

**Potential of *Eisenia foetida* as a protein source for broiler chickens and its effect on growth performance, digestive organs, bone strength and meat characteristics**

By

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*Together in Excellence*

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## Notes

This Thesis is presented in the format prescribed by the Department of Livestock and Pasture Science at the University of Fort Hare. It is structured to form six chapters, which are presented as introduction chapter, literature review, and four experimental chapters and concluded with general discussion and recommendations.

Language, style and references used are in accordance with the requirements of Department of Livestock and Pasture Science. This Thesis represents a compilation of manuscripts where each chapter is an individual entity, and repetition between chapters has therefore been avoided.

Result of the study have been presented at the following symposium:

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- iii. **Gunya B.**, Masika P.J., and Muchenje V. The effect of *Eiseina foetida* on carcass characteristics and meat quality of broilers. Submitted to *Livestock Science*


## Declaration

I, **Gunya Busisiwe**, declare that this Thesis has not been submitted to any University and that it is my original work conducted under the supervision of Prof P.J. Masika and Prof V. Muchenje. The research was approved by the University of Fort Hare Research Ethics Committee (Certificate No.: MAS021SGUN01). All assistance towards the production of this work and all references contained herein have been duly accredited

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## **Abstract**

### **Potential of *Eisenia foetida* as a protein source for broiler chickens and its effect on growth performance, digestive organs and bone strength and chicken meat characteristics**

**By**

**Gunya Busisiwe**

This study was conducted to determine the nutrient composition of *Eisenia foetida* earthworm meal and its effects as a protein source on growth performance, carcass characteristics and meat quality of broilers. Protein content was higher in freeze-dried earthworm meal while drying methods did not influence fat content. Most minerals (macro and micro) of *E. foetida* meal were significantly different except for calcium ( $P < 0.05$ ) with freeze-dried *E. foetida* meal having the predominant minerals than oven-dried earthworms. Most essential fatty acids were significantly higher in oven-dried *E. foetida* meal than in freeze-dried earthworm meal. A total of 180 day old Cobb broilers were randomly allocated to five dietary treatments as follows: T1 (0%), T2 (1%), T3 (3%), T4 (5%) and T5 (10%) earthworm meal inclusion. The FCR was significantly influenced by dietary treatments at 0-21d of age, with T1 birds had the best FCR than all dietary treatments. At 22-28 days of age, significant dietary effects ( $P < 0.05$ ) were observed on ADG and ADFI. The highest ADG was recorded in T3 birds (89.9g), the least ADG was seen in T5 (60.9g). All growth traits were significantly different ( $P < 0.05$ ) across dietary treatments at 29-35 days of age. Birds in T4 recorded the highest values of BWG (1137.9g) and ADG (162.5g) and the least BWG and ADG of 882.9g and 126.1g, respectively, were observed in T3 while, ADFI was highest in T3 birds (199.4g) and the least

was recorded in T5 (164.4g). Furthermore, birds in T4 had the highest (1.6) FCR and birds in T1 recorded the least value (1.2). At 1- 35 days of age no significance difference ( $P > 0.05$ ) was observed on ADG, ADFI, and FCR among different inclusion levels of *E. foetida* meal. The dietary effect was observed on BWG ( $P < 0.05$ ) and birds fed 5% inclusion of earthworm meal (T4) had the highest body weight gain of 2590.4g. However, no significant difference ( $P > 0.05$ ) was observed in the dressing percentage for birds fed with or without *E. foetida* meal. Birds in T3 had the highest (2.1kg) body weight, while the least body weight was recorded for birds in T5 (1.7 kg). Dietary treatments did not significantly ( $P > 0.05$ ) influence gizzard pH. However, gizzard weight and intestine weight were significantly different ( $P < 0.05$ ) among dietary treatments. Birds in T2 exhibited the highest gizzard weight (42.5g) and birds in T4 recorded the least weight of 36.1g. The highest intestine weight of 92.2g was observed in birds in T3, while the least weight of 80.1g was observed in birds in T5. Dietary treatments significantly influenced bone strength, where birds in T1, exhibited the highest strength and those in T2 exhibited the lowest bone strength. Bone ash percentage was influenced by dietary treatments. Birds in T2 had the highest ash percentage (70.2%) where those in T3 and T4 had the least bone ash percentage. Wing, thigh, and drumstick yield were significantly ( $P < 0.05$ ) higher in T3 birds, whereas the breast yield was the highest in T5 birds. Liver and gizzard yield were significantly higher in birds in T5, while the least values were seen in birds in T3. Furthermore, there were no significant differences ( $P > 0.05$ ) observed with heart and spleen yield among the birds fed different treatments. The highest values for  $L^*$  and  $b^*$  were found in T4 birds while the highest values for  $a^*$  were found in T1 (control) birds. The pH values of breast meat were affected ( $P < 0.05$ ) by the dietary treatments at 1 and 48 hours *post-mortem*. However, at one hour *post-mortem*, the highest pH values were observed in breast meat of birds in T3 (6.6) and T5 (6.6) while at 48 hour *post-mortem*, the highest values were seen in T1 (5.8) birds. Dietary treatments had a significant

influence ( $P < 0.05$ ) on cooking loss; even though, there were no differences ( $P > 0.05$ ) observed on shear force values among the dietary treatments. The highest cooking loss value was observed in T5 (12.0 %) and the lowest value in T3 (7.2). There were no significant differences ( $P > 0.05$ ) on chicken aroma and metallic aroma scores of breast meat across the dietary treatments. Moreover, dietary effect ( $P < 0.05$ ) was observed on first bite scores of breast meat; where meat from T2 had the least score of 2.6, while meat from T5 had the highest score of 3.5. However, breast meat from T5 was found to have the highest scores (3.9) for the initial juiciness and sustained juiciness ( $P < 0.05$ ), while the lowest scores (2.5) were observed in T2. Chicken and metallic flavor scores of breast meat were not influenced ( $P > 0.05$ ) by the dietary treatments, contrary to toughness scores ( $P < 0.05$ ). Breast meat from T5 exhibited the highest scores (3.5) of toughness, whereas the least scores (2.3) were from birds in T2. It was, therefore, concluded in the current study that *E. foetida* can be considered as an alternative source of protein as it seems to be particularly suitable in broiler nutrition.

**Keyword:** *Eisenia foetida*, broilers, bone, growth performance, meat quality, sensory scores

## **Dedication**

I dedicate the Thesis to my lovely families (Gunya and Ncapayi) for giving me support to fulfill my dream of becoming an academic Doctor.

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## List of symbols

%	Percentage
°C	Degree Celsius
g	Grams
Kg	Kilograms
KJ	Kilojoules
mm	Millilitre
mg	Milligrams
N	Newton
cm <sup>3</sup>	Cubic centimetre

## List of Abbreviations

a*	Redness
ADFI	Average daily feed intake
AD G	Average daily gain
ADF	Acid detergent fibre
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemistry
b*	Yellowness
C*	Chroma
CL%	Cooking loss percentage
Ca	Calcium
Cu	Copper
d	Day
CP	Crude protein
CF	Crude fibre
EE	Ether extract
DP	Dressing percentage
FA	Fatty acid
FCR	Feed conversion ratio
Fe	Iron
H	Hours
H*	Hue angle

L*	Lightness			
Mg	Magnesium			
Mn	Manganese			
MUFA	Monounsaturated fatty acids			
Na	Sodium			
NDF	Neutral detergent fibre			
n-3	Omega three			
n-6	Omega six			
P	Phosphorous			
PUFA	Polyunsaturated fatty acid			
SAS	Statistical Analysis System			
SFA	Saturated fatty acid			
T	Treatment			
WBSF	Warner	Bratzler	Shear	Force

## CHAPTER: 1 Introduction

### 1.1. Background

The greatest challenge confronting the poultry industry is that boilers require more input, in particular feed. Feed is the most expensive input in poultry production. Tiroesele and Moreki (2012) stated that feed cost for broiler production is about 70% of their production. Presently, the protein sources for chickens such as fish meal and soya bean are expensive (Prayogi, 2011). Commercial farmers use many conventional protein supplements such as soya bean meal, fish meal, and sunflower but are still a major limiting factor because they are scarce and expensive. Thus, the demand for low-cost feed is high, due to the rising costs and a limited supply of commercial feeds (Tiroesele and Moreki, 2012). Furthermore, the conventional protein supplements are not only consumed by chickens but also by human beings, thus bringing about competition between chickens and human beings. Therefore, it is important to explore alternative sources of proteins that are relatively cheap and readily available for chickens.

*Eisenia foetida* can be used as an alternative non-conventional protein source (Tiroesele and Moreki, 2012) because they can be raised in the households with minimal management practices being required (Muchadeyi *et al.*, 2007), are palatable to chickens (Tiroesele and Moreki, 2012) besides being natural feed for the birds. Moreover, Earthworms can grow on a wide range of composting organic materials, from fruit to vegetables to kitchen wastes, rendered fish, and poultry, pigs, and cattle manure, thus being potentially interesting in reducing environmental criticisms by transforming waste invaluable biomass (Nguyen *et al.*, 2015). This was also substantiated by Tuan and Focken (2000) that *E. foetida* can be produced and harvested with ease, and they can be produced by simple methods from many

kinds of organic material. Currently, consumers prefer organic products. Chickens fed on insects had a proffered taste and high market price comparable to those fed on conventional protein source. This has supported the use of non-conventional animal protein sources like Earthworms as an organic feed supplement to Chickens (Ncobela and Chimonyo, 2015). These organic supplements can be offered to chickens with least cost or no cost by using freely available Earthworms such as *E. foetida*.

Moreover, advantage of using Earthworm meal as a source of protein for Livestock especially for Chickens is that they have an excellent chemical composition (Reinbecke *et al.*, 1991). Moreover, they can be used as a substitute for a fish meal and soya bean, due to their high protein content which ranges between 64-76% (Prayogi, 2011). This protein percentage is superior to that of fish (45%) and meat (51%) (Prayogi, 2011). Furthermore, *E. foetida* protein content has essential amino acids, especially lysine which is limiting in many food stuffs (Anitha and Jayraaj, 2012). It has been reported that the amount of lysine in Earthworm meal is sufficient lysine requirement by growing chickens (Vielma *et al.*, 2003). Tirosele and Moreki (2012) specified that Earthworms contain a large amount of protein (64.5 to 72.9%), essential amino acids, total fatty acids (6.6 to 10.5 mg/g), calcium (1020-7070 µg/g) and iron (1050-2990 µg/g). Therefore, *E. foetida* is a potential ingredient and a good source of protein for poultry diet that can be used to improve growth performance and meat quality.

## **1.2. Problem statement**

High-cost feed, especially protein source limits the performance of the broiler. Broiler feed consist of 23 % protein for a starter, 20 % protein for grower and 18 % protein in finisher feed respectively (NRC, 1994). The industry depends on fish meal for protein

source which has a physiological effect on the internal organs of broilers. It has high histamine content which causes gizzard defect (Miculec *et al.*, 2003).

Fish meal is a commonly used source of protein worldwide due to its high protein content, and more than half of it is being consumed by humans; hence the demand for fishmeal has increased (Naylor *et al.*, 2009; Tacon and Metian, 2009). This has resulted in a sharp price increase for fishmeal (Hardy, 2010). Despite the feed cost of broilers, there is also less to no information on the performance of broilers supplied earthworms meal as an alternative protein source.

### **1.3. Justification**

Despite the high nutritional content of earthworm, there is little information regarding its utilization in poultry feeding as an alternative protein feed. Such information is needed to identifying feeding strategies, to improve broiler production considering that the use of synthetic growth promoters and biotics have been banned in some countries. It has been demonstrated, that natural occurring insects like *Eisenia foetida* earthworms are rarely used in animal diets (Cross *et al.*, 2011) because knowledge on their use is still limited (Windisch *et al.*, 2008).

The commonly used source of proteins fishmeal is very expensive. Identifying naturally-occurring alternatives, such as earthworm meal is a possible alternative. According to Sales and Janssens (2004), the poultry industry requires an alternative source of protein due to the massive import costs of high-quality fishmeal, especially in developing countries. Therefore, the effect of such alternatives on broiler performance is significant for the poultry industry.

Therefore, research on *E. foetida* used as a source of protein and its effect on growth performance, carcass characteristics and meat quality on chickens is imperative.

#### **1.4. Objectives**

The main objective of this study was to determine the potential of *E. foetida* as a protein source for chickens and its effects on their growth, digestive organs, bone strength, carcass characteristics and quality of meat. Therefore, the specific objectives of this study were to:

1. Determine the effect of preparation method on nutrient composition and fatty acid profiles of *E. foetida*;
2. Determine the effect of *E. foetida* meal supplementation on growth performance, digestive organs, bone strength and carcass characteristics of broilers;
3. Determine the effect of *E. foetida* meal supplementation on carcass yield and physicochemical attribute of broiler meat;
4. Assess the effect of *E. foetida* on meat quality sensory scores of broiler meat.

#### **1.5. Hypothesis**

The null hypothesis tested in this study was that there is no level of *E. foetida* at which supplementation will affect growth performance, digestive organs, bone strength, carcass characteristics and quality of broilers Therefore the specific null hypotheses were:

1. The preparation methods have no effect on nutrient composition and fatty acid profiles of *E. foetida* value;
2. *Eisenia foetida* has no effect on growth performance, digestive organs, and bone strength and carcass characteristics of broilers;

3. *Eisenia foetida* has no effect on carcass characteristics and physicochemical attributes of broiler breast meat;

4. *Eisenia foetida* has no effect on sensory scores of broiler breast meat

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## CHAPTER 2: Literature Review

### 2.1. Introduction

Feed constitutes about 60-70 percent of total production costs of poultry meat (Bhatti *et al.*, 2002). The protein content of a feed ingredient is a factor of great importance. Protein is the basic nutrient that cannot be compromised in the preference of ingredients for feed formulation and preparation (Sogbensen and Ugwamba, 2008) because it is essential in poultry diets for growth and to repair tissues. Due to the high costs associated with high quality protein sources, it has become crucial to research into the nutrient composition of some of the easily culturable and less expensive animal protein sources.

Protein can be obtained from many feedstuffs such as meat and fish meals, cereal grains and legume by-products such as soybean meal. Usually fish meal is commonly used as the most popular protein source for poultry production, and it is frequently the preferred source of protein because of its balance of essential amino acids (Advisory Committee on Animal Feeding Stuffs, 2001). Nevertheless, the fish meal also has a nutritional constraint in its usage, for example, the high content of histamine that can cause a defect on the gizzard of the poultry (Miculec *et al.*, 2004). The fish meal also at times, causes tainting of chicken meat depending on quantities used and stages of feeding (Swick, 1999).

Feed cost has constituted about 60-70% of poultry production (Hinrichs and Steinfield, 2007; Tiroesele and Moreki, 2012). In the least cost feed formulation, the protein content of feed ingredient is the most expensive feed ingredient because conventional feeds are scarce and

limited. Though, nutritionists choose ingredients that are high in protein to meet nutrient requirements of chickens of different categories (Bhatti *et al.*, 2002).

Fishmeal and soybean have been used worldwide as a source of protein for poultry. They are the conventional sources of animal protein in the poultry industry. They have been treasured for their balanced amino acids, and palatability (Davis *et al.*, 2005; Antolovic *et al.*, 2012). Due to the rise in prices of high quality conventional animal feed required for poultry and competition for feed in human diets as well as animal husbandry, these feedstuffs are becoming limited and scarce.

Since there is a restriction on availability of conventional feed for chickens, researchers have come up with alternative protein sources of poultry feed. These alternatives are able to supply adequate essential and non-essential amino acids to enable their synthesis (Jogia *et al.*, 2009). Moreover, it has been reported that the possible use of some alternative animal protein feed stuff to substitute fishmeal such as earthworms can be a solution (Istiqomah *et al.*, 2009; Ogello *et al.*, 2014).

Earthworms have been found to be a good source of protein (Korstecka and Pączka, 2006; Sogbesan and Ugwumba, 2008). Earthworms with an important high protein component are used to feed chickens, pigs, rabbits, and as a dietary supplement for fish species (Akiyama *et al.*, 1984; Stafford and Tacon, 1985; Sabine, 1986; Mason *et al.*, 1992). The nutritive potential and utilization of earthworm *E. foetida* as poultry feed ingredients have not been documented in South Africa. Therefore, the objective of this review is to create attentiveness on the nutritional value of *E. foetida* as a source of protein for poultry.

## **2.2. *Eisenia foetida***

### **2.2.1. Characteristics of *E. foetida***

*Eisenia foetida* worm belongs to the family of *lumbericidae* and genus *Eisenia*, which is known by other names such as the tiger worm, garlic worm, flatworm, cadillac worms and worm for fishing bait (Fadaee, 2012). These worms are red, purple or brown in color and yellowish in their abdomen. The number of its segment is about 80-110 and it is between 23-130 mm in length. During puberty, the genital belt reaches to 7-9 pieces in between parts 24, 25, 26 or 32.

### **2.2.2. Growth of *E. foetida***

*Eisenia foetida* growth depends on the population density and food rationing (Singh *et al.*, 2013). Their weight increases faster with a decrease in the population density (Garg *et al.*, 2005). On the other hand, Ton *et al.* (2009) observed that the growth rate of earthworms depends heavily on the type and quality of the substrate. Normally, the growth cycle of worm *E. foetida* takes 40-60 days for the juvenile to develop into a mature or adult worm (Fadaee, 2012). Adult worm weight is approximately 1.5 g at 50 to 55 days. After coming out of the cocoon they are able to reproduce. Adult worms can create a cocoon every three days on average and after 23 days, one- third of newborns come out of the cocoon. For optimum growth of worms, they require temperatures that range between 15 to 20 °C, moisture content from 89 to 90%, oxygen, ammonia content of waste lower than 0.5mg/g, salt content that is less than 0.5% and pH that ranges from >5 and <9 (Sherman, 2003).

### **2.2.3. Reproduction of *E. foetida***

*Eisenia foetida* is hermaphroditic which means that each worm has both female and male reproductive organs (Dynes, 2003). *Eisenia foetida* can reproduce by both sexual and asexual reproduction. The majority of the species reproduce by cross-fertilisation, although some reproduce cocoon parthenogenetically. In sexual reproduction, worms mate by joining their clitella together with the heads pointing in opposite directions. After copulation and long after the worms separate each worm secretes three eggs or their cocoon from clitellum. It takes approximately four days for the formation of cocoon after mating has occurred (Reinecke and Alberts, 1988). Incubation lasts for about 23 days for worms to hatch (Fig. 2.2.1). Three hatchlings are produced from each cocoon and it takes 20 to 40 days for mature worms to develop clitella (Reinecke and Alberts, 1988). According to Edwards and Bohlen (1996), a cocoon takes about 3 to 5 months to hatch.

### **2.2.4. Feeding earthworms**

Earthworms feed on organic matter (Yadav and Garg, 2009), including animal waste, plant waste, and urban waste. Animal manure is the main source of feed for *E. foetida* (Gunadi and Edward, 2003). This was also substantiated by Sherman (2003) who specified that cow, horse, rabbit, swine, dairy and beef manure are excellent feed for earthworms, but poultry manure is not recommended because of its high protein and mineral content. It has been found by many authors that they grow faster in cattle manure (Atiyeh *et al.*, 2000; Nagavallmma *et al.*, 2004; Singh *et al.*, 2013). Cow dung has lower ammonia levels (Ton *et al.*, 2009) and it is rich in nitrogen which enhances the rapid growth of *E. foetida* (Sharma *et al.*, 2005). They are usually found in areas rich in organic matter, such as the upper topsoil layer, in the forest under piles of leaves or decaying logs, or in piles of manure.

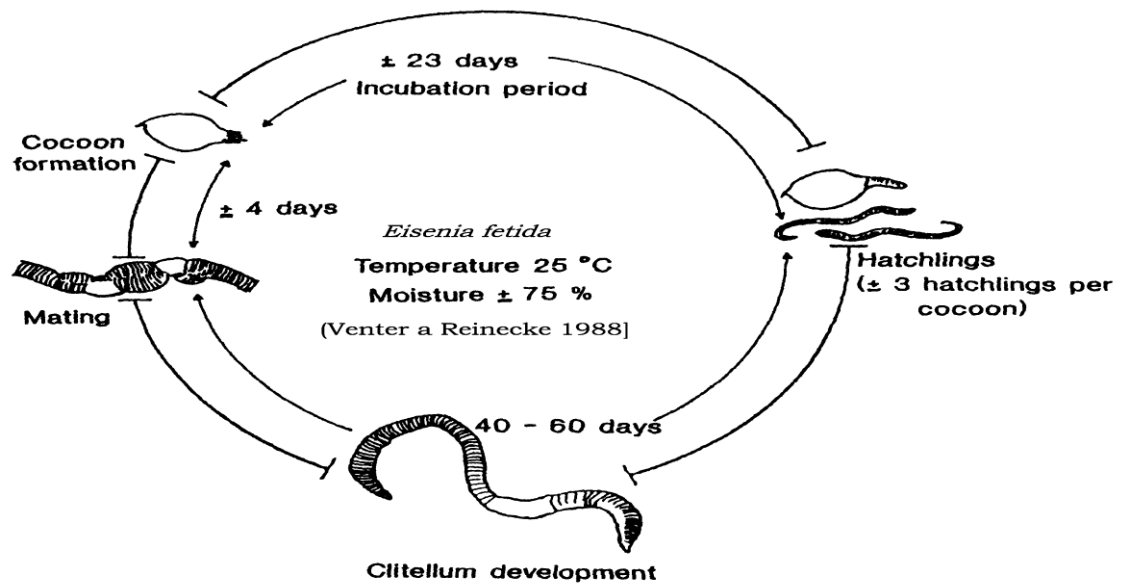


Figure 2.2 1 Life cycle of *Eisenia foetida* (Reinecke, 1988)

### **2.3. *Eisenia foetida* as a source of feed for Chickens**

*Eisenia foetida* are natural feed for chickens. They can be used as an alternative non-conventional protein source (Bou-Maroun *et al.*, 2013) due to their palatability to chickens (Tiroesele and Moreki, 2012). They can be produced and harvested with ease, and they can be produced by simple methods from many kinds of organic material (Tuan and Focken, 2009). Several studies have reported that *E. foetida* are very nutritious for poultry (Barcelo, 1988; Reinecke and Viljoen 1991; Istiqomah *et al.*, 2009; Tiroesele and Moreki, 2012). It is one of the substitutes that can be used instead of fishmeal and soybean because they have a protein content of 64-76% which is greater than the one for fishmeal (45%) and meat meal (51%) (Resnawati, 2004). Moreover, it has been reported that the protein content of earthworm meal is close to that of Peru fishmeal and higher than that of Chinese fish meal, hen egg and soybean meal (Dedeke *et al.*, 2013).

The use of *E. foetida* as a sustainable protein-rich feed ingredient in poultry diet is precisely feasible. They can be reared on low-grade bio-waste and can turn this bio-waste into high-quality protein. In addition using earthworm meal as a source of protein for livestock especially for chickens is that they have an excellent chemical composition (Reinbeck *et al.*, 1991), including essential amino acids, especially lysine which is not found in other feed stuff (Anitha and Jayraaj, 2012; Tiroesele and Moreki 2012). It has been found that earthworms contain a large amount of protein (64.5 - 72.9%), essential amino acids (4.95-5.70g/100g) (Dedeke *et al.*, 2010) total fatty acids (6.6 to 10.5 mg/g), calcium (1020-7070 µg/g) and iron (1050-2990 µg/g) for chickens. This was also supported by Korstecka and Pączka (2006) and Sogbesan and Ugwumba (2008) who reported that *E. foetida* is an excellent source of protein, rich in essential amino acids and vitamins. Moreover, they can be used as a substitute for fish

meal and soya bean meal, due to their high protein content which ranges between 64-76% (Prayogi, 2011). The dietary use of worms has already been analysed for poultry because they are already part of their natural diet. According to Reinecke *et al.* (1991), *E. foetida* can be harvested, dried and utilised as a protein source for poultry.

There is a contradiction in results found by some researchers regarding earthworm use as poultry feed. Reinecke *et al.* (1991) found it to be a satisfactory source of protein for growing broilers whereas Koh *et al.* (1984) reported decreased performance. This difference may be due to the different inclusion levels of earthworm meal used as a protein supplement. The earthworm *E. foetida* has high-quality protein content along with antimicrobial and antioxidant that could be used as a feed supplement in broiler diet (Rezaeipour *et al.*, 2014). Moreover, it has been reported that it has no anti-nutrient factor which declines performance of broilers (Reinecke *et al.*, 1991). Furthermore, it has been found that growth performance of broilers fed diets containing earthworm meal is equal to that fed fishmeal (Rezaipour *et al.*, 2014). This is in agreement with the observation by Boushy *et al.* (2000) who found that birds fed on earthworm meal as the major source of protein in the diets have all grown at rates equal to or better than those fed conventional protein meal. Resnawatti (2004) observed that earthworm *E. foetida* meal in feed up to 5% level has no significant effect on broiler body weight. Dried worm meal included up to 10% in broiler starter diet based on sorghum and soybean meal could be used without negative effects on feed consumption, weight gain and feed efficiency (Ramos-Elonduy *et al.*, 2002). The improvement of body weight of broilers fed with earthworm meal may be attributed to the antibacterial characteristics of earthworm meal (Julendra *et al.*, 2012).

## **2.4. *Eisenia foetida* nutrient composition**

### **2.4.1. Protein Content of *Eisenia foetida***

*Eisenia foetida* have high nutrient values as shown in Table 2.4.1. *Eisenia foetida* are mostly featured by their high protein content that ranges between 58% and 71% dry weight (Zhenjun *et al.*, 1997; Tiroesele and Moreki, 2012). *Eisenia foetida* has been found to have the highest crude protein content than other earthworm species (Tiroesele and Moreki, 2012). *Eisenia foetida* has 66.8 % of crude protein while *E. cagina* has 58.38% and *P. excavates* has 61.68%. This differs with the study of Sogbesan and Ugwamba (2008) who found that the crude protein for *H. euryaulis* is lower than 69.8% for *P. excavates* (Guerrero,1983) and 67.68% for *E. foetida* (Reinecke and Alberts, 1987) but higher than 56.4% and 61.8 for *E. foetida* reported by Tacon (1994). This variation of protein content may be attributed to the different mediums used to grow earthworms.

### **2.4.2. Amino Acid content of *Eisenia foetida***

*Eisenia foetida* has been found to have high amino acid values as compared to other earthworm species (Tiroesele and Moreki, 2012). It is better quality in all quantities of the amino acid as shown in Table 2.4. 2. Earthworm protein is high in essential amino acids compared to other common feeds (Zhenjun *et al.*, 1997). *Eisenia foetida* contains 20 out of 24 major amino acids, including the ten essential amino acids (Albarran, 1996). Segovia (1996) reported that the content of amino acid may be practically 6% of the worm's dry weight hence; *E. foetida* is one of the best-known sources of amino acids. *Eisenia foetida* is rich in lysine which is limiting in many feed stuff and methionine and isoleucine (Albarran, 1996; Anitha and Jayraaj, 2012). Moreover, it has been also reported by Gabriel *et al.* (2010) that earthworms have an amino acid composition which is very similar to that of fishmeal and

potentially superior to meat meal and contains essential amino acids as phenylalanine, leucine, lysine, methionine and valine (Gabriel *et al.*, 2010).

#### **2.4.3. Fatty Acid profile of *Eisenia foetida***

*Eisenia foetida* contains appropriate levels of desirable fatty acids and a high amount of omega 3 that distinguish it from other non-conventional feeds (Fadaee, 2012). The fat content of worm meal has been reported to range from 5-20% of dry matter (Edward, 1988). A number of mono and polyunsaturated fatty acids are in fairly high concentration in worm meal. These fats are of special interest of nutrition benefits to poultry feed.

#### **2.4.4. Minerals and vitamins of *Eisenia foetida***

*Eisenia foetida* contains an adequate mineral content and an excellent range of vitamins which are a valuable component of poultry feed (Vielma *et al.*, 2001). Essential elements such as Copper (Cu), Manganese (Mn) and Zinc (Zn) are about one to six times higher in earthworm meal than in soybean and fishmeal (Zhenjun *et al.*, 1997). Furthermore, Zhenjun *et al.* (1997) have reported that the iron content of earthworm fluid is ten times than that of soybean and fishmeal. This feature of earthworm fluid could be exploited to produce special iron (Fe) supplementation for poultry feed production (Zhenjun *et al.*, 1997). Earthworm body is rich in vitamin A and vitamin B compounds 0.25 mg vitamin B<sub>1</sub> and 2.3 mg vitamin B<sub>2</sub> per 100 g. It can be concluded that the earthworm *E. foetida* can be used for mineral and vitamin supplementation in addition to protein supplementation, in poultry feed.

**Table 2.4.1** Nutrient composition of *Eisenia foetida*

Nutrients	Sogbesan & Ugwaba, 2008	Fadaee, 2012	Istiqoma <i>et al.</i> , 2009 (Dry matter basis)	Zhenjun <i>et al.</i> , 1997
Crude protein (%)	63.0	59.0	63.06	54.6
Crude lipid (%)	5.9	11	18.5	7.34
Crude fibre (%)	1.9	-	0.19	
Ash	8.9	17	5.81	21.2
Nitrogen	11.8	15	12.41	
Extract (%)				
Moisture	-	-	9.03	
Gross energy (kJ/100g)	1943	-	-	
Ca	0.53 (g/100g)	-	-	1.55
P	0.94 (g/100g)	0.33 (%)	-	2.75
K	0.62 (g/100g)	0.98 (%)	-	

Source: Istiqoma *et al.*, 2009; Sogbesan and Ugwaba, 2008 and Zhenjun *et al.* 1997

**Table 2.4. 2** Amino Acid composition of *Eiseinia foetida*

<b>Amino Acid</b>	<b>Sogbensan (2008)</b>	<b>Ugwumba, Reineck, 1991</b>	<b>Zhenjun <i>et al.</i>, 2007</b>
Threonine	4.43	4.47	3.40
Serine	-	4.44	-
Valine	4.43	6.00	2.89
Methionine	5.30	1.80	1.13
Isoleveine	2.04	4.60	-
Leucine	4.11	9.80	-
Tryosine	—	3.50	-
Phenylalanine	6.26	3.58	2.38
Histidine	1.47	3.37	1.56
Lysine	1.47	7.76	4.17
Arginne	2.83	9.56	4.07

Source: Sogbensan and Ugwaba, 2008; Zhenjun, 2006 and Reineck, 1991

## **2.5. Preparation methods used for earthworms for animal feed**

For practicality, earthworms are often dried and used as a powder in the different application including freeze drying, oven drying, air drying at room temperature, ensiling earthworm with formic acid, incorporation of an earthworm with molasses and acetone immersion of earthworm (Edward and Arancon, 1994) to make them suitable for application to poultry feed. The most commonly used drying methods are freeze, oven drying and air drying for poultry feed. It has been reported that the choice for the method used depends mainly on the use to which the protein is to be put, the animal that is to be fed and the cost of a processing method in relation to the feed value of the protein. Drying worms is a very crucial step because it may cause off-flavour and/or create new odorant compounds (Bou-Maroun *et al.*, 2013). The type of processing methods may influence the nutrient availability of *E. foetida* for animal feed. Table 2.5.3 demonstrates how earthworm meal is prepared.

### **2.5.1. Freeze drying**

Freeze-drying is a method of removing water by sublimation of ice crystals from frozen material (Khairnar *et al.*, 2013). The advantage of the freeze-drying processing method is that it ensures the long shelf life of high-value protein products (Bou-Morroun *et al.*, 2013) such as *E. foetida*. Despite its increasing the shelf life of protein products, the low temperature of freeze drying has a negative influence on protein stability because some proteins denature in low temperatures (Edward and Arancon , 1994) and others can be degraded or decomposed during processing (Bou-Morroun *et al.*, 2013). Furthermore, freeze drying is expensive in terms of time and energy. Therefore freeze drying can only be used to dry high-value protein. Furthermore, methods used to affect the amount of total and essential amino acids in the feed very little; however, the lysine content decreases slightly by freeze drying as compared with the other methods (Edward and Arancon, 2004).

### **2.5.2. Oven drying**

Oven-drying is the most relevant preparation method of earthworms for poultry nutrition (Boush, 2000). It requires high ambient temperatures. In oven drying a dry earthworm meal is produced by killing and drying worms in trays in a large oven at 80°C for 2 to 4 hours (Edward and Arancon, 1994). A number of effects are observed during the heat drying of protein containing product like earthworm *E. foetida*, the main effect being the thermal denaturation of the protein. Heat causes rapid thermal motion of atoms of worms leading to rupture of the hydrogen bonds that link them together. Under suitable conditions, thermal denaturation leads to coagulation (Bou-Morroun *et al.*, 2013). During oven drying, peptide chains are propagated and form agglomerates.

### **2.5.3 Air drying**

In air drying, earthworm meal is produced by blanching worms in boiling water for a minute and then dried in air and ground (Boushy and van der Poel, 2000). Edward and Bohlen (1996) reported that using air drying has disadvantages because it is a very slow process and requires high ambient temperatures. High temperatures used in air-drying result in denaturation of earthworm protein.

**Table 2.5.3** Process for the preparation of earthworm meal

	Freeze drying	Oven Drying	Air drying
<b>Rearing Process</b>	Rearing of earthworms in organic waste ↓		
<b>Harvesting Process</b>	Harvest using hand picking method or using pyramidal method ↓		
<b>Killing</b>	Kill by placing worms in boiling water for a minute ↓		
<b>Fraction</b>	Grind earthworms using a household blender ↓	Cut earthworm using knife ↓	Cut earthworm using knife ↓
<b>Drying Process</b> (Temperature & Time)	Chill at - 35°C for 30 min & Freeze dry for 24 hours ↓	Oven-dried at 80 to 90°C for 4 hours ↓	Air dry at room temperature 25°C for 24 hours ↓
<b>Chemical processes</b>	Solidification ↓ Ice sublimation ↓ Desorption of unfrozen water ↓	Vapor of latent vaporization ↓ Removing of water by steaming ↓	Evaporation of moisture ↓
<b>Product</b>	Earthworm powder	Dried worms	Dried worms
<b>Storage</b>	Stored in airtight plastic container at 4°C till used.		

## **2.6 *Eisenia foetida* and chicken meat**

### **2.6.1. Effect of *Eisenia foetida* diet on chicken meat characteristics and quality**

Due to the high protein content, that ranges from 60 to 70 % in *E. foetida*, supplementing it to chickens results in increasing weight gain of broilers and specifically high breast muscle of chickens (Bahadori *et al.*, 2015; Rezaeipour *et al.*, 2014). This is in agreement with the result by Vu *et al.* (2009) who specified that diet containing 2% *E. foetida* earthworm led to weight gain and increased the percentage of breast muscle and leg in broilers. According to Harwood (1976), chickens fed with worms had similar growth rate but lower feed consumed than other diets. This denotes that chickens fed by worms had better muscle growth or muscle development, therefore, had the higher performance of meat production (Vu *et al.*, 2009). It has been found that supplementing *E. foetida* in the diet resulted in higher carcass yield (Ton *et al.*, 2009). It has been found that the different inclusion levels of earthworm in diet supplementation have no effect on meat quality attributes such as pH, color, drip loss and cooking loss. There are no negative effects of *E. foetida* that have been reported on chicken meat quality.

## **2.7. Effect of *E. foetida* on Digestive organs**

### **2.7.1. Gizzard weight**

A gizzard is an organ found in the digestive tract of a chicken. Similar to a stomach, the gizzard is used to grind foods the bird eats. Gizzards are considered a delicacy in certain cultures and provide a healthy dose of certain vitamins and minerals. Fresh commercial chicken gizzards weigh roughly 22 grams. A 100-gram chicken gizzards, which is equal to about 3.5 ounces, contains 2.68 grams of total fat, less than 1 gram of which is saturated (Maiti and Ahlawat, 2011). A diet low in saturated fat and cholesterol but high in protein such as earthworms can reduce the risk of diseases. Earthworms increase protein on gizzards

which make them gain weight. Animal-based proteins, like those found in earthworms, are complete proteins, since they provide sufficient amounts of all of the essential amino acids.

Each 3-ounce of gizzards contains 15 grams of protein, which is about 30 percent of the daily value for a protein of 50 grams. It is clear that a high-quality protein with balanced amino acid promotes more weight gain per unit of protein consumed than low-quality protein (Paoletti *et al.*, 2003). Proteins constitute about 80% of the kaolin weight, with high levels of glutamic and aspartic acids, leucine and arginine (Pivnenko *et al.*, 1998).

### **2.7.2. Gizzard pH**

Earthworm meal contains 65.6% crude protein (Damayanti *et al.*, 2008). The feed that is rich in protein causes a definitely higher acidity in the gizzard than the food lower in protein (Prayogi, 2011). Foods rich in protein cause the secretion of a larger quantity of stomach juice. Probably this is compensated by the promotion of digestion through strong movements of the gizzard. The gastric juice secreted from the proventriculus has to have a pH around 2. However, the amount, retention time, and chemical characteristics of the feed in the gizzard or proventriculus result in a more variable and usually higher pH (Khajavi *et al.*, 2015).

The pH of gizzard contents from broiler chickens varies between 1.9 and 4.5, with an average value of 3.5. The average values for broiler chickens are between 3 and 4 for normal pelleted diets (Svihus *et al.*, 2013). The high calcium carbonate content in the diet causes pH values for gizzard contents to be between 4 and 5 (Walk *et al.*, 2012). It is imperative that broiler GI pH is kept at a constant optimal level as small changes outside the normal pH ranges (gizzard

1.2–4 and duodenum 5.7–6.5) as it is also shown in Figure 2.7.1. The increase in the size of the gizzard when the diet contains structural components in the form of coarse fibers or cereals with high protein improves digestive function both through an increased retention time, a lower pH, and better grinding. This, probably combined with a better synchronization of feed flow, has been shown to improve nutrient utilization.

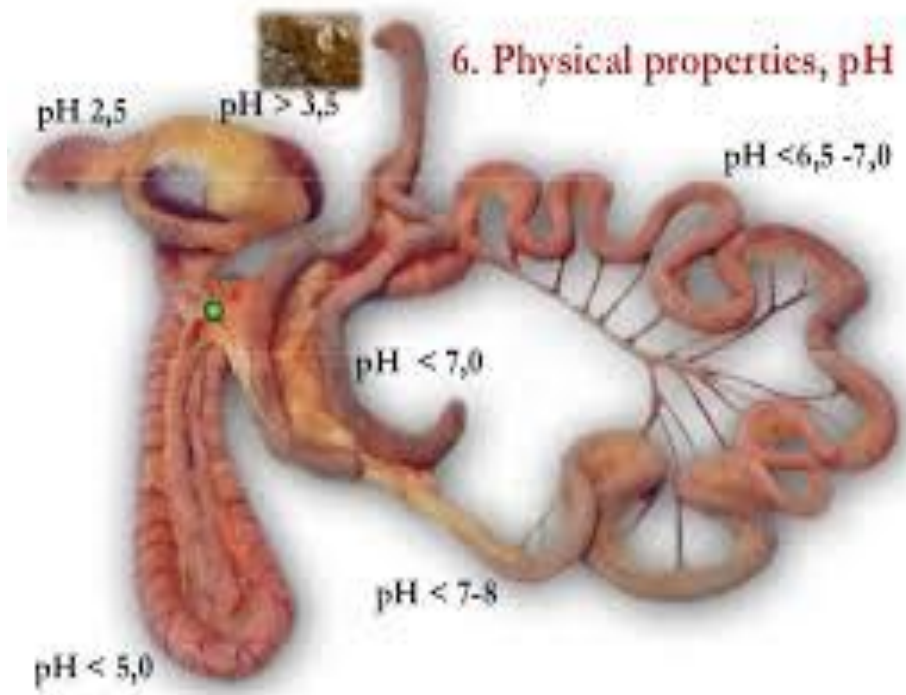
### **2.7.3. Intestines**

The small intestine helps to absorb digestion products (proteins, carbohydrates, and fats) the first part of the small intestine loops around the pancreas (called the duodenal loop). Amino acids such as glutamine, glutamic acid (Ebadiasl, 2011) and threonine (Rezaeipour *et al.*, 2014) have an important role in intestine histology of broilers. Ignacio *et al.* (1993) indicated that earthworm meal is a good source of glutamic acid (glutamate) for broilers. Glutamine can be formed by the combination of an amino group and glutamate by the action of Glutamine synthetases. Soltan (2009) observed that broilers fed a diet containing glutamine have bigger weight villi in duodenum and jejunum compared to the control group. Villi are finger-like projection which greatly expands the surface area of intestinal lining (mucosa), which in the avian gut exist throughout the length of the small and large intestine.

Earthworm (*E. foetida*) also has lubricin, which contains antimicrobial activity (Liu *et al.*, 2004). So, antibiotic characteristics of earthworm meal could thin the intestinal mucosa and increase the nutrient absorption and feed efficiency (Olawumi and Fagbuaro, 2011). Changes in the functionality of the small intestine are more difficult to assess. The weight of the small intestine is sometimes recorded, but effects are often not seen and interpretation of changes is often difficult.

Murakami *et al.* (2007) showed that on day 14 post-hatch, broilers fed a diet supplemented with 10 mg vitamin E along with 1% glutamine had longer villi and deeper crypts in the duodenum and deeper crypts in jejunum than broilers fed a diet containing vitamin E without glutamine. Increase of crypt depth can be caused by the effect of the content of glutamic acid in earthworm meal (Popovic *et al.*, 2005). There is a dearth of reports linking the effects of earthworm *E. foetida* meal on the gastrointestinal morphology of broilers.

Earthworms are high in protein and high true protein content (230 g/kg) that remains available in the intestine in a proportion of 33%, as well as adequate levels of all essential amino acids. Intestinal microflora population is a mixture of bacteria, fungi, and protozoa, but predominantly bacteria (Yegani and Korver, 2008) and is present in the avian small intestine within 24 hours following hatch (Miles *et al.*, 2006). The lower intestine of warm-blooded animals has been described as a site of intense protein turnover. Although proteins and amino acids provide a less significant energy source in the lower intestine, their importance lies mainly in the formation of potential systemic toxins and carcinogens as a result of protein or amino acid fermentation by putrefactive bacteria (Bingham, 2000).



**Figure 2.7. 1** Internal organs of broiler pH (Torok *et al.*, 2011)

Despite the name, the large intestine is actually shorter than the small intestine (Buwjoom *et al.*, 2010). Both the small and large intestines normally are populated with beneficial organisms (bacteria, yeast, etc.), referred to as microflora (Hossain *et al.*, 2012). These microflora aid in digestion. The large intestine's primary function is the absorption of water and electrolytes.

## **2.8. Bone characteristics**

Bone breakage leads to mortality, low productivity and carcass condemnation, hence it is important to understand the bone strength and factors that influence it (Rath *et al.*, 2000). Moreover, bone problems are regarded as major production and health concerns in broilers (Rath *et al.*, 2000). Leg weakness, lameness, and other bone abnormalities as a result of metabolic disorders are problems in broilers, leading to considerable production losses and having a negative effect on broiler welfare (Julian, 2005; Dibner *et al.*, 2007). In addition, Sanotra *et al.* (2001) also reported that the rapid growth of broilers causes skeletal deformities including problems caused by leg weakness that result in lameness and consequently poor animal welfare. The inclusion of protein source such as *E. foetida* in broiler diets plays an important role in their bone breaking strength since protein is a building block of bones.

## **2.9. Meat quality and Feeding**

### **2.9.1. Meat color**

Meat color is the most important meat attribute that determines the choice or rejection of the meat by consumers (Ponsano *et al.*, 2003). The color of carcass skin affects the acceptability

of broiler carcasses and its product (Karaoğlu *et al.*, 2006) Broiler skin meat is influenced by a number of factors such as live production, slaughter, processing, handling and packaging (Petraccia and Fletcher, 2002). Furthermore, meat color is associated with variation in intramuscular fat, level, and state of myoglobin and moisture content.

Chicken color has been classified into three groups: lighter than normal ( $L^* < 53$ ), normal ( $48 < L^* < 53$ ) and darker than normal ( $L^* < 48$ ) (Qiao *et al.*, 2001). Extreme variations in chicken color, especially in the darker than normal color ranges, may adversely have effects on final product color (Fletcher *et al.*, 2000). Broiler carcasses with dark breast are often condemned for cyanosis. DFD condition may be due to other causes such as ascites or emaciation. Moreover, darker-coloured breasts are from birds that were allowed to struggle freely during slaughter when compared to breast meat from anesthetized birds. Struggling before slaughter depletes muscle glycogen and less lactic acid accumulates in the muscle during post-mortem glycolysis, resulting in higher ultimate meat (Muchenje *et al.*, 2008).

PSE is the acronym for Pale, Soft and Exudative, which indicate that meat is pale or yellowish, flaccid or soft, and exudative or wet (Garcia *et al.*, 2010). This condition is explained by a pH lower than 5.8 combined with high muscle temperature higher than 35°C at the beginning of rigor mortis. This is due to the rapid metabolic transformation of glycogen into lactic acid, which results in achieving ultimate pH before carcass cools, causing protein denaturation, and consequently, meat becomes pale, soft and exudative and have its qualities compromised (Garcia *et al.*, 2010).

Supplementation of protein by *E. foetida* increases protein myoglobin of broilers, myoglobin protein that is richly pigmented. Meat becomes redder or darker when there is more myoglobin in the cells. However, there have been few studies investigating dietary-protein-related changes in meat quality in broilers. Decreased light reflectance in broiler breast fillets with increasing dietary lysine has been found by Corzo *et al.* (2002). Berri *et al.* (2007) showed that final breast pH rose when dietary lysine concentration was increased from 8-3 to 10-3 g/kg. This result confirms previous results of Berri *et al.* (2005) who reported that muscle glycogen reserves at death were associated with pH.

### **2.9.2. Meat pH**

The ultimate pH of meat is directly influenced by the amount of glycogen present in the muscle. There is a linear relationship between raw meat color and its pH, as well as the higher  $R^2$  for lightness ( $L^*$  value) as a function of pH (Fletcher *et al.*, 2000). Poultry meat with low pH has been associated with low water-holding capacity, which results in increase cook-loss and drip loss. Meat with low pH has also been reported to decrease tenderness.

Protein intakes can influence muscle pH. Furthermore, for chicken meat, characteristic pH for drumsticks after slaughter is 6.54 (Liu and Niu, 2008). Increasing the level of lysine in the diet which is high in *E. foetida*, of broilers improves breast meat yield and reduce drip loss during storage by increasing its pH (Berri *et al.*, 2007). This result was consistent with the general hypothesis that breast muscle hypertrophy in broilers is accompanied by a decrease in glycogen storage (Berri *et al.*, 2007). When levels of protein intake are increased meat pH increases and breast muscle glycogen content decreases, higher meat pH will give a darker color and less drip loss, this improves meat quality because it gives meat juiciness (Zhao *et al.*, 2012).

### **2.9.3. Tenderness**

Tenderness is an integrated textural property composed of mechanical, particulate, and chemical components (Brewer and Novakofski, 2008). Mechanical characteristics include hardness, cohesiveness, and elasticity; particulate characteristics include grittiness and fibrousness; chemical characteristics include juiciness and oiliness (Bourne, 1992). Tenderness of meat is one of the main sensorial attributes that determines global acceptability (Garcia *et al.*, 2010). According to Thu (2006), meat tenderness is a most difficult trait to be predicted, though it is of importance for meat quality and consumer acceptance. Tenderness is based on ease of chewing that is contributed by many factors. It can be influenced by several production factors (genetic, feeding system) and processing techniques (chilling, marinating, cooking). The improvement of tenderness in meat is mainly caused by changes in the structure of connective tissue solubilised by heat, while at the same time heat-denaturation of myofibrillar protein cause meat toughness (Barbanti and Pasquini, 2005).

It is well known that change of muscle structure plays a vital role in meat tenderness (Maltin *et al.*, 2003) and the increasing of the protein content in the diet by supplementation of *E. foetida* can result in firmer meat. Eventually, muscles become soft again, which means that they are tender when cooked. Meat tenderness is indirectly affected by protein supplementation of *E. foetida* because it affects growth rate (Maltin *et al.*, 2003).

### **2.9.4. Cooking loss**

The cooking loss is the degree of shrinkage of meat during cooking. The total loss that occurs during the cooking of meat includes the losses known as drippings and the volatile losses. When the muscle is cooked, it causes the proteins in the muscle to change in shape and

conformation. This change in conformation causes them to shift and lose the ability to bind and hold onto water. This loss of ability allows for the cooking loss to occur.

The cooking loss is affected by temperature, pH of the muscle, and method of cooking. Higher final cooking temperatures increase the amount of water that is driven by the muscle as well as the amount of fat or lipid that is liquefied and allowed to excrete. The pH of the muscle also has a large role in the amount of cooking loss of a muscle. The pH is directly related to that amount of negative charges on the muscle proteins that bind the water molecules. When pH is close or at the isoelectric point, the point in which in the net charge of the protein is equal, less water is allowed to bind.

## **2.10. Sensory attributes**

### **2.10.1. Aroma**

The aroma that is released when meat is cooked influence the acceptability of meat (Resconi *et al.*, 2013) due to the complex interaction of precursors derived from both the lean and fat composition of meat-generating volatile flavor compounds that contributes to meat aroma (Mottram, 1991). Aroma is detected by sniffing the meat since the response to aroma occurs in the olfactory cells of the nasal surface and conveyed to the brain for interpretation (Lawrie, 1998; McWilliams, 2001).

### **2.10.2. Juiciness**

Juiciness is one of the most sensory attributes that most influence meat acceptability (Muchenje *et al.*, 2008) depends on the quality and composition of fat in the meat (Muchenje *et al.*, 2008). Generally, consumers prefer the meat that is juicy. Meat juiciness is from

moisture release by meat during chewing, and moisture from saliva. Moreover, it not only influenced by fat content only but also physiological and psychological factors inherent to individual taste (Juarez *et al.*, 2011).

### **2.10.3. Flavour**

Flavour has been found as the most important sensory attribute that influences consumers' buying habits and preference (Jayasena *et al.*, 2013). Flavour is made of taste and aroma. Chicken meat flavor is influenced by many factors including breed, the diet of bird, amino acids, nucleotides, irradiation, high-pressure treatment, cooking, antioxidants, pH and aging (Jayasena *et al.*, 2013). Among many factors that influence the flavor of chicken meat; the diet of bird also has a significant contribution to meat flavor (Perez- Alvarez *et al.*, 2010). It has been found that diet can affect the flavor of chicken meat either positively or negatively (Jayasena *et al.*, 2010). A study by Jayasena *et al.*, 2010) who fed chickens with *Allium sativum* (garlic) found that meat had a repulsive odor and taste. However, Jang *et al.* (2008) found that breast meat from broilers in a diet containing medical herb extract mix has better flavor scores as compared to non-supplemented birds.

### **2.10.4. Tenderness**

Tenderness is regarded as the most important sensory attribute in consumer perception of palatability or quality of meat. Tenderness of meat in sensory evaluation is determined by scores of first bite and toughness (American Meat Science Association, 2015). Myofibrillar component, connective tissue, and juiciness of meat influence meat tenderness. Juiciness also influences meat tenderness because it provides lubrication to the consumers (Owens *et al.*, 2004).

### **2.11. Effect of *E. foetida* diet on chicken consumption**

Chickens supplemented with *E. foetida* have muscular fiber, fat, and minerals. Most consumers prefer meat that is very muscular but very nutritious as meat from chickens fed on *E. foetida* earthworms. It has been found that *E. foetida* does not contain levels of lead and mercury. Hence, *E. foetida* protein could be used as a feed supplement in animal diets, which then can be consumed by humans without risk of contamination by infection agents or metals (Medina *et al.*, 2003). Therefore *E. foetida* could then have many different nutritional applications, as animal feed and as an ingredient in food products for humans (Medina *et al.*, 2003).

### **2.12. Constraints of using earthworm in Chicken diets**

Despite their importance, it has been reported that earthworms have limitations as poultry feed. Above 5% worm meal in diets, some researchers have reported a reduction of feed consumption to chickens (Yaqub, 1997). Earthworms are the main intermediate of the internal parasite in free range chickens (LeeMaster, 2007). Chickens may pick up parasites directly by ingesting earthworms which carry the eggs of internal parasites specifically roundworms and cecal worm (Butcher and Miles, 2012). Internal parasites have a negative impact to chickens that can result in poor growth rate and feed efficiency, decrease egg production and even death in severe infections (Gabanakgosi *et al.*, 2012). Earthworm production is economically feasible with high technology rearing methods (Edward and Arancon, 2010). However, current methods of harvesting earthworms from organic waste are labour-intensive. This has been regarded as the greatest barrier to the successful commercial production of earthworm protein. Hence, earthworm as a source of protein has not been adopted commercially in developing countries (Edward and Arancon, 2010). The earthworm meal is produced at an economical price, and additionally, the value of vermicompost

produced can also be taken into account as complimentary income (Edward and Arancon, 1994). Therefore, there is a need to come up with most efficient earthworm harvesting methods for the reason that its economic value is extremely valuable as feed for poultry as fishmeal because of its amino acids, fatty acids, minerals and vitamins content (Edward and Arancon, 1994).

### **2.13. Conclusions**

The advantage of utilising *E. foetida* as chicken feed are that they grow very rapidly, low inputs are required and they convert organic matter diet into high-quality protein feed. It can be concluded that earthworm *E. foetida* has a potential to be used as a protein source to poultry. It has a crude protein content that ranges between 60-70%, which is more than of fish and meat meal currently used in the poultry industry. Besides its good protein, it is better in quality in all amino acids and lysine which is limiting in many feed stuff. *E. foetida* contain vitamins and minerals, hence it can also be used as mineral and vitamin supplements. Chickens on *E. foetida* diet have better muscular growth resulting into high carcass yield with *E. foetida* meal has no negative effects on chicken meat quality.

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## CHAPTER 3: Nutrient composition and fatty acid profile of oven dried and freeze dried

### *Eisenia foetida* earthworm

(Published in *Journal of Food and Nutrition Research*)

#### **Abstract**

The nutrient and fatty acid composition of *Eisenia foetida* earthworm when freeze dried and oven dried were investigated. The earthworms were oven or freeze-dried, then analysed for nutrient composition (protein, fat, and minerals) according to the AOAC method and fatty acids using gas chromatography. Protein content was higher in freeze-dried earthworms while fat content of earthworms was not influenced by drying methods used. Most minerals (macro and micro) of *E. foetida* were significantly different except for calcium ( $P < 0.05$ ) with freeze-dried *E. foetida* having the predominant minerals than oven-dried earthworms. Most of the essential fatty acids were significantly higher in oven-dried *E. foetida* than in freeze-dried earthworms. No significant differences ( $P > 0.05$ ) were observed on Margaric, Vaccenic, Arachidic, Tricosanoic, omega-3, SFA, MUFA, n-3, PUFA: SFA and PUFA/MUFA between oven-dried and freeze-dried samples. The study revealed that freeze-dried *E. foetida* can serve as a better source of nutrients than oven-dried earthworms whereas fatty acids were better in oven-dried *E. foetida* than freeze-dried *E. foetida*.

**Keywords:** Chemical composition, drying methods, *Eisenia foetida*, fatty acids

### 3.1. Introduction

Earthworms *E. foetida* are commonly used as a protein source to feed chickens, pigs, rabbit and fish (Kosteck and Packza, 2006; Sogbesan and Ugwaba, 2008) and it has been found to be a substitute for fishmeal and soya bean in animal diets (Anitha and Jayraaj, 2012). Moreover, they are a good source of essential amino acids, calcium, iron and fatty acids (Tiroesele and Moreki, 2012).

Earthworms are fed to animals in dried form. Frequently, they are dried using oven-drying and freeze drying methods (Edward and Arancon, 1994). Freeze-drying is a method of removing water by sublimation of ice crystals from frozen material (Khairnar *et al.*, 2013). The advantage of using freeze-drying is that there are minimal losses in volatile chemicals and heat-sensitive nutrients (Khairnar *et al.*, 2013) Moreover it ensures long shelf-life of the protein product (Bou Morroun *et al.*, 2013). Oven-drying is the commonly used method. Despite its frequent use, it does not enhance nutrient stability and bioavailability (Shadung *et al.*, 2012).

The value of earthworms as a supplement in animal dietary formulation had been rated high by nutritionists (Lourdumary and Uma, 2012). Therefore it is a necessity to determine the chemical composition and fatty acid profile of *E. foetida*, which could help in the formulation of diets according to nutrient requirements of chickens. Information on the nutrient composition of earthworm *E. foetida* is limited, making it complex to include them into feed formulation by nutritionists and farmers. Furthermore, determination of the fatty acid profile of a diet is of importance to know the quality of the fat fraction of the diet (Baiao and Lara, 2005) and may also be indicative of the absorbability of the lipids. Previous studies focused

on nutrient composition of *E. foetida* (Sogbesan and Ugwaba, 2008; Fadae, 2012; Lourdmay and Uma, 2012; Medina *et al.*, 2003; Zhenjun *et al.*, 2012) however, these studies did not provide in-depth comparison of fatty acid and nutrient composition of freeze-dried vs. oven-dried *E. foetida*. Therefore the objective of this study was to determine the effects of oven drying and freeze drying on nutrient composition and fatty acid profile of *E. foetida*.

## **3.2. Materials and Methods**

### **3.2.1. Study site**

Earthworms were obtained from Soil Science Department, University of Fort Hare, Alice. The worms were raised in Fort Hare farm, University of Fort Hare, Alice, South Africa. The farm lies along longitude 32° 78'E and latitude 26°85'S at an altitude of 450-500m above sea level. The farm is located in the False Thornveld which is characterised by mean annual rainfall of 480mm and mean annual temperature of 18.7 °C.

### **3.2.2. Worm management**

Earthworms were fed a diet of organic waste compost twice a week specifically shredded paper and cow manure. The feed was spread on top of compost and water was sprinkled on it and then feed was thoroughly mixed with compost. In order to guarantee optimum growth conditions, optimum temperature 12-24°C, moisture 80-90% and pH >5 <9 of the compost were kept under control (Bou-Maroun *et al.*, 2013).

### **3.2.3. Processing of earthworms**

Earthworms were harvested with hand picking method. The harvested worms were thoroughly rinsed in water and kept in a bowl for 30 min to evacuate their guts (Akpodiete and Okagbare, 1999). They were killed with boiling water for a minute (Bou-Maroun *et al.*, 2013) and after they were dried with oven and freeze drying method.

#### **3.2.3.1. Oven drying**

Earthworms were evenly spread on trays covered with foil to avoid fixing of earthworms on trays. Earthworms were dried in a conventional laboratory oven (Heraeus, Model no. T5050) at 90 °C for four hours (Boush and van der Poel, 2000). Dried samples were milled into a powder using a mortar. The resultant dried powder was packed in airtight containers and refrigerated until they were analysed.

#### **3.2.3.2. Freeze drying**

Earthworms were meshed for a minute with a household blender machine (Sunbeam Deluxe Glass Blender, Model no. SGB150). Samples were diluted with distilled water in a ratio of one kg of earthworm in two liters of distilled water (Ishii and Hisashi, 1992). The samples were then filtered through filter paper to remove dirt and soil particles. Earthworm samples were placed in 500ml vacuum bottles and then chilled at -35°C using a chilling machine (Vir Tis Benchtop K, Vir Tls Co., Gardner, NY, USA) up until they were frozen. Then they were placed in a freeze dryer (Vir Tis Benchtop K, Vir Tls Co., Gardner, NY, USA) for 24 hours. The resultant dried powder was placed in airtight containers and refrigerated until it was analysed.

#### **3.2.4. Nutritional composition determination**

The nutrient composition of freeze-dried and oven-dried *E. foetida* was analysed to determine the dry matter, crude protein, fat, calcium, magnesium, potassium, phosphorous, zinc, copper, iron, manganese, selenium and sodium. The proximate composition of earthworms was determined according to the AOAC (1993) method. Dry matter (moisture content) was determined by differential weighing of dried and fresh samples. Crude protein was determined by the micro Kjeldahl procedure while crude lipid was determined using Soxhlet extraction method. Five macro (Calcium, Potassium, Sodium, Phosphorus and Magnesium) and four micro minerals (Iron, Zinc, Copper, and Manganese) were determined using an Atomic Absorption Spectrophotometer.

#### **3.2.5. Fatty acid profile determination**

A lipid aliquot (20 mg) of earthworm lipid was transferred into a Teflon-lined screw-top test tube by means of a disposable glass Pasteur pipette. Fatty acids were transesterified to form methyl esters using 0.5 N NaOH in methanol and 14 % boron trifluoride in methanol (Park and Goins, 1994). FAMES from earthworm were quantified using a Varian 430 flame ionization GC, with a fused silica capillary column, Chrompack CPSIL 88 (100 m length, 0.25 mm ID, 0.2 µm film thicknesses). The analysis was performed using an initial isothermic period (40°C for 2 minutes). Thereafter, the temperature was increased at a rate of 4°C/minute to 230°C. Finally, an isothermic period of 230°C for 10 minutes followed. FAMES in n-hexane (1µl) were injected into the column using a Varian CP 8400 Autosampler. The injection port and detector were both maintained at 250°C. Hydrogen, at 45 psi, functioned as the carrier gas, while nitrogen was employed as the makeup gas. Galaxy Chromatography Software recorded the chromatograms.

Fatty acid methyl ester samples were identified by comparing the retention times of FAME peaks from samples with those of standards obtained from Supelco (Supelco 37 Component Fame Mix 47885-U, Sigma-Aldrich Aston Manor, Pretoria, South Africa). All other reagents and solvents were of analytical grade and obtained from Merck Chemicals (Pty Ltd, Halfway House, and Johannesburg, South Africa). Fatty acids were expressed as the proportion of each individual fatty acid to the total of all fatty acids present in the sample. The following fatty acid combinations were calculated: omega-3 (n-3) fatty acids, omega-6 (n-6) fatty acids, total saturated fatty acids (SFA), total monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), PUFA/SFA ratio (P/S) and n-6/n-3 ratio (Folch *et al.*, 1957).

### **3.2.6 Statistical Analysis**

The effect of drying methods (freeze drying and oven drying) on fatty acid profile and nutrient composition of *E. foetida* were analysed statistically using one-way analysis of variance (ANOVA) of SAS (2003). Differences among means were tested for significance using the Duncan Multiple Range test. Differences were considered significant when  $P < 0.05$ .

## **3.3. Results**

### **3.3.1. Nutrient composition of freeze-dried and oven-dried *E. foetida***

The results for the effects of preparation method on nutrient composition of *E. foetida* are presented in Table 3.3.1. Drying methods used on *E. foetida* had no ( $P > 0.05$ ) significant effect on the fat percentage of *E. foetida*. Significant differences were observed on the fat-free dry matter of earthworms where oven-dried worms had significantly higher (79.9 %) content than freeze-dried worms (36.4 %). Significant impact ( $P < 0.05$ ) on most of the macro elements (Potassium, Phosphorous, and Magnesium) was observed, except for calcium

( $P > 0.05$ ) where freeze dried *E. foetida* had the higher values. Similarly, there was a significant difference ( $P < 0.05$ ) on trace elements between the two drying methods, where freeze-dried *E. foetida* had higher values for all microelements (zinc, copper, manganese, iron and aluminum) measured than oven-dried samples.

**Table 3.3.1** Nutrient composition of freeze-dried and oven-dried *Eisenia foetida*

<b>Nutrients</b>	<b>Freeze dried</b>	<b>Oven dried</b>
Crude protein (%)	66.2 <sup>b</sup> ±0.63	59.7 <sup>a</sup> ±0.63
Calcium (%)	0.82 <sup>a</sup> ±0.02	0.82 <sup>a</sup> ±0.02
Magnesium (%)	0.3 <sup>b</sup> ±0.01	0.1 <sup>a</sup> ±0.01
Potassium (%)	2.2 <sup>b</sup> ±0.03	0.9 <sup>a</sup> ±0.03
Phosphorous (%)	1.2 <sup>b</sup> ±0.02	0.9 <sup>a</sup> ±0.02
Zinc (mg/Kg)	317.0 <sup>b</sup> ±5.02	150.7 <sup>a</sup> ±5.02
Copper (mg/Kg)	812.1 <sup>b</sup> ±11.23	22.3 <sup>a</sup> ±11.3
Manganese (mg/Kg)	116.6 <sup>b</sup> ±2.21	26.0 <sup>a</sup> ±2.21
Iron (mg/Kg)	1498 <sup>b</sup> ±7±23.35	495.3 <sup>a</sup> ±23.3
Aluminium (mg/Kg)	1117.7 <sup>b</sup> ±31.51	86.0 <sup>a</sup> ±31.51

<sup>a, b</sup> Means in the same row with different superscripts are significantly different (P < 0.05).

### 3.3.2. Fatty acid composition of freeze-dried and oven-dried *E. foetida*

Results of the fatty acid composition of freeze and oven dried *E. foetida* are summarised in Table 3.3.2. Drying methods used on *E. foetida* had no ( $P > 0.05$ ) significant effect on the fat percentage of *E. foetida*. Significant differences were observed in moisture content and fat-free dry matter of earthworms, with freeze-dried had higher moisture content and lower fat-free dry matter than oven-dried *E. foetida*. Almost all fatty acids for freeze-dried and oven-dried *E. foetida* samples were significantly different ( $P < 0.05$ ), except for Margaric, Vaccenic, Arachidic, Tricosanoic, omega-3, SFA, MUFA, PUFA: SFA and PUFA/MUFA. Results showed that lipids of freeze-dried earthworms were composed of 48.1% of saturated fatty acids (SFAs), 24.4% of monounsaturated fatty acids (MUFAs) and 28.5% of polyunsaturated fatty acids (PUFAs) while oven-dried earthworms were composed of 45.8% of SFAs, 22.2% of MUFAs and 31.0% of PUFAS. Among the SFAs which occurred in higher proportions in freeze-dried earthworms were Palmitic and Stearic acid and the rest were higher in oven-dried earthworms. Oleic was the main fatty acid among MUFAs observed in *E. foetida*, and a higher proportion of Oleic was seen in oven dried earthworms. Significant differences ( $P < 0.05$ ) were observed in PUFAs between oven-dried and freeze-dried earthworms. The oven drying method induced an increase of PUFAs between oven-dried and freeze-dried *E. foetida*. A higher level of omega six (n-6) was recorded in oven dried, whereas the proportions of omega three (n-3) among oven-dried and freeze-dried earthworms were similar.

**Table 3.3.2** Fatty acid composition of freeze-dried and oven-dried earthworms

<b>Sample ID</b>	<b>Freeze dried</b>	<b>Oven dried</b>
<b><u>Proximate analysis (%)</u></b>		
Fat (%)	10.0 <sup>a</sup> ±0.44	9.5 <sup>a</sup> ±0.44
Fat free dry matter (%)	36.4 <sup>a</sup> ±2.08	79.9 <sup>b</sup> ±2.08
Moisture (%)	53.5 <sup>b</sup> ±1.66	10.5 <sup>a</sup> ±1.66
<b><u>Fatty acids</u></b>		
C12:0	3.4 <sup>a</sup> ±0.04	8.4 <sup>b</sup> ±0.04
C13:0	3.7 <sup>b</sup> ±1.74	1.4 <sup>a</sup> ±1.74
C14:0	2.6 <sup>a</sup> ±0.02	3.8 <sup>b</sup> ±0.02
C15:0	1.0 <sup>a</sup> ±0.01	1.2 <sup>b</sup> ±0.01
C15:1c10	1.8 <sup>b</sup> ±0.11	1.4 <sup>a</sup> ±0.11
C16:0	14.8 <sup>b</sup> ±1.03	6.7 <sup>a</sup> ±1.03
C17:0	4.1 <sup>a</sup> ±0.09	4.6 <sup>a</sup> ±0.09
C17:1c10	1.3 <sup>b</sup> ±0.23	0.1 <sup>a</sup> ±0.23
C18:0	19.9 <sup>b</sup> ±0.54	17.9 <sup>a</sup> ±0.54
C1:1t9	1.6 <sup>b</sup> ±0.01	0.0 <sup>a</sup> ±0.01
C18:1c9	3.8 <sup>a</sup> ±0.05	4.7 <sup>b</sup> ±0.05
C18:1c7	13.4 <sup>a</sup> ±0.34	14.5 <sup>a</sup> ±0.34
C18:2c9,12(n-6)	6.2 <sup>a</sup> ±0.13	10.2 <sup>b</sup> ±0.13
C20:0	0.4 <sup>a</sup> ±0.02	0.3 <sup>a</sup> ±0.02
C18:3c9,12,15(n-3)	1.2 <sup>b</sup> ±0.04	0.0 <sup>a</sup> ±0.04
C20:2c11,14(n-6)	3.4 <sup>b</sup> ±0.24	2.6 <sup>a</sup> ±0.24
C22:0	0.4 <sup>a</sup> ±0.03	1.3 <sup>b</sup> ±0.03
C20:2c11,14(n-6)	2.4 <sup>b</sup> ±0.04	1.6 <sup>a</sup> ±0.04
C20,3c11,14,17(n-3)	5.3 <sup>b</sup> ±0.19	0.0 <sup>a</sup> ±0.19
C20:4c5,8,11,14(n-6)	7.3 <sup>a</sup> ±0.41	9.3 <sup>b</sup> ±0.41
C23:0	0.1 <sup>a</sup> ±0.03	0.0 <sup>a</sup> ±0.03
C20:5c5,8,11,14,17(n-3)	2.6 <sup>a</sup> ±0.77	8.8 <sup>b</sup> ±0.77
C24:0	0.4 <sup>b</sup> ±0.12	0.0 <sup>a</sup> ±0.12
C22:5c7,10,13,16,19(n-3)	0.5 <sup>b</sup> ±0.03	0.0 <sup>a</sup> ±0.03
<b><u>Fatty acid ratios</u></b>		
SFA	48.1 <sup>a</sup> ±1.40	45.8 <sup>a</sup> ±1.40
MUFA	23.4 <sup>a</sup> ±0.36	22.2 <sup>a</sup> ±0.36
PUFA	28.5 <sup>a</sup> ±1.37	31.9 <sup>b</sup> ±1.37
n-6	18.6 <sup>a</sup> ±0.59	23.5 <sup>b</sup> ±0.59
n-3	9.4 <sup>a</sup> ±1.30	8.3 <sup>a</sup> ±1.03
PUFA: SFA	1.2 <sup>a</sup> ±0.06	1.4 <sup>a</sup> ±0.06
PUFA/MUFA	2.0 <sup>a</sup> ±0.21	2.9 <sup>a</sup> ±0.21

<sup>a, b</sup> Means in the same row with different superscripts are significantly different (P < 0.05)

### 3.4. Discussion

The potential of *E. foetida* as a protein source for animal feed has previously been reported by many researchers (Yaqub, 1997; Lieberman, 2002; Sogbesan and Ugwaba, 2008; Lourdumary and Uma, 2012). The results of this study established that protein content of *E. foetida* is influenced by the processing method used where freeze-dried *E. foetida* had higher protein content than oven-dried *E. foetida*. The findings of the current study are consistent with the report by Bou-Maroun *et al.* (2013) who found that protein content of *E. foetida* decreased during oven drying as compared to freeze drying. The high protein content of freeze-dried *E. foetida* may be influenced by low temperatures used in freeze drying that ensure long-term stability of high-value protein (Bou-Maroun *et al.*, 2013).

The diminishing protein content of oven-dried *E. foetida* was expected due to the thermal denaturation of earthworm protein. In addition, the loss of protein could be attributed to the heating effect associated with oven drying condition which results in the unzipping of hydrophobic forces leading to a partial distribution of structure of the protein molecule (Abioye *et al.*, 2014). Nevertheless, the current results contradict the findings on *Sternocera orrissa* by Shadugu *et al.* (2012) who found that oven drying increased protein content. This deviation may be due to the temperature variation of these studies. In the current study, the temperature used was 90°C and in a study by Shadung *et al.* (2012) a temperature of 66°C was used. *Eisenia foetida* has been known to have a high protein content that ranges between 58 % and 71 % dry weight/ matter basis (Zhenjum *et al.*, 2012; Tiroesele and Moreki, 2012) and growing chickens require only 20% of crude protein for their growth (NCR, 1994). The crude protein supplied by *E. foetida* is greater than the protein requirement of chickens thus making it a good protein supplement.

The methods used to prepare and preserve food and feed may affect the concentration and availability of minerals and other essential compounds (Gyamfi *et al.*, 2011). Out of two preparation methods used, freeze-dried *E. foetida* retained most of the macro minerals (magnesium, potassium and phosphorous). This may be due to the fact that freeze drying method is able to keep hold of most of the nutrients since during freeze drying, external influences are minimum and oxidizable substances are well protected under vacuum conditions (Khairnar *et al.*, 2013). Oven drying reduced the nutrient value of *E. foetida* either through chemical modification or direct loss of minerals by direct heating through inducing biochemical and nutritional variation in earthworm composition.

Calcium content between oven-dried and freeze-dried *E. foetida* was not different. This similarity of calcium content between oven-dried and freeze-dried *E. foetida* may be attributed to the fact that calcium can be used as a drying agent (Burfield *et al.*, 1977) hence no differences were found between the two drying methods. The current results are in accordance with finding on injera from pre-fermented flour by Abiyu *et al.* (2013) who found no differences in calcium content of freeze and oven dried injera from pre-fermented flour. Calcium and phosphorus are essential for the formation and maintenance of the skeleton. Most of the calcium in the diet of the growing bird is used for bone formation, whereas in the mature laying fowl most dietary calcium is used for egg shell formation (NRC, 1994). Magnesium is found in the natural feed of poultry; hence deficiency is rare. Although it has been reported that once newly hatched chicks fed a diet totally devoid of magnesium live only a few days (The Merck Veterinary Manual, 2015). Potassium is important to control

blood pressure, anxiety and stress, enhance muscle strength, water balance, electrolytic function and nervous system (Bhaskarachary, 2011).

The earthworm *E. foetida* is also capable of providing substantial quantities of trace elements that are essential for chickens, in particular, iron, zinc, and copper. Freeze-dried *E. foetida* contained peak concentration levels of all trace elements. This is in line with the report by Castro and Garcia (2002) who reported that freeze drying avoids losses of the trace element. The high levels of trace elements of freeze-dried earthworms may be due to the low temperature used in the freeze-drying process which prevented most of the microbial reactions with the final product having excellent quality (Ratti, 2001). Decreases in a number of trace elements in oven-dried *E. foetida* may be due to the heat applied during the process, which enhances nutrient diminish. Zinc is important for the immune system, sexual maturity and reproductive capacity (Avitech Scientific Bulletin, 2002). In addition, Zinc is well known for its anti-viral, anti-bacteria; anti-fungal and anti-cancer properties (Brisible *et al.*, 2009). Iron is a constituent of hemoglobin and myoglobin for oxygen transport and is a component of many enzymes containing protein (Avitech Scientific Bulletin, 2002). It is also needed for normal functioning of the central nervous system and in the oxidation of carbohydrates, protein, and fats (Umar *et al.*, 2007). Copper is a necessary component of a number of enzymes, which function in increased structural strength, the elasticity of connective tissues and blood vessels (Avitech Scientific Bulletin, 2002). Moreover, copper is required for antibody development and lymphocyte replication (Burker and Miller, 2006).

The moisture content of *E. foetida* was influenced by drying methods used in this study where freeze-dried *E. foetida* had higher moisture than oven-dried *E. foetida*. The high

moisture content of freeze-dried *E. foetida* may be attributed to the fact that freeze-dried products can be easily rehydrated much more quickly as the process leaves microscopic pores hence; freeze-dried worms had higher moisture content than oven-dried *E. foetida*.

The present findings revealed that fat content of *E. foetida* is not influenced by drying method used. This could be attributed to the temperature (90°C) used in this study for oven drying that did not cause a decrease of the fat content hence there was no variation between the two drying methods. The results of the current study are comparable with previous studies, which indicated that the fat content of *E. foetida* ranges between 9 % and 10 % (Fadaee, 2012). Fats are required in poultry diets to absorb fat-soluble vitamins (vitamin A, D, E and K) (Folorunso *et al.*, 2014). Furthermore, fats are added in poultry diets to improve the palatability of the feed (Baiao and Lara, 2005). However, the current findings are different to the report by Shandung *et al.* (2012) who found that the oven drying increased the fat content of African metallic wood boring beetle.

Oven-dried *E. foetida* had a higher amount of omega six as compared to freeze dried. The increase of omega six in oven-dried *E. foetida* may be attributed to the fact that hot air from oven drying induces an increase of PUFAs (Wu and Mao, 2008) such as omega six. Hence oven dried *E. foetida* have higher omega six as compared to freeze-dried *E. foetida*. Findings of this study are in line with the report by Dynes (2003) and Grdisa *et al.* (2013) who reported that earthworms are rich in omega six. In poultry, omega six is very important for brain and heart function and in growth and development (Franzen-Castle and Ritter-Gooder, 2010).

The unsaturated omega-3 fatty acids were higher in the freeze-dried samples. This may be attributed to milder freeze drying process. Furthermore, oven drying is a more aggressive

process with the samples exposed to air that may lead to oxidation and destruction of long chain unsaturated omega-3 fatty acids like  $\alpha$ -Linoleic and Eicosatrienoic. The exposure to heat in oven drying may enhance dehydration of earthworms and cause considerable loss of fatty acids (Telahigue *et al.*, 2012). Similarly, results were seen on chicken sausage by Abdulhammed *et al.* (2014) who found that unsaturated fatty acids decreased significantly in oven drying due to lipid oxidation that was initiated by the presence of oxygen, which attacks the double bond of unsaturated fats.

Even though there are different fatty acids present in poultry diets, chickens have a specific requirement for one fatty acid which is Linoleic (Watkins, 1991). The findings of the current study are in agreement with the report by Stafford (1984) who found that *E. foetida* had high proportions of essential fatty acid including Linoleic and  $\alpha$ -Linoleic. Linoleic was found to be higher in oven-dried samples than in the freeze-dried in this study. This may be due to the fact that oven drying minimally affects the fatty acids (Telahigue *et al.*, 2012). This can be related to the lower dehydration produced in oven drying (Moradi *et al.*, 2008). Linoleic is regarded as of particular importance in poultry diets (Shin *et al.*, 2011). Once chickens have a deficiency in Linoleic it causes retarded growth, increased water consumption, reduced resistance to disease, an enlarged liver with increased lipid content and elevated concentrations of Eicosatrienoic (Watkins, 1991). Hence, diets for poultry should contain an adequate amount of Linoleic.

As expected, freeze-dried *E. foetida* contained most of the fatty acids such Tridecenoic, Pentadecenoic, Palmitic, Heptadecenoic, Stearic acid, Elaidic, Lignoceric, and Docosapentaenoic though retained nonessential fatty acids. The predominance of some fatty acids in freeze-dried *E. foetida* may be due to the minimal changes that occur during the

process because the growth of microbes and enzyme effects cannot be exerted under low temperatures (Khairna *et al.*, 2013). Moreover, this may be due to the non-existence of water and low temperature employed during freeze drying process, which stops most microbial reactions, with the final product having an excellent quality (Ratti, 2001).

### **3.5. Conclusions**

The study shows that effects of two drying methods (freeze drying and oven drying) had significant differences in nutrient composition and fatty acid profile of *E. foetida*. Freeze drying method recorded higher concentrations of nutrients and fatty acids than oven drying method. Therefore, freeze drying could be preferred drying method for *E. foetida*. Therefore, further research is needed to determine the potential of *E. foetida* as a supplement on growth performance of broilers, as it has been found as excellent in nutrient composition and fatty acid profiles.

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## **CHAPTER 4: The potential of *Eisenia foetida* meal on the growth performance, digestive organs, bone strength and carcass characteristics of broilers**

### **Abstract**

The effects of *Eisenia foetida* meal as fishmeal replacement on body weight gain (BWG), average daily gain (ADG) average daily feed intake (ADFI) and feed conversion ratio (FCR), carcass characteristics, digestive organs and bone strength of broilers was investigated. A total of 180 day old Cobb broilers were randomly allocated to five dietary treatments as follows: T1 (0%), T2 (1%), T3 (3%), T4 (5%) and T5 (10%) earthworm meal inclusion. Each treatment was presented by three replicates with 12 birds per replicate. The trial lasted for 35 days, which was divided into three growth phases including; starter (1-21d), grower (22-28d) and post finisher (29-35 d). At day 35 of age, 75 broilers, 15 birds per treatment, five per replicate were slaughtered and used to measure carcass characteristics, digestive organs, and bone strength. Data was analysed with One Way Analysis of Variance. There was no significant difference ( $P > 0.05$ ) on growth performance traits of birds in different dietary treatments except for FCR ( $P < 0.05$ ) at 0 -21 days of age. The FCR of T1 birds was the best compares to the all dietary treatments used in the current study but was statistically similar to birds in T2. At 22-28 days of age, significant dietary effect ( $P < 0.05$ ) was observed on ADG and ADFI. The highest ADG was recorded in T3 birds (89.9g) though was statistical similar with birds in T1, T2, and T3, the least ADG was seen in T5 (60.9g). All growth traits were significantly different ( $P < 0.05$ ) across dietary treatments at 29-35 days of age. Birds in T4 recorded the highest values of BWG (1137.9g) and ADG (162.5g) and the least BWG and ADG of 882.9g and 126.1g respectively were observed in T3 while, ADFI was highest in T3 birds (199.4g) and the least was recorded in T5 (164.4g). Furthermore, birds in T4 had the highest (1.6) FCR and birds in T1 recorded the least value (1.2). At 1- 35 days of age no

significance differences ( $P > 0.05$ ) were observed on ADG, ADFI, and FCR among different inclusion levels of *E. foetida* meal. The dietary effect was observed on BWG ( $P < 0.05$ ) and birds fed 5% inclusion of earthworm meal (T4) had the highest body weight gain of 2590.4g. Birds in T2 had the least body weight gain, though; they were statistically similar to T1 and T3 birds. Furthermore, results revealed significant differences ( $P < 0.05$ ) of live and carcass weights of birds across the dietary treatments. However, no significant difference ( $P > 0.05$ ) was observed in the dressing percentage for birds fed with or without *E. foetida* meal. Birds in T3 had the highest (2.1kg) body weight, while the least body weight was recorded for birds in T5 (1.7 kg). Gizzard pH was not significantly ( $P > 0.05$ ) influenced by dietary treatments. However, gizzard weight and intestine weight were significantly different ( $P < 0.05$ ) among dietary treatments. Birds in T2 exhibited the highest gizzard weight (42.5g) whereas those, in T4 recorded the least weight of 36.1g. The highest intestine weight of 92.2g was observed in birds in T3 while the least weight of 80.1g was seen in birds in T5. Dietary treatments significantly influenced bone strength, with birds in T1, exhibited the highest bone strength whereas birds in T2 exhibited the lowest bone strength. Bone ash percentage was influenced ( $P < 0.05$ ) by dietary treatments. Birds in T2 had the highest ash percentage (70.2%) and birds in T3 and T4 had the least bone ash percentage. It can be concluded that, earthworm meal can replace fishmeal at 5 % inclusion level for an improved weight gain of broilers without adverse effects on the performance of the birds Furthermore, it reveals the effectiveness of *E.foetida* meal on carcass characteristics, digestive organs, and bone strength.

**Keywords:** Average Daily Gain, Average Daily Feed Intake, Bone, Body Weight Gain, carcass, chickens, *Eisenia foetida*, digestive organs Feed Conversion Ratio

#### 4.1. Introduction

The major constraint for poultry production is the feed cost since the cost of feed is about 75% of poultry production (Mupeta *et al.*, 2003). Commonly used protein feed sources for chickens are fishmeal and soya bean. These protein feed sources are restrictive (Veldkamp *et al.*, 2012) because they are much expensive. Therefore there is a need to come up with alternative or replacement with available sources of protein that can substitute fishmeal.

One such class of comparable alternatives is *E. foetida*. It has a good nutritional profile (Gunya *et al.*, 2016). Many researchers have found earthworm *E. foetida* having a potential to be used as an alternative animal protein (Yaqub, 1991; Edward, 1988; Sogbensan and Ugwaba, 2008), since it has high protein content of 64-76% and contains 20 out of 24 major amino acids (Zhenjun *et al.*, 1997) which are very important in poultry production. Furthermore, *E. foetida* contains lysine which is limiting in many feedstuffs (Anitha and Jaryjaaj, 2012). Moreover, *E. foetida* is high in the amount of fatty acids and omega 3 and contains an adequate mineral content and excellent range of vitamins which are valuable components of poultry feed (Vielma *et al.*, 2001). Therefore the inclusion of *E. foetida* in feeds for poultry production seems to be promising innovation because of their high efficiency in making valuable nutrients.

The potential of *E. foetida* as a protein source for poultry feed and its effects on growth performance has previously been reported by many researchers (Rezaeipour *et al.*, 2014; Mahmoud *et al.*, 2015; Mohanta *et al.*, 2015; Ncobela and Chimonyo, 2015; Zivar *et al.*, 2015). Although the focus of researchers is on use of *E. foetida* meal as an alternative source

of protein for poultry, little has been done on how digestive organs and bone strength are affected. The leg weakness and broken bones are serious challenges in the broiler industry, they result into large economic losses estimated at 1.1% bird mortality, and an additional 2.1% due to down-grading of carcasses during processing (Anyang *et al.*, 2003; Atlatl *et al.*, 2009). It has been reported that bone strength is directly influenced by diet. Therefore the objective of this study was to investigate the effect of *E. foetida* as a replacement of fish meal on growth performance, digestive organs, and bone strength and carcass characteristics of broilers.

## **4.2. Materials and Methods**

### **4.2.1. Study site**

The study was conducted at the Fort Cox Farm, Alice, South Africa. The farm lies along longitude 32° 78'E and latitude 26° 85'S at an altitude of 450-500m above sea level. The farm is located in the False Thornveld which is characterised by mean annual rainfall of 480mm and mean annual temperature of 18.7 °C.

### **4.2.2. Preparation of *Eisenia foetida* meal**

*Eisenia foetida* were obtained from commercial suppliers Ado cruise earthworm farm in Port Elizabeth, Eastern Cape, South Africa. Fresh *E. foetida* earthworms were harvested by hand picking and then were dried in a controlled oven at 90°C for four hours. After two days of drying the worms were ground to powder form with a mortar. The earthworm meal was then placed in airtight polyethene plastic bags until they were used.

#### **4.2.3. Birds used**

A total of 180 Cobb from Buffalo Chick Hatchery (Belyn, East London, South Africa) broilers were used. Broilers were reared from day old to five weeks.

#### **4.2.4. Bird Management**

The chicks were reared together on deep litter in an open house. The house floors were covered with six cm of wood shavings as litter material. These experimental pens were constructed within a house in which a 1 m high net wall was covered with wire mesh. The wire mesh was allowed for ventilation and natural light. The diets were then randomly allocated to the pens (Kingori *et al.*, 2003). Chicks were inspected daily and dead birds were removed. Feed and fresh water were accessible *ad libitum* throughout the whole production cycle.

#### **4.2.5. Dietary treatments**

The feeding program consisted of a starter (1 to 21 days broilers), finisher (22 to 28days broilers), and post finisher (29-35 days) basal diets formulated on Win-Feed 3.0 Formulation Software to be isocaloric and iso-nitrogenous to meet the bird's dietary nutrient requirements (NRC, 1994). Five dry feeds were formulated based on the protein of the major feed ingredient mainly earthworm meal, canola oil cake, and soya oilcake. Each basal feed were split into 5 treatment (T) groups, fish meal were replaced in the diet with increasing inclusion levels of earthworm meal at 0% (Diet 1: control); 1% (Diet 2); 3% (Diet 3); 5% (Diet 4) and 10% (Diet 5). The ingredients and chemical composition of dietary treatments are shown in Table 4.2.2.

Proximate analysis for moisture, crude protein, ash and ether extract and mineral composition were performed on all experimental diets and on *E. foetida* samples according to methods of the Association of Official Analytical Chemists (AOAC, 2000).

#### **4.2.6. Experimental Design**

A total of 180 broiler day-old unsexed Cobb chicks were randomly allocated to 15-floor pens. The experiment was a completely randomised design divided into 5 dietary treatments with 3 replicates and 12 birds per replication.

#### **4.2.7. Evaluation of growth performance and nutrient utilization**

The birds were weighed weekly using a weighing scale (LBK, 12) on Tuesdays at 10:00 am to observe their rate of gain. Body weight gain (BWG), feed intake (FI), average daily gain (ADG), average daily feed intake (ADFI) and then feed conversion efficiency (FCE) of the chickens were recorded at the beginning of each week. Body weight gain for each chicken was measured as final weight minus initial weight

$$\text{BWG} = \text{final body weight} - \text{initial body weight}$$

To determine the Average Daily Gain for each chicken was measured as weekly average

$$\text{ADG} = \frac{\text{final wt} - \text{initial wt}}{\text{no. of days between weighing}}$$

To measure the Average Daily Feed Intake, the amount of feed provided and the amount of feed that is left as waste every day were weighed to calculate ADFI as:

$$\text{ADFI} = \text{feed provided} - \text{feed left as waste}$$

Subsequently, the Feed Conversion Efficiency was determined as a measure of the amount of feed required to attain one unit of weight gain. That is:

$$\text{FCE} = \text{ADFI} / \text{ADG}$$

**Table 4.2. 1** Chemical composition of *Eisenia foetida*

<b>Ingredients</b>	<b>Nutrient Composition</b>
<b>Proximate analysis (%)</b>	
Protein	51.62
Moisture	6.75
Fat	7.76
Fibre	3.80
Ash (%)	19.74
Starch	0.0
Sugar	0.31
NDF	7.70
ADF	2.81
Total fat	8.21
<b>Minerals</b>	
Ca (%)	5.03
P (%)	1.21
Na (%)	1.09
Salt (%)	3.12
Mg (%)	0.25
K (%)	2.04
Cu (mg/Kg)	420.91
Mn (mg/Kg)	0.0
Fe (mg/Kg)	73245
Zn (mg/Kg)	183

**Table 4.2. 2** Chemical ingredients of the experimental diets

<b>Ingredient</b>	<b><u>Starter</u></b>					<b><u>Finisher</u></b>					<b><u>Post finisher</u></b>				
	<b>T1</b>	<b>T2</b>	<b>T3</b>	<b>T4</b>	<b>T5</b>	<b>T1</b>	<b>T2</b>	<b>T3</b>	<b>T4</b>	<b>T5</b>	<b>T1</b>	<b>T2</b>	<b>T3</b>	<b>T4</b>	<b>T5</b>
Yellow Maize	65.20	65.68	66.05	65.70	63.81	69.50	69.63	69.90	69.41	65.9	73.29	73.51	72.48	70.52	61.46
Soya Oilcake	24.16	22.15	20.30	18.25	15.91	18.51	17.80	16.37	14.18	10.50	14.83	13.90	13.27	12.55	7.76
Sunflower Oilcake	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Canola Oilcake	2.84	3.50	3.27	3.85	0.0	4.84	4.57	4.02	4.85	5.00	5.00	5.00	5.00	5.00	5.00
Worms	0.00	1.00	3.00	5.00	10.00	0.00	1.00	3.00	5.00	10.00	0.00	1.00	3.00	5.00	10.00
Wheat Middlings	0.00	0.00	0.00	0.00	3.34	0.00	0.00	0.00	0.00	2.75	0.265	0.16	0.00	0.64	5.00
Canola Oil	0.33	0.23	0.03	1.21	0.00	0.29	0.23	0.19	0.00	0.56	0.260	0.16	0.19	0.43	2.07
Limestone Powder	1.41	1.34	1.27	0.51	1.12	1.10	1.34	1.06	0.93	0.87	0.949	0.92	0.90	0.88	0.69
Monocalcium Phosphate	0.63	0.61	0.56	0.32	0.38	0.35	0.61	0.34	0.25	0.03	0.137	0.11	0.05	0.00	0.00
Salt Fine	0.34	0.33	0.32	0.20	0.29	0.34	0.33	0.34	0.32	0.29	0.347	0.34	0.33	0.32	0.29
Methionine Powder	0.20	0.21	0.20	0.13	0.19	0.16	0.21	0.16	0.15	0.13	0.132	0.13	0.12	0.10	0.08
Tryptophan	0.03	0.08	0.11	0.05	0.16	0.07	0.08	0.08	0.12	0.09	0.061	0.07	0.04	0.00	0.00
Threonine	0.06	0.06	0.06	0.42	0.04	0.05	0.06	0.05	0.03	0.00	0.029	0.03	0.01	0.00	0.00
Lysine	0.41	0.43	0.43	0.00	0.38	0.42	0.43	0.41	0.39	0.31	0.383	0.38	0.32	0.27	0.22
Choline Chloride Powder	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.086	0.090	0.09	0.09	0.09
Vit/Min Premix	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.200	0.20	0.20	0.20	0.20
Aviax Plus	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.00	0.00	0.00	0.00	0.00
Surmax	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.0	0.00	0.00		0.00
Gluten 20%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.15

**Table 4.2. 3** Analysed nutrient composition of the experimental diets on dry basis

<b>Dietary treatments</b>	<b>ME (MJ/Kg)</b>	<b>CP (%)</b>	<b>CF (%)</b>	<b>EE (%)</b>	<b>Moisture (%)</b>	<b>Ash (%)</b>
<b><u>Starter</u></b>						
<b><u>(0-21 days)</u></b>						
T1	12.8	21.3	3.2	5.4	9.4	4.7
T2	12.6	22.2	2.3	5.6	9.5	4.8
T3	12.4	23.2	2.7	5.7	11.0	5.0
T4	12.9	23.4	3.3	5.3	9.8	4.1
T5	13.0	23.6	3.5	5.4	9.5	4.4
<b><u>Finisher</u></b>						
<b><u>(22-28days)</u></b>						
T1	12.9	19.3	2.0	5.4	11.7	5.0
T2	12.8	18.9	2.9	5.6	11.2	4.7
T3	12.7	19.2	1.8	5.5	10.8	5.2
T4	12.7	19.2	4.7	5.6	10.6	3.9
T5	13.0	19.5	4.6	5.5	10.8	4.4
<b><u>Post finisher</u></b>						
<b><u>(29-35 days)</u></b>						
T1	13.0	17.2	1.9	5.4	10.9	4.0
T2	13.0	17.3	2.7	5.4	10.8	3.6
T3	13.0	17.4	2.3	5.4	11.3	5.2
T4	13.0	17.3	3.3	5.4	10.1	3.5
T5	13.0	17.7	3.9	5.4	10.4	5.0

T1, T2, T3, T4 T5 contained graded levels of E. foetida meal at 0, 1,3,5 10% of DM intake, respectively

**Table 4.2.** 4Analysed mineral composition of the experimental diets, on dry matter basis

Dietary treatments	P	Ca	Mg	K	Na	Cu	Fe	Mn	Zn
	%				mg/Kg				
<b>Starter</b>									
<b>(0-21 days)</b>									
T1	0.53	0.81	0.08	0.85	148	5	128	91	186
T2	0.49	0.71	0.05	0.60	10	6	196	83	396
T3	0.48	1.04	0.16	0.95	164	5	157	146	436
T4	0.38	0.43	0.06	0.56	206	6	88	43	374
T5	0.32	0.60	0.06	0.66	195	6	654	101	208
<b>Finisher</b>									
<b>(22-28days)</b>									
T1	0.53	0.60	0.09	0.87	94	8	108	65	233
T2	0.48	0.40	0.07	0.53	42	7	81	41	89
T3	0.50	0.63	0.10	1.02	117	8	131	90	225
T4	0.56	1.45	0.11	0.77	23	8	195	106	311
T5	0.76	1.32	0.26	0.91	149	7	327	130	128
<b>Post finisher</b>									
<b>(29-35days)</b>									
T1	0.54	0.40	0.12	0.91	128	4	146	115	111
T2	0.35	0.39	0.10	0.81	69	2	177	71	38
T3	0.56	0.50	0.11	0.79	82	4	77	38	257
T4	0.56	0.40	0.08	0.47	276	6	185	69	97
T5	0.52	0.57	0.19	0.84	113	4	163	131	136

T1, T2, T3, T4 T5 contained graded levels of *E. foetida* meal at 0, 1, 3, 5 10% of DM intake, respectively

#### **4.2. 8. Slaughter procedure**

At 35 days of age, 75 birds were randomly selected, 15 birds per treatment and fasted for six hours with water offered *ad libitum*. The chickens were stunned individually on the head using 70 volts prongs, heads were decapitated from the neck using a sharp knife.

#### **4.2.9. Carcass characteristics**

At the processing plant, birds were reweighed before slaughter to determine their live weights. After bleeding, scalding, plucking and washing, the feet, head, and neck were removed. Gizzards and visceral organs (liver, heart, and spleen) were removed by hand through an incision made around the vent and sternum. Dressing percentage (carcass weight/Bodyweight) was calculated and expressed as a percentage.

#### **4.2.10. Digestive organs size measurements**

Twelve birds per treatment were randomly selected, and the internal organs were removed from each chicken, plastic bags which were carrying internal organs were marked according to their treatment types. The scale was used to weigh intestines and the gizzard weight individually in grams.

#### **4.2.11. Gizzard pH measurement**

The digestive tract with digest was removed aseptically and then the gizzard was excised and the contents were collected. The pH of the gizzards for each chicken was measured using a portable pH meter (Crison pH 25 CRISON Instruments SA, Spain) after calibration with pH 4, pH7 and pH9 standards solutions (CRISON Instrument, SA, and Spain).

#### **4.2.12. Preparation of bone samples**

At the end of the experimental period three birds from each treatment were slaughtered, the carcass identified and then frozen at -20°C for further analysis. Carcasses were carefully dissected into primal cuts. The left and right tibia of each bird were removed with the drumsticks and flesh undamaged. The drumsticks were labeled and immersed in boiling water (100°C) for 10 minutes (Nkukwana, 2012). After cooling to room temperature, the flesh was manually removed from the drumsticks and then air dried for 7 hours at room temperature.

#### **4.2.13. Bone strength determination**

The breaking strength of each tibia was determined using Instron (Model (3344), Universal Testing apparatus machine with default specimen dimension setup as circular geometry, 10mm diameter, an anvil height of 30 mm and a crosshead speed of 50 mm/min. The vertical hydraulic force was applied at the midpoint of the bone shaft to minimize splintering. The load, defined as force (Newton, N) of cross-section area, represented bone strength, while the modulus measured rigidity, as related to stress and strain (Nkukwana, 2012).

#### **4.2.14. Ash percentage**

The crucibles and lid were weighed, and then the combined mass recorded as weight one, weight two was obtained by weighing approximately 1g of the ground dry sample then the weight was recorded (i.e. combined mass of crucible, lid, and sample). Lastly, weight three was recorded after the samples were ashed in a muffle furnace for 24 hours at 600 °C (Kalebo

and Strid, 1988). The percentage ash was determined relative to dry weight of ground tibia using the formula below:

$$\text{Ash (\%)} = \frac{w_3 - w_1}{w_2 - w_1} \times 100$$

#### 4.2.15. Data analysis

The effect of dietary treatments on BWG, FI, ADG, AFI, FCE, bone characteristics and digestive organs of broiler chickens were analysed using one-way ANOVA, using General Linear Model (GLM) procedure of SAS (SAS, 2006). The model used was

$$Y_{ij} = \mu + \alpha_i + e_{ij}$$

Where:  $Y_{ij}$  = response variable,

$\mu$  = the common mean

$\alpha$  = the effect of dietary treatment (T1, T2, T3, T4, and T5) and

$e_{ij}$  = random error

Mean separation was done using LSD test option of SAS (2006).

### 4.3. Results

#### 4.3.1. Body weight

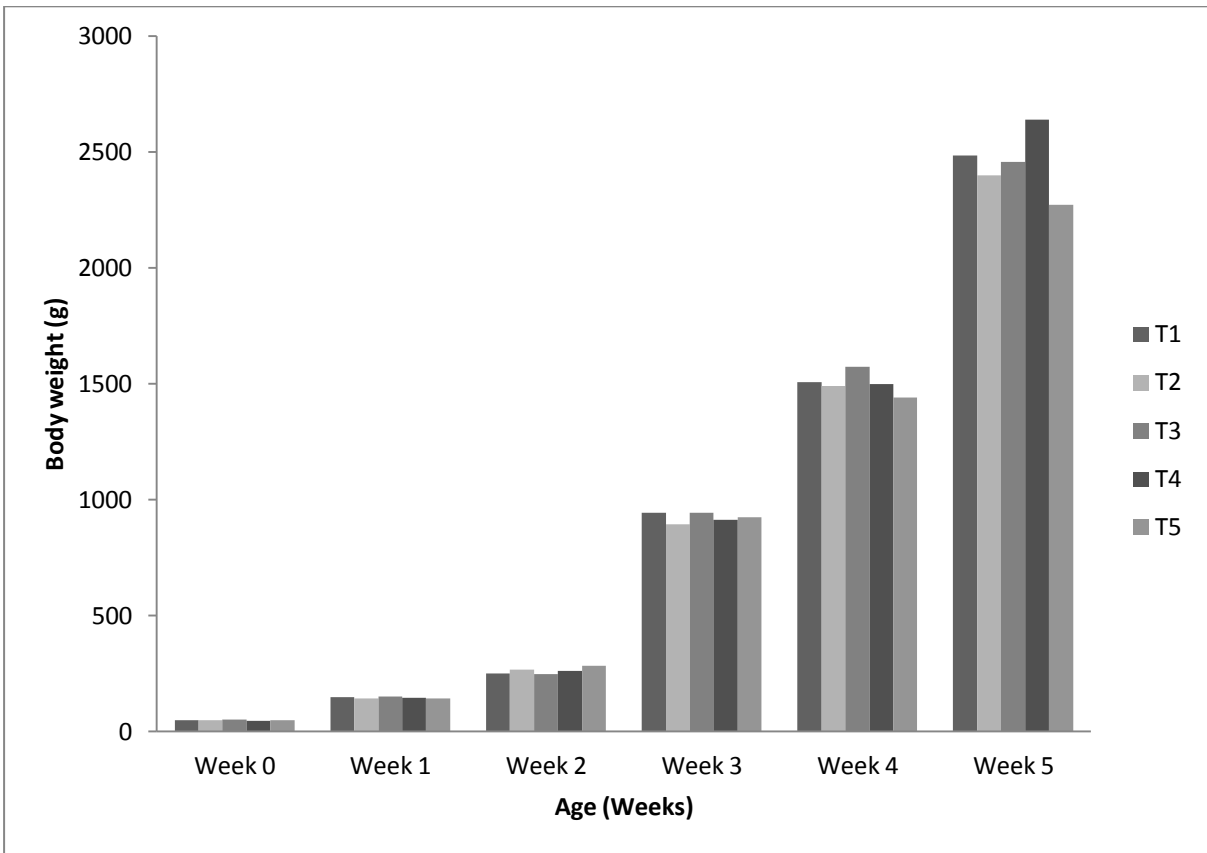
Figure 4.3.1 shows the effect of *E. foetida* inclusion levels on body weight of birds. There was no significant difference in body weight of birds across dietary treatments from week 0 to week three. However, the dietary effect on body weight was observed in week 4 and week 5. In week 4, birds in T3 had the highest body weight but was statistical similar with birds in T1 and T4 and the least body weight was seen in T5. Significant highest body weights in week 5 were observed in birds in T4 and the least body weight was found in T5.

### **4.3.2. Body gain of broilers**

The effect of different inclusion levels of *E. foetida* meal on body weight gain (BWG) of birds is shown in Figure 4.3.2. No significant difference ( $P > 0.05$ ) was observed on body weight gain of birds among dietary treatments at 1-21d and 22-28 d. However, dietary effect ( $P < 0.05$ ) was observed on body weight gain at the last phase of growth (29-35 days of age) and at 0-35 days of age with birds fed 5 % inclusion of *E. foetida* (T5) meal recorded the highest body weight gain of 1137.9g and 2590.4g, respectively.

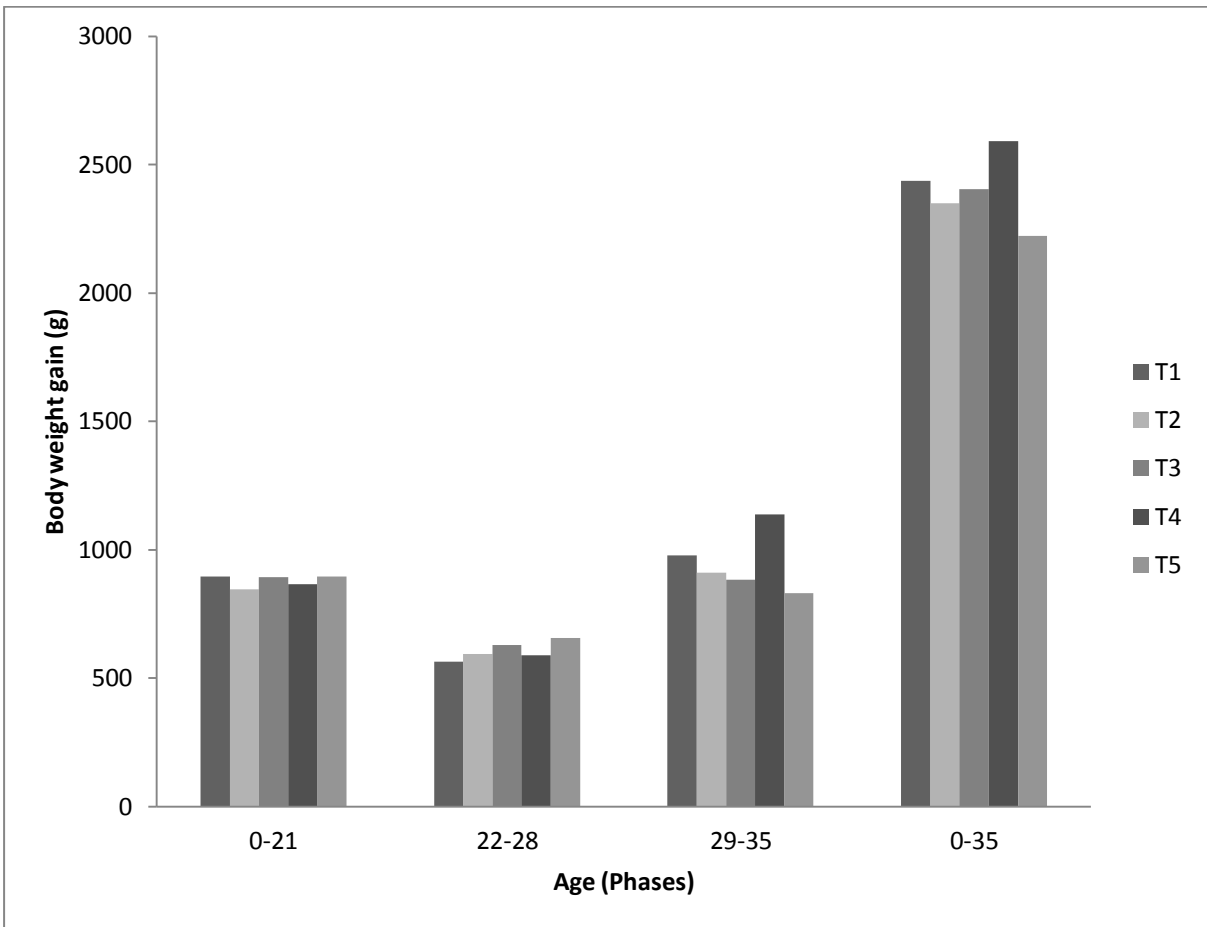
### **4.3.3. Average Daily Gain**

The average daily gain of broilers fed different inclusion levels of *E. foetida* is presented in Figure 4.3.3 No significant difference ( $P > 0.05$ ) was observed on an average daily gain during the first phase (1-21 d) and during the whole trial (1-35d). However, the average daily gain was significantly different ( $P < 0.05$ ) across dietary treatments in the last two phases (22-28d and 29-35). At 22-28d, birds in T1, T2, T3 and T4 had an improved average daily gain of 80.40g, 84.90g, 89.90g and 83.90g respectively more than their counterparts in T5 that had an average daily gain of 60.90g. The highest average daily gain (74.0g) of birds was recorded in birds in T4 and the least values (63.5g) were seen in T5 birds at 0-35d.



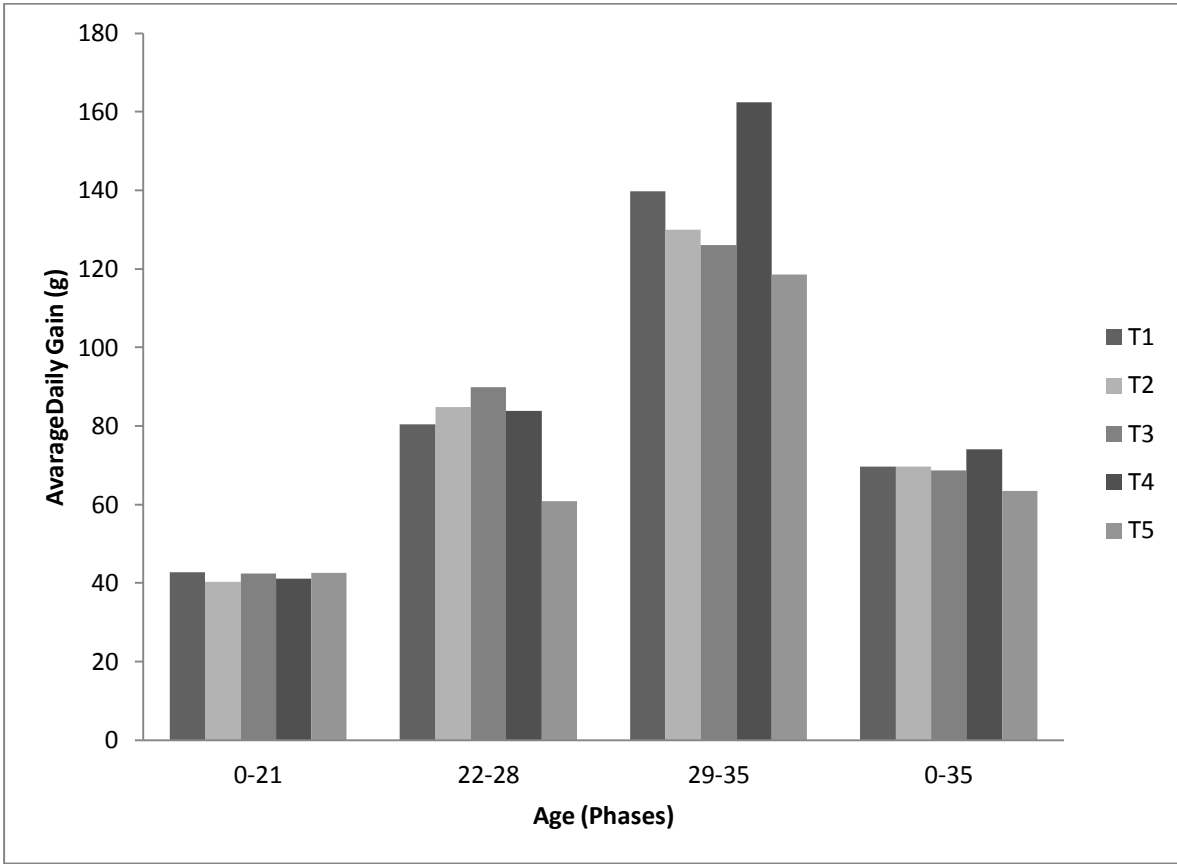
**Figure 4.3 1** Body weight (BW) of birds fed different inclusion levels of *E. foetida* meal

T1, T2, T3, T4 and T5 contained graded levels of *E. foetida* 0%, 1%, 3%, 5% and 10% of DM intake, respectively



**Figure 4.3 2** Body weight gain (BWG) of birds fed different inclusion levels of *E. foetida* meal

(T1 control, T2, T3, T4, and T5 contained graded levels of *E. foetida* 0%, 1%, 3%, 5% and 10% of DM intake, respectively)



**Figure 4.3 3** Average daily gain (ADG) of birds fed different inclusion levels of *E. foetida* meal

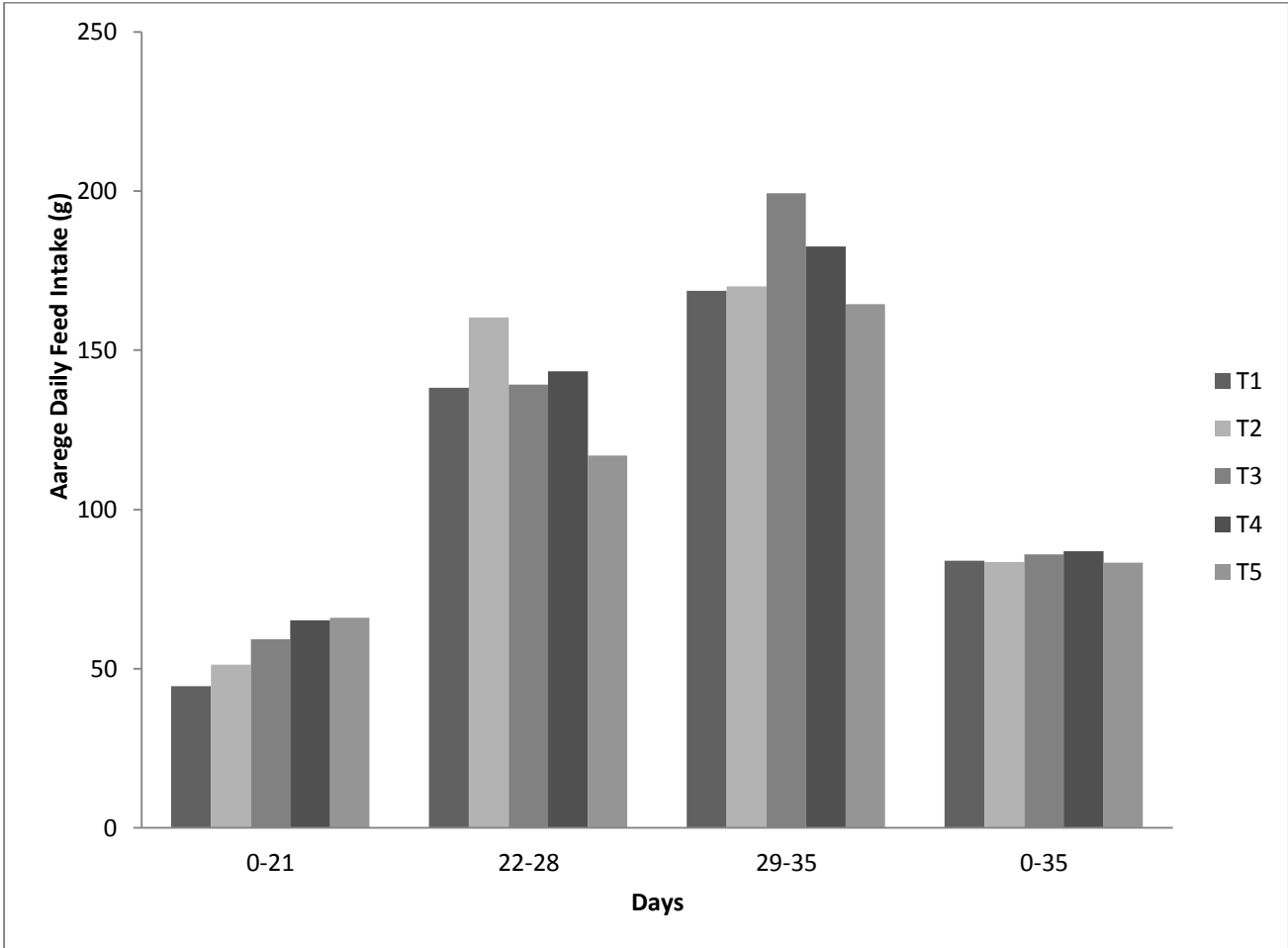
(T1 control, T2, T3, T4 and T5 contained graded levels of *E. foetida* 0%, 1%, 3%, 5% and 10% of DM intake, respectively)

### 4.3.3 Average daily feed intake

The effect of *E. foetida* meal inclusion levels on average daily feed intake is presented in Figure 4.3.4. No dietary effect ( $P > 0.05$ ) was observed on ADFI of birds at 0-21 d and 0-35d, although dietary influence ( $P < 0.05$ ) was found at 22-28d and 29-35d. At 22-28 d the highest average daily feed intake values were noticed for birds in T2 (160.4), T3 (139.2) and T4 (143.5), while the least average daily feed intake was recorded for T5 (117.0) birds, though, they did not differ significantly ( $P > 0.05$ ) with T1 (control) birds. Bird fed 3% *E. foetida* meal inclusion (T3) recorded the highest ADFI of 199.4g and the least was seen in the T5 bird (164.4g).

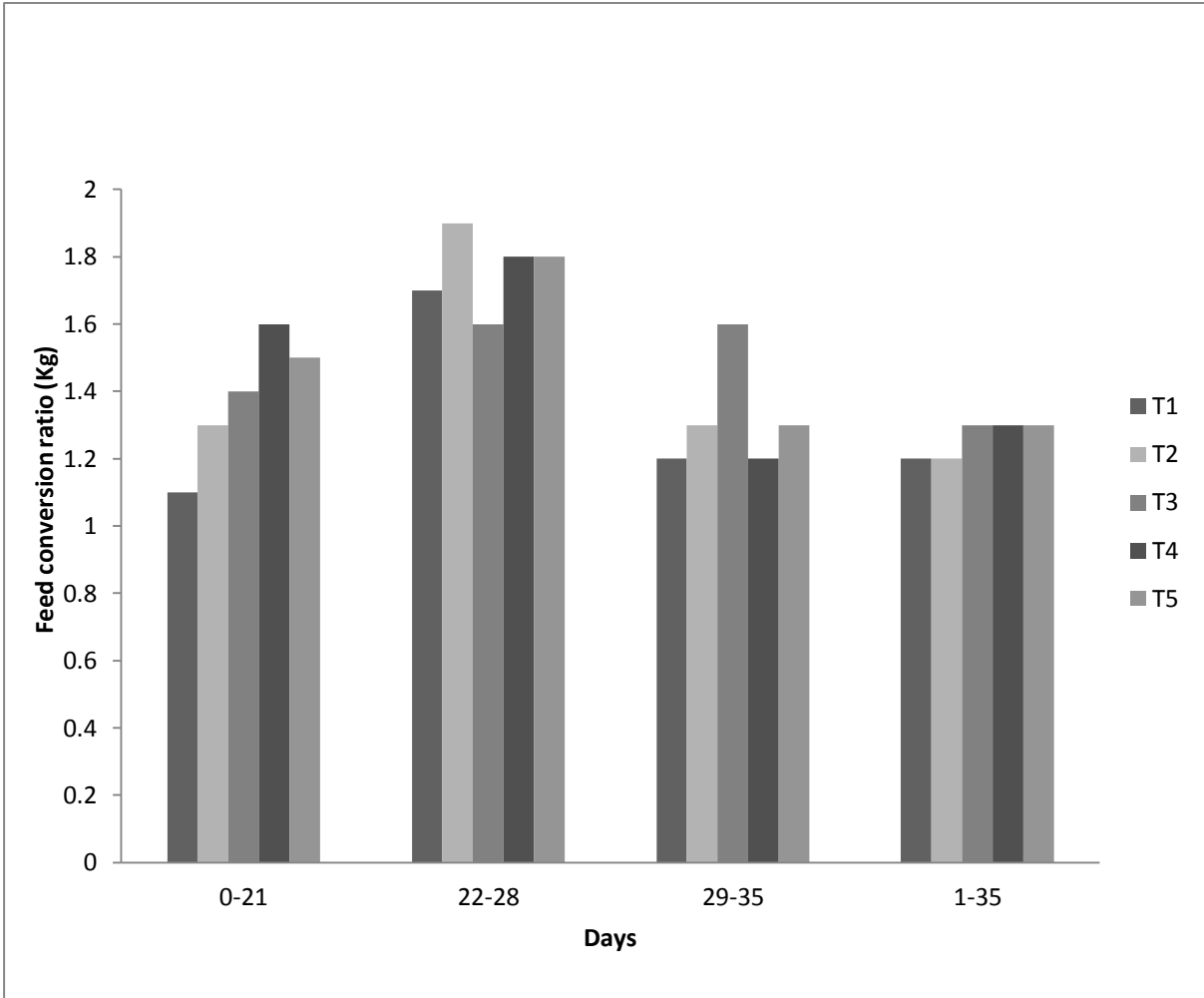
### 4.3.4. Feed conversion ratio

Feed conversion ratio of birds fed different inclusion levels of *E. foetida* meal is shown in Figure 4.3.5. Dietary effect ( $P < 0.05$ ) of FCR was seen at 1-21 d and 0-35d, whereas no significant difference was seen at 22-28d. The FCR of birds in T1 (control diet) was better among all the treatments used in the study but was statistically similar to birds in T2 (1%) while the highest FCR was observed in T5 birds at 1-21d. However, at 1-35d FCR was highest in birds in T3 and the least was recorded in birds in T1 (1.2), T2 (1.3), T4 (1.2) and T5 (1.3).



**Figure 4.3 4** Average daily feed intake (ADG) of birds fed different inclusion levels of *E. foetida* meal

(T1 control, T2, T3, T4 and T5 contained graded levels of *E. foetida* 1%, 3%, 5% and 10% of DM intake, respectively).



**Figure 4.3 5** Feed conversion ratio (FCR) of birds fed different inclusion levels of *E. foetida* meal

(T1 control, T2, T3, T4, and T5 contained graded levels of *E. foetida* 1%, 3%, 5% and 10% of DM intake, respectively)

#### **4.3.5. Carcass characteristics**

Table 4.3.1 depicts the effect of the inclusion level of earthworm meal on carcass characteristics of broilers. All carcass traits were significantly ( $P < 0.05$ ) influenced by dietary treatments except for dressing. As the inclusion level of earthworm increased, the live weights of birds decreased. Furthermore, birds in treatment 2 and 3 recorded the highest ( $P < 0.05$ ) live weight of 2.00 kg and 2.10 kg respectively but they were statistically similar to birds in T1 with a live weight of 1.90 kg. The lowest live weight was observed in birds fed T4 (1.80 kg) and T5 (1.70kg). Birds in treatment 4 and 5 had the lowest pluck weight of 1.6 kg and carcass weight of 1.3 kg and 1.4 kg, respectively. Birds in T2 had the highest (1.8 kg) pluck weight although they were similar to T1, T2, and T3. On the other hand, birds in T3 had the highest (1.5 kg) carcass weight even though they were statistically similar to birds fed T1 and T2.

#### **4.3.6. Digestive organs**

The effect of different inclusion levels of earthworm meal on gizzard weight, gizzard pH, and intestine weight is shown in Table 4.3.2. There was a significant difference ( $P < 0.05$ ) on gizzard weights among dietary treatments. It was found that birds in T5 had the least gizzard weights while the highest gizzard weights were found in T2. However, there was no significant difference ( $p > 0.05$ ) on gizzard pH among dietary treatments, but birds in T5 were found to have the highest gizzard pH than other treatments and birds in T3 had the lowest gizzard pH. Intestine weights were significantly different ( $P < 0.05$ ) among the dietary treatments with birds in T3 having the highest intestine weight than other treatments whereas the lowest intestine weights were found to be in T5.

#### **4.3.7. Bone strength**

The effect of *E. foetida* inclusion levels on the bone strength of birds is shown in Figure 4.3.6. Bone strength values of broilers were significantly different ( $P < 0.05$ ) among dietary treatments. The bone strength of broilers increased as the inclusion levels of *E. foetida* earthworm meal increased. The results showed an increase from  $T_2 = 78.7\text{N}$  which is the least minimum to  $T_5 = 132.1\text{N}$  which is the maximum excluding the two controls  $T_1$  and  $T_2$ . The highest bone strength values were observed in birds in  $T_1$  (control) and the lowest values were seen in  $T_2$  (1%).

#### **4.3.8. Bone ash content**

The bone ash content of broilers fed different inclusion levels of *E. foetida* meal is presented in Table 4.3.2. Bone ash content was significantly different ( $P < 0.05$ ) across dietary treatments. The bone ash content decreased as the inclusions of *Eisenia foetida* earthworm meal increased. The highest values of bone ash content were seen in birds in  $T_2$  (1%) and the least values were observed in birds in  $T_1$  (control).

**Table 4.3 1** Carcass characteristics of broilers fed different inclusions of *E. foetida*

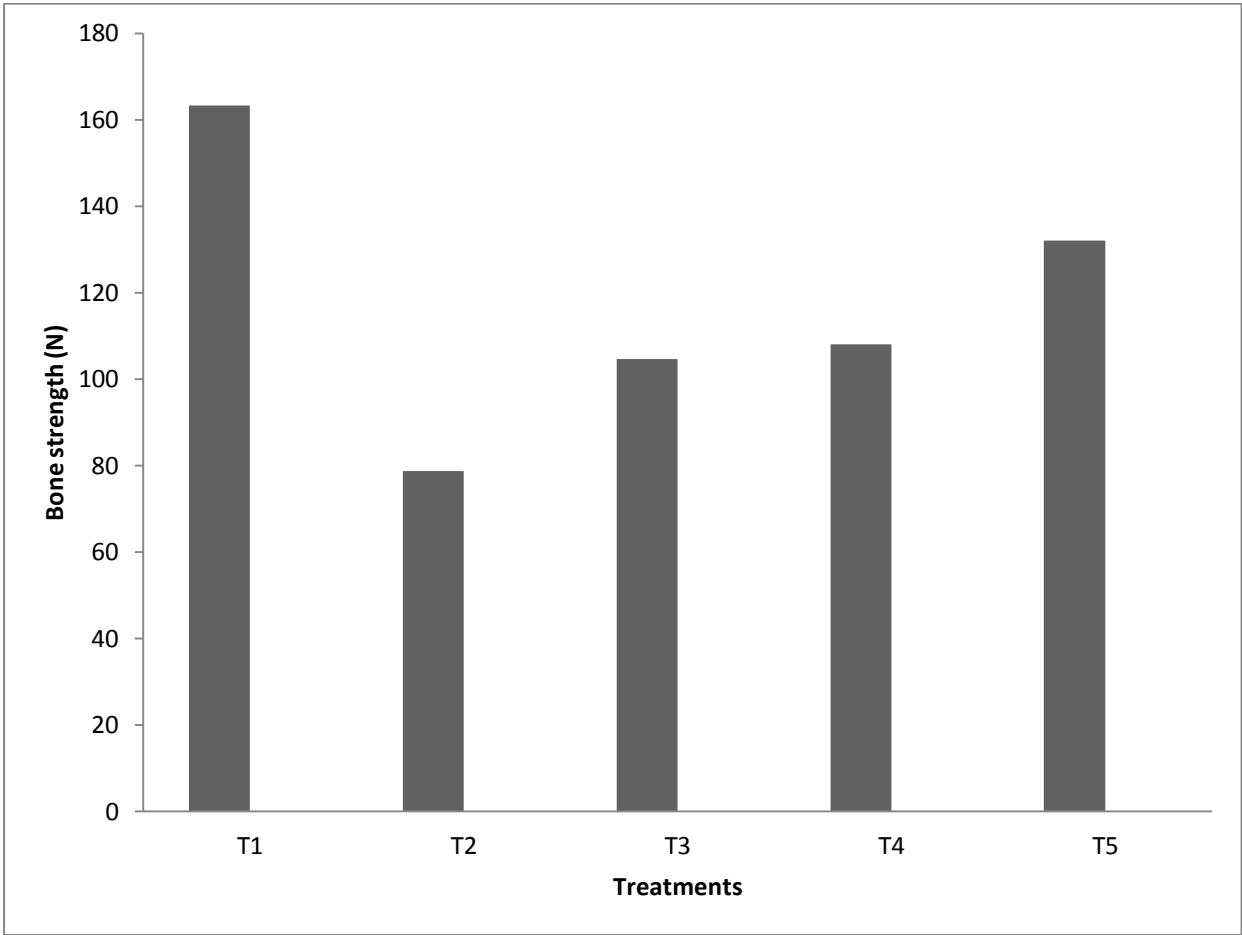
<b>Carcass characteristics</b>	<b>Dietary treatments</b>				
	<b>T1</b>	<b>T2</b>	<b>T3</b>	<b>T4</b>	<b>T5</b>
Live weight (Kg)	1.9 <sup>ab</sup> ±0.06	2.0 <sup>b</sup> ±0.06	2.1 <sup>b</sup> ±0.06	1.8 <sup>a</sup> ±0.06	1.7 <sup>a</sup> ±0.06
Pluck weight (Kg)	1.7 <sup>bc</sup> ±0.05	1.8 <sup>c</sup> ±0.08	1.7 <sup>bc</sup> ±0.05	1.6 <sup>a</sup> ±0.05	1.6 <sup>a</sup> ±0.06
Carcass weight (Kg)	1.4 <sup>ab</sup> ±0.05	1.4 <sup>ab</sup> ±0.05	1.5 <sup>b</sup> ±0.05	1.3 <sup>a</sup> ±0.05	1.4 <sup>a</sup> ±0.05
% Dressing	76.3 <sup>a</sup> ±1.78	73.9 <sup>a</sup> ±1.78	75.1 <sup>a</sup> ±1.86	75.1 <sup>a</sup> ±1.86	75.5 <sup>a</sup> ±1.86

<sup>a,b,c</sup>Mean within the same row that do not share a common superscript are significantly different (p<0.05)

T1 control; T2, T3, T4 and T5 contained graded levels of *E. foetida* 1%, 3%, 5% and 10% of DM intake, respectively.

% - percentage

Kg- kilograms



**Figure 4.3 6** Bone strength of birds fed different inclusion levels of *E. foetida* meal

(T1 control, T2, T3, T4, and T5 contained graded levels of *E. foetida* 1%, 3%, 5% and 10% of DM intake, respectively)

**Table 4.3 2** Ash content of tibia bones obtained from broilers fed different inclusions of *Eisenia foetida* earthworm meal

<b>Treatment</b>	<b>Ash (%)</b>
T1	37.1 <sup>a</sup> ±6.84
T2	70.2 <sup>b</sup> ±6.84
T3	39.3 <sup>a</sup> ±6.84
T4	39.3 <sup>a</sup> ±6.84
T5	42.3 <sup>a</sup> ±6.84

<sup>a,b,c</sup>Mean within the same row that do not share a common superscript are significantly different (p<0.05)

T1 control; T2, T3, T4 and T5 contained graded levels of *E. foetida* 1%, 3%, 5% and 10% of DM intake, respectively

**Table 4.3 3** The effect of inclusion level of *E. foetida* meal on gizzard pH, weight and intestine weight

<b>Treatment</b>	<b>Gizzard pH</b>	<b>Gizzard weight</b>	<b>Intestine weight</b>
T1	5.7±0.04	40.1 <sup>ab</sup> ±2.15	90.6 <sup>b</sup> ±4.78
T2	5.7±0.04	42.5 <sup>b</sup> ±2.15	90.8 <sup>b</sup> ±4.78
T3	5.6±0.04	38.2 <sup>ab</sup> ±2.15	92.2 <sup>c</sup> ±4.78
T4	5.7±0.05	36.1 <sup>a</sup> ±2.15	80.5 <sup>a</sup> ±4.78
T5	5.8±0.05	37.7 <sup>ab</sup> ±2.15	80.1 <sup>a</sup> ±4.78

<sup>a,b,c</sup> Means within the same row that do not share a common superscript are significantly different (p<0.05), T1 control; T2, T3, T4, and T5 contained graded levels of *E. foetida* 1%, 3%, 5% and 10% of DM intake, respectively.

## Discussion

In this study, a significant dietary effect was observed during 29- 35 days, where birds in the diet with 5% inclusion of earthworm meal had a higher significant weight gain and birds fed a diet containing 10% earthworm meal inclusion had the least body weight gain. Improved body weight gain in broilers fed diets containing earthworm meal may be due to the antibacterial characteristics of earthworm meal (Julendra *et al.*, 2012). This means that, at the end of the trial, birds fed treatment four (5% inclusion level of earthworm) were heavier compared to birds fed other treatments used in this study.

The inclusion of *E. foetida* meal in diet of birds in this study, enhanced average daily gain in the last phase of growth (29-35 days of age) and during the whole trial (1-35 days of age) with birds fed 5 % inclusion of *E. foetida* (T5) meal recorded the highest average daily gain and birds in diet containing 10% *E. foetida* meal inclusion level had the least average daily gain. Current findings are in accordance with those observed by Resnawatti (2004) who reported that inclusion of earthworm meal up to 5% level improves broiler ADG. Nevertheless, they are contrary to results of Rezaeipour *et al.* (2014) and Hwangbo *et al.* (2009) who found that weight gain was improved in quail and broiler diets with 10 % earthworm meal. Moreover, Jankovic *et al.* (2015) found that birds fed with fresh earthworms had lower weight gain as compared to control group. This variation in our findings may be attributed to the type of earthworm (*Lumbricus rubellus*) and state of earthworm (fresh earthworm) used which resulted in decreasing of feed intake, thus decrease weight gain of birds.

The improved ADG of birds fed 5 % inclusion could be due to the higher protein content of the diets which is efficiently metabolised for growth (Safa and Tazi, 2012). In addition, antimicrobial and antioxidant in *E. foetida* earthworm (Rezaeipour *et al.*, 2014) and no anti-nutrient factor which improves the performance of broilers (Reinecke *et al.*, 1991) are likely the

main cause of enhancement in weight gain in birds fed 5 % inclusion level of earthworm meal (T4). Even though, birds in T4 had the low average feed intake, the birds recorded the high weight gain than other treatments. This may be explained by high protein content (50-70%) and fat content (7-10%) of earthworms that meets the nutrient requirements of birds. Hence, birds consumed smaller quantities of feed exhibited the great growth.

The decrease in average feed intake in birds in T5 might account for the low body weight gain in birds in the diet with 10% inclusion of *E. foetida* which might be caused by unpalatable feed with more earthworm meal. This is consistent with the findings of Resnawatti (2004) who reported that inclusion of earthworm meal above 5% inclusion level results in depressing performance of broilers.

In this study, dietary *E. foetida* meal inclusion influenced the daily feed intake of birds during the last stages of production (22-28d and 29-35d). In contrast with our findings, Dehghani and Jahanian (2012) in their study observed that birds fed organic acids containing different crude protein levels had no effect on ADFI throughout the feed trial. These contradictory results may be due to different sources of proteins used in these studies. During day 22-28, the highest daily feed intake of birds was recorded in birds fed low-level *E. foetida* meal (1%) while the lowest intake was seen in birds fed 10% inclusion of earthworm meal. The decrease in feed intake in birds fed a diet with 10% inclusion of earthworm may be attributed to the dull color of diet with high earthworm meal as earthworms are dark, even more after processing (drying). According to Khan *et al.* (2016) broilers are very sensitive to the color of feed, and bright feed attracts birds then resulting in high feed intake while dull feed reduces feed intake of birds, thus birds fed 10% of earthworm meal had the least feed intake because of high earthworm meal in the diet. Moreover, it has been reported that earthworms contain coelomic fluid, which could make feeds unpalatable when large quantities of earthworm accumulate in the feed (Edward and

Lofty 1977; Ngoc *et al.*, 2015) which consequently inhibited the birds consuming adequate feed in T5. The results corroborate the findings of Vu *et al.* (2009), Prayogi (2012) and Khan *et al.* (2016) who found that earthworm meal reduces feed intake of birds. The increased ADFI in birds fed low inclusion of *E. foetida* meal may be due to the fact that the diet was palatable to birds, thus increase in ADFI of broilers.

Our findings are in support of the findings of Dairo *et al.* (2010) who found that feed intake of birds increase with low protein inclusion in diet and decrease with high protein inclusion. In the current study, on the last phase of production (29-35d), birds in a diet containing 3% inclusion level of earthworm meal had the highest ADFI whereas birds in 5% inclusion recorded the least ADFI. Current findings are consistent with the results found by Rezaiepour *et al.* (2014) who found minimum feed consumption in birds in a diet containing 5% *E. foetida* meal level.

Feed conversion ratio is used to determine the efficiency in livestock production (Halloran *et al.*, 2016). Even though no differences were observed on BWG, ADG, and ADFI during the first phase of growth, feed conversion ratio (FCR) was influenced by diets provided in this study. Birds fed diet without *E. foetida* (T1) and those in the least inclusion level of earthworm (T2) were able to convert their feed to the meat better than birds that consume higher inclusion levels of earthworm meal (T3, T4, and T5). This is in agreement with Valsala (2016) who reported that the birds consumed and managed diet with less inclusion level of non-conventional feed better unlike when the level of non-conventional feed is increased. Furthermore, Ljiljan *et al.* (2015) also witnessed similar results, where broilers fed on earthworm meal showed no difference in weight gain at the beginning of the feeding trial. Ljiljan *et al.* (2015) argued that normal weight gain was achieved later in life, but the imbalance of minerals in the earthworm meal as its level of inclusion increases, prevent accelerated gains and efficiency of feed conversion to meat at the beginning of production. Das *et al.* (1990) also

reported that after replacing fish meal with earthworm meal, a non-significant effect in feed intake was observed during the early growth days. This could be due to the slow development of the crop which plays a very important role in softening of the feed.

In the last phase of production, the feed conversion ratio was also influenced by dietary treatments used in this study with birds in T4 had the best FCR. This is in agreement with the findings of Prayogi (2011) who reported that the use of 5% earthworm meal provides better feed conversion of quail. General, broiler producers aim to produce feed with a low intake, high conversion ratio which can then result in high body weight gains. Treatment 4 (5 % *E. foetida* meal inclusion) seemed to be a solution for such objective as birds in T4 had low feed intake, best feed conversion efficiency and increase body weight gains.

The inclusion of 3% of *E. foetida* meal in the diet of broilers provided superior results in terms of live weight, carcass dressing percentage and carcass yield as compared to the control group. This could be attributed to the high feed intake, the growth rate of the birds in T3 resulting in better muscle growth, hence had the higher performance of carcass characteristics. The current finding contradicts with the report by Khatun *et al.* (2005) who observed a linear increase of body weight with increasing silk worm pupae, but 8% inclusion of silkworm pupae had little effect on the live weight. Dressing percentage had a similar trend among treatments, finding in line with a report by Safa and Tazi (2014) who didn't find differences on dressing percentage of broiler chickens fed different inclusion levels of *Moringa oleifera* leaf meal. However, our findings are contrary to findings of Khan *et al.* (2015) who found that birds received a higher level of maggot meal had significantly better dressing percentage and carcass weight.

Gizzard size is of importance in digestion of birds because an increased gizzard size will not only increase the grinding action but also increase the incidence of gastric reflexes that serve to re-expose the digesta to pepsin in the proventriculus, enhance the mixing of digestive enzymes, improves digestion and also discourage microbial proliferation, which may cause disease or compete for nutrients (Ferket, 2000; Gabriel *et al.*, 2003; Wu and Ravindran, 2004). The finding from this study contradicts with the results by Maiti and Ahlwat (2011) who found that fresh commercial chicken gizzards weigh roughly 22g, as birds in T1 had higher gizzard weights comparable to 22g. The inclusion of *E. foetida* meal in diets influenced gizzard weights in this study, whereby birds in T2 (1% inclusion) had the highest gizzard weight and birds in T4 had the least weights. Gizzard weights of birds diminished with the increase inclusion level of *E. foetida* earthworm meal. The reduction of feed intake in birds that received high inclusion of *E. foetida* meal in this study could explain the increase in gizzard weight with decrease inclusion level of *E. foetida* meal in the diet. Probably the reduction of gizzard weight with high protein inclusion in diet, could be attributed to the lack of mechanical stimulation by the feed (Lv *et al.*, 2015) and this is consistent with the report by Swatson *et al.* (2002), who found that increase in inclusion level of protein source in a diet reduced gizzard weight.

Gizzard pH influences nutrient bioavailability (Pang and Applegate, 2007) and the intestinal microbiota (Hajati and Rezeial, 2010). Moreover, Pang and Applegate (2007) reported that it is important that gastrointestinal pH should be kept at a constant optimal level as small changes outside the normal pH ranges (1.2-4) to improve digestion. This was also substantiated by Svuhus (2015) who specified that the pH of gizzard content from broilers should range between 1.9 and 4.5, with an average of 3.5. A low gizzard pH improves pepsin activity and nitrogen retention and increases the solubility of the mineral fraction of the feed (Guinotte *et al.*, 1995) which then might favor its absorption (Mabelebele *et al.*, 2013). In the current study, the

gizzard pH values were higher than 4.5. The high pH in this study could be due to the low calcium carbonate content in the diets (Svihus *et al.*, 2013) which caused pH values for gizzard contents to range between 4 and 5. No dietary effects on gizzard pH were observed. Our findings are in line with results by Dahike *et al.* (2003), Morgan *et al.*, (2014), Qaisrani *et al.* (2014) who also found no dietary effect on gizzard pH. Current results differ from many studies that reported a dietary influence on gizzard pH (Bhuiyan *et al.*, 2013). Nevertheless, the current results contradict with the report by Damayanti *et al.* (2008) who found that a diet rich in protein causes a definitely higher acidity in the gizzard than the feed poorer in protein.

Earthworm meal inclusion in diets had a positive effect on intestine weight; the high intestinal weights were exhibited from birds in 1 to 3% inclusion levels of *E. foetida* earthworm meal diets. Our results are similar to the report by Rezaeipour *et al.* (2014) who found that *E. foetida* meal inclusion in diet improved intestinal weights of birds. Furthermore, Ignacio *et al.* (1993) indicated that increase intestine weight is caused by earthworm meal, which is a good source of glutamic acid (glutamate) for broilers. It causes bigger weight villi in duodenum and jejunum. Moreover, Murakami *et al.* (2007) reported that broilers fed a diet supplemented with 10 mg vitamin E along with 1% glutamine had longer villi and deeper crypts in the duodenum and deeper crypts in jejunum than broilers fed a diet containing vitamin E without glutamine. Furthermore, Soltan (2009) observed that broilers fed diet containing glutamine had bigger weight villi in duodenum and jejunum compared to the control group.

The bone strength of broilers is regarded as an important attribute for production since leg weakness, lameness and other bone abnormalities as a result of metabolic disorders are problems in broilers, leading to considerable production losses and having a negative effect on broiler welfare (Julian, 2005; Dibner *et al.*, 2007). Moreover, weak bones are an undesired trait

in broilers because results to 1.1% mortality of birds and 2.1 downgrading of carcasses during processing (Talaty *et al.*, 2009) which then result into economic losses. Breaking strength of tibia of birds in the current study was positively influenced by the inclusion of *E. foetida meal* in the diet. The breaking strength of tibia improved with the increase of inclusion level of *E. foetida meal* in the diet. This could be attributed to the fact that *E. foetida* is rich in protein which is needed for bone strength. Similarly to results obtained in the present study, Panda *et al.* (2006) found the inclusion of *Lactobacillus sporogenes* improved the bone breaking strength of birds. In contrast to our finding Yalcin *et al.* (1998) reported that no dietary effect on bone parameters fed different inclusion levels of protein.

Bone ash content is used to assess the bioavailability for calcium and phosphorous and (Shaw *et al.*, 2010) and the actual weight of tibia is best indicator of the amount of available calcium and phosphorous in the diet (Hall *et al.*, 2003). Birds in T2 had the highest bone ash percentage as compared to other dietary treatments. The increase of bone ash content of birds may be due to high mineral content but these minerals were not Ca and P because if it was these two also the bone breaking strength would have been improved, it may also have been accelerated by unnatural factors such as degree of ashing however there is no experimental evidence to support this. Birds in T2 showed high ash content but initially had low bone strength this means that the contradiction between these two bone characteristics may have been brought about by nutritional factors such as high concentration of minerals within the bones.

#### **4.4. Conclusions**

It can be concluded from the results obtained in this study that; fishmeal can be replaced at the level of 5 % by *E. foetida* earthworm meal for an improved weight gain of broilers without

adverse effects on the performance of the birds. Furthermore, it was revealed the effectiveness of *E. foetida* meal on carcass characteristics, digestive organs, and bone strength. Further investigation is necessary to determine the consequence of inclusion levels of *E. foetida* on carcass characteristics, meat yield, and quality.

#### 4.6. References

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## CHAPTER 5: The effect of *Eisenia foetida* meal as a fish meal replacement on carcass characteristics and physicochemical attributes of broiler meat

### Abstract

The effect of *Eisenia foetida* (earthworm) meal as a replacement for a fish meal on carcass characteristics and physicochemical attributes of broiler breast meat was investigated. A total of 180, un-sexed day old Cobb broiler chicks were randomly assigned to five dietary treatments (T) with three replicates as follows: 0% (T1); 1% (T2); 3% (T3); 5% (T4) and 10% (T5) earthworm meal inclusion. At day 35 of age, 75 broilers, 15 birds per treatment, five per replicate were slaughtered, carcasses were weighed, and the left side breast muscles were removed from each carcass for the measurements of meat pH and colour ( $L^*$ ,  $a^*$ ,  $b^*$ ,  $C^*$  and  $H^*$ ) at 1, 24 and 48 hours *post-mortem*. The right breast muscles were cooked to measure cooking loss and shear force values. Results revealed significant differences ( $P < 0.05$ ) of wing, thigh and drumstick yield and were significantly higher in T3 birds, whereas the breast yield was the highest in T3 birds. Liver and gizzard yield were significantly higher in birds in T5, while the least values were seen in birds in T3. Furthermore, there was no significant difference ( $P > 0.05$ ) observed with heart and spleen yield among the birds fed different treatments. The results indicated that dietary treatments effects ( $P < 0.05$ ) on the  $L^*$ ,  $a^*$ ,  $b^*$ ,  $C^*$  and  $H^*$  of breast muscles over time. The highest values for  $L^*$  and  $b^*$  were found in T4 birds while the highest values for  $a^*$  were found in T1 (control) birds. The pH values were affected ( $P < 0.05$ ) by the dietary treatments at 1 and 48 hours *post-mortem*; however, no dietary effect ( $P > 0.05$ ) was observed in pH values of breast meat at 24 hours *post-mortem*. However, at one-hour *post-mortem*, the highest pH values were observed in breast meat of birds in T3 (6.6) and T5 (6.6) while at 48-hour *post-mortem*, the highest values were seen in T1 (5.8) birds. Dietary treatments had a significant influence ( $P < 0.05$ ) on cooking loss; even though, there were no differences ( $P > 0.05$ ) observed on shear force values among the dietary treatments. The highest cooking loss

value was observed in T5 (12.0 %) and the lowest value in T3 (7.2 %). This study concludes that among the dietary treatments, birds fed a diet supplemented with a 3% inclusion level of *E. foetida* beneficially influenced the physic-chemical attributes and carcass characteristics of breast meat while visceral organs were better in a diet containing 10% *E. foetida* meal.

**Keywords:** *Eisenia foetida*, breast meat, Chroma intensity, dietary treatment, pH, Warner braztler shear force

## 5.1. Introduction

Chicken is one of the most consumed meats in the world (van de Poel *et al.*, 2013). The perceived health related issues attached to red meat by consumers have increased the demand for chicken meat. There is need to improve chicken production in order to meet the huge demand. The major challenge in poultry production is the availability of good quality feed at cheaper prices. Commercial poultry production is dependent on scarce and expensive conventional feed ingredients such as fish meal. Thus, this has resulted in the increase of the production cost of broilers (Tiroesele and Moreki, 2012).

Many alternative sources of protein as animal feed were explored including house fly maggots, termites, snails, grasshoppers, silk worm caterpillars and earthworms. Earthworms feed on organic waste, have high propagative rates, easy to process and stored. The quality of earthworm varies with and within the species, *Eisenia foetida* has been found to be better in nutrient composition than *Allolobora coligonosa*, *Pheretima gullemi*, *Eudrilus eugentae* and *Pertony excavate* (Ncobela and Chimonyo, 2015).

Hence, *Eisenia foetida* meal can be a solution to the limiting and high cost of protein source for chicken feed. Many authors reported that *E. foetida* is a good source of protein for chickens (Barcelo, 1988; Reinecke *et al.*, 1991; Istiqomah *et al.*, 2009; Tiroesele and Moreki, 2012; Gunya *et al.*, 2016). Its protein content ranges from 50 to 70% which makes it a better protein supplement than fish meal and meat meal (Zhenjum *et al.*, 1997). Naturally, free range chickens are known to feed on earthworms, therefore it can be easily used as a protein supplementation for chickens.

Zlender *et al.* (1995) reported that the meat quality of chickens depends on genetic factors (genetic line, sex) age at slaughter and environmental conditions specifically feeding. Diet composition and feed composition specifically protein source can affect the carcass characteristic and physicochemical composition of muscle tissue (Bogosavljevic-Boskoviv *et al.*, 2010) such as meat pH, color, cooking loss and tenderness.

Recently many researchers focused on the use of alternative sources of proteins such as edible insects for animal feed (Hwangbo *et al.*, 2009; Tao *et al.*, 2012; Pieterse *et al.*, 2014; Culler *et al.*, 2016; Mohanta *et al.*, 2016). Although the focus is on alternative sources of proteins, little has been done on how the quality of the end product is affected. To our knowledge, only one study reported on the effect of earthworm *Perionyx excavates* on physicochemical attributes of chicken meat (Vu *et al.*, 2009). There seems to be a lack of information in all the literature accessed on how *E. foetida* meal influences broiler meat quality. Therefore, considering its high protein content, there is a need to investigate the effect of *E. foetida* meal on carcass characteristics and physicochemical attributes of chicken meat. The objective of this study was to investigate the effect of inclusion levels of *E. foetida* meal as a replacement for a fish meal on carcass characteristics and meat quality.

## **5.2. Materials and Methods**

### **5.2.1. Preparation of *E. foetida* meal and dietary treatments**

The preparation of *E. foetida* meals is described in Chapter 4, Section 4.2.2.

### **5.2.2. Bird Management and dietary treatments**

Bird management and dietary treatments are also described in Chapter 4, Section 4.2.4 and 4.2.5, respectively.

### **5.2.3. Slaughter method**

The slaughter method of birds is described in Chapter 4, Section 4.28.

### **5.2.4. Carcass yield and digestive organ size**

After bleeding, scalding, plucking and washing, the feet, head, and neck were removed. Gizzards and visceral organs (liver, heart, and spleen) were removed by hand through an incision made around the vent and sternum. Visceral organs were weighed individually and expressed as a percentage of the live weight. Carcasses were dissected into drumsticks, wings, thighs, and breasts, then cuts were weighed and yield was calculated.

### **5.2.4. Meat pH**

The pH of the left breast muscle for each chicken was measured using a portable pH meter (Crison pH 25 CRISON Instruments SA) after calibration with pH 4, pH7 and pH9 standards solutions (CRISON Instrument, SA, and Spain). Measurements were done at approximately 1, 24 and 48 hours after slaughter and thereafter the chickens were refrigerated at 0-3 °C.

### **5.2.5. Meat colour**

The colour of the meat ( $L^*$  = Lightness,  $a^*$  = Redness and  $b^*$  = Yellowness) was determined on 75 left side breast muscles of individual chicken carcasses at one, 24 and 48 hours after slaughter using a colour guide 45/0 BYK-Gardener GmbH machine, with a 20 mm diameter measurement area and illuminant D65-day light, 10° standard observer. Three readings were taken by rotating the Colour Guide 90° between each measurement in order to obtain a representative average value of the color. The guide was calibrated each using the green standard before measurements.

#### **5.2.6. Cooking loss**

Seventy-five freshly cut right chicken breasts were individually sliced in 50 mm thick (maximum) pieces. Individual pieces were placed in thin-walled plastic bags which were placed in warm water-bath, with the bag opening extending above the water surface. Samples were cooked to a defined internal temperature of 85°C for 45 minutes. Thereafter, samples were removed from the water bath and cooled, removed from bags, blotted dry and weighed. Cooking loss was calculated using the following formula:

$$\text{Cooking loss} = [(\text{weight before cooking} - \text{weight after cooking}) \div \text{weight before cooking}] \times 100$$

#### **5.2.7. Tenderness**

Tenderness of the broiler right breast meat was determined using the Instron- Warner-Bratzler Shear Force (WBSF). Three sub-samples measuring 10 mm core diameter were cored parallel to the grain of the meat. The samples were sheared perpendicular to the fiber direction using a Warner Bratzler Shear device mounted on Instron (Model 3344), Universal Testing apparatus. The mean maximum load (N) was recorded.

#### **5.2.8. Statistical Analysis**

The effects of different inclusion levels *E. foetida* meal on carcass yield, meat color, pH, cooking loss and tenderness were analysed statistically using General Linear Model Procedure of SAS (2003). The statistical model used was:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where  $Y_{ij}$  = response variables = carcass yield, digestive organs size, meat color, meat pH, cooking loss, tenderness

$\mu$  = overall mean,

$T_i$  = effect of dietary treatments (T1, T2, T3, T4 and T5),

$e_{ij}$  = random error.

The differences among means were tested for significance ( $P < 0.05$ ) using LSD range test.

### **5.3. Results**

#### **5.3.1. Carcass yield**

The effect of inclusion of *E. foetida* meal on carcass yields is represented in Table 5.3.1. The wing percentage was higher for birds in T1, T3, and T4 but similar to birds in T2. Birds fed T1, T4 and T5 had the highest breast percentage but similar to birds fed T2. Thigh and drumstick percentage were best in T3 birds but they were significantly similar ( $P > 0.05$ ) to birds in T1 and T2 with the least thigh and drumstick seen in birds fed T5. The gizzard percentage was significantly higher in T5 (3.1%) birds and significantly lower in birds in T3 (2.6 %). The liver percentage was higher for T4 and T5 birds but was similar to T1 and T2, while the least percentages found in T3 birds.

**Table 5.3 1** Carcass yield of broilers fed with different inclusion levels of *Eisenia foetida*

Carcass characteristics	Dietary Treatments				
	T1	T2	T3	T4	T5
Breast (%)	27.6 <sup>b</sup> ±0.99	25.5 <sup>ab</sup> ±0.99	24.4 <sup>a</sup> ±1.03	26.3 <sup>b</sup> ±1.03	26.2 <sup>b</sup> ±0.99
Wing (%)	4.4 <sup>b</sup> ±0.16	4.0 <sup>ab</sup> ±0.16	4.4 <sup>b</sup> ±0.16	4.2 <sup>b</sup> ±.17	3.7 <sup>a</sup> ±0.16
Thigh (%)	5.5 <sup>ab</sup> ±0.25	5.1 <sup>ab</sup> ±0.2	5.6 <sup>b</sup> ±0.25	5.0 <sup>ab</sup> ±0.26	4.9 <sup>a</sup> ±0.26
Drumstick (%)	4.2 <sup>ab</sup> ±0.17	4.2 <sup>ab</sup> ±0.16	4.4 <sup>b</sup> ±0.16	3.8 <sup>a</sup> ±0.18	3.9 <sup>a</sup> ±0.18
Gizzard (%)	2.8 <sup>b</sup> ±0.11	2.8 <sup>b</sup> ±0.11	2.6 <sup>b</sup> ±0.11	2.7 <sup>b</sup> ±0.11.	3.1 <sup>c</sup> ±0.11
Liver (%)	1.9 <sup>ab</sup> ±0.09	1.9 <sup>ab</sup> ±0.09	1.7 <sup>a</sup> ±0.09	2.0 <sup>b</sup> ±0.09	2.0 <sup>b</sup> ±0.09
Heart (%)	0.5±0.02	0.5±0.02	0.5±0.02	0.5±0.02	0.5±0.02
Spleen (%)	0.1±0.01	0.1±0.01	0.1±0.01	0.1±0.01	0.1±0.01

<sup>a,b,c</sup>Mean within the same row that do not share a common superscript are significantly different (p<0.05)

T1 control; T2, T3, T4 and T5 contained graded levels of *E. foetida* 1%, 3%, 5% and 10% of DM intake, respectively.

%- percentage

Kg- kilograms

### 5.3.2. Color values

The color coordinates of the breast meat from birds fed different dietary inclusion levels of *E. foetida* meal at 1, 24 and 48 hours *post-mortem* are presented in Table 5.3.2. Dietary treatments had significant effects on the lightness of breast muscles, with birds fed T4 having the highest lightness values (45.6, 49.6, and 49.0, respectively) while T5 had the lowest values (42.9, 44.7, and 46.1, respectively). There were no significant ( $P > 0.05$ ) differences in the  $a^*$  values of the breast meat among the treatments at 1 and 24-hour *post-mortem*. However, the  $a^*$  values of the breast meat at 48 hours after slaughter were significantly different ( $P < 0.05$ ) across the dietary treatments, with birds in T1 having the highest value of 4.1 followed by T2 (4.9), T3 (4.3), and T4 (3.5). At 1 and 24 hours *post-mortem*, T4 indicated the highest significant value of yellowness value among other treatments. However, at 48 hours *post-mortem*, there was no significant difference ( $P > 0.05$ ) in the  $b^*$  values across the dietary treatments. The highest value of Chroma ( $C^*$ ) at 1-hour *post-mortem* was found in T2 (1.4), T3 (1.4) and T4 (1.4), while the lowest values were observed in T1 (1.3). At 24 hours *post-mortem*, the highest value of  $C^*$  was observed in T3 (1.4) and T4 (1.4) treatments compared to the others. The breast meat from T4 had the highest hue values at 1 and 24 hours *post-mortem* of 16.6, and 17.1, respectively. Dietary treatments had no significant ( $P > 0.05$ ) effects on the hue angle of the breast meat at 48-hour *post-mortem*.

**Table 5.3 2** Effect of *Eisenia foetida* meal as protein source on color coordinate

Color coordinates	Time	Dietary treatments				
		T1	T2	T3	T4	T5
<b>L* (Lightness)</b>						
	<b>1</b>	44.4 <sup>bc</sup> ±0.77	44.0 <sup>bc</sup> ±0.77	45.0 <sup>bc</sup> ±0.77	45.6 <sup>c</sup> ±0.77	42.9 <sup>ab</sup> ±0.77
	<b>24</b>	45.3 <sup>ab</sup> ±0.77	45.9 <sup>ab</sup> ±0.77	47.0 <sup>b</sup> ±0.77	49.6 <sup>c</sup> ±0.77	44.7 <sup>a</sup> ±0.77
	<b>48</b>	45.7 <sup>ab</sup> ±0.77	45.7 <sup>ab</sup> ±0.77	47.9 <sup>cd</sup> ±0.77	49.0 <sup>d</sup> ±0.77	46.1 <sup>bc</sup> ±0.77
<b>a* (Redness)</b>						
	<b>1</b>	3.7±0.68	2.7±0.68	3.2±0.68	2.8±0.68	3.4±0.68
	<b>24</b>	3.6±0.68	3.3±0.68	3.1±0.68	3.4±0.68	3.4±0.68
	<b>48</b>	4.7 <sup>ab</sup> ±0.68	4.9 <sup>ab</sup> ±0.68	4.0 <sup>ab</sup> ±0.68	3.5 <sup>a</sup> ±0.68	4.3 <sup>ab</sup> ±0.68
<b>b*(Yellowness)</b>						
	<b>1</b>	14.3 <sup>ab</sup> ±0.73	13.7 <sup>a</sup> ±0.73	14.9 <sup>ab</sup> ±0.73	16.1 <sup>b</sup> ±0.73	15.2 <sup>ab</sup> ±0.73
	<b>24</b>	14.1 <sup>ab</sup> ±0.73	13.3 <sup>a</sup> ±0.73	15.2 <sup>abc</sup> ±0.73	16.7 <sup>c</sup> ±0.73	13.8 <sup>ab</sup> ±0.73
	<b>48</b>	15.5 <sup>a</sup> ±0.773	16.4 <sup>a</sup> ±.73	16.5 <sup>a</sup> ±0.75	16.8 <sup>a</sup> ±0.73	15.2 <sup>a</sup> ±0.73
<b>C* (Chroma)</b>						
	<b>1</b>	1.3 <sup>ab</sup> ±0.03	1.4 <sup>b</sup> ±0.03	1.4 <sup>b</sup> ±0.03	1.4 <sup>b</sup> ±0.03	1.4 <sup>b</sup> ±0.03
	<b>24</b>	1.3 <sup>ab</sup> ±0.03	1.3 <sup>ab</sup> ±0.03	1.4 <sup>b</sup> ±0.03	1.4 <sup>b</sup> ±0.03	1.3 <sup>ab</sup> ±0.03
	<b>48</b>	1.3 <sup>ab</sup> ±0.03	1.2 <sup>ab</sup> ±0.03	1.3 <sup>b</sup> ±0.03	1.4 <sup>c</sup> ±0.03	1.3 <sup>abc</sup> ±0.03
<b>H* (Hue)</b>						
	<b>1</b>	14.8 <sup>ab</sup> ±0.86	14.0 <sup>a</sup> ±0.86	15.3 <sup>ab</sup> ±0.86	16.6 <sup>b</sup> ±0.86	15.6 <sup>ab</sup> ±0.86
	<b>24</b>	14.6 <sup>ab</sup> ±0.86	13.8 <sup>a</sup> ±0.86	15.6 <sup>abc</sup> ±0.86	17.1 <sup>c</sup> ±0.86	14.3 <sup>ab</sup> ±0.8
	<b>48</b>	16.3 <sup>a</sup> ±0.86	17.2 <sup>a</sup> ±0.86	17.0 <sup>a</sup> ±0.86	17.2 <sup>a</sup> ±0.86	15.8 <sup>a</sup> ±0.6

Means within the same row that do not share a common superscript are significantly different ( $p < 0.05$ ); T1 –control; T2, T3, T4 and T5 contained graded levels of *E. foetida* 1%, 3%, 5% and 10% of DM intake, respectively

### **5.3.3. pH values**

The pH values of the breast meat from birds fed different inclusion levels of *E. foetida* meal at one, 24 and 48 hours *post-mortem* are shown in Table 5.3.3. There were dietary effects on pH values of the breast meat at one and 48 hours *post-mortem*. At one hour *post-mortem*, the highest pH values were observed in T3 (6.6) and T5 (6.6) while the least was in T2 (6.2), whereas the highest pH value at 24 *post-mortem* was found in T1 (5.8) and the lowest in T4 (5.6). Dietary treatments had the same effects ( $P > 0.05$ ) on the pH of the breast meat at 24 hours *post-mortem*.

### **5.3.4. Cooking loss and Tenderness**

Cooking loss and shear force values of the breast meat are shown in Table 5.3.4. Dietary treatments had a significant influence on the cooking loss of breast meat. The highest cooking loss value was observed in T5 (12.0) while the lowest values were observed in T3 (7.2). There were no significant differences observed among dietary treatments on the shear force values of breast meat ( $P > 0.05$ ). However, T1 had the lowest tenderness values compared to the other treatments.

**Table 5.3 3** Effect of *Eisenia foetida* meal on pH values in broilers

Dietary treatment	pH values (unit)		
	1 hour	24 hours	48 hours
<b>T1</b>	6.4 <sup>b</sup> ±0.06	6.1±0.06	5.8 <sup>b</sup> ±0.06
<b>T2</b>	6.2 <sup>a</sup> ±0.06	6.2±0.06	5.7 <sup>ab</sup> ±0.06
<b>T3</b>	6.6 <sup>b</sup> ±0.06	6.2±0.06	5.7 <sup>ab</sup> ±0.06
<b>T4</b>	6.4 <sup>b</sup> ±0.06	6.2±0.06	5.6 <sup>a</sup> ±0.06
<b>T5</b>	6.6 <sup>b</sup> ±0.06	6.2±0.06	5.7 <sup>ab</sup> ±0.06

<sup>a,b</sup>Mean within the same column that do not share a common superscript are significantly different (p<0.05). T1

control; T2, T3, T4 and T5 contained graded levels of *E. foetida* 1%, 3%, 5% and 10% of DM intake, respectively

**Table 5.3 4** Cooking loss and Tenderness values of broilers fed different levels of *Eisenia foetida*

<b>Dietary Treatment</b>	<b>Cooking Loss (%)</b>	<b>Tenderness (N)</b>
<b>T1</b>	7.3 <sup>a</sup> ±1.45	5.5±0.71
<b>T2</b>	9.7 <sup>ab</sup> ±1.23	6.7±0.61
<b>T3</b>	7.2 <sup>a</sup> ±1.18	7.0±0.58
<b>T4</b>	8.8 <sup>a</sup> ±1.18	5.4±0.58
<b>T5</b>	12.0 <sup>b</sup> ±1.18	6.2±0.58

<sup>a,b</sup>Mean within the same column that do not share a common superscript are significantly different (p<0.05). T1 control, T2, T3, T4, and T5 contained graded levels of *E. foetida* 1%, 3%, 5% and 10% respectively

#### 5.4. Discussion

The current results are in accordance with those observed by Vu *et al.* (2009) who found that increasing worm level supplemented resulted in greater carcass yield. The increase in carcass cut yield in birds fed with *E. foetida* meal may be due to the increase in weight gain (Khan *et al.*, 2016). Breast yield was highest in control group (T1) although it was statistical similar with birds in T4 and T5. Our findings are contrary with of Rezaeipour *et al.* (2014) who reported that the breast yield increased with the increase of inclusion level of *E. foetida*. This deviation could be due to the different inclusion levels of *E. foetida* meal in diets.

The findings in this study are contrary to the results found by Ologhobo *et al.* (2014) who reported that the heavier the birds at slaughter the greater the dressing percentage and the higher the eviscerated yield than lighter birds. Gizzard yield in this study was influenced by dietary treatments and the high yield of gizzard that was observed in birds fed 10% inclusion level of *E. foetida* meal, could be due to the increasing frequency of contraction of this organ (Sobayo *et al.*, 2012) due to more protein inclusion. Current findings are in accordance with the report by Sobayo *et al.* (2012) and Malik *et al.* (2013) who observed that high protein inclusion diets increase gizzard yield. The decreased gizzard yield in a bird in T3 may be attributed to the partial hydrolysis and destruction of cell wall components of feed ingredients, thereby reducing the grinding action of gizzard and its relative weight (Jahanian and Golshadi, 2015). Moreover, birds that received 3% inclusion of *E. foetida* recorded the highest live weight and carcass weight, and the lower gizzard yield in this group could be due to the body weights because the organs have been expressed as the relative weights (Dehghani-Tafti and Jahanian, 2016).

The liver is a crucial organ in avian metabolism and is very sensitive to nutritional modifications (Azadmanesh and Jahanian, 2014). In accordance with a previous report (Djavinov *et al.*, 2005), the significant increase in the liver yield as increased protein inclusion.

Nevertheless, the liver percentage was higher for T4 and T5 birds but was statistically similar to birds in T1 and T2. The increase of the liver yield in the lower-protein diet (T2) may be attributed to the fact that liver is an organ that is used for lipogenesis in birds. This assertion suggests that the tendency for increased liver weight in the low-protein diet may be due to increased lipogenic activity (Aletor *et al.*, 2000).

The findings of the current study are in line with the results by Bahadori *et al.* (2015) and Resnawati (2002) who reported the non-significant effect of earthworm meal on the heart and spleen percentage of broilers. This may be attributed to the fact that the spleen and heart are associated with an immune function (Mushtaq *et al.*, 2014) which explains the current findings where there was no dietary effect on them.

The findings showed that the  $L^*$  values of the breast meat was influenced by dietary different inclusion levels of *E. foetida* worm meal. According to Woelfel *et al.* (2002) and Corzo *et al.* (2009), meat lightness is an important attribute to determine the incidence of paleness or the pale, soft and exudative (PSE) condition in broiler breast meat. Petracchi *et al.* (2004) reported that normal  $L^*$  values of breast meat range between 50 and 56, pale meat having values greater than 56 and darker meat having  $L^*$  value less than 50. In the current study,  $L^*$  values were less than 50 thus breast portions were darker and this could be associated with high pH, higher than 5.9 (Garcia *et al.*, 2010) recorded in this study. The darker meat has a high ultimate pH value ( $\text{pH} > 6.0$ ) accompanied by lower levels of glycogen, glucose, hexo phosphate, trio phosphate and lactate (Pethick *et al.*, 1995). The high pH recorded in this study may be due to antemortem stress experienced by the birds during the time of feed deprivation, handling, before and after slaughter.

The  $a^*$  values of the breast meat were influenced by the dietary treatment in this study. The  $a^*$  values at 1 and 24 hours post mortem were within the normal range according to Fletcher *et al.* (2000) which then, increased gradually at 48 hours after slaughter. The current finding is in line with the study of King and Whyte (2006) who stated that  $a^*$  value increases with storage time explain reflection of myoglobin concentration in meat (Mancini and Hunt, 2005). According to Jiang *et al.* (2014), a higher  $a^*$  value of meat is always favored by customers. Chroma and Hue angle were influenced by different inclusion levels of earthworm meal and birds in T4 had the highest values of both chroma and hue angle.

This study revealed that inclusion of earthworm in broiler feed had a positive influence on the color coordinates of breast meat. However, the findings of the current study differ from those found by Vu *et al.* (2000) who reported that there was no significant difference in breast color among birds fed different inclusion levels of earthworm meal. This deviation may be due to low inclusion levels of worm meal, and the type of worm *Perionyx excavates* used.

Van Lack and Lane (2000) asserted that normal breast meat pH for broilers is 5.7, although they were within the normal range as reported by Karaoglu *et al.* (2004) and Abdulla *et al.* (2016). However, in the current study, pH values were slightly higher than the normal values that Van Lack and Lane (2000) indicated. The higher pH values found in this study may be due to the presence of lysine in diets (Schroeder *et al.*, 1954). Increasing the level of lysine in the diet which is high in *E. foetida*, of broilers improves breast meat yield which then reduces drip loss during storage by increasing its pH (Berri *et al.*, 2007). Moreover, it could be protein intake, since protein intake increases meat pH by decreasing breast muscle glycogen content. Breast meat pH values significantly decreased gradually with time (from one to 48 hours *post-mortem*), due to glycolysis, lactic acid formation and a decrease of oxygen in muscle. This finding is in accordance with, Santos *et al.* (2004). The normal pH range found in this study

could be evidence of a good quality meat from birds fed with *E. foetida* meal. Dietary treatments had a significant effect on pH values observed in the current study at 1 and 24 hours *post-mortem*. The difference of pH values among dietary treatments in this study may be due to glycogen in breast meat manipulated by dietary composition (Rosenvold *et al.*, 2003). At one-hour *post-mortem* pH values were between 6.6 and 6.2 with T5 and T3 having the highest pH values. Our findings are similar to the results of Corzo *et al.* (2009) who observed no significant difference between pH values at 24 hours *post-mortem*.

The findings of this study showed that cooking loss values were influenced by different dietary inclusion levels of *E. foetida*. Birds in T3 can be considered to have a better meat quality than those in other dietary treatments since they had the least cooking loss. The low cooking loss in breast meat may be a result of the low loss of protein into the water during cooking (Abu *et al.*, 2015). The high cooking loss observed in birds fed with 10 % of *E. foetida* meal may be due to the low ability of meat from the broiler to hold on the water application of external force (Abu *et al.*, 2015). No differences were observed on tenderness of breast meat among birds fed different inclusion levels of *E. foetida* meal. Shear force values among all treatments were below 30 N, an indicator of very tender meat that is acceptable to consumers (Schilling *et al.*, 2003).

## **5.5. Conclusions**

This study showed that, birds fed with a diet supplemented with 3% inclusion level of *E. foetida* meal beneficially influenced carcass characteristics of breast meat while the visceral organs were better in the diet of 10% *E. foetida*. Thus, it is suggested that 3% inclusion level of *E. foetida* meal can replace fishmeal for broilers without deleterious effect on carcass characteristics and meat quality attributes. Further research is needed to determine the effect of

*E. foetida* meal on sensory scores of breast meat as to identify the acceptance of chicken meat by consumers fed with different inclusion levels.

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## **Chapter 6: The effect of *Eisenia foetida* meal as a replacement of fish meal on sensory scores of broiler meat**

### **Abstract**

The effect of *E. foetida* inclusion levels at 0% (T1), 1% (T2), 3% (T3), 5% (T4), and 10% (T5) on sensory scores of broiler meat was investigated. At day 35 of age 15 birds, three birds per treatment were randomly selected for the determination of sensory scores of the breast meat. Sensory attributes, which included aroma, initial juiciness, chicken flavor, sustained juiciness, metallic aftertaste, toughness, and residues, were evaluated by a semi-trained sensory panel. The findings of this study revealed that there were no significant differences ( $P > 0.05$ ) on chicken aroma and metallic aroma scores of breast meat across the dietary treatments. Moreover, the dietary effect ( $P < 0.05$ ) was observed on first bite scores of breast meat; where meat from T2 had the least score of 2.6, while meat from T5 had the highest score of 3.5. However, the breast meat from T5 was found to have the highest scores (3.9) for the initial juiciness and sustained juiciness ( $P < 0.05$ ), while the lowest scores (2.5) were observed in T2. Chicken and metallic flavour scores of breast meat were not influenced ( $P > 0.05$ ) by the dietary treatments, contrary to toughness scores ( $P < 0.05$ ). Breast meat from T5 exhibited the highest scores (3.5) of toughness, whereas the least scores (2.3) were from birds in T2. In conclusion, the *E. foetida* inclusion levels positively influenced sensory scores of broiler breast meat, where 10 % (T5) showed the best influence as compared to the other dietary treatment groups used in this study.

**Keywords:** Aroma, *Eisenia foetida*, flavor, juices, breast meat, tenderness

## 6.1. Introduction

Chicken producers are challenged to produce meat that is of good quality, palatable, accepted, and capable of providing adequate nutrition for humans. Other indicators used in assessing the quality of meat include; aroma, flavor, juiciness, tenderness, and taste. Furthermore, the ultimate pH, color, water holding capacity and tenderness are also indicators of meat quality evaluation. Despite the knowledge of the meat quality evaluation indicators, meat producers still have to consider the response of sensory attributes towards the product, because of sensory evaluation influences satisfaction, preference, and/or repurchase intent from consumers (Pieterse *et al.*, 2014). Thus, sensory evaluation is much important than instrumental meat quality measurements in determining the acceptability of meat.

Coetzee and Hoffman (2003) reported that sensory attributes of chicken meat are directly influenced by their diet. Currently, poultry producers are faced with a challenge of reducing feed costs, especially the cost of protein supplementation. The increased cost and limited supply of conventional protein sources, such as fishmeal, has resulted in research aimed at providing alternative non-conventional protein sources, which could be readily available and cheaper to sustain poultry production. One such alternative protein sources are *E. foetida* earthworm, which has a potential to be a protein source for broiler chickens that is comparable to that of fishmeal.

In poultry, diet provides direct influence on the sensory attributes of meat (Pieterse *et al.*, 2014) and information about consumer preferences and limitations for using earthworms as an animal feed is still lacking. However, researchers' interest in using edible insects and earthworms as an alternative source of protein for animal feed has grown due to their high nutrient composition, especially protein content. To date, it is unknown whether adding *E. foetida* meal to broiler

diets is also effective in maintaining sensory meat quality of breast meat as other protein sources such as house fly maggots, termites, snails, grasshoppers, silk worm caterpillars, and earthworms. Furthermore, to our knowledge, there are no studies that have been recorded to compare the effect of *E. foetida* meal inclusion levels on sensory scores of breast meat. Such information is crucial because the assessment of sensory parameters could provide information about the acceptance or rejection of the meat. Therefore, the objective of this study was to determine the effects of inclusion levels of *E. foetida* meal on sensory scores of broiler meat.

## **6.2. Materials and Methods**

### **6.2.1. Birds management and dietary treatment**

These sections were described in Chapter 4, Section 4.2.4 and 4.2.5, respectively.

### **6.2.2. Animal slaughter**

This section was described in Chapter 4, Section 4.2.8.

### **6.2.3. Sampling procedure**

Fifteen birds, three per treatment, were submitted for sensory analysis at the end of the feeding trial. The breast meat was harvested from the respective carcasses and skins were removed. Samples were vacuum packed, labeled and stored in a freezer at about -20°C for 24 hours before analysis.

### **6.2.3. Preparation of chicken**

Sensory evaluation was carried out in a Nutrition Laboratory, Department of Livestock Science, University of Fort Hare, with individual booths, which consisted of a counter top with three side walls. The booths were made in such a way that the panelists could not influence one

another. Ten panelists were trained for sensory characteristics of meat, by a pre-test before evaluation started. Training was undertaken in order to familiarise the assessors with attributes before beginning the process of meat evaluation. No information was given to the panelist regarding the treatments used. Prior to analysis, samples were defrosted in a refrigerator at 4°C for 12h. Each sample was cut into 10 mm thick slices, vacuum packed, labeled and cooked for 50 minutes at 85°C in a water bath with no spices or additives. Samples from each experimental group were served three times from each experimental group, and the serving order was randomised according to sample, replicate and panelist. Prepared samples of chicken breast were served on white glass plates, to each panelist in individual booths. Water was served in between treatments to neutralise the taste.

#### **6.2.5. Sensory analysis**

The five treatments were profiled using the quantitative description technique (Lawless and Heyman, 2010). A semi-trained panel consisting of ten sensory evaluation judges was used to assess meat. Each panelist received 1 cm<sup>3</sup> cubes of meat without skin from five treatments. The panel decided on the following sensory attributes: chicken aroma, juiciness, first bite, and sustainable impression of juiciness, chicken flavor, metallic aftertaste, toughness, and the amount of residues. The scale used for evaluation of sensory attributes ranging from the worst of each attribute (score one) to the best of each attribute (score five) as described in Table 6.2.1.

#### **6.2.6. Statistical analysis**

Data collected on sensory quality traits of different dietary treatments were analysed statistically using the software SAS (2003). Analysis of variance was performed by an NPAR1WAY Procedure of SAS (2003). The model included dietary treatments as a factor. Differences among means were deemed to be significant at  $P \leq 0.05$ .

**Table 6.2 1** Description of sensory attributes

<b>Sensory attributes</b>	<b>Rating</b>	<b>Description</b>
Chicken Aroma	1= Extremely bland 5 = Extremely intense	Intensity of the chicken meat
Metallic aroma	1= Extremely bland 5 = Extremely intense	Intensity of a metallic aroma
Initial impression of juiciness	1= Extremely dry 5 = Extremely juicy	The amount of fluid exude on the cut surface when pressed between thumb and forefinger
First bite	1= Extremely tough 5 = Extremely tender	The impression that you form on the first bite
Sustainable impression of juiciness	1= Extremely dry 5 = Extremely juicy	The impression of juiciness that you form as you start chewing
Chicken flavor	1= Extremely bland 5 = Extremely intense	Intensity of the chicken flavor
Metallic aftertaste	1= Extremely bland 5 = Extremely intense	Intensity of the metallic aftertaste
Toughness	1= Extremely tough 5 = Extremely tender	Toughness/ tenderness of the sample as measured by number of chews before the sample is ready to swallow
Residues	1= Abundant 5 = None	Amount of connective tissue remain in teeth after swallowing

### 6.3. Results

Effects of *E. foetida* earthworm meal dietary inclusion levels on the sensory scores of breast meat are presented in Table 6.3.1. The results show that the inclusion of *E. foetida* meal to diets had no significant effect ( $P > 0.05$ ) on chicken aroma and metallic aroma scores of breast meat. Significant dietary effect ( $P < 0.05$ ) was observed on initial impression of juiciness and sustainable juiciness scores of breast meat. Birds that were fed with 10% *E. foetida* diet (T5) had the highest initial and sustainable juiciness scores of 3.9 and 3.4 respectively, while the lowest score of 2.5 was found in birds fed with the 1% inclusion level (T2). Increasing the dietary inclusion level of *E. foetida* meal was found to increase the initial and sustainable juiciness scores of breast meat. The first bite scores were found to be significantly influenced ( $P < 0.05$ ) by dietary treatments, with breast meat from birds in T1 having the highest scores (3.5) and breast of birds in T2 with the least scores (2.6). There were no significant differences ( $P > 0.05$ ) among diets for chicken flavor and metallic flavor of breast meat. However, there were differences ( $P < 0.05$ ) found in toughness scores of breast meat among the dietary treatments where breast meat from birds fed T5 had the highest score (3.5) and birds in T2 had the least scores (2.3). Furthermore, toughness scores increased with increased inclusion levels of *E. foetida* meal in diets. Residues were significantly different across the dietary treatments, with birds in T2 having abundant connective tissues and birds from T5 having the least amount of connective tissues.

**Table 6.3.1** The effect of *Eisenia foetida* meal inclusion levels on sensory scores in broilers

Attributes	<u>Dietary Treatments</u>					P-value
	T1	T2	T3	T4	T5	
Chicken	2.6	2.8	3.1	3.0	3.2	0.21
Aroma						
Metallic	3.1	2.7	3.4	2.9	2.9	0.07
Aroma						
Initial	3.1	2.5	3.0	3.2	3.9	<0.001
juiciness						
First bite	3.5	2.6	3.2	3.3	3.4	0.003
Sustainable	3.1	2.5	2.9	3.1	3.4	0.002
juiciness						
Chicken flavor	2.6	2.6	3.1	2.8	2.9	0.241
Metallic flavor	2.7	2.6	2.7	2.7	2.6	0.991
Toughness	3.3	2.3	3.1	3.2	3.5	<0.001
Residues	3.8	3.0	3.4	3.8	3.9	0.003

T1 negative control, T2, T3, T4 and T5 contained graded levels of *E. foetida* 1%, 3%, 5% and 10% of DM intake, respectively. P value > 0.05 not significant and P- value < 0.05 significant different

#### 6.4. Discussion

Aroma is an important sensory attribute of meat because it gives the first impression of the product. It has been reported that the palatability of meat is a result of its aroma and taste (Jayasena *et al.*, 2013). However, aroma is sensed more easily than taste. This is also affirmed by Winiarska-Mieczan *et al.* (2016) who assert that; aroma is a more important sensory attribute of meat. Thus, Ba *et al.* (2012) also indicate that aroma is crucial in consumption, acceptance, and preference by consumers. Furthermore, the current findings are in accordance with the findings observed by Silva *et al.* (2015); Pieterse *et al.* (2014) and Hashim *et al.* (2013) who found that there is no dietary effect on the aroma of meat fed. Even though there was no significance in the aroma, the scores of chicken aroma increased with the increased levels of *E. foetida* meal. This may be due to a high fatty acid composition that was found by Gunya *et al.* (2016) in *E. foetida* worms. The increased fatty acid composition was also reported by Ramarathnam *et al.* (1993) to be directly influencing meat aroma.

In addition, metallic aroma is a sensory attribute that is found by consumers to be undesirable because of its accumulation in meat that has a negative impact on consumer acceptance and is a threat to its marketability (Mahmoud and Buettner, 2016). The presence of polyunsaturated fatty acids in meat leads to peroxidation (Winiarska-Mieczan *et al.*, 2016) which then results into the metallic aroma. However, there was no dietary influence observed on metallic aroma scores of breast meat in this study. This differs with the findings by Pieterse *et al.* (2014) who observed the differences on metallic aroma scores of breast meat of chickens fed a diet that contains *Musca domestica*, larvae meal, fish meal, or soya bean meal. This deviation may be due to the different sources of protein used in these studies, and also different types and nature of ingredients insects, fish, and plant, which have different PUFA, in the study of Pieterse *et al.* (2014).

Meat juiciness is also an important attribute of meat because it influences the texture of meat. The juiciness of meat depends on the quality and composition of fat (Muchenje *et al.* 2009). According to Teye *et al.* (2015), juiciness is composed of two organoleptic components including the impression of wetness during first chewing produced by the rapid release of meat fluid and sustainable juiciness largely due to the stimulatory effect of fat on salivation. Both initial impression and sustainable impression of the juiciness of breast meat were significantly influenced by dietary treatment. The juiciness scores of breast meat were improved as the inclusion levels of *E. foetida* increased. Moreover, birds that received 10% level of *E. foetida* diet scored the highest initial and sustainable juiciness values, while birds on diet containing 1% of *E. foetida* meal scored the lowest values. These findings are in contrast with Alson *et al.* (2010) who found out that juiciness improved with a decrease in dietary protein level. The high scores of juiciness in birds fed T5 diet may be attributed to the high-fat accumulation in breast muscle, and increased levels of fatty acids in the breast meat of broilers fed with *E. foetida* diet compared to other diets (Overland *et al.*, 2005). The current findings differ from reports by Winiarska- Mieczan *et al.* (2016); Pieterse *et al.* (2014) and Williams and Damron (1998) who did not find any dietary effect on the juiciness of breast meat. This deviation may be caused by the different sources of protein included in diets of the chickens used in these studies.

Moreover, improvement of tenderness in meat is mainly caused by changes in the structure of connective tissue solubilised by heat, while heat-denaturation of myofibrillar protein causes meat toughness (Barbanti and Pasquini, 2005). Tenderness is one of the important sensory attributes that cannot be compromised because it also influences the acceptance of meat. Tenderness of meat in sensory evaluation is determined by scores of first bite and toughness (American Meat Science Association, 2015). Furthermore, it can be influenced by several production factors such as genetic makeup, feeding system, and processing techniques that

include chilling, marinating, and cooking (Adam and Abugroun, 2015). In this study, tenderness scores were influenced by the dietary treatments used. The inclusion of *E. foetida* in broiler diet improved the first bite score of breast meat. Birds with higher *E. foetida* inclusion level (10%) produced meat, which was more acceptable to consumers. Increased *E. foetida* inclusion levels may have resulted in improvements of collagen and myofibrillar solubility, in turn improving tenderness, due to improvement in the juiciness of the meat, recorded in this study. The current findings contradict the results by Alson *et al.* (2010) and Teye *et al.* (2006) who found out that, low-protein level in the diet increases the tenderness of the meat.

Flavour comprises mainly of taste and aroma and it influences consumer purchasing behavior and preferences (Dinesh *et al.*, 2013). It has been reported that flavor is affected by ante- and post-mortem factors, including breed, aging, cooking method and diet. Poultry flavor could be improved by manipulating the diet (Fanatico *et al.*, 2007) positively or negatively (Jayasena *et al.*, 2013). Perez- Alvarez *et al.* (2010) reported that the type of diet offered to bird contributes to the flavor of the meat. Nevertheless, the current results found no dietary effect on the chicken flavour. Current findings are in line with the report of Lyon *et al.* (2004) but contradict the findings by William and Damron (1998). This deviation may be due to the different meat portions used in these studies since the sensory attributes were measured on the thigh in the study of William and Damron (1998). Many of flavor components of poultry are fat soluble and would be more abundant in the thigh meat than breast meat, which was used in this study.

## **6.5. Conclusions**

In conclusion, *E. foetida* meal inclusion levels influenced sensory scores of breast meat. Sensory scores were improved as the inclusion level of *E. foetida* meal increased in the diet. Among the dietary treatments used in the current study, birds that were fed a diet that was

supplemented with 10% inclusion level of *E. foetida* meal beneficially influenced the sensory scores of breast meat. Thus, it is suggested that 10% inclusion level of *E. foetida* meal could be used to replace fishmeal for broiler diets without deleterious effects on sensory scores.

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## CHAPTER 7 General Discussion and Recommendations

### 7.1. General Discussion

The demand for poultry products especially broiler meat has increased over the years. The broiler industry is however, met with high costs of production, especially due to high feed costs mostly protein source. This has increased the significance and potential nonconventional, less expensive and readily available ingredients for use in animal feed. Hence, there is a growing interest in edible insects as animal feed worldwide, due to their potential as a source of protein. One such alternative is the *E. foetida* earthworm. Therefore, the objective of this study was to determine the inclusion level of *E. foetida* meal as a source of protein at which supplementation will affect growth performance, carcass characteristics, digestive organs, bone strength, meat yield, and quality of meat and sensory scores of broiler meat.

In Chapter 3 of this thesis, the nutrient composition and fatty acid profiles of freeze-dried and oven-dried earthworm *E. foetida* were determined. The earthworms were oven or freeze-dried, then analysed for nutrient composition (protein, fat, and minerals) according to the AOAC method and fatty acids using gas chromatography. Protein content was higher in freeze-dried earthworms while fat content of earthworms was not influenced by drying methods used. These findings are consistent with the report by Bou-Maroun *et al.* (2013) who found that protein content of *E. foetida* decreased during oven drying as compared to freeze drying. The low protein content in oven-dried earthworms was expected due to protein denaturation. Most minerals (macro and micro) of *E. foetida* were significantly different except for calcium with freeze-dried *E. foetida* having the predominant minerals than oven-dried earthworms. During freeze drying, external influences are minimum and oxidizable substances are well protected

under vacuum conditions (Khairna *et al.*, 2013) hence more minerals were retained in freeze-dried earthworms. Oven drying reduced the nutrient value of *E. foetida* by direct heating through inducing biochemical and nutritional variation in earthworm composition. Most of the essential fatty acids were significantly higher in oven-dried *E. foetida* than in freeze-dried earthworms. This can be related to the lower dehydration produced in oven drying (Moradi *et al.*, 2009). No significant differences were observed on Margaric, Vaccenic, Arachidic, Tricosanoic, omega-3, SFA, MUFA, n-3, PUFA: SFA and PUFA/MUFA between oven-dried and freeze-dried samples. It became evident that *E. foetida* is excellent in nutrient composition and fatty acid profile for chickens.

The objective of Chapter 4 was to determine the effect of inclusion level of *E. foetida* meal on growth performance, digestive organs and bone strength of broilers. A total of 180 day old Cobb broilers were randomly allocated to five dietary treatments as follows: T1 (0%), T2 (1%), T3 (3%), T4 (5%) and T5 (10%) earthworm meal inclusion. Each treatment was presented by three replicates with 12 birds per replicate. The trial lasted for 35 days, which was divided into three growth phases including; starter (1-21d), grower (22-28d) and post finisher (29-35 d). At day 35 of age, 75 broilers, 15 birds per treatment, and five per replicate were slaughtered and used to measure carcass characteristics, digestive organs, and bone strength. The results revealed that birds fed a diet with no *E. foetida* meal inclusion had lower values of BW, ADFI, ADG and FCR than birds fed with *E. foetida* meal inclusion levels. At the first phase of production, only FCR was influenced by dietary treatments with birds in T1 (control) having the best FCR comparable to bird in diets with *E. foetida* meal. However, birds in T4 had the highest BWG, ADG but exhibited low feed intake. Generally, broiler producers are interested in producing feed with a low intake, high conversion ratio which can then result in high body weight gains. Treatment 4 (5 % *E. foetida* meal inclusion) seemed to be a solution for such aim

as birds in T4 had low feed intake, best feed conversion efficiency and increase body weight gains. ADFI of birds decreased with increase in the inclusion level of earthworm meal in the diet. This may be due to dull feed when more earthworms are included in diets as birds are very sensitive to the color of diet (Khan *et al.* 2016). The highest ADFI was observed in birds in diet containing 3% inclusion (T3) level of earthworm meal.

Gizzard weights of birds decreased with the increase of inclusion level of *E. foetida* earthworm meal. The reduction of feed intake in birds that received high inclusion of *E. foetida* meal in this study could explain the increase in gizzard weight with decrease inclusion level of *E. foetida* meal in the diet. This was also supported by the findings of Swatson *et al.* (2002) who found that increase in inclusion level of protein source in a diet reduced gizzard weight. Svihus (2015) reported that the pH of gizzard content from broilers should range between 1.9 and 4.5, with an average of 3.5. Nevertheless, in the current study, the gizzard pH was above 4.5 and this could be due to the low calcium carbonate content in the diets (Svihus *et al.*, 2013) which caused pH values for gizzard contents to increase. Furthermore, no dietary effect was observed on gizzard pH, in this study. Earthworm meal inclusion in diets had a positive effect on intestine weight; the high intestinal weights were exhibited from birds in 1 to 3% inclusion levels of *E. foetida* earthworm meal diets. The increase in intestine weight in birds in T3 could be due to the higher feed intake of birds which then increase the surface area of intestines for feed absorption. Our findings are in line with the report by Rezaeipour *et al.* (2014) who found that earthworm inclusion in broiler diet increase intestine weights of birds.

In this study, the bone breaking strength of bird's tibia was positively influenced by the inclusion of *E. foetida meal* in the diet, whereby it improved with the increase in inclusion levels. This could be attributed to the fact that protein is a building block of bones, hence the

high inclusion of *E. foetida* improved bone breaking strength of birds. Our findings are consistent with the results by Panda *et al.* (2006) who found that the increase of protein source in birds' diet improved their bone breaking strength. In addition, bone ash content used to assess the bioavailability for calcium and phosphorous and (Shaw *et al.*, 2010) and the actual weight of tibia are the most used indicators of amounts of available calcium and phosphorous in the diet (Hall *et al.*, 2003). Birds in T2 had the highest bone ash percentage as compared to other dietary treatments. The increase of bone ash content of birds may be due to high mineral content but these minerals were not calcium and phosphorous.

In Chapter 5, the effect of inclusion level of *E. foetida* meal on carcass characteristics, meat yield and physicochemical attributes of broiler meat was investigated. At day 35 of age, 75 broilers, 15 birds per treatment, five per replicate were slaughtered, carcasses were weighed, and the left side breast muscles were removed from each carcass for the measurements of meat pH and colour ( $L^*$ ,  $a^*$ ,  $b^*$ ,  $C^*$  and  $H^*$ ) at 1, 24 and 48 hours *post-mortem*. The right breast muscles were cooked to measure cooking loss and shear force values. Birds that were subjected to 3% inclusion level of *E. foetida* meal provided better results in terms of live weight, carcass dressing percentage and carcass yield as compared to the control group and other treatments. Birds in T3 were heaviest; they had the highest body weight, while the least body weight was recorded for birds in T5. Birds that received 3% inclusion level also had an improved meat yield. This could be attributed to the high feed intake of the birds in T3 resulting in better muscle growth, and hence higher performance of meat production. Moreover, wing, thigh and drumstick yield were also significantly higher in T3 birds. Nevertheless, liver and gizzard yields were significantly higher in birds in T5, while the least values were seen in birds in T3.

The pH of meat is highly dependent on the amount of glycogen present in the muscle (Allen *et al.*, 1997). In the current study, pH values were slightly higher than the normal values.

Muchenje *et al.* (2008) reported pre-slaughter stress increases meat pH. When birds are stressed, there is a rapid release of cortisol through adrenal cortex which is stimulated by the HPA axis resulting in reduced muscle glycogen. The high pH observed resulted into darker meat portions. The L\* values were less than 50 thus breast portions were dark. Dietary treatments had a significant influence on cooking loss; even though, there were no differences observed in shear force values among the dietary treatments. Birds in T3 can be considered to have a better meat quality than those in other dietary treatments since they had the least cooking loss.

Sensory evaluation is a very reliable tool that is used to determine acceptance of an end product. In addition, the assessment of sensory attributes could provide additional information about how people perceive food products (Moussaou and Verala, 2010). Therefore, in Chapter 6, the effects of inclusion levels of *E. foetida* meal on the sensory scores of broilers were investigated. Sensory attributes, which included aroma, initial juiciness, chicken flavor, sustained juiciness, metallic aftertaste, toughness, and residues, were evaluated by a semi-trained sensory panel. Even though the high inclusion of *E. foetida* meal (10%) in broiler diets didn't have excellent results on physicochemical attributes of breast meat, but the sensory attributes of breast meat were improved as inclusion level of *E. foetida* increased. Birds in high inclusion level (10%) of *E. foetida* meal diet improved sensory scores of broiler meat more than birds in the diet with no *E. foetida* meal. No dietary effects were observed on chicken aroma and metallic aroma in this study. Current findings are in line with reports by Silva *et al.* (2015) and Pieterse *et al.* (2014) who didn't observe dietary influence on aroma score of broilers. The initial and sustained impression of juiciness scores of breast meat were improved as the inclusion levels of *E. foetida* increased. Meat juiciness is a very important sensory attribute of chicken meat that cannot be compromised since it directly influences the texture of meat. The

inclusion of *E. foetida* in broiler diet improved the first bite and toughness score of breast meat. Birds with higher *E. foetida* inclusion level (10%) produced meat, which was more tender comparable to other dietary treatments. Both metallic and chicken flavors were not affected by dietary treatments used in this study, in agreement with the findings by Lyon *et al.* (2004).

## **7.2. Conclusions**

It was, therefore, concluded in the current study that *E. foetida* can be considered as an alternative source of protein as it seems to be particularly suitable in broiler nutrition. It became evident that *E. foetida* is excellent in nutrient composition and fatty acid profile for chickens. This was evident as improved growth performance, carcass characteristics, meat yield, meat quality and sensory scores of broiler meat from broilers subjected to 3%, 5% and 10 % *E. foetida* inclusion levels than those in the control treatment (0% *E. foetida*).

## **7.3. Recommendations**

Birds supplemented with *E. foetida* levels had significantly higher performance, with regards to growth, carcass characteristics, meat yield, meat quality and sensory scores of broilers. The utilisation of earthworm could be cost effective to broiler farmers as they are readily available and cheap to a rise. These effects need to be validated with further research:

1. The diet treatment and the meat fatty acid profile should be conducted
2. Bone mineralisation analysis should be conducted as they influence the performance of broilers.

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## Appendix 1 Ethical Clearance Certificate



**University of Fort Hare**

*Together in Excellence*

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

Certificate Reference Number: MAS021SGUN01

Project title: **The potential of *Eisenia foetida* as a protein source for village and broiler chickens and its effect on growth performance and chicken meat characteristics**

Nature of Project: PHD

Principal Researcher: Busisiwe Gunya

Supervisor: *Co-supervisor:*

Prof PJ Masika

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:

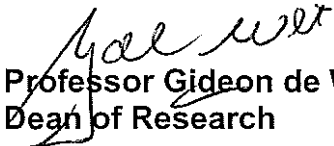
Note: The UREC is aware of the provisions of s71 of the National Health Act 61 of 2003 and that matters pertaining to obtaining the Minister's consent are under discussion and remain unresolved. Nonetheless, as was decided at a meeting of the National Health Research Ethics Committee and stakeholders 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
  - Any unethical principal or practices are revealed or suspected o
  - Relevant information has been withheld or misrepresented
  - Regulatory changes of whatsoever nature so require
  - 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

10

June

2015

## Appendix 2 Fatty Acids

Common Name	Abbreviation
Lauric	C12:0
Tridecoic	C13:0
Myristic	C14:0
Pentadecylic	C15:0
Pentadecenoic	C15:1c10
Palmitic	C16:0
Palmitoleic	C16:1c9
Margaric	C17:0
Heptadecenoic	C17:1c10
Stearic acid	C18:0
Elaidic	C18:1t9
Oleic	C18:1c9
Vaccenic	C18:1c7
Linoleic	C18:2c9,12(n-6)
Arachic	C20:0
$\alpha$ -Linolenic	C18:3c9,12,15(n-3)
Eicosadienoic	C20:2c11,14(n-6)
Eicosatrienoic	C20:2c11,14(n-3)
Arachidonic	C20:0
Tricosanoic	C23:0
Eicosopentaenoic	C20:5c5,8,11,14,17(n-3)
Lignoceric	C24:0
Docosapentaenoic	C22:5c7,10,13,16,19(n-3)

**Appendix 3** Sensory evaluation form

Characteristics	Rating	T1			T2			T3			T4			T5		
		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
<b>Chicken Aroma</b> (Intensity of chicken meat)	1.Extremely bland 2.Very bland 3.Fairly intense 4. Very intense 5.Extremely intense															
<b>Metallic aroma</b> (Intensity of metallic aroma)	1.Extremely bland 2.Very bland 3.Fairly intense 4. Very intense 5.Extremely intense															
<b>Initial impression of Juiciness</b> (The amount of fluid exude on the cut surface when	1.Extremely dry 2.Very dry 3.Fairly juicy															

pressed between thumbs and forefingers)	4. Very juicy 5. Extremely juicy														
<b>First bite</b> (The impression that you form on the first bite)	1. Extremely tough 2. Very tough 3. Fairly tender 4. Very tender 5. Extremely tender														
<b>Chicken flavor</b> (intensity of chicken flavor)	1. Extremely bland 2. Very bland 3. Fairly intense 4. Very intense 5. Extremely intense														
<b>Metallic aftertaste</b> (Intensity of metallic after taste)	1. Extremely bland 2. Very bland 3. Fairly														

	intense 4. Very intense 5. Extremely intense															
<b>Toughness</b> (Toughness/tenderness of the sample as measured by number of chews before the sample is ready to swallow)	1. Extremely tough 2. Very tough 3. Fairly tender 4. Very tender 5. Extremely tender															
<b>Amount of connective tissue</b> (Amount of connective tissue remain in teeth after swallowing)	1. Extremely abundant 2. Very abundant 3. Moderate 4. Trace 5. None															

