



**Effect of *Tenebrio molitor* larvae as a protein source on growth performance, carcass
yield and meat quality of broiler chickens**

By

MNGQI SINETHEMBA CENSUS

**A dissertation submitted in fulfilment of the requirements for the degree in
Masters of Science in Agriculture (Animal Science)**

Department of Livestock and Pasture Science

Faculty of Science and Agriculture

University of Fort Hare

Alice

2015

SUPERVISOR : Prof. V. MUCHENJE

Declaration

I, Sinethemba Census Mngqi, vow that this dissertation has not been submitted to any University and that it is my original work conducted under the supervision of Prof V. Muchenje. All assistance towards the production of this work and all the references contained herein have been duly acknowledged.

.....
Sinethemba Census Mngqi

.....
Date

Approved as to style and content by:

.....
Prof. V. Muchenje

.....
Date

Effect of *Tenebrio molitor* larvae as a protein source on growth performance, carcass yield and meat quality of broiler chickens

Abstract

This study was conducted to assess the effect of including *Tenebrio molitor* larvae (*T. molitor* L) as a protein source in different diets on broiler performance, carcass characteristics and meat quality. A total of 144 day-old Cobb-500 chicks were randomly allocated into three treatment groups, each was allocated 16 birds and reared in 9 identical pens. Experimental diets used were as follows: T1 Control (no *T. molitor* L inclusion); T2 and T3 contained levels of *T. molitor* L at 5% and 10% of dry matter (DM) intake, respectively. Body weights (BW), average daily gain (ADG), feed intake (FI), and Feed conversion ratio (FCR) were measured for the 1st experimental chapter. For the second experimental chapter; live weights (LW) of broilers were recorded before slaughter and thereafter carcass weights (CW), meat yield (weights of breast, thigh, drumstick and wing) were recorded. The dressing percentage (DP %) was also calculated. Breast muscles were sampled for meat pH and colour measurements. The LW of birds from T1 (0% *T. molitor* L) were significantly different ($P<0.05$) from both T2 (5% *T. molitor* L) and T3 (10% *T. molitor* L) which were similar to each other, with T2 exhibiting the highest live weights (2166g) and the control treatment exhibiting the lowest live weights (2018.3g). In CW, T1 was significantly different ($P<0.05$) from T2 while it was similar ($P>0.05$) to T3. The dressing % of T1 was significantly different ($P<0.05$) from T2 and T3 which were similar to each other, with T2 having the highest dressing percentage (78, 2%) and T1 having the lowest DP% (66%). The breasts in T2 were significantly higher and different ($P<0.05$) from both T1 and T3 which were similar to each other. The drumsticks in T3 were significantly different ($P<0.05$) from T1 while they were similar to T2 with values with T2 having highest values. After 45 minutes of slaughter, a significant difference ($P<0.05$) was

observed in L^* among all treatments. In redness (a^*), T3 was significantly different from T1 but was similar to T2 and all treatments in this study exhibited a darker red meat, with T3 muscles exhibiting darker red colour than the other treatments. Similar results were observed in yellowness (b^*), where the breast muscles from T3 were more yellow than the other treatments. After 24 hours of slaughter, T2 L^* values were significantly different ($P < 0.05$) from both T1 and T3 which were similar to each other. It was also found that the broiler chickens given diet with no *T. molitor* L inclusion (T1) had lower values of BW, FI, ADG and FCR throughout the experiment than those that were in T2 and T3 with 5% and 10% *T. molitor* L inclusion levels, respectively. However, it was also found that although broilers with 5% *T. molitor* L inclusion (T2) in their diet had high ABW and ADG than the broilers with 10% *T. molitor* L inclusion (T3), the T3 birds compared favourably to T2 birds as they required low feed intake to reach the same slaughter weight due to high FCR. It was, therefore, concluded that *T. molitor* L meal can be incorporated into the diets of broilers to produce heavy birds either at 5 or 10%. However, although 5% *T. molitor* L inclusion yields heavier carcasses, the 10% *T. molitor* L inclusion compared favourably to 5% inclusion since it required low feed intake to reach the same slaughter weights and there were slight differences on meat quality attributes between the two treatments. Thus *T. molitor* L at 10% inclusion levels was the best inclusion level to enhance broiler growth performance, carcass yield, meat yield and meat quality.

Keywords: Growth performance, meat colour, nutrition, ultimate pH

List of abbreviations

a*	Redness of meat
ADG	Average daily gain
AFI	Average feed intake
b*	Yellowness of meat
CW	Carcass weight
D%	Dressing percentage
FCR	Feed conversion ratio
L*	Lightness of meat
<i>T. molitor L</i>	<i>Tenebrio molitor larvae</i>

Acknowledgements

I humbly give praise and honour to my Lord and saviour Jesus Christ who strengthened me through this study. I would never have made it this far without his everlasting Grace. My sincere gratitude goes to my supervisor Prof V. Muchenje who guided me through the whole process of this Masters Project, for being patient with me and giving me all the support I needed. This project would have never been successful without the financial assistance from the National Research Foundation Research and Technology Fund (NRF-RTF) grant, the National Research Foundation Scarce skills scholarship and the Moringa and Poultry meat project (T319).

It is with great pleasure that I thank Mr T. Mabusela and the Berlin Epol company for always opening their doors for me to conduct the laboratory work. I am also grateful to the technical staff of the Department of Livestock and Pasture Science who were always present whenever I needed assistance. To my fellow colleagues and caring friends from the Department of Livestock and Pasture Science, the word “thank you” can never fully express how grateful I am for your help, support and helpful ideas. Lastly I would never end without thanking my family for all their support, prayers and encouragement.

Dedication

This project is dedicated to Mngqi Family (My family) in particular my grandmother and my late grandfather.

Table of content

Effect of <i>Tenebrio molitor</i> larvae as a protein source on growth performance, carcass yield and meat quality of broiler chickens	1
Declaration	i
List of abbreviations	iv
List of Tables	ix
List of Figures	x
Chapter 1: Introduction	1
1.1. Background	1
1.2. Problem statement	2
1.3. Justification	2
1.4. Objective	3
1.5. Hypothesis	3
1.6. References	4
Chapter 2: Literature review	6
2.1. Introduction	6
2.2. Nutrient requirements of broiler chickens	7
2.3. Feeding diets containing insects to poultry	8
2.4. Nutritional value of insects	8
2.4.1. Protein content of insects	9
2.5. Major insect species used as feed	9
2.5.1. Silkworms	10
2.5.2. Mealworms	10
2.5.3. Mopane worms	12
2.5.4. Grasshoppers	12
2.5.5. Fly maggots	13
2.5.6. Earthworms	14
2.6. Current status of insect production	14
2.7. Disease risk and food safety of insects	15
2.8. Effect of using insects as protein source on carcass and meat quality	16
2.8.1. Meat colour	16
2.8.2. Meat pH	17
2.9. Effect of insects on broiler carcass characteristics and meat colour	17

2.10. Summary.....	18
2.11. References.....	19
Chapter 3: The effect of <i>Tenebrio molitor</i> larvae (meal worms) as a protein supplement on growth performance of broiler chickens.....	29
3.1 Introduction.....	33
3.2. Materials and Methods.....	33
3.2.1 Ethical consideration.....	33
3.2.2. Experimental site description.....	34
3.2.3. Animal management.....	34
3.2.4. Feed composition.....	35
3.2.5. Experimental diet preparation.....	38
3.2.6. Measurements.....	38
Growth performance (Body weight gain, feed intake and feed conversion).....	38
3.3. Statistical analysis.....	39
3.4. Results and discussion.....	40
3.4.1. Feed intake.....	40
3.4.2. Body weight.....	43
3.4.3. Average daily gain.....	45
3.4.4. Feed conversion ratio.....	47
3.5. Conclusion.....	49
3.6. References.....	50
Chapter 4: The effect of <i>Tenebrio molitor</i> larvae (meal worm) supplementation on carcass yield and meat quality of broiler chickens.....	52
4.1 Introduction.....	54
4.2. Materials and Methods.....	55
4.2.1. Description of study sites.....	55
4.2.2. Animals and experimental procedure.....	55
4.2.3. Slaughter procedure.....	55
4.3. Data collection.....	56
4.3.1. Carcass characteristics and meat yield measurements.....	56
4.3.2. Meat pH measurement.....	56
4.3.3. Determination of colour.....	56
4.4. Statistical analysis.....	57
4.5 Results and discussion.....	58
4.6. Conclusion.....	62
4.7. References.....	63

Chapter 5: General discussion, conclusions and recommendations	64
5.1. General discussion	64
5.2. Conclusions	65
5.3. Recommendations	66
5.4 References	67

List of Tables

Table 3.1: Nutritional composition (DM basis) of Tenebrio molitor larvae meal.....	35
Table 3.2: Dietary composition of the three treatments (control, 5% Tenebrio molitor larvae and 10% Tenebrio molitor larvae.....	36
Table 4.1: Least square means \pm STD error of carcass characteristics, meat yield and meat quality as affected by Tenebrio molitor larvae at different inclusion levels (0%, 5% and 10%).....	60

List of Figures

Figure 3.1: Weekly feed intake measured in the groups T1 (0% <i>T. molitor</i> L), T2 (5% <i>T. molitor</i> L) and T3 (10% <i>T. molitor</i> L).....	42
Figure 3.2: Average body weight measured in the groups T1 (0% <i>T. molitor</i> L), T2 (5% <i>T. molitor</i> L) and T3 (10% <i>T. molitor</i> L).....	44
Figure 3.3: Average daily gain measured in the groups T1 (0% <i>T. molitor</i> L), T2 (5% <i>T. molitor</i> L) and T3 (10% <i>T. molitor</i> L).....	46
Figure 3.4: Feed conversion ratio in the groups T1 (0% <i>T. molitor</i> L), T2 (5% <i>T. molitor</i> L) and T3 (10% <i>T. molitor</i> L).....	48

Chapter 1: Introduction

1.1. Background

Broiler chickens play an important role as a leading market in the poultry industry (FAO, 2009) and in human nutrition by providing lean and healthy meat for protein. It is, therefore, of paramount significance to satisfy the nutrient requirements of broilers in order to ensure optimal productivity (Klasing *et al.*, 2000). This may ultimately require increased inclusion levels of high quality protein sources such as soybean meal, fish meal and sunflower meal especially in the starter and grower phases of broiler chickens. However, the continuous demands for high quality proteins in broiler diets poses some challenges which may include among others “the competition between humans and animals for the same protein sources and the increased demand for renewable protein resources in animal nutrition” (Pretorius, 2011). The commercial poultry production is also facing major challenges such as high costs and shortage of feed stuffs mainly the protein sources like soybean and fish meal (Adeniji, 2007).

These challenges may, however, be overcome by using insects such as *T. molitor* L as a protein source in broiler diets. Insects require relatively little input in terms of energy, feed, space, and time (Ramos-Elorduy, 2005). Thus it is possible to provide large quantities at relatively low cost. They also create relatively few outputs in terms of environmental wastes and are a very concentrated source of protein (Ramos-Elorduy, 2005; Verkerk *et al.*, 2007) along with containing high levels of essential fatty acids and other micro-nutrients (vitamins/minerals) (Bukkens, 1997; Banjo *et al.*, 2006; Xiaoming *et al.*, 2010). The *T. molitor* L insects have also been proposed as high quality, efficient and sustainable alternative protein source (Klasing *et al.*, 2000). The Food and Agriculture Organisation (FAO) (2009) suggested that using insects

as a protein source can also contribute to global food security as indirect food source for humans

1.2. Problem statement

The poultry industry is faced with a major problem of limited protein sources. Fish and soybean meal which are the commonly used protein sources in poultry diets are continuously increasing in prices. Since 2005 the basket commodity price index (CPI) rose about 50% and prices of soybean, fish meal, corn and wheat rose by 67, 55, 284, 255 and 180 percent respectively (FAO, 2009). There is a high demand for cheap poultry feed due to increasing costs & limited supply of commercial feeds (Mupeta *et al.*, 2003). Moreover, the competition between people and animals for plant resources used for food and feed is expected to further increase the commercial poultry feed price (Mengesha, 2012), forcing producers to look for alternative locally available feeds for their chickens (NRC, 1994). The rise in food and feed prices in the future will influence the exploration of alternate protein sources.

1.3. Justification

The production of soybean meal and fish meal are mainly linked to land acidification and climate change (Mungkung *et al.*, 2013). It is important to find alternative good quality renewable protein sources that can replace or substitute current protein sources that will increase broiler live-weight and growth rate. Therefore, using *T. molitor* L as an alternative protein source can lower the cost of broiler diets and reduce the amount of wild fish used as protein. This may consequently reduce the competition between humans and animals for the same protein source. This study therefore sought to investigate the potential of *T. molitor* L as

protein source in broiler diets and to make necessary recommendations on improved broiler production at comparatively low costs.

1.4. Objective

The main objective of this study was to determine the effects of *T. molitor* L meal on growth performance, carcass characteristics and meat quality of broiler chickens.

1.4.1.

The specific objectives were to:

- Determine the effect of including *T. molitor* L meal as a protein source to broiler diets on feed intake, growth rate, and feed conversion efficiency of broiler chickens.
- Determine the effect of including *T. molitor* L meal as a protein source to broiler diets on slaughter weight, carcass weight, dressing percentage and physico-chemical shelf-life indicators (pHu and colour) of broiler meat of broiler chickens.

1.5. Hypothesis

The null hypotheses tested were:

- Feeding *T. molitor* L meal as a protein source to broilers has no effect on feed intake, growth rate and feed conversion efficiency.
- Feeding *T. molitor* L meal as a protein source to broilers has no effect on slaughter weight, carcass weight, dressing percentage and physico-chemical shelf-life indicators (pHu and colour) of broiler meat.

1.6. References

Adeniji, A.A. 2007. Effect of replacing groundnut cake with maggot in the diet of broilers. *International Journal of Poultry Science*, **6**:822-825.

Banjo, A.D., Lawal, O.A. and Songonuga, E.A. 2006. The nutritional value of fourteen species of edible insects in South West Nigeria. *Africa Journal Biotechnology*. **5**: 289-301.

Bukkens, S.G.F. 1997. The nutritional value of edible insects. *Ecology of Food and Nutrition journal*, **36**:287-319.

Food and Agriculture Organisation (FAO). 2009. How to Feed the World in 2050. Italy. http://www.fao.org/fileadmin/templates/wsfs/docs/expert_paper/How_to_Feed_the_World_in_2050.pdf (accessed 24 June 2015).

Mengesha, M. 2012. Feed resources and chicken production in Ethiopia. *World's Poultry Science Journal*, **68**: 491–502.

Mungkung, R., Aubin, J., Prihadi, T.H., Slembrouck, J., van der Werf, H.M.G. and Legendre, M. 2013. Life cycle assessment for environmentally sustainable aquaculture management: a case study of combined aquaculture systems for carp and tilapia. *Journal of Cleaner Production*, **57**: 249-256.

Mupeta, B., Coker, R. and Zaranyika, E. 2003. The added value of sunflower performance of indigenous chickens fed a reduce-fibre sunflower cake diet in pens and on free range. Accessed on 04 December 2015 from www.dfid.gov.uk/r4d/PDF/outputs/R7524e.pdf

National Research Council (NRC). 1994. Nutrient Requirements of Poultry. Ninth Revised ed. National Academy Press. Washington DC, USA.

Pretorius, Q. 2011. The evaluation of larvae of *Musca domestica* (common house fly) as protein source for broiler production. MSc. thesis, Department of Animal Science, Stellenbosch University, South Africa.

Ramos-Elorduy, J. 2005. Insects: a hope food source. In: Ecological implications of minilivestock (potential of insects, rodents, frogs, and snails) Eds. Science Publisher, INC: 263-291.

Verkerk, M.C., Tramper, J., Van Trijp, J.C. and Martens, DE. 2007. Insect cells for human food. *Biotechnology Advances*, **25**: 198-202.

Xiaoming, C., Ying, F., Hong, Z. and Zhiyong, C. 2010. Review of the nutritive value of edible insects. Food and Agricultural Organization of the United Nations, Regional Office for Asia and Pacific, Bangkok, Thailand.

Chapter 2: Literature review

2.1. Introduction

The nutrition of broiler chickens has been subjected to a steady improvement in the previous years. According to Van Huis *et al.* (2013) the world feed production in 2011 was about 870 million tonnes which generated approximately US \$350 billion globally. The Food and Agriculture Organisation (2009) estimated that there will be an increase of 70 percent on world food and feed production. Poultry and livestock feed supplementation include fishmeal, soybean meal and several other grains (Van Huis *et al.*, 2013) which may require high inclusion levels for optimum productivity. However, a major constraint for further improvement in the poultry industry is the high-priced feedstuffs including meat meal, fish meal, and soybean meal which are estimated to be 70 percent of production expenses.

The poultry industry is currently aiming at producing broilers at low feed cost and feed intake while improving feed efficiency (Kumaran *et al.*, 2014). Broiler growth performance and muscle growth of broilers can be stimulated by improved feed quality with increased protein levels. (Firman and Boling, 1998). Various studies revealed that using different protein sources as supplements in broiler rations has positive effects on broiler performance and meat production (Aftahi *et al.*, 2006; Kermanshahi and Rostami, 2006). Protein is one of the very important nutrients in poultry diets. It is beneficial in maintenance and tissues repairing in animals and it promotes proper growth and development (Bondari and Sheppard, 1981). In Poultry Science, protein supplements are divided into animal and plant proteins (Abro *et al.*, 2012). Soybean and cotton seed are the major plant proteins sources in poultry feeds (Park *et al.*, 2002; Anwar *et al.*, 2008) while insects, fish meal and bone meal are animal sources. Protein from animal sources is having a higher nutritive value (larger amounts of essential

amino acids) when compared to protein from plants (Yen, 2009). According to Olomu (1995) insects have rich sources of energy and are significant for body weight gains. Insects' protein is a complete protein that includes essential amino acids compared to plant protein which include soybean meal. *T. molitor* L (mealworms) contains protein which is between 30% and 70% on a dry matter basis (Kirk *et al.*, 2000), have a high nutritive value and are also rich in fats, minerals and vitamins (Chapman, 1998; Finke, 2013). However, consumer reactions in feeding housefly larvae to broilers are of high debate due to health concerns. Nonetheless the processing of housefly larvae limit the chances of unhealthy benefits to consumers.

2.2. Nutrient requirements of broiler chickens

According to Leeson and Summers (2001), nutrient requirement is the minimum quantity of the nutrient needed by animals to maximise growth and utilization efficiency as well as to improve meat quality. The high rate of productivity in poultry results in relatively high nutrient needs. Proteins, lipids and carbohydrates are utilised as the sources of metabolic machinery and are essential requirements for growth and energy. Growth involves deposition of bones, muscle and fat with each trait showing an individual pattern of development (Carlson, 2009). As the chickens grow, the weekly weight increases become substantially less although feed requirements increases (Mgnon-Grasteaus *et al.*, 2001).

There is, therefore, a need to formulate diets which fulfils all the nutrient requirements, including limiting essential amino acid for growth. Chickens need protein for growth, meat and egg production. Protein deficiency in a feed results in reduced broiler growth performance (Chinrasri, 2004). This is mainly because feeding broilers with diets below their energy or protein requirements has a negative influence on growth and nutrient efficiency.

2.3. Feeding diets containing insects to poultry

A major problem facing the poultry industry is the provision of feeds that will contain all the necessary dietary components for birds to grow rapidly within a short period (Oyegoke *et al.*, 2006). There is a need for supplementing cereal based rations with a high quality animal protein. The importance of fish meal in a complete poultry diet will supplement any amino acid deficiency in vegetable proteins such as soybean, groundnut cake and cotton seed cake (Miles and Jacobs, 1997). Most of the published trials have been carried out with broilers fed housefly larvae meal.

The results showed that housefly larvae can be added at approximate dietary levels of 25% (Pretorius, 2011) without any negative effect on weight gain, feed intake and feed efficiency. This suggests that maggot meal can efficiently replace other protein sources, e.g. soy bean meal, fish meal and groundnut cake. Ijaiya and Eko (2009) observed no differences among the dietary treatments of *Anaphe infracta* (7%), fishmeal and silkworm as protein sources in broiler performance, on feed intake, body weight and feed conversion ratio. Thus insects' protein compares favourably with conventional sources of protein.

2.4. Nutritional value of insects

Insects at all life stages are rich sources of animal protein. Various studies have dedicated on exploring the nutritional value of numerous alternative foodstuffs that has potential of being used as protein or mineral supplements for broilers chickens. The main research efforts have focussed on (*Hermetia illucens*) which is a Black soldier fly, the maggot and pupae of the housefly (*Musca domestica*), the larvae of the mealworm (*T. molitor* L), and insect families

belonging to the order Orthoptera including locusts, grasshoppers and crickets (Makkar *et al.*, 2014; Sánchez-Muros *et al.*, 2014).

According to Ramos-Elorduy (2005) and Bukkens (2005), insects are branded by a high nutritive value in terms of protein, fats, minerals, and vitamins. Protein is the most expensive ingredient in poultry diets and insect inclusion in the diets seems to be an economically viable option. Ravindran and Blair (1993) stated that essential amino acids derived from animal protein supplements are superior to those obtained from plant protein (legumes and grains) supplements in poultry feed formulation. Anand *et al.* (2008) stated that insects belonging to the Acridids have a higher proportion of protein content in comparison to conventional fish and soybean meals.

2.4.1. Protein content of insects

According to Rumpold and Schluter (2013), the average protein content of insects varies between 50 and 82 % dry matter (DM) depending on the insect's species or on the method of processing the insects. However, the protein content of a good quality fish meal can reach up to 73 % while soybean meal contains up to 50% of protein. The protein content of insects therefore compares favourably with the conventional sources of proteins and has a potential to be used as an alternative cheap source of protein.

2.5. Major insect species used as feed

Insects have always been a natural food sources for poultry, which is reflected by wild birds. The most hopeful insect species for poltry industry feed production are the black soldier fly

larvae, common housefly larvae, silkworms and *T. molitor* L (yellow mealworms). Grasshoppers and termites are also viable, but to lesser extent (Carlson, 2009).

2.5.1. Silkworms

Silkworms are the caterpillars of the *Bombyx mori* moth and are protuberant at the beginning of rainy seasons and prefer eating green leaves (Ijaiya and Eko, 2009). Silkworm pupae has good quality protein and crude fat (503g/kg and 164 g/kg) which makes it to be highly recognized nutrient source and comprise of fibrous chitin (Jintasataporn, 2012). Ijaiya and Eko (2009) studied the likelihood of substituting fishmeal (25%, 50%, 75% and 100%) inclusion levels with the silkworm caterpillar meal for growth performance. There were no differences witnessed in growth performance on feeding intake, body weight gain and feed conversion ratio between the treatments. Thus the growth performance of chickens was not affected by the incorporation of silkworm caterpillar meal at different inclusion levels. However, Silkworm caterpillar meal proved to be cheaper than conventional fishmeal, which makes it economically appropriate substitute. Safety assessment of silkworm pupae which was done by Zhou and Han (2006) with the use of mutagenicity test in rodents showed the silkworm pupae to be toxic free. Dutta *et al.* (2012) also reported there are no toxicological effects that were allied with silkworm feeding on broilers.

2.5.2. Mealworms

Mealworms are the larvae of beetles from the family *Tenebrionidae* and *Tenebrio obscurus Fabricius*. Mealworm beetles are native to Europe and now they are found all over the world. *T. Molitor* L is a pest that feeds on cereal grains and is commonly found around grain stores (Ramos- Elorduy *et al.*, 2002). Mealworms can be easily bred and are commonly fed to pets

and fish live but dried and powder forma are also available (Aguilar *et al.*, 2002; Hardouin and Mahoux, 2003; Veldkamp *et al.*, 2012). The adult *T. molitor* beetle life span is about 1 to 3 months.

Mealworms feed on any plant and animal products but they are often fed cereals. (Ramos-Elorduy *et al.*, 2002). These worms normally feed on cereals like oats gains, wheat grains and corn and for protein they are fed milk powder and soybean flour and for moister the provided with vegetables and fruits (Hardouin and Maoux, 2003; Aguilar *et al.*, 2002). The diet should, however, be balanced to contain about 20% protein on dry matter basis (Ramos- Elorduy *et al.*, 2002). Mealworms are able to utilise small amounts of water contained in dry feed and it has to be noted that the productivity of water deprived mealworms is low. Hence, it is necessary to provide them with a source of water for better productivity and in order to prevent cannibalism. Relative humidity is linked positively with fertility and adult activity (Hardouin and Mahoux, 2003). Mealworms are capable of producing high quality feed from recycling waste quickly (Kamran *et al.*, 2008). Mealworm (*T. molitor* L) are already raised on an industrial scale. They can also be produced on low nutritive waste products and fed to broiler chickens. Thus mealworms are promising alternative to conventional protein sources, particularly soybean meal and fishmeal.

Furthermore, the on-going increase in feed prices, especially protein sources for example soybean meal, fishmeal and many more has placed more emphasis on the exploitation of alternative protein sources. In previous studies the use of mealworms was compared with other protein sources for the use in animal nutrition where the effect of larvae and pupae meal was evaluated as supplement for other protein sources commonly used in animal feed (Khusro, 2012). Mealworm was suggested as a suitable nutritional composition and can serve as a

replacement for fish meal as well as other protein sources normally used in animal nutrition. However, these protein sources can be used together to complement each other in the animal feed industry since amino acids vary in the protein sources.

2.5.3. Mopane worms

The Mopane caterpillar is the larval stage of the Mopane moth, (*Lepidoptera saturniidae*). The mopane caterpillar derives its name from the host plant, *Colophospermum mopane* and feeds on leaves (Motshegwe *et al.*, 1998). These caterpillars of the emperor moth emerges in southern Africa's summer, a time when other staple food can be in short supply (Vogt, 2010). Traditionally, mopane used to be an important food source mainly in north-eastern Botswana. However, mopane is now consumed all over Botswana and has become a much sought after delicacy in Southern Africa (Motshegwe *et al.*, 1998) and is among the cheapest sources of animal protein in most African countries.

Mopane can also be used as an animal feed, especially in Botswana where there is inadequate supply of animal protein for livestock feeds. Though seasonal mopane is a more readily available and cheaper source of animal protein. Mopane worms contain higher protein, fat, carbohydrate and mineral content than beef and chicken. According to Siame *et al.* (1989) and Madibela *et al.* (2007), mopane contains about 50% crude protein (CP) and is abundant in the wilderness during its season of availability.

2.5.4. Grasshoppers

Grasshoppers are scientifically called (*Orthoptera: Acrididae*) and most other *Acrididae* family members have high nutritive value and are mostly utilized by people (Blásquez *et al.*,

2012). Feed made of grasshoppers can contain up to 76% crude protein but with poor amino acid profile (Ravindran and Blair, 1993). Grasshoppers contain high level of calories (4.7–7 kcal/gm), crude fat content of (6–7.5%) and carbohydrate content of (3.6–7.5 (Anand *et al.*, 2008). Most edible insects are collected in the wild. In Mexico, for instance, farmers collect young grasshoppers from their maize and alfalfa fields, where they would otherwise cause some damage in plants (Vogt, 2010). Anand *et al.* (2008) reported that protein content of acridids ranges from 60 to 66% while Blasquez *et al.* (2012) reported that it ranges from 43.9 to 77.1%. The protein content of acridids compares favourably with that of soybean meal (48%) and fish meal (50 to 55%) (Anand *et al.* 2008).

2.5.5. Fly maggots

Maggots (*Diptera muscidae*) can be obtained from housefly (*Musca domestica*) and then prepared into poultry meal, which is called maggot meal and are always obtainable in every time of the year (Moreki *et al.*, 2012). The advantages of utilizing maggot meal in poultry nutrition are recycling of poultry waste (El-Boushy, 1991) and sustainable environmentally friendly management of poultry wastes (Akpodiete *et al.*, 1998). Several studies have shown that maggot meal is of high nutritive value. For instance, the percentage of lipids in maggots ranges from 12.5 to 21%, and crude fibre from 5.8 to 8.2% (Ogunji *et al.*, 2006).

The crude protein for maggot meal ranges from 37.5% (Ogunji *et al.*, 2006) to 40-61.4% dry matter basis (Teotia and Miller, 1973; Spinelli *et al.*, 1979). Olele (2011) showed that maggots have 44.5% CP, 10% ash and 24% lipid, whereas Aniebo *et al.* (2008) showed that maggot meal contains 47.1% crude protein, 25.3% fat, 7.5% crude fibre and 6.25% ash. The amino acid profile of maggot meal contains 17 amino acids among which are nine essential amino acids with tryptophan not identified (Aniebo *et al.*, 2008). Furthermore, lysine and methionine

were found to be higher in the maggot meal (6.04% and 2.28%, respectively) compared to other conventional protein sources including fish meal.

2.5.6. Earthworms

Earthworms are tube shaped insects belonging to the phylum Annelida and are mostly found in soil and dead organic matter which they feed on. Earthworms have a potential of being used as an alternative protein source in poultry industry (Ibáñez *et al.*, 1993). They contain a good amino acid profile together and high protein levels with high lysine content. The nutrient quality of earthworms differs according to their species (Zhenjun *et al.*, 1997). Reinecke *et al.* (1991) reported that *Eisenia fetida*, *Eudrilus eugeniae*, and *Perionyx excavates* have 661 g/kg, 584 g/kg and 616 g/kg levels of crude protein, respectively.

2.6. Current status of insect production

Practically speaking, no established methods for the large-scale industrial production of mealworms currently exist. That is to say, the methods that are currently being used are at a scale inconsistent with anticipated future demand (Gracer, 2010). Those methods are primarily aimed at producing mealworms to supply the demand of the pet food industry and, to a lesser degree, scientific research and as chicken feed. Additionally, fringe sector of private corporate interests that are developing methods of large-scale production exist, however, these methods are being protected as proprietary and therefore inaccessible to the public (Kok *et al.*, 1988).

The mass rearing of insects has become a subject of formal study although not at a point in its development where one can expect to see implementation in the immediate future. As an example, Kok *et al.* (1988) have published the results of their trials with what they refer to as

an “insect farm.” Their aims were to develop reliable methods for the mass production of the larvae of *Tenebrio* and *confusum* flour beetle (a close relative of *T. molitor*) as a human food product. Although they indicated moderate success in rearing, there remain aspects of the process that have prevented its immediate implementation. Notably, there is a need to increase the output of the system, incorporate the use of an inexpensive, cellulose feed, analyse the economics of the process and identify a consumer for the waste generated

2.7. Disease risk and food safety of insects

Insects are high in nutrients and moisture content which is capable of providing a favourable environment for microbial survival and growth (Klunder *et al.*, 2012). A range of pathogens such as bacteria, protozoa, fungi, and viruses have been reported to be present in insects (Vega and Kaya, 2012). Banjo *et al.* (2006) reported the presence of the pathogenic bacteria such as *Staphylococcus aureus*, *Rosenbach*, *Pseudomonas aeruginosa* (Schroeter), *Migula* and *Frankland bacillus cereus*, in edible rhinoceros beetle species in West Africa, which poses risk to consumers (Ekraekene and Igeleke, 2007). The mopane caterpillar is associated with *Penicillium* and *Fusarium* fungi and are also reported to produce aflatoxins sometimes above the maximum safety level set by FAO (Mpuchane *et al.*, 1996).

Potential pathogens in edible insects need to be assessed along the entire farm-to-table food chain (production, processing, distribution, sale, handling and consumption) (Braide, 2012). Insects also pose a threat of allergies, toxicological and chemical hazards. Increased consumption of chitin which is contained in insects can lead to asthmatic symptoms and allergies. (Gouli *et al.*, 2011). Heavy metals, pesticides as well as mycotoxins can be accumulated in substrates used for insect rearing and constitute a risk if carried over through the food chain to insects, poultry, fish and humans. However, the risk of transmitting zoonotic

infections to humans is likely to be lower because insects are taxonomically much more distant from humans than livestock (Van Huis, 2013).

2.8. Processing, storage and packaging of insects

There is a need for insects to undergo postharvest processing as the main objective of providing safe nutritious diet to maintain a healthy life. Insects as food and feed require special processing and packaging to protect them for the required storage life (Verghese *et al.*, 2013). The products' packing and labelling, aids on product tracking (Bollen *et al.*, 2006; Opara, 2003).

2.8. Effect of using insects as protein source on carcass and meat quality

2.8.1. Meat colour

The meat colour is important for both the consumer's selection of fresh meat at the retail level and for the consumer's final evaluation and acceptance of a meat product at time of consumption (Fletcher *et al.*, 2000; Muchenje *et al.*, 2009; Dyubele *et al.*, 2010). Factors such as age, sex, strain, diet, intramuscular fat, meat moisture content, pre-slaughter conditions and processing variables have an effect on broiler meat quality (Northcutt, 1997). Meat colour is the result of numerous chemical and physical processes within the muscle (Barbut, 2009). Breast meat is expected to have a pale pink colour when it is raw, while thigh and leg meat are expected to be dark red when raw (Fletcher *et al.*, 2000). Meat colour alterations such as PSE (pale, soft and exudative) and DFD meat (dark, firm and dry) which are common in pork are rare in chickens (Pelicano *et al.*, 2003).

Hwangbo *et al.* (2009) studied the influence of larvae meal on broiler breast meat colour. The results showed that larvae meal supplementation had no significant effect ($P>0.05$) on meat colour regarding the CIElab L^* , a^* and b^* values. This suggests that the use of insects on broiler diets may either produce the same meat colour as conventionally used sources of proteins or even produce better colour when included at different levels or when other insects are investigated. Therefore, there is still a room for research on the effects of feeding insects on broiler meat quality.

2.8.2. Meat pH

The variations in broiler breast meat colour are as a result of changes in muscle pH, and may be related to differences in the shelf-life meat (Allen *et al.*, 1997). At post-mortem, anaerobic metabolism reduces the pH from about 7.2 in muscle to 5.8 in meat as a result of rigor mortis (Fletcher *et al.*, 2000). The ultimate pH of meat is highly dependent on the amount of glycogen present in the muscle, with the implication that pre-slaughter stress is related to muscle pH (Allen *et al.*, 1997). Fletcher *et al.* (1999) reported significant linear relationships between raw meat colour and its pH.

2.9. Effect of insects on broiler carcass characteristics and meat colour

A study by Hwangbo *et al.* (2009) showed that larvae meal supplementation in the diets of broilers led to significantly better carcass characteristics, such as; dressing percentage yield as well as breast muscle and thigh muscle yield as percentage of carcass weight. It was noticed that broilers receiving larvae meal supplementation had a significantly higher dressing percentage, breast muscle and thigh muscle yields when compared to the control group

(Hwangbo *et al.*, 2009). These results suggest that insects can be used as an alternative cheap protein source to produce heavy broilers at relatively low costs.

2.10. Summary

The limited protein sources for animal feed are a major problem in the poultry industry. The cost of the particular protein plays a very important role in the selection of appropriate protein sources used in animal nutrition. With the lack of renewable protein sources together with the rise in protein feed costs, it is becoming increasingly important to find alternative good quality renewable protein sources that can replace or substitute current protein sources used in poultry nutrition. This provides opportunities to explore other possible means of protein production in animal nutrition and such possible means can come from various organisms such as insects. Insects play a nutritional role on both animals and humans. Most edible insects are cheap, available and can provide good source of protein and minerals needed by poultry. This can help farmers increase their broiler productivity at relatively low costs. The *Tenebrio molitor Larvae* is among the insects with a potential to provide good quality protein source to chickens. Thus this study aims at investigation the *Tenebrio molitor Larvae* as an alternative protein source to broiler chickens.

The protein sources used for broilers in the poultry industry had a short fall since 2005 while these protein sources are expected to be included at high inclusion levels for optimum production. The price of these process is another main problem as they continuously being expensive to both commercial and small holder farmers. Thus coming up with alternative protein sources such as *T. molitor* meal could improve broiler production and at the same time improve the economy of the country through the poultry industry

2.11. References

- Abro, M. R., Sahito, H. A., Memon, A., Soomro, R. N., Soomro, H. and Ujjann, N. A.** 2012. Effects of various protein source feed ingredients on the growth performance of broiler. *International Journal Medical Plant*, **1**: 38-43.
- Aftahi, A., Munim, T., Hoque, M. A. and Ashraf, M. A.** 2006. Effects of yoghurt and protexin boost on broiler performance. *International journal of Poultry Science*, **5**: 651-655.

- Aguilar-Mirand, E.D., Lopez, M.G., Escamilla-Santana, C. and De La Rosa, B.A.P.** 2002. Characteristics of maize flour tortilla supplemented with ground *Tenebrio molitor* Larvae. *Journal of Agricultural Science and Food chemistry*, **50:192-195**.
- Akpodiete, O.J., Ologhobo, A.D. and Onifade, A.A.** 1998. Maggot meal as a substitute for fish meal in laying chicken diet. *Ghana Journal of Agricultural Science*, **31:137-142**.
- Allen, C.D., Russell, S.M. and Fletcher, D.L.** 1998. Department of Poultry Science. University of Georgia, Athens. *Poultry Science*, **76:1042-108**.
- Anand, H., Ganguly, A. and Haldar, P.** 2008. Potential value of acridids as high protein supplement for poultry feed. *International journal of Poultry Science*, **7: 722-725**.
- Aniebo, A.O., Erondy, E.S. and Owen, O.J.** 2008. Proximate composition of house fly larvae (*Musca domestica*) meal generated from mixture of cattle blood & wheat bran. Department of Animal Science & Fisheries, University of Port Harcourt. Nigeria.
- Anwar, M. S., Khan, M. Z., Javed, K. A. and Saleem, M. K.** 2008. Pathological effects of cottonseed meal with and without ferrous sulphate in male Japanese quails (*Coturnix Japonica*). *Pakistan Veterinary Journal*, **28:51-56**.
- Banjo, A.D., Lawal, O.A. and Songonuga, E.A.** 2006. The nutritional value of fourteen species of edible insects in South West Nigeria. *Africa Journal Biotechnology*. **5: 289-301**.
- Blásquez, J., Ramos-Elorduy, Moreno, J.M.P. and Camacho, V.H.M.** 2012. Could Grasshoppers Be a Nutritive Meal? *Food and Nutrition Sciences*, **3: 164-175**.
- Bollen, A.F., Riden, C.P. and Opara, L.U.** 2006. Traceability in postharvest quality management. *International Journal of Postharvest Technology and Innovation*, **1: 93-105**.
- Bondari, K. and Sheppard, D.C.** 1981. Soldier fly larvae as feed in commercial fish production. *Aqua-culture*, **24: 103-109**.

Braide, W., 2012. Perspectives in the microbiology of the leaves of three plant species as food for an edible caterpillar of an emperor moth. *International Journal of Research in Pure and Applied Microbiology*, **2**: 1-6.

Bukkens, S.G.F. 2005. Insects in the human diet: nutritional aspects. In: Ecological implications of mini livestock (potential of insects, rodents, frogs and snails) Eds. Paoletti, M.G. (Science Publisher, INC): 545-577.

Carlson, I.R. 2009. Growth regulators. In: Animal growth and nutrition. Eds. Hafez, E.S. E and Dryer, I.A. Publishers: Lea and Febiger, Philadelphia, U.S.A. pp. 138-155.

Chapman, R.F. 1998. The insects: structure and function. Cambridge University Press, Four Edition, 69-91

Chinrasri, A. 2004. Poultry Production Technology. Apichart Printing Press, Mahasarakam Province, Thailand. pp 206.

Dutta, A., Dutta, S. and Kumari, S. 2012. Growth of poultry chicks fed on formulated feed containing silk worm pupae meal as protein supplement and commercial diet. *Online Journal of Animal and Feed Research*, **2**:303–307.

Dyubele, N.L., Muchenje, V., Nkukwana, T.T. and Chimonyo, M. 2010. Consumer sensory characteristics of broiler and indigenous chicken meat: A South African example. *Food Quality and Preference*, **21**: 815-819.

Ekrakene, T. and Igeleke, C.L. 2007. Microbial isolates from the roasted larva of the palm weevil (*Rhynchophorus phoenicis*) from Edo and Delta states of Nigeria. *Australian Journal of Basic and Applied Sciences*, **1**: 763-768.

El-Boushy, A.R. 1991. House-fly pupae as poultry manure converters for animal feed: A review. *Bioresources and Technology*, **38** (1): 45-49.

- Food and Agriculture Organisation (FAO).** 2009. How to Feed the World in 2050. Italy. http://www.fao.org/fileadmin/templates/wsfs/docs/expert_paper/How_to_Feed_the_World_in_2050.pdf (accessed 24 June 2015).
- Finke, M.D., Sunde, M.L. and Defoliar, G.R.** 2003. An evaluation of the protein quality of Mormon cricket (*Anabrus simplex* H.) when used as a high protein feedstuff for poultry. *Poultry science*, **64**: 708-712.
- Firman, J.D. and S. D. Boling.** 1998. Ideal protein in Turkeys. *Poultry science*, **77**: 105-110.
- Fletcher, D.L.** 1999. Broiler breast meat colour variation, pH, and texture. *Poultry science*, **78**:1323-1327.
- Gouli, V.V., Gouli, S.Y. and Marcelino, J.** 2011. Common infectious diseases of insects in culture: diagnostic and prophylactic methods – Springer Briefs in animal sciences. Springer Netherlands, Dordrecht, the Netherlands.
- Gracer, D.** 2010. Filling the plates: serving insects to the public in the United States. Pages 217-220 in PB Durst, DV Johnson, RN Leslie and K Shono, editors. Forest insects as food: humans bite back. Food and Agricultural Organization of the United Nations, Regional Office for Asia and Pacific, Bangkok, Thailand.
- Hardouin, J. and Mahoux, G.** 2003. Zootechnie d'insectes – Elevage et utilisation au bénéfice de l'homme et de certains animaux. Bureau pour l'Echange et la Distribution de l'Information sur le Mini-élevage (BEDIM), pg 164-166.
- Hwangbo, J., Hong, E.C., Jang, A., Kang, H.K., Kim, B.W. and Park, B.S.** 2009. Utilization of house fly-maggots, a feed supplement in the production of broiler chickens. *Journal of Environmental Biology*. **30**: 609-614.

- Ibáñez, I.A., Herrera, C.A., Velásquez, L.A., Hebel, P.,** 1993. Nutritional and toxicological evaluation on rats of earthworm (*Eisenia fetida*) meal as protein source for animal feed. *Animal Feed Science and Technology*, **42**: 165–172.
- Ijaiya, A.T. and Eko, E.O.** 2009. Effects of replacing dietary fish meal with silkworm (*Anaphe infrecta*) caterpillar meal on performance, carcass characteristics and haematological parameter of finishing broiler chickens. *Pakistan Journal Nutrition*, **8**: 850-855.
- Kamran, Z., Sarwar, M., Nisa, M., Nadeem, M.A., Mahmood, S., Babar, M.E. and Ahmed, S.** 2008. Effects of low protein diets having constant energy to protein ration on performance and carcass characteristics of broiler chickens from one to thirty-five days of age. *Poultry Science*, **87**: 468-474.
- Kermanshahi, H. & Rostami, H.** 2006. Influence of supplements dried mealworms on broiler performance and Cecal Flora. *Internal Journal of Poultry Science*, 5(6), 538-543.
- Khusro, M., Andrew, N.R. and Nicholas, A.** 2012. Insects as poultry feed: a scoping study for poultry production system in Australia. *Worlds Poultry Science Journal*, **68**: 435-446.
- Kirk, C.K., Paul, T., Melvin, A.L. and Christopher C.C.** 2000. Increasing the calcium content of mealworms (*Tenebrio molitor*) to improve their nutritional value for bone mineralisation of growing chicks. *Journal of Zoo and wildlife Medicine*, **31**: 512-517.
- Klunder, H.C., Wolkers-Rooijackers, J., Korpela, J.M. and Nout, M.J.R.** 2012. Larvae meal as a supplement for swine. *Journal of Animal Science*, **44**: 395-400.
- Kok, R., Lomaliza, K., Shavhare, U. S.** 1988. The design and performance of an insect farm/chemical reactor for human food production. *Canadian Agricultural Engineering*, **30**: 307-317.

Leeson, S. and J. D. Summers. 2001. Nutrition of the Chicken. 4th ed. University Books, Guelph, Ontario, Canada.

Makkar, H.P.S., Tran, G., Heuzé, V. and Ankers, P. 2014: State-of-the-art on use of insects as animal feed. *Animal Feed Science and Technology*, **197**: 1–33.

Mignon-Grasteau, S., Piles, M., Varona, L., de Rochambeau, H., Poivey, J.P., Blasco, A. and Beaumont C. 2000. Genetic analysis of growth curve parameters for male and female chickens resulting from selection on shape of growth curve. *Journal of Animal Science*, **78**: 2515-2524.

Miles, R.D. and Jacob, J.P. 1997. Fish meal understanding why this feed ingredient is so valuable in poultry diets. University of Florida Cooperative and Extension Service. Institute of food and Agricultural Science, USA.

Moreki, J.C., Tiroesele, B. and Chiripasi, S.C. 2012. Prospects of utilizing insects as alternative sources of protein in poultry diets in Botswana. *Journal of Animal Science Advances*, **2**: 649–658.

Motshegwe, S.M., Holmback, J. and Yeboah, S.O. 1998. General properties and fatty acid composition of the oil from Mophane caterpillar, *Imbrasia belina*. *Journal of the American Oil Chemists' Society*, **75**: 725-728.

Madibela, O.R., Seitiso, T.K. and Letso, M. 2007. Effect of traditional processing methods on chemical composition and in vitro true dry matter digestibility of Mopane worm (*Imbrasia belina*). *Journal of Arid Environments*, **68**: 492-500.

Mpuchane, S., Gashe, B.A., Allotey, J., Siame, B., Teferra, G. and Dithogo, M. 2000. Quality deterioration of phane, the edible caterpillar of an emperor moth *Imbrasia belina*. *Food Control*, **11**: 453–458.

Muchenje, V., Dzama, K., Chimonyo, M., Strydom, P.E. and Raats, J.G. 2009 Relationship between stress responsiveness and meat quality in three cattle breeds. *Meat Science*, **81**: 653-657.

Northcutt, J.K.1997. Factors affecting poultry meat quality. Tech. Bull. No. 1157. University of Georgia, Athens.

Ogunji, J.O., Kloas, W., Wirth, M., Schulz, C. and Rennert, B., 2006. Housefly maggot meal (Magleal): an emerging substitute of fishmeal in Tilapia Diets. In: Conference on International Agricultural Research for Development; Deutscher Tropentag. Accessed on 04 December 2015 from Bonn, Germany <http://www.tropentag.de/2006/abstracts/full/76.pdf>

Olele, N.F. 2011. Comparative study on the use of natural and artificial based feeds for the culture of *Clarias gariepinus* Fingerlings. Asian Research Publishing Network. *Journal of Agricultural and Biological Science*, **6**: 9-13.

Olomu, J. M. 1995. Principles and practice. Monogastric animal nutrition pp. 67-128.

Opara, L.U. 2003. Traceability in agriculture and food supply chain: a review of basic concepts, technological implications, and future prospects. *Journal of Food, Agriculture and Environment*, **1**: 101-106.

Oyegoke, O.O., Akintola, A.J. and Fasoranti, J.O., 2006. Dietary potential of the edible larvae of *Cririna forda* (Westwood) as a poultry feed. *Africa Journal Biotechnology*, **5**: 1799-1802.

Park, Y. H., Kim, H. K., Kim, H. S., Lee, H. S., Shin, I. S., & Whang, K. Y. (2002). Effects of three different soybean meal sources on layer and broiler performance. *Asian-Australia Journal Animal Science*, 15(2), 254-265.

Pelicano, E.R.L., Souza, P.A., Souza, H.B.A., Oba, A., Norkus, E.A., Kodawara, L.M., Lima, T.M.A.2003.Morfometria e ultra-estrutura da mucosa intestinal de frangos de corte alimentados com dietas contendo diferentes probióticos. *The Portuguese Society of Veterinary Sciences*, **98**:124-134.

Pretorius, Q. 2011. The evaluation of larvae of *Musca domestica* (common house fly) as a protein source for broiler production. MSc. thesis, Department of Animal Science, Stellenbosch University, South Africa.

Ramos-Elorduy, J. 2005. Insects: a hope food source. In: Ecological implications of minilivestock (potential of insects, rodents, frogs, and snails) Eds. Science Publisher, INC: 263-291.

Ravindran, V., Blair, R., 1993. Feed resources for poultry production in Asia and the Pacific. III. Animal protein sources. *World's Poultry Science Journal*. **49**: 219–235.

Reinecke, A.J., Hayes, J.P. and Cilliers, S.C.1991. Protein quality of three different species of earthworms. *South African Journal of Animal Science*. **21**: 99–103.

Rumpold, B.A. and Schlüter, O.K.2013. Potential and challenges of insects as an innovative source for food and feed production. *Innovative Food Science and Emerging Technologies*, **17**: 1-11.

Sánchez-Muros, M.J., Barroso, F.G. and Manzano-Agugliaro, F. 2014: Insect meal as renewable source of food for animal feeding: a review. *Journal of Cleaner Production*, **65**: 16 27.

Siame, A.B., Teferra, G., Allotey, J., Mpuchane, S.F. and Gashe, B.A. 1989. Nutritional quality of Mophane worms, and the microorganisms associated with the worms. The Department of Biological Sciences and Kalahari Conservation Society. Gaborone, Botswana.

Spinelli, J., Mahnken, C. and Steinberg, M. 1979. Alternative sources of protein for fish meal in Salmonid diets. Proc. World Symp. Finfish Nutrition and Fish feed Technology, Hamburg 20 - 23 June, 1978. Vol. II. Berlin Heinemann GMBH. p. 132 - 143.

Teotia, J.S. and Miller, B.F. 1974. Nutritive content of house fly pupae and manure residue. *British Poultry Science*, **15**: 177-182.

van Huis, A., Van Itterbeeck, J., Klunder, H., Mertens, E., Halloran, A., Muir, G. and Vantomme, P. 2013. Edible insects - Future prospects for food and feed security. FAO Forestry Paper 171.

Vega, F. and Kaya, H. 2012. Insect pathology. Elsevier/Academic Press, Amsterdam, the Netherlands, 490 pp.

Veldkamp, T., Van Duinkerken, G., Van Huis, A., Lakemond, C.M.M., Ottevanger, E., Bosch, G. and Van Boekel, M.A.J.S. 2012. Insects as a sustainable feed ingredient in pig and poultry diets- a feasible study. Rapport 638- Wageningen Livestock Research.

Vergheese, K., Lewis, H., Lockrey, S. and Williams, H. 2013. The role of packaging in minimizing food waste in the supply chain of the future. *CHEP Australia* **3**: 50-61.

Vogt, G. 2010. For More Protein, Filet of Cricket: Could an African Caterpillar be the new beefsteak? *Science*, **327**: 811-815.

Yen, A.L. 2009. Edible insects: Traditional knowledge or western phobia. *Journal of the Entomological Research Society*, **39**: 289-298.

Zhenjun, S., Xianchun, L., Lihui, S. and Chunyang, S. 1997. Earthworm as a potential protein resource. *Ecology of Food and Nutrition*, **36**: 221–236.

Zhou, J. and Han, D. 2006. Safety evaluation of protein of silkworm (*Antheraea pernyi*) pupae. *Food and Chemical Toxicology*, **44**: 1123–1130.

Chapter 3: The effect of *Tenebrio molitor* larvae (meal worms) as a protein supplement on growth performance of broiler chickens

Abstract

The effect of different inclusion levels of *Tenebrio molitor* larvae (*T. molitor* L) meal on growth performance of broiler chickens was determined. Three experimental diets: T1 Control (0% *T. molitor* L inclusion); T2 (5% *T. molitor* L) and T3 (10% *T. molitor* L) of dry matter (DM) intake were used. Body weights (BW) feed intake (FI), average daily gain (ADG) and Feed conversion ratio (FCR) were determined. No significant differences ($P > 0.05$) were found in FI among treatments in weeks 1 and 2, while in week 3 significant differences ($P < 0.05$) were

only found between T1 and T3 with T3 having low FI. In week 4, the FI for T3 was significantly different ($P<0.05$) from those of T1 and T2, and exhibited low FI. In week 5, the FI for T2 was significantly different ($P<0.05$) from both T1 and T3, and exhibited high FI. From week 1 to week 4, no significant differences ($P>0.05$) were found in BW among the treatments. However, notable significant differences ($P<0.05$) were found in week 5 where BW for T1 were different from both T2 and T3, while T2 and T3 compared favorably with each other. In ADG significant differences were found in week 2 where T1 was significantly different ($P<0.05$) from T3, while T2 and T3 compared favorably with each other. During the first two weeks the ADG increased as the inclusion levels increased. However, in week 3 the ADG decreased as the inclusion levels increased with T3 being significantly different ($P<0.05$) from both T2 and T1. Similar results were obtained in week 4, but T2 and T3 compared favorably with each other. Opposing results were obtained in week 5, where the ADG increased as the inclusion levels increased and significant differences were found among all treatments. The FCR was high in T3 throughout the trial, while T2 compared favorably with both T3 and T1 for up to 3 weeks. In week 4 and 5 the FCR for T3 was significantly different from both treatments 1 and 2. It was concluded that while broilers supplemented with 10% *T. molitor* L had lower FI, they had high FCR and consequently attained higher BW than in other diets. Thus 10% *T. molitor* L was the best inclusion level for improving broiler growth performance.

Keywords: Average daily gain, body weight, feed conversion ratio, feed intake

1 **3.1 Introduction**

2 Protein is a very important nutrient that has been required in broiler nutrition with amino acid
3 profile stressed (Mupeta *et al.*, 2003). However, the limited protein sources for animal feed are
4 a major problem in the poultry industry (Bollitoc and Sun, 2013). The cost plays a vital role in
5 the selection of an appropriate protein sources to be used in animal nutrition. With the scarcity
6 renewable protein sources and the rise in feed cost it is impoertant to search for alternate high
7 quality protein sources (Téguia *et al.*, 2002). In the wild insects are a source of food to various
8 types of animals. Though they are often underestimated, they have a lot to provide. (Anand *et*
9 *al.*, 2008).

10

11 Most edible insects readily available and cost little to produce and are capable of providing
12 good source of protein (Ifie *et al.*, 2011). Yellow mealworms (*T. molitor* L) are commonly used
13 as treats for pets and other small animals and are a very good protein source wiyh protein
14 content ranging from 44%-70% (Ramos- Elorduy, 1987). Mealworm contains high quality of
15 nutrients (lipids, carbohydrates, vitamins and proteins (De Foliart *et al.*, 2009). These insects
16 can, therefore, also serve as a potential substitute for protein source for chickens. Moreover,
17 the possibility of insects being used as animal feed could have an environmental healthy impact
18 (Pimental *et al.*, 1975). This study, therefore, sought to investigate the effects of different levels
19 of *T. molitor* L as a protein supplement on the growth performance of broiler chickens.

20 **3.2. Materials and Methods**

21

22 **3.2.1 Ethical consideration**

23 Ethical principles were deliberated in the study to adhere to the national and international
24 standards governing this kind of research with the usage of animals and humans. Authorization

25 for the use of animals and humans was attained from the Ethical Clearance Committee of the
26 University of Fort Hare (Reference Number: MUS061SMNG01) (Appendix 1).

27

28 **3.2.2. Experimental site description**

29 The study was conducted at the University of Fort Hare, Alice, Eastern Cape, South Africa.
30 The site is 520 m above sea level and is located 32.48°S and 26.53°E. The average rainfall is
31 approximately 480 mm per year, with most rainfall occurring mainly during summer. Mean
32 temperature is about 18.7°C per year. The topography of the area is generally flat with a few
33 steep slopes.

34

35 **3.2.3. Animal management**

36 One hundred and forty-four day-old Cobb-500 broiler chicks were distributed into three groups
37 randomly with each treatment having three replicates and there were 16 chicks in each
38 replicate. Prior to the experiment the chicken houses and equipment were cleaned and
39 disinfected. The houses were ventilated and contained pens with were of (1.5 x 2 x 1.5 m),
40 infrared light bulbs were fitted in each pen for warmth and light. A layer of 10 cm wood
41 shavings was spread for the litter. The chickens were fed *ad libitum* and water was always
42 offered permanently throughout the investigate. The chicks All broiler chicks were inoculated
43 with Gumboro which was mixed into the drinking water against Infectious Bursal disease and
44 PoulShot B1+IB as well as against Newcastle disease. Care and management of birds was in
45 accordance with principles of animal care in experimentation (NRC, 1985).

46

47 **3.2.4. Feed composition**

48 Tables 1 and 2 shows the ingredients and the chemical compositions of the rations containing
49 different inclusion levels of meal worms. These dietary rations were balanced formulated feed
50 containing meal worms (*T. molitor* L). The same percentages of *T. molitor* L (0, 5 and 10%)
51 were given to the starter, grower and finisher diets.

52 **Table 3.1:** Nutritional composition (DM basis) of *Tenebrio molitor* larvae meal. (Also see
53 appendix 2).

Proximate analysis	Larvae meal
Gross Energy (MJ/kg)	8.15
Crude Protein (%)	50.68
Crude Fat (%)	27.2
Crude Fibre (%)	9.7
NDF (%)	27.84
Ash (%)	5.59
Mineral Content	
Phosphorus (%)	1.29
Potassium (%)	1.70
Calcium (%)	0.03
Magnesium (%)	0.07
Sodium (%)	0.67

54

55

56 **Table 3.2:** Dietary composition and proximate analysis of the three treatments (Diet 1: contro
57 (0% *Tenebrio molitor* larvae), Diet 2: 5% *Tenebrio molitor* larvae and Diet 3: 10% *Tenebrio*
58 *molitor* larvae. (Also see appendix 3).

59

Ingredients (% of diet)	Starter Phase			Grower Phase			Finisher Phase		
	Diet 1	Diet 2	Diet 3	Diet 1	Diet 2	Diet 3	Diet 1	Diet 2	Diet 3
Yellow Maize	63.42	60.70	57.03	67.03	64.14	60.93	70.62	67.68	62.55
Gluten 20 %	0.96	0.97	2.52	2.09	1.43	4.58	3.15	2.31	6.69
Wheat Middlings	21.61	5	5	17.28	5	5	13.05	5	5
Soya Oilcake	10	1439	11.89	10	10.96	6.27	10	6.98	9.04
Sunflower Oilcake	1.45	10	10	0	10	10	0	10	4
Worms	1.18	5	10	1.3	5	10	1.34	5	10
Limestone Powder	0.36	1.48	1.65	0.94	1.35	1.44	0.58	1.39	1.75
Mono-calcium Phosphate (KK)	0.20	1.01	0.82	0.37	0.76	0.57	.37	0.4	0.2
Salt Fine	0.04	0.31	0.25	0.17	0.31	0.25	0.14	0.32	0.25
Methionine DL Powder	0.04	0.16	0.17	0.06	0.17	0.15	0.08	0.14	0.11
Tryptophan	0.34	.34	0.22	0.34	0.14	0.17	0.34	0.31	0.04
Lysine HCL Powder	0.07	0.07	0.01	0.06	0.32	0.25	0.05	0.04	0.10
Choline Chloride Powder 60%	0.09	0.09	0.09	0.09	0.06	0.09	0.09	0.09	0.09
Vit/Min premix	0.2	0.2	0.2	0.2	0.02	0.2	0.2	0.2	0.2
Elancoban 20%	0.05	0.05	0.05	0.05	0.2	0.05	-	-	-

Zinc Bacitracin 15%	0.03	0.03	0.03	0.03	0.05	0.03	-	-	-
Proximate analysis									
Gross Energy (Mj/kg)	11.69	11.91	10.77	11.75	12.23	11.98	11.73	11.55	10.55
Crude Protein	18.42	21.3	23.02	18.14	19.91	20.02	17.76	20.24	20.27
Crude Fat (%)	2.40	3.59	6.33	2.68	4.11	3.35	2.65	4.41	4.25
Crude Fibre (%)	4.10	4.54	4.06	4.38	4.54	4.19	4.55	4.96	4.27
Ash	4.99	4.89	5.18	4.39	4.38	4.59	4.70	4.39	5.18
Minerals									
Phosphorus	0.67	0.64	0.74	0.60	0.63	0.66	0.65	0.64	0.76
Calcium	0.90	0.73	1.08	0.87	0.77	0.80	0.97	0.79	1.09
Sodium	0.16	0.17	0.13	0.13	0.11	0.15	0.12	0.11	0.13

60 **3.2.5. Experimental diet preparation**

61 The chicks were assigned to three different treatment diets. The diets were formulated so that
62 the chicks were maintained on the minimum nutrient specifications as provided by Ross
63 International (2009). The treatment diets were formulated in three phases to contain 0%
64 mealworm inclusion (control), 5% and 10% mealworm inclusion levels on (treatment 2 and 3,
65 respectively).

66

67 **3.2.6. Measurements**

68 **Growth performance (Body weight gain, feed intake and feed conversion ratio)**

69 The body weights of the chickens were measured once a week using an electric balanced scale.
70 The feed intake (FI) was determined as the difference between the amounts of feed provide and
71 feed left by the chickens using the following formula;

$$72 \text{ Feed intake} = \text{Feed given (g)} - \text{Feed left (g)}$$

73

74 Body weight gain (BWG) was determined by subtracting the final body weight (g) from the
75 initial body weight (g) using the following formula;

$$76 \text{ Body weight gain} = \text{Final body weight (g)} - \text{Initial body weight (g)}$$

77

78 Average daily gain (ADG) was measured at a fixed time in the morning (7 am) before the
79 chickens were provided feed. The ADG was calculated by subtracting week 2 weights from the
80 weights of week 1 divided by the number of days between as shown in the following formula;

$$81 \text{ Average Daily Gain} = \frac{\text{Average Live Weight per Chick (g)}}{\text{Age (days)}}$$

82 Feed conversion efficiency (FCR) was also calculated by dividing average feed intake (g) by
83 the average body weights (g) as shown in the following formula;

$$84 \text{ Feed Conversion Ratio} = \frac{\text{Cumulative Feed Intake (g)}}{\text{Average LiveWeight per Chick(g)}}$$

85

86 **3.3. Statistical analysis**

87 The data was analysed using the Statistical Analysis Systems (SAS) package of 2009. The
88 General linear model procedure (PROC GLM) was used to test the effect of different levels of
89 *T. molitor* L on growth performance (FI, BWG, ADG and FCR). The significant difference
90 between least square means was compared using fishers' least significant difference (LSD) and
91 differences were considered to be significant at $P < 0.05$.

92

93 The following model was used:

$$94 Y_{ij} = \mu + T_i + E_{ij} \text{ where;}$$

95 Y_{ij} – Response variables (FI, BWG, ADG and FCR)

96 μ - Overall mean

97 T_i – i^{th} effect of different levels of *Tenebrio molitor* larvae (T1 0% inclusion; T2 5% inclusion;
98 T3 10% inclusion)

99 E_{ij} – Random error

100 **3.4. Results and discussion**

101 **3.4.1. Feed intake**

102 As expected, the feed intake was increasing per week on the quantity of feed consumed in each
103 group (Figure 1). These results suggest that the feed intake was directly proportional with the
104 age of birds, thus, the feed intake increased with age. However, no significant differences ($P <$
105 0.05) were found on feed intake (FI) among treatments in week 1 and week 2. These results
106 suggest that there was no rejection of the *T. molitor* L which can be attributed to texture or
107 palatability at early stages of the birds' growth phase. The results are similar to those reported
108 by Ramos-Elorduy *et al.* (2002) who also found no rejection due to texture or palatability of
109 the *T. molitor* L meal in broilers diets of up to 10% inclusion rate.

110

111 In week 3, the FI of T3 (10% inclusion) was significantly different from T1 (0% inclusion)
112 with T1 having high FI. These results suggest that as the birds were growing older they
113 exhibited some preferences on the different diets and were shown to prefer the diet with 0%
114 inclusion of *T. molitor* L in week 3. However, the FI for T3 (10% inclusion) was not different
115 from T2 (5% inclusion) and both T2 and T3 had lower FI than T1 in week 3. These results
116 agree with the previous findings by Kermanshahi and Rostami (2006) which also showed no
117 significant differences on feed intake on chickens fed diets containing mealworms at different
118 levels. These results suggest that the inclusion of *T. molitor* at either 5 or 10 % levels does not
119 affect the feed preference of the broilers.

120

121 In week 4, T3 was significantly different from T1 and T2, and exhibited low FI than T2 and
122 T1. These results suggest that the preference of the diets among the birds decreased as the
123 inclusion levels of *T. molitor* L increased. These results contradict to those reported by Bovera

124 *et al.* (2015) which showed that dried meal worms had sufficient palatability for broilers also
125 when the inclusion rate was near 30% of the diet. The decrease in the palatability of diet with
126 high inclusion level was further shown in week 5, where T2 (5% inclusion) was significantly
127 different from both T1 (0% inclusion) and T3 (10% inclusion), and exhibited high FI than the
128 other treatments. These results may also be attributed to the appetite of the chickens for
129 different levels (Aftahi *et al.*, 2006). In the current study it was, therefore, shown that broiler
130 chickens had high feed intake at 5% inclusion level of *T. molitor* L during the last growth
131 period (week 5).

132 Treatment 1 had the same performance throughout the trial from week 1 to week 4, where it
133 was mostly preferred by the birds than T2 and T3. However, in week 5 both T2 and T3 were
134 mostly preferred by the birds with T3 over performing. The results suggest that age is a
135 determining factor of feed preference as the birds preference changed as they grew older.

136

137

138

139

140

141

142

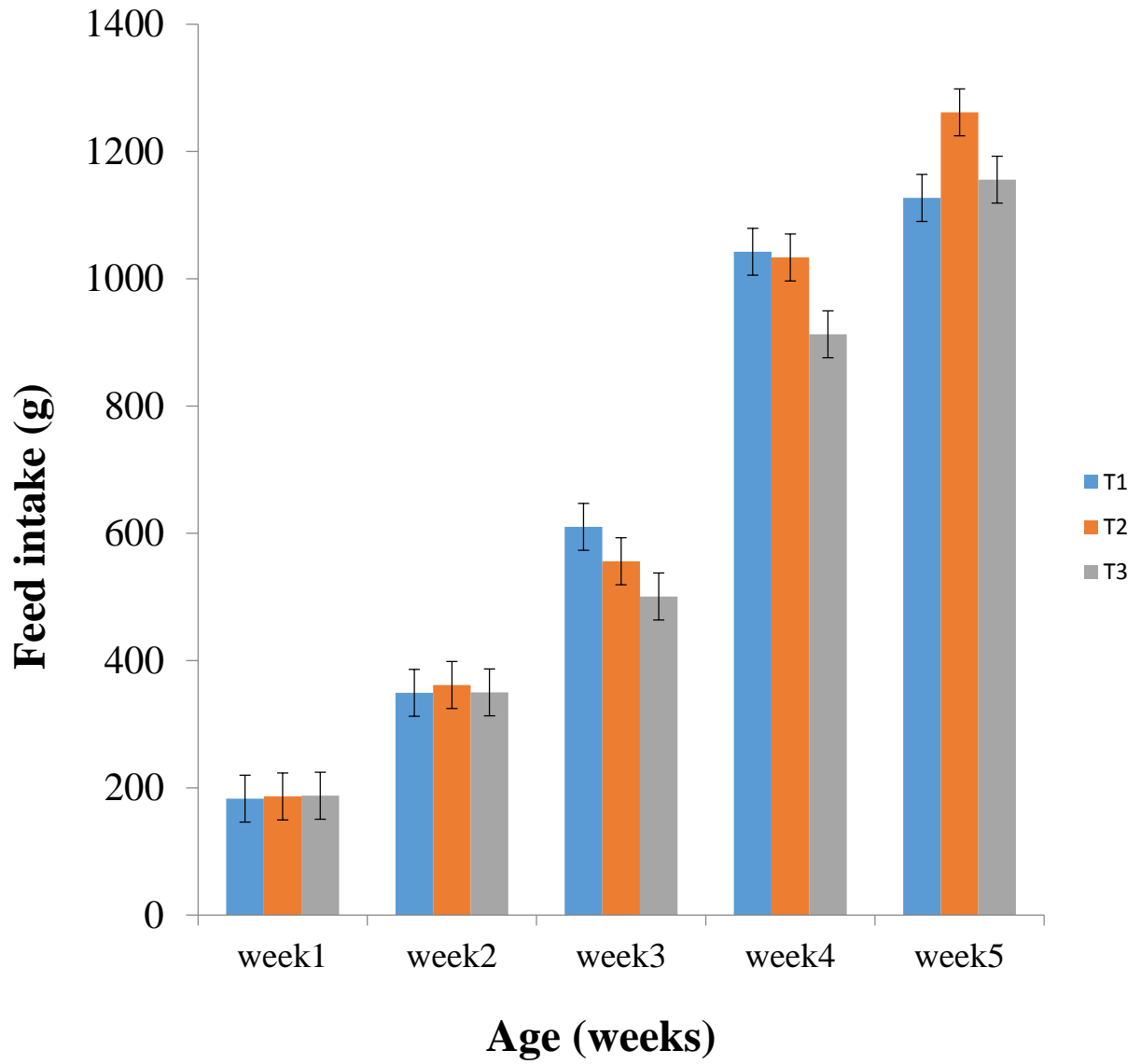
143

144

145

146

147



148

149

150 **Figure 3.1:** Weekly feed intake measured in the groups T1 (0% *T. molitor* L), T2 (5% *T.*
151 *molitor* L) and T3 (10% *T. molitor* L).

152

153

154

155 **3.4.2. Body weight**

156 In every seventh day of the experiment (day 7,14,21,28 and 35), the body weight of each broiler
157 chicken was measured and the mean body weights were calculated for each group. From day
158 7 to 35 all groups showed a trend of increasing body weights (Figure 2). These results are as
159 expected since broilers are bred to be very fast growing chickens. From week 1 to week 4, no
160 significant differences ($P>0.05$) were found in body weights among the treatments. However,
161 significant differences were noted in week 5, where T1 (0% inclusion) was significantly
162 different from both T2 (5% inclusion) and T3 (10% inclusion), and T2 exhibited high body
163 weights.

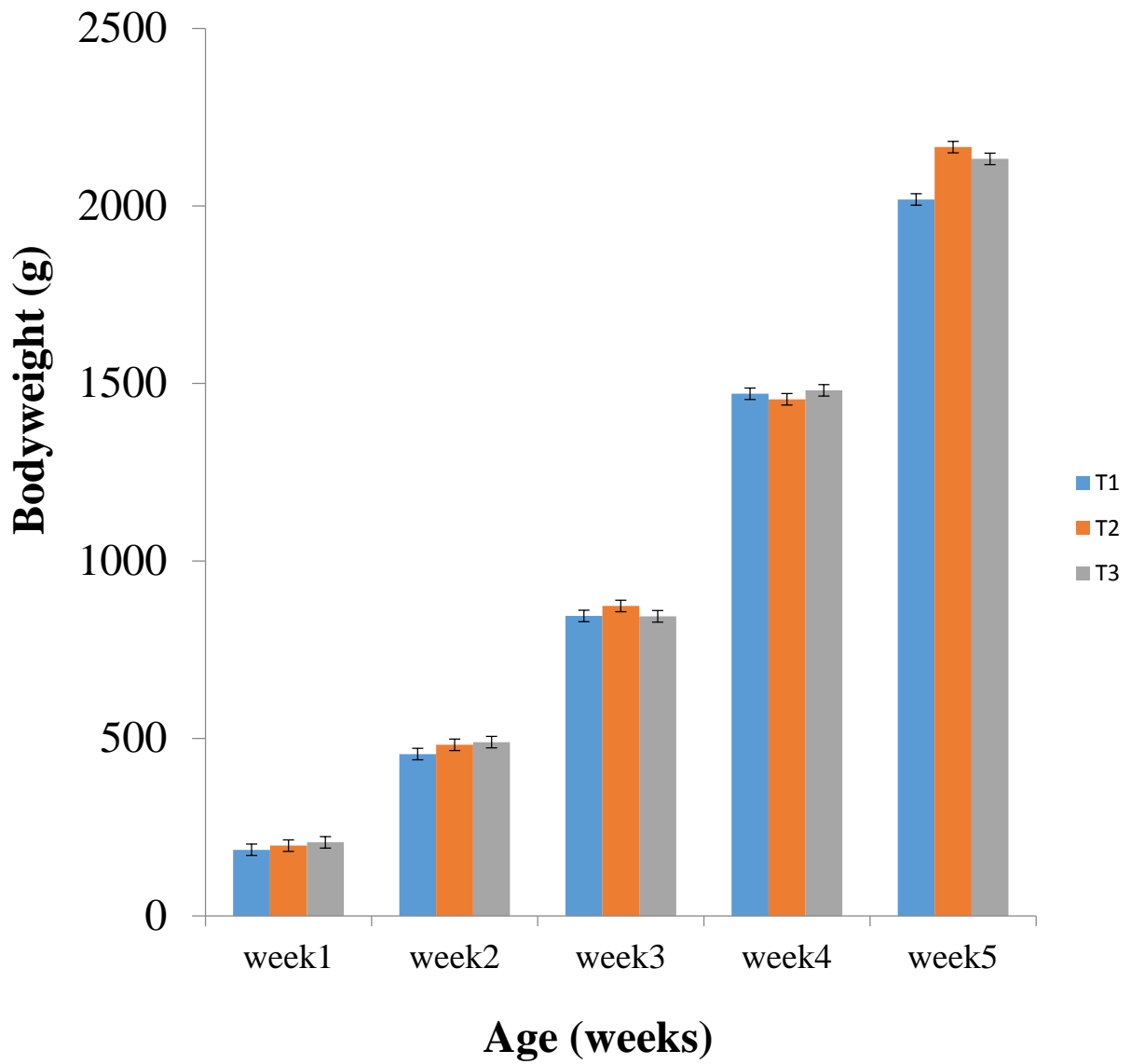
164

165 These results concur with the previous results reported in the current study on feed intake,
166 which showed that in week 5 there was a high feed intake of T2 (5% inclusion) followed by
167 T3 (10% inclusion). Hence, there were high body weights in week 5 on T2. Nonetheless,
168 although the FI on T3 was lower than that of T2, there was no significant difference ($P>0.05$)
169 on body weights of birds between these two treatments. Since the *T. molitor* L was fed at the
170 larval stage, these results may be attributed to high protein levels contained in the diets with 5
171 and 10% supplementation. Ghally and Alkoaik (2009) also reported that the larval stages of *T.*
172 *molitor* L are rich in protein, this may consequently trigger the birds into high body weights at
173 increased inclusion levels.

174

175

176



179 **Figure 3.2:** Average body weight measured in the groups T1 (0% *T. molitor* L), T2 (5% *T.*
180 *molitor* L) and T3 (10% *T. molitor* L).

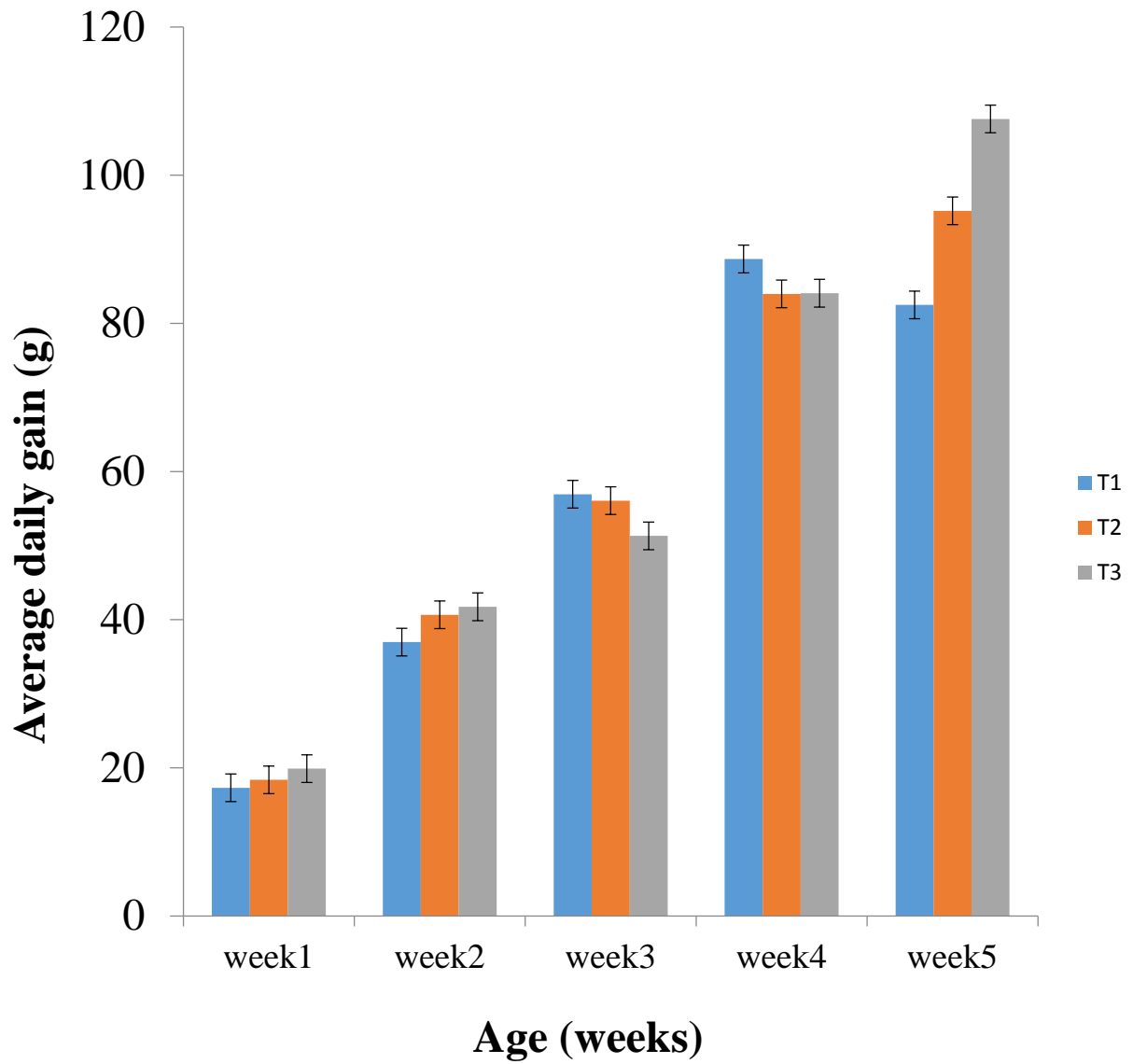
185 3.4.3. Average daily gain

186 Figure 2 displays the effect of mealworm supplementation on average daily gains of the
187 different groups. There was no significant difference ($P>0.05$) found among all treatments in
188 week 1 on average daily gain (ADG). In week 2, T1 was significantly different ($P<0.05$) from
189 T3, however, T3 was not significantly different ($P>0.05$) from T2. It was noted that during the
190 first two weeks the ADG increased as the inclusion levels increased. Similar findings were
191 reported by Hwangbo *et al.* (2009), where broilers which received diets with mealworms
192 supplement at 10% and 15% had significantly higher weight gains than the broiler receiving
193 no mealworms 0% (control). In week 3 it was, however, noted that the ADG decreased as the
194 inclusion levels increased with T3 being significantly different ($P<0.05$) from both T2 and T1,
195 and exhibited low ADG.

196

197 Similar results were obtained in week 4, where the ADG decreased as the inclusion levels
198 increased except for the slight increase on T3. However, on week 5, opposing results were
199 obtained where the ADG increased as the inclusion levels increased. Although in the current
200 study it has been previously reported that the birds mostly preferred the 5% *T. molitor* L diets,
201 it can be noted that the high ADG can be obtained at 10% *T. molitor* L diets, however, factors
202 such as palatability may inhibit the birds from feeding on 10% *T. molitor* L. These results,
203 therefore, are in agreement with the results by Ramos-Elorduy *et al.* (2002) which indicated
204 that *T. molitor* L can be used as a protein source up to 10% inclusion in the diet of broilers.

205



206

207 **Figure 3.3:** Average daily gain measured in the groups T1 (0% *T. molitor* L), T2 (5% *T. molitor*
 208 L) and T3 (10% *T. molitor* L).

209

210 **3.4.4. Feed conversion ratio**

211 Figure 4 shows the effect of *T. molitor* L on Feed conversion ratio (FCR). The FCR was found
212 to be lower in treatments T2 (5%inclusion) and T1 (0% inclusion) and higher in T3 (10%
213 inclusion) throughout the experiment although T2 had a slightly higher FCR than T1. The FCR
214 was further shown to be significantly higher in all the feed supplemented with *T. molitor* L
215 when compared with the control. Hwangbo *et al.* (2009) attributes these differences to the
216 essential amino acid profile of the *T. molitor* L. These results contradict with the findings by
217 Awonoyi *et al.* (2003); Adeniji (2007) and Teguaia *et al.* (2002) who found no significant effect
218 of *T. molitor* L supplementation on weight gain and feed conversion ratio (FRC) of broilers.
219 The results from the current study also oppose those by Bollitoc and Sun (2013) who showed
220 a decreasing trend in feed conversion ratio of broilers from 0% to 10% *T. molitor* inclusion.

221

222 The differences in the feed conversion ratio in the current study may, therefore, be attributed
223 to the enhanced feed digestibility due to the high amino acid profile of *T. molitor* worms. The
224 inclusion of the *T. molitor* worms stimulated high digestibility of the feed and high absorption
225 of the necessary nutrients. Hence the FCR was high in both T2 and T3 than the control
226 treatment.

227

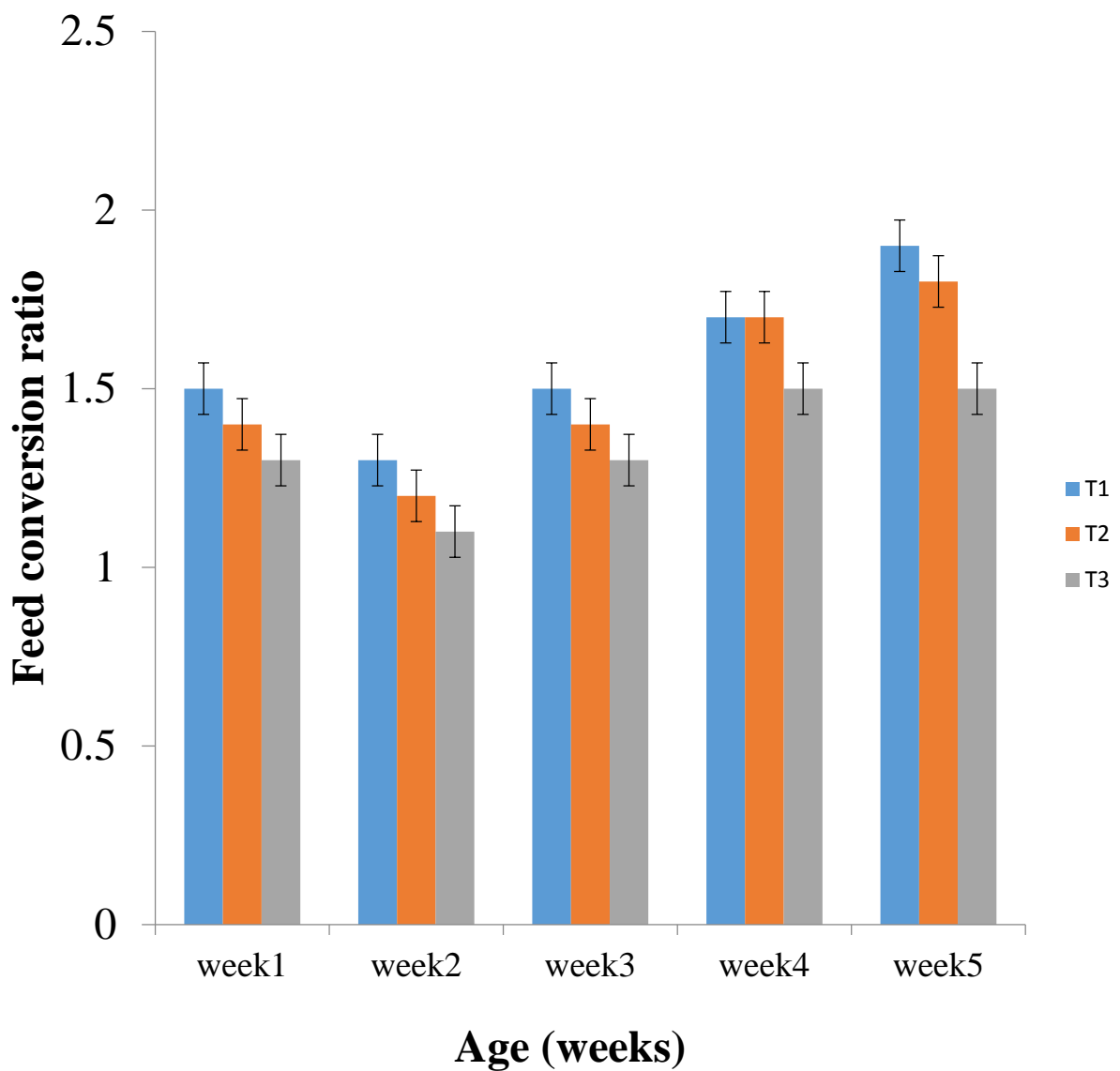
228 The poultry industry currently aims at producing a feed with low intake but high conversion
229 ratio which can consequently result in high body weight gains. Treatment 3 (10% *T. molitor* L
230 inclusion) seemed to be particularly suitable for such objective as this treatment had low FI but
231 high conversion ratio which consequently resulted in high body weight gains. This higher feed
232 conversion ratio in broilers supplemented with 10% *T. molitor* L indicate its potential

233 advantage to be considered as an alternative to commonly used protein sources such as fishmeal
234 and soybean meal.

235 .

236

237



238

239 **Figure 3.4:** Feed conversion ratio in the groups T1 (0% *T. molitor* L), T2 (5% *T. molitor* L)
240 and T3 (10% *T. molitor* L).

3.5. Conclusion

It was concluded from this study that *T. molitor* L illustrates a great potential of being used as an alternative protein source that can replace the commonly used protein sources in poultry nutrition. Broilers fed diets supplemented with 10% *T. molitor* L had significantly lower feed intake, but gained significantly higher body weight and had a lower feed conversion ratio when compared with the chickens fed with 5% *T. molitor* L and the chickens fed control diets (0% *T. molitor* L). Thus T3 (10% *T. molitor* L) was the best inclusion level in the current study. However, the current study has also shown that palatability factors affect the intake of diets if the inclusion levels of *T. molitor* L are increased. Further investigations with more different inclusion levels of *T. molitor* L supplementation to broilers feeds are also recommended to come up with the most appropriate inclusion level of *T. molitor* L.

3.6. References

- Adeniji, A.A.** 2007. Effect of replacing groundnut cake with maggot in the diet of broilers. *International Journal of Poultry Science*, **6**:822-825
- Aftahi, A., Munim, T., Hoque, M.A. and Ashraf, M.A.** 2006. Effects of yoghurt and protexin boost on broiler performance. *International journal of Poultry Science*, **5**: 651-655.
- Anand, H.A.** 2008. Potential value of acridids as high protein supplement for poultry feed. *International of poultry science*, **7**: 722-725.
- Awonoyi, T.A.M., Aletor, V.A. and Aina, J.M.** 2003. Performance of broiler –chickens fed on maggot meal in place of fishmeal. *International Journal Poultry Science*, **2**: 271-274.
- Ballitoc, D.A. and Sun S.**2013Ground yellow mealworms (*Tenebrio molitor* L.) feed supplementation improves growth performance and carcass yield characteristics in broilers. Accessed on 04 December from Open Science Repository Agriculture, Doi:10.7392/openaccess.23050425.
- DeFoliart, G., Dunkel, F.V. and Gracer, D.** 2009. The food insects’ newsletter–chronicle of changing culture. Salt Lake City: Aardvark Global Publishing, p. ix + 414.
- Ellinger, G.M.** 1958. The importance of protein quality in animal feeding. *Proceedings of the Nutritional Society*, **17**:100-106.
- Ghaly, A.E. and Alkoaik, F.N.** 2009. The yellow mealworms as a novel source of protein. *American Journal of Agricultural and Biological Sciences*, **4**:319-331
- Hwangbo, J., Hong, E.C., Jang, A., Kang, H.K., Kim, B.W. and Park, B.S.** 2009. Utilization of house fly-maggots, a feed supplement in the production of broiler chickens. *Journal of Environmental Biology*, **30**: 609-614.

Ifie, I. and Emeruwa, C.H. 2011. Nutritional and anti-nutritional characteristics of the larvae of *Oryctes monoceros*. *Agriculture and Biology Journal of North America*, **2**:42-46

Kermanshahi, H. and Rostami, H. 2006. Influence of supplements dried mealworms on broiler performance and Cecal Flora. *Internal Journal of Poultry Science*, **5**: 538-543.

National Research Council. 1985. National Research Council (Guide for the Care and Use of Laboratory Animals), Publication No. 85 – 23. National Academy Press, Washington DC, USA.

Pimental, D., Dritschilo, W., Krummel, J. and Kutzman, J. 1975. Energy and land constraints in food protein production. *Science*, **190**:754–61.

Ramos-Elorduy, J. 2005. Insects: a hope food source. In: Ecological implications of mini livestock (potential of insects, rodents, frogs, and snails) Eds. Science Publisher, INC: 263-291.

Statistical Analyses System (SAS). 2009. SAS/STAT Software 9.2 Release TS1M0. SAS Institute Inc., Cary, NC, USA.

Téguia, A., Mpoame, M. and Mba, J.A.O. 2002. The production performance of broiler birds as affected by the replacement of fish meal by maggot meal in the starter and finisher diets. *Tropicultura*.**20**: 187-192.

Chapter 4: The effect of *Tenebrio molitor* larvae (meal worm) supplementation on carcass yield and meat quality of broiler chickens

Abstract

The objective of this study was to evaluate the effect of different inclusion levels of *Tenebrio molitor* larvae (*T. molitor* L) supplementation on carcass characteristics, meat yield and meat quality of broiler chickens. The treatments comprised of three graded levels of the *T. molitor* L at 0% (control), 5% and 10% respectively. Live weights (LW) of broilers were recorded before slaughter and thereafter carcass weights (CW), meat yield (weights of breast, thigh, drumstick and wing) and dressing percentage (DP %) were recorded. Breast muscles were sampled for meat pH and colour measurements. The LW of birds from T1 (0% *T. molitor* L) were significantly different ($P < 0.05$) from both T2 (5% *T. molitor* L) and T3 (10% *T. molitor* L) which were similar to each other, with T2 exhibiting the highest live weights (2166g) and the control treatment exhibiting the lowest live weights (2018.3g). In CW, T1 was significantly different ($P < 0.05$) from T2 while it was similar ($P > 0.05$) to T3. The DP% of T1 was significantly different ($P < 0.05$) from both T2 and T3 which were similar to each other, with T2 having the highest dressing percentage (78, 2%) and T1 having the lowest DP% (66%). The breasts in T2 were significantly higher and different ($P < 0.05$) from both T1 and T3 which were similar to each other. The drumsticks in T3 were significantly different ($P < 0.05$) from T1 while they were similar to T2. After 45 minutes of slaughter, a significant difference ($P < 0.05$) was observed in L^* among all treatments. In redness (a^*) T3 was significantly different from T1 but was similar to T2 and all treatments in this study exhibited a dark red meat, with T3 muscles exhibiting dark red colour than the other treatments. Similar results were observed in yellowness (b^*), where the breast muscles from T3 were more yellow than the other treatments. After 24 hours of slaughter T2 L^* values were significantly different ($P < 0.05$) from both T1 and

T3 which were similar to each other. It was, therefore, concluded that *T. molitor* L meal can be incorporated into the diets of broilers to produce heavy birds either at 5 or 10%. However, although 5% *T. molitor* L inclusion yields heavier carcasses, the 10% *T. molitor* L inclusion compared favourably to 5% *T. molitor* L since it required low feed intake to reach the same slaughter weights and there were slight differences on meat quality attributes between the two treatments. Thus 10% *T. molitor* L was the best inclusion level in the current study.

Keywords: Breast muscle, drumstick, meat colour, meat pH, thigh, wing

1 **4.1 Introduction**

2 Broiler chickens have been improved in many traits such as daily weight gain, feed efficiency
3 and resistance to diseases. However, the high selection intensity for growth rate has caused
4 many problems; especially the decreasing development of meat quality attributes (Rance *et al.*,
5 2002). The main factors that determine broiler meat quality can be divided into the appearance
6 and physical characteristics of the meat and these factors are exclusively determined by the
7 consumer (Allen *et al.*, 1998; Van Laack *et al.*, 2000 Qiao *et al.*, 2001; Swatland, 2004; Huff-
8 Lonergan and Lonergan, 2005). The major parameters considered in the assessment of meat are
9 attributes, such as juiciness, tenderness, drip loss, cooking-loss, ultimate pH and shelf-life
10 (Muchenje *et al.*, 2009). Meat colour, drip and others act as a determining factor of whether the
11 products will be satisfactory or not to the consumers (Huff-Lonergan and Lonergan, 2005).

12

13 Meat pH is an important factor that has an influence on meat colour, tenderness and water
14 holding capacity (Van Laack *et al.*, 2000; Huff-Lonergan and Lonergan, 2005). During the
15 process of rigor mortis when muscle is converted to meat, the acidification of meat takes place.
16 (Allen *et al.*, 1998; Qiao *et al.*, 2001; Swatland, 2004). The measuring of meat pH helps gives
17 an indication of the degree of meat acidification after slaughter and an indirect measure of the
18 meat quality. (Huff-Lonergan Lonergan, 2005). In the study by Hwangbo *et al.* (2009) on
19 broiler carcass colour of chicks that received diets containing insect larvae meal as a protein
20 source., the results revealed that house fly larvae meal supplementation had no significant
21 effect ($P>0.05$) on breast meat colour. According to Allen *et al.* (1998) dark coloured broiler
22 meat have higher pH values than lighter coloured meat, but the darker meat have a reduced
23 shelf-life that could be attributed to the increased number of psychotropic bacteria that colonize
24 the darker meat.

25 Published results show that insect larvae meal supplementation has a significant influence on
26 various carcass characteristics, such as; dressing percentage as well as breast muscle and thigh
27 muscle yield as percentage of the carcass weight (Hwangbo *et al.*, 2009). This research was
28 conducted to investigate how carcass characteristics are affected by the supplementation of
29 larvae meal, while the current study sought to investigate how meat and carcass quality are
30 affected at different inclusion levels of larvae meal.

31

32 **4.2. Materials and Methods**

33 **4.2.1. Description of study sites**

34 The information on the study site, experimental animals and management of the chickens is
35 described in Section 3.2.1 and 3.2.2.

36

37 **4.2.2. Animals and experimental procedure**

38 The same birds which were used on the first trial (Chapter 3) were further used for the second
39 trial. After the evaluation of growth performance (first trial) the broilers were slaughtered at 35
40 days of age for the evaluation of meat quality, carcass characteristics and meat yield. Eighteen
41 chickens from each treatment (Control; 5% *T. molitor* L and 10% *T. molitor* L, inclusion levels)
42 were slaughtered. The birds were slaughtered according to acceptable commercial standards.

43

44 **4.2.3. Slaughter procedure.**

45 Feed was withdrawn for 12 hours before slaughter while water was provided *ad libitum*.
46 Slaughtering was done following the normal procedures of the abattoir. Prior to slaughter, the
47 birds were first stunned with a (50-70 volts) electrical stunner under the beak for 5 seconds

48 before their throats were cut for exsanguination. Plucking of feathers was done after slaughter
49 and thereafter were fully dressed. The carcasses were then separated into breast, thigh, feet,
50 head and the internal organs (viscera).

51

52 **4.3. Data collection**

53 **4.3.1. Carcass characteristics and meat yield measurements**

54 Before and after slaughter broiler live and carcass weights were recorded and thereafter,
55 dressing percentage was calculated as proportion of carcass weight to live weight. The
56 carcasses were further portioned to obtain commercial cut yields (breast, thigh, drumstick and
57 wing). The breast muscles were removed by cutting from the clavicle furcula bone alongside
58 the carina (keel) bone.

59

60 **4.3.2. Meat pH measurement**

61 The pH measurements were taken on the breast muscle of the chickens. The pH was determined
62 with a CRISON pH 25 (CRISON Instruments SA, Spain) which was calibrated before each
63 measurement using pH4, pH7, and pH9 standard solutions. The pH meter probe was placed
64 directly into the left breast muscle and the instrument was given time to stabilize before the pH
65 reading was taken. Between each measurement the probe was rinsed with distilled water and
66 rested in a 3M KCl electrolytic solution. The measurements were done approximately 45
67 minutes and 24 hours after slaughter.

68

69 **4.3.3. Determination of colour**

70 Colour was measured according to the CIE L* a* b* colour system using a colour-guide 45°/0°
71 colorimeter (BYK-Gardner GmbH, Geretsried, Germany) with a 20mm diameter measurement

72 area and illuminant D65-day light and 10° standard observer on the breast muscle. Three
73 readings were taken on each muscle by rotating the Colour Guide 90° between each
74 measurement, in order to obtain a representative average value of the colour. The machine was
75 first calibrated using the green standard before each measurement.

76

77 **4.4. Statistical analysis**

78 The data was analysed using the Statistical Analysis Systems (SAS) package of 2009. The
79 General linear model procedure (PROC GLM) was used to test the effect of different levels of
80 *T. molitor* L on meat quality (meat pH and colour) carcass characteristics (dressing%, live
81 weight, carcass weight) and meat yield (breast, thigh, drumstick and wing). The significant
82 difference between least square means was compared using fishers' least significant difference
83 (LSD) and differences were considered to be significant at $P < 0.05$.

84

85 The following model was used:

86 $Y_{ij} = \mu + T_i + E_{ij}$ where;

87 Y_{ij} – Response variables (meat pH, colour, dressing%, live weight, carcass weight and meat
88 yield)

89 μ - Overall mean

90 T_i – i^{th} effect of different levels of *Tenebrio molitor* larvae (T1 0% inclusion; T2 5% inclusion;
91 T3 10% inclusion)

92 E_{ij} – Random error

93

94 **4.5 Results and discussion**

95 Table 4.1 shows the effect of *T. molitor* L on meat quality, carcass characteristics and carcass
96 yield. The live weights in the control treatment T1 (0% *T. molitor* L) were significantly
97 different ($P < 0.05$) from both T2 (5% *T. molitor* L) and T3 (10% *T. molitor* L) which were
98 similar to each other, with T2 exhibiting the highest live weights (2166 g) and the control
99 treatment exhibiting the lowest live weights (2018.3 g). In carcass weights T1 was
100 significantly different ($P < 0.05$) from T2 while it was similar ($P > 0.05$) to T3. However, T3 was
101 similar ($P > 0.05$) to both T1 and T2 with values (1509.3 g, 1346.6 g and 1661.7 g, respectively).

102

103 The dressing % of T1 was significantly different ($P < 0.05$) from T2 and T3 which were similar
104 to each other, with T2 having the highest dressing percentage (78, 2%) and T1 having the
105 lowest DP% (66%). These results are similar to those reported by Hwangbo *et al.* (2009) that
106 broilers receiving larvae meal in their diets have significantly better dressing percentages than
107 the control group. The results from the current study further suggest that although the birds that
108 were subjected to 5% *T. molitor* L diet (T2) had the highest LW, CW and DP% than the other
109 treatments (T1 and T3), T3 compared favourably to T2.

110

111 Carcass yield was evaluated based on the mass of different portions. The breasts in T2 were
112 significantly higher and different ($P < 0.05$) from both T1 and T3 which were similar to each
113 other. No significant differences were observed on thighs drumsticks and wings among all
114 treatments. These results are similar to those observed in carcass characteristics, suggesting
115 that although the birds that were subjected to 5% *T. molitor* L diet (T2) had the highest masses
116 of breast, thigh and wing than the other treatments (T1 and T3), T3 compared favourably to
117 T2.

Table 4.1: Least square means \pm STD error of carcass characteristics, meat yield and meat quality as affected by *Tenebrio molitor* larvae at different inclusion levels (0%, 5% and 10%)

	Treatment 1	Treatment 2	Treatment 3
	Control	5% Meal worms	10% Meal worms
Live weight (g)	2018.3 ^a \pm 29.67	2166 ^b \pm 29.67	2105.3 ^b \pm 29.67
Carcass weight (g)	1346.8 ^a \pm 82.96	1661.7 ^b \pm 82.96	1509.3 ^{ab} \pm 82.96
Dressing percentage (%)	66.0 ^a \pm 3.96	78.2 ^b \pm 3.96	68.7 ^a \pm 3.96
Body portion masses (grams) Right side			
Breast	244.3 ^a \pm 9.04	289.1 ^b \pm 9.04	261.7 ^a \pm 9.04
Thigh	201.0 ^a \pm 6.87	210.4 ^a \pm 6.87	207.8 ^a \pm 6.87
Drumstick	90.9 ^a \pm 6.09	97.4 ^{ab} \pm 6.09	95.4 ^b \pm 6.09
Wing	76.0 ^a \pm 4.83	87.9 ^a \pm 4.83	79.9.8 ^a \pm 4.83
Colour and pH measurements Breast muscle after 45 minutes			
L*	43.8 ^a \pm 0,34	44.5 ^a \pm 0,34	42.8 ^b \pm 0,34

a*	3.1 ^a ± 0,14	3.0 ^a ± 0,14	3.3 ^a ± 0,14
b*	13.3 ^a ± 0,27	13.5 ^a ± 0,27	13.8 ^a ± 0,27
pH	6.1 ^a ± 0,02	6.1 ^a ± 0,02	6.2 ^b ± 0,02
Hue degrees	77 ^a ± 0,56	77.1 ^a ± 0,56	76.7 ^a ± 0,56
Chroma	13.7 ^a ± 0,27	13.9 ^a ± 0,27	14.21 ^a ± 0,27
Colour and pH measurements Breast muscle after 24 hours			
L*	46.7 ^a ± 0,34	46 ^a ± 0,34	46.8 ^a ± 0,34
a*	3.7 ^a ± 0,14	4.09 ^a ± 0,14	3.52 ^a ± 0,14
b*	13.8 ^a ± 0,26	14.2 ^a ± 0,26	13.6 ^a ± 0,26
pH	5.8 ^a ± 0,02	5.8 ^a ± 0,02	5.8 ^a ± 0,02
Hue degrees	74.9 ^a ± 0,56	73.4 ^b ± 0,56	75.6 ^c ± 0,56
Chroma	14.3 ^a ± 0,27	14.8 ^a ± 0,27	14,1 ^a ± 0,27

^{a, b} Means with different superscripts within the same row differ significantly at (P<0.05); L*- lightness, a*- redness, b*- yellowness

After 45 minutes of slaughter, a significant difference ($P < 0.05$) was observed in L^* among all treatments. According to Van Laack *et al.* (2000) the normal meat colour of the CIE L^* , a^* and b^* measurements are 55.1, 2.2 and 9.6 respectively. In the current study the breast muscle colour L^* had the following values; T1 (43.8), T2 (44.5) and T3 (42.8). These results revealed that all the chickens produced meat with darker colour than described by Van Laack *et al.* (2000). The results suggest that the consumers will not prefer chicken with darker colour, as colour is the first used technique to detect consumer acceptability of meat or meat products in the retail outlets. In redness (a^*) T3 was significantly different from T1 but was similar to T2. According to the (a^*) values of normal meat as described by Van Laack *et al.* (2000), all treatments (T1 3.1; T2 3.0; and T3 3.3) in this study exhibited a darker red meat, with T3 muscles exhibiting a higher darker red colour than the other treatments. Similar results were observed in yellowness (b^*), where the breast muscles from T3 were more yellow than the other treatments with values T1 (13.3), T2 (13.5) and T3 (13.8).

No significant differences were observed in pH, hue degrees and chroma values among all treatments. The pH values observed in the current study were; T1 (6.1), T2 (6.1) and T3 (6.2). Van Laack *et al.* (2000) reported that the pH of normal meat is 5.96, thus, the results reported in the current study showed that the breast muscle pH among all the treatments was slightly above normal. Since there were no significant differences on the pH values among all treatments, the results further suggest that *T. molitor* L can be included at 5% and 10 % without having an effect on the broiler meat pH.

After 24 hours of slaughter T2 L^* values were significantly different ($P < 0.05$) from both T1 and T3 which were similar to each other. No significant differences were observed in a^* , b^* , pH and

chroma among all treatments, while the hue degrees were different among all treatments. These results also suggest that *T. molitor* L can be included either at 5% or 10 % with fewer differences on the meat quality attributes as they were observed to be similar in most cases.

4.6. Conclusion

The inclusion of *T. molitor* L affects live and carcass weights, dressing percentage as well as meat yield of broiler chickens. Broilers that received 5% and 10% *T. molitor* L inclusion in their diets were heavier than the birds which were in the control treatment and had higher DP% and meat yield. However, slight variations were observed between the inclusion levels (5 and 10%), where T2 birds (5% inclusion) exhibited heavier live and carcass weights as well as high DP% with heavier portions such as breast, thigh and wing than the T3 birds (10% inclusion). In meat quality, variations among the treatments on L^* , a^* and b^* were evident 45 minutes after slaughter, while only variations on L^* were evident 24 hours after slaughter. It can therefore be concluded that *T. molitor* L meal can be incorporated into the diets of broilers to produce heavy birds either at 5 or 10% without affecting meat quality characteristics. Nonetheless 10% *T. molitor* L can be recommended as the best inclusion level since it compared favourably to 5% inclusion level at low intake.

4.7. References

- Allen, C.D., Russell, S.M. and Fletcher, D.L.** 1998. Department of Poultry Science. University of Georgia, Athens. *Poultry Science*, **76**:1042-108.
- Huff-Lonergan, E. and Lonergan, S.M.** 2005. Mechanisms of water-holding capacity of meat: The role of post mortem biochemical and structural changes. *Meat Science*, **71**: 194-204.
- Hwangbo, J., Hong, E.C., Jang, A., Kang, H.K., Oh, J.S., Kim, B.W. and Park, B.S.** 2009. Utilization of housefly-maggots, a feed supplement in the production of broiler chickens. *Journal of Environmental Biology*, **30**: 609-614.
- Muchenje, V., Dzama K., Chimonyo, M., Strydom, P.E. and Raats, J.G.** 2009. Relationship between stress responsiveness and meat quality in three cattle breeds. *Meat Science*, **81**: 653-657.
- Qiao, M., Fletcher, D.L., Smith, D.P. and Northcutt, J.K.,** 2001. The effect of broiler breast meat colour on pH, moisture, water-holding capacity, and emulsification capacity. *Poultry Science*, **80**: 676- 680.
- Rance, K.A., Mcentee, G.M. and Mcdevitt, R.M.** 2002. Genetic and phenotypic relationships between and within support and demand tissues in a single line of broiler chicken. *British Poultry Science*, **43**: 518-527.
- Statistical Analyses System (SAS).** 2009. SAS/STAT Software 9.2 Release TS1M0. SAS Institute Inc., Cary, NC, USA.
- Swatland, H.J.** 2004. Progress in understanding the paleness of meat with a low pH. *South African Journal of Animal Science*, **34**:1-7.
- Van Laack, R.L.J.M., Liu, C.H., Smith, M.O. and Loveday, H.D.** 2000. Characteristics of pale, soft, exudative broiler breast meat. *Poultry Science*, **79**:1057–1061.

Chapter 5: General discussion, conclusions and recommendations

5.1. General discussion

The broad objective of the study was to determine the growth performance, meat yield, meat pH and colour of broiler chickens given diets with different levels of *T. molitor* L as a protein source. One hundred and forty-four unsexed day-old Cobb-500 broiler chicks 35 days of age were slaughtered. The growth performance of broilers given diets containing different levels of *T. molitor* L was determined in Chapter 3 where average body weights (ABW), average feed intake (AFI), average daily gain (ADG) and feed conversion ratio (FCR) were evaluated. Furthermore, the effect of *T. molitor* L as a protein source on live and carcass weights, dressing percentage (DP %), meat yield and meat quality characteristics (meat colour, pH) of broilers was determined in Chapter 4.

In Chapter 3, it was found that the broiler chickens given diet with no *T. molitor* L inclusion (T1) had lower values of BW, FI, ADG and FCR throughout the experiment than those that were in T2 and T3 with 5% and 10 % *T. molitor* L inclusion levels, respectively. However, it was also found that although broilers with 5% *T. molitor* L inclusion (T2) in their diet had high BW and ADG than the broilers with 10% *T. molitor* L inclusion (T3), the T3 birds compared favourably to T2 birds as they required low feed intake to reach the same slaughter weight due to high FCR. Other studies also reported that the groups with increasing percentages of ground yellow mealworm inclusion in the diets the feed conversion ratio was significantly lower (Bollitoc and Sun, 2013).

In chapter 4 it was found that the inclusion of *T. molitor* L affects the carcass and meat quality characteristics as well as meat yield of broiler chickens, where birds that were subjected to 5%

and 10% *T. molitor* L diet were found to have heavier live weights (LW), carcass weights (CW), high DP% and meat yield (measured on the basis of breast, drumstick, thigh and wing) than those in 0% *T. molitor* L diet. However, slight variations on these characteristics were also observed between the 5 and 10% inclusion levels. Broilers that were subjected to 5% *T. molitor* L diet were found to have heavier LW and CW, high DP% and meat yield than those that were in 10% *T. molitor* L diet.

Since these variations were also quite small it was, therefore, also suggested in the referred chapter that although T2 birds had higher LW, CW, DP% and meat yield, T3 birds compared favourably to T2 birds. In similar study done by Okah and Onwunjiariri (2012) where they used maggot meal as fish meal replacement in broiler diets and measured the performance they found out that replacement of a 4.0% dietary fish meal with 50% maggot meal resulted to improved performance characteristics, while Hwangbo *et al.* (2009) found that maggots inclusion in diets significantly improved dressing percentage, breast muscle and thigh muscle of broiler chickens.

It was also found that some meat quality attributes (L^* , a^* and b^*) varied among all treatments at 45 minutes after slaughter, although these variations were only evident on L^* 24 hours after slaughter. It was therefore, suggested that the *T. molitor* L can be included in boiler diets either at 5 or 10% with less effect on meat quality characteristics.

5.2. Conclusions

It was, therefore, concluded in the current study that *T. molitor* L can be considered as an alternative protein source to broiler chickens as it seems to be particularly suitable in broiler

nutrition. This was evident as improved growth performance, carcass characteristics, meat yield and meat quality attributes were achieved from broilers subjected to 5 and 10% *T. molitor* L inclusion levels than those that were in the control treatment (0%*T. molitor* L). While the inclusion level of 5% gave the best results.

5.3. Recommendations

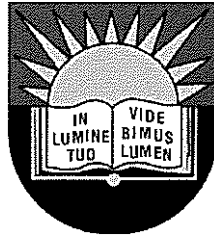
It can, however, be recommended that poultry farmers should include *T. molitor* L at 10% inclusion levels since broilers can consume feed with this particular inclusion at low feed intake but reach the same slaughter weights as those in 5% inclusion which consequently results in high meat yield. This might be cost effective for the farmers, thus improving their profitability as these worms are also cheaper than the normal broiler diets.

5.4 References

Ballitoc, D.A. and Sun, S.2013. Ground yellow mealworms (*Tenebrio molitor* L.) feed supplementation improves growth performance and carcass yield characteristics in broilers. Accessed on 04 December 2015 from Open Science Repository Agriculture/Doi:10.7392/openaccess.23050425.

Hwangbo, J., Hong, E.C., Jang, A., Kang, H.K., Oh, J.S., Kim, B.W. and Park, B.S. 2009. Utilization of housefly-maggots: a feed supplement in the production of broiler chickens. *Journal of Environmental Biology*, **30**: 609-614.

Okah, U. and Onwujiariri, E.B. 2012. Performance of finisher broiler chickens fed maggot meal as a replacement for fish meal. *Journal of Agricultural Science and Technology*, **8**:471-477.



University of Fort Hare
Together in Excellence

ETHICAL CLEARANCE CERTIFICATE
REC-270710-028-RA Level 01

Certificate Reference Number: MUS061SMNG01

Project title: **Black Soil fly larvae as an alternative protein supplement feed for improved growth and meat quality of indigenous chickens**

Nature of Project: Masters

Principal Researcher: Sinethemba Mngqi

Supervisor: Prof V Muchenje

Co-supervisor:

On behalf of the University of Fort Hare's Research Ethics Committee (UREC) I hereby give ethical approval in respect of the undertakings contained in the above-mentioned project and research instrument(s). Should any other instruments be used, these require separate authorization. The Researcher may therefore commence with the research as from the date of this certificate, using the referencenumber indicated above.

Please note that the UREC must be informed immediately of

- Any material change in the conditions or undertakings mentioned in the document
- Any material breaches of ethical undertakings or events that impact upon the ethical conduct of the research

The Principal Researcher must report to the UREC in the prescribed format, where applicable, annually, and at the end of the project, in respect of ethical compliance.

Special conditions: Research that includes children as per the official regulations of the act must take the following into account:

Note: The UREC is aware of the provisions of s71 of the National Health Act 61 of 2003 and that matters pertaining to obtaining the Minister's consent are under discussion and remain unresolved. Nonetheless, as was decided at a meeting between the National Health Research Ethics Committee and stakeholders on 6 June 2013, university ethics committees may continue to grant ethical clearance for research involving children without the Minister's consent, provided that the prescripts of the previous rules have been met. This certificate is granted in terms of this agreement.

The UREC retains the right to

- Withdraw or amend this Ethical Clearance Certificate if
 - o Any unethical principal or practices are revealed or suspected
 - o Relevant information has been withheld or misrepresented
 - o Regulatory changes of whatsoever nature so require
 - o The conditions contained in the Certificate have not been adhered to
- Request access to any information or data at any time during the course or after completion of the project.
- In addition to the need to comply with the highest level of ethical conduct principle investigators must report back annually as an evaluation and monitoring mechanism on the progress being made by the research. Such a report must be sent to the Dean of Research's office

The Ethics Committee wished you well in your research.

Yours sincerely



Professor Gideon de Wet
Dean of Research

27 April 2015

Feed formulation chart of three diets containing different levels of *Tenebrio molitor* larvae (0%, 5% and 10 %)

STARTER

5% worms		Control	
Yellow maize	60,703	Yellow maize	63,415
Gluten 20	0,968067	Wheat Middlings	0,959037
Wheat Middlings	5	Soya Oilcake	21,61143
Soya Oilcake	14,3855	Sunflower Oilcake	10
Sunflower Oilcake	10	Limestone Powder	1,449859
Worms	5	Monocalcium Phosphate(KK)	1,182452
Limestone Powder	1,475297	Salt Fine	0,364256
Monocalcium Phosphate(KK)	1,012126	Methionine DL Powder	0,200169
Salt Fine	0,308522	Tryptophan	0,039958
Methionine DL Powder	0,205646	Lysine HCL Powder	0,340747
Tryptophan	0,156171	Threonine	0,066878
Lysine HCL Powder	0,3442	Choline Chloride Powder 60%	0,086406
Threonine	0,071377	Vit/Min premix	0,2
Choline Chloride Powder 60%	0,086406	Elancoban 20%	0,05
Vit/Min premix	0,2	Zinc Bacitracin 15%	0,0334
Elancoban 20%	0,05		
Zinc Bacitracin 15%	0,0334		
10% Worms			
Yellow maize	57,024		
Gluten 20	2,518207		
Wheat Middlings	5		
Soya Oilcake	11,88705		
Sunflower Oilcake	10		
Worms	10		
Limestone Powder	1,645443		
Monocalcium Phosphate(KK)	0,822447		
Salt Fine	0,250736		
Methionine DL Powder	0,172374		
Tryptophan	0,077578		
Lysine HCL Powder	0,218515		
Threonine	0,013731		
Choline Chloride Powder 60%	0,086406		
Vit/Min premix	0,2		
Elancoban 20%	0,05		
Zinc Bacitracin 15%	0,0334		

GROWER

NOWORMS		5% WORMS	
Yellow Maize	67,031	Yellow Maize	64,137
Wheat Middlings	2,087863	Gluten 20%	1,432046
Soya Oilcake	17,27319	Wheat Middlings	5
Sunflower Oilcake	10	Soya Oilcake	10,95532
Worms	0	Sunflower Oilcake	10
Limestone Powder	1,295691	Worms	5
Monocalcium Phosphate(KK)	0,938129	Limestone Powder	1,347539
Salt Fine	0,369134	Monocalcium Phosphate(KK)	0,762302
Methionine DL Powder	0,167721	Salt Fine	0,312555
Tryptophan	0,061956	Methionine DL Powder	0,165104
Lysine HCL Powder	0,342201	Tryptophan	0,142823
Threonine	0,06306	Lysine HCL Powder	0,320702
Choline Chloride Powder 60%	0,086406	Threonine	0,054793
Vit/Min premix	0,2	Choline Chloride Powder 60%	0,086406
Elancoban 20%	0,05	Vit/Min premix	0,2
Zinc Bacitracin 15%	0,0334	Elancoban 20%	0,05
VOLUME %	100	Zinc Bacitracin 15%	0,0334
10% WORMS			
Yellow Maize	60,925		
Gluten 20%	4,583782		
Wheat Middlings	5		
Soya Oilcake	6,264522		
Sunflower Oilcake	10		
Worms	10		
Limestone Powder	1,44321		
Monocalcium Phosphate(KK)	0,572123		
Salt Fine	0,253319		
Methionine DL Powder	0,146338		
Tryptophan	0,16558		
Lysine HCL Powder	0,254122		
Threonine	0,021562		
Choline Chloride Powder 60%	0,086406		
Vit/Min premix	0,2		
Elancoban 20%	0,05		
Zinc Bacitracin 15%	0,0334		

FINISHER

10% WORMS		5% WORMS	
Yellow Maize	62,553	Yellow Maize	67,682
Gluten 20%	6,684526	Gluten 20%	2,31225
Wheat Middlings	5	Wheat Middlings	5
Soya Oilcake	9,042331	Soya Oilcake	6,975932
Sunflower Oilcake	4	Sunflower Oilcake	10
Worms	10	Worms	5
Limestone Powder	1,74524	Limestone Powder	1,391006
Monocalcium Phosphate(KK)	0,195101	Monocalcium Phosphate(KK)	0,399975
Salt Fine	0,247766	Salt Fine	0,316925
Methionine DL Powder	0,1083	Methionine DL Powder	0,135613
Tryptophan	0,034563	Tryptophan	0,153576
Lysine HCL Powder	0,102351	Lysine HCL Powder	0,307423
Choline Chloride Powder 60%	0,086406	Threonine	0,038882
Vit/Min premix	0,2	Choline Chloride Powder 60%	0,086406
		Vit/Min premix	0,2
NOWORMS			
Yellow Maize	70,618		
Wheat Middlings	3,149371		
Soya Oilcake	13,04753		
Sunflower Oilcake	10		
Worms	0		
Limestone Powder	1,334965		
Monocalcium Phosphate(KK)	0,581112		
Salt Fine	0,374703		
Methionine DL Powder	0,141822		
Tryptophan	0,077858		
Lysine HCL Powder	0,336005		
Threonine	0,052386		
Choline Chloride Powder 60%	0,086406		
Vit/Min premix	0,2		

EPOL CENTRAL LABORATORY



Sample 612014

BERLIN-08/12/2015-7

Status: Authorized **Login By:** PHUMZILE
Product: F00001
Description: Miscellaneous Feed
Location: BERLIN **Login Date:** 2015-12-08
Customer: EPOL_BER **Date Completed:** 2015-12-15
Site: BERLIN **Date Reviewed:** 2015-12-15
Supplier: EPOL_BER **Reviewer:** AUTO
Source: BERLIN
Delivery Note: For there
Mix Number: Finisher Sample 1

Comments:

For there Student Feed

Test / Rep	Status	Component	Result Value	Analyst
ASH	Authorized	ASH %	4.70 %	NOLWAZI
CA	Authorized	Calcium(%)	0.97 %	NOLENE
FAT	Authorized	%Fat(F)	2.65 %	BONGIWE
FIBRE	Authorized	% Fibre	4.55 %	NOLWAZI
MST_OVN_CL	Authorized	%LOD ECL	11.73 %	NOLWAZI
NA	Authorized	Sodium(%)	0.12 %	NOLENE
PHOS	Authorized	Phos(%)	0.65 %	NOLENE
PROT_LECO	Authorized	Protein	17.76 %	LABSTATION

EPOL CENTRAL LABORATORY



Sample 612015

BERLIN-08/12/2015-8

Status: Authorized **Login By:** PHUMZILE
Product: F00001
Description: Miscellaneous Feed
Location: BERLIN **Login Date:** 2015-12-08
Customer: EPOL_BER **Date Completed:** 2015-12-15
Site: BERLIN **Date Reviewed:** 2015-12-15
Supplier: EPOL_BER **Reviewer:** AUTO
Source: BERLIN
Delivery Note: For there
Mix Number: Finisher Sample2

Comments:

For there Student Feed

Test / Rep	Status	Component	Result Value	Analyst
ASH	Authorized	ASH %	4.39 %	NOLWAZI
CA	Authorized	Calcium(%)	0.79 %	NOLENE
FAT	Authorized	%Fat(F)	4.41 %	BONGIWE
FIBRE	Authorized	% Fibre	4.96 %	NOLWAZI
MST_OVN_CL	Authorized	%LOD ECL	11.55 %	NOLWAZI
NA	Authorized	Sodium(%)	0.11 %	NOLENE
PHOS	Authorized	Phos(%)	0.64 %	NOLENE
PROT_LECO	Authorized	Protein	20.24 %	LABSTATION

EPOL CENTRAL LABORATORY



Sample 612016

BERLIN-08/12/2015-9

Status:	Authorized	Login By:	PHUMZILE
Product:	F00001		
Description:	Miscellaneous Feed		
		Login Date:	2015-12-08
Location:	BERLIN	Date Completed:	2015-12-15
Customer:	EPOL_BER	Date Reviewed:	2015-12-15
		Reviewer:	AUTO
Site:	BERLIN	Comments:	
Supplier:	EPOL_BER		For there Student Feed
Source:	BERLIN		
Delivery Note:	For there		
Mix Number:	Finisher Sample 3		

Test / Rep	Status	Component	Result Value	Analyst
ASH	Authorized	ASH %	5.18 %	NOLWAZI
CA	Authorized	Calcium(%)	1.09 %	NOLENE
FAT	Authorized	%Fat(F)	4.25 %	BONGIWE
FIBRE	Authorized	% Fibre	4.27 %	NOLWAZI
MST_OVN_CL	Authorized	%LOD ECL	10.55 %	NOLWAZI
NA	Authorized	Sodium(%)	0.13 %	NOLENE
PHOS	Authorized	Phos(%)	0.76 %	NOLENE
PROT_LECO	Authorized	Protein	20.27 %	LABSTATION

EPOL CENTRAL LABORATORY



Sample 565878

BERLIN-04/06/2015-11

Status:	Authorized	Login By:	PHUMZILE
Product:	R00001	Login Date:	2015-06-04
Description:	Miscellaneous Raw Material	Date Completed:	2015-06-12
Location:	BERLIN	Date Reviewed:	2015-06-12
Customer:	EPOL_BER	Reviewer:	AUTO
Site:	BERLIN	Comments:	For there University
Supplier:	EPOL_BER		
Source:	BERLIN		
Delivery Note:	For there		
Mix Number:			

Test / Rep	Status	Component	Result Value	Analyst
ADF_NIR_BR	Authorized	ADF %	10.09 %	LABSTATION
ASH	Authorized	ASH %	5.59 %	NOLWAZI
ASH_NIR_BR	Authorized	Ash NIR BR (%)	0.68 %	LABSTATION
CA	Authorized	Calcium(%)	0.03 %	NOLENE
CA_NIR_BR	Authorized	Calcium(%)	0.00 %	LABSTATION
FAT	Authorized	%Fat(F)	31.00 %	BONGIWE
FAT_NIR_BR	Authorized	%Fat	26.5 %	LABSTATION
FATT_NIRBR	Authorized	Total Fat %	27.2 %	LABSTATION
FIB_NIR_BR	Authorized	% Fibre	9.7 %	LABSTATION
FIBRE	Authorized	% Fibre	8.46 %	NOLWAZI
K_NIR_BR	Authorized	Potassium NIR BR %	1.70 %	LABSTATION
MG_NIR_BR	Authorized	Magnesium(%)	0.07 %	LABSTATION
MST_NIR_BR	Authorized	Moisture	8.78 %	LABSTATION
MST_OVN_CL	Authorized	%LOD ECL	8.15 %	NOLWAZI

EPOL CENTRAL LABORATORY



NA	Authorized	Sodium(%)	0.08 %	NOLENE
NA_NIR_BR	Authorized	Sodium(%)	0.67 %	LABSTATION

Sample 565878 BERLIN-04/06/2015-11, cont.

Test / Rep	Status	Component	Result Value	Analyst
NDF_NIR_BR	Authorized	NDF %	27.84 %	LABSTATION
PHOS	Authorized	Phos(%)	0.74 %	NOLENE
PHOS_NIR_B	Authorized	Phos(%)	1.29 %	LABSTATION
PROT_LECO	Authorized	Protein	50.68 %	LABSTATION
PRT_NIR_BR	Authorized	Protein	48.60 %	LABSTATION
STAR_NIR_B	Authorized	Starch NIR BR %	0.00 %	LABSTATION
SUGR_NIR_B	Authorized	Sugars NIR BR %	9.37 %	LABSTATION



Sample 611992

BERLIN-08/12/2015-1

Status:	Authorized	Login By:	PHUMZILE
Product:	F00001		
Description:	Miscellaneous Feed		
Location:	BERLIN	Login Date:	2015-12-08
Customer:	EPOL_BER	Date Completed:	2015-12-15
		Date Reviewed:	2015-12-15
Site:	BERLIN	Reviewer:	AUTO
Supplier:	EPOL_BER	Comments:	
Source:	BERLIN		For there Student Feed
Delivery Note:	for there		
Mix Number:	Grower 1		

Test / Rep	Status	Component	Result Value	Analyst
ASH	Authorized	ASH %	4.39 %	NOLWAZI
CA	Authorized	Calcium(%)	0.87 %	NOLENE
FAT	Authorized	%Fat(F)	2.68 %	BONGIWE
FIBRE	Authorized	% Fibre	4.38 %	NOLWAZI
MST_OVN_CL	Authorized	%LOD ECL	11.75 %	NOLWAZI
NA	Authorized	Sodium(%)	0.13 %	NOLENE
PHOS	Authorized	Phos(%)	0.60 %	NOLENE
PROT_LECO	Authorized	Protein	18.14 %	LABSTATION



Sample 611994

BERLIN-08/12/2015-2

Status:	Authorized	Login By:	PHUMZILE
Product:	F00001		
Description:	Miscellaneous Feed		
Location:	BERLIN	Login Date:	2015-12-08
Customer:	EPOL_BER	Date Completed:	2015-12-15
		Date Reviewed:	2015-12-15
Site:	BERLIN	Reviewer:	AUTO
Supplier:	EPOL_BER	Comments:	
Source:	BERLIN		For there Student Feed
Delivery Note:	for there		
Mix Number:	Grower		

Test / Rep	Status	Component	Result Value	Analyst
ASH	Authorized	ASH %	4.38 %	NOLWAZI
CA	Authorized	Calcium(%)	0.77 %	NOLENE
FAT	Authorized	%Fat(F)	4.11 %	BONGIWE
FIBRE	Authorized	% Fibre	4.54 %	NOLWAZI
MST_OVN_CL	Authorized	%LOD ECL	12.23 %	NOLWAZI
NA	Authorized	Sodium(%)	0.11 %	NOLENE
PHOS	Authorized	Phos(%)	0.63 %	NOLENE
PROT_LECO	Authorized	Protein	19.91 %	LABSTATION

