EFFECT OF USING RED-CLAW CRAYFISH (*Cherax quadricarinatus*) MEAL, BLOOD MEAL OR BLACK SOLDIER FLY (*Hermetia illucens*) LARVAE MEAL AS REPLACEMENT FOR FISHMEAL ON THE GROWTH AND SURVIVAL RATE OF THE THREE SPOTTED TILAPIA (*Oreochromis andersonii*)

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LUSAKA

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DECLARATION

I, **Chilufya Kabati**, to the best of my knowledge, declare that this research has not been done or presented for a degree in this or any other university. The results shown herein are a true reflection of what was obtained from the study.

Signature……………………………..… Date……………………………

CERTIFICATE OF APPROVAL

This dissertation of Chilufya Kabati is approved as fulfilling the requirements or partial fulfilment of the requirements for the award of the Degree of Master of Science in Animal Nutrition by the University of Zambia.

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ABSTRACT

Aquaculture is fastest food producing sector in the world with most of the production coming from small and medium income countries, Unfortunately, the development of aquaculture in Africa has largely lagged behind Asian countries due to the high cost and scarcity of fishmeal, which is an important animal protein source used in the formulation of good quality fish feed. The objective of this study was to compare the effect of red-claw crayfish meal, bloodmeal or black soldier fly larvae meal as replacement of fishmeal on growth performance, survival and whole-body composition of *Oreochromis andersonii*. Four diets containing 35% crude protein were provided at 5% live body weight to four groups of *O. andersonii* fingerlings of 2.92 ± 0.1g replicated 3 times for 90 days; Diet 1 (control) contained fishmeal (FM), Diet 2 contained Red-claw crayfish meal (CFM), Diet 3 contained bloodmeal (BM), Diet 4 contained black soldier fly larvae meal (BSFM). The final mean weight varied from 20.87±0.16g(BM) to 23.39±0.25g(FM) and Weight gain varied from 17.92±0.16g(BM) to 20.50±0.27g(FM) respectively, FM was significantly different ($P < 0.05$) from CFM and BM but was not significantly different ($P > 0.05$) from BSFM with respect to final mean weight and weight gain of fish respectively. Specific growth rate (SGR) ranged from 2.17±0.02g(BM) to 2.33±0.02g(FM), in this category FM did not differ significantly ($P > 0.05$) from BSFM and it was significantly higher in these two treatments, however, FM was significantly different ($P < 0.05$) from CFM and BM. Feed conversion ratio (FCR) showed significant differences ($P < 0.05$) across the treatments with BSFM(1.24±0.01) performing significantly better than other treatments followed by FM, BM(2.06±0.02) was significantly lower. The results showed that the best growth performance and good feed utilization was noticed in FM and BSFM and the least was observed in BM. Furthermore, survival rate of fish ranged from 89±0.05% (BM) to 98±0.02% (CFM) and it did not differ significantly ($P > 0.05$) across the treatments. Crude protein ranged from 40.47±0.52% (CFM) to 56.19±0.72% (BM), fish fed FM was significantly different ($P < 0.05$) from those fed CFM and BSFM but it was not significantly different ($P > 0.05$) from that fed BM. Crude fat varied from 27.33±0.21% (FM) to 40.41±0.41% (BSFM), significant differences ($P < 0.05$) were observed in crude fat across all the treatments. The Ash content varied from 15.44±0.35%(CFM) to 16.55±0.23%(FM), significant differences ($P < 0.05$) in ash content were observed between fish fed FM and those fed CFM and BM but fish fed FM was not significantly different ($P > 0.05$) from that fed BSFM, however among the treatments, fish fed CFM and BM were not significantly different ($P > 0.05$) from each other. The results of this study shows that black soldier fly larvae meal (BSFM) can be used to replace fishmeal (FM) in fish feeds without having any adverse effect on growth performance, survival rate and whole-body composition of *Oreochromis andersonii*.

Key words: Red-claw Crayfish, Black soldier fly larvae, blood meal, Fishmeal, *Oreochromis andersonii.*

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LIST OF ABBREVIATIONS

UN	:	United Nations
FAO	:	Food and Agriculture Organization
AOAC	:	Association of Official Analytical Chemist
CRD	:	Completely Randomise Design
IW	:	Initial Weight
FW	:	Final Weight
WG	:	Weight Gain
SGR	:	Specific Growth Rate
FI	:	Feed Intake
FCR	:	Feed Conversion Ratio
BSFM	:	Black Soldier Fly Larvae
DO	:	Dissolved Oxygen
pH	:	Potential Hydrogen
ANOVA	:	Analysis of Variance
ANCOVA	:	Analysis of Covariance
SPSS	:	Statistically Package for Social Science
MFL	:	Ministry of Fisheries and Livestock
DoF	:	Department of Fisheries
UNZA	:	University of Zambia
CARS	:	Chilanga Aquaculture Research Station

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Aquaculture is the farming of aquatic organisms including fish, molluscs, crustaceans and some plants. Farming implies some form of human intervention in the rearing process such as changes in regular stocking rates, supplementary feeding or protection of farmed species from natural predators in order to enhance productivity (FAO, 2011). Aquaculture is the fastest food producing sector in the world (FAO, 2018), with most of the production coming from small and medium income countries (Belton *et al*., 2018). According to Gephart (2015), fish provides more than a billion people with most of their daily animal protein needs and in regions with the greatest number of resource-poor and vulnerable people, fish is often the primary source of animal protein foods. Thus, aquaculture contributes significantly to rural household food security, income generation and employment creation (Genschick *et al*., 2018).

Unfortunately, the development of aquaculture in Africa has largely lagged behind Asian countries with the continent producing only 2.3% of global output and when Egypt; Africa's biggest producer is excluded, the output for sub-Saharan countries is less than 1% (FAO, 2016: Genschick *et al*., 2017). According to Genschick *et al*., (2017) and Kobayashi *et al*., (2015); despite recent improvements in some African countries, projections for the continent predict a decline in fish production and consumption for up to 2030, a scenario that will negatively impact on food and nutrition security on the continent.

Zambia's total annual fish production for 2021 was 159, 043 metric tonnes, which showed an increase of 12% from 2020's overall production of 140, 613 metric tonnes. This increase was registered from aquaculture production, with a 28% increase of 63, 418 metric tonnes in 2021 and 94, 924 metric tonnes from capture fisheries (DoF, 2022). Fish feed forms the single largest cost in aquaculture production, it is estimated that about 30–70% of the total operational cost of an average fish farm is due to fish feed (El-Sayed, 2004). The cost of fish feed is driven by the need to include proteins in the formulated feeds and fishmeal has been the principal source of proteins in formulated fish feeds globally. However, inclusion of fishmeal in fish feeds is increasingly becoming unsustainable due to the growing aquaculture industry and reduced supply of the requisite fish-based products (Kefi *et al.*, 2013). To reduce costs of fish feeds, there are substantial interests in substituting the expensive and competitive fishmeal with other animal protein sources including processing by-products or insects that are not used as human foods (Makkar *et al*., 2014; Makinde, 2015).

1.2 Statement of the problem

Non-availability of good quality feeds is the main limitation to the development of smallholder aquaculture in many developing countries including Zambia (Genschick *et al*., 2017). This is because most of the commercially available fish feeds are based on imported feed ingredients like fishmeal, mineral and vitamin premixes that make these feeds too expensive, especially for small scale fish farmers. The other set back is the competition for fishmeal by other sectors like the pig and poultry industries (Tacon, 2012). As a result, small scale farmers resort to using available feed resources that are not nutritionally balanced (Mzengereza *et al*., 2014;

Obirikorang *et al*., 2015). This results in feed wastage and pollution of fish ponds leading to heavy economic losses for the farmer.

To alleviate these losses and for fish to grow to reach market size within the shortest possible time, there is need to provide a high quality and nutritionally balanced feed (Gabriel *et al*., 2007) and the feed should be based on cheap, locally available and easily produced animal protein sources. Unfortunately, use of locally available plant-based protein sources is unlikely to replace high value feed ingredients such as fishmeal (Makinde, 2015). For now, fishmeal remains important in the diets of fish, However, the concerns about the sustainability of these ingredients and their growing prices on the global market incentivize a search for alternative sources of these ingredients. hence the use of alternative sources of high-quality proteins (Nasopoulou, 2012; Schalekamp, 2016). Many scientists (Tacon *et al*., 1983; Fagbenro, 1993; Aladetohun and Sogbesan, 2013) have reported the possible use of some alternative animal protein feedstuffs to fishmeal.

The high cost and scarcity of fishmeal in formulated feeds has led to the use of other protein source such as earthworms, insects, snails, maggots, and frogs (Tacon *et al*., 1983; Lim and Dommy, 1989). Therefore, this research is aimed at formulating fish feeds using red-claw crayfish meal, blood meal or black soldier fly larvae meal as replacement for fish meal in the diets of *O. andersonii*.

1.3 Justification of the study

According to the United Nations (UN, 2019), the world population is projected to increase to 9.7 billion people by 2050. An increase in human population entails more people that will need more food for survival while socio-economic advancements often lead to increased demand for high quality processed foods mostly of animal origin (Herrero *et al*., 2009). Increased world population also means increased conversion of natural or farming lands for human settlements (Delgado *et al*., 1999). Thus, the increase in demand for foods of animal origin must be produced under declining land and water resources. Among the major agricultural sectors earmarked to meet the increase in demand for food and animal protein needs is aquaculture (FAO, 2016). This is because aquaculture is the only sector that has the capacity to produce a kilogram of animal protein from 1-2 kilograms of feed unlike other livestock species like poultry and pigs that require to 2-3 kilograms and 3-6 kilograms of feed to produce a kilogram of meat respectively (FAO, 2016). Efforts to meet fish demand from capture fisheries is hampered by over fishing and other climate change related factors and this leaves aquaculture as the only viable means to satisfy the need for animal protein requirements for the growing human population (Genschick *et al*., 2018).

The animal protein sources (i.e., bloodmeal, black soldier fly larvae meal and crayfish meal) to be used in this study are readily available, easy to produce and process. These protein sources especially black soldier fly larvae meal and crayfish meal are an excellent source of protein, micro nutrients and has complete amino acid profile; that is highly digestible (Badmus *et al*, 2012). According to Wangari (2008) and Nakayama *et al*. (2010), the red-claw crayfish (*Cherax quadricarinatus*) has established a large and rapidly growing population in the Kafue River and Siavonga on the Zambia shore of Lake Kariba. Recently *C. quadricanatus* has established itself in the Barotse floodplains on the upper Zambezi River (Nunes *et al*., 2016) According to the study by Mukuka (2019), it was established that as much as 24 percent of the total catch of fishers around Mongu is crayfish, which is normally discarded due to cultural beliefs. Blood meal a by-product after slaughter of cattle, is readily available in abattoirs and offer alternative cheaper protein source to fishmeal. According to Agbebi, *et al*. (2009) much can be achieved by intensifying research on blood meal inclusion in fish feed for better balanced diets. Black soldier fly larvae are easy to produce and process (Aniebo and Owen 2010; Anene *et al.,* 2013), and relatively cheaper than other sources of animal protein (Ajani *et al.*, 2004).

1.4 Objective

1.4.1 Overall objective

To compare the effect of red-claw crayfish meal, bloodmeal or black soldier fly larvae meal as replacement of fishmeal on the growth, survival and whole-body composition of *Oreochromis andersonii* fish.

1.4.2 Specific objectives

i. To compare the dietary effect of replacing fishmeal with red-claw crayfish, blood or black soldier fly larvae meals on the growth of *Oreochromis andersonii*.

ii. To compare the dietary effect of replacing fishmeal with red-claw crayfish, blood or black soldier fly larvae meals on the survival of *Oreochromis andersonii.*

iii. To compare the dietary effect of replacing fishmeal with red-claw crayfish, blood or black soldier fly larvae meals on the body composition of *Oreochromis andersonii*.

1.5 Statistical hypothesis

1.5.1 Null hypotheses

i. Replacing fishmeal with red-claw crayfish, bloodmeal or black soldier fly larvae meal had no effect on the growth performance of *Oreochromis andersonii*.

ii. Replacing fishmeal with red-claw crayfish, bloodmeal or black soldier fly larvae had no effect on the survival of *Oreochromis andersonii*.

iii. Replacing fishmeal with red-claw crayfish, bloodmeal or black soldier fly larvae had no effect on the whole-body composition of *Oreochromis andersonii*.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Introduction to aquaculture

Aquaculture is the practice of artificially rearing aquatic organisms in an enclosed water body for economical or recreational exploitation. Reared organisms may include plant species such as Phyto-planktons, seaweeds and lilies and/or animal species including different types of fish, crocodiles and oysters (FAO, 2016). Production systems range from extensive type of management that mostly involve subsistence production of fish in water ponds by rural small-scale farmers to intensive large-scale commercial production that involve rearing of monoculture or polyculture aquatic species (FAO, 2016). In between the two extremes, there is a diverse class of medium and emerging large-scale commercial enterprises with a wide range of sophistication in investments (Hernandez *et al*., 2017). Most intensive large-scale commercial production systems are done in floating cages installed on large natural water bodies such as lakes and rivers (Brummet *et al*., 2008; Genschick *et al*., 2018). They may also be done on especially constructed ponds or water gateways that are usually equipped with automated facilities for easy management and feeding of fish. The most common aquaculture fish species reared in many parts of the world including Soutrhern Africa are the breams that include 3 spotted tilapia (*Oreochromis andersonii*), longfin tilapia (*Oreochromis macrochir*) and the red breast tilapia (*Coptodon rendalli*) (Genschick *et al*., 2017). Other farmed fishes are the common carp (*Cyprinus carpio*), Nile tilapia (*Oreochromis niloticus*).

2.2 Importance of aquaculture

Fish is mostly produced to meet animal protein needs for food and nutrition security in addition to income generation, employment creation and recreation; thereby contributing to socio-economic welbeing of local communities (Belton *et al*., 2018). Since, early 2000s, the aquaculture sector has continued to grow at 8.2 percent every year that is faster than any other major food producing sector in the world (FAO, 2016). With the ever-increasing world human population and increasingly demanding consumers, fish production from aquaculture is bound to be the only sustainable means for supplying proteins to meet this increasing demand (Makinde, 2015; Genschick *et al*., 2017). This is supported by the stagnation in recent years in fish production from capture fisheries throughout the world (FAO, 2016). Production from capture fisheries is mostly limited by overfishing and variations in weather patterns as influenced by climate change and global warming. The average per capita fish consumption in the African region is currently about 11 kilograms, which is far less than the average 20 kilograms and there is likely to be more demand as more and more nations attain improved socio-economic development (Farahiyah *et al*., 2015). It is therefore imperative that if fish consumption has gone up, there must be a matched increase in investments for intensification of aquaculture systems and commercialization of the fish value chain (Kassam and Dorward, 2017; Genscheick *et al*., 2018). It must also be appreciated that fish is a special food in that it is low in saturated fats, carbohydrates and cholesterol but very rich in proteins, vitamins, minerals and polyunsaturated or omega 3 fatty acids (Belton *et al*., 2018). This means fish posses all key nutrients that are not only required for better health of individuals, but also nations at large. Many studies have also made a case for aquaculture in alleviating poverty among resource poor rural and urban dwellers (Hernandez *et al*., 2018). This has mostly been demonstrated through income, employment and fish consumption pathways (Genschick *et al*., 2018). Aquaculture is also responsible for increasing the supply of fish and in stabilizing prices for both rural and urban consumers (Belton *et al*., 2018).

2.3 Limitations to development of aquaculture

Despite recent breakthroughs in the development of aquaculture in many parts of the world, many African countries have generally lagged behind Asian countries in aquaculture production (Belton *et al*., 2018; FAO, 2018). At present, the continent only produces about 2.3 percent of global production (FAO, 2016), with more than 50 percent of this production coming from Egypt alone (Genschick *et al*., 2018). The main limitation to increased productivity of Aquaculture in Sub-Saharan Africa is non-availability and supply of good quality fish feeds (Obirikorang *et al*., 2015; Admasu *et al*., 2017) that make up to more than 70 percent of total variable cost of production. This is because commercial fish feed production relies on imported feedstuffs that are subject to global supply and price fluctuations thus; making fish feeding very expensive especially for rural small-scale farmers (Admasu *et al.*, 2017). The manufacturing of fish feeds also presents a special challenge to traditional feed processing due to the aquatic nature of the medium in which the feed has to be delivered and consumed by the usually small sized fish. Sometimes the feed disintegrates and leaks the nutrients into the pond before being consumed by the intended fish (Ighwela *et al*., 2013).

To minimize costs and improve effeciency in the production of fish on small scale farms, current efforts are aimed at finding locally produced replacements for imported feed ingredients (Belghit *et al*., 2018). It must be noted that there is no single feed ingrediet that can be consumed to meet all nutritional requirements for best growth and performance of fish, which calls for use of formulated diets consisting of several feed ingredients (Admasu *et al*., 2017). The choice of feed ingredients also greatly affects environmental sustainability. Dietary protein is the main nutrient limiting aquaculture production as protein requirments for fish are generally higher than that of telesterial animals such as poultry and pigs. This means to meet protein requirements in aquaculture, some level of animal protein sources must be included in the diet. As indicated earlier, use of animal protein sources such as fishmeal and other animal processing by-products is hindered by limited supply and high cost (Devic *et al*., 2013). Use of some feed ingrediets is also limited by competition in that they may also be required as human foods. This calls for the exploitation of non human food animal products such as worms, maggots and termites (Devic *et al*., 2013; Makkar *et al*., 2014). Although insects may not currently be produced in sufficient amounts for commercial exploitation in fish feeds, they hold great promise for the future of the aquaculture industry (Makkar *et al*., 2014; Tran *et al*., 2015).

2.4 Animal protein sources in diets of fish

Fish feed forms the single largest cost in semi-intensive and intensive fish culture operations. It is estimated that about 30–70 percent of the total operational cost of an average fish farm is due to fish feed (El-Sayed, 2004). Fish meal is currently one of the major sources of animal protein used in diets for fish (Sá *et al*., 2013). Much of the raw materials used in the production of fish meal comes from pelagic fishing (Tacon and Metian, 2008). Fish meal is obtained by cooking, pressing, drying and milling fresh raw fish or fish trimmings (IFFOO, 2006), there are several types of fish meal on the market depending on the source of fish or fishery by-product used and on the processing technology involved. However, on the market the fishmeal available in abundance is processed from trimmings and offals left over when fish is processed for human consumption (IFFOO, 2006). Protein is the most expensive ingredient in the diet, and its quality is a very important nutritional aspect in aquaculture (El-Sayed, 2004) and according to El-Sayed (1998), he stated that many nutritionists have extensively studied other animal protein sources for partial or total fish meal replacements in tilapia feeds.

2.4.1 Crayfish meal in diets of fish

Red-claw crayfish (*Cherax quadricarinatus*) is a freshwater crustacean belonging to the family Parastacidae (Austin *et al*., 2010; FAO, 2011). Feral populations have been established in most tropical and subtropical freshwater systems across the globe from its native range of the tropical river catchments of Northern Australia and South Eastern Papua New Guinea (Belle *et al*., 2011). They are physically robust and capable of adapting their feeding habits to their new habitats (Ruscoe, 2002).

Figure 1: Male red-claw crayfish (Cherax quadricarinatus)
Source: Australian Fish Photos, Seafood Photography and Information

Red-claw Crayfish (*Cherax quadricarinatus*) accounts for 40-70 percent of crude protein and they are an excellent source of micro nutrients which can impart nutritive value to foods (Ibironke *et al.*, 2014). El-sayed (1998), concluded that crayfish meal can totally replace fishmeal in practical diets for Nile tilapia (*Oreochromis niloticus*) without any significant differences on growth rate, feed conversion ratio (FCR) and protein efficiency ratio (PER) and Similar results were reported by Davies *et al*. (1989) on Mozambique tilapia (*Oreochromis mossambicus*). According to a study by Ojewole and Annah (2006) and Asafa *et al.* (2012) it was observed that total replacement of fishmeal with crayfish meal promoted growth of broiler chicken and had not adverse effects on its health, at the same time it reduced the cost of feed. Chemical composition and nutritive value of Crayfish revealed that Cray fish is a good source of protein and minerals with low content of fat and cholesterol, however, have high levels of lead and zinc which if not handled properly can be poisonous in a long run despite this limitation, Crayfish can provide a pollution free source of protein, perfectly safe since all the poisonous material absorbed by Crayfish is stored in the shell, which itself shed six times a year (Zaglol and Eltadawy, 2009).

2.4.2 Bloodmeal in diets of fish

Blood meal which is an animal waste product and readily available in abattoirs is an alternative cheaper protein source. The development of a more sustainable aquaculture feed production will depend on identifying and establishing alternative feedstuffs to fishmeal (Olukayode and Emmanuel, 2012). Kirimi *et al.* (2016) observed that bloodmeal had no effect on the survival rate of fish at 50 percent inclusion level, he further went on to state that it can substitute fishmeal without any effect on growth rate and it would be economically sustainable to use locally available bloodmeal to raise Nile tilapia in semi-intensive system. The results of Kirimi *et al.* (2016) were in agreement with Agbebi *et al*. (2009) who reported that fishmeal can be replaced completely (100 percent) by bloodmeal with no adverse effect on growth, survival and feed conversion of *Clarias gariepinus* juvenile. Aladetohun and Sogbesan (2013) also recorded no mortality and concluded that bloodmeal can perform very well as a feedstuff at inclusion rate of 100 percent and can replace fishmeal in Tilapia diet with no adverse effect on growth and survival of *Oreochromis niloticus* fingerlings. Davies *et al*. (1989) reported that bloodmeal could effectively replace up to 75 percent of the fishmeal in the diets fed to Mozambique tilapia (*Oreochromis mossambicus*) fry over in seven-week period. The major limitation to the use of Bloodmeal in fish feed for some fish species such as the *Oreochromis species* is that when the inclusion rate exceeds 50%, it is deleterious to the animal and the growth rate is reduced significantly (Njieassam, 2016).

2.4.3 Black soldier fly larvae meal in diets of fish

Black soldier fly (*Hermatia illucens*) larvae are capable of turning decaying organic matters into nutritionally rich food of animal origin (Hussein *et al.,* 2017). They are easy to produce and process (Aniebo and Owen 2010; Anene *et al.,* 2013) and relatively cheaper than other sources of animal protein (Ajani *et al*, 2004). black soldier fly larvae can grow on various organic wastes such as pig dung (Pastor *et al.,* 2011), cattle blood and wheat bran (Aniebo and Owen 2010), poultry manure (Hwangbo *et al.,* 2009), cattle gut and rumen content (Anene *et al.,* 2013). The larvae are fairly nutritious with relatively high amounts of micronutrients (Anene *et al.,* 2013; Hussein *et al.,* 2017). There are many systems used for culturing black soldier fly larvae but the system commonly used consists of two (2) chambers: the top and the bottom, the top chamber of the unit is the larvae culturing chamber which must be open at the top for access to flies for laying their eggs on the exposed substrate. The base of the chamber is screened with 3 mm galvanized wire mesh net to allow dropping of the larvae. The bottom chamber, with a covered outlet, is the collection chamber where the larvae are collected. Partially or completely replacing costly fishmeal in fish diets with the black soldier fly larvae meal showed that it had no effect on the growth and survival of Tilapia fish (Idowu and Afolayan, 2013; Sogbesan, 2014). Ajani *et al.* (2004) reported that replacement of fishmeal up to 100 percent black soldier fly larvae meal in diets of Nile tilapia (*Oreochromis niloticus)* improved growth and also led to a reduction in the cost of production by 18 to 28 percent. Aniebo *et al.* (2009) in the study of replacing fishmeal with black soldier fly larvae meal concluded that larvae meal is a viable alternative protein source to fish meal in catfish diets, this is especially so in developing countries where fishmeal is imported at an exorbitant cost. The only limitation of black soldier fly larvae meal is during production, black soldier fly larvae require a warm environment during its biodegradation because of this, during cold seasons it becomes a bit difficult to produce large quantities of black soldier fly larvae.

Table 1: Essential amino acid profile in fish ingredients

Amino acids	Fishmeal	Crayfish meal	Bloodmeal	BSF meal
Arginine	6.28	3.82	3.75	5.8
Histidine	2.44	1.45	5.14	3.9
Isoleucine	4.39	2.66	1.04	3.06
Leucine	7.27	4.48	10.82	6.35
Lysine	7.51	6.5	7.45	6.04
Methionine	2.81	2.31	2.32	2.28
Phenylalanine	3.85	4.35	8.47	3.96
Threonine	4.16	2.31	3.76	2.03
Tryptophan	1.32	0.97	1.33	0.98
Valine	4.91	5.3	7.48	3.61

Source: N.R.C, (1993); El-sayed (1998); Ojewole and Annah, (2006)

Table 2: Crude protein, crude fat, crude fiber and ash levels in fish ingredients

Protein source	Crude protein	Crude fat	Crude fiber	Ash
Crayfish meal	65.27	4-5	2.23	8-11
Bloodmeal	70.39	1.07	-	3.32
Black soldier fly larvae meal	56.3	22.3	7.5	6.25

Source: N.R.C, (1993); El-sayed (1998); Ojewole and Annah, (2006)

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area

This research was conducted at Chilanga Aquaculture Research Station (15°34’05” South and 28°16’12” East) in Chilanga district which is 20 kilometres south of Lusaka Central Business District the capital city of Zambia.

3.2 Experimental facilities

Five (5) concrete experimental ponds of size (6m x 2m x 1.5m) and twelve (12) plastic fish tanks of size (0.85m x 0.60m x 0.45m) were used for the experiment. The experimental ponds were rehabilitated and the plastic fish tanks were fitted with bird nets on top to prevent the fish from being eaten by fish eating birds.

Figure 2: Experimental ponds and fitting of bed nets on the plastic fish tanks

3.3 Feed ingredients acquisition and preparation

Fishmeal, bloodmeal, maize bran and minerals and vitamin premixes were purchased from Livestock Services Cooperative Limited in Lusaka, red-claw crayfish was sourced from Kafue Gorge in Kafue district, while cassava flour and vegetable oil were purchased from City Market in Lusaka district. Black soldier fly larvae meal was cultured in Luanshya district at Mr. John Davies's Farm by using the following culturing method:

i. The eggs of the black-soldier fly laid by mature flies were collected and put in trays made from cutting empty 20 litres containers filled with organic matter (brewery wastes and cow manure) then covered with a net to build a colony.

ii. After the colony was established, a stack of cut-out carton box (30 cm x 1cm) was placed in each tray for the flies to lay eggs on after the eggs are laid, the stacks of cut-out carton box were transferred to the feeding container.

iii. After growing for 10 plus days, the newly hatched larvae were transferred to the vertical stacking system (stacks of trays) to grow further.

iv. Then finally transferred to a large container after 20 plus days, where the larvae grew further until there were harvested.

Figure 3: Culturing of black soldier fly larvae

Figure 4: Grinding of Black soldier fly larvae into a meal

3.4 Experimental fish

A total number of 300 sex reversed *O. andersonii* fingerlings of 2.92g ± 0.1g was procured from Chilanga Aquaculture Research Station (CARS) and stocked in a pond (6m x 2m x 1.5m) for 7 days for acclimatization, then 180 sex reversed fish were randomly selected from the total population (first each tank was assigned a random number then 5 fish were randomly picked from the total population using a scope net and placed in the tank whose random number was drawn. The process was repeated until all the tanks had 15 fish each), weighed and randomly assigned to 12 plastic fish tanks (0.95m x 0.65m x 0.45m) placed in 4 experimental ponds at a density of 15 fish per tank.

3.5 Feed Formulation

Proximate Analysis (according to the procedure of AOAC, 2000) was conducted for all the ingredients and feed formulation was based on the results of Proximate Analysis. The results of the analysis for each ingredient were inputted into WinFeed feed formulation software (version 2.8) then respective ingredients were selected from the feed store of the software to come up with the quantity to use for each diet at 35% crude protein level. These are the ingredients that were used in the formulation of diets; fishmeal, bloodmeal, crayfish meal, black soldier fly larvae meal, maize bran, cassava flour, vegetable oil, minerals and vitamin premixes. All the ingredients were weighed appropriately, mixed thoroughly then pelleted using a meat mincer No. 22 with 2 mm screen. 2 mm sized pellets were formulated and then kept in air-tight labelled bags and stored at room temperature, when it was time to feed the fish, the pellets were reduced to grumbles so that the feed can be utilized by small sized fish.

Figure 5: Mixing of formulated fish feed

Figure 6: Pellets of formulated fish feed

3.6 Experimental treatments (diets)

Four (4) diets of the same crude protein (35%) were formulated; Diet 1 contained fishmeal (FM) which was the control diet, Diet 2 contained crayfish meal (CFM), Diet 3 contained bloodmeal (BM), Diet 4 contained black soldier fly larvae meal (BSFM).

Table 3: Fish feed formulation for each treatment in percentages (%)

Ingredients	FM	CFM	BM	BSFM
Fish meal	49	-	-	-
Crayfish meal	-	46.5	-	-
Blood meal	-	-	41.8	-
Black Soldier Fly Larvae meal	-	-	-	58.5
Maize bran	46	47.5	51.8	35.5
Cassava flour	2.5	2.5	2.5	2.5
Palm oil	1	1	1	1
Salt	1	1	1	1
Vitamin	0.8	0.75	1	0.75
Minerals	0.8	0.75	1	0.75
Crude protein level (%)	35	35	35	35

Note: FM = Fishmeal, CFM = Crayfish meal, BM = Bloodmeal & BSFM = Black Soldier Fly Larvae meal

3.7 Experimental design

A Complete Randomized Design (CRD) was used in this experiment. 180 *Oreochromis andersonii* fish were randomly selected from the previously acclimated population and fasted for a day. Thereafter, the fish were weighed and randomly assigned to the 12 plastic fish tanks (0.95m x 0.65m x 0.45m) placed in 4 concrete experimental ponds at a stocking rate of 15 fish per 0.62 m^2. Four (4) treatments (diets) were randomly assigned to the fish tanks containing experimental fish using random numbers and each treatment was replicated 3 times.

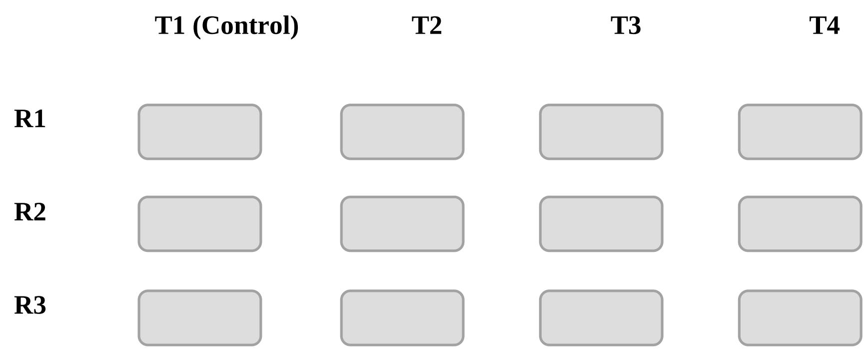

Figure 7: Layout of the experiment

3.8 Feeding and Sampling of fish

The feeding of fish was done at 5 percent live body weight 2 times a day, at 09:00 AM and 3:00 PM (NRC, 1993). The fish were weighed every 2 weeks of the experimental period and the feed quantities readjusted according to the change in live body weight. Due to the destructive nature of sampling, 5 fish, which is 30 percent sampling intensity was used as per recommendations by Fisher and Belle (1993), At the end of the study all the fish were weighed and measured. The water quality parameters such as Dissolved oxygen, Temperature, pH was monitored using multi-parameter water quality meter, model Horiba Hachit Kit U – 10 and Ammonia levels were monitored 2 times a week using a BIOZYM Multi-Test 3 in 1 test kit, model BT301.

3.9 Determination of growth, survival and feed utilisation parameters

Weight measurement, survival rate and feed utilisation of the fish were computed at the end of the experiment and the following; growth, survival rate and feed utilization parameters were determined according to the formulae outlined by Sogbesan and Ugwumba (2008) and Iheanacho *et al*. (2017).

a. **Growth parameters**

i. **Weight gain (WG)**

Weight gain (WG. g) = *final weight (g) – initial weight (g)*

ii. **Specific growth rate (% per day)**

SGR = ($In\ Final\ weight - In\ Initial$ weight)/ $Time$ ($days$) × 100

Where: *In* is the Natural logarithm

b. **Survival Rate (SR)**

Survival rate (%) = ((Number of fish at the end of the experiment)/ Number of fish at the start of the experiment) x 100

c. **Feed utilization**

Feed conversion ratio (FCR) = *feed intake (g)/ weight gain (g)*

d. **Body composition**

At the beginning of the experiment, 100 fish samples were cleaned then stored in the freezer and at the end of the experiment, the same procedure was repeated for the remaining fish in each treatment and the fish samples were dried in an oven set at 150^0C then homogenised and grinded to powder before being subjected to proximate analysis to determine the whole-body composition of the fish. Proximate Analysis for the crude protein, crude fat, dry matter, moisture, ash, and Metabolizable energy were conducted according to the procedures as described by the Association of Official Analytical Chemists (AOAC, 2000).

3.10 Data analysis

The data that was collected (initial weight of fish, final weight of fish, feed given and fish mortality) was used to calculate the following parameters: body weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR) and survival rate (SR); these parameters were analysed using Analysis of Variance (ANOVA) to determine presence of significant ($P < 0.05$) differences between treatment means. The significantly different means were separated using Duncan's test of significance at 0.05 significance level. Analysis of Covariance (ANCOVA) was also used to determine if the initial weight had an effect on the final weight. All statistical analyses were conducted using the statistical software package SPSS version 16. Microsoft excel was used in the production of figures and graphs.

The predictor variable was diet and the response variables were growth, survival and Feed utilization. Completely randomize design (CRD) statistical model which was used:

$$Yij=\mu+ \tau_i +\varepsilon ij$$

Where:

Yij = j^{th} growth, survival, feed utilization parameters or body composition parameters of the i^{th} diet

μ = is the overall mean

τ_i = effect of i^{th} diet

εij = are random and independent error associated with the j^{th} growth, survival, feed utilization parameters or body composition parameters on i^{th} diet.

CHAPTER FOUR

4.0. RESULTS

The nutrient composition obtained using proximate analysis for the ingredients (protein sources) and the formulated diets are shown in table 4 and 5.

Table 4: Nutrient composition of ingredients (protein sources) before proximate analysis

Nutrients composition	**FM**	**CFM**	**BM**	**BSFM**
Dry matter %	90.18	85.13	92.42	91.94
Moisture %	9.82	14.87	7.58	8.06
Crude protein %	64.55	52.83	75.83	57.97
Crude fibre %	0.75	0.26	0.46	10.86
Crude fat %	8.63	2.93	0.30	10.96
Ash %	9.37	6.23	1.32	15.76
Calcium %	10.42	1.28	1.07	3.49
Phosphorus %	1.39	0.12	0.22	0.84
Sodium chloride %	0.36	1.61	0.97	1.15
Metabolizable energy (kcal/kg)	3,019.13	2,938.67	3,267.70	3,266.33

Note: FM = Fishmeal, CFM = Crayfish meal, BM = Bloodmeal and BSFM = Black Soldier Fly Larvae meal

Table 5: Nutrient composition of the formulated diets after proximate analysis

Nutrients composition	FM	CFM	BM	BSFM
Dry matter %	90.40	87.90	91.37	91.25
Moisture %	9.60	12.31	8.63	8.75
Crude protein %	37.10	35.21	37.60	37.10
Crude fibre %	2.50	2.20	2.61	7.90
Crude fat %	9.20	6.50	5.90	10.30
Ash %	6.22	4.80	2.74	4.97
Calcium %	5.40	1.10	0.92	2.30
Phosphorus %	3.20	2.60	2.97	2.50
Sodium chloride %	0.51	1.03	0.81	0.88
Metabolizable energy (kcal/kg)	3,431.60	3,112.96	3,273.30	3,190.60

Note: FM = Fishmeal, CFM = Crayfish meal, BM = Bloodmeal, BSFM = Black Soldier Fly Larvae meal

4.1 Water quality parameters

Water quality parameters ranged from 20.87^{0}C to 21^{0}C for temperature, 6.60±0.16mg/L to 6.72±0.16 mg/L for dissolved oxygen (D.O.), 7.61±0.20 to 7.68±0.17 for pH, and 0.09±0.00 mg/L to 0.10±0.00 mg/L for ammonia. No significant differences ($p > 0.05$) were observed in all the selected water quality parameters (see table 6).

Table 6: Water quality parameters during the rearing of *O. andersonii* fed experimental diets

Water parameter	**FM**	**CFM**	**BM**	**BSFM**
Temperature (^oC)	20.87±0.67	20.89±0.69	20.93±0.70	21.00±0.69
DO (mg/L)	6.72±0.16	6.65±0.14	6.60±0.16	6.63±0.17
pH (log (mg/L)	7.66±0.21	7.61±0.20	7.63±0.19	7.68±0.17
Ammonia (N, mg/L)	0.09±0.00	0.10±0.00	0.10±0.00	0.09±0.00

Note: FM = Fishmeal, CFM = Crayfish meal, BM = Bloodmeal, BSFM = Black Soldier Fly Larvae meal

4.2 Growth performance and feed utilization

Growth performance and feed utilization parameters of *Oreochromis andersonii* fish fed diets containing different animal protein sources over the study period are shown in table 7.

Table 7: Growth, feed utilization and survival parameters of *O. andersonii*

Parameters	Diets			
	FM	**CFM**	**BM**	**BSFM**
IW (g)	2.86 ± 0.03^{a}	2.99 ± 0.03^{b}	2.96 ± 0.03^{b}	2.85 ± 0.02^{a}
FW (g)	23.39 ± 0.25^{c}	21.60 ± 0.21^{b}	20.87 ± 0.16^{a}	22.93 ± 0.22^{c}
WG (g)	20.50 ± 0.27^{c}	18.59 ± 0.21^{b}	17.92 ± 0.16^{a}	20.07 ± 0.22^{c}
SGR (%/day)	2.33 ± 0.02^{b}	2.20 ± 0.02^{a}	2.17 ± 0.02^{a}	2.32 ± 0.01^{b}
FI (g)	28.58 ± 0.02^{c}	27.77 ± 0.01^{b}	36.89 ± 0.01^{d}	24.71 ± 0.01^{a}
FCR	1.40 ± 0.02^{b}	1.50 ± 0.02^{c}	2.06 ± 0.02^{d}	1.24 ± 0.01^{a}
SR (%)	93 ± 0.04^{a}	98 ± 0.02^{a}	89 ± 0.05^{a}	96 ± 0.03^{a}

Values are means ± S.E. (n = 15)
$^{a\text{-}e}$ Means with different superscripts within each parameter differ ($p < 0.05$)
IW = Initial weight, FW = Final weight, WG = Weight gain, SGR = Specific growth rate, FI = Feed Intake,
FCR = Feed conversion ratio, SR = Survival rate

Figure 8 shows a bi-weekly weight gain in grams of fish fed formulated diets i.e., fish meal (D1), red-claw crayfish (D2), bloodmeal (D3) and black soldier fly larvae meal (D4) over a period of twelve weeks (90 days).

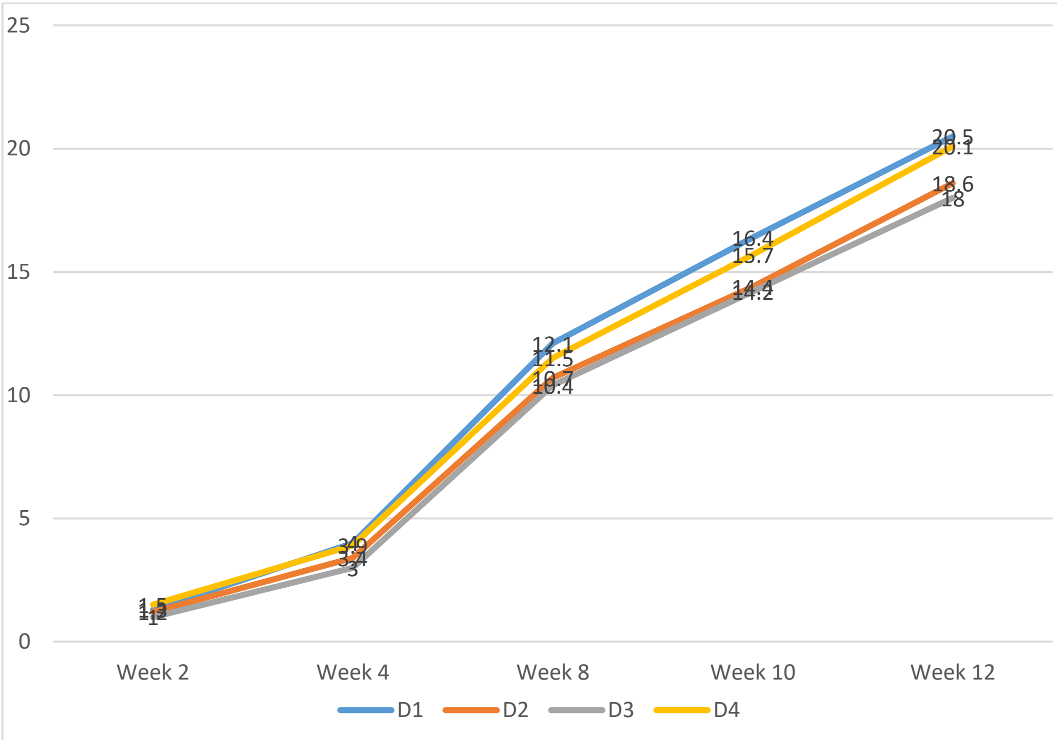

Figure 8: Bi-weekly weight gain (in grams) of fish fed formulated diets for 90 days

To determine whether significant differences occurred between variables an analysis of variance (ANOVA) was conducted. The initial weight ranged from 2.85±0.02g to 2.99±0.03g among diets. Significant differences ($P < 0.05$) were observed and an analysis of covariance (ANCOVA) was conducted to test if the initial weight was going to affect the final weight. The test results indicated a p-value of 0.096 ($P > 0.05$) showing that the initial weight did not significantly affect the final weight (See Annex 2).

The final mean weight varied from 20.87±0.16g to 23.39±0.25g among diets with FM been the highest and BM the lowest. The final mean weight showed significant differences ($P < 0.05$) between the control diet FM containing fishmeal and diets CFM and BM containing red-claw crayfish meal and bloodmeal respectively. No significant differences ($P > 0.05$) were observed between the control diet FM and diet BSFM containing black soldier fly larvae meal. In the experimental diets, the highest final weight was observed in diet BSFM with a mean weight of 22.93±0.22g and the lowest observed in diet BM with a mean weight of 20.87±0.16g.

The weight gain ranged from 17.92±0.16g to 20.50±0.27g among diets with FM been the highest and BM the lowest. Significant differences ($P < 0.05$) were observed in the weight gain in diets CFM and BM from the control diet FM. No significant differences ($P > 0.05$) were observed between the control diet FM and diet BSFM. Fish fed on diet FM (20.50±0.27g) recorded the highest weight gain but among the experimental diets, diet BSFM recorded the highest weight gain of 20.07±0.22g and the lowest was observed in diet BM with a weight gain of 17.92±0.16g.

The specific growth rate (SGR) ranged from 2.17±0.02g to 2.33±0.02g among the four (4) formulated diets. Significant differences ($P < 0.05$) were observed across diet FM, diet CFM and diet BM. No significant differences ($P > 0.05$) were observed between diet BSFM and the control diet (FM). The highest SGR was observed in diet BSFM and diet FM with growth rates of 2.32±0.01 % and 2.33±0.02 %, respectively. The lowest SGR was observed in diet BM with an SGR of 2.17±0.02 %.

Feed utilization of *Oreochromis andersonii* was estimated by Feed conversion ratio using feed intake and weight gain. Feed intake had an average mean of 29.35±0.34g ranging from 24.71±0.01g to 36.89±0.01g with BM been the highest and BSFM the lowest. Feed intake showed significant differences ($P < 0.05$) between the control diet FM and the rest of the experimental diets. The FCR ranged from 1.24±0.01 to 2.06±0.02. The highest FCR was observed in diet BM recorded at 2.06±0.02. The lowest FCR was observed in diet BSFM with a conversion ratio recorded at 1.24±0.01. The lowest FCR of 1.24 was indicative of efficient utilization of fish feed containing BSFM when compared to the control diet FM which had an FCR of 1.4. Significant differences ($P < 0.05$) were observed in all the diets across the treatment.

4.3 Survival rate

Figure 9 shows the survival rates of all the fish that were administered diets containing fish meal (D1), red-claw crayfish (D2), bloodmeal (D3), and black soldier fly larvae (D4) during the experimental period.

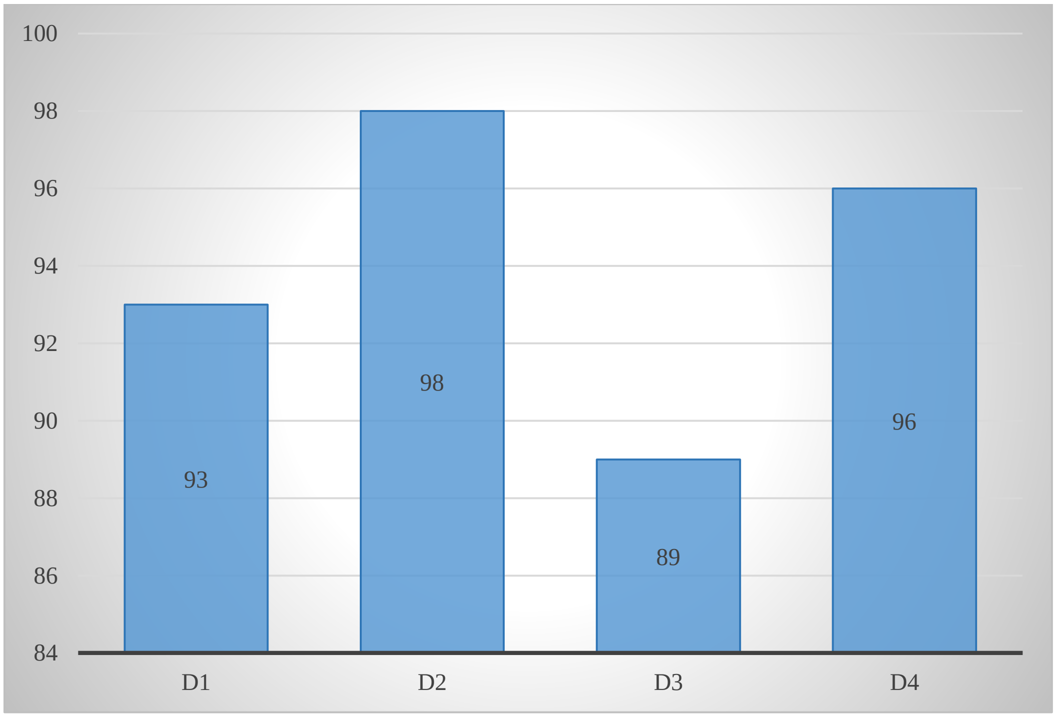

Figure 9: Survival rate (%) of fish fed formulated diets for 90 days

All the fish fed on different formulated diets showed high survival rates ranging from 89±0.05% to 98±0.02 % (see table 7). The highest survival rate (%) was recorded in fish fed on diet CFM and the lowest observed in fish fed on diet BM. No significant differences ($P > 0.05$) were observed across the four (4) formulated diets.

4.4 Whole body composition

The whole-body proximate composition at the end of the study period is presented in table 8 below.

Table 8: Whole-body proximate composition of formulated diets administered to *O. andersonii*

Components	**FM**	**CFM**	**BM**	**BSFM**
Dry Matter (%)	93.65±0.18[a]	94.29±0.20[b]	93.79±0.14[ab]	94.29±0.21[b]
Moisture (%)	6.35±0.18[b]	5.71±0.20[a]	6.21±0.14[ab]	5.71±0.21[a]
Crude Protein (%)	54.13±0.64[c]	40.47±0.52[a]	56.19±0.72[c]	50.33±0.88[b]
Crude Fat (%)	27.33±0.21[a]	31.48±0.37[c]	29.34±0.55[b]	40.41±0.41[d]
Ash (%)	16.55±0.23[b]	15.44±0.35[a]	15.53±0.29[a]	16.44±0.18[b]
M. Energy (Kcal/kg)	3954.33±12.39[a]	4217.67±17.70[b]	4109.67±13.04[ab]	4231.67±13.30[b]

Values are means ± S.E. (n = 15)
[a-e] Means with different superscripts within each parameter differ ($p < 0.05$)

Figure 10 shows the whole-body composition of fish fed formulated diets for moisture, crude protein, crude fat and ash containing fishmeal (D1), red-claw crayfish (D2), bloodmeal (D3) and black soldier fly larvae meal (D4).

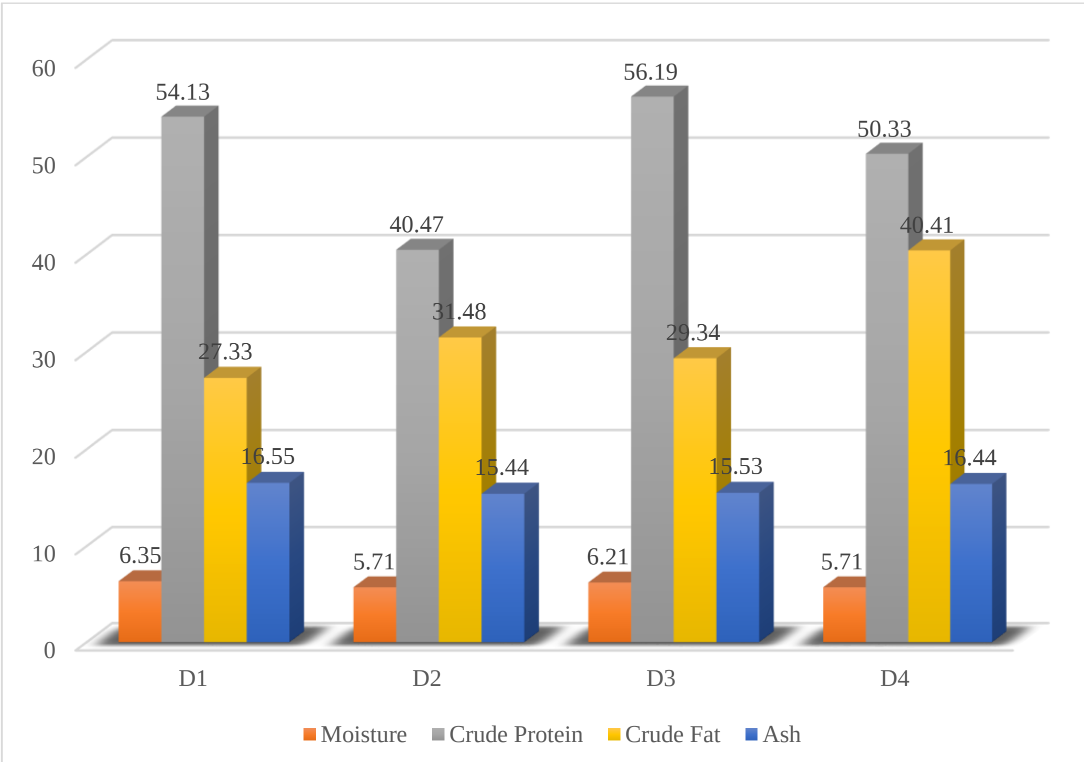

Figure 10: Whole-body composition of fish fed formulated diets

The dry mater content ranged from 93.65±0.18% to 94.29±0.21% with the highest dry matter content 94.29±0.20 % observed in diet CFM and the lowest 93.65±0.18% in diet FM. No significant differences ($P > 0.05$) in dry matter were observed across the formulated diets. Moisture content ranged from 5.71±0.20% to 6.35±0.18% with the highest (6.35±0.18 %) observed in diet FM and the lowest (5.71±0.20 %) in diet CFM. A similar pattern was also observed in Moisture content showing no significant differences ($P > 0.05$) across the formulated diets. Crude protein ranged from 40.47±0.52% to 56.19±0.72% among the diets with the highest crude protein (CP) observed in BM with a value of 56.19±0.72% and the lowest observed in CFM with a value of 40.47±0.52%. Significant differences ($P < 0.05$) in crude protein were observed between the control diet FM and diets CFM and BSFM. Nevertheless, the control diet was not significantly different ($P > 0.05$) from the diet containing BM.

Crude fat ranged from 27.33±0.21% to 40.41±0.41% among the diets with the highest crude fat with a value of 40.41±0.41% observed in diet BSFM and the lowest crude fat 27.33±0.21% observed in diet FM. Significant differences ($P < 0.05$) were observed in crude fat across all the formulated four diets. The Ash content ranged from 15.44±0.35% to 16.55±0.23% among the diets with the highest ash observed in diet FM and the lowest observed in diet CFM. Significant differences ($P < 0.05$) in ash content were observed between the control FM and experimental diets CFM and BM but diet FM was not significantly different ($P > 0.05$) from diet BSFM, however among the experimental diets, diet CFM and diet BM were not significantly different ($P > 0.05$) from each other.

Metabolizable energy ranged from 3,954.33±12.39 Kcal/kg to 4,231.67±13.30 Kcal/kg among diets with the highest energy observed in diet BSFM and the lowest in the control diet FM, Significant differences ($P < 0.05$) were observed between the control FM and the rest of the experimental diets but among the experimental diets, diet CFM and BSFM were not significantly different ($P > 0.05$) from each other.

CHAPTER FIVE

5.0 DISCUSSION

To properly examine the sustainability of utilizing different dietary treatments of either red-claw crayfish meal, blood meal or black soldier fly larvae meal as an alternative replacement for fish meal, this study provides evidence to determine the possibility of using these potential replacements in formulating fish feeds to achieve the highest growth performance, feed utilization, survival rate and whole-body composition of *O. andersonii*.

5.1 Nutrient composition in feed formulation

Nutrient requirements particularly for protein, lipid and energy ranged between 35.21-37.60%, 5.90-10.30% and 3,112.96–3,431.60Kcal/kg after formulating the dietary treatments. Formulation of feed that is balanced is crucial for optimal growth of any fish species, in this study the protein, lipid and energy levels were within the recommended ranges for culture of tilapia species. Halver and Hardy (2002) reported that protein quality of dietary protein sources depends on the amino acid composition and their digestibility. Though, the same authors showed that deficiency of an essential amino acid leads to poor utilization of the dietary protein and consequently reduces growth and decreases feed efficiency. Hasan (2001) observed that the nutrient requirements for a fish species under culture must be met to ensure production goals of the system utilized are optimized. Other findings by Wantanabe *et al.*, (1990) showed that tilapia fish production increased through the utilization of high amounts of protein with about 35% CP inclusion being more profitable. In the present study, a range of CP 35.21-37.60% was utilized in the formulated diets that fell within these recommended ranges.

5.2 Water quality parameters

Water quality parameters have an impact on the growth performance of fish, feed utilization, survival rate and whole-body composition. Furthermore, water quality parameters are regarded as the most crucial variables in the success of any aquatic system as fish are sensitive to changes in parameters such as temperature, pH, dissolved oxygen and ammonia (Al-Zahrani *et al*, 2023). All water quality parameters in the study tanks were within the recommended ranges. Since the study was conducted from May 12 to August 11, during the cold season, water quality parameters ranged from 20.87^0C to 21^0C for temperature, 6.60±0.16 mg/L to 6.72±0.16 mg/L for dissolved oxygen (D.O.), 7.61±0.20 to 7.68±0.17 for pH, and 0.09±0.00 mg/L to 0.10±0.00 mg/L for ammonia. These findings agree with other studies whose water quality parameters ranged between 0.09-0.85 mg/L for ammonia, 3.0 to 5.0 mg/L for dissolved oxygen and temperatures of 21^oC to 25^0C and 6.7 – 7.4 for pH in aquaponic systems, earthen ponds and concrete tanks (Shoko *et al*, 2014; Al-Zahrani *et al*, 2023).

5.3 Growth performance and feed utilization

The present study revealed that different formulated dietary levels of fish meal, red-claw crayfish meal, blood meal or black soldier fly larvae meal affected the Final Weight (FW), Weight Gain (WG), Specific Growth Rate (SGR) and Feed Conversion Ratio (FCR) of *Oreochromis andersonii*. The results were in agreement with similar results by Kefi *et al*, (2013) for *O. andersonii* cultured in concrete tanks where the dietary levels of formulated feeds affected the growth performance of the fish. Results for the present study revealed that fish fed diet CFM which contained crayfish meal as a main protein source showed a significantly lower weight gain and specific growth rate than FM and BSFM, this may be attributed to the fact that of all the protein sources used in this study Crayfish meal had a lower crude protein content and this was also seen in the nutrient composition of the formulated diet. These results agree with results of Mohsen *et al*. (2017), who reported that *Clarias gariepinus* fed on diets formulated with crayfish meal showed that weight gain, specific growth rate decreased significantly with increase in crayfish meal. It is clear that complete replacement of fishmeal with crayfish meal in African catfish, caused reduction of growth performance and feed efficiency, this may be attributed to increase crude fiber or chitin in diet.

The present study also showed that when *Oreochromis andersonii* fish was administered a diet with total replacement of bloodmeal as a source of protein, it produced the lowest final mean weight, weight gain and specific growth rate, it further revealed high feed intake and high feed conversion ratio than other diets in the experiment. This low performance can be attributed to the fact that the fish could not utilize diet BM due to its low palatability and bloodmeal has a poor amino acid balance although it has a high crude protein content. The results for the present study agree with a study on fingerlings of *O. niloticus* conducted by Otubusin (1987) where fish was fed different levels of replacing fishmeal with bloodmeal, it was observed that the diet with the highest levels of bloodmeal had the poorest performance in terms of growth (weight gain and specific growth rate) and survival of fish, due to the imbalanced amino acid profile. Compensating for this imbalance when formulating diets of partial or complete replacement of fishmeal may increase the utilization efficiency of the bloodmeal in fish diets. The results also agree with other studies that were conducted on other fish species by Hamed *et al.* (2017), Kirimi *et al.*, (2016), Agbebi *et al.*, (2009) and Lee *et al.*, (2001): who observed that bloodmeal had no effect on the growth and survival rate of fish when used in combination with fishmeal but when fishmeal is completely replaced with bloodmeal the growth performance and survival rate was negatively affected.

Black soldier fly larvae meal (BSFM) had a significantly higher weight gain (WG) and specific growth rate (SGR) and also lower feed intake (FI) and a better feed conversion ratio (FCR) than crayfish meal (CFM) and bloodmeal (BM) respectively but it was not significantly different from fish fed the control diet FM that had a similar growth rate. This can be attributed to an excellent amino acid profile that was highly palatable and was efficiently utilized for growth. The present study was in agreement with a study conducted by Gbai *et al*., (2019) on feeding trials of *Oreochromis niloticus* fed on fishmeal as the control diet, commercial diet and black soldier fly larvae meal, they observed the highest growth rate in the fish fed on BSFM meal and having a lower FCR. These results show that the feed formulated with black soldier fly larvae meal is of high interest to aquaculture, an indication that black soldier fly larvae meal protein was easily converted into growth. Similar values of growth performance (final weight, weight gain and specific growth rate) obtained in fish fed with black soldier fly larvae meal and fishmeal feed were in agreement with those obtained by Samuel and Nyambi (2013) and Ogunji *et al.*, (2021). These authors reported that *Clarias gariepinus* fingerlings had better weight gain and specific growth rates when fishmeal was totally replaced by black soldier fly larvae meal in their diets. Ajani *et al*., (2004) reported that complete replacement of fishmeal with black soldier fly larvae meal in diets of Nile tilapia (*Oreochromis niloticus*) improved growth attributed to the excellent amino acid profile and palatability of black soldier fly larvae meal. These findings were further supported by findings of Idowu and Afolayan, (2013) and Sogbesan (2014). Similar results were reported by Faskin *et al*., (2005) and Kirimi *et al*., (2016).

Feed utilization of the four (4) treatment diets indicated a feed intake across the dietary treatments with an average mean intake of 29.35±0.34g. The lowest FCR of 1.24 was observed in a diet replaced with black soldier fly larvae meal and the highest FCR of 2.06 recorded from a diet consisting of bloodmeal. Significant differences ($P < 0.05$) were observed in feed intake and feed conversion ratio of the formulated diets with black soldier fly larvae meal being the efficient feed. The results from the growth experiment indicated that better growth rates were observed using black soldier fly larvae meal. This may be attributed to the high digestibility of black soldier fly larvae and the composition of providing a more balanced diet containing the amino acid profile, sufficient vitamins and/or minerals to support growth.

5.4 Survival rate

The survival rate of the fish in the study ranged from 89% observed in bloodmeal diet to 98% observed in crayfish meal. In this study, the complete inclusion of bloodmeal in a diet recorded the lowest survival rate of 89% indicative of bloodmeal requiring a supplement of fishmeal to obtain good survival rates. The highest survival rate was observed in fish fed on crayfish meal with a survival rate of 98%. Despite these differences, no significant differences ($P > 0.05$) were observed in survival rate of *O. andersonii* fed on different dietary feeds. These findings were in agreement with studies that were done by Agbebi *et al.,* (2009), Mohsen *et al*., (2017) and Gbai *et al.,* (2019) and closely comparable to those reported by Liti *et al*., (2002), Chaula *et al*., (2002) and Kang'ombe and Brown (2007) being at over 90%. These findings could be attributed to reduced stress caused by handling during sampling, suitable water quality parameters and zero predation (Kang'ombe and Brown, 2007). Another attribute could be due to the implementation of good aquaculture practices (GAP) during the entire study period. Kirimi *et al*., (2016) observed that fish fed on a diet containing bloodmeal without any supplement of fishmeal affected survival rate of fish supporting the findings of our study contrary to the findings of Agbebi *et al*., (2009) who reported that fishmeal could be completely replaced with bloodmeal with no adverse effects on the survival rate of *Clarias gariepinus* juvenile.

5.5 Whole body composition

Results of the present study showed no significant differences in the fish fed different dietary feeds containing fishmeal (control), crayfish meal, bloodmeal or black soldier fly larvae meal for dry matter and moisture but significant differences were observed in crude protein, ash and metabolized energy. Ingredients used in this experiment showed significant influence on the whole-body composition of *Oreochromis andersonii* corresponding to different diets, Oreochromis *andersonii* fed CFM containing crayfish meal showed a decrease in crude protein when compared with the control (FM) and other treatments. These findings can be attributed to the poor ability of fish, in some cases, in utilizing a single feed ingredient. Studies on partial and complete replacement of fishmeal with crayfish on the growth performance of the Nile tilapia by Abd-El *et al.* (1998), and on other fish species by Agouz and Tonsy (2003) and Abeer et al., (2007) supported the findings of the present study. However, fish subjected to diet BM had a higher crude protein content than all the fish that was fed experimental diets although it was not significantly different from the fish fed the control diet (FM), these findings were supported by the findings of Ali *et al.,* (2000) who observed an increase in the nutritional value of the fish when subjected to bloodmeal. Although the results of the present study were much higher than those reported by Njieassam (2016), a difference in species cultured and facilities could have influenced the differences. The crude protein content of fish in the experiment was ranging from 40 to 57% was below that obtained by Otubusin (2009) who recorded 70% crude protein content. This figure together with the ash content (14 to 17%) were within the normal range which according to Drew *et al*. (2007), may vary from 40 to 70% and 10 to 21%, respectively, depending on fish species, the source and processing method.

The percentage of crude fat across the diets was significantly different and this was observed to be higher in fish fed on black soldier fly larvae meal and lowest in fishmeal. However, fish fed on BSFM was significantly lower than fish fed the control (FM) with respect to crude protein but suffice to say, the crude protein in fish fed BSFM was within the normal recommended values for fish according to Drew *et al*. (2007), this can be attributed to the high palatability of BSFM which was shown by a better FCR than all the diets in this experiment. The present study results agree with Ajani *et al*., (2004) who reported that complete replacement of fishmeal with black soldier fly larvae meal in diets of Nile tilapia (*Oreochromis niloticus*) improved the whole-body composition of the fish attributed due to the high palatability of the fish fed on black soldier fly larvae meal efficiently utilizing the lipids and other nutrients. Black soldier fly larvae meal in addition to the fishmeal-soybean meal-based diet did not significantly influence crude protein and ash contents in the whole body of gibel carp, but the black soldier fly larvae meal fed fish showed significantly higher content of crude lipid than those in the control group (Dong et al., 2013). However, the results obtained are in disagreement with the results reported by Qiao *et al*. (2019), Wang *et al*., (2017) and Wen *et al*., (2013) indicated that the addition of dietary black soldier fly larvae meal did not significantly influence lipid and ash contents in the whole-body and muscle of the fish.

Black soldier fly larvae meal is a protein-rich feed ingredient that can replace fishmeal in fish feed, based on the results of this study, BSFM have been found to be efficient for farmed fish and it can be concluded that black soldier fly larvae meal (BSFM) can effectively replace fish meal in fish feed without any adverse effect on growth performance, survival rate and whole-body composition. The use of black soldier fly larvae meal as an alternative source for protein can reduce the cost of aquaculture feed without reducing the quality of fish.

CHAPTER SIX

6.0. CONCLUSSION AND RECOMMENDATIONS

6.1 CONCLUSSION

Based on the research results and discussion it can be concluded that:

i. Black soldier fly larvae (BSFL) meal can be used to replace fishmeal (FM) without significantly affecting the growth performance of *Oreochromis andersonii.* It was observed that black soldier fly larvae meal as protein source in the diet of *Oreochromis andersonii* yields better results than the other two protein sources on growth performance of fish, this can be seen from high significant final weight, specific growth rate and an excellent feed conversion ratio.

ii. There is no negative effect in replacing fishmeal with crayfish meal, bloodmeal or black soldier fly larvae meal on the survival of *Oreochromis andersonii.* The present study showed no significant differences among the treatments and the survival rate was achieved at 89 percent.

iii. Black soldier fly larvae meal and bloodmeal as protein sources in the diet of *Oreochromis andersonii* had no negative effect on the whole-body composition of the fish, therefore, based on this study, black soldier fly larvae meal and bloodmeal as animal protein sources can completely replace fishmeal in the diet of *Oreochromis andersonii* without negatively affecting the whole-body composition of the fish.

6.2 RECOMMENDATIONS

It is the recommendation of this study that;

Black soldier fly larvae meal can be used as a complete replacement of fishmeal as a protein source in the diet of *Oreochromis andersonii* without negatively affecting growth performance, survival and whole-body composition of the fish.

Further studies

i. Due to the scope of this study, there is need to perform an economical study on cost of producing and processing of black soldier fly larvae into black soldier fly larvae meal, so as to know how much it can cost to produce 1 kilogram of black soldier fly larvae meal in comparison with fishmeal.
ii. There is also need to determine the optimal levels of black soldier fly larvae meal on the growth, survival and whole-body composition of the *Oreochromis andersonii.*

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APPENDICES

Annex 1: ANOVA *for morphometric parameters*

		Sum of Squares	df	Mean Square	F	Sig.
Initial weight (g)	Between Groups	.687	3	.229	6.200	.000
	Within Groups	6.499	176	.037		
	Total	7.186	179			
Final weight (g)	Between Groups	169.865	3	56.622	29.235	.000
	Within Groups	319.572	165	1.937		
	Total	489.437	168			
Weight Gain (g)	Between Groups	184.808	3	61.603	30.569	.000
	Within Groups	332.512	165	2.015		
	Total	517.319	168			
S.G.R (%/day)	Between Groups	.802	3	.267	25.444	.000
	Within Groups	1.733	165	.011		
	Total	2.535	168			
F I (g)	Between Groups	3335.829	3	1111.943	132178.782	.000
	Within Groups	1.388	165	.008		
	Total	3337.217	168			
F. C. R	Between Groups	15.809	3	5.270	419.029	.000
	Within Groups	2.075	165	.013		
	Total	17.884	168			
S. R	Between Groups	.194	3	.065	1.126	.340
	Within Groups	10.133	176	.058		
	Total	10.328	179			

Annex 2 Tests of between-subjects effects

Dependent Variable: Final weight (g)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	491.746[a]	4	70.249	26.89	0.000
Intercept	739.513	1	739.513	283	0.000
Initial weight	**7.274**	**1**	**7.274**	**2.784**	**0.096**
Treatment	474.504	3	79.084	30.27	0.000
Error	760.338	291	2.613		
Total	156515.57	299			
Corrected Total	1252.084	298			

a. R Squared = .393 (Adjusted R Squared = .378)

Annex 3 ANOVA for body composition

		Sum of Squares	df	Mean Square	F	Sig.
C. Protein	Between Groups	438.088	3	146.029	98.328	.000
	Within Groups	11.881	8	1.485		
	Total	449.969	11			
C. Fat	Between Groups	299.192	3	99.731	203.636	.000
	Within Groups	3.918	8	.490		
	Total	303.110	11			
Ash	Between Groups	3.102	3	1.034	4.795	.000
	Within Groups	1.725	8	.216		
	Total	4.827	11			
Moisture	Between Groups	.997	3	.332	3.306	.078
	Within Groups	.804	8	.101		
	Total	1.801	11			
Dry Matter	Between Groups	.997	3	.332	3.306	.078
	Within Groups	.804	8	.101		
	Total	1.801	11			
M. Energy	Between Groups	147848.000	3	49282.667	4.877	.000
	Within Groups	80842.667	8	10105.333		
	Total	228690.667	11			

Annex 4 ANOVA for water quality parameters

		Sum of Squares	df	Mean Square	F	Sig.
Temperature	Between Groups	0.22	6	0.037	0.01	1.000
	Within Groups	438.17	77	5.691		
	Total	438.39	83			
pH	Between Groups	0.097	6	0.016	0.04	1.000
	Within Groups	33.161	77	0.431		
	Total	33.257	83			
Dissolved oxygen	Between Groups	0.147	6	0.025	0.07	0.998
	Within Groups	25.395	77	0.33		
	Total	25.542	83			
Ammonia	Between Groups	0.001	6	0	1.34	0.267
	Within Groups	0.002	35	0		
	Total	0.003	41			

Annex 5 Laboratory proximate analysis results for feed ingredients before feed formulation

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DEPARTMENT OF ANIMAL SCIENCE

P. O. BOX 32379

LUSAKA

DATE:...26/03/2021....

RESULT SHEET

S/N	SAMPLE	ANALYSIS	RESULTS (%)		
1	FM	CRUDE PROTEIN	66.36	63.29	64.01
		CRUDE FIBRE	0.79	0.80	0.65
	Fish Meal	CRUDE FAT	8.66	9.59	7.63
		ASH	9.16	9.86	9.10
		CALCIUM	10.13	11.87	9.25
		PHOSPHORUS	1.40	1.35	1.43
		SODIUM CHLORIDE	0.35	0.32	0.41
		MOISTURE	9.60	10.20	9.65
		DRY MATTER	90.40	89.80	90.35
		METABOLISABLE ENERGY	3589 kCal/kg	2824kCal/kg	2594kCal/kg
2	CM	CRUDE PROTEIN	55.00	52.50	51.00
		CRUDE FIBRE	0.19	0.39	0.20
	Crayfish meal	CRUDE FAT	2.95	2.92	2.92
		ASH	6.21	6.14	6.35
		CALCIUM	1.28	1.27	1.29
		PHOSPHORUS	0.11	0.13	0.12
		SODIUM CHLORIDE	1.43	1.52	1.87
		MOISTURE	15.10	14.80	14.70
		DRY MATTER	84.90	85.20	85.30
		METABOLISABLE ENERGY	2932 kCal/kg	2944kCal/kg	2940kCal/kg
3	MM	CRUDE PROTEIN	58.90	56.00	59.00
		CRUDE FIBRE	10.65	11.15	10.79
	Maggot meal	CRUDE FAT	10.89	11.08	10.91
		ASH	16.35	14.74	16.20
		CALCIUM	3.26	3.58	3.62
		PHOSPHORUS	0.80	0.82	0.88
		SODIUM CHLORIDE	1.05	1.25	1.14
		MOISTURE	8.19	7.99	8.00
		DRY MATTER	91.81	92.01	92.00
		METABOLISABLE ENERGY	3171 kCal/kg	3244kCal/kg	3184kCal/kg
4	BM	CRUDE PROTEIN	77.00	74.50	76.00
		CRUDE FIBRE	0.61	0.19	0.59
	Blood meal	CRUDE FAT	0.39	0.22	0.29
		ASH	1.75	0.60	1.60
		CALCIUM	1.02	1.08	1.12
		PHOSPHORUS	0.21	0.27	0.19
		SODIUM CHLORIDE	1.01	0.93	0.96
		MOISTURE	7.20	7.50	8.05
		DRY MATTER	92.81	92.50	91.95
		METABOLISABLE ENERGY	3258 kCal/kg	3312 kCal/kg	3231 kCal/kg
5	MB	CRUDE PROTEIN	12.00	11.00	12.00
		CRUDE FIBRE	4.36	3.72	4.55
	Maize meal	CRUDE FAT	11.02	9.66	10.48
		ASH	3.69	3.64	2.59
		CALCIUM	0.87	0.98	0.86
		PHOSPHORUS	5.51	5.52	5.48
		SODIUM CHLORIDE	0.73	0.47	0.99
		MOISTURE	10.24	11.30	9.99
		DRY MATTER	89.75	90.00	89.67
		METABOLISABLE ENERGY	3554 kCal/kg	3458 kCal/kg	3578 kCal/kg
6	SM	CRUDE PROTEIN	33.00	35.00	34.00
		CRUDE FIBRE	11.46	10.40	9.50
	Soya meal	CRUDE FAT	1.46	1.25	1.33
		ASH	5.25	5.15	4.85
		CALCIUM	1.18	1.22	1.19
		PHOSPHORUS	0.42	0.41	0.41
		SODIUM CHLORIDE	0.15	0.35	0.20
		MOISTURE	7.14	7.63	7.44
		DRY MATTER	92.86	92.37	92.56
		METABOLISABLE ENERGY	3184 kCal/kg	3161 kCal/kg	3182 kCal/kg

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Annex 6 Laboratory proximate analysis results for formulated feeds

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RESULT SHEET

S/N	SAMPLE	ANALYSIS	RESULTS (%)		
1	C	CRUDE PROTEIN	51.00	51.01	49.99
		CRUDE FIBRE	0.00	0.00	0.00
		CRUDE FAT	26.04	26.00	26.05
		ASH	14.95	14.85	14.90
		CALCIUM	8.16	8.16	8.15
		PHOSPHORUS	12.20	12.21	12.20
		SODIUM CHLORIDE	0.52	0.52	0.50
		MOISTURE	5.75	5.70	5.76
		DRY MATTER	94.25	94.30	94.24
		METABOLISABLE ENERGY	4, 124KCal/kg	4, 123KCal/kg	4, 120KCal/kg
2	D1	CRUDE PROTEIN	54.10	55.24	53.04
		CRUDE FIBRE	0.00	0.00	0.00
		CRUDE FAT	27.58	27.50	26.92
		ASH	16.56	16.14	16.95
		CALCIUM	5.97	4.90	6.01
		PHOSPHORUS	8.10	8.25	8.12
		SODIUM CHLORIDE	1.08	1.34	1.10
		MOISTURE	6.15	6.20	6.70
		DRY MATTER	93.85	93.80	93.30
		METABOLISABLE ENERGY	3, 979KCal/kg	3,944KCal/kg	3,940KCal/kg
3	D2	CRUDE PROTEIN	39.80	41.50	40.10
		CRUDE FIBRE	0.00	0.00	0.00
		CRUDE FAT	31.80	30.74	31.91
		ASH	15.27	14.94	16.10
		CALCIUM	6.14	6.25	6.62
		PHOSPHORUS	10.19	10.82	10.88
		SODIUM CHLORIDE	4.43	4.19	3.94
		MOISTURE	5.80	5.34	6.00
		DRY MATTER	94.20	94.66	94.00
		METABOLISABLE ENERGY	4,225KCal/kg	4, 244KCal/kg	4, 184KCal/kg
4	D3	CRUDE PROTEIN	57.50	55.00	56.07
		CRUDE FIBRE	0.00	0.00	0.00
		CRUDE FAT	29.48	30.22	28.34
		ASH	15.99	15.60	15.00
		CALCIUM	6.33	6.08	6.12
		PHOSPHORUS	11.54	11.27	11.19
		SODIUM CHLORIDE	0.95	0.93	0.96
		MOISTURE	6.09	6.50	6.05
		DRY MATTER	93.91	93.50	93.95
		METABOLISABLE ENERGY	4, 086KCal/kg	4, 112KCal/kg	4, 131KCal/kg
5	D4	CRUDE PROTEIN	52.00	49.00	50.00
		CRUDE FIBRE	0.00	0.00	0.00
		CRUDE FAT	41.09	39.66	40.48

		ASH	16.09	16.64	16.59
		CALCIUM	6.40	6.98	6.86
		PHOSPHORUS	8.78	8.52	8.48
		SODIUM CHLORIDE	1.11	1.47	0.99
		MOISTURE	5.85	5.30	5.99
		DRY MATTER	94.15	94.70	94.01
		METABOLISABLE ENERGY	4, 109KCal/kg	4, 458KCal/kg	4, 128KCal/kg
6	D5	CRUDE PROTEIN	54.33	55.00	54.10
		CRUDE FIBRE	0.00	0.00	0.00
		CRUDE FAT	39.19	39.25	39.33
		ASH	15.46	15.15	14.85
		CALCIUM	12.91	12.22	12.19
		PHOSPHORUS	10.82	10.41	10.41
		SODIUM CHLORIDE	1.05	0.35	0.20
		MOISTURE	5.84	5.63	5.44
		DRY MATTER	94.15	94.37	94.56
		METABOLISABLE ENERGY	4, 546KCal/kg	4, 361KCal/kg	4, 582KCal/kg
7	D6	CRUDE PROTEIN	56.12	54.30	55.23
		CRUDE FIBRE	0.00	0.00	0.00
		CRUDE FAT	27.90	27.55	26.96
		ASH	15.37	14.98	15.00
		CALCIUM	6.38	6.20	6.46
		PHOSPHORUS	9.10	9.67	9.30
		SODIUM CHLORIDE	1.34	1.09	1.15
		MOISTURE	5.99	5.10	5.54
		DRY MATTER	94.01	94.90	94.46
		METABOLISABLE ENERGY	4, 041KCal/Kg	4,104KCal/Kg	4, 090KCal/Kg

8	D7	CRUDE PROTEIN	51.05	55.25	53.15
		CRUDE FIBRE	0.00	0.00	0.00
		CRUDE FAT	28.74	27.98	28.30
		ASH	16.12	16.54	16.20
		CALCIUM	6.63	6.20	6.57
		PHOSPHORUS	9.13	9.09	9.07
		SODIUM CHLORIDE	0.73	0.42	0.68
		MOISTURE	5.79	5.48	5.87
		DRY MATTER	94.21	94.52	94.13
		METABOLISABLE ENERGY	3, 758KCal/Kg	3, 986KCal/Kg	3, 948KCal/Kg

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