PERFORMANCE OF R0SS 308 BROILERS ON MAIZE-SPROUTED COWPEAS GROWER-FINISHER DIETS FORTIFIED WITH BLACK SOLDIER FLY (*HERMATIA ILLUCENS*) LARVAE MEAL

BY

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DECLARATION

I, **Thivhalitshi Thomas Muleya of student number: 15013751**, hereby declare that this dissertation is submitted in fulfilment of the requirements for the Master of Science in Agriculture (MSCANS) Department of Animal Science, in the Faculty of Science, Engineering, and Agriculture, University of Venda, and has not been previously in part or in its entirety been submitted to any University for any other degree. I further declare that this is my own work in design and execution, and that all reference material contained herein has been duly acknowledged.

Signed: .... Date: 22 February 2023

DEDICATION

This dissertation honours my parents, Mr. and Mrs. T.A. Muleya, as well as my siblings Pfano, Thinavhuyo, Grace, Tshedza, and Sarah.

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Abstract

The objectives of the study were to evaluate the efficacy of sprouted cowpea (*Vigna unguiculata)*-maize (*Zea mays*) grower and finisher broiler diets, and of 5%, versus 10% fortification of the diets with full-fat Black Soldier fly (*Hermatia illucens*) larvae meal (BSFLM). The trial used 360 -day-old Ross 308 broiler chicks reared in an open, deep litter house. Chicks were placed in 30 wire mesh experimental pens of 150 cm length × 144 cm width. Chicks were uniformly managed during the starter (21 days) phase, feeding on a commercial starter. The chicks received supplementary stress vitamins (Virbac© Samrand Business Park, Centurion, Pretoria, South Africa) during days 1-6. On day-22, sexed broilers were randomly allotted at 12 birds per pen in a 5 (diet) X 2 (sex) factorial arrangement with three replications per treatment. Grower phase (days 22-35) and finisher phase (days 36-42) dietary treatments were: 0% BSFLM sprouted cowpea negative control (NC) diets, positive control (PC) commercial diets, low (LF 5% BSFLM) and high (HF 5% BSFLM) fat sprouted cowpea diets with 5% BSFLM diets, and 10% BSFLM sprouted cowpea diets. Feed intake (FI), body weight gain (BWG), feed conversion ratio (FCR) and mortality rates were measured on a weekly basis. Upon slaughter, the weight of the warm dressed carcass, carcass components, visceral organs and abdominal fat were measured while meat quality (pH, drip loss and meat colour) were evaluated. During the grower phase, broilers on the PC consumed more ($p<0.05$) feed and achieved higher weight gain ($p<0.05$) than those on the NC and LF 5% BSFLM diets. The PC birds achieved lower ($p<0.05$) FCR compared to broilers on the NC and HF 5% BSFLM. During the finisher phase, broilers on the PC consumed more ($p<0.05$) feed than those on the 10% BSFLM diet. Cumulatively throughout the grower-finisher phases, broilers on the PC had higher feed intake and weight gain ($p<0.05$) than on the NC and HF 5% BSFLM diets and attained higher ($p<0.05$) final live weight with lower ($p<0.05$) FCR compared to broilers on the NC. Male broilers consumed more ($p<0.05$) feed during the grower phase and by slaughter, with higher $p<0.05$) weight gain during finishing, which resulted in heavier ($p<0.05$) live weight at slaughter. There was no ($p>0.05$) sex * diet interaction on parameters for measuring the growth performance. Broilers on the PC had larger ($p<0.05$) breast weights compared to birds on the NC and the 10% BSFLM diets and had higher ($p<0.05$) thigh weights than those on all other treatments. Broilers on the PC had a larger ($p<0.05$) spleen than birds on the NC and the 5% BSFLM diets. The females had lower thigh and liver weights compared to the males ($p<0.050$). Birds on the 10% BSFLM had lower drip loss compared to those on the negative control ($p<0.05$). In conclusion, broilers fed the sprouted cowpea-maize diet without BSFLM had low feed intake, weight gain and high FCR during the growing phase, and these effects were cumulatively reflected at slaughter. Compared to the NC and PC diets, BSFLM fortification of sprouted cowpea-maize diets resulted in intermediate parameters for broiler growth performance. Based on the growth performance, it was concluded that dietary efficacy was in the order NC <5 % LF<5% HF< 10 % BSFLM < PC fortification.

Key words: Ross 308 broilers, sprouted cowpeas, Black soldier fly larvae meal.

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ABBREVIATIONS AND ACRONYMS

%	Percentage
a*	CIE red (+)/ green (-) colour attributes
ADF	Acid detergent fibre
ANF	Anti-nutritional factors
BSFLM	Black soldier fly larvae meal
BSF	Black soldier fly
b*	CIE yellow (+) / blue (-) colour attributes
BW	Body weight
BWG	Body weight gain
C*	Chroma
Ca	Calcium
C_D	Colour difference
DM	Dry matter
FCE	Food conversion efficiency
FCR	Feed conversion ratio
FI	Feed intake
FAO	Food and Agriculture Organisation
g	Grams
g/b/d	Gram per bird per day
g/kg	Gram per kilogram
GLM	General linear model
HF BSFLM	High fat Black soldier fly larvae meal
h*	Hue

kg	Kilogram
L*	CIE lightness coordinate
LF	Low fat
LF BSFLM	Low fat Black soldier fly larvae meal
NC	Negative control
NDF	Neutral detergent fibre
PC	Positive control
P	Phosphorus
SEM	Standard error mean
TMR	Total mixed ration

CHAPTER 1

INTRODUCTION

1.1. Background

A sustained upward global trend in the consumption of animal products is increasing demand for feed ingredients (Belghit *et al*., 2018). Predicted climate-change related decline in the production of conventional feed grains demands urgent, concerted search for suitable alternatives (Belghit *et al*., 2018). Globally, insects are increasingly considered important, quality feed protein source (van Huis & Oonincx, 2017). Insects are good protein complement for potentially low quality novel diets (Makkar, 2018). The potential of insects as alternative protein feeds lies in efficient mass production to facilitate competitive pricing in the feed supply chain (Sánchez-Muros, *et al*., 2014). In addition, mass insect production for stock feeding can also be coupled to environment friendly organic waste processing (Smetana, Schmitt & Mathys, 2019). Several insect species have been tested, with the most promising being the Black Soldier fly (BSF) (*Hermetia illucens*), the yellow mealworm (*Tenebrio molitor*), and the common House fly (*Musca domestica*) (Laconisi *et al*., 2017; Schiavone *et al*., 2017; Secci *et al*., 2018). Black Soldier Fly larvae meal (BSFLM) is rich in protein (40-44%), which is of superior quality compared to soya bean meal (Lee *et al*., 2018). The BSFLM is rich in lysine (6-8g/100g), Ca (5-8%), P (0.6-1.5%) and fat (15-49%) (Sheppard *et al* 1998, cited by Lee *et al*., 2018).

The cowpea (*Vigna unguiculata*) is a herbaceous annual legume plant which is native to arid-semi arid tropical regions (Kirse & Karklina, 2015). In these regions, in the advent of climate change, the cowpea (*Vigna unguiculata*) is increasingly considered an ecologically competitive, futurist plant protein source for poultry (Kirse & Karklina, 2015). With 23-32% protein and 50-60% carbohydrates (Sirelkhatim Balla Elharadallou, 2013; Cruz, de Almeida & dos Santos, 2014; Kirse & Karklina, 2015; Ehirim *et al*., 2018), the cowpea is relatively more nutritious, and the most cost-effective of the native tropical grain legumes (Defang *et al*., 2008).

Typical of the legumes, without efficient processing, cowpea antinutrients may limit dietary efficacy for poultry (Ehirim *et al*., 2018). Sprouting is considered an efficient, practical low-cost processing solution in poorly resourced settings (Ehirim *et al*., 2018). For example, sprouting reduced cowpea antinutrients, and increased the nutrient content (Ehirim *et al*., 2018). Inclusion of BSFLM could complement the nutritive benefit of sprouting cowpeas for broiler feeding.

Therefore, the study evaluated the effects of BSFLM inclusion in maize-sprouted cowpea growing and finishing diets.

1.2. Problem statement

With an estimated 8,0 billion people on the planet in 2022, the demand for animal protein is expected to rise even faster as developing nations' economies expand and the ever-wealthier population changes its eating preferences. This means that production of soya beans, which is used extensively as animal feed, must increase beyond current production levels, or should be complemented with alternative sources. Relative to soya bean, cowpea is of inferior protein quality, such that diets formulated using cowpea as the sole protein source can limit broiler growth performance, the yield of prime carcass components and meat quality. Bioprocessing of cowpeas potentially retains residual anti-nutritional factors (ANF) such as trypsin inhibitors that might still affect protein utilization. There is limited information on the use of BSFLM as supplementary protein to bio-processed cowpea diets for broiler chickens diets in terms of optimal levels, and the implications on dietary efficacy. These questions were investigated in this study.

1.3. Justification of the Study

Protein is the most expensive component of broiler diets and therefore any reduction in the price of protein will cause a significant financial relief to both producers and consumers. Projections by the food and Agriculture Organization (FAO) suggest a significant increase in demand for soya bean meal in 30 years to come, with the current production being 1.24 million metric tons (Herforth *et al.*, 2020). The existing production, including of the supplementary protein sources, both animal and plant derived, are not projected to meet the future demand. Regardless of the ecological adaptability and agronomic potential, alternative legumes such as cowpea are not yet produced in significant quantities to contribute to the commercial stockfeed supply chain (Osipitan *et al.,* 2021). Research which explores and successfully justifies significant dietary replacement of conventional feeds by native grains such as cowpeas might drive cost-effective future production and increase their demand.

1.4 Research Objectives

1.4.1 Main objective

To evaluate the efficacy of supplementing Black Soldier fly larvae (BSFL) meal in sexed Ross broiler chickens fed maize-sprouted cowpea diets during the grower and finisher phases.

1.4.2 Specific objective(s)

To compare the effects of the following on Ross 308 broiler growth, slaughter, and meat quality parameters.

i) 5% versus 10% inclusion of BSFL meal in maize-sprouted cowpea broiler diets
ii) Sex

1.5 Research Hypotheses

The following do not affect Ross 308 broiler growth, slaughter, and meat quality parameters.

i) 5% versus 10% inclusion of BSFL meal in maize-sprouted cowpea broiler grower and finisher diets
ii) Sex

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Global population development has resulted in altered lifestyles and eating choices as well as an increase in global food consumption patterns, which has raised the demand for animal protein. This will have an impact on the demand for livestock feeds and will put great strain on the already scarce supplies (Van Huis, 2013). As a result, there is an urgent need for alternative sources of proteins for animals. Insects have recently started being studied as innovative feed ingredients for aquaculture (Belforti *et al*., 2015; Gasco *et al*., 2016; Secci *et al*., 2018) and poultry since the two main derived products (BSFL meal and fat) may be suitable for substituting/integrating the conventional protein and fat sources (soybean/fish meals, vegetable oils) (Bovera *et al*., 2015; Biasato *et al*., 2016; Cullere *et al*., 2016). Among insect species, the Diptera, *Hermetia illucens* has been indicated as one of the most promising candidates for industrial production in the world (Veldkamp & Bosch, 2015). BSF larvae are rich in nutrients, depending on the quantity of fat and protein that they can store in their bodies during the larval stage (Surendra *et al*., 2016). Cowpea (Vigna unguiculata) called "white gold" is cultivated in many agro-ecological zones of the world and is well integrated into local population feeding habits. Recent research work in Cameroon has revealed that cowpeas hold promising potential as a feedstuff for poultry (Bouba *et al*., 2016). In fact, cowpea contains 63% carbohydrates, and 14-24% crude protein. The limiting aspect in the use of legumes in animal feed is the presence of antinutritional factors (Teguia & Beynen., 2005; Abu Hafsa *et al.,* 2022). Grain legumes like cowpea contain many anti-nutritional factors such as chymotrypsin inhibitors, amylase inhibitors, tannins and phytic acids (Teguia & Beynen, 2005). Abu Hafsa *et al*., (2022) indicated that the presence of certain anti-nutritional factors and non-digestible components might be the reason for their underutilization. However, recent research shows that there is a possible solution to reduce the level of anti-nutritional factors and non-digestible components of dietary cowpea.

2.2 Methods for processing cowpeas for broiler diets

2.2.1 Effects of sprouting

Sprouting, the practice of germinating seeds, is one of the most adopted methods for legume processing. Sprouting is a process whereby the seeds are soaked in water overnight and allowed to germinate at room temperature for 2 days. The sprouts will then be air dried for 24 hours and oven dried at 60°C until a constant weight is obtained (Liyanage *et al*., 2018). During this process several metabolic enzymes, such as proteinases, are activated. This leads to the release of amino acids and peptides to synthesize new proteins (Devi, Kushwaha & Kumar, 2015). As a consequence, nutritional quality of proteins may be enhanced by sprouting in legumes and other seeds (Uppal & Bains, 2012). Sprouting rate gives information about the age of seeds (Gulewicz *et al*., 2008). During sprouting, the water intake of seeds varies. Agrahar-Murugkar & Jha. (2010) observed that the moisture content range in raw seeds was 4.8% in 'DSb1' to 5.7% in 'JS9305'. The moisture content on germination increased about 10 times after 48 h. Thereafter it increased in the sprout marginally at 72 h in all the varieties. Uwaegbute, Iroegbu & Eke., (2000) observed a moisture increase from 15.6 to 17.6 % after sprouting cowpea. Usually during sprouting, seeds absorb water by a process called imbibition (Nonogaki, Bassel & Bewley., 2010). Uppal and Bains., (2012) observed 43.19 % decrease in phytic acid in cowpea after sprouting for 24 hours.

2.3 Performance of broilers on cowpea diets

2.3.1 Feed intake

In a study by Akanji, Fasina & Ogungbesan., (2016) which investigated the effect of raw cowpea, dehulled cowpea, dehulled cooked cowpea and dehulled roasted cowpea grains on growth and haematological profiles of broiler chickens, feed intake was reduced in birds fed raw cowpea and dehulled cowpea. Abdelgani *et al*., (2018) reported that broiler chicks can tolerate up to 15% cowpea in the diet without adverse effect and dietary cowpea seems acceptable and palatable to the birds. When raw cowpea was included in different ration levels along with the constant substitution of soybean meal, an insignificant impact on dry matter intake was observed among dietary treatments (Adino, Wondifraw & Addis., 2018). Substitution of different levels of cooked cowpea grain was proven to have no effect on the dry matter (DM) intake of broilers alternatively, it can improve the mean daily and cumulative feed consumption of broilers. In a study by Kur *et al*., (2013), the inclusion of cooked cowpea in broiler diets at various percentages did not affect the overall feed consumption. In another investigation that was investigating the influence of diets containing raw or roasted cowpeas on the performance and gut health of broiler chickens, the results observed showed no effect on feed intake on day 5 of the experiment (Anjos *et al*., 2012). Abdelgani *et al*., (2018) reported that incorporation of different levels of cowpea had no significant effects on feed intake. Georgeta, Vasilachi & Ropotă., (2022) found that inclusion of cowpea at 10-20% had no significant effect on feed intake. When using enzyme addition or roasted cowpea, Dousa *et al.,* (2013) found that the feed intake of broilers was similar in all treatments.

2.3.2 Feed conversion ratio

The feed conversion ratio (FCR) is the amount of feed ingested by an animal which can be converted into one kilogram of live weight, FCR allows for an estimate of the feed that is required in the growing cycle. In terms of FCR, Kur *et al*., (2013) and (Abdelgani *et al*., (2018) reported better performance for cowpea incorporated diets than for those with 0% cowpea inclusion. In the study by Kana, Teguia & Fomekong. (2012) found diets containing 0% cowpea inclusion to perform better in terms of FCR than cowpea incorporated diets, however, diets with cooked cowpea showed a better FCR compared to birds fed raw cowpea. Similarly, in a study conducted by Georgeta, Vasilachi & Ropotă., (2022) investigating the effect of cowpea and chickpea on growth performance, blood parameters and breast meat fatty acids in broiler chickens, inclusion of 10-20 % cowpea in diets had no significant effect on FCR. When using soaked and roasted cowpea, the dry matter conversion ratio showed a non-significant difference among the dietary treatments (Adino, Wondifraw & Addis., 2018). Adino, Wondifraw & Addis (2018) reported that dietary inclusion of up to 100% cowpea had no significant effect on FCR. In an investigation carried out by Kana, Teguia & Fomekong., (2012) reported a FCR that was lower for the birds fed raw cowpea supplemented with Canarium charcoal, while those on diets without cowpea recorded the best FCR. This clearly indicates that raw cowpea interfered with digestion which led to a poor conversion ratio. In a study by Akanji *et al*., (2016) which investigated the effect of raw and processed cowpea on growth and haematological profiles of broiler chickens, feed conversion efficiency (FCE) was significantly reduced in birds fed raw cowpea and dehulled cowpea, respectively. Akanji *et al.,* (2016) attributed the depression in growth to the presence of ANF (i.e., protease inhibitors) as they interfere with the digestion of protein and utilisation of minerals.

2.3.3 Weight gain

In a study conducted by Adino *et al*., (2018) in which the replacement of soyabean grain with raw cowpea grain (*Vigna unguiculata*) as protein supplement in Sasso x Rir crossbred chicks' diet, the control diet resulted in a significantly higher body weight gain in broiler chickens than in diets containing 30% cowpea. Adino *et al*., (2018) further argued that incorporation of cowpea in Sasso x Rir crossbred chick rations above 22.5% in total mixed ratio (TMR), resulted in a decrease in mean body weight gain. Georgeta, Vasilachi & Ropotă., (2022) found that inclusion at 10-20% had no significant effect on weight gain. Incorporation of cowpea at 22.5% percentage of raw cowpea in TMR, resulted in a progressive decline of the mean daily body weight gain. Based on these findings, cowpea grain can replace soyabean grain up to 75% without affecting body weight gain (BWG) (Heuzé V *et al*., 2012).

In a study by Akanji *et al*., (2016) which investigated the effect of raw and processed cowpea on growth and haematological profiles of broiler chickens, birds fed dehulled roasted cowpea had marginal reductions in weight gain, when compared to those fed dehulled cooked cowpea-based diet. In a study by Embaye *et al.,* (2018), wherein the effect of cowpea grain on growth performance of Cobb 500 broiler chickens was being studied, there were no significant differences on the final body weight gain and average daily weight gain in the starter phase. Final body weight gain in finisher phase was similar among treatments. There were no significant differences in final body weight gain and average daily weight gain between treatments across growth phases.

2.3.4 Mortality

A study by Embaye *et al.* (2018), investigating the effect of cowpea grain on growth performance of Cobb 500 broiler chickens reported sudden death syndrome of chickens on diets containing 5% and 10% of cowpea grains. In all treatments, a 5% mortality rate occurred at the finisher phase. Lameness also occurred in all treatments.

2.3.5 Carcass components

Awah-Ndukum *et al*. (2008) reported higher weights of the heart, liver and gizzard for birds fed with 14% boiled cowpea compared to the control diet. The increase in the size of liver and gizzard was related to increased activity to overcome the effect of toxic anti- nutritive compounds in the diets (Teguia *et al*., 2003 and Awah-Ndukum., 2008). In the study by Kana, Teguia and Fomekong (2012), the lowest gizzard relative weight was recorded with control birds as compared with birds on cowpea incorporated diets.

2.3.6 Meat quality

Laudadio & Tufarelli (2010) reported differences in the values of redness (a*) and the yellowness (b*) of breast and leg muscles, which were higher in broilers fed a diet with the inclusion of other legume plants (i.e., faba bean) compared to broilers on Black Soldier Fly larvae meal (BSFLM) based diets. Biesek *et al*., (2020) on pea or lupin as a substitute for SBM into broilers' diets also reported that the yellowness (b*) of leg muscles was significantly higher for birds on these protein sources. The change of protein source in the diet significantly influenced the colour of breast and thigh muscle (Georgeta, Vasilachi & Ropotă., 2022).

2.4 Black soldier fly larvae as feed for broilers

2.4.1 Biology of the Black soldier fly

The Black soldier fly *(Hermatia illucens)* is a fly (Diptera) of the *stratiomyidae* family. The adult fly is black, wasp-like and is 15-20 mm long (Rindhe *et al*., 2019). The larvae can reach 27 mm in length and 6 mm in width and weigh up to 220 mg in their last larval stage. The larvae have a dull and whitish colour. The larvae can feed effectively on rotting fruits and vegetables, coffee bean pulps, distiller grains, fish offal and animal manure (Van Huis *et al*., 2013). The BSF is naturally found in Asia and South America and can adapt to different environmental conditions. Natural habitats of the BSF are suitable for larval development, examples being damp places that contain animal wastes or organic decaying matter (Li *et al*., 2011).

Life cycle of the Black soldier fly

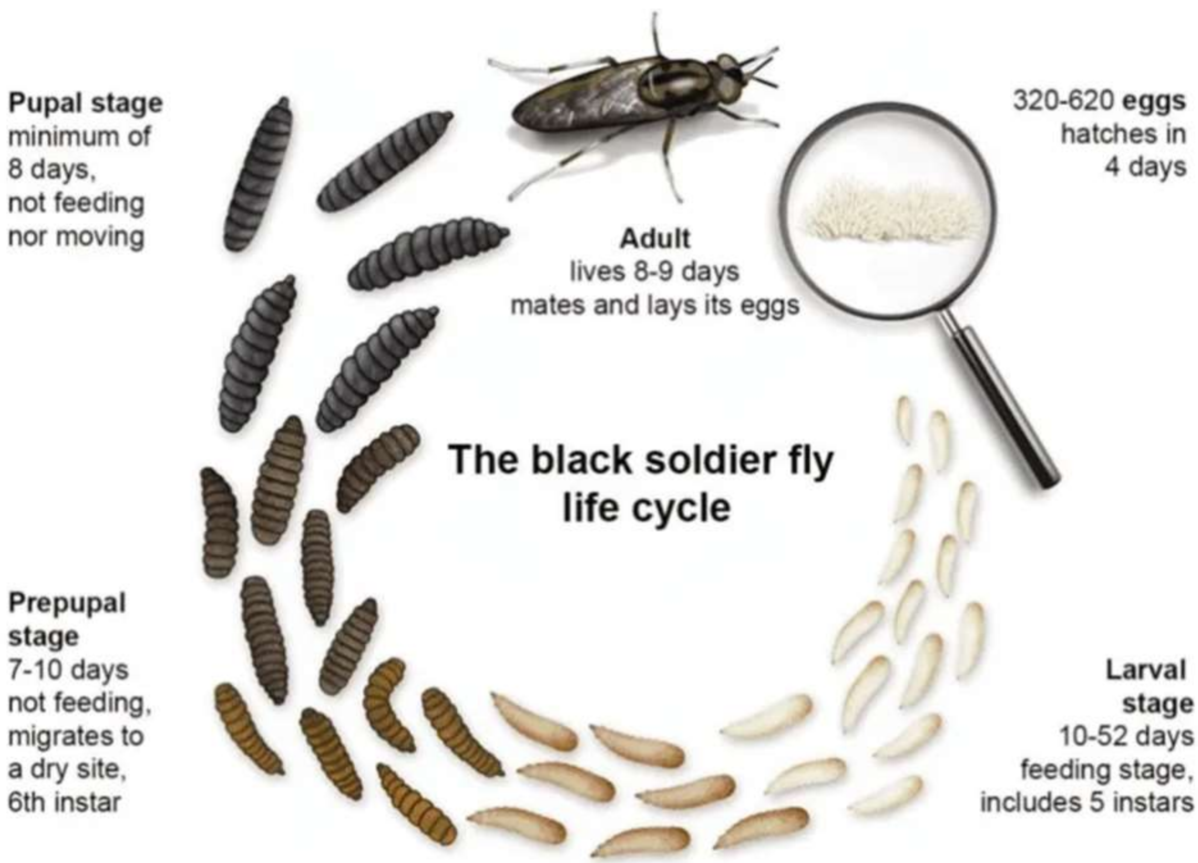

Figure 1: Life cycle of the black soldier fly (*Hermatia illucens*) larvae. Adopted from: Life stages of black soldier fly larvae (Chris, 2020).

2.4.2 Production of Black soldier fly larvae

2.4.2.1 Preparing adult Black soldier fly for egg laying

A fly cage is highly recommended (figure 2 below), for efficient breeding of the BSF, as it allows maximum amount of direct sunlight available to the setup which is key to successful mating. In the Southern hemisphere, when breeding indoor, it is crucial to ensure that the colony gets the window that is facing north, to maximise the amount of direct sunlight that is accessible to the cage. Temperature is kept above 23 degrees Celsius all the time for successful mating, and above 13 degrees Celsius to keep the flies alive. Humidity is kept around 50% (Rindhe *et al*., 2019).

Figure 2: Cages for breeding adult black soldier flies. Adopted from: Intensive Black Soldier Fly Farming (Rindhe *et al*., 2019).

2.4.2.2 Eggs harvesting, hatching and larval growing

In the process of egg harvesting, hatching and larval growing, eggies (figure 3) are supplied into the cages, which satisfy the requirements of the flies regarding a safe location (i.e., sheltered cavities) for egg deposition, as well as an “attractant” which mimics decomposing organic matter that attracts the female to lay eggs close by. Once the egg packages are deposited into the eggies, they are harvested before any larvae hatch (Dortmans *et al.,* 2017).

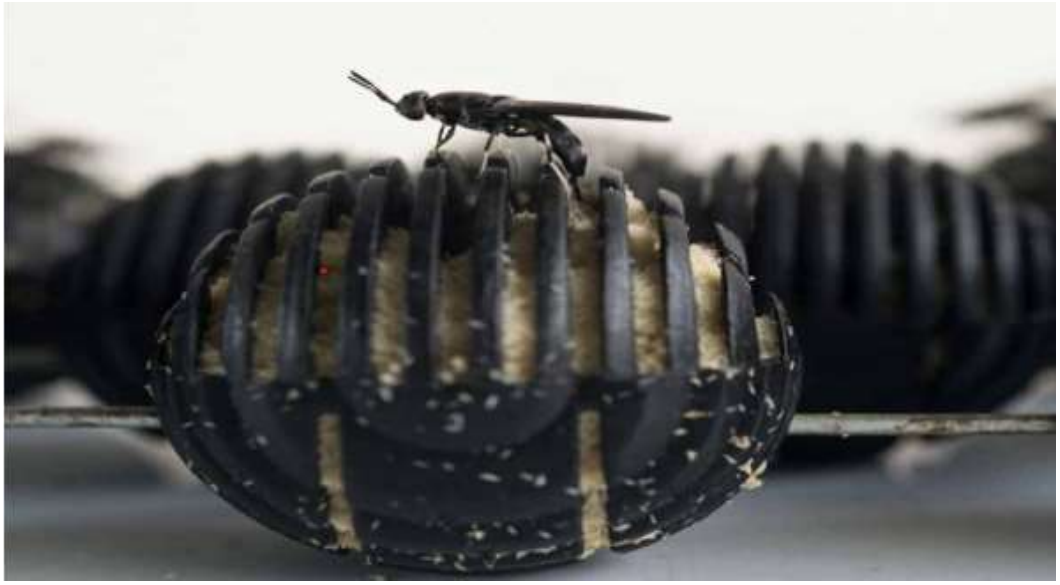

Figure 3: An image depicting "Eggies" for laying of eggs by the female BSF. Adopted from: Black Soldier Fly Biowaste Processing (Dortmans *et al.,* 2017)

The harvested "eggies" are placed together over an open "hatchling container" (Figure 4) with a high-quality food source. The closeness of the eggs to the decomposing organic matter ensures that the larvae have their first food source nearby after hatching. The larvae hatch over a period of several days, after hatching, the larvae fall from the "eggies" into the hatchling container below where they will start feeding immediately. The larvae feed voraciously on the decomposing organic matter and grow from a few millimetres size to around 2.5 cm length and 0.5 cm width with a cream-like colour. The growth of the larvae requires a period of 14-16 days. (Dortmans *et al.,* 2017)

Figure 4: An image showing hatching container for collected eggs with "Eggies". Adopted from: Black Soldier Fly Biowaste Processing (Dortmans *et al.,* 2017)

2.4.3 Harvesting and processing of Black soldier fly larvae meal

Dortmans *et al.,* (2017) indicated that BSFL can be harvested from the rearing substrates after 12 days. At this stage, the larvae would have reached their maximum weight, but have not yet transformed into pupae. Their nutritional value is, therefore, at its maximum. Harvesting is the process in which the larvae are separated from the frass. This can be done by using a manual or automated shaking sieve by which the larvae are easily separated from the residue. With a higher shaking frequency, the mesh size of the sieve can be bigger. This is because the larvae have difficulties to position themselves and cannot crawl through the mesh when there is a high shaking frequency. Automated shaking sieves can achieve higher shaking frequencies than manual sieves.

In a study done by Pasotto *et al.* (2020) killing of the BSFL larvae was performed by means of freezing at -20°C for 24h. Larvae were removed from the freezer and allowed to defrost at room temperature before drying in a ventilated oven for 24 h at 65°C until a constant weight was achieved. After drying, the larvae were milled through a 3 mm sieve using a Foss Knifetec 1095 sample mill (Höganäs, Sweden). The milled samples were stored at -20°C until mixing of the treatment diets.

2.5 Broiler responses to supplementary Black soldier fly larvae meal

2.5.1 Feed intake

The inclusion of increasing levels of partially defatted BSFL meal obtained by processing larvae reared on a vegetable by-product substrate for broilers (Ross 308), showed no differences in daily feed consumption during the periods from 10 to 35 days of age on (Dabbou *et al.,* 2018). Mohammed *et al.* (2017) reported that when 4% of BSFL meal was included in grower and finishing diets, they observed a feed intake of 152.2 (g/bird/day) which was similar to that of the control diet of 152.6 (g/bird/day), thus inclusion of BSFL meal in grower and finisher diets did not affect the feed intake. Onsongo *et al* (2018) found that insect diets had no effect on dietary intake in birds when fed at inclusion level of up to 15%. Feed intake increased when the level of inclusion of BSFL was increased (Schiavone *et al.* 2017). In contrast, Sumbule *et al.,* (2021) reported reduced feed intake with incremental levels of BSFLM in broiler diets.

2.5.2 Feed conversion ratio

A non-significant difference between the control diet and the diets containing 50% and 100% BSFL meal on feed conversion ratio (FCR) was observed in a study by (Schiavone *et al*., 2017). When different percentages of BSFL meal were included for the entire feeding cycle of broiler chickens (starter, grower, and finisher) there was no significant difference observed between the treatments. Amao *et al*. (2010) reported that replacing fishmeal with BSF larvae meal significantly increases the FCR values of experimental birds. However, Onsongo *et al*. (2018) reported that inclusion of up to 15% BSFLM in broiler diets did not significantly affect FCR. Similarly, Sumbule *et al*., (2021) found that the incorporation of BSFLM in grower diets did not show any significant effect on the overall FCR of the birds subjected to the various feed regimes. BSFLM inclusion levels decreased the feed conversion ratio by 10% in broilers fed on diets containing 20% BSFLM.

2.5.3 Weight gain

A linear decrease in daily/weekly weight gain was reported for diets containing BSFLM as the level of supplementation increased from 0 to 15% during the finisher period (Dabbou *et al,* 2018). Schiavone *et al.,* (2017), when replacing 50% and 100% of soyabean meal in broiler diets with Black Soldier Fly larvae meal (BSFLM) found that there was no difference in body weight gain for all treatments. Dietary inclusion of 4% BSFL meal in grower and finisher diets did not affect weight gain of the broiler chicken (Mohammed *et al.,* 2017). Inclusion of partially defatted and full fat BSFL meal at 5%-15% in the diet of broilers (Ross 308) did not significantly affect BWG. Onsongo *et al* (2018) found that replacing soyabean and fish meal with defatted BSFLM (4%, 8% and 12%) resulted in the 4% BSFLM inclusion diet attaining the highest feed intake in the grower phase. Replacing soyabean and fish meal with defatted BSFLM (4%, 8% and 12%) resulted in the control diet attaining the highest weight gain in the grower phase (Onsongo *et al*., 2018).

2.5.4 Mortality

In a study involving substitution of 50% and 100% soyabean meal with BSFL meal Schiavone *et al.,* (2017), reported that the chickens remained healthy (absence of clinical signs) for coccidiosis, respiratory infections and bacterial infections throughout the study and no mortalities were reported during the trial. Dabbou *et al.,* (2018), found that during the whole experimental period, the birds displayed all their vitality (no signs of illness were observed), and the mortality rate was zero in all the broiler groups fed graded levels of BSFL from day one through the finishing phase. Inclusion of the BSF meal (partially defatted and full fat BSFL meal at 5%) in the diet of broilers (Ross 308) resulted in no mortality, while the control group had a higher mortality rate of 7.55 % for the whole experimental period (Popova *et al*, 2020).

2.5.5 Carcass components

The inclusion of BSF meals in the broiler diet had no influence on the dressed weight of carcasses as well as the relative weight of internal organs i.e liver, heart, spleen, and gizzard (Nayohan *et al*., 2022). In a study conducted by Schiavone *et al.,* (2017), carcass traits were not affected by partial or total replacement of soya bean oil with BSLF.

2.5.6 Meat quality

BSFLM inclusion reduced meat pH of quail pectoralis major at 10-15% inclusion rate (Cullere *et al*., (2016). In a study by Cullere *et al.* (2016) breast meat had lower pH and a higher dripping loss. Meat with a pH close to the isoelectric point (5.2 to 5.5) of its constituting proteins has a lower water holding capacity, thus giving a more intense cooking loss (Cullere *et al.,* 2016).

Cullere *et al*., (2016) reported a non-significant difference in L and b values, however the redness index was high in 10% BSFLM inclusion and lower at 15% inclusion. Contrarily, breast meat obtained from broilers fed 15% dietary BSF larvae meal showed higher redness (a*) index, while birds that were fed 5% and 10% exhibited middle levels in comparison to the control (Abd El-Hack *et al*., 2020). Abd El-Hack *et al*., (2020) reported a linear decrease for the yellowness value (b*) with increasing BSF larvae meal content. No differences were observed between larvae-fed and chickens on the control feed regarding the colour of breast muscles (Moula *et al*., 2018). Schiavone *et al*., (2017) reported a linear increase of meat redness to a maximum inclusion of 15% BSF and a decrease in yellowness with an increase in BSFLM inclusion.

2.6 Summary of literature review

Broiler diets that contain different levels of BSFL meal and processed cowpea do not have significant effects on feed intake and feed conversion ratio, but raw cowpea has negative effects on the feed intake and feed conversion ratio, which results in low weight gain in broiler chickens. Feeding BSFL meal in place of processed cowpeas produces higher body weight gain in broilers. Overall, most researchers did not report any mortalities when including different levels of BSFL meal and cowpea in broiler diets. BSFL meal has higher crude protein when compared to cowpea meal. Feeding BSFL meal results in higher carcass weight of broilers as compared to those fed soyabean meal-based diets. It can be said in general, that carcasses of broilers fed cowpea-based were of significantly poor quality as compared to the carcasses of broilers fed soyabean based diets.

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Location and description of the study area

The study was conducted in March 2022, at the University of Venda experimental farm located in Thohoyandou, Limpopo Province, South Africa, coordinates 22.9761° S, 30.4465° E and 596m above sea level. The mean daily temperature is in the range 25°C-40°C in summer, and 12°C-26°C in winter. The annual average rainfall is 800mm, which is highly temporally variable, with 95% occurring between October and March (Mzezewa & Gwata, 2012).

3.2 Preparation of experimental diets

3.2.1 Cowpea processing

The cowpeas used in the study were sourced from a local retailer. The bulk grain was manually cleaned of dirt and damaged grains, prior to sterilization in batches by soaking for 30 minutes in a 2.5% sodium hypochlorite solution. Residual bleach was washed off, and the grain soaked in tap water over night (about 12 hours), prior to a 4-day irrigated open-air sprouting at ambient conditions. Sprouting was terminated by rapid sun-drying of sprouts thinly spread on black plastic sheeting laid on a concrete floor.

3.2.2 Black soldier fly larvae production

The Black soldier fly larvae used in the study were sourced from AEGIS Environmental (Pty) Ltd, Gauteng, South Africa. The production process is multi-step; a breeding unit for maintaining the breeder flies, hatchery where fly eggs are hatched into larvae at 32°C and 80% relative humidity and stay for 4 days, transfer of the larvae to the rearing unit for growing in a medium of vegetable waste, larvae harvesting after 13 days of growth, larvae drying to constant weight in a ventilated oven at 65°C.

3.2.3 Formulation and processing of diets

Prime Broiler Starter (Product V17767, Meadow Feeds (Pty) Ltd, Delmas, South Africa, containing 200.0 g/Kg/Min protein, 25.0 g/Kg/Min fat, 50.0 g/Kg/Max fiber and 12.0 g/Kg/Min total Lysine) was used to feed the 360 experimental birds for the first 21 days. Experimental diets were then prepared for evaluation during the growing and finishing phases. The basal dietary formulation used maize meal as the energy source, and sprouted cowpea meal as the protein source, with 0% as the negative control (NC), 5% or 10 BSFLM for the different treatments. Grower and finisher diets were formulated to match the nutrient composition of the respective positive controls (PC), which were Meadow Feeds' Budget Broiler Grower (Product V17768, 180.0 g/Kg/Min protein, 25.0 g/Kg/Min fat, 50.0 g/Kg/Max fibre and 10.0 g/Kg/Min total Lysine) and Budget Broiler Finisher (Product: V17810, 160.0 g/Kg/Min protein, 25.0 g/Kg/Min fat, 70.0 g/Kg/Max, 60.0 g/Kg/Max fibre and 9.0 g/Kg/Min total Lysine) commercial diets. To evaluate the effects of contrasting fat content between the NC and the 10% BSFLM diet, two diets at the mid (5%) BSLFM dietary inclusion level were formulated to contain low fat (LF)) versus high fat (HF), which respectively approximated the contrast in fat content between the 0 and 10 % BSFLM diets. Diets were formulated and fortified with micronutrient additives to meet the minimum feeding standards for grower and finisher broiler diets (National Research Council (NRC), 1998). To avoid clogging the hammer mill sieve, the dried larvae were first mixed into the total diet along with all other feed ingredients prior to milling through a 3-mm sieve. The chemical composition of the dietary ingredients is presented in Table 3.1. The ingredients and chemical contents of the test diets are presented in Tables 3.2.

Table 3.1: Analyzed chemical composition of feed ingredients (DM Basis)

Components	Maize	Raw Cowpea	[1]Sprouted Cowpeas	[2]Black Soldier Larvae Meal
Dry matter (g kg $^{-1}$)	863.0	926.0	876.0	927.0
Ash (g kg $^{-1}$)	32.4	42.0	73.7	85.2
Crude protein (g kg $^{-1}$)	94.3	258.6	297.4	375.1
Fat (g kg $^{-1}$)	60.2	15.7	12.7	422.8
Neutral detergent fibre (g kg $^{-1}$)	210.9	372.8	357.8	325.0
Acid detergent fibre (g kg $^{-1}$)	42.1	124.9	155.5	86.9
Ca (g kg $^{-1}$)	0.1	0.7	1.3	11.7
P (g kg $^{-1}$)	3.6	4.0	5.2	6.4
Essential amino acids (g/100g)				
Arginine	0.05	0.15	0.20	0.22
Alanine	0.06	0.07	0.12	0.27
Asparagine	0.05	0.14	0.35	0.32
Glutamine	0.15	0.27	0.43	0.42
Glycine	0.04	0.07	0.13	0.24
Histidine	0.04	0.07	0.11	0.13
Isoleucine	0.03	0.08	0.12	0.18
Leucine	0.10	0.18	0.23	0.30
Lysine	0.03	0.15	0.12	0.18
Methionine	0.01	0.02	0.05	0.11
Phenylalanine	0.04	0.10	0.20	0.23
Proline	0.07	0.07	0.13	0.23
Serine	0.04	0.09	0.14	0.17
Threonine	0.03	0.06	0.11	0.15
Tyrosine	0.03	0.03	0.12	0.42
Valine	0.04	0.09	0.14	0.24

[1]Soaked for 12 hours, 4-day open-air sprouting, rapidly sun-dried. [2]Full-fat, Produced by AEGIS Environmental (Pty) Ltd, Gauteng, South Africa – eggs hatched into larvae at 32°C and 80% relative humidity, larvae transferred to rearing unit after 4-days, cultured on mixed vegetable waste, harvested after 13 days growth, dried to constant weight in a ventilated oven at 65°C.

Table 3.2: Ingredient and analysed chemical composition of test diets

Composition	[1]Diets							
	Grower				Finisher			
	0% BSLM	HF5% BSFLM	LF 5% BSFLM	10% BSFLM	0% BSFLM	HF 5% BSFLM	LF 5% [3]BSFLM	10% BSFLM
Ingredients (% DM basis)								
[2]Sprouted cowpeas	50.3	43.0	41.7	33.5	40.4	32.5	32.5	24.0
Maize	41.0	41.5	45.5	48.0	52.4	53.4	54.0	57.5
Vegetable oil	0.7	1.7	0.0	0.0	0.4	1.8	0.0	0.0
[3]Grower macro pack	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Sand	0.8	1.9	0.9	2.5	0.3	0.8	2.0	1.9
BSF Meal	0.0	5.0	5.0	10.0	0.0	5.0	5.0	10.0
Lysine	0.0	0.0	0.0	0.2	0.0	0.1	0.1	0.2
Methionine	0.6	0.6	0.6	0.0	0.4	0.4	0.4	0.4
Mono Dicalcium Phos	1.5	1.5	1.5	1.3	1.5	1.5	1.5	1.7
Limestone	1.1	0.8	0.8	0.5	0.6	0.5	0.5	0.3
Salt	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Chemical composition (g kg $^{-1}$ DM)								
Ash	169.0	130.0	159.7	125.0	169.0	130.0	159.7	125.0
Fat	40.5	42.9	29.3	54.0	40.5	42.9	29.3	54.0
Acid detergent fibre	77.2	88.9	79.2	76.8	77.2	88.9	79.2	76.8
Neutral detergent fibre	258.5	278.9	289.0	300.3	258.5	278.9	289.0	300.3
Crude protein	183.0	195.8	214.7	184.5	183.0	195.8	214.7	184.5
Ca	14.0	14.5	12.7	32.1	14.0	14.5	12.7	32.1
P	10.2	8.7	12.0	9.1	10.2	8.7	12.0	9.1

[1]BSFLM- Black Soldier fly larvae meal, full fat, produced by AEGIS Environmental (Pty) Ltd, Gauteng, South Africa – eggs hatched into larvae at 32°C and 80% relative humidity, larvae transferred to rearing unit after 4-days, cultured on mixed vegetable waste, harvested after 13 days growth, dried to constant weight in a ventilated oven for 24 h at 65°C. HF-High fat, LF- Low fat-LF. [2]Soaked for 12 hours, 4-day open-air sprouting, rapidly sun-dried. [3] Trouw Nutrition South Africa (Pty) Ltd: Broiler Grower Macro pack: Vitamin A -294117.700UI, Vitamin D3 -58823.530UI, Vitamin E -882.353UI, Vitamin K -58.824 mg, Vitamin B1 -58.824mg, Vitamin B2 -161.765mg, Vitamin B6 -117.647mg, Niacin -1029.412mg, Calcium Pantothenate- 323.529mg, Biotin -2941.177mcg, Folic acid -23.529mg, Vitamin B12 -588.235mcg, Choline Chloride -7639.412mg, 6-Phytase (FTU) -29411.770FTU, Lasalocid -2647.059mg, Zinc bacitracin -661.765mg, Limestone (as carrier)-485.294g, Salt -117.647g, Mono-dicalcium phosphate -294.118g, Cobalt from cobalt sulphate -14.706mg, Copper from Copper sulphate -220.588mg, Iron from Ferrous sulphate -588.235mg, Iodine from Potassium iodide -29.412mg, Manganese from Manganese sulphate -2352.941mg, Zinc from Zinc sulphate -1470.588mg, Selenium from Sodium selenite -8.824mg, Lysine -5.882g, Methionine -38.235g. Enzyme Natuphos E 10000 G -2.941g. [4]Trouw Nutrition South Africa (Pty) Ltd: Broiler finisher Macro pack:. Vitamin A -303030.300UI, Vitamin D3 -60606.060UI, Vitamin E -757.576UI, Vitamin E Equivalent -151.515UI, Vitamin K -60.606mg, Vitamin B1 -60.606mg, Vitamin B2 -166.667mg, Vitamin B6 -121.212mg, Niacin -1060.606mg, Calcium Pantothenate -333.333mg, Biotin -3030.303mcg, Folic acid -24.242mg, Vitamin B12 -606.061mcg, Choline Chloride -7870.909mcg, 6 -Phytase (FTU)-30303.030FTU, Lasalocid -2727.273mg, Zinc bacitracin -681.818mg, Limestone (as carrier) -544.182g, Salt -121.212g, Mono-dicalcium phosphate -272.727g, Cobalt from cobalt sulphate -15.152mg, Copper from Copper sulphate -227.273mg, Iron from Ferrous sulphate -606.061mg, Iodine from Potassium iodide -30.303mg, Manganese from Manganese sulphate -2424.242mg, Zinc from Zinc sulphate -1515.151mg, Selenium from Sodium selenite -9.091mg, Methionine -3.030g, Enzyme Natuphos E 10000 G -3.030g.

3.3 Housing, experimental design and broiler management

The trial used 360-day-old Ross 308 broilers reared in an open, deep litter house. The birds were subjected to brooding, lighting, vaccination, hygiene, and biosecurity management to simulate local small-scale production, as previously described by Benyi *et al*. (2015). The broiler house measured 17.0 m × 9.0 m, with the trial confined to a section partitioned by wire mesh into 30 experimental pens of 150 cm length × 144 cm width. The chicks were uniformly managed throughout the starter phase (day 1 - 21), including the same starter diet, and supplementary stress vitamins (Virbac© Samrand Business Park, Centurion, Pretoria, South Africa) during days 1-6. During week 3 after placement, the chicks were sexed and allocated by sex to experimental pens at a stocking density of 12 birds per pen. During the grower (days 22-35) and finisher phases (day 36-42), the sexed broilers, were randomly allocated to treatments for a 5 (diets) X 2 (sex) factorial experiment which was replicated three times. Each pen an adjustable height, 390 mm diameter tube feeder, and a 360 mm diameter bell drinker. A coccidiostat (Esb3, Reg. No./Nr. G1305) was administered in drinking water from day 22-39 at 14g per 14 litres of water since the feed formulated in this study was not medicated with a coccidiostat. Birds were fully vaccinated as per recommendations (Table 3.3)

Table 3.3: Vaccination programme of the birds during the experiment

Age in days	Vaccination	Administration route
8	Infectious Bronchitis	Drinking water
12	Gumboro	Drinking water
18	Gumboro	Drinking water
23	Newcatle Disease	Drinking water
30	Newcastle Disease	Drinking water

3.4 Measurements

3.4.1 Growth, slaughter performance and meat quality evaluation

Pen feed intake, body weight (random 4-birds/pen) gain, and the calculated feed conversion ratio were evaluated weekly. The results were calculated as daily feed intake, daily weight gain. Mortality was recorded whenever deaths occurred. On the 38th day, birds were left for 12hrs without feed but with access to water in preparation for slaughter on the 39th day. At slaughter, three (3) random chickens from each replicate were picked for carcass measurements. The chickens were slaughtered humanely, dressed and the carcass components dissected as described by Benyi *et al*., (2015). Weighments of the carcass, thighs, breasts, and wings were taken, as well as those of the visceral organs and tissues. Carcass components and viscera were scaled to percentages of the warm carcass and the live weight, respectively.

Meat colour was evaluated on 30 mm thick x 20 mm wide x 10 mm long samples dissected from a fresh breast fillet. Quality was evaluated using a Lab Scan-XE (Hunter Associates Laboratory Inc. USA) spectrophotometer to measure lightness (L*), Redness (a*), and Yellowness (b*), and to calculate the colour difference according to Mohamed *et al*. (2008). Drip loss was estimated using the bag-loss procedure (Bowker and Zhuang, 2015). Samples 20 mm thick x 30 mm wide x 50 mm long dissected from the center of one fresh breast fillet were placed in a zip-sealable biodegradable plastic bag and stored at 4◦C for re-weighing 72 hours postmortem. Breast meat pH (basic 20 pH meter, Crimson Instruments, SA EU) was measured from 10g tissue slurries produced using a homogenizer (stomacher, model pb international. L7) in 90 ml distilled water (Zhang *et al*., 2012). Breast samples were weighed using a digital scale (Precision balance, J series RS323, South Africa) before being refrigerated for 3 days at 4˚C according to a procedure by Bowker and Zhuang. (2015).

3.4.2 Chemical analysis

Feed and dietary chemical components were analysed using official AOAC (2000) methods for dry matter (DM) (method 934.01), Crude protein (method 954.01), Crude fibre (method 978.10), Crude fat (method 920.39) and Ash (method 942.05). Acid detergent fibre (ADF) and Neutral detergent fiber (NDF) were determined using the method of Licitra, Hernandez & Van Soest. (1996).

3.5 Statistical analysis

The General Linear Model (GLM) procedures of SAS (SAS, 2017) was used to analyse the 1-week finisher phase, and all slaughter parameters in a 5 (diet) x 2 (sex) factorial arrangement within a complete randomized design (Model I), and the 2-week grower phase, and the cumulative three-week growth performance parameters in a 5 (diet) x 2 (sex) factorial arrangement in a randomized complete block ("week") design (model II);

Model 1

$$\boldsymbol{Yijk = \mu + Di + Si + (DS)ij + \epsilon ijk}$$

Where:

y_{ijk} = k^{th} Observation on birds on the i^{th} Diets and of the j^{th} sex

μ = the overall mean,

D_i = the effect of diets ($i_{1,2,3,4,5}$),

S_i = the effect of the j[th] sex ($j=_{1,2}$),

$(DS)_{ij}$ = respective diets and sex interaction,

e_{ijk} = the random error.

Model 2

$$Yijk = \mu + Di + Sj + Wk + (DS)ij + (DW)ik + (SW)jk + (DSW)ijk + \in ijk$$

Where:

$\mathbf{Y}_{ijkl}$ = The l[th] observation on birds on the i[th] diets, of the j[th] sex, during the k[th] week

μ = the overall mean,

D_i = the effect of diets ($i_{1,2,3,4,5}$),

s_i = the effect of the j[th] sex ($j_{1,2}$),

W_k = Effect of the k[th] week ($k_{4,5,6}$),

$(DS)_{ij}$ $(DW)_{ik,}$ $(SW)_{jk}$,$(DSW)_{ijk}$ = respective sex, diets and week interactions,

e_{ijkl} = the random error.

3.6 Ethical Considerations

Ethical approval was granted by the University of Venda, research ethics committee (certificate number: FSEA/21/ANS/02/0203).

CHAPTER 4

RESULTS

. Initial live weight was similar ($p>0.05$) across dietary treatments. During the grower phase, broilers on the PC consumed more ($p<0.05$) feed with higher weight gain ($p<0.05$) than those on the NC and LF 5% BSFLM diets and achieved lower ($p<0.05$) FCR compared to broilers on the NC and HF 5% BSFLM. During the finisher phase, broilers on the PC consumed more ($p<0.05$) feed than those on the 10% BSFLM diet. Cumulatively, through the grower-finisher phases, broilers on the PC had higher feed intake and weight gain ($p<0.05$) than those on the NC and HF 5% BSFLM diets and attained higher ($p<0.05$) final live weight with lower ($p<0.05$) FCR compared to broilers on the NC. Male broilers consumed more ($p<0.05$) feed during the grower phase and by slaughter, with higher ($p<0.05$) weight gain during finishing, which resulted in heavier ($p<0.05$) live weight at slaughter. There was no significant ($p>0.05$) sex * diet interaction on parameters measuring the growth performance.

Table 4.2 shows scaled weights for dietary effects of carcass components, visceral organs, and tissues. Broilers on the PC had higher ($p<0.05$) breast weights compared to birds on the NC and the 10% BSFLM diets and had higher ($p<0.05$) thigh weights than those on all other treatments. Broilers on the PC had a larger ($p<0.05$) spleen compared to birds on the NC and the 5% BSFLM diets. The females had smaller thighs and livers compared to the males ($p<0.05$).

Tables 4.3 and 4.4 respectively present treatment effects on parameters which describe breast meat keeping quality, and the meat colour. The NC diet had higher dripping loss compared to the 10% BSFLM diet ($p<0.05$).

Table 4.1: Effects of maize-sprouted cowpea diets fortified with Black soldier fly larvae meal and sex on production performance of Ross 308 broiler chickens.

Treatments		Initial LW (g/bird)	Growth Parameters												
			Grower (22 to 35 days)				Finisher (36 to 42 days)				Slaughter				
			Weight gain (g/bird/day)	Feed Intake (g/day)	FCR	Mortality (%)	Weight gain (g/bird/day)	Feed intake (g/bird/day)	FCR	Mortality (%)	Final live Weight (g/bird)	Weight gain (g/bird/day)	Feed intake (g/day)	FCR	Mortality (%)
Diets															
	NC	1009.2	37.85^{b}	103.41^{b}	2.80^{a}	0.69	64.86	127.81^{ab}	2.00	1.39	1703.8^{b}	46.86^{b}	111.55^{b}	2.53^{a}	0.92
	PC	962.3	57.70^{a}	114.98^{a}	2.01^{b}	0.69	69.09	155.57^{a}	2.31	0.00	1988.7^{a}	61.50^{a}	128.51^{a}	2.11^{b}	0.00
	5% LF BSFLM	940.0	44.65^{b}	101.44^{b}	2.37^{ab}	0.00	60.62	129.38^{ab}	2.27	8.33	1722.9^{ab}	49.98^{b}	112.45^{b}	2.34^{ab}	2.77
	5% HF BSFLM	924.6	46.03^{ab}	114.65^{a}	2.55^{a}	0.00	78.68	136.44^{ab}	1.76	6.94	1841.3^{ab}	56.91^{ab}	121.91^{ab}	2.28^{ab}	2.31
	10% BSFLM	933.5	46.86^{ab}	108.69^{ab}	2.46^{ab}	0.00	69.44	126.38^{b}	1.82	5.56	1785.8^{ab}	54.38^{ab}	114.59^{b}	2.25^{ab}	2.31
	SEM	23.000	3.030	2.430	0.130	0.444	5.580	6.870	0.160	2.560	63.80	2.550	2.700	0.100	0.910
Sex															
	F	913.80^{b}	46.52	103.70^{b}	2.35	0.27	62.30^{b}	130.86	2.16	2.78	1735.8^{b}	51.78	113.43^{b}	2.28	1.11
	M	994.00^{a}	46.71	113.57^{a}	2.53	0.27	74.77^{a}	139.37	1.90	6.11	1881.2^{a}	56.07	122.17^{a}	2.32	2.22
	SEM	14.600	1.920	1.540	0.080	0.280	3.530	4.340	0.100	1.620	40.40	1.610	1.700	0.060	0.580
Sex	**Diets**														
	NC	951.2	37.65	100.39	2.76	0.00	58.47	123.63	2.1	0.00	1654.2	44.59	108.14	2.54	0.93
	PC	947.1	59.12	110	1.89	0.00	63.88	151.89	2.41	2.78	1921.7	60.71	123.96	2.06	0.00
Female	5% LF BSFLM	932.9	42.36	98.75	2.4	0.00	55.41	129.39	2.52	2.78	1659.2	46.71	112.35	2.44	0.93
	5% HF BSFLM	870	45.91	109.04	2.5	0.00	68.88	125.11	1.83	5.56	1732.5	53.57	114.4	2.27	1.85
	10% BSFLM	867.9	47.58	100.3	2.22	1.39	64.86	124.3	1.91	2.78	1711.7	53.34	108.3	2.11	1.85
	NC	973.3	38.06	106.43	2.85	1.39	71.25	131.99	1.9	0.00	1753.3	49.12	114.95	2.53	0.93
	PC	1071.3	56.28	119.97	2.14	0.00	74.3	159.25	2.19	0.00	2055.8	62.29	133.07	2.15	0.00
	5% LF BSFLM	947.1	46.94	104.13	2.35	0.00	65.83	129.37	2.01	13.89	1786.7	53.24	112.54	2.23	4.63
Male	5% HF BSFLM	979.2	46.14	120.26	2.61	0.00	88.47	147.76	1.68	8.33	1950.0	60.25	129.43	2.3	2.78
	10% BSFLM	999.2	46.13	117.08	2.71	0.00	74.02	128.47	1.73	8.33	1860.0	55.43	120.88	2.38	1.85
	SEM	32.60	4.290	3.440	0.180	0.630	7.890	9.710	0.230	3.620	90.300	3.600	3.810	0.140	1.300
P Values															
Diet		0.834	0.001	0.000	0.002	0.138	0.256	0.039	0.083	0.332	0.000	0.004	0.000	0.121	0.241

Sex	0.117	0.945	0.000	0.128	0.161	0.021	0.182	0.093	0.252	0.000	0.093	0.000	0.727	0.171
Diet x Sex	0.903	0.930	0.476	0.637	0.389	0.965	0.815	0.932	0.736	0.316	0.948	0.610	0.673	0.314

For each factor or combination of factors, means in the same column not sharing a common superscript are significantly different (P<0.05). [1]Diets; Negative control= Sprouted cowpea-based diet; Positive control= Meadow Feeds' Budget Broiler Grower (Product V17768) and Budget Broiler Finisher (Product: V17810) commercial diets, BSFLM- Black Soldier fly larvae meal, full fat, produced by AEGIS Environmental (Pty) Ltd, Gauteng, South Africa – eggs hatched into larvae at 32°C and 80% relative humidity, larvae transferred to rearing unit after 4-days, cultured on mixed vegetable waste, harvested after 13 days growth, dried to constant weight in a ventilated oven for 24 h at 65°C, 5% LF BSFLM= 5% Black soldier fly larvae meal low fat diet; 5% HF BSFLM= 5% Black soldier fly larvae meal high fat diet; 10% BSFLM= 10% Black soldier fly larvae diet; SEM: Standard Error of Mean

Table 4.2: Effect of maize-sprouted cowpea diets fortified with Black soldier fly larvae and sex on weight (grams) of carcass components, visceral organs, and tissues of Ross 308 broiler chickens.

Treatments		Carcass components (% Dressed weight)			Viscera (% Live weight)				
		Breast	Thigh	Wing	Heart	Spleen	Gizzard	Liver	Abdominal fat
Diet									
	Negative control	27.86^{b}	11.46	5.35	0.63	0.15	3.19^{ab}	2.68^{a}	1.98^{ab}
	Positive control	31.11^{a}	11.44	4.94	0.63	0.18	2.97^{b}	2.18^{b}	1.43^{b}
	5% LF BSFLM	29.14^{b}	11.07	5.37	0.62	0.15	3.65^{a}	2.77^{a}	2.13^{a}
	5% HFBSFLM	29.73^{ab}	10.99	5.02	0.65	0.15	3.33^{ab}	2.66^{a}	2.16^{a}
	10% BSFLM	29.01^{b}	11.26	5.14	0.62	0.18	3.45^{a}	2.54^{a}	2.21^{a}
	SEM	0.443	0.166	0.154	0.025	0.012	0.108	0.062	0.135
Sex									
	Female	29.84^{a}	11.00^{b}	5.19	0.63	0.16	3.34	2.57	2.12^{a}
	Male	28.77^{b}	11.48^{a}	5.14	0.64	0.16	3.29	2.57	1.85^{b}
	SEM	0.280	0.105	0.098	0.016	0.007	0.686	0.039	0.086
Interactions									
Sex	**Diet**								
Females	Negative control	27.44^{c}	11.25	5.63	0.62	0.15	3.14	2.78	1.93^{ab}
	Positive control	31.38^{a}	11.33	4.97	0.65	0.18	3.08	2.23	1.60^{ab}
	5% LF BSFLM	29.89^{abc}	10.83	5.49	0.61	0.15	3.65	2.69	2.31^{a}
	5% HFBSFLM	31.27^{ab}	10.55	4.87	0.65	0.15	3.44	2.62	2.38^{a}
	10% BSFLM	29.26^{abc}	11.05	4.99	0.63	0.18	3.4	2.53	2.38^{a}
Males	Negative control	28.28^{abc}	11.66	5.08	0.65	0.14	3.25	2.58	2.03^{ab}
	Positive control	30.85^{ab}	11.56	4.91	0.62	0.18	2.87	2.14	1.27^{b}
	5% LF BSFLM	28.39^{abc}	11.31	5.25	0.63	0.15	3.64	2.85	1.96^{ab}
	5% HFBSFLM	28.24^{bc}	11.43	5.18	0.66	0.14	3.21	2.7	1.94^{ab}
	10% BSFLM	28.77^{abc}	11.47	5.29	0.63	0.17	3.49	2.54	2.05^{ab}
	SEM	0,626	0.235	0.218	0.036	0.176	0.153	0.087	0.192
P Values									
Diets		0.000	0.003	0.092	0.051	0.003	0.043	0.024	0.046
Sex		0.336	0.005	0.052	0.138	0.783	0.087	0.024	0.002
Diet x Sex		0.808	0.985	0.560	0.892	0.983	0.382	0.601	0.034

For each factor or combination of factors, means in the same column not sharing a common superscript are significantly different (P<0.05). [1]Diets; Negative control= Sprouted cowpea-based diet; Positive control= Meadow Feeds' Budget Broiler Grower (Product V17768) and Budget Broiler Finisher (Product: V17810) commercial diets, BSFLM- Black Soldier fly larvae meal, full fat, produced by AEGIS Environmental (Pty) Ltd, Gauteng, South Africa – eggs hatched into larvae at 32°C and 80% relative humidity, larvae transferred to rearing unit after 4-days, cultured on mixed vegetable waste, harvested after 13 days growth, dried to constant weight in a ventilated oven for 24 h at 65°C, 5% LF BSFLM= 5% Black soldier fly larvae meal low fat diet; 5% HF BSFLM= 5% Black soldier fly larvae meal high fat diet; 10% BSFLM= 10% Black soldier fly larvae diet; SEM: Standard Error of Mean

Table 4.3: Effect of maize-sprouted cowpea diets fortified with Black soldier fly larvae meal and sex on meat quality (meat pH and dripping loss) of Ross 308 broiler chicken.

	Treatments	Meat quality parameter	
		pH	Drip Loss
Diet			
	Negative control	5.95	2.84[a]
	Positive control	5.94	2.48[ab]
	LF 5% BSFLM	5.96	2.62[ab]
	HF 5% BSFLM	5.98	2.26[ab]
	10% BSFLM	6.02	1.87[b]
	SEM	0.041	0.193
Sex			
	Female	5.98	2.37
	Male	5.96	2.36
	SEM	0.026	0.122
Interaction			
Sex	Diets		
Females	Negative Control	5.95	2.91
	Positive Control	5.91	2.38
	LF 5% BSFLM	5.98	2.60
	HF 5% BSFLM	5.97	2.32
	10% BSFLM	6.08	1.63
Males	Negative Control	5.94	2.76
	Positive Control	5.97	2.59
	LF 5% BSFLM	5.95	2.64
	HF 5% BSFLM	5.98	1.69
	10% BSFLM	5.97	2.11
	SEM	0.059	0.272
P Values			
Diet		0.633	0.028
Sex		0.679	0.960
Diet x Sex		0.701	0.506

For each factor or combination of factors, means in the same column not sharing a common superscript are significantly different ($P<0.05$).
[1]Diets; Negative control= Sprouted cowpea-based diet; Positive control= Meadow Feeds' Budget Broiler Grower (Product V17768) and Budget Broiler Finisher (Product: V17810) commercial diets, BSFLM- Black Soldier fly larvae meal, full fat, produced by AEGIS Environmental (Pty) Ltd, Gauteng, South Africa – eggs hatched into larvae at 32°C and 80% relative humidity, larvae transferred to rearing unit after 4-days, cultured on mixed vegetable waste, harvested after 13 days growth, dried to constant weight in a ventilated oven for 24 h at 65°C, 5% LF BSFLM= 5% Black soldier fly larvae meal low fat diet; 5% HF BSFLM= 5% Black soldier fly larvae meal high fat diet; 10% BSFLM= 10% Black soldier fly larvae diet; SEM: Standard Error of Mean

Table 4.4: Effect of maize-sprouted cowpea diets fortified with Black soldier fly larvae meal and sex on meat color parameters of Ross 308 broiler chickens.

Treatments		Breast Meat Colour Parameters					
		a*	b*	L*	Chroma	Hue	Colour difference
Diets							
	Negative control	9.33	19.97	36.10	20.17	65.10	19.85
	Positive control	8.77	17.66	32.09	19.80	62.50	22.95
	5% LF BSFLM	9.24	20.28	35.84	22.38	65.83	21.79
	5% HF BSFLM	9.48	19.71	33.67	21.95	64.54	23.91
	10% BSFLM	8.98	19.97	36.48	21.99	65.89	21.15
	SEM	0.750	0.751	1.350	1.250	1.720	1.280
Sex							
	F	9.35	20.14	35.55	21.53	65.31	22.24
	M	8.97	18.89	34.12	20.98	64.24	21.63
	SEM	0.474	0.475	0.851	0.790	1.090	0.808
Interaction							
Sex	**Diets**						
Female	Negative control	9.13	20.5	36.16	18.61	65.91	20.17
	Positive control	8.94	18.89	34.99	20.96	64.99	20.89
	5% LF BSFLM	10.43	19.47	36.66	23.65	64.13	21.43
	5% HF BSFLM	10.07	19.92	33.07	22.41	63.53	27.35
	10% BSFLM	8.21	20.36	36.89	22.01	68.03	21.34
Male	Negative control	9.53	19.45	36.04	21.96	64.31	19.53
	Positive control	8.61	16.47	29.27	18.64	60.03	25.02
	5% LF BSFLM	8.06	19.47	35.02	21.11	67.54	22.15
	5% HF BSFLM	8.90	19.50	34.26	21.48	65.56	20.47
	10% BSFLM	9.75	19.58	36.08	21.96	63.76	20.97
	SEM	1.060	1.060	1.900	1.770	2.430	1.810
P Values							
Diets		0.936	0.134	0.140	0.486	0.632	0.234
Sex		0.577	0.077	0.247	0.628	0.490	0.601
Diets X Sex		0.438	0.899	0.448	0.517	0.352	0.081

For each factor or combination of factors, means in the same column not sharing a common superscript are significantly different (P<0.05). [1]Diets; Negative control= Sprouted cowpea-based diet; Positive control= Meadow Feeds' Budget Broiler Grower (Product V17768) and Budget Broiler Finisher (Product: V17810) commercial diets, BSFLM- Black Soldier fly larvae meal, full fat, produced by AEGIS Environmental (Pty) Ltd, Gauteng, South Africa – eggs hatched into larvae at 32°C and 80% relative humidity, larvae transferred to rearing unit after 4-days, cultured on mixed vegetable waste, harvested after 13 days growth, dried to constant weight in a ventilated oven for 24 h at 65°C, 5% LF BSFLM= 5% Black soldier fly larvae meal low fat diet; 5% HF BSFLM= 5% Black soldier fly larvae meal high fat diet; 10% BSFLM= 10% Black soldier fly larvae diet; SEM: Standard Error of Mean

CHAPTER 5

DISCUSSION

The present study evaluated the efficacy of sprouted cowpea-maize grower/finisher broiler diets, and of their fortification with BSFLM at 5 versus 10% dietary inclusion, with two 5% BSFLM treatments which contrasted in fat content. Broiler growth and slaughter parameters of broilers on custom sprouted cowpea maize test diets were compared to standard commercial grower/finisher diets as the positive controls. The study used Ross 308 broilers to evaluate dietary effects on growth performance during the growing (22-35 days) and finishing (36-42 days) phases, and the slaughter performance. The feeding, management and production conditions simulated a typical small-scale production scenario. Compared to the standard, maize-soybean commercial diet, during the grower phase, the sprouted cowpea-maize diet was inefficient without BSFLM fortification, considering the low intake, low weight gain, with high FCR. Compared to the positive and negative control diets, BSFLM fortification of the sprouted cowpea-maize diets induced statistically intermediate, though quantitatively positive influences on overall broiler growth performance, with some diet-specific effects. The high fat 5% BSFLM and 10% BSFLM diets improved weight gain in the grower phase, finisher phase and at slaughter. The 5% low fat BSFLM diet resulted in poor weight gain in the grower phase n lower weight gain at slaughter. Improvement in feed intake in the grower stage was only recorded in the 5% high fat and 10% BSFLM diet. All cowpea incorporated diets resulted in smaller breast and thigh weights. The standard, maize-soybean commercial diet increased in spleen weights compared to the unfortified sprouted-cowpea-maize diets, and to the 5% BSFLM fortified sprouted cowpea-maize diets. Increasing BSFLM inclusion to 10% resulted in intermediate improvement of the spleen weights. The birds on the 10 % BSFLM had lower drip loss compared to other diets, while birds on the negative control diets recorded the highest drip loss. The positive control, 5% low, and high fat BSFLM recorded moderate drip loss. Male broilers were more productive in terms of faster growth rate, which resulted in higher slaughter weights.

In the literature, thermal processing is more widely investigated compared to sprouting. However, assuming similar product quality regardless of the processing method, then findings from thermally processed cowpeas provide useful insight into the potential for using differently processed cowpeas in maize-based broiler diets. At 20% dietary inclusion, roasted cowpeas did not affect broiler intake (Anjos et al., 2012), Previously, at the same dietary level, cooked cowpeas did not affect growth and slaughter performance (Kur et al., 2013). However, studies on diets containing graded levels of thermally processed cowpeas suggest an opportunity for substantial dietary inclusion of processed cowpea. For example, in a study by Chakam et al., (2010), up to 20% dietary inclusion of cooked cowpeas into broiler finisher diets did not affect growth and the slaughter performance. Feed intake was not affected when birds were fed up with 15% soaked and roasted cowpea (Kur et al., 2013). In the study by Georgeta et al., (2022), 10-20% dietary raw cowpeas resulted in feed intake similar to that of a maize-soyabean meal basal diet. (Ehirim et al., 2018) reported broiler chick tolerance of up to 15% cowpea dietary inclusion. In the present study, feeding broiler chickens at 0% BSFLM-cowpea (maize-soyabean meal base) inclusion resulted in higher overall feed consumption compared to other diets. This contradicted findings by (Adino et al., 2018), where 25-100% substitution of soaked cowpeas for soybean meal did not affect feed intake.

Though the literature is also scant of studies on BSFLM inclusion in wholly sprouted cowpea-based broiler diets, the evidence in this study points to substantial benefit of supplementary defatted and full-fat BSFLM in conventional maize-soybean broiler diets. A 20% BSFLM dietary inclusion in a soybean diet reduced 42-day broiler FCR and improved the immune response (de Souza Vilela *et al*., 2021).

The negative impact on feed efficiency due to cowpea inclusion as recorded in this study is in line with the results by Akanji *et al*., (2016) who investigated the effect of raw and processed cowpea on growth and haematological profiles of broiler chickens. They reported low feed conversion efficiency (FCE) in birds fed raw and dehulled cowpea diets. Similar results were reported by Kana *et al*. (2012) for diets which contained 0% cowpea had better FCR than diets with 20% cooked and raw cowpea. Kana *et al*., (2012) reported low FCR when birds were fed raw cowpea supplemented Canarium charcoal compared to diets without cowpeas. In contrast, 100% dietary replacement of soya bean meal with soaked or roasted cowpeas did not affect the FCR (Adino *et al*., 2018).

In the present study, the negative impact of feeding a sprouted cowpea diet on weight gain was similar to findings on a 30% cowpea diet by Adino *et al*.,(2018). Heuzé *et al*. (2012) reported progressive decline in mean daily body weight gain above inclusion of 22.5% cowpeas in total mixed ration . In a study by Georgeta *et al*., (2022) dietary inclusion of 10-20% cowpea did not affect weight gain. Relative to the negative and positive controls, the intermediate weight gain in maize-sprouted cowpea diets fortified with BSFLM suggested partial advantage of the extra protein. Dietary supplements of insect feed of up to 15% did not improve broiler growth performance (Onsongo *et al*., 2018). In a study by Schiavone *et al*., (2017), 50% and 100% replacement of soyabean meal with BSFLM maintained the weight gain. In contrast, Schiavone *et al*. (2017) reported a linear decrease in weight gain with incremental, (0-15% BSFM) supplementation during the finisher period. Four percent BSFLM dietary inclusion did not improve weight gain during the grower and finisher phase (Mohammed *et al*., 2017).

In the present study, compared to the control, cowpea-based diets reduced the breast and thigh yields, with no effects of BSFLM inclusion on these parameters, as well as on sizes of the visceral organs. However, the standard, maize-soybean commercial diet increased the spleen size compared to the 0% BSFLM diet. Increasing the BSFLM inclusion to 10% resulted in intermediate improvement of spleen size. Nayohan *et al*., (2022) and Schiavone *et al*., (2017) reported that breast, thighs yield, and the size of the heart, liver, and gizzard were not affected by partial or /total replacement of soybean oil cake with BSLF. Defang *et al*., (2008) reported higher heart, liver and gizzard sizes in birds fed 14% boiled cowpea compared to the ones fed raw cowpea. Sprouting might have been successful lowering the content of toxic anti-nutritional compounds of cowpea in this study. The increase in the size of liver and gizzard is related to increased activity to overcome the effect of toxic anti- nutritive compounds in the diets (Defang *et al*., 2008; Teguia & Beynen, 2005). Kana *et al*., (2012), reported increased gizzard sizes on 20% raw cowpea diets.

In the present study, birds on the 10 % BSFLM had lower drip dross compared to other diets. The negative control recorded the highest drip loss. The positive control, 5% low fat and high fat BSFLM recorded moderate drip loss. Therefore, feeding BSFLM to broiler chickens did not affect the colour of breast meat (Moula *et al*., 2018). Breast meat of broilers fed a 15% BSFLM larvae meal diet showed higher meat redness (a*) index compared to birds on 5% and 10% BSFLM, with least redness on the standard, control diet (Abd El-Hack *et al*., 2020). Abd El-Hack *et al*. (2020) also reported a linear decrease in the yellowness value (b*) with increasing dietary BSFLM. Schiavone *et al*., (2019) observed a linear increase in meat redness to a maximum on 15% BSF inclusion. Findings by Laudadio & Tufarelli (2010) and Biesek *et al*., (2020) suggested the dietary legume protein source affects meat redness (a*) and the yellowness (b*) of breast muscles.

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Broilers fed the sprouted cowpea-maize grower diet without BSFLM fortification had low feed intake, weight gain and high FCR during the growing phase, and these effects were cumulatively reflected at slaughter. Compared to the positive and negative control diets, BSFLM fortification of the sprouted cowpea-maize diets resulted in intermediate parameters of broiler growth performance, which suggested partial benefit in supplementing BSFLM to a maize-sprouted cowpea diet. Based on the growth phase, and the cumulative intake, growth and FCR, it was concluded that dietary efficacy was in the order 0 %<5 % LF<5% HF< 10 % BSFLM fortification.

6.2 Recommendations

Depending on the relative cost of cowpeas, BSFLM and soybean cake, and, up to 10 % of BSFLM fortification of maize-sprouted cowpeas diets is recommended, subject to further research to investigate the possibility for higher levels of fortification.

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