested diets (**Table 3**) was high, between 92.2% and 97.7% for larvae grown on WB 100 and WS 25/CF 75, respectively (**P3**). Owing to the low density of mealworms in a box (50 larvae per box) and the sufficient amount of feed, the mortality was low. The present results on mortality are very low compared to the literature [44].

The larvae fed on WB 25/CF 75 started to pupate after 73 days (**Table 3**). The longest larval period (115 days) was recorded for mealworms fed on the WS 100 diet. The development time from hatchling to pupation of mealworm larvae grown on CF (control diet) was 76.8 days. The duration of the larval stage depends on a number of factors, including the temperature, the feed used, and its nutritional characteristics. The larval stage of mealworms fed on 50:50 distillers dried grain mixed with wheat bran, and on 100% wheat bran lasts for around 43.61 and 49.77 days, respectively [45]. In another study [19], pupation occurred 60 days after hatching among mealworms fed on wheat bran, corn stover soybean meal and distiller grains.

Table 3. Survival and larval stage duration (from hatching to pupation) of larvae grown on different agro-industrial byproducts.

No.	*Diet*	*Survival (%)*	*Larval Period (Days) **
1.	CF (control)	96.0 ± 2.6	76.8 ± 3.2
2.	RM 100	95.3 ± 2.8	78.5 ± 0.5
3.	RM 75/CF 25	96.9 ± 1.4	76.0 ± 1.0
4.	RM 50/CF 50	96.7 ± 2.4	76.0 ± 1.0
5.	RM 25/CF 75	96.6 ± 1.9	76.0 ± 1.0
6.	WB 100	92.0 ± 6.7	74.7 ± 0.3
7.	WB 75/CF 25	96.0 ± 3.7	76.0 ± 1.0
8.	WB 50/CF 50	95.8 ± 2.5	76.0 ± 1.0
9.	WB 25/CF 75	95.8 ± 2.3	73.0 ± 2.0
10.	WS 100	95.8 ± 3.2	115.0 ± 1.0
11.	WS 75/CF 25	96.7 ± 2.4	81.5 ± 4.5
12.	WS 50/CF 50	96.2 ± 1.5	76.5 ± 1.5
13.	WS 25/CF 75	97.7 ± 2.2	75.0 ± 0.0
	Mean	96.0 ± 3.1	79.3 ± 0.6

CF (chicken feed), RM (rapeseed meal), WB (wheat bran) and WS (willowleaf sunflower); ± - standard deviation; * average of two trials.

4.3.2. Individual fresh weight

The significantly highest final fresh larval weight (**Figure 9**) was found for mealworms fed with WB 100, WB 75/CF 25, and WB 50/CF 50 (140.4, 136.9, and 135.5 mg f.m., respectively) (**P3**). The fresh weight of mealworms fed on the control diet was statistically similar (131.5 mg f.m. per larvae). The final fresh larval weight was not significantly different between the

diets containing RM, and ranged between 113.6 and 118.6 mg f.m., for RM 75/CF 25 and RM 25/CF 75, respectively. Therefore, it can be concluded that diets with RM (sole) or mixed with CF can be used in any proportion with respect to the final fresh larval weight. The same situation was found in a preliminary trial (**P2**), in which the weights differed slightly between larvae grown on RM diets. In the case of WS diets, it was observed that a higher CF ratio led to the higher weight of mealworms. The lowest larval fresh weight was determined for insects grown on WS 100 diet (76.6 mg f.m.).

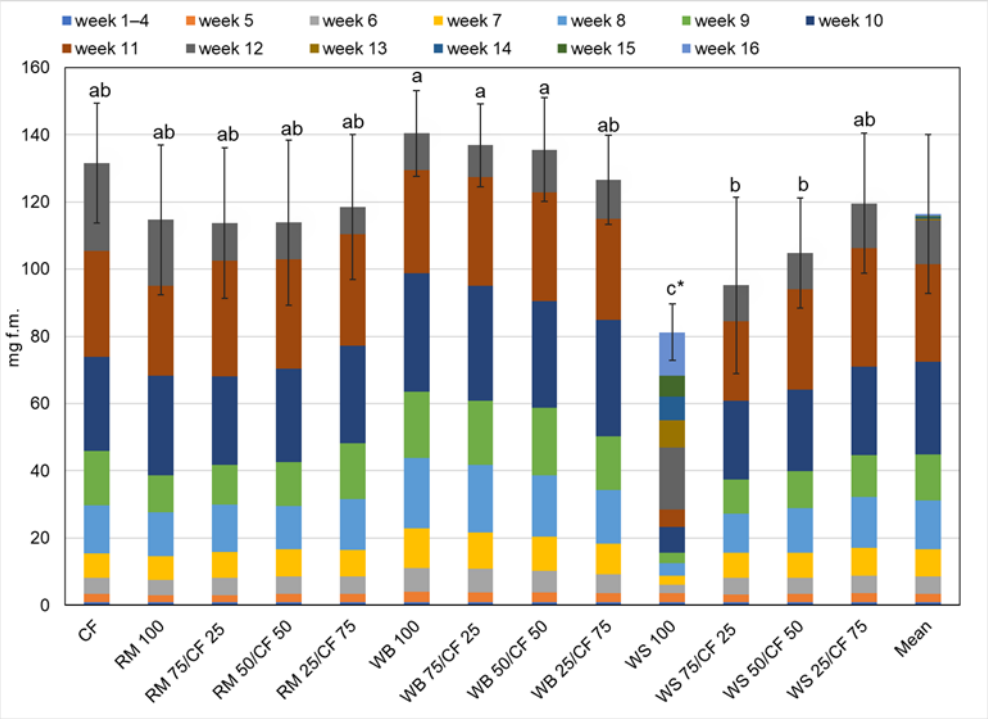


Figure 9. Individual fresh weight of yellow mealworm larvae depending on the week and the experimental diet; CF (chicken feed), RM (rapeseed meal), WB (wheat bran) and WS (willowleaf sunflower); a, b, c… - letters indicate that values are statistically different (Tukey's test at $p < 0.05$), (* the mean of 2 trials).

4.3.3. Feed conversion efficiency

The FCR of larvae differed significantly between the tested diets (**Figure 10**) (**P3**). The significantly highest FCR (3.31) was recorded for insects fed with WS 100. These data are much lower compared to the data obtained previously (4.42) on single-trial study (**P2**) as larvae were kept until the pupation. Significantly the lowest FCR was found for insects fed with all RM/CF diets and on the sole CF diet, where it ranged from 1.53 to 1.59 for RM 75/CF 25 and CF 100, respectively. The current results suggest that the protein content of feed influenced the indicators of insect development. In the current study, the FCR of larvae grown on the highest protein content diets (RM diets) was the lowest compared with larvae grown on the diets with less protein (especially on WS 100 diet). The scientific literature discusses relationships

between the protein content of a feed ingredient and the FCR; this aspect is carefully evaluated when formulating various diets from byproducts [17, 18].

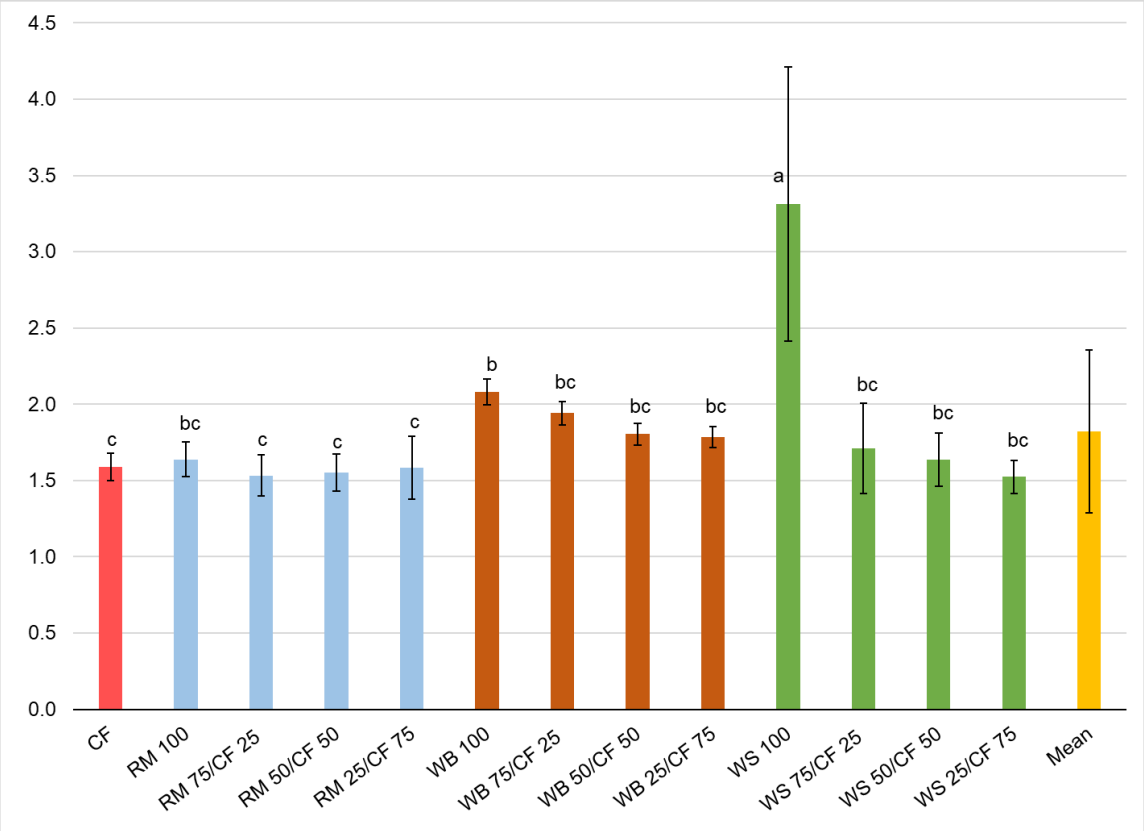


Figure 10. Mealworm feed conversion ratio (FCR); CF (chicken feed), RM (rapeseed meal), WB (wheat bran) and WS (willowleaf sunflower); a, b, c… - letters indicate that values are statistically different (Tukey's test at $p < 0.05$).

4.3.4. Efficiency of ingested feed

It is obvious that there is an inverse relationship between the FCR and ECI, also confirmed by the literature [18]. The ECI values were different between the mealworm grown on different diets (**Figure 11**) (**P3**). It was found that a higher ratio of CF in diets was correlated with higher ECI. The significantly lowest ECI (the worst) (6.9%) was recorded for mealworms grown on WS 100. The ECI of larvae fed on WS 100 improved compared to the data on preliminary results (5.9.%) obtained on single trial (**P2**). Therefore, an inadequate or insufficiently nutritious diet will be ingested by insects in a larger amount but with lower efficiency. This was especially observed in the case of mealworms fed on the WS 100 diet, in which both ECI and FCR values were unacceptable. Other researchers found that a higher fat content in diets entailed a higher ECI [18]. Both FCR and ECI are influenced also by the mealworm strains. Thus, the best ECI (21.0, 19.9 and 19.7%, respectively) was for Italian, Turkish and Greek strains of mealworms [46].

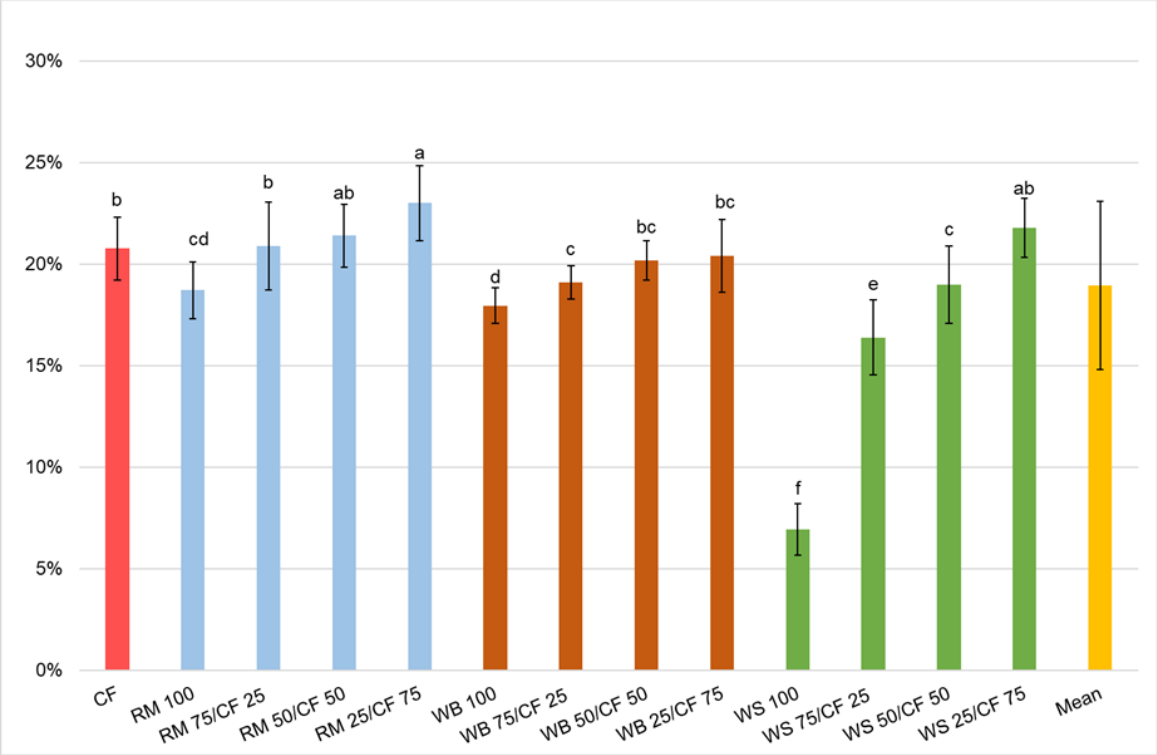


Figure 11. Mealworm efficiency of ingested feed (ECI) conversion; CF (chicken feed), RM (rapeseed meal), WB (wheat bran) and WS (willowleaf sunflower); a, b, c … - letters indicate that values are statistically different (Tukey's test at $p < 0.05$).

4.4. Influence of different drying methods on the yellow mealworm features (P4)

The results of one-way ANOVA (**P4**) revealed significant differences in all laboratory-analyzed parameters ($p < 0.001$ for all parameters). **Figure 12** presents samples of mealworm larvae after processing. Moisture content was significantly lowest in larvae dried by methods B2T4 (3.15%) and B1T4 (3.22%) (**Table 4**). Similar moisture content was noted in samples dried by method B1T3 (3.28%). Moisture content was significantly highest in larvae dried by method B1FD1 (5.47%), followed by method B2FD1 (4.79%). The moisture content of the analyzed samples was not always correlated with the water activity (a_w) of larvae dried by different methods. Water activity was significantly lowest in sample B1FD2 (0.04), followed by samples B2T4 (0.06) and B2T2 (0.07). Water activity was highest in samples B1FD1 (0.55) and B2FD1 (0.49).



Figure 12. Larvae processed during the experiment. From left to right: B1T1, B1T2, B1FD1.

Table 4. Parameters and composition of mealworm larvae dried by different methods.

Method	*Moisture Content (%)*	*Water Activity*	*Protein (% DM)*	*Crude Fat (% DM)*	*Crude Fiber (% DM)*	*Ash (% DM)*
B1T1	4.05 ± 0.06 c	0.14 ± 0.0 d	54.5 ± 0.39 a	30.9 ± 0.10 d	5.49 ± 0.06 b	3.52 ± 0.02 a
B1T2	4.07 ± 0.04 c	0.12 ± 0.001 f	53.7 ± 0.48 ab	30.9 ± 0.51 d	5.95 ± 0.13 a	3.52 ± 0.10 a
B1T3	3.28 ± 0.01 f	0.13 ± 0.001 de	49.4 ± 0.10 c	38.7 ± 0.39 bc	4.18 ± 0.09 de	2.80 ± 0.11 c
B1T4	3.22 ± 0.01 fg	0.13 ± 0.002 e	48.6 ± 0.04 cd	39.4 ± 0.13 b	4.39 ± 0.14 d	2.87 ± 0.02 bc
B2T1	3.64 ± 0.05 d	0.08 ± 0.001 g	48.7 ± 0.35 cd	39.9 ± 0.29 a	4.21 ± 0.08 de	2.94 ± 0.11 bc
B2T2	3.66 ± 0.02 d	0.07 ± 0.001 h	49.0 ± 0.23 c	39.5 ± 0.31 ab	4.60 ± 0.11 cd	2.77 ± 0.03 c
B2T3	3.49 ± 0.06 e	0.12 ± 0.007 f	49.4 ± 0.01 c	39.8 ± 0.56 a	4.62 ± 0.02 cd	3.00 ± 0.03 b
B2T4	3.15 ± 0.04 g	0.06 ± 0.001 h	49.4 ± 0.30 c	39.7 ± 0.30 ab	4.81 ± 0.07 c	2.91 ± 0.03 bc
B1FD1	5.47 ± 0.06 a	0.55 ± 0.004 a	53.3 ± 0.15 b	29.5 ± 0.13 e	5.35 ± 0.09 b	3.55 ± 0.02 a
B1FD2	4.08 ± 0.05 c	0.04 ± 0.002 i	49.4 ± 0.29 c	38.4 ± 0.56 bc	5.26 ± 0.13 b	2.98 ± 0.05 b
B2FD1	4.79 ± 0.04 b	0.49 ± 0.002 b	47.1 ± 0.33 e	38.0 ± 0.46 c	4.05 ± 0.05 e	2.98 ± 0.04 b
B2FD2	4.04 ± 0.01 c	0.23 ± 0.001 c	48.1 ± 0.17 d	37.8 ± 0.17 c	4.69 ± 0.08 c	2.89 ± 0.04 bc

a, b, c … - letters means that values are statistically different (Tukey's test at $p < 0.05$).

Protein content was significantly highest in larvae dried by methods B1T1 (54.5% DM) and B1T2 (53.7% DM) (**Table 4**). Protein content was also high in B1FD1 samples. The analyzed parameter was significantly lowest in insects processed by method B2FD1 (47.1% DM), and it was only somewhat higher in B2FD2 samples (48.1% DM).

Fat content was significantly highest in larvae dried by methods B2T1 (39.9% DM) and B2T3 (39.8% DM), and highly similar values were noted in samples B2T4 (39.7% DM) and B2T2 (39.5% DM). Fat content was considerably lower (30.9% DM) in yellow mealworms processed by methods B1T1 and B1T2, and it was lowest in samples dried by method B1FD1 (29.5% DM).

The analyzed samples also differed significantly in the content of crude fiber (**Table 4**). Crude fiber content was significantly the highest in insects dried by method B1T2 (5.95% DM) and significantly the lowest in larvae dried by method B2FD1 (4.05% DM).

Larvae dried by different methods were divided into two distinct groups in the dendrogram generated based on the results of agglomerative hierarchical clustering. The results were additionally interpreted with the use of a heat map (**Figure 13**). High values of the analyzed parameters were marked in red, whereas low values were marked in green. The first group in the dendrogram was formed by larvae processed by convective drying methods B1T1 and B1T2 as well as larvae that were freeze-dried by method B1FD1. This group was characterized by a high content of ash, protein, and crude fiber, and low-fat content. The second group was composed of larvae processed by the remaining drying methods, and it was characterized by a lower content of ash, protein and crude fiber, and high fat content. In both groups, larvae dried by methods B1FD1 and B1FD2 were characterized by considerably higher moisture content and water activity than the remaining samples. Despite the above, these samples did not form a separate group. It should also be noted that larvae forming the first group originated from the first batch of insects, and they were younger and lighter (**Table 2**) than the larvae dried by other methods, which could explain the differences in their fat and protein content. The accumulation of fat in the insects of prepupal stage was noticed in the literature [47].

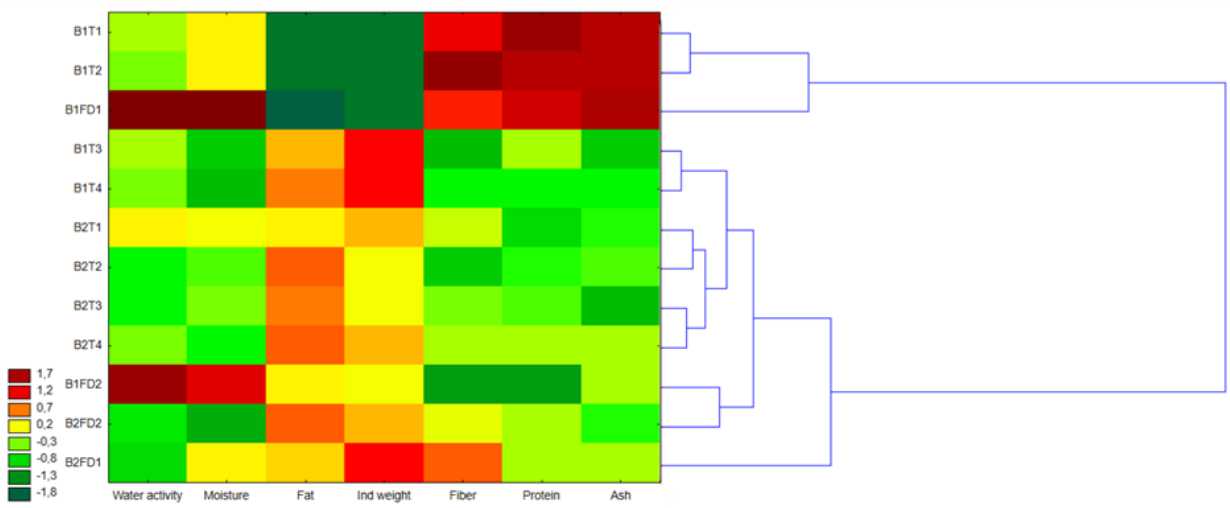


Figure 13. Heat map of drying methods and the analyzed parameters of yellow mealworms.

CONCLUSIONS

Based on reviewed literature (P1) it was concluded that the interest in mealworms as a source of food and feed has been increasing steadily in Europe due to the high nutritional value of mealworms, ease of production and lower environmental impact in comparison with livestock. Our analysis shows that number of research studies on *Tenebrio molitor* increased in recent years, especially since 2015, when European Union defined insects as novel food. The easy handling and unpretentious growing conditions contribute to the extending of use of yellow mealworm larvae for biomass conversion, as they are able to convert low-energy feeds into food products with a high value and they can mitigate the environmental pressure exerted by livestock.

The results obtained on single trial study on yellow mealworm larvae fed on 13 different diets (P2) showed that the individual dry larvae weight was the highest for mealworms fed WB diets, followed by those fed CF and RM diets, which confirm that the best "common feed" from residues for mealworms should be cereal residues. The WS diet containing 75% of chicken feed also demonstrated high dry weight and it can probably be attributed to the self-selection of the chicken feed component rather than the WS residues. Larvae grown on a WS 100 diet displayed very low final dry weights (only 3.9 mg d.m.). The FCR for all diets, except for WS 100, was at a similar level and ranged from 1.55 to 2.08. This is a satisfactory result and can be compared with the FCR of poultry. The highest FCR was found for mealworms fed the WS 100 diet (4.42).

Long term results (P3) of yellow mealworm experiment, on the same diets as in the first trial (P2), showed that larvae survival was high and not influenced by the experimental diets. The length of the larval period was typically from 73 to 82 days (for WB 25/CF 75 and WS 75/CF 25, respectively), except for larvae fed on WS 100 (115 days). The highest final fresh and dry weight of harvested larvae was found for those grown on WB diets, being confirmed in previous preliminary study (P2). The results also shown that a diet based only on WS was not sufficient nutritionally for their efficient growth and resulted in a long larval stage, low larval fresh and dry weight, slow larval weight gain, and unfavourable FCR and ECI values. Adding CF into the diets significantly improved the FCR compared to the sole diets. The same tendency was observed for the ECI, where larvae used the feed more efficiently. Based on the results, it can be concluded that the most efficient, rational and balanced diets related to both FCR and ECI are those that contain RM mixed with CF (confirmed in preliminary study). On the other

hand, all the tested production residues (including lignocellulosic biomass of willowleaf sunflower) mixed with chicken feed also gave satisfactory results in terms of the feed use efficiency.

Results of mealworm processing and drying (P4) shown that blanching inhibits enzymatic processes that lead to browning, and larvae have a lighter color that is more appealing to consumers. The moisture content and water activity of dried larvae were significantly affected by the drying method. However, no significant changes in the crude protein, fiber, or fat content of insects were found when drying parameters were modified, which corroborates the findings of other authors. However, it was noticed that the age of insects significantly influenced the examined larva features.

Acknowledgments

I would like to thank my supervisors Michał Krzyżaniak and Stanisław Czachorowski. Also, many thanks to prof. Mariusz J. Stolarski who made it possible for me to be admitted to the doctoral program. I am grateful for the opportunity to take part in such an innovative study.

I would like to thank the technical staff of Department of Genetics, Plant Breeding and Bioresource Engineering and Department of Bioresource Engineering for their technical support and constructive collaboration throughout the experiment.

I thank the ladies who took care of the administrative work and guided me to fill in all the forms correctly over the years, Ms. Ewa, Ms. Monika, Ms. Sylwia. Also, last but not least, I want to thank my devoted family and friends who have always been with me online in the most difficult moments related to the doctorate far from them. Dumitru, thank you for all the help in the laboratory, for the harshest criticism, and the strongest encouragement. Thank you for not leaving me alone when I wasn't sure of myself.

Funding

The results presented in this thesis were obtained as part of a comprehensive study financed by the University of Warmia and Mazury in Olsztyn, Faculty of Agriculture and Forestry, Department of Genetics, Plant Breeding and Bioresource Engineering (grant No. 30.610.007-110).

Project financially supported by the Minister of Education and Science under the program entitled "Regional Initiative of Excellence" for the years 2019-2023, Project No. 010/RID/2018/19, amount of funding 12.000.000 PLN.

Anna Bordiean participated in the Interdisciplinary Doctoral Studies in Bioeconomy (POWR. 03.02.00-00-I034/16-00), funded by the European Social Fund.

REFERENCES

[1] R.A. Houghton. Biomass. in: *Encyclopedia of Ecology*. S.E. Jørgensen, B.D. Fath (Eds.), Academic Press, Oxford, pp. 448-453, **2008**.

[2] Scarlat, N., Dallemand, J., Taylor, N. and Banja, M. Brief on biomass for energy in the European Union. Sanchez Lopez, J. and Avraamides, M. (Eds), Publications Office of the European Union, Luxembourg, **2019**, doi:10.2760/49052.

[3] P. Gurria Albusac, H. Gonzalez Hermoso, N. Cazzaniga, G. Jasinevičius, S. Mubareka, V. de Laurentiis, C. Patinha Caldeira, S. Sala, G. Ronchetti, J. Guillen Garcia. Biomass Flows in the European Union. Publications Office of the EU, Luxeombourg, **2020**, doi:10.2760/14342.

[4] FAO. The future of food and agriculture – Trends and challenges. Food and Agriculture Organization, Rome, Italy, **2017**.

[5] FAO. Crop Prospects and Food Situation – Quarterly Report No. 2. July **2022**. Rome, Italy, 46 p., https://doi.org/10.4060/cc0868en.

[6] FAO. Global food losses and food waste – Extent, causes and prevention, SAVE FOOD: An Initiative on Food Loss and Waste Reduction. Food and Agriculture Organization, Rome, Italy, **2011**.

[7] World Health Organization. The state of food security and nutrition in the world 2018: building climate resilience for food security and nutrition. Food and Agriculture Organization, Rome, Italy, **2018**.

[8] H. Ritchie. How much of the world's land would we need in order to feed the global population with the average diet of a given country? Our World in Data. October 3, **2017**. https://ourworldindata.org/land-use (accessed 5 September 2022).

[9] M. Van Peer, L. Frooninckx, C. Coudron, S. Berrens, C. Álvarez, D. Deruytter, G. Verheyen, S. Van Miert. Valorisation potential of using organic side streams as feed for *Tenebrio molitor*, *Acheta domesticus* and *Locusta migratoria*. Insects,12(9), 796, **2021**.

[10] T.-K. Kim, H.I. Yong, Y.-B. Kim, H.-W. Kim, Y.-S. Choi. Edible insects as a protein source: A review of public perception, processing technology, and research trends. Food science of animal resources, 39(4), 521, **2019**.

[11] A. Van Huis. Potential of insects as food and feed in assuring food security. Annual Review of Entomology, pp. 563-583, **2013**.

[12] Y. Jongema. List of edible insects of the world, 2017, Wageningen University, Wageningen, the Netherlands, **2018**. https://www.wur.nl/en/Research-Results/Chair-groups/Plant-Sciences/Laboratory-of-Entomology/Edible-insects/Worldwide-species-list.htm (accessed on 20 July 2022).

[13] EFSA Scientific Committee. Scientific Opinion on a risk profile related to production and consumption of insects as food and feed. EFSA Journal 13, (10): 4257, **2015**.

[14] European Commission. Commission Regulation (EU) 2021/1372 of 17 August 2021 amending Annex IV to Regulation (EC) No 999/2001 of the European Parliament and of the Council as regards the prohibition to feed non-ruminant farmed animals, other than fur animals, with protein derived from animals In L 295/1; European Commission. Official Journal of the European Union: Brussels, Belgium, **2021**, Volume 2021/1372; p. 21.

[15] EFSA Panel on Nutrition. D. Turck, T. Bohn, J. Castenmiller, S. De Henauw, K.I. Hirsch-Ernst, A. Maciuk, I. Mangelsdorf, H.J. McArdle, A. Naska. Safety of frozen and dried formulations from whole yellow mealworm (*Tenebrio molitor* larva) as a novel food pursuant to Regulation (EU) 2015/2283, EFSA Journal 19(8), **2021**.

[16] J. Ramos-Elorduy, E.A. González, A.R. Hernández, J.M. Pino. Use of *Tenebrio molitor* (Coleoptera: Tenebrionidae) to recycle organic wastes and as feed for broiler chickens. Journal of economic entomology 95(1), 214-220, **2002**.

[17] D.G.A.B. Oonincx, S. van Broekhoven, A. van Huis, J.J.A. van Loon. Feed conversion, survival and development, and composition of four insect species on diets composed of food by-products. PLoS ONE 10(12), **2015**.

[18] S. van Broekhoven, D.G.A.B. Oonincx, A. van Huis, J.J.A. van Loon. Growth performance and feed conversion efficiency of three edible mealworm species (Coleoptera: Tenebrionidae) on diets composed of organic by-products. Journal of Insect Physiology 73, 1-10, **2015**.

[19] X. Zhang, H. Tang, G. Chen, L. Qiao, J. Li, B. Liu, Z. Liu, M. Li, X. Liu. Growth performance and nutritional profile of mealworms reared on corn stover, soybean meal, and distillers' grains. European Food Research and Technology 245 (12) 2631-2640, **2019**.

[20] R. Melis, A. Braca, R. Sanna, S. Spada, G. Mulas, M.L. Fadda, M.M. Sassu, G. Serra, R. Anedda. Metabolic response of yellow mealworm larvae to two alternative rearing substrates. Metabolomics 15(8), 113, **2019**.

[21] C.I. Rumbos, D. Bliamplias, M. Gourgouta, V. Michail, C.G. Athanassiou. Rearing *Tenebrio molitor* and *Alphitobius diaperinus* larvae on seed cleaning process byproducts. Insects 12(4), 293, **2021**.

[22] A. Bordiean, M. Krzyżaniak, M.J. Stolarski, D. Peni, A. Marek. The influence of different diets on growth and nutritional composition of yellow mealworm. IFW - Insects to Feed the World, Quebec City, Canada, **2022**.

[23] G. Chemello, M. Renna, C. Caimi, I. Guerreiro, A. Oliva-Teles, P. Enes, I. Biasato, A. Schiavone, F. Gai, L. Gasco. Partially defatted *Tenebrio molitor* larva meal in diets for grow-out rainbow trout, *Oncorhynchus mykiss* (Walbaum): Effects on Growth Performance, Diet Digestibility and Metabolic Responses. Animals 10(2), 229, **2020**.

[24] I. Biasato, L. Gasco, M. De Marco, M. Renna, L. Rotolo, S. Dabbou, M.T. Capucchio, E. Biasibetti, M. Tarantola, L. Sterpone. Yellow mealworm larvae (*Tenebrio molitor*) inclusion in diets for male broiler chickens: effects on growth performance, gut morphology, and histological findings. Poultry Science 97(2), 540-548, **2018**.

[25] P. Miech, Å. Berggren, J.E. Lindberg, T. Chhay, B. Khieu, A. Jansson. Growth and survival of reared Cambodian field crickets (*Teleogryllus testaceus*) fed weeds, agricultural and food industry by-products. Journal of Insects as Food and Feed 2(4), 285-292, **2016**.

[26] G.P. Waldbauer. The consumption and utilization of food by insects. In: Advances in insect physiology. Elsevier **1968**, pp. 229-288.

[27] R.T. Gahukar. Entomophagy and human food security. International Journal of Tropical Insect Science 31(3), 129-144, **2011**.

[28] S. Marono, G. Piccolo, R. Loponte, C. Di Meo, Y.A. Attia, A. Nizza, F. Bovera. In vitro crude protein digestibility of *Tenebrio molitor* and *Hermetia illucens* insect meals and its correlation with chemical composition traits. Italian Journal of Animal Science 14(3), 3889, **2015**.

[29] M. Kulma, V. Plachý, L. Kouřimská, V. Vrabec, T. Bubová, A. Adámková, B. Hučko. Nutritional value of three *Blattodea* species used as feed for animals. J. Anim. Feed Sci 25(4), 354-360, **2016**.

[30] E. Dreassi, A. Cito, A. Zanfini, L. Materozzi, M. Botta, V. Francardi. Dietary fatty acids influence the growth and fatty acid composition of the yellow mealworm *Tenebrio molitor* (Coleoptera: Tenebrionidae). Lipids 52(3), 285-294, **2017**.

[31] E. Zielińska, B. Baraniak, M. Karaś, K. Rybczyńska, A. Jakubczyk. Selected species of edible insects as a source of nutrient composition. Food Research International 77, 460-466, **2015**.

[32] T. Grau, A. Vilcinskas, G. Joop. Sustainable farming of the mealworm *Tenebrio molitor* for the production of food and feed. Zeitschrift für Naturforschung C 72(9-10), 337-349, **2017**.

[33] E. Simon, E. Baranyai, M. Braun, I. Fábián, B. Tóthmérész. Elemental concentration in mealworm beetle (*Tenebrio molitor* L.) during metamorphosis. Biological Trace Element Research 154(1), 81-87, **2013**.

[34] E. Siemianowska, A. Kosewska, M. Aljewicz, K.A. Skibniewska, L. Polak-Juszczak, A. Jarocki, M. Jedras. Larvae of mealworm (*Tenebrio molitor* L.) as European novel food. Agricultural Sciences, 4, 287-291, **2013**. doi: 10.4236/as.2013.46041.

[35] N. Ribeiro, M. Abelho, R. Costa. A review of the scientific literature for optimal conditions for mass rearing *Tenebrio molitor* (Coleoptera: Tenebrionidae). Journal of Entomological Science 53(4), 434-454, **2018**.

[36] J.D. Bjørge, J. Overgaard, H. Malte, N. Gianotten, L.-H. Heckmann. Role of temperature on growth and metabolic rate in the tenebrionid beetles *Alphitobius diaperinus* and *Tenebrio molitor*. Journal of Insect Physiology 107, 89-96, **2018**.

[37] A. Adámková, M. Adámek, J. Mlček, M. Borkovcová, M. Bednářová, L. Kouřimská, J. Skácel, E. Vítová. Welfare of the mealworm (*Tenebrio molitor*) breeding with regard to nutrition value and food safety. Potravinarstvo Slovak Journal of Food Sciences 11(1), 460-465, **2017**.

[38] C. Liu, J. Masri, V. Perez, C. Maya, J. Zhao. Growth performance and nutrient composition of mealworms (*Tenebrio molitor*) fed on fresh plant materials-supplemented diets. Foods 9(2), 151, **2020**.

[39] J.A. Morales-Ramos, M.G. Rojas, D.I. Shapiro-Ilan, W.L. Tedders. Self-selection of two diet components by *Tenebrio molitor* (Coleoptera: Tenebrionidae) larvae and its impact on fitness. Environmental Entomology 40(5), 1285-1294, **2011**.

[40] L. Li, Z. Zhao, H. Liu. Feasibility of feeding yellow mealworm (*Tenebrio molitor* L.) in bioregenerative life support systems as a source of animal protein for humans. Acta Astronautica 92(1), 103-109, **2013**.

[41] S. Urrejola, R. Nespolo, M.A. Lardies. Diet-induced developmental plasticity in life histories and energy metabolism in a beetle. Revista Chilena de Historia Natural 84(4), 523-533, **2011**.

[42] V. Smil. Eating meat: Evolution, patterns, and consequences. Population and Development Review 28(4), 599-639, **2002**.

[43] A. van Huis. Edible insects contributing to food security? Agriculture and Food Security 4(1), **2015**.

[44] J. Mlček, M. Adámek, A. Adámková, J. Matyáš, M. Bučková, M. Mrázková, R. Vícha, R. Vychodil, I. Knížková, Z. Volek. Feed parameters influencing the breeding of mealworms (*Tenebrio molitor*). Sustainability 13(23), 12992, **2021**.

[45] S.Y. Kim, H.G. Kim, H.J. Yoon, K.Y. Lee, N.J. Kim. Nutritional analysis of alternative feed ingredients and their effects on the larval growth of *Tenebrio molitor* (Coleoptera: Tenebrionidae). Entomological Research 47(3), 194-202, **2017**.

[46] C.I. Rumbos, C. Adamaki-Sotiraki, M. Gourgouta, I.T. Karapanagiotidis, A. Asimaki, E. Mente, C.G. Athanassiou. Strain matters: strain effect on the larval growth and performance of the yellow mealworm, *Tenebrio molitor* L. Journal of Insects as Food and Feed 7(8), 1195-1205, **2021**.

[47] M.D. Finke. Complete nutrient composition of commercially raised invertebrates used as food for insectivores. Zoo Biology 21(3), 269-285, **2002**.