UNIVERSITY OF NAIROBI

EVALUATING MEALWORM (*Tenebrio* sp.) PERFORMANCE ON KENYAN AGRI-BYPRODUCTS: GROWTH, NUTRITION AND BIOCONVERSION

BY

PETER MUSEMBI JOHN

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A Thesis submitted in partial fulfilment of the requirements for the award of the Degree of Master of Science in Agricultural Entomology of the University of Nairobi

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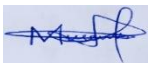
DECLARATION

I certify that this is my original work and has not been submitted to any institution for study, an exam, a degree, or publication. Other people's work has been properly acknowledged and referenced.

Signature:  **Date**: 25/10/2023

Peter Musembi John

I58/38810/2020

Department of Biology

University of Nairobi

This Thesis is submitted for examination with our approval as research supervisors.

	Signature	**Date**
Dr. E. A. Owino		25/10/2023
Department of Biology		
University of Nairobi		
P.O Box 30197-00100 Nairobi, Kenya		
Email: eaowino@uonbi.ac.ke		
Prof. F. A. Oyieke		25/10/2023
Department of Biology		
University of Nairobi		
P.O Box 30197-00100 Nairobi, Kenya		
Email: foyieke@uonbi.ac.ke		
Dr. C.M. Tanga		25/10/2023
Head of Insects for Food, Feed and Other Uses (INSEFF) Program,		
International Centre of Insect Physiology and Ecology,		
P. O. Box 30772- 00100, Nairobi, Kenya		
Email: ctanga@icipe.org		

DEDICATION

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LIST OF ABBREVIATIONS AND ACRONYMS

AOAC	Association of Official Analytical Chemists
GHGs	Greenhouse gases
WB	Wheat bran
PW	Potato peels
PP	Pineapple peels
CL	Cabbage leaves
CW	Cotton wool
ANOVA	Analysis of variance
EP	Edible portion
EV	Energy value
PUFA	Polyunsaturated fatty acids
MUFA	Monounsaturated fatty acids
SFA	Saturated fatty acids
EAA	Essential amino acids
NDF	Neutral detergent fiber
ADF	Acid detergent fiber
CP	Crude protein
ILW	Initial larval weight
FLW	Final larval weight
IFW	Ingested feed weight
WG	Weight gain
FCR	Feed conversion ratio
ECI	Efficiency of conversion of ingested feed

ABSTRACT

The current study aimed at evaluating the effect of replacement of wheat bran, a popular but scarce mealworm diet with potato waste, pineapple peels and cabbage leaves for efficient rearing of *Tenebrio* sp. The first experimental trial involved inclusion of potato wastes on wheat bran with fresh cabbage leaves provision to supplement water supply. The second experimental trial composed of wheat bran, cabbage leaves and pineapple peels at different ratios, and wet cotton wool provision in one of the diet. Both experiments were replicated four times and repeated once. Nutritional composition of the diets and harvested larvae were analysed using standard chemical procedures. The data for the first experiment demonstrated that replacement of wheat bran with potato waste is viable, as it yields comparably high survival rate (91 – 95%), favoured efficient conversion of ingested feed and yields the highest larval crude fat and energy contents. Moreover, the larvae reared on mixtures of the two ingredients were longer and heavier than those raised on sole wheat bran and sole potato wastes. However, the crude protein, most amino acids, acid detergent fiber contents as well as feed conversion ratio of the larvae remain favoured by wheat bran. On the other hand, the data for the second experiment showed that larvae raised on wheat bran diet supplied with wet cotton wool favored all growth, bioconversion rate, fat, and energy content levels. The survival remained high across all the diets. Whereas the diet comprising wheat bran, potato wastes, pineapple peels and cabbage leaves can be used to farm the mealworms, more readily available substrates with comparable protein and ash contents as wheat bran need to be evaluated for further improvement of the diet.

CHAPTER ONE

1.0 Background Information for the study

1.1 Introduction

Globally, an estimate of 2 billion people consume at least 1900 insect species (Halloran *et al.*, 2015; Zielińska *et al.*, 2015). Insect consumption is highly practiced in Asia, Africa and Latin America (Raheem *et al.*, 2019) due to their nutritive value and taste (Nonaka, 2009). Insects orders commonly consumed worldwide include coleoptera (31%), Lepidoptera (18%), Orthoptera (13%), Hemiptera (10%), Isoptera and Odonata (estimated to be 3%), Diptera (2%) and other insect orders making 5% of the totals consumed (Van Huis *et al.*, 2013; Raheem *et al.*, 2019). Mealworms forms part of human diet in Australia, Americas, Asia and Africa (Alves *et al.*, 2016). They are also used in space missions as bioregenerative life support systems (Li *et al.*, 2016). In European Union, mealworms is produced as feed alongside black soldier fly, common housefly, locusts, grasshoppers, katylids and crickets (Józefiak *et al.*, 2016).

Over 470 species of insects are eaten throughout the African continent, with Central Africa producing the most (256 species), followed by Southern Africa (164 species), Eastern Africa (100 species), Western Africa (91 species), and Northern Africa (8 species) (Kelemu *et al.*, 2015). The commonly African consumed insect orders include Lepidoptera (41%), Orthoptera (23%), Coleoptera (15%), Blattodea (12%), Hemiptera (4%) with other orders such as Hymenoptera, Mantodea, Diptera contributing less than 1% (Womeni *et al.*, 2009; Dzerefos, Witkowski and Toms, 2013; Riggi *et al.*, 2013; Kelemu *et al.*, 2015; Hlongwane, Slotow and Munyai, 2020). In Africa, mealworm farming is only practiced by poultry small scale farmers in South Africa (Selaledi, Maake and Mabelebele, 2021).

In Kenya, cultures, ethnicity and beliefs influence insect consumption (Münke-Svendsen *et al.*, 2016). Different communities consume different insects such as black ants (Ayieko *et al.*, 2012), lake flies (Ayieko and Oriaro, 2008; Ayieko, Oriaro and Nyambuga, 2010), crickets (Christensen *et al.*, 2006), grasshoppers/ locusts (Kinyuru *et al.*, 2010), termites (Kinyuru *et al.*, 2013; Alemu *et al.*, 2015), moths and honey bees (Münke-Svendsen *et al.*, 2016). Communities in Western, Eastern, Coastal and Central Kenya consume insects with 88% adult termites, grasshoppers adult 28%, 8.3% larval Saturniids in Kilifi, 6.8% adult crickets in Homabay, Siaya and Kwale, 3% larvae compost grubs in Kakamega and Vihiga , and 1.5% adult lake flies in Homa Bay and Siaya (Tanga *et al.*, 2021). However, there is no evidence for mealworm (*Tenebrio* sp.) consumption/ production in Kenya, thus this is relatively new species that farmers are currently willing to adopt for poultry and aquaculture production.

Generally, edible insects are rich in energy, proteins, minerals such as manganese, iron, phosphorus, copper, magnesium, selenium and zinc, fats, antioxidants, fibre and vitamins such as pantothenic acid, biotin, riboflavin and folic acid (Rumpold and Schlüter, 2013; Tao and Li, 2018; Di Mattia *et al.*, 2019). As a result of their novel nutritional composition, they are considered to be an efficient, effective and a substitute food and feed source (Van Huis *et al.*, 2013). The production of mealworms is environmentally sustainable as they efficiently convert bio-waste to useful products (Veldkamp *et al.*, 2012), have high feed conversion rate (Bordiean *et al.*, 2020), release fewer greenhouse gases (GHGs) (Oonincx and de Boer, 2012) and require less land and water (Oonincx and de Boer, 2012; Miglietta *et al.*, 2015).

1.2 Statement of the problem

Previous findings demonstrate the significance of mealworms (*Tenebrio* sp.) as nutritious food and feed source with high energy value, proteins, fats both polyunsaturated fatty acids, saturated fatty acids and monounsaturated fatty acids, carbohydrates, crude fibre, ash content, vitamin B complex among other micronutrients (Nowak *et al.*, 2016). Economically, mealworm sustainability is not yet fully competitive when compared to other protein sources for animal and humans. However, many efforts are suggested to develop a cost-effective balanced diets, designed to fully supplement the nutritional needs and production system in order to optimize product quality, increase production yield and minimize manual labor (Heckmann *et al.*, 2018).

Numerous research works recommend wheat bran as the best diet on mealworm production. However, wheat bran is limited and expensive commodity to non-wheat growing countries. Some literature suggests agricultural by-products as potential alternative diets (Harsányi *et al.*, 2020). The potential of organic wastes performance in mealworm production is not yet fully investigated. Therefore, there is need to investigate readily available wastes, in order to make mealworm production process more efficient, sustainable and affordable to Kenyan farmers and other farmers across the world.

1.3 General objective

To develop a cost effective diet for mealworm production to Kenyan farmers, based on locally available agricultural by–products by comparing their growth performance, survivorship, bioconversion and nutritional composition.

1.3.1 Specific objectives

1 To evaluate the impact of incremental replacement of wheat bran with potato waste in the diet on development, survival, bioconversion and nutritional value of mealworm

2 To assess the effect of inclusion of cabbage leaves and pineapple peels wastes in the diet on survival, development, bioconversion and nutritional composition of the mealworms

1.4 Research hypothesis

1 Incremental replacement of wheat bran with potato waste in the mealworm diet doesn't influence growth, survival, bioconversion and proximate value of mealworms

2 Inclusion of cabbage leaves and pineapple peels waste in the diet do not enhance growth, survival, bioconversion and proximate value of mealworms.

1.5 Research Questions

1. Does replacement of wheat bran with potato wastes influence growth performance, survivorship, bioconversion and nutritional composition for mealworm?

2. Do cabbage leaves and pineapple peels waste inclusion in mealworm diet influence their growth, survival, bioconversion and nutrients composition?

1.6 Justification of the study

As food insecurity is gaining momentum in developing countries, more alternative underutilized food resources particularly edible insects are also needed. Shifting from livestock production to edible insects' production is essential to cater for social, environmental and economical sustainability. Mealworm production has socially and environmentally shown to be efficient and effective in different aspects. The economical sustainability is still in development whereby a

readily available, efficient and cost–effective diet for commercial mealworm production is needed. Although, mealworm production is not yet initiated in developing countries, farmers' willingness to promote its usage in poultry and fish production accelerate need for an affordable diet. Given the scarcity and expensive importation of wheat bran to Kenya from Ukraine and Russia, the organic wastes from local markets or restaurants is essential in replacing wheat bran use in production process for sustainable protein supply in order to supplement other potential protein sources derived from animal or plants' origin. The study is meant to help farmers use readily and affordable diet that caters both quality and quantity mealworm produce and minimize losses that might occur.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The Biology and ecology of mealworms

Mealworms belongs to order Coleoptera, Tenebrionidae family (darkling beetles), genus *Tenebrio*. Globally, the Tenebrionidae family is highly diversified group containing more than 30000 described species (Condamine *et al.*, 2014) with 4122 genus groups, whereby are 33 extinct and 2307 valid genera. The Tenebrioninae subfamily contains 349 genera (Bouchard *et al.*, 2021). The majority of larvae and adults are saprophagous in nature (feed on decayed vegetation) with few tribes having predatory and mycotophagous larvae.

Mealworms (*Tenebrio* sp.) are cosmopolitan species native to Europe, but globally distributed (Ramos-Elorduy *et al.*, 2002). They primarily feed on farinaceous materials and products from animals such as feathers and meat (Ribeiro, Abelho and Costa, 2018). They are found in barns, flour mills hence considered pest of economic importance on those facilities (Ramos-Elorduy *et al.*, 2002). Naturally, males and females are polygynandrous, with males portraying post-copulatory mate guarding behaviour (Carazo *et al.*, 2004). Mealworms are characterized by rapid growth, easy handling with low breeding requirements, hence highly bred and traded species.

2.2 The mealworm Lifecycle

The mealworm is holometabolic insect. The entire lifecycle takes place in same ecosystem, and the developmental rate is influenced by ambient temperature, relative humidity, population density and quality of the diet given. An average of 250 – 500 eggs are produced singly or in small clusters and are found attached to trays' floor/ walls or to substrate (Ghaly and Alkoaik, 2009). Eggs take several days to hatch ranging from 4 days (26 ℃ to 30 ℃), 34 days at 15℃ (Kim *et al.*, 2015).

The larvae duration varies from 57 days under controlled conditions to 629 days in natural ambient conditions with several molts (9 to 22 molts) (Ribeiro, Abelho and Costa, 2018). The pupal stage takes 6 to 20 days (Ghaly and Alkoaik, 2009) before adult emergence. The adults lasts for 16 – 173 days, with an average of 31 to 62 days. At optimal conditions, the entire life cycle usually lasts from 75 to 90 days.

2.3 Mealworm water intake

Mealworms can live under extreme dry conditions due to their adaptation to take water from ingested food and from atmosphere (Fraenkel and Blewett, 1944). They efficiently absorb atmospheric water at high relative humidity levels. The larval development is greatly influenced by water intake, with fast growth experienced under high moist conditions. At artificial rearing conditions, water is mainly supplemented by adding fresh vegetables and fruits to the diet (Ortiz *et al.*, 2016). Supplementing diets with water increases growth performance by increasing survivorship rate by more than 80%, increasing moisture content and subsequently reducing developmental time from 145 – 151 days to 91 – 95 days (Oonincx *et al.*, 2015).

2.4 Environmental and Physical conditions

The performance and development of the mealworms are regulated by physical and environmental elements namely temperature, relative humidity, photoperiod, diets and population density. The diet composition also influences nutritional value

2.4.1 Temperature

The optimal temperature conditions for mealworm rearing mainly ranges from 25 to 30 ℃ (Koo *et al.*, 2013; Kim *et al.*, 2015). The minimum and maximum mealworm temperatures regime for

their growth is at 10 ℃ and 35 ℃ respectively (Punzo and Mutchmor, 1980). For the normal growth and development, the extreme minimum and maximum values lies at 17 ℃ and 30 ℃ respectively (Koo *et al.*, 2013). The lethal maximum and minimum temperatures ranges at 40 - 44 ℃ (Martin, Rivers and Cowgill, 1976) and 7 – 8 ℃ respectively for 24 hours exposure (Mutchmor and Richards, 1961).

2.4.2 Relative humidity

Mealworms are flexible insects with wide range of relative humidity values. The optimal humidity levels varies from 60% to 75% (Punzo and Mutchmor, 1980). Although, very high relative humidity favour molds development on substrate, many authors suggest positive correlation whereby, an increase in relative humidity lead to increase in mealworm development for instance, relative humidity above 70% (Fraenkel, 1950) or 90 – 100%.

2.4.3 Population density

The mealworm population density affects larval molts number and duration. High population density results in small-sized larvae and fewer larval instars and significant reduction in female progeny and reproductive output (Morales-Ramos *et al.*, 2012; Morales-Ramos and Rojas, 2015). Larval metabolism in overcrowded scenario lead to increased temperature whereby, to some extend it may be lethal.

2.4.4 Photoperiod

Naturally, mealworms are negatively phototropic with large larvae and adults at daylight positioning below substrates' surface and come above the surface at dark. The photoperiod influence development and growth in mealworms with optimal larval development during long-

days, reduced development in 14L:10D (Kim *et al.*, 2015) and unrhythmic development at constant conditions (Cloudsley-Thompson, 1953). Photopheriodic conditions also influences the rate of molting with long day conditions of 14L:10D constituting high rates in comparison to short day regime of 10L:14D with lower rates of 45.5% and 24.2% respectively (Kim *et al.*, 2015), with constant regime of 12L:12D inducing 100% pupation rate at 25 ℃ (Kim *et al.*, 2015).

2.5 Benefits of mealworm production

2.5.1 Environmental benefits

Generally, rearing edible insects is an alternative to supplement supply of essential necessities to both humans and animal. Mealworms are easily bred and reared in trays, and their production on large scale basis is environmentally and socially important strategy for food and feed. They can be cultured on dried organic waste of vegetable, fruit origin, wastes from bread production, potato processing or beer brewing (Oonincx *et al.*, 2015; Van Broekhoven *et al.*, 2015). They are capable of reducing and converting low nutritive wastes to high protein content with other insects such as *Hermetia illucens* L. and *Musca domestica* with bioconversion rate of 1.3 billion per annum (Veldkamp *et al.*, 2012) and feed conversion rate of 3.4 – 6.1 kilograms of ingested feed per larval harvested kilogram (Bordiean *et al.*, 2020).

Mealworm production is considered to be mini–livestock production system requiring less land and little water compared to livestock production. For instance, *Zophobas morio* and *Tenebrio* sp. production require 0.2% of the overall land (Oonincx and de Boer, 2012) with 99% of the total land associated very small fraction of water (Miglietta *et al.*, 2015). In chicken production, total land required to produce 1g of edible protein is 2 to 3 times and water required is 50% more in comparison to mealworm production (Oonincx and de Boer, 2012; Miglietta *et al.*, 2015). For beef

production, 1 gram of edible protein requires approximately upto 14 times land as well as 5-fold water when compared to mealworms production.

Collectively, rearing migratory locust, mealworms, house cricket and orange spotted cockroach (*Blaptica dubia*) can generate less amounts of GHGs (CO_2) per meat kilogram, compared to pig and bovine rearing (Premalatha *et al.*, 2011). Beef cattle emits 6-13 times carbon dioxide, and broiler chicken emit 32 – 167% more carbon dioxide, compared to mealworm emission on basis of edible protein (Oonincx and de Boer, 2012).

2.5.2 Mealworms as feed and food source

Mealworms are nutritious sustainable food and feed source to humans, poultry, pets and fish. They are able to transform low nutritive organic wastes to a diet rich in protein and fat, with low environmental impact (Ramos-Elorduy *et al.*, 2002; Bordiean *et al.*, 2020). As human food, they are used as whole (Ghaly and Alkoaik, 2009; Zhao *et al.*, 2016) or ground to flour (Aguilar-Miranda *et al.*, 2002).

In recent studies, mealworm commercial farming as protein feed source for livestock (De Marco *et al.*, 2015; Biasato *et al.*, 2016; Benzertiha *et al.*, 2019; Gasco *et al.*, 2019) and fish (Ng *et al.*, 2001; Barroso *et al.*, 2014; Belforti *et al.*, 2015; Gasco *et al.*, 2016) has become popular around the world particularly in USA (Yang *et al.*, 2018), Spain (Reyes *et al.*, 2020), France (Thévenot *et al.*, 2018) and China (Bovera *et al.*, 2015; De Marco *et al.*, 2015; Biasato *et al.*, 2017). In 2020, the larval mealworm market price ranged from USD 10.8 – 14, 8.4 – 9.3, 65 – 70, and 12.9 – 20 per kg in USA, China, South Korea and European Union respectively, that were higher compared to price of soybean meal and fish meal that retailed at USD 0.34 per kg and USD 1.2 – 1.3 per kg, respectively (Hong and Han, 2020).

Mealworms are usually ground to flour then added in the diet. Mealworm inclusion as fishing bait /pet feed in different species showed improved performance, for instance, the European sea bass (*Dicentrarchus labrax*) juveniles growth performance was facilitated when fed on a diet with 30% of fish meal substitution with mealworms compared conventional diet (Mastoraki *et al.*, 2020). In Nile Tilapia juveniles (*Oreochromis niloticus*) study, mealworm inclusion at 5%, 10%, 15% and 20% in the diets led to increased feed intake, final weight, weight gain, high FCR and specific growth rate compared to control diet (Tubin *et al.*, 2020). In accordance to (Belforti *et al.*, 2015), mealworm inclusion at 25 % and 50 % in rainbow trout (*Oncorhynchus mykiss*) diet improved , feed conversion ratio, specific growth rate and protein efficiency ratio.

In poultry production, mealworms inclusion in small quantities was found to significantly replace soybean meal (Hong and Han, 2020). For instance, mealworm inclusion in broiler chicks of Ross 708 breed (male) rate of 5 %, 10 % and 15 % with control diet formulated based on soy bean meal, corn gluten meal and corn meal, showed significant increase in body weight (12 to 25 days old), feed conversion ratio and daily feed intake (Biasato *et al.*, 2018). The addition of 0.2 % and 0.3 % of mealworms on broiler chickens (Ross 308 breed females) with basal diet comprising of soybean meal, soybean oil, wheat, fish meal and rye, increased daily feed intake, weight gain and feed conversion ratio (Benzertiha *et al.*, 2020). The study on barbary partridge (*Alectoris barbara*) (Loponte *et al.*, 2017), 25 % inclusion of mealworms on corn soybean meal (control) showed high live weight at 64 day and high feed conversion efficiency experienced both in 25 % and 50 % inclusion rate. The mealworm feed conversion efficiency and body weight was significantly improved in Japanese quails (*Coturnix japonica*) (Zadeh, Kheiri and Faghani, 2019) when soybean oil and fish meal was substituted with 22.5 and 30 g/kg of mealworms.

2.5.3 Nutritional value of mealworms

Mealworms contain significant amount of protein that vary depending on developmental stage and diet provided. The protein content ranges from 47.76 to 53.13% (Bovera *et al.*, 2015), 43.3% to 66.8% (Ghaly and Alkoaik, 2009; Jin *et al.*, 2016) in dry matter basis with almost all EAAs present (Ramos-Elorduy *et al.*, 2002; Barroso *et al.*, 2014; Zielińska *et al.*, 2015; Jin *et al.*, 2016), 50% (Mancini *et al.*, 2019), 47.18 – 49.43 (Rumpold and Schlüter, 2013), 47% on dry matter basis (Van Broekhoven *et al.*, 2015) and 187 g/kg (Finke, 2002). The edible portion (EP) ranges from 13.68 to 22.32 g/100 g EP thus they are rich in protein (Nowak *et al.*, 2016).

Mealworm fats/ lipids contents also varies depending on developmental stage with 30 – 35% (Mancini *et al.*, 2019), 19 – 28% (Van Broekhoven *et al.*, 2015), 27.25 – 38.26% (Bovera *et al.*, 2015), 35.17 – 43.08% (Rumpold and Schlüter, 2013), 35 – 60% (Finke, 2002), 17 – 42.48% (Siemianowska *et al.*, 2013; Adámková *et al.*, 2016) on dry matter basis, 25% with palmitic acid content of 16% (Van Broekhoven *et al.*, 2015). The edible portion ranges from 8.9 – 19.94 g/100 g EP (Nowak *et al.*, 2016).

Mealworm contain significant amounts of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). However, fatty acids values vary greatly. The PUFA content ranges from 21 – 62% of the total lipids which is equivalent to 3.17 – 6.75 g/100 g EP (Nowak *et al.*, 2016). In another study (Rumpold and Schlüter, 2013), the mealworm fatty acid contents weren't reported, only mean value of 27.14 % PUFA, 35.72% MUFA and 38.49% SFA was reported, and wide range of 2.78 – 65.29 %, 0.72 – 66.60% and 3.05 – 95.77% respectively of total lipids of different species in order Coleoptera.

The mineral composition for mealworms are highly variable and include magnesium, sodium, copper, potassium, phosphorus, selenium, zinc and iron (Siemianowska *et al.*, 2013; Zielińska *et al.*, 2015; Nowak *et al.*, 2016) with very low calcium levels 12 – 65 mg/100 g EP (Nowak *et al.*, 2016). Generally, ash content ranges from 3.00 – 3.08% on dry matter basis (Rumpold and Schlüter, 2013), 9 g/ kg (Finke, 2002). The larval mealworms contain less dietary fibre ranging from 5.00 – 14.96% compared to adults 16.30 – 20.22% (Rumpold and Schlüter, 2013). They contain mean crude fibre of 2.1 g/100 g EP, neutral detergent fibre (NDF) of between 2.9 – 7.3 g/100 g EP (Nowak *et al.*, 2016), 57 g/kg (Finke, 2002) and acid detergent fibre (ADF) ranging from 2.13-2.5 g/100 g (Nowak *et al.*, 2016), 25 g/kg (Finke, 2002), with no dietary fibre essential for human consumption in accordance with the AOAC Prosky method (Nowak *et al.*, 2016).

The larval mealworms are labelled as vitamins source as they are rich in riboflavin 0.81 – 1.61 mg/100 EP, vitamin B12, B6, E, C, A, E biotin, thiamine, folate, pyridoxine, panthotenic and niacin at different levels. However, they contain low panthotenic and vitamin E contents (Nowak *et al.*, 2016). The mealworms carbohydrates content ranges from 0.01 – 3.86% (Rumpold and Schlüter, 2013) with the energy values ranging between 379 – 573 kcal/ 100 g (Bovera *et al.*, 2015), 539.63 – 577.44 kcal/ 100 g (Rumpold and Schlüter, 2013), 160 – 283 kcal/100 g EP (Nowak *et al.*, 2016) and metabolizable energy of 2056 kcal/ kg (Finke, 2002).

2.6 Mealworm growth performance based on diet nutritional composition.

Diet rich in protein influences mealworm lifecycle particularly in larval development, survivorship, weight and adult fertility. Supplementing mealworm diets with high protein content of 33-39% dry mass at temperature and relative humidity of 28℃ and 70% respectively reduces pupation time to 116 – 144 days from 191 – 227 days, with an increase in survival rate to 67 –

79% from 19-52% (Oonincx *et al.*, 2015). At 28℃ and 65% RH, the pupation time is reduced from 95-168 days to 79-95 days and survivor rate increase from 84 – 88% to 88 – 92% (Van Broekhoven *et al.*, 2015). In terms of weight gain, addition of protein to mealworm diets is highly detected in pupal stage with increment of weight to 238 mg/g from 123 mg/g and fertility increase from average of 3 eggs per day (protein free diet) to an average of 6 – 7 eggs per day in protein enriched diet (Morales-Ramos *et al.*, 2013) and from 117 – 145 mg (diet with low protein of 5% yeast) to 146-161 mg (diet with high protein content 40% yeast) (van Broekhoven *et al.*, 2015).

Mealworm fat content remains constant even when fed with different diets. Adding lipids to mealworm diets is favourable at low concentrations, with high concentration being unfavourable (Morales-Ramos *et al.*, 2013). Fats enriched diets promotes substrates agglomeration thus reducing the aeration and mealworm movement, further resulting to respiration difficulties (Alves *et al.*, 2016).

Mealworm growth performance in diets deficient of carbohydrates is almost zero. In many scenarios, the diet rich in carbohydrate is usually supplemented with protein source, whereby the ratio of the two diets influences the entire life cycle with optimal reproductive success and lifespan at 1:1 ratio (Rho and Lee, 2016). Diet rich in Vitamin B complex is essential for mealworm growth and development, with no impact of vitamin A, C, D, E and K (Martin and Hare, 1942).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area

The study was conducted at International Centre of Insect Physiology and Ecology (*icipe*), Animal Rearing and Quarantine unit in Kasarani – Nairobi, positioned at approximately S 01º 13′14.6″ latitude and E 036º 53′ 44.5″ longitude, at an elevation of around 1,612 meters above sea level.

3.2 Mealworm Stock culture

The mother stock colony of mealworms (*Tenebrio* sp.) was carefully maintained on a diet of wheat bran. The rearing and maintenance of this colony closely followed established procedures detailed by Ramos-Elorduy *et al.* (2002), Ortiz *et al.* (2016), Morales-Ramos *et al.* (2013), and Ribeiro, Abelho and Costa (2018), albeit with some minor adjustments. To initiate this process, eggs obtained from the stock colony were carefully transferred into rectangular plastic trays, each measuring 56 cm × 38 cm × 10 cm, and these trays contained 500 grams of wheat bran. To sustain the optimal conditions for the mealworms' development, the wheat bran diet was consistently supplemented with market fruits and vegetables. This was done to ensure that the moisture content, approximately 70 ± 2%, was maintained, and this moisture level was validated using a specialized moisture sensor that had two lengthy probes, each measuring 12 cm in length (HydroSenseTM CS620; Campbell Scientific, Inc., Logan, UT, USA). The growth of the mealworm larvae within the rearing environment was meticulously monitored on a daily basis. As described by Ortiz *et al.* (2016), the pre-pupal stages were singled out from the substrate and subsequently placed into separate transparent rectangular plastic containers (Kenpoly Manufacturer Limited, Nairobi, Kenya). These containers were sized at 18.4 cm × 12.6 cm × 6.7 cm and were furnished with moist

wood shavings (sawdust) to serve as the substrate for pupation. Each lid of these containers possessed an opening measuring 14.5 cm × 8.3 cm, which was covered with a fine mesh made of organza material. This mesh served to effectively contain the emerging adult darkling beetles. The environmental conditions within the mealworm stock culture rearing facility were rigorously controlled, maintaining a temperature of 28 ± 2.5 ºC, a relative humidity level of 70 ± 2%, and a photoperiod regime of L12:D12 (light to dark hours). It is noteworthy that this colony had been actively managed for approximately two years, spanning more than 13 generations. The design of the photoperiodic regime was informed by prior research works highlighted by Oonincx *et al.* (2015) and van Broekhoven *et al.* (2015).

3.3 Experimental mealworms

The experimental mealworm larvae were obtained from a mealworm stock culture that has been growing for 2 years (with over 13 generation) on wheat bran at *icipes*' Animal Rearing and Quarantine Unit. To make handling easier when introducing the freshly hatched larvae into the test diets, the diet of wheat bran was permitted to continue until 14 days after hatching. The experimental larvae were transferred to another rearing facility, away from stock culture, whereby rearing conditions were kept at 28.8 ℃ to 30.5 ℃, 65% – 70% relative humidity, and 12L:12D photoperiodic conditions, respectively.

3.4 Diets sources and preparation

The experimental wheat bran was bought from Pembe Flour Mills Limited, along Lunga Lunga Road Nairobi, Kenya (S 01º 18′26.316″; E036º 52′ 25.138″). The potato waste were sourced from Propack Kenya Ltd Company, Baba Dogo Road, Nairobi, Kenya (S 01º 14′44.484″; E 036º 52′ 35.147″). The cabbage leaves and pineapple peels were collected from Githurai market, Nairobi,

Kenya (S 01º 11′6.1296″; E036º 55′ 50.3652″). The wastes were milled into fine particles using a grinding mill (Rhino Brand F-35ZS, JB/T6270, Nyagah Mechanical Engineering Limited, Kenya). They were dried at *icipes*' green house at 28.5 ± 1.5 ℃ and 60 ± 2.5% of temperature and relative humidity respectively for a week. To standardize the feed composition, their moisture state was evaluated, and the actual amounts provided based on dry matter. The empty aluminum foil sample cups were weighed. The weight of the cups was also measured before and after drying the samples at 60 ℃ for 24 hours in an oven (WTC Binder FD 115, Tuttlingen, Germany). These were carried out in triplicate, and dry matter values averaged. The following equations were used to compute the samples' % dry matter;

Equation 1: Moisture (%) content computation

$$\text{Moisture }(\%) = \frac{(\text{WFS}-\text{CW})-(\text{WDS}-\text{CW})}{\text{WFS}-\text{CW}} \times 100 \quad \ldots\ldots (1)$$

Whereby: CW – cup weight; WFS – cup and fresh sample weight; WDS – weight of the dried sample with cup.

Equation 2: Feed dry matter (%) calculation

$$\text{Feeds dry matter }(\%) = 100 - \text{moisture }(\%) \quad \ldots\ldots (2)$$

3.5 Experimental Design

3.5.1 Diets formulations

Experiment 1: The experimental design employed 5 × 4 Completely Randomized Design (CRD), that is, five- dietary treatments replicated four times. The dietary treatments were as follows; WB100 – 100% wheat bran (control); WB75/PW25 – 75% wheat bran and 25% potato waste; WB50/PW50 – 50% wheat bran and 50% potato waste; WB25/PW75 – 25% wheat bran and 75% potato waste; and PW100 – 100% potato waste. The diets' nutritional composition are as presented in Table 1

Table 1: Substrate nutritional composition [means (± standard error)] for raising *Tenebrio* sp. (n = 3)

Nutritional content	WB (control)	WB75/PW25	WB50/PW50	WB25/PW75	PW100	df	F	P
Dry matter (%)	92.7 ± 0.7a	92.3 ± 0.3a	92.3 ± 0.3a	92.3 ± 0.7a	91.0 ± 0.0a	4,10	1.9	0.187
Crude protein (%)	15.5 ± 0.1c	12.8 ± 0.1b	13.1 ± 0.2b	14.9 ± 0.1c	12.1 ± 0.1a	4,10	109.2	< 0.001
Crude fat (%)	3.9 ± 0.4a	2.9 ± 0.4a	2.2 ± 0.0a	2.9 ± 0.8a	4.0 ± 0.7a	4,10	2.3	0.13
Ash (%)	6.1 ± 0.3a	17.7 ± 0.7c	13.4 ± 1.0b	10.5 ± 0.4b	4.4 ± 0.6a	4,10	67.74	< 0.001
Crude fiber (%)	0.34 ± 0.03a	0.39 ± 0.03a	0.40 ± 0.04a	0.42 ± 0.01a	0.41 ± 0.01a	4,10	1.548	0.262
Carbohydrates (%)	74.1 ± 0.5bc	66.3 ± 1.1a	70.9 ± 1.3ab	71.3 ± 1.2ab	79.1 ± 1.4c	4,10	17.4	< 0.001
Energy (kcal/100 g)	394.7 ± 2.7d	342.9 ± 1.0a	356.6 ± 4.0b	371.8 ± 3.0c	401.7 ± 2.0d	4,10	83.09	< 0.001

Within each row, means followed by the same lowercase letter indicate no significant different, whereas, different lowercase letters within each row indicate larval significant differences in different treatments at $\alpha = 0.05$. Where, WB100 – 100% wheat bran (control); WB75/PW25 – 75% wheat bran and 25% potato waste; WB50/PW50 – 50% wheat bran and 50% potato waste; WB25/PW75 – 25% wheat bran and 75% potato waste; and PW100 – 100% potato waste.

Experiment 2: In this scenario, five dietary formulations were used: WB – wheat bran (Control); WB/CL - Wheat bran and cabbage leaves at 2:1; WB/PP – Wheat bran and pineapple peels at 2:1; WB/CL/PP – Wheat bran, cabbage leaves and pineapple peels at 2:0.5:0.5; WB/CW – Wheat bran and wet cotton wool. Each treatment was replicated four times. The nutritional composition for the formulated diets are as shown in Table 2.

Table 2: Diets proximate composition used for raising larval mealworms

Diet	Dry matter (%)	Crude protein (%)	Crude fat (%)	Ash (%)	Crude fiber (%)	Carbohydrates (%)	Energy (kcal/ 100 g)
WB (Control)	93.7 ± 0.42a	15.3 ± 0.11d	2.14 ± 0.02a	6.05 ± 0.34a	0.37 ± 0.01a	76.1 ± 0.25b	385.7 ± 1.36bc
WB/CL	93.3 ± 0.33a	14.1 ± 0.06c	3.21 ± 0.01b	10.0 ± 0.70b	0.33 ± 0.02a	72.3 ± 0.68a	375.4 ± 2.86a
WB/PP	92.0 ± 1.53a	11.7 ± 0.10a	3.26 ± 0.05a	6.17 ± 0.43a	0.36 ± 0.02a	78.5 ± 0.56c	390.9 ± 1.55c
WB/CL/PP	91.7 ± 1.45a	13.3 ± 0.20b	3.27 ± 0.05b	8.73 ± 0.14b	0.36 ± 0.00a	74.4 ± 0.39ab	380.7 ± 0.30ab
WB/CW	–	–	–	–	–	–	–
df	3, 8	3, 8	3, 8	3, 8	3, 8	3, 8	3, 8
F	1.242	137.5	204.2	18.78	1.818	27.68	14.14
P	0.341	< 0.001	< 0.001	< 0.001	0.222	< 0.001	0.001

WB (Control) – wheat bran; WB/CL – wheat bran and cabbage leaves at ratio of 2 : 1; WB/PP – wheat bran and pineapple waste at ratio of 2 : 1; WB/CL/PP – wheat bran, cabbage leaves and pineapple waste at ratio of 2 : 0.5 : 0.5 and WB/CW – wheat bran and wet cotton wool (water source). In the same column, values in each diets represent means (± standard error), with same letters showing no significance differences at $p < 0.05$. n = 3

3.5.2 Mealworm rearing protocol

Twenty thousand (20,000) 14 day–old larvae were randomly selected from the stock colony and divided into 20 groups, each of which contained 1000 larvae. A subset of 40 larvae was randomly chosen from each group and reared on smaller plastic food mate containers (Rectangle Food Mate No.1, Kenpoly Manufacturers Limited) with dimensions of 18.4 × 12.6 × 6.7 cm (Length × Width × Height) for fortnightly measurements. These containers were positioned within the larger plastic trays (Acme Containers Limited, Nairobi – Kenya) measuring 56 × 38 × 10 cm (Length × Width × Height), which held the remaining 960 larvae. Each of the five diets was replicated four times and experiments repeated once for each group. A 500 g chunk of the feed was given to the 960 larvae, while 20.83 g of the diet per 40 larvae was given (first experiment). Fresh green cabbage leaves weighing 38.4 g and 1.6 g were given to the 960 and 40 larvae respectively every week based on Kim *et al.* (2016) and Ortiz *et al.* (2016). In the second experiment, the diets were provided in ratios as highlighted in diet formulation.

3.5.3 Growth performance and survival

Mealworms growth performance was computed fortnightly for 40 larvae per treatment. The dead larvae were regularly removed to prevent the risk of transmitting pathogenic microbes to live larvae. The experiment was terminated upon the appearance of first pupa and observable latency for most larvae, with final computations done per treatment. The final substrates' (residues) and larval weight was also computed per treatment.

3.5.4 Bioconversion

Based on the overall final larval fresh weight for all harvested larvae and residual feed weight in each treatment, the ingested feed weight (IFW), weight gain (WG), feed conversion ratio (FCR)

as well as conversion efficiency of ingested feed (ECI) was computed. The FCR computation was based on Miech *et al.* (2016) whereas ECI estimation was in accordance to Waldbauer (1968) with the use of following equations;

Equation 3: Ingested feed weight computation

$$\text{IFW} = \text{Initial weight (feed)} - \text{residual weight (feed)} \quad \ldots\ldots (3)$$

Equation 4: Weight gain computation

$$\text{WG} = \text{final larval weight} - \text{Initial weight (larvae)} \quad \ldots\ldots (4)$$

Equation 5: Feed conversion ratio calculation

$$\text{FCR} = {}^{\text{ingested feed wweight}}/_{\text{weight gain}} \quad \ldots\ldots (5)$$

Equation 6: Formula for calculating efficiency of conversion of ingested feed

$$\text{ECI} = \frac{\text{Final larval weight}}{\text{Ingested feed weight}} \quad \ldots\ldots (6)$$

3.6 Sample preparation for proximate analysis

Fully grown mature larvae were subjected to a 24-hour starvation period to facilitate the removal of body waste and subsequently rendered inactive through freezing at a temperature of -80 ℃. Following this, the specimens were subjected to a drying process using an oven (WTC Binder, FD 115, Tuttlingen, Germany) set at 60 ℃, and this procedure continued until a consistent weight was achieved, a process which typically spanned around 48 hours. The desiccated larval specimens were then ground using a laboratory blender (KM – 400 mrc) and securely stored in hermetically sealed Ziplock bags. These bags were housed within a freezer unit (Samsung Freezer) that was maintained at a temperature of –20 ℃, ensuring their preservation until the point of nutritional analysis. Prior to the analysis, the samples were allowed to thaw at room temperature.

3.7 Nutritional analysis

Following Association of Official Analytical Chemists (AOAC, 1990) standard procedures, the diets' and larval dry matter, crude protein, crude fat and ash were determined. The crude fiber was determined in accordance to Weende (2019). The diets' and larval carbohydrates and energy contents were computed based on equations 20 and 21 respectively. The larval neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined in accordance to Goering and Van Soest (1970) and Van Soest, Robertson and Lewis (1991).

3.7.1 Dry Matter determination

The dry matter was determined using AOAC (1990), Method 930.15. Crucibles were weighed (W0) and a 1 g ground sample was weighed (WS) alongside the crucible. The samples were desiccated to room temperature after being oven dried (WTC Binder, FD 115, Tuttlingen, Germany) at 135 ℃ for two hours. Finally, oven dried weight (WS2) was obtained. The following equation was used to compute the percentage of dry matter:

Equation 7: Computation of diets and larval dry matter (%)

$$\%\mathrm{DM} = \frac{\mathrm{WS2}-\mathrm{W0}}{\mathrm{WS}-\mathrm{W0}} \times 100 \quad (7)$$

3.7.2 Ash content determination

The samples ash content was determined following AOAC, Method 942.05. This involved weighing dried crucible (W_0), adding 1 g of ground sample (W_S) and placing them in muffle furnace (Heraeus-Kundendienst, Dusseldoerf, Germany) at 550 ℃ for two hours. Furnace temperature was adjusted to 135 ℃, allowed to drop. The samples were cooled desiccator for 20

minutes and final sample weight recorded (W_{S2}). The percentage ash content was computed and expressed on dry matter basis as follows;

Equation 8: Larval and diets ash content calculation

$$\%\text{Ash} = \frac{WS2-W0}{WS-W0} \times 100 \quad \ldots\ldots (8)$$

Equation 9: Ash content in dry matter basis

$$\%\text{Ash DM} = \frac{\%\ \text{Ash}}{\text{Sample DM (fraction)}} \quad \ldots\ldots (9)$$

3.7.3 Determination of crude protein

The crude protein was determined using copper catalyst Kjeldahl method, AOAC, Method 984.13. Briefly, 1 g sample was weighed, 7.5 g catalyst (consisting of a mixture of potassium sulphate and copper sulphate at the ratio of 9:1) and 15 ml concentrated sulfuric acid added to the sample, including blanks. The samples were digested in DKL 20 Automatic Heating Digester, programmed as 32–P 1 at different temperature and time range of 200 ℃ for 15 minutes, 250 ℃ for 15 minutes, 350 ℃ for 30 minutes, and finally at 420 ℃ for 1 hour. Digested samples were cooled to room temperature and transferred to UDK 159 Automatic Distillation and Titration System, Velp Scientifica, Europe, for nitrogen concentration determination, whereby a nitrogen-protein conversion factor of 5.41 (Boulos, Tännler and Nyström, 2020) was used to compute crude protein content. The following equations were used to compute crude protein;

Equation 10: Crude protein (%) computation from % nitrogen concentration; F = 5.41

$$\%\ \text{CP} = \%\text{N} \times \text{F};\ \text{with F} = 5.41 \quad \ldots\ldots (10)$$

Equation 11: Crude protein (%) calculation on dry matter basis

$$\%\text{CP DM} = \frac{\%\ \text{CP}}{\text{Sample DM (fraction)}} \times 100 \quad \ldots\ldots (11)$$

3.7.4 Crude fat determination

The crude fat extraction was done using Randall Technique following AOAC, Method 920.29. The Soxhlet extractor (SER 148 RS 232, Velp Scientifica, Europe) and 70 ml diethyl ether (solvent) was used to extract fats. The extraction cups were dried for 30 minutes at 105 ℃, cooled in desiccator and weighed (W_0). The solvent was put in each extraction cup. One gram samples were weighed (W_S), tied in filter papers and then put in extraction thimbles. Thimble containing samples were immersed in boiling solvent for 30 minutes. The sample were washed for 60 minutes by raising thimble out of the solvent for further test sample extraction by continuous flow of condensed solvent. The solvent was again recovered through evaporation for 30 minutes. The extraction cups with fats were dried for another 30 minutes at 105 ℃ to remove last solvent traces and moisture. Samples were then desiccated and weighed (W_{S2}) and computation done as follows;

Equation 12: Crude fat (%) determination on dry matter basis

$$\%\ \text{Crude fat (DM)} = \frac{\text{WS2} - \text{W0}}{\text{WS} \times \text{DM (fraction)}} \times 100 \quad \ldots\ldots (12)$$

3.7.5 Crude fiber determination

The crude fiber was extracted using fiber analyzer (FIWE Raw Fiber Extractor, Velp Scientifica-Europe) in accordance to (Weende, 2019), AOAC 978.10 by solubilizing non-cellulosic compounds using sulfuric acid and sodium hydroxide solutions. In this case, 1 g sample and oven dried glass crucibles (dried at 135 ℃ for 2 hours) were weighed (F0) and placed in fiber analyzer. Sulfuric acid (1.25%) was added up to 150 ml notch and 5 drops of octan-1-ol (antifoam) added. The samples were preheated until onset of boiling upon which samples were heated for 30 minutes. The sulfuric acid was drained, and samples washed thrice with hot deionized water (each wash per sample 30 ml was used). This procedure was repeated in same samples using 1.25% sodium

hydroxide solution. Cold deionized water was used after washing thrice with hot deionized water, and finally 25 ml acetone used thrice to wash samples. The samples were oven dried for 1 hour at 105 ℃, cooled in desiccator and weighed (F1). Samples were then ashed for 3 hours in muffle furnace at 550 ℃ and reweighed after cooling (F2). The percent crude fiber computation was based on the following equation;

Equation 13: Crude fiber (%) computation

$$\text{Crude fiber (\%)} = \frac{\text{F1} - \text{F2}}{\text{F0}} \times 100 \quad \ldots\ldots (13)$$

3.7.6 Determination of Neutral Detergent Fibre (NDF)

Oven dried crucible were weighed (W_0), 1 g sample recorded and weighed together with crucibles (W_S) and fixed in fiber extractor. Neutral detergent solution (100 ml) was added and samples preheated until boiling onset upon which samples were heated for 1 hour at 100 ℃, filtered and washed three times with boiling water and twice with cold acetone. The samples were dried at 135 ℃ for 2 hours, cooled in desiccator and weighed (W_{ND}). They were ashed in muffle for 2 hours at 550 ℃, cooled in desiccator and weighed (W_A). The computations were done as follows

Equation 14: Neutral detergent fiber computation on dry matter basis

$$\text{NDF \% DM} = \frac{\text{WND} - \text{W0}}{(\text{WS} - \text{W0}) \times \text{DM(fraction)}} \times 100 \quad \ldots\ldots (14)$$

Equation 15: Neutral detergent solubles computation

$$\text{Neutral detergent solubles (NDS\%)} = 100 - \text{NDF \% DM} \quad \ldots\ldots (15)$$

Equation 16: Insoluble ash in NDF calculation

$$\text{Insoluble Ash in NDF} = \frac{\text{WA} - \text{W0}}{(\text{WS} - \text{W0}) \times \text{DM (fraction)}} \times 100 \quad \ldots\ldots (16)$$

3.7.7 Determination of Acid Detergent Fibre (ADF)

Similar procedure used in NDF determination was used for ADF determination. However, in ADF, an acid detergent solution (100 ml) was added instead of neutral detergent solution. The ADF computations was as follows

Equation 17: Acid detergent fiber (%) determination on dry matter basis

$$\%\ \text{ADF DM} = \frac{\text{WAD}-\text{W0}}{(\text{WS}-\text{W0})\times\text{DM(fraction)}} \times 100 \quad \ldots\ldots (17)$$

Equation 18: Insoluble ash in acid detergent fiber

$$\text{Insoluble Ash in ADF} = \frac{\text{WA}-\text{W0}}{(\text{WS}-\text{W0})\times\text{DM(fraction)}} \times 100 \quad \ldots\ldots (18)$$

Equation 19: Hemicellulose determination (%) on dry matter basis

$$\text{Hemicellulose}\ \%\ \text{DM} = \text{NDF}\ \%\ \text{DM} - \text{ADF}\ \%\text{DM} \quad \ldots\ldots (19)$$

The contents of carbohydrates were computed by subtracting crude fat, crude fiber, crude protein, as well as ash contents from 100%, with all expressed in dry matter basis.

Equation 20: Carbohydrates (%) computation

$$\%\ \text{Carbohydrates (CHO)} = 100 - \text{fat}\ \% - \text{Ash}\ \% - \text{Protein}\ \% - \text{crude fibre}\ \% \quad \ldots\ldots (20)$$

For the energy value (EV) given as kcal/100 g, the following formula will be used (Manzi, Aguzzi and Pizzoferrato, 2001);

Equation 21: Energy value calculation

$$\text{EV} = 4 \times \text{protein}\ \% + 4 \times \text{carbohydrate}\ \% + 2 \times \text{fibre}\ \% + 9 \times \text{lipid}\ \% \quad \ldots\ldots (21)$$

3.7.8 Amino acid determination

The analysis of the amino acid profile involved the following steps: 100 mg of each sample was carefully measured and placed into digestion vials. These samples were then subjected to hydrolysis by adding 1.5 ml of 6N HCl, followed by a one-minute vortexing, and digestion at 110 ℃ for a duration of 24 hours in GC oven (5890, Series II Gas Chromatography, Hewlett Packard). After the hydrolysis process, the resulting samples were transferred to Eppendorf tubes, subjected to centrifugation at 14000 rpm for 15 minutes, and then filtered before undergoing analysis using LC–MS.

The LC−MS procedure was executed with specific operational parameters: A quaternary LC pump (Model 1200) was coupled with Agilent MSD 6120–Single quadruple MS, equipped with an electrospray source in Palo Alto, CA. The chromatographic separation was carried out using an Agilent system 1100 series (MA, USA) with a ZORBAX SB–C18, 4.6 250 mm, 3.5 µm column, maintained at a constant temperature of 40 ℃. The mobile phases employed for separation were water (A) and a solution of 0.01% formic acid in acetonitrile (B). The gradient for separation included the following time intervals: 0 – 8 minutes, 10% B; 8 – 14 minutes, 10% – 100% B; 14 – 19 minutes, 100% B; 19 – 21 minutes, 100% – 10% B and 21 – 25 minutes, 10% B. The injection volume was 3 µL, and the flow rate was consistently maintained at 0.5 ml/min. The mass spectrometer, operating in ESI-positive mode, covered a mass range of m/z 50 – 600 with a cone voltage of 30 eV.

In order to facilitate external quantification, similar LC–MS analyses were performed on serial dilutions of an amino acid standard. This standard contained 18 amino acids in the range of 1-100 ng/µl and was sourced from Sigma-Aldrich in St. Louis, MO, USA. Linear calibration curves,

plotting peak area against concentration, were generated from these standard analyses. Additionally, three more amino acid analyses were conducted using various batches of samples for further evaluation and verification.

The chromatographic separation was carried out on an Agilent system 1100 series (MA, USA) with a ZORBAX SB-C18, 4.6 250 mm, 3.5 µm column, operated at 40 °C. The mobile phases used were water (A) and 0.01% formic acid in acetonitrile (B). The gradient used was 10% B for 0–8 min, 10% B for 8 – 14 min, 10% B for 14 – 19 min, 100% B for 19 – 21 min, 100% B for 100 – 10 min, and 10% B for 21 – 25 min. The flow rate was held constant at 0.5 ml per minute and the injection volume was 3 µL. The LC was interfaced with a triple mass spectrometer. The mass spectrometer was run in ESI-positive mode with a mass range of m/z 50 – 600 and a cone voltage of 70 eV.

Similar LC-MS studies were carried out on repeated dilutions of the real standard (1 – 105 g/l, Sigma-Aldrich, St. Louis, MO, USA), which comprised 18 amino acids. These analyses resulted in linear calibration curves (peak area vs. concentration), which were used for external quantification. Using different batches of samples, three more analyses of amino acids were carried out.

3.8 Data Analysis

For growth, survival and bioconversion performance, data from experimental cycles 1 and 2 were pooled during statistical analysis, giving a total of eight replications per treatment. The data were tested for normality using the Shapiro–Wilk test and homogeneity of variance using Bartlett test. Data that were normally distributed with homogenous variances were subjected to one-way Analysis of Variance to determine diet effects on mealworm growth and bioconversion performance as well as nutritional quality. The heterogeneous data were analyzed using Welch F test which takes into account unequal variances. No data transformation applied for non-normal data. Survival analysis was done using Generalized Linear Model fitted with negative binomial distribution, whereby the data were modelled to binary data. The GLM was applied since in survival, the distribution is normally screwed. Computation of least squares means was done using "lsmeans" package, followed by mean separation using adjusted Tukey's method at $p \leq 0.05$, implemented using "cld" function from the "multicompView" package. The standard error computation in survival was based on the following formula $\sqrt{p(1-p)/n}$ whereby, p = proportion of live larvae and n = sample size. The data were analyzed using R software version 4.2.1 for windows (R Core Team 2022).

CHAPTER FOUR

4.0 RESULTS

4.1 Experiment one

4.1.1 Effect of potato waste inclusion in wheat bran diet on *Tenebrio* sp. length, weight and survival

Incorporating potato waste into a diet based on wheat bran had a significant impact on the length and weight of mealworm larvae ($p < 0.001$), while it did not affect their survival ($p > 0.05$) (Table 3). Larvae reared on a diet consisting of wheat bran with 25%, 50%, and 75% inclusion of potato waste exhibited approximately a 2 mm increase in length and were 1- 2 times heavier compared to those raised solely on wheat bran or potato waste alone. The survival rate of the larvae in all treatments remained consistently high, ranging from 92.5% to 93.8% at the time of harvest.

Table 3: *Tenebrio* sp. [means (± standard error)] larval length, weight, and survival rate reared on wheat bran (WB) with different inclusion levels (25, 50, 75 and 100%) of potato waste (PW) (n = 8)

Parameter	Diet	Time (weeks)				
		2	4	6	8	9
Larval length (mm)	WB100 (Control)	6.35 ± 0.04a	8.48 ± 0.06a	12.59 ± 0.10a	15.08 ± 0.10b	16.29 ± 0.11b
	WB75/PW25	6.32 ± 0.04a	8.77 ± 0.06b	14.30 ± 0.11d	16.68 ± 0.10c	17.81 ± 0.11c
	WB50/PW50	6.37 ± 0.04a	8.95 ± 0.06b	13.55 ± 0.11c	16.83 ± 0.11c	17.62 ± 0.11c
	WB25/PW75	6.45 ± 0.04a	8.84 ± 0.06b	13.08 ± 0.10b	16.71 ± 0.10c	17.66 ± 0.11c
	PW100	6.45 ± 0.04a	8.92 ± 0.06b	13.21 ± 0.10bc	14.50 ± 0.10a	15.27 ± 0.09a
	df	4, 1595	4, 1521	4, 1501	4, 1491	4, 1487
	F	2.453	9.264	37.82	116	114.1
	p	0.044	< 0.001	< 0.001	< 0.001	< 0.001
Larval weight (mg)	WB100 (Control)	1.63 ± 0.07a	3.32 ± 0.12a	11.41 ± 0.45a	23.00 ± 0.59a	27.26 ± 0.52b
	WB75/PW25	1.59 ± 0.08a	3.63 ± 0.11a	19.48 ± 0.46c	37.24 ± 0.34b	40.90 ± 0.37c
	WB50/PW50	1.53 ± 0.07a	3.84 ± 0.15a	20.17 ± 0.44c	35.32 ± 0.42b	39.77 ± 0.79c
	WB25/PW75	1.75 ± 0.07a	3.84 ± 0.16a	21.85 ± 1.05c	35.65 ± 1.12b	38.70 ± 1.39c
	PW100	1.56 ± 0.10a	3.84 ± 0.16a	16.75 ± 0.25b	22.32 ± 0.55a	22.86 ± 0.67a
	df	4, 35	4, 35	4, 35	4, 35	4, 35
	F	1.131	2.716	47.15	123	100.1
	p	0.358	0.045	< 0.001	< 0.001	< 0.001
Survival (%)	WB100 (Control)	100 ± 0	95 ± 0.04	94.4 ± 0.04	93.4 ± 0.04	93.4 ± 0.04
	WB75/PW25	100 ± 0	95.6 ± 0.03	93.4 ± 0.04	93.4 ± 0.04	93.1 ± 0.04
	WB50/PW50	100 ± 0	95.9 ± 0.03	94.4 ± 0.04	94.4 ± 0.04	93.8 ± 0.04
	WB25/PW75	100 ± 0	95.3 ± 0.03	94.1 ± 0.04	93.8 ± 0.04	93.4 ± 0.04
	PW100	100 ± 0	95 ± 0.04	93.4 ± 0.04	92.5 ± 0.04	92.5 ± 0.04
	df	4	4	4	4	4
	X^2	0	0.022	0.031	0.962	0.031
	p	1	0.999	0.999	0.916	0.999

Within each column, means followed by the same lowercase letter show no significant different, whereas, different lowercase letters within each column indicate larval significant differences for different treatments at $\alpha = 0.05$. Where, WB100 – 100% wheat bran (control); WB75/PW25 – 75% wheat bran and 25% potato waste; WB50/PW50 – 50% wheat bran and 50% potato waste; WB25/PW75 – 25% wheat bran and 75% potato waste; and PW100 – 100% potato waste.

4.1.2 Bioconversion performance of *Tenebrio* sp. fed on different formulated diets.

The final weight of the larvae, weight gain, ingested feed weight, efficiency of conversion of ingested feed, and feed conversion ratio were all significantly influenced ($p < 0.001$) by the varying ratios of wheat bran and potato waste in the diets of the larvae (Table 4). Notably, the initial weight of the larvae showed no significant variation ($F_{4,35} = 0.4$, $p = 0.809$). At the time of harvest, the final weights of the larvae and their weight gain were comparable in diets with 25% and 50% potato waste, where they were approximately 1 to 2 times heavier. The quantity of feed consumed by the larvae decreased with an increase in potato waste proportion. Additionally, the larval feed conversion ratio was 1 to 2 times higher in larvae raised on a diet consisting solely of wheat bran compared to diets with potato waste.

Table 4: Feed conversion parameters [means (± standard error)] of *Tenebrio* sp. larvae raised on wheat bran (WB) with different inclusion levels of irish potato wastes (PW) (n = 8).

Diets	WB100 (Control)	WB75/PW25	WB50/PW50	WB25/PW75	PW100	df	F	p
Initial weight (g)	1.37 ± 0.003a	1.36 ± 0.003a	1.37 ± 0.005a	1.37 ± 0.005a	1.37 ± 0.003a	4, 35	0.4	0.809
Final weight (g)	34.35 ± 0.55b	47.40 ± 0.74d	47.43 ± 0.87d	43.96 ± 0.82c	23.46 ± 0.57a	4, 35	206	< 0.001
Weight gain (g)	32.98 ± 0.55b	46.04 ± 0.74d	46.06 ± 0.87d	42.59 ± 0.82c	22.10 ± 0.56a	4, 35	207	< 0.001
IFW (g)	110.6 ± 2.01d	105.5 ± 3.13cd	98.89 ± 0.69c	80.82 ± 1.35b	45.30 ± 0.46a	4, 35	215	< 0.001
FCR	3.26 ± 0.10c	2.29 ± 0.07b	2.15 ± 0.05ab	1.90 ± 0.03a	2.06 ± 0.04ab	4, 35	73.5	< 0.001
ECI (%)	31.13 ± 0.80a	45.14 ± 1.17b	47.99 ± 1.00bc	54.42 ± 0.88d	51.77 ± 1.04cd	4, 35	84.9	< 0.001

Within each row, means followed by the same lowercase letter show no significant different, whereas, different lowercase letters within each row indicate larval significant differences for different treatments at $\alpha = 0.05$. Where, IFW – Ingested feed weight, FCR – Feed conversion ratio and ECI – Efficiency of Conversion of Ingested Feed. WB100 – 100% wheat bran (control); WB75/PW25 – 75% wheat bran and 25% potato waste; WB50/PW50 – 50% wheat bran and 50% potato waste; WB25/PW75 – 25% wheat bran and 75% potato waste; and PW100 – 100% potato waste.

4.1.3 Variations in the proximate composition of *Tenebrio* sp. larvae across diverse feeding regimens

The larval composition in terms of dry matter, ash, crude fiber, and neutral detergent fiber exhibited no significant variations ($p > 0.05$) based on the type of diet, as indicated in Table 5. However, the content of crude protein (CP) in the larvae was significantly influenced by the dietary compositions ($p < 0.001$). As the proportion of potato waste increased in the diet, the CP content decreased. Furthermore, the acid detergent fiber content was 2 to 4 times higher in larvae raised solely on wheat bran. In contrast, the crude fat content in the larvae remained similar when fed diets with 75% and 100% potato waste. It is worth noting that the energy content was highest in the larvae reared on a diet comprising 75% potato waste.

Table 5: Nutritional composition [means (± standard error)] of *Tenebrio* sp. larvae fed on wheat bran (WB) with different inclusion levels (25, 50, 75 and 100%) of irish potato waste (PW), with n = 4 for dry matter, crude protein, crude fat, ash and the rest n = 3).

Nutritional contents	WB (Control)	WB75/PW25	WB50/PW50	WB25/PW75	PW100	df	F	p
Dry matter (%)	88.8 ± 1.3a	90.3 ± 0.5ab	89.8 ± 0.9ab	90.8 ± 0.9ab	92.5 ± 0.7b	4,15	2.625	0.076
Crude protein (%)	55.4 ± 1.2c	50.1 ± 0.5b	48.3 ± 0.5b	47.8 ± 0.4b	43.3 ± 0.8a	4,15	34.18	< 0.001
Crude fat (%)	34.9 ± 1.0a	37.4 ± 0.6a	38.4 ± 0.7a	47.7 ± 1.4b	44.6 ± 1.6b	4,15	22.56	< 0.001
Ash (%)	13.8 ± 1.1a	10.8 ± 2.4a	9.7 ± 0.9a	9.9 ± 1.4a	11.1 ± 2.0a	4,15	0.976	0.45
Crude fiber (%)	0.22 ± 0.00a	0.22 ± 0.04a	0.21 ± 0.01a	0.23 ± 0.00a	0.25 ± 0.02a	4,10	0.676	0.624
Carbohydrates (%)	0.0 ± 0.0a	4.3 ± 1.2b	2.3 ± 0.4b	0.0 ± 0.0a	4.5 ± 2.4b	4,10	7.993	0.004
Energy (kcal/ 100 g)	517.9 ± 4.3a	550.7 ± 5.9b	553.8 ± 3.6b	609.0 ± 1.3d	580.0 ± 7.6c	4,10	46.45	< 0.001
NDF	14.7 ± 0.8a	12.6 ± 1.3a	14.0 ± 1.1a	12.8 ± 0.6a	12.9 ± 0.5a	4,10	0.971	0.465
ADF	30.3 ± 3.1b	15.6 ± 0.7a	13.3 ± 1.4a	8.0 ± 1.2a	10.1 ± 0.4a	4,10	28.68	< 0.001

Within each row, means followed by the same lowercase letter show no significant different, whereas, different lowercase letters within each row indicate larval significant differences for different treatments at $\alpha = 0.05$. Where, NDF – Neutral Detergent Fiber, ADF – Acid Detergent Fiber. WB100 – 100% wheat bran (control); WB75/PW25 – 75% wheat bran and 25% potato waste; WB50/PW50 – 50% wheat bran and 50% potato waste; WB25/PW75 – 25% wheat bran and 75% potato waste; and PW100 – 100% potato waste.

4.4 *Tenebrio* sp. amino acid profile across different treatments

Among the 14 detected amino acids in the larvae, six (arginine, histidine, lysine, threonine, glycine, and alanine) exhibited varying levels across the different dietary conditions, as outlined in Table 6. Notably, isoleucine and leucine content was significantly elevated in the larvae reared on a diet solely based on wheat bran. Methionine and proline concentrations were notably higher in the larvae fed a diet comprising 75% wheat bran and 25% potato waste. Phenylalanine content was significantly greater in larvae that received a wheat bran-based diet compared to those fed diets containing 0% to 50% wheat bran. Additionally, glutamic acid levels were higher in larvae nourished by diets consisting of either wheat bran exclusively or a wheat bran-based diet with 25% potato waste substitution.

Table 6: Mean ± standard error of mean of *Tenebrio* sp. amino acid composition; and the analysis of variance (ANOVA) summary at 0.05 significance level

Amino acid (mg/100 g)	Diet					df	*F*	*P*
	WB100	WB75/PW25	WB50/PW50	WB25/PW75	PW100			
Arginine*	1.88 ± 0.06a	1.91 ± 0.03a	1.86 ± 0.06a	2.06 ± 0.06a	1.90 ± 0.14a	4, 15	0.968	0.454
Histidine*	1.28 ± 0.05a	1.37 ± 0.04a	1.36 ± 0.08a	1.42 ± 0.02a	1.26 ± 0.06a	4, 15	1.429	0.272
Isoleucine*	1.30 ± 0.04b	1.13 ± 0.02ab	1.02 ± 0.10a	1.14 ± 0.03ab	1.17 ± 0.03ab	4, 15	3.487	0.033
Leucine*	2.02 ± 0.08b	1.78 ± 0.04ab	1.38 ± 0.17a	1.63 ± 0.07ab	1.53 ± 0.06a	4, 15	6.623	0.003
Lysine*	1.89 ± 0.02a	1.71 ± 0.08a	1.59 ± 0.14a	1.97 ± 0.02a	1.91 ± 0.13a	4, 15	2.84	0.062
Methionine*	0.67 ± 0.02ab	0.67 ± 0.01b	0.51 ± 0.07a	0.60 ± 0.04ab	0.63 ± 0.03ab	4, 15	3.249	0.042
Phenylalanine*	1.73 ± 0.09b	1.54 ± 0.04ab	1.07 ± 0.19a	1.26 ± 0.09a	1.17 ± 0.06a	4, 15	6.427	0.003
Threonine*	0.91 ± 0.02a	0.95 ± 0.01a	0.81 ± 0.08a	0.86 ± 0.03a	0.86 ± 0.03a	4, 15	1.57	0.233
Valine*	1.44 ± 0.02c	1.38 ± 0.02bc	1.11 ± 0.11a	1.20 ± 0.04ac	1.17 ± 0.05ab	4, 15	5.985	0.004
Alanine	2.23 ± 0.05a	2.27 ± 0.06a	1.88 ± 0.20a	2.09 ± 0.08a	2.24 ± 0.09a	4, 15	2.181	0.121
Glycine	2.42 ± 0.07b	2.30 ± 0.05ab	1.88 ± 0.23a	2.21 ± 0.10ab	2.18 ± 0.06ab	4, 15	2.748	0.068
Glutamic acid	2.16 ± 0.04b	2.22 ± 0.03b	1.51 ± 0.21a	1.80 ± 0.11ab	1.82 ± 0.09ab	4, 15	6.547	0.003
Proline	1.08 ± 0.02ab	1.13 ± 0.04b	0.89 ± 0.09a	0.94 ± 0.02ab	0.96 ± 0.03ab	4, 15	4.706	0.012
Tyrosine	1.56 ± 0.01c	1.52 ± 0.04c	1.25 ± 0.11ab	1.40 ± 0.06bc	1.08 ± 0.03a	4, 15	10.84	< 0.001

Asterisks indicate essential amino acids. WB100 – 100% wheat bran; WB75/PW25 – 75% wheat bran and 25% potato waste; WB50/PW50 – 50% wheat bran and 50% potato waste; WB25/PW75 – 25% wheat bran and 75% potato waste; and PW100 – 100% potato waste.

4.2. Second experiment

4.2.1 Effect of dried pineapple peels and cabbage leaves inclusion in wheat bran diet on mealworm growth and survival

The introduction of wet cotton wool as a water source in the mealworm wheat bran diet had a significant impact ($p < 0.001$) on the weight and length of the larvae, while their survivability remained largely unaffected ($p > 0.05$) (Table 7). In contrast, the addition of pineapple waste and cabbage waste to the mealworm wheat bran diet resulted in larvae with comparable weight and length. Larvae raised on a wheat bran diet supplemented with wet cotton were notably longer by approximately 5 mm and 2 to 3 times heavier when compared to those reared on alternative diets. As of the harvest, the survival of mealworms in all treatment groups fell within the range of 91.6% to 95.3%.

Table 7: *Tenebrio* sp. [means (± standard error)] larval length, weight, and survival rate reared on wheat bran (WB), cabbage leaves and pineapple peels at different inclusion ratios.

Parameter	Diet	Time (weeks)				
		2	4	6	8	10
Larval length (mm)	WB	6.42 ± 0.04ab	8.61 ± 0.04a	11.3 ± 0.06a	12.3 ± 0.08ab	14.2 ± 0.09a
	WB/CL	6.55 ± 0.04b	9.05 ±0.27ab	11.0 ± 0.06a	12.2 ± 0.08a	13.9 ± 0.09a
	WB/PP	6.35 ± 0.04a	9.14 ± 0.05b	12.4 ± 0.10c	13.0 ± 0.09c	14.2 ± 0.34a
	WB/CL/PP	6.40 ± 0.04a	8.96± 0.05ab	11.7 ± 0.08b	12.7 ± 0.08bc	13.7 ± 0.09a
	WB/CW	6.39 ± 0.04a	8.97 ± 0.05ab	13.5 ± 0.12d	16.0 ± 0.13d	19.0 ± 0.14b
	df	4, 1595	4, 1528	4, 1524	4, 1514	4, 1496
	F	4.578	2.525	127.4	264.7	162.9
	p	0.001	0.039	< 0.001	< 0.001	< 0.001
Larval weight (mg)	WB	1.65 ± 0.07a	3.15 ± 0.14a	7.32 ± 0.33a	12.5 ± 0.79a	19.5 ± 0.83a
	WB/CL	1.69 ± 0.09a	3.34 ±0.16ab	8.62 ± 0.26a	13.1 ± 0.26a	17.4 ± 0.38a
	WB/PP	1.53 ± 0.06a	3.88 ± 0.13b	12.5 ± 0.35b	16.6 ± 0.27a	19.4 ± 0.68a
	WB/CL/PP	1.59 ± 0.08a	3.52 ±0.18ab	9.80 ± 0.22ab	14.0 ± 0.31a	17.3 ± 0.55a
	WB/CW	1.63 ± 0.07a	3.69 ±0.11ab	16.7 ± 1.65c	32.2 ± 2.42b	53.9 ± 3.87b
	df	4, 35	4, 35	4, 35	4, 35	4, 35
	F	0.671	3.944	22.43	50.89	76.63
	p	0.617	0.009	< 0.001	< 0.001	< 0.001
Survival (%)	WB	100 ± 0	95.6 ± 0.03	95.0 ± 0.04	94.7 ± 0.04	94.1 ± 0.04
	WB/CL	100 ± 0	95.4 ± 0.03	95.0 ± 0.04	94.1 ± 0.04	91.6 ± 0.05
	WB/PP	100 ± 0	96.5 ± 0.03	96.3 ± 0.03	96.3 ± 0.03	95.3 ± 0.03
	WB/CL/PP	100 ± 0	95.0 ± 0.04	94.7 ± 0.04	93.8 ± 0.04	91.9 ± 0.04
	WB/CW	100 ± 0	97.5 ± 0.03	96.9 ± 0.03	95.9 ± 0.03	95.3 ± 0.03
	df	4	4	4	4	4
	X^2	0	0.206	0.120	0.167	0.452
	p	1	0.998	0.998	0.997	0.978

WB (Control) – wheat bran; WB/CL – wheat bran and cabbage leaves at ratio of 2: 1; WB/PP – wheat bran and pineapple waste at ratio of 2: 1; WB/CL/PP – wheat bran, cabbage leaves and pineapple waste at ratio of 2: 0.5: 0.5 and WB/CW – wheat bran and wet cotton wool (water source). In the same column, the same letters show no significance differences at $p = 0.05$. n = 8

4.2.2 Effect of dried cabbage and pineapple peels inclusion in wheat bran on mealworm bioconversion

The final weight, weight gain, feed ingestion, feed conversion ratio, and ingested feed conversion efficiency of *Tenebrio* sp. were all influenced by the diet type (Table 8) with p-values less than 0.001. The initial larval weight, on the other hand, exhibited no significant difference ($p = 0.302$) among the groups. Notably, mealworms reared on a wheat bran diet with wet cotton supplementation displayed a weight gain and final weight that were 2-3 times higher, as well as ingesting 1-2 times more feed and achieving greater efficiency in converting ingested feed compared to their counterparts raised on other tested diets. Furthermore, the feed conversion ratio was lower for mealworms in this group in comparison to those on the alternative diets.

Table 8: Feed conversion parameters [means (± standard error)] of *Tenebrio* sp. larvae raised on wheat bran (WB) with different inclusion levels cabbage leaves and pineapple peels (n = 8).

Diets	Initial weight (g)	Final weight (g)	Weight gain (g)	Ingested feed weight (g)	Feed conversion ratio	Efficiency of conversion of ingested feed (%)
WB	1.37 ± 0.00a	21.8 ± 0.37a	20.5 ± 0.37a	120.1 ± 3.01ab	5.88 ± 0.13b	18.2 ± 0.42a
WB/CL	1.37 ± 0.00a	19.3 ± 0.73a	18.0 ± 0.73a	113.2 ± 0.84a	6.38 ± 0.27b	17.1 ± 0.69a
WB/PP	1.36 ± 0.00a	21.4 ± 0.90a	20.0 ± 0.90a	140.4 ± 1.42c	7.12 ± 0.35b	15.3 ± 0.69a
WB/CL/PP	1.37 ± 0.00a	20.1 ± 0.99a	18.7 ± 0.99a	131.4 ± 1.41bc	7.16 ± 0.40b	15.3 ± 0.76a
WB/CW	1.36 ± 0.00a	49.2 ± 4.28b	47.8 ± 4.28b	200.2 ± 6.77d	4.40 ± 0.36a	24.5 ± 1.93b
df	4, 35	4, 35	4, 35	4, 35	4, 35	4, 35
F	1.265	39.28	39.29	100.9	12.64	13.32
p	0.302	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

WB (Control) – wheat bran; WB/CL – wheat bran and cabbage leaves at ratio of 2: 1; WB/PP – wheat bran and pineapple waste at ratio of 2: 1; WB/CL/PP – wheat bran, cabbage leaves and pineapple waste at ratio of 2: 0.5 : 0.5 and WB/CW – wheat bran and wet cotton wool (water source). In the same column, the same letters show no significance differences at $p = 0.05$. n = 8

4.2.3 Mealworm nutritional composition reared on different formulated substrates

Tenebrio sp. did not display significant differences ($p > 0.05$) in dry matter, carbohydrates, crude fiber, ash, and neutral detergent fiber content when subjected to various diets, as shown in Table 9. However, the composition of the diets significantly impacted larval crude fat, crude protein, and energy content (kcal/100 g), with a minor distinction in acid detergent fiber ($p = 0.045$). Notably, the larvae reared on a wheat bran diet with wet cotton supplementation exhibited the highest levels of energy and crude fat content.

Table 9: Nutritional composition [means (± standard error)] of *Tenebrio* sp. larvae fed on wheat bran (WB), cabbage leaves (CL) and pineapple peels (PP) at different inclusion ratios

Diet	WB	WB/CL	WB/PP	WB/CL/PP	WB/CW	df	F	P
Dry matter (%)	96.3 ± 0.25a	96.5 ± 0.29a	96.8 ± 0.49a	96.8 ± 0.49a	97.5 ± 0.29a	4, 15	1.591	0.228
Crude protein (%)	56.1± 0.59bc	57.1 ± 0.65c	54.5 ± 0.21b	55.5± 0.21bc	48.2 ± 0.15a	4, 15	70.16	< 0.001
Crude fat (%)	25.2±0.82ab	23.6 ± 0.61a	26.9 ± 0.45b	24.6±0.37ab	34.6 ± 0.90c	4, 15	45.37	< 0.001
Ash (%)	8.83 ± 1.09a	5.95 ± 1.06a	6.98 ± 0.80a	5.42 ± 0.47a	5.39 ± 0.49a	4, 15	3.075	0.049
Crude fiber (%)	0.26 ± 0.00a	0.29 ± 0.00a	0.29 ± 0.01a	0.29 ± 0.01a	0.26 ± 0.02a	4, 10	1.71	0.224
Carbohydrates (%)	9.26 ± 0.92a	13.1 ± 1.36a	10.8 ± 1.49a	14.3 ± 0.39a	11.3 ± 1.12a	4, 10	3.061	0.069
Energy (kcal/ 100 g)	483.5 ± 5.46a	493.2± 4.16ab	506.3 ± 1.90b	499.5± 4.51ab	547.4 ± 6.44c	4, 10	26.92	< 0.001
NDF	19.7 ± 6.27a	13.8 ± 3.47a	16.9 ± 1.91a	14.1 ± 1.88a	13.7 ± 0.66a	4, 10	0.591	0.677
ADF	18.7 ± 1.20ab	16.9 ± 1.19ab	16.2 ± 2.35ab	20.3 ± 0.88b	13.4 ± 0.60a	4, 10	3.608	0.045

WB (Control) – wheat bran; WB/CL – wheat bran and cabbage leaves at ratio of 2: 1; WB/PP – wheat bran and pineapple waste at ratio of 2: 1; WB/CL/PP – wheat bran, cabbage leaves and pineapple waste at ratio of 2: 0.5: 0.5 and WB/CW – wheat bran and wet cotton wool (water source). The same letters within the same row show no significance differences at $p = 0.05$. n = 3

4.2.4 The mealworm amino acid composition based on reared diets

Among the 14 amino acids examined, the levels of isoleucine, lysine, threonine, alanine, glycine, and tyrosine in mealworms were not significantly ($p > 0.05$) influenced by diet composition (Table 10). Conversely, mealworms raised on a diet that combined wheat bran, pineapple waste, and cabbage leaves exhibited elevated levels of methionine, leucine, phenylalanine, and valine. In contrast, *Tenebrio* sp. fed exclusively with wheat bran demonstrated higher glutamic acid and proline contents, while those nourished with wheat bran supplemented by wet cotton wool had increased arginine and histidine levels.

Table 10: Mean ± standard error of mean of *Tenebrio* sp. amino acid composition; and the analysis of variance (ANOVA) summary at 0.05 significance level

Amino acid (mg/100 g)	Diet					df	*F*	*P*
	WB	WB/CL	WB/PP	WB/CL/PP	WB/CW			
Arginine*	1.81 ± 0.04ab	1.73 ± 0.03ab	1.59 ± 0.07a	1.77 ± 0.05ab	1.91 ± 0.05b	4, 15	5.278	0.007
Histidine*	1.30 ± 0.03bc	1.23 ± 0.01ac	1.10 ± 0.04a	1.18 ± 0.04ab	1.37 ± 0.06c	4, 15	7.557	0.002
Isoleucine*	1.24 ± 0.04a	1.24 ± 0.05a	1.24 ± 0.04a	1.11 ± 0.05a	1.21 ± 0.06a	4, 15	1.535	0.242
Leucine*	1.89 ± 0.02ab	1.85 ± 0.09ab	2.03 ± 0.08b	1.58 ± 0.07a	1.73 ± 0.09ab	4, 15	5.121	0.008
Lysine*	1.64 ± 0.02a	1.67 ± 0.02a	1.39 ± 0.04a	1.60 ± 0.11a	1.79 ± 0.19a	4, 15	2.066	0.136
Methionine*	0.68 ± 0.01b	0.68 ± 0.04b	0.70 ± 0.02b	0.57 ± 0.03a	0.59 ± 0.02ab	4, 15	5.525	0.006
Phenylalanine*	1.56 ± 0.00ab	1.58 ± 0.11ab	1.71 ± 0.07b	1.25 ± 0.07a	1.48 ± 0.09ab	4, 15	4.601	0.010
Threonine*	0.96 ± 0.03a	0.92 ± 0.03a	0.92 ± 0.04a	0.81 ± 0.05a	0.90 ± 0.03a	4, 15	2.367	0.099
Valine*	1.43 ± 0.02ab	1.37 ± 0.08ab	1.48 ± 0.03b	1.22 ± 0.04a	1.28 ± 0.05ab	4, 15	4.59	0.013
Alanine	2.01 ± 0.05a	2.02 ± 0.12a	2.09 ± 0.05a	1.81 ± 0.09a	1.90 ± 0.14a	4, 15	1.264	0.327
Glycine	2.41 ± 0.06a	2.43 ± 0.14a	2.42 ± 0.10a	2.05 ± 0.10a	2.41 ± 0.16a	4, 15	2.076	0.135
Glutamic acid	2.08 ± 0.07b	1.89 ± 0.10ab	2.01 ± 0.04b	1.59 ± 0.10a	1.95 ± 0.11ab	4, 15	4.797	0.011
Proline	1.09 ± 0.02b	1.02 ± 0.04ab	1.06 ± 0.02b	0.84 ± 0.07a	0.99 ± 0.05ab	4, 15	4.612	0.013
Tyrosine	1.51 ± 0.05a	1.40 ± 0.06a	1.53 ± 0.08a	1.36 ± 0.05a	1.50 ± 0.03a	4, 15	1.88	0.165

Asterisks indicate essential amino acids. WB – wheat bran; WB/CL – Wheat bran and cabbage leaves at 2:1; WB/PP – Wheat bran and pineapple peels at 2:1; WB/CL/PP – Wheat bran, cabbage leaves and pineapple peels at 2:0.5:0.5; WB/CW – Wheat bran and wet cotton wool. The same letters within the same row show no significance differences at $p = 0.05$. n = 3

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATION

In this study, the *Tenebrio* sp. performance on diets that contained various ratios of wheat bran, potato wastes, cabbage leaves and pineapple peels was assessed. In each experiment set, consistency and similar outputs was observed. The diets standardization, constant maintenance of rearing conditions may be attributed to results consistency. The wheat bran-potato waste mixture diets led to improved growth performance, pronounced nutritional quality, and higher survival across all the treatments. On the other hand, the mealworm performance when reared using organic wastes from fruits and vegetable origin showed similar growth performance, survival and slight variation in nutritional contents. However, the provision of wet cotton wool in diet significantly led to improved length, weight, fat, energy and bioconversion performance.

Despite having similar amounts of crude fiber, crude fat, and dry matter, examination of the various diets studied showed that mixed meals were higher in ash (the mineral component) and lower in carbohydrate and calories than single diets. According to Chapman (2012), insects need certain minerals as coenzymes and as metalloenzymes. More research is necessary to establish which individual minerals and in what amounts are essential for raising *Tenebrio* sp. while avoiding metal bioaccumulation (Pinotti and Ottoboni, 2021).

Not all sugars are useable by all insects, and some monosaccharides can be harmful because they compete with other necessary sugars, which may explain *Tenebrio* sp. poor performance in the carbohydrate and energy dense sole diets (Kraus *et al.*, 2019). Therefore, an optimum level of energy and carbohydrates in mealworm diets should be established. Potato waste had the lowest protein level, but in the mixed diets, this was considerably improved, with the diet consisting of

25% wheat bran and 75% potato waste matching the protein content of pure wheat bran. The diet comprising of mixture of wheat bran, pineapple peels and cabbage leaves also had poor protein composition compared to wheat bran alone. In insects, protein is essential for a variety of biological processes such cell construction, enzymes, storage and transport, or receptor molecules (Kraus *et al.*, 2019). Future research on replacing wheat bran in the diet of *Tenebrio* sp. should carefully investigate other protein-rich locally accessible substrates because potato peels, cabbage leaves and pineapple peels were poor in protein.

In first experiment, the mealworms raised on wheat bran -potato waste were noticeably longer and heavier than those raised solely on wheat bran and potato waste. The larvae raised on mixed wheat bran diets likely balanced the nutritional contents in different proportionate diets provided (Morales-Ramos *et al.*, 2011, 2013, 2020). In second experiment, the larvae raised on wheat bran diet with wet cotton wool supplementation experienced higher length and weight performance. The provision of wet cotton wool as water source facilitated larval growth hence growing much longer and heavier.

In the first experimental set up, the larval mealworm weight at harvest in the mixed diets ranged from 38.7 to 40.9 mg per larva. This supports the findings of a study whereby mealworms were raised on organic vegetable wastes until they reached an average weight of 41 mg (Harsányi *et al.*, 2020). However, mealworms raised utilizing high protein concentrations (Van Broekhoven *et al.*, 2015) and cookies and brewer's spent grain at a ratio of 1:1 (Mancini *et al.*, 2019) were reported to have astonishingly higher average larval weights of 140 mg and 168 mg per larva, respectively. Additionally, Kim *et al.* (2016) reported a higher average mealworm weight ranging from 176 mg to 198 mg when fed on a mixture of wheat bran and brewer's spent grain. The wheat and potato waste mixed diets produced longer larvae 17 mm to 18 mm at harvest stage considering. In second

experimental, the larvae raised on wheat bran diet with wet cotton supply recorded remarkably high weight of 53.9 mg, that was 2 – 3 times higher compared to other diets tested. Lengthwise, similar diet produced longer larvae of 19 mm. Thus, for better mealworm growth, water provision in critical in rearing system. Unfortunately, there is no available literature comparing mealworm length performance based on provided diets. There is a need for additional research to improve the performance of the mealworms, based on various diets and study locations.

The investigated feeding treatments had no statistically significant impact on larval *Tenebrio* sp. survival rates, which was high and ranged from 92.5% to 93.8% (first experiment) and 91.6% to 95.3% (second experiment). The remarkable survival rate in this study can be due to sufficient food supply and stocking density per treatment, which remarkably lead to low intraspecific competition for the limited food and space. The mealworms capacity to use a variety of agricultural organic wastes is as well a crucial factor in the high survival rates seen in this study. The survival of mealworm larvae in this study closely supports the findings of Bordiean *et al.* (2022), who found that 92% to 98% of mealworms survived when raised on wheat bran 100% and mixture of Willowleaf sunflower 25% and 75% chicken feed. As opposed to this, (van Broekhoven *et al.*, 2015) reported a wider range of of 71 – 91% when mealworms were fed on commercial diets (Control B–Tm/Za and B–Ad from insect rearing companies) and a high-protein/low starch diet (comprising of spent grains, bread remains, beer yeast, and maize distillers' dried grains with solubles at 30%, 10%, 40%, and 20%, respectively). Divergent results on the impact of diet on mealworm survival have also been reported by Oonincx *et al.* (2015) who found significant survival reduction by 15 – 19% on diets with low protein and high fat, and 52–80% survival rate on larval fed on diets with high protein and low fat as reported by Mlček *et al.* (2021). Silva *et al.* (2021) reported that the survival rate for mealworms fed on poultry litter, which is made up of

chicken waste and rice husks, ranged from 66.8% to 81.3%, when the control diet (barley, milk, chicken feed, oats, and wheat bran at a ratio of 1:1:2:3:3) was substituted with the other four poultry litter diets at 25%, 50%, 75%, and 100%. According to Deruytter and Coudron (2022), weekly mealworm feeding with freshly formulated diets and regular frass removal decreased mealworm survival from 97.4% to 87%. Given these conflicting results regarding the performance of mealworms on wheat bran and other diet compositions in different locations and rearing practices, there is a need for more in-depth research on the factors influencing the insect's performance based on diet, location, rearing practices, and environmental conditions

In many scenarios, the diet composition (Scriber and Slansky, 1981) and insect use (either animal feed or human food) determines how efficient feed is converted to body mass (Oonincx *et al.*, 2015), with insects for food being less efficient converters compared to animal feed insect species. The larval *Tenebrio* sp. raised on wheat bran mixes and solely raised on potato waste efficiently utilized provided diets, with FCR ranging from 1.90 to 2.29 and ECI between 45.14% to 54.42% as opposed to larvae raised solely on wheat bran diets. The higher ECI values on diet mixes meant that larvae exposed to those diets used ingested feed efficiently converting it to body mass.

In the second experiment, feed consumption was high and conversion of ingested feed to body mass was low as indicated by higher FCR values ranging from 4.40 to 7.16 and lower ECI of 15.3% to 24.5%. The higher ECI in wheat bran diet with wet cotton supplementation meant that, water provision in mealworm diets significantly improves nutrients intake. A study by Bordiean *et al.* (2020) reported mealworms FCR ranging from 1.57 – 2.08 when reared on chicken feed and 100 % wheat bran respectively, with diets containing wheat bran having slightly higher values as opposed to willowleaf sunflower diet that contributed significantly highest FCR of 4.42. Similar FCR of 2.62 to 6.05 were reported by van Broekhoven *et al.* (2015) depending on diet composition.

Huge divergent FCR of 3.8 to 19.1 were observed by Oonincx *et al.* (2015) when mealworms were fed on diets made up of different proteins, fats proportions, and carrot supplementation.

Generally, mealworm ECI values are influenced by diet quality, insect species and protein content whereby, a higher protein leads to high ECI values as observed in this study and in comparison to study carried out by Bordiean *et al.* (2022). Water provision in form wet cotton led to increased ingested feed conversion hence water is essential for nutrients intake. van Broekhoven *et al.* (2015) reported mealworm ECI of 16.8 – 28.9% when fed on low protein- high starch and high protein-high starch respectively. Bordiean *et al.* (2020) reported larval mealworm ECI values of 23.3% and 23.9%, on mealworms reared using 25% willowleaf sunflower/ 75% chicken feed and 25% rapeseed meal/ 75% chicken feed respectively. Bordiean *et al.* (2022) reported mealworm ECI values of 40.1%, 49.4% and 50.1% for mealworms fed on 100% rye bran, rapeseed meal and rapeseed cake respectively. In comparison to other species, a higher ECI of 59% was reported for house crickets (Collavo *et al.*, 2005). Oonincx *et al.* (2015) reported different ECI ranges of 3 – 9% (control 12%), 17 – 24% and 16 – 30% (control 14%) on house crickets, black soldier fly and Argentinean cockroach respectively. Different ECI findings from various studies demonstrates need for careful consideration and assessment of best diet combinations that facilitating uniform nutrients uptake.

In the first experiment, while the dry matter, ash, crude fiber, and neutral detergent fiber levels of the mealworm larvae wheat bran - potato waste mixes were comparable, the crude protein content and acid detergent fiber was favored by the wheat bran, while crude fat and energy contents were favored by sole potato waste diets. Similar trend was observed for the second experiment, whereby mealworms fed on wheat bran diet mixtures and wheat bran with no water provision, had comparable the ash, dry matter, crude fiber, neutral detergent fiber and carbohydrates contents,

with larvae raised on a mixture of wheat bran and cabbage leaves portraying significantly higher and low crude protein and crude fat respectively. Meanwhile, larvae raised on wheat bran supplemented with wet cotton wool were enriched with energy content, fat and acid detergent fiber Therefore, depending on the caliber of the foods offered, mealworms contain different nutritional profiles.

Generally, the mealworm crude protein (CP) content ranges from 47% to 60.2%, with an approximated average value of 52.4% comparable to soybean meal with CP of 49.4% (Hong and Han, 2020). In this study, the nitrogen-protein conversion factor of 5.41 (Boulos, Tännler and Nyström, 2020) was employed, whereby *Tenebrio* sp. fed solely on wheat bran recorded high CP of 55.43% (first experiment) and trend declined in other larvae depending on the amount of substituted wheat bran. This suggests that the protein content of mealworms was significantly influenced by a wheat bran diet. The CP range of 43.27 to 55.43% (first experiment) and 48% to 56% (second experiment) established concurs with 51.93% reported by Bovera *et al.* (2015), 47.8% (Yoo *et al.*, 2019) on dried mealworms were fed to pigs, 47.7% (Ramos-Elorduy *et al.*, 2002) for mealworm fed on feed, 46.07% (Ghosh *et al.*, 2017) with a conversion factor of 5.41 for the commercially raised mealworms using wheat bran and Chinese cabbage as the water supply among other studies. In this regard, based on larval protein composition, there is need to establish best diets made up of exclusively locally available agricultural by products that can replace wheat bran diet in mealworm production system.

All treatments in first experiment had significant levels of crude fat in *Tenebrio* sp., ranging from 34.9% to 47.7%. Diets with higher potato waste proportions had a bigger impact on mealworm fat composition. This suggests that, in comparison to wheat bran, ground potato waste are fattier. These findings are comparable (higher) to the fat content reported by Yoo *et al.* (2019) of 34.6%,

37.7% by Ramos-Elorduy *et al.* (2002), 34.54% by Ghosh *et al.* (2017), 32.70% (Ravzanaadii *et al.*, 2012), 31.6% (Ao *et al.*, 2020), 36.06% (Hussain *et al.*, 2017), and very low content of 19.12% reported by Heidari-Parsa *et al.* (2018). In the second experiment, larvae raised on wheat bran diet enriched with cabbage leaves significantly low fat content of 23.6% and a high fat levels of 34.6% for mealworms on wheat bran enriched with wet cotton wool that also portrayed high energy

In the first experiment, the mealworms energy content ranged from 517.9 to 609 kcal/100 g with larvae raised solely on wheat bran diet having the least energy. This energy content is within the range of the measurements made which were 539.63 kcal/100 g and 577.44 kcal/100 g (Rumpold and Schlüter, 2013) and 554.3 kcal/100 g (Ramos-Elorduy *et al.*, 2002). The second experimental higher energy content of 547.4 kcal/100 g was observed in larvae reared on wheat bran with wet cotton supplementation. Thus, the provision of water in mealworms greatly influence fat content, with mealworms deprived of water having low fat and energy contents. The energy content obtained in two experiments are comparable to conventional meat and can attributed to high fats and proteins composition (Rumpold and Schlüter, 2013).

The diets carbohydrate was significantly reduced to yield larvae with high protein and fats contents as evidenced in proximate analysis. The mealworm carbohydrates of 2.3% to 4.5% (first experiment) conforms to the results obtained by Ramos-Elorduy *et al.* (2002) ranging from 0.98% to 7.09%. However, a higher carbohydrate content ranging from 9.26% to 14.29% was observed in second experiment.

Mealworm ash content (mineral composition) of 9.73 – 13.84% (first experiment) and 5.39 – 8.83% (second experiment) was higher and somehow comparable that reported values of 2.86%

(Ravzanaadii *et al.*, 2012), 4.04% (Ghosh *et al.*, 2017), 4.20% (Heidari-Parsa *et al.*, 2018), 6.70% (Yoo *et al.*, 2019), and 3.00% (Ao *et al.*, 2020).

The range of crude fiber discovered in this study was 0.21 to 0.25% (first experiment) and 0.26 to 0.29% (second experiment). This content is considerably less than that of Yoo *et al.* (2019) of 6.1%, 5% (Ramos-Elorduy *et al.*, 2002), 6.26% (Ghosh *et al.*, 2017), 4.58% (Ravzanaadii *et al.*, 2012), 4.90% (Ao *et al.*, 2020), 4.19% (Hussain *et al.*, 2017), and 22.35% (Heidari-Parsa *et al.*, 2018). Low fiber content could be attributed to diet provided and higher conversion of ingested feed to body mass. The ADF values for mealworms ranged from 8.03 to 30.34% (first experiment) and 13.4 to 20.3% (second experiment) is comparable to ADF content of 22.3 g/kg reported by Finke (2015), and low ADF of 7.66% reported by Bovera *et al.* (2016). In this investigation, larval mealworm NDF content of 12.6 –14.7% (first experiment) and 13.7 to 19.7% (second experiment) is comparable to raw NDF mealworm value of 17.4% reported by Poelaert *et al.* (2016).

The high quality mealworm amino acid profile facilitates quality protein supply. There were found to be 14 amino acids in total, with 9 essential and 5 non-essential amino acids. The amino acids lysine and threonine that are missing from regularly consumed cassava, wheat, maize, and rice foods are given the most attention (DeFoliart, 1992). Higher lysine (1.59 –1.97 mg/100 g) and low threonine (0.81 – 0.95 mg/100 g) levels in the first experiment and 1.39 to 1.79 mg/100 g (Lysine) and 0.81 – 0.96 mg/100 g (Threonine) contents in second experiment were revealed in this research. As described by DeFoliart (1992); Ravzanaadii *et al.* (2012); Heidari-Parsa *et al.* (2018), low methionine content ranging 0.51 – 0.67 mg/100 g (first experiment) and 0.57 – 0.70 mg/100 g (second experiment) was found across treatments, but no cysteine was found. This concurs with the mealworm methionine of 0.52% (Heidari-Parsa *et al.*, 2018), 0.60% (Wu *et al.*, 2020) and 0.67% (Ravzanaadii *et al.*, 2012). Children cannot synthesis arginine in their bodies, hence the

availability of arginine 1.86 – 2.06 mg/100 g (first experiment) and 1.59 to 1.91 mg/100 g (second experiment) in mealworms is crucial for their growth, and arginine levels here concurs with reported 1.89% (Wu *et al.,* 2020). In contrast to contents published by (Ravzanaadii *et al.,* 2012; Ghosh *et al.*, 2017; Heidari-Parsa *et al.*, 2018; Ao *et al.*, 2020), this study's valine, tyrosine, leucine, lysine, alanine, glycine and glutamic acid were lower. More studies should be carried out on mealworm amino acids composition based on given side streams to ascertain diet that produces larvae with more and high amounts of essential amino acids.

In summary, mealworms are versatile creatures with various applications, from serving as a source of nutrition for animals and with potential role in waste reduction and scientific research. As awareness of the environmental impact of traditional livestock production grows, mealworms and other edible insects are increasingly considered as a more sustainable protein source for the future.

CONCLUSION

1. Substituting potato waste for wheat bran in mealworm growth is viable because it results in similar high larval survival rates and encourages effective conversion of ingested feed, crude fat, and energy contents of the larvae.
2. Combining wheat bran and potato waste as *Tenebrio* sp. diet produces noticeably larger larvae than using only wheat bran and potato peels alone.
3. Wheat bran continues to be preferred conventional diet for mealworm due to the crude protein, majority of amino acids, acid detergent fiber content, and feed conversion ratio of the larvae.
4. The wheat bran substitution with pineapple peels and cabbage leaves is also viable as mealworm diet as a result of similar performance in survivorship, larval weight and length performance and facilitates feed conversion efficiency and approximately equal protein, crude fats, ash, crude fiber, energy, ADF and NDF contents.
5. Incorporating water in mealworm diet improves growth performance, bioconversion, crude fats and energy content composition.

RECOMMENDATIONS

1. There is need to assess other mealworm potential organic matter that can substitute wheat bran or portray similar performance as observed in wheat bran – potato waste mixtures.
2. Information dissemination to the farmers on mealworm production, benefits and use of readily available by products in Kenya to strengthen livestock and human nutrition and enhance food security.
3. There is need to carry out mealworm farming acceptance study to different communities in Kenya in order to assess willingness and views in incorporating mealworm production since it's relatively new species in Kenya with no evidence of its utilization.
4. For safety in human nutrition, it's important to assess possible microbes and chemical contaminants that may be associated with the use of potato peels, cabbage leaves, pineapple peels associated with mealworm production.
5. It's also necessary to determine the optimal point for waste inclusion to mealworm diet to facilitate sustainable mealworm production.
6. Based on the observation made during mealworm rearing (experiment 2), there is need to develop mechanism that minimize fungal and moulds build up on substrates offered to mealworm, particularly when wheat bran is mixed with cabbage and pineapple peels

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APPENDICES

Appendices 1: Feed standardization

Substrates	Rep	W1	WS	WS2	Moisture (%)	Dry matter (%)	Average Dry matter (%)
First trial							
Wheat bran	1	2.58	18.51	16.42	13.12	86.88	
Wheat bran	2	1.93	16.32	14.42	13.20	86.80	86.86
Wheat bran	3	2.17	15.69	13.92	13.09	86.91	
Potato peels	1	2.36	14.14	12.97	9.93	90.07	
Potato peels	2	2.5	14.66	13.44	10.03	89.97	90.07
Potato peels	3	2.75	14.36	13.22	9.82	90.18	
Cabbage leaves	1	2.32	16.67	15.05	11.29	88.71	
Cabbage leaves	2	3.46	21.93	19.88	11.10	88.90	88.88
Cabbage leaves	3	2.66	19.08	17.28	10.96	89.04	
Pineapple peels	1	2.53	17.05	15.78	8.75	91.25	
Pineapple peels	2	2.83	14.60	13.52	9.18	90.82	91.12
Pineapple peels	3	2.01	17.36	16.02	8.73	91.27	
Second trial							
Wheat bran	1	2.07	11.64	10.46	12.33	87.67	
Wheat bran	2	1.88	10.38	9.32	12.47	87.53	87.62
Wheat bran	3	2.04	12.98	11.63	12.34	87.66	
Potato peels	1	1.86	11.35	10.66	7.27	92.73	
Potato peels	2	1.51	9.45	8.87	7.30	92.70	92.71

Potato peels	3	1.96	10.86	10.21	7.30	92.70	
Cabbage leaves	1	1.77	8.39	7.83	8.46	91.54	
Cabbage leaves	2	2.12	9.62	9	8.27	91.73	91.78
Cabbage leaves	3	1.93	10.49	9.81	7.94	92.06	
Pineapple peels	1	1.64	9.32	8.87	5.86	94.14	
Pineapple peels	2	2.01	9.17	8.62	7.68	92.32	92.97
Pineapple peels	3	1.47	7.56	7.1	7.55	92.45	

$$\text{Moisture (\%)} = \frac{(WS-W1)-(WS2-W1)}{WS-W1} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots..$$

$$\text{Feeds dry matter (\%)} = 100 - \text{moisture (\%)} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots$$

Whereby: W1- cup weight; WS- sample and cup weight; DSW- Dried sample and cup weight.

Appendices 2: Actual weights of feed given to larvae in different treatments (first experiment)

Experiment 1		
Diets	Actual amount of weight (g) equivalent to 500 g dry weight (given to 960 larvae)	Actual amount of weight (g) equivalent to 20.83 g dry weight (given to 40 larvae)
WB100	WB = 575.64 g	WB = 23.99 g
WB75/PW25	WB = 431.73 g	WB = 17.99 g
	PW = 138.78 g	PW = 5.78 g
WB50/PW50	WB = 287.82 g	WB = 11.99 g
	PW = 277.56 g	PW = 11.57 g
WB25/PW75	WB = 143.91g	WB = 5.99 g
	PW = 416.34 g	PW = 17.35 g
PW	PW = 555.12 g	PW = 23.13 g
Second trial		
WB	WB = 570.65 g	WB = 23.78 g
WB75/PW25	WB = 429.98 g	WB = 17.92 g
	PW = 134.83 g	PW = 5.62 g
WB50/PW50	WB = 285.32 g	WB = 11.89 g
	PW = 269.66 g	PW =11.24 g
WB25/PW75	WB = 142.66 g	WB = 5.94 g
	PW = 404.49 g	PW = 16.85 g
PW	PW = 539.32 g	PW = 22.47 g
Cabbage leaves	960 Larvae = 38.4 g	
	40 larvae = 1.6 g	

Appendices 3: Actual weights of feed given to larvae in different treatments (second experiment)

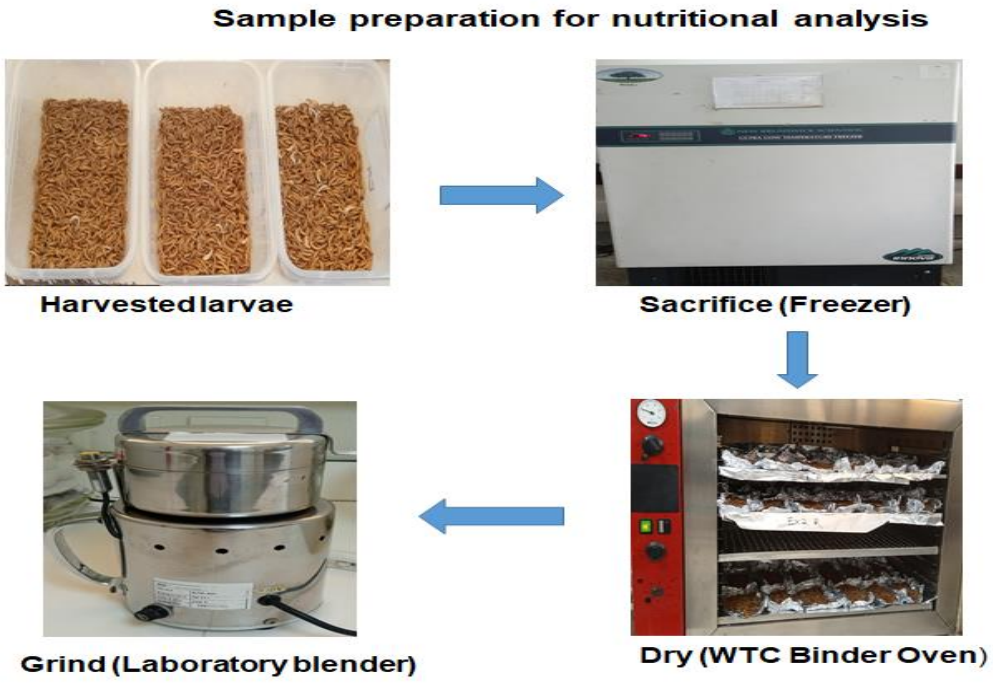
Experiment 2		
Diets	Actual amount of weight (g) equivalent to 500 g dry weight (given to 960 larvae)	Actual amount of weight (g) equivalent to 20.83 g dry weight (given to 40 larvae)
WB	WB = 575.64 g	WB = 23.99 g
WB/CL	WB = 383.76 g	WB = 15.99 g
	CL = 187.52 g	CL = 7.81 g
WB/PP	WB = 383.76 g	WB = 15.99 g
	PP = 182.91 g	PP = 7.62 g
WB/CL/PP	WB = 383.76 g	WB = 15.99 g
	CL = 93.76 g	CL = 3.91 g
	PP = 91.45 g	PP =3.81 g
WB/ Wet cotton	WB = 575.64 g	WB = 23.99 g
Second trial		
WB	WB = 570.65 g	WB = 23.78 g
WB/CL	WB = 380.43 g	WB = 15.85 g
	CL = 181.6 g	CL = 7.57 g
WB/PP	WB = 380.43 g	WB = 15.85 g
	PP =179.27 g	PP = 7.45 g
WB/CL/PP	WB = 380.43 g	WB = 15.85 g
	CL = 90.75 g	CL = 3.78 g
	PP = 89.63 g	PP = 3.73 g
WB/ Wet cotton	WB = 570.65 g	WB = 23.78 g

Appendices 4: Rearing conditions

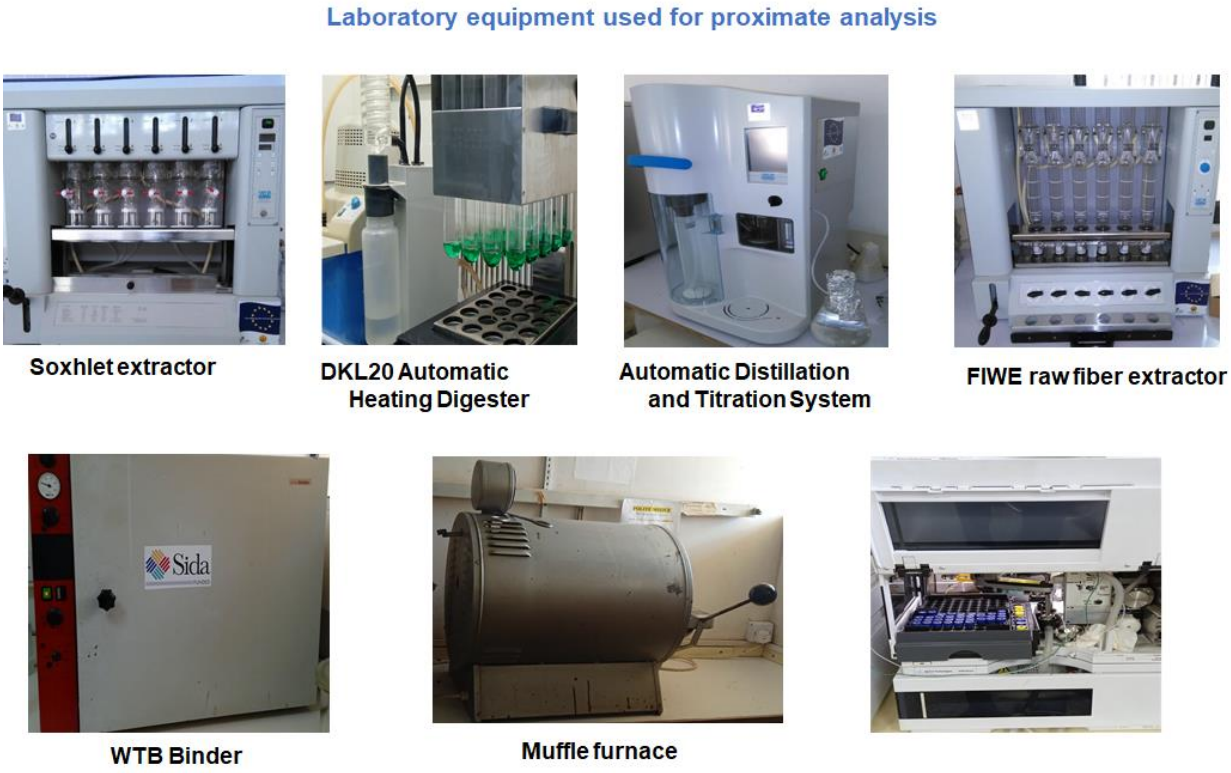
Temperature (0C)	29	29.2	29.3	29.6	30.4	29.2	29	29.2	29.3	29.1	29.4	30.5	30.2	30.3	30.2	30.3	29.2	29.1	28.8	29.2
RH (%)	63.4	63	63	63.1	58.2	63.4	62.5	63.1	63.6	63.5	63.3	59.7	54.6	56.8	54.4	54.2	62.7	62.9	63.1	62.5
Photoperiod	12L: 12D																			

Appendices 5: Diet and experimental preparation

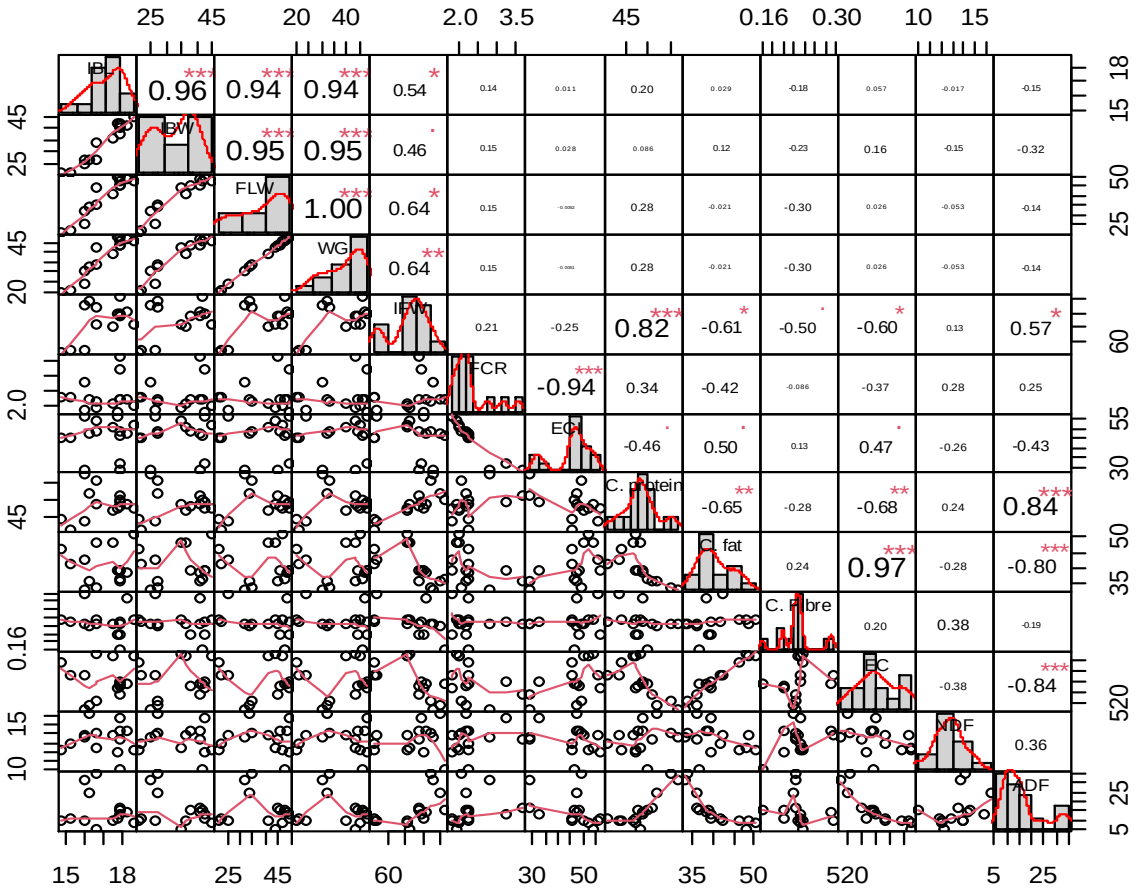
Appendices 6: Sample preparation for nutritional analysis



Appendices 7: Laboratory equipment for nutritional analysis



Appendices 8: The correlation chart matrix with bivariate scatter plots and fitted line displayed on bottom of the diagonal and correlation values with significance level on top of the diagonal.

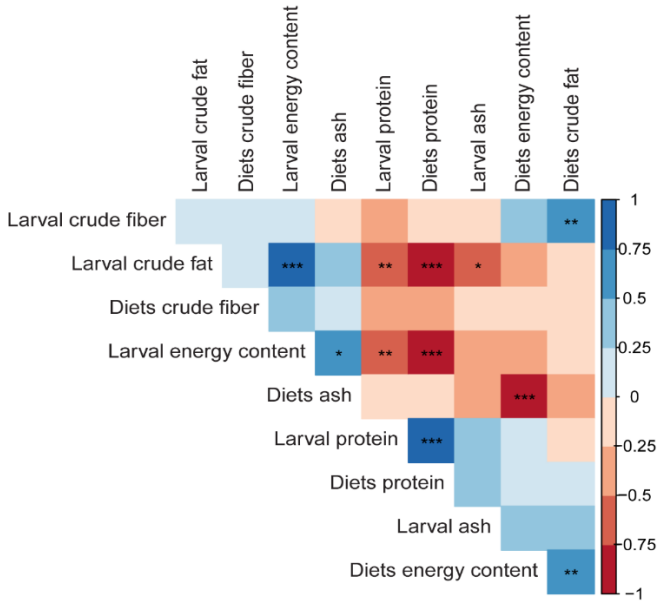

IBL
IBW
FLW
WG
IBW
FCR
EC
C. protein
C. fat
C. Fibre
EC
NDF
ADF

Appendices 9: Overall correlational analysis table for all tested parameters

	IBL	IBW	FLW	WG	IFW	FCR	ECI	CP	CF	CFib	EC	NDF	ADF
IBL	1.000	0.964	0.938	0.938	0.539	0.138	0.011	0.204	0.029	-0.184	0.057	-0.017	-0.149
1BW	0.964	1.000	0.953	0.953	0.464	0.147	0.028	0.086	0.118	-0.231	0.161	-0.154	-0.317
FLW	0.938	0.953	1.000	0.999	0.641	0.146	-0.005	0.284	-0.021	-0.299	0.026	-0.053	-0.143
WG	0.937	0.953	0.999	1.000	0.641	0.146	-0.005	0.284	-0.021	-0.300	0.026	-0.053	-0.143
IFW	0.539	0.463	0.641	0.641	1.000	0.210	-0.255	0.815	-0.606	-0.501	-0.601	0.132	0.572
FCR	0.138	0.147	0.146	0.146	0.210	1.000	-0.942	0.338	-0.421	-0.086	-0.374	0.275	0.255
ECI	0.011	0.028	-0.005	-0.005	-0.255	-0.942	1.000	-0.458	0.496	0.134	0.470	-0.262	-0.427
CP	0.204	0.086	0.284	0.284	0.815	0.338	-0.458	1.000	-0.646	-0.277	-0.680	0.242	0.838
CF	0.029	0.118	-0.021	-0.021	-0.606	-0.421	0.496	-0.646	1.000	0.236	0.966	-0.283	-0.800
CFib	-0.184	-0.231	-0.299	-0.300	-0.501	-0.085	0.134	-0.277	0.236	1.000	0.199	0.382	-0.191
EC	0.057	0.161	0.026	0.026	-0.600	-0.374	0.470	-0.680	0.966	0.199	1.000	-0.381	-0.845
NDF	-0.017	-0.154	-0.053	-0.053	0.132	0.275	-0.262	0.242	-0.283	0.382	-0.380	1.000	0.357
ADF	-0.149	-0.316	-0.144	-0.144	0.573	0.254	-0.427	0.838	-0.799	-0.191	-0.844	0.357	1.000

The IBL- Individual body length; IBW- individual body weight, FLW- final larval weight; WG- weight gain; IFW- Ingested feed weight; CP- crude protein; CF- crude fat; CFib- crude fiber; EC- Energy content; NDF- neutral detergent fiber; ADF- acid detergent fiber

Appendices 10: Relationships between various Substrates and larval T. molitor are depicted in a correlogram. Significant correlations are denoted by asterix, with *** denoting $p < 0.001$, ** denoting $p < 0.01$ and * denoting $p < 0.05$ (Expt. 1).



Appendices 11: Relationships between various Substrates and larval T. molitor are depicted in a correlogram. Significant correlations are denoted by asterix, with *** denoting $p < 0.001$, ** denoting $p < 0.01$ and * denoting $p < 0.05$ (Expt.2)