EFFECTS OF SPROUTED COWPEA *(VIGNA UNGUICULATA*) DIETARY INCLUSION WITH RONONZYME ® ProAct SUPPLEMENTATION ON BROILER PERFORMANCE

BY

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DECLARATION

I, Nancy Mariba, hereby declare that this thesis is submitted in fulfilment of the requirements for the Master of Science in Agriculture at the Department of Animal Science, Faculty of Science, Engineering and Agriculture, University of Venda by me has not previously been submitted for a degree at this or any other university, and that it is my own work in design and in execution and that all reference material contained therein has been duly acknowledged.

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Student: ____________________ Date: 27/02/2023

N. Mariba

DEDICATION

This dissertation is dedicated to my lovely daughter, mother, sisters, brother and grandmother.

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ABSTRACT

The study evaluated the efficacy of maize-sprouted cowpea (*Vigna unguiculata*) diets when fed with supplementary exogenous enzymes on the growth (live weight, live weight gain, feed conversion ratio) and carcass parameters of Ross-308 broilers. Cowpeas were screened for viable seed and sterilised by 30-minute immersion in 2% sodium hypochlorite aqueous solution. Germinated by 12-hour soaking in tap water prior to 4-day open-air, 2-hourly irrigated sprouting on steel screens, and rapid, hot sun-drying to 35% DM spread on black plastic sheeting laid on a concrete surface. Balanced [160 g CP kg-1 DM] cowpea-based grower and finisher diets were mixed at 0, 50, 100% with iso-nutrient respective commercial feed mixes (controls). Duplicates of the experimental diets were fortified with 200 g/tonne of Rononzyme ® ProAct (75,000 PROT units g-1 serine protease). Nine hundred chicks were randomly allotted at 30 birds/pen in a 3 (diet) x 2 (enzyme) factorial experiment replicated five times. Random sample (8 birds/pen) live weight were evaluated on a weekly basis. Treatments were subjected to the analysis of variance using Minitab Statistical package version 18 (Minitab, 2017). Treatment means were separated using Tukey's test at 5% level of significance. The maize-sprouted cowpea inclusion rate had no effect ($P>0.05$) on feed consumption (g/b/d). Grower feed with 100 maize-sprouted cowpea inclusion had a significantly ($P<0.05$) lower live weight gain (45.0 g/b/d) (LWG) and consequently live weight at day 35 (LW_{35}) compared to SCG0 and SCG50 which were not different. Enzyme fortification had no effect on all growth parameters in both grower and finisher phases. During the finisher phase, birds on maize-cowpea diets had significantly higher ($P<0.05$) feed consumption (g/b/d) compared to the control diet (SCF0). Diet SCF100 had the lowest ($P<0.05$) live weight at day 42 (LW_{42}) compared to SCF0 and SCF50, and eventually had the highest feed conversion ratio (FCR). Cumulatively, grower-finisher (day 22-42) live weight gain ($LWG_{22\text{-}42}$) was in the dietary order SCG0> SCG50>SCG100 ($P<0.05$). The feed conversion ratio ($FCR_{22\text{-}42}$) was in the dietary order SCG0< SCG50<SCG100 ($P<0.05$). Broilers on the SCG0-SCF0 dietary regime had larger carcasses and the proportionate breast ($P<0.05$). Broilers on the SCG100-SCF100 dietary regime had smaller proportions of wings and thighs ($P<0.05$). The treatments did not ($P>0.05$) affect the abdominal viscera. The enzyme had no effect ($P>0.05$) on the slaughter parameters except the proportional weight (%) of the heart. The maize-sprouted cowpea diets resulted in a low value for the meat redness coordinate (a) ($P < 0.05$). The yellowness coordinate (b) was in the order SCG0-SCF0>SCG50-SCF50>SCG100-SCF100 ($P < 0.05$). Meat water holding capacity and the shear force were higher on the SCG0-SCF0 compared to the SCG100-SCG100-SCF100 feeding regime ($P<0.05$). In conclusion, dilution of the control with the sprouted cowpea diet reduced the live weight gain, feed efficiency ratio and carcass weight, and caused adverse effect on meat quality, with more adverse effects at the 100%, compared to the 50% dilution level. Adverse metabolic and physiological effects were

indicated by the enlargement of the liver and gizzard at the high inclusion of sprouted cowpea in broiler diets.

Keywords: Cowpea, broilers, exogenous enzyme, exogenous, performance, sprouting, meat quality

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LIST OF ABBREVIATIONS

%	Percentage
^{0}C	Degrees celcius
a*	redness
ADF	Acid detergent fibre
FI	Feed intake
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemistry
b*	yellowness
LWT	Live weight thickness
Ca	Calcium
Cm	Centimetres
CP	Crude protein
(ΔE)	Colour change
FCR	Feed conversion ratio
G	grams
Kg	Kilograms
L*	lightness
ME	Metabolizable energy
MJ	Mega joules
NDF	Neutral detergent fibre
NRC	National Research Council
Ns	Not significant
OM	Organic matter
P	Phosphorus
SC	Sprouted cowpea
SCG	Sprouted cowpea for grower phase
SCF	Sprouted cowpea finisher phase

CHAPTER 1: INTRODUCTION

1.1. Background

Global consumption of poultry products (meat & eggs) has increased consistently since the past 50 years (Nkukwana, 2018), a trend which is expected to continue, marking sustained sector growth (Mottet and Tempio, 2017). Projected rapid human population growth (Kim *et al.,* 2019; Dorper *et al.,* 2021) is causing deficits in the supply of conventional protein feeds (Minocha *et al.,*2019), particularly in developing countries (Andreoli *et al.,* 2021), which necessitates the search for alternative quality protein sources (Rzymski *et al.,* 2021).

The cowpea is herbaceous, annual legume plant, that is grown in many tropical and subtropical international locations (Oyewale and Bamaiyi, 2013). It is among native leguminous plant protein sources with similar protein quality to soybean (Atawodi *et al*., 2008, Algam, 2012 and Hervé *et al.,* 2020). The crop can be cultivated on a different range of soil conditions, even in marginal areas by poor resource farmers (Horn *et al.,* 2022). Cowpeas can be successfully included in poultry diets (Alonso *et al.,* 2000; Fouad, 2015). Cowpea contains 63% of carbohydrates, 14-24% crude protein (Chathuni et al., 2018) while the metabolizable energy value varies from 3375.7 to 3606.7kcal/kg, methionine (0.28-0.32g/kg) and lysine (1.52-1.67g/kg) (Hervé *et al.,* 2020).
Cowpe grain has digestibility of amino acids of,72.8 to 81.0% (Devi *et al.,*2015), with high digestibility of amino acid and phosphorus (Cowieson *et al*., 2004; Ciurescu *et al.,* 2020).

Several methods have been employed to reduce anti-nutritive factors in cowpeas to improve the nutritional value as a protein source in poultry feed. These include cooking, heating and fermentation (Edens, 2003) and sprouting (Maidala, 2015; Popova and Mihaylova, 2019). Processing such as sprouting is advantageous in not requiring energy and given the short duration of 4 Days required for optimal effects. Nutrient digestion and utilization are improved by sprouting, which also raises the bioavailability of nutrients (Kumar *et al.,* 2010; Handa *et al.*,2017). During sprouting, enzymes which degrade complex compounds to simpler forms are activated, thereby improving nutritional quality (Shakuntala *et al.,* 2011; Ravi Kiran *et al.,* 2012; Nkhata *et al.,* 2018).

Exogenous proteases carbohydrases and phytases are increasingly used in poultry diets (Attia *et al*.,2012; Alagawany *et al.,*2018). Application in grain-based diets can improve productivity by increasing nutrient availability (Attia, 2003; Abudabos, 2012; Nourmohammadi *et al.,* 2012) and the dietary energy value (Park *et al.,* 2020). Phytase increased the bioavailability of calcium, phosphorus, protein and metabolizable energy, with the benefit of minimal excretion of nutrients to the environment (Avila *et al.,* 2010; Hussain *et al.,* 2020).

Combination of legume sprouting (Mahmoud and El-Anany, 2014; Rubio *et al.,* 2003; Afsharmanesh *et al.,* 2016) with exogenous enzymes (Mathlouthi *et al.,* 2003; Afsharmanesh et al., 2016) are potentially additively beneficial to the efficacy for cheaper, novel poultry diets. In South Africa, there is limited research on the use of typically chemically compiled novel diets, and on the benefit of exogenous enzymes in such diets.
This current study evaluated the effects on Ross 308 Broiler performance of maize -sprouted cowpea (*Vigna unguiculate*) diets supplemented with Rononzyme ® ProAct.

1.2. Problem statement

In South Africa and globally, there is heavy dependence on expensive soybean as the main plant protein source in stock feeds (Algam *et al.,* 2012; Murithi *et al.,* 2016). It is, therefore, imperative to search for locally readily available, economical, high-quality substitutes. Cowpeas could be a good substitute for soybeans in broiler diets given similar amino acid profiles and energy value. Coulibaly *et al.* (2002) and Chakam *et al.,* (2010) observed that the protein in cowpeas is high in lysine and tryptophan, but deficient in methionine and cystine, which would probably be a limiting factor in maize-cowpea broiler diets. Common to grain legumes, cowpeas contain anti-nutritional factors particularly haemagglutinins and trypsin inhibitors, (Amaefule *et al.,* 2005; Teguia *et al.,* 2008), which limit protein utilization. Processing cowpeas through sprouting, complemented by exogenous enzymes, could allow for greater dietary inclusion for efficient broiler growth. There is a need to evaluate the efficacy of these interventions to facilitate wider adoption into modern precision feeding systems. The current broiler feeding trial investigated effects on growth, carcass and meat characteristics of different levels of dietary inclusion of sprouted cowpeas, and the effects of supplementary Rononzyme ® ProAct.

1.3. Justification

Increasing market deficits and costs of maize and Soybean meal (SBM) have long compelled poultry nutritionists to look for suitable alternative energy and protein sources (Baurhoo *et al.,* 2011; Dabbou *et al.,* 2018). Substitution of these conventional stockfeed with cheaper, locally available feed ingredients may significantly reduce the cost of poultry production without risk to productivity (Bamgbose *et al.,* 2004; Murawska *et al.,* 2021). Cowpea *(Vigna unguiculate)* grain contains, on an average, 23–25 % protein and 50–67 % carbohydrate (Devi, *et al.*,2015). Cowpeas have a similar amino acid profile and metabolizable energy to SBM (Indriani and

Murwani, 2005; Moawia, 2015). Previous research on alternative grain legumes for poultry diets supports the need for processing to improve nutrient availability and removal of anti-nutritive factors (Mubarak, 2005; Afiukwa *et al.,* 2012; Soetan, 2012).

Sprouting cowpeas (Maidala, 2015) and application of exogenous enzymes may be viable solutions to the risks associated with high levels of inclusion in poultry diets (Ledvinka *et al.,* 2022). Sprouting increases bioavailability and utilization of nutrients (Kumar *et al.,* 2010) obviating costly chemical and or physical processing, and without energy cost. The use of exogenous cocktails of phytase, xylanase, and protease enzymes to improve the efficacy of broiler diets has increased over the last few years (Alqhtani *et al.,* 2022) with the added benefit of reduction of excretions to the environment (Avila *et al.,* 2010).

The determination of threshold levels of sprouted cowpea inclusion and establishing the potency of the currently available exogenous enzymes should assist help rural chicken farmers to increase poultry production for greater income and better livelihoods. Accordingly, the study will provide solutions tailor-made for the local environment.

1.4. Objectives

1.4.1. Main objective

The main objective of this study was to investigate the potential of using sprouted cowpeas as a substitute for the increasingly scarce and costly soybean cake in broiler diets.

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1.4.2. Specific objectives

The specific objective of this study was to determine the effects of including sprouted cowpeas in grower and finisher broiler diets, and of supplementary Ronozyme ® ProAct on the following:

a) Feed intake
b) Growth
c) Feed conversion ratio
d) Mortality
e) Carcass characteristics (carcass weight, carcass weight, dressing percentage, breast, wing and shark)
f) Meat quality (color characteristic, pH water holding capacity (WHC) texture profile and (hardness)

1.5. Hypothesis

Replacing soybean with sprouted cowpea in broiler grower and finisher diets does not affect:

a) feed intake
b) Growth
c) Feed conversion ratio
d) Mortality
e) Carcass characteristics
f) Meat quality

Ronozyme ® ProAct has no effect on the utilization of sprouted cowpea broiler grower and finisher diets.

CHAPTER 2: LITERATURE REVIEW

2.1. Introduction

Throughout the last decade, persistent pressure on grain markets has compelled poultry producers to think about using less expensive alternatives in place of the cereal and legume grains now used in poultry diets (Akinola and Sese, 2011; Watson *et al.,* 2017; Ciurescu *et al.*, 2020). The nutritional risks in the use of non-conventional feed ingredients on precision feeding systems, and on animal productivity, and broader implications on the viability and environmental impact of the industry are major concerns. Given similar amino acid profiles, cowpeas (*Vigna Unguiculata)* are a potential alternative vegetable source of poultry dietary protein to expensive oil extracted soybean (Tshovhote *et al.,* 2003; Adino *et al.,* 2018). However, cowpeas contain anti-nutritional factors that reduce the nutritional value in the diet (Bedford and Cowieson, 2012: Samtiya *et al.*, 2020). Thus, appropriate processing should be geared towards good quality cowpeas. Sprouting legumes enhances the nutritional quality of legume seeds by increasing the bioavailability of nutrients as reflected in improved digestibility and utilization of nutrients (Kumar *et al.,* 2010; Sharma, 2021). Supplementation of exogenous enzymes is potentially a complementary strategy to further increase nutrient availability (Rutherfurd *et al.,* 2004; Chapman *et al.,* 2018). The use of enzymes technology in food processing can be traced back to about 10,000 years (Bedford & Partridge, 2001, Kaiser *et al.,* 2020) and has significantly advanced to the huge current global industry (Chapman *et al.,* 2018). If correctly matched to monogastric dietary feed substrates, exogenous feed enzymes allow flexibility in diet formulation, for lower feed cost, improved digestibility and reduced environmental pollution (Zakaria *et al.* 2010).

2.2. Botanical, agronomic characteristics and uses of cowpea

Cowpea *(Vigna unguiculate*) is an herbaceous, short term annual legume plant, which is grown in many tropical and subtropical countries (Ameen *et al.,* 2005). Cowpea is a member of the *Phaseoleae* tribe of the *Leguminosae* family. Several of the economically significant warm season grain and oilseed legumes, such as soybean *(Glycine max*), common bean *(Phaseolus vulgaris)*, and mungbean, are members of the *Phaseoleae* family *(Vigna radiata).*

Cowpeas are generally grown for their seed, although they are also used as a cover crop, fodder, and a vegetable (for leafy greens, green pods, fresh shelled green peas, and shelled dried peas). On a dry mass basis, the protein content of grain ranges from 29% to 34.9%, with younger leaves having the highest nitrogen concentration (Enyiukwu *et al.,* 2018). The cowpea has the highest energy (16.3-18.2 MJ/kg) among vegetative foods (Gonçalves *et al.,* 2016). The crop provides higher quality fodder than cereals or forage grass (Akyeampong, 2012). Cowpeas are nutritious components in human diet and livestock feed, providing an important human source of protein, fat, fibre, carbohydrates and vitamins (Mekonnen *et al.,* 2022). It is mainly consumed by rural and peri-urban people in developing countries (Asiwe, 2009).

Cowpea is an important legume and a versatile crop, also commonly known as southern pea, black eye pea, crowder pea, lubia, niebe, coupeor Frijole (Devi *et al.,* 2015). It is widely distributed in sub-Saharan Africa, where it is believed to have originated (Nkomo *et al.,* 2021). However, some studies show that it also originated from Asia, Central, and South America, USA and some parts of southern Europe (AATF., 2005).
A variety of soil types, including marginal locations, can support the crop's growth (Natasha Muchemwa *et al*., 2022).

2.3. Nutrient Composition of cowpea

Locally, small-scale farmers in rural areas produce cowpeas for its high protein content (20-25%), palatability and relatively low toxicity (Aveling, 2000). Recent studies (Hervé *et al.,* 2020) have shown that cowpeas have promising potential as feedstuff for poultry. Its incorporation in broiler diets may reduce the feed cost without compromise on productivity (Chakam *et al.,* 2008, Defang *et al.,* 2008).

Cowpeas are an excellent and inexpensive source of protein, fatty acid, essential amino acid, vitamins and minerals (Adino *et al.,* 2018). Coulibaly *et al.,* (2002) reported 20- 23% DM protein content, adequate to complement maize as protein source in livestock feed, being rich in lysine and tryptophan, though deficient in methionine and cystine. Adeyoju *et al.,* (2021) reported 22.30%-26.73% protein, 2.10%-2.30% fat, 4.10%-1.02% fibre, 3.77%-3.87% ash and 60%-59% DM carbohydrates. Data from the USDA (2008) shows that cowpeas contain an average of 24% crude protein and 7g lysine per 100g protein. Ayana *et al.* (2013) reported ranges of about 48% to 90% globulins, 3% to 15% albumin, 5% to 13% prolamins and 7% to 23% glutelins. In contrast, Tran *et al.,* (2015) found 71% albumin and 11% globulin proteins. Similarly, Tshovhote *et al.* (2003) reported 67% globulins and by 25% albumins.

2.4. Anti-nutritional factors in cowpeas

Feed anti-nutritional factors (ANF) negatively affect poultry nutrient utilization and therefore limit the dietary inclusion level. The composition of anti-nutritional factors such as protease inhibitor, lectins, phytic acid, alkaloids, cyanogens and indigestible carbohydrates vary with plant species (Yadahally *et al.,* 2012). The most abundant serine protease inhibitors in cowpeas are trypsin inhibitor known as Kuniz inhibitors (KSTI) and Bowman-Birk inhibitor (BBI) (Rehder *et al.,* 2021). Trypsin inhibitors inactivate the digestive enzyme, trypsin and chymotrypsin, through binding it to the active proteases, and depressing activity in the gut (Vagadia *et al.,*2018). The inhibition can result in overstimulated secretion of digestive enzymes from the pancreases causing pancreatic hypertrophy (Lephale *et al.,* 2012; Jeyakumar & Lawrence. 2022).

Udensi *et al.* (2007) and Nadimi *et al.,* (2022) described the activities of lectins as an anti-nutritional factor in legumes. These glycoproteins were shown to have the ability to bind to cell surface through specific oligosaccharide or glycol-peptides. Furthermore, research indicate that glycoprotein can bind to the epithelium of small intestine and result in the impairment of the brush border (Zhao *et al.,* 2019). Excessive accumulation of these glycoproteins result in villous atrophy (Ileke *et al.,* 2013), likely a major cause for elevated endogenous nitrogen losses and depressed growth rate in young animals (Coudray *et al.,* 2003).

2.5. Potential of cowpea-based diets for poultry

Despite being possess some undesirable properties common to other legumes, cowpeas appear to be viable for use in poultry feeds due to their composition being similar to that of plant protein sources like lupins and field peas (Sulaiman *et al.,* 2012; Ciurescu *et al.,* 2022). Tshovhote *et al.* (2003) evaluated the chemical composition and poultry digestibility of three cultivars of cowpeas, which had relatively narrow range of protein concentrations (253.5 to 264.3 g/kg). The amino acid (AA) profile varied among the cultivars. Dietary crude fibre levels range from 51.5 to 58.1 g/kg. The cultivars were almost devoid of lipid and calcium but, were relatively high in phosphorus. Between 9.88 and 10.02 MJ/kg DM and 10.29 and 10.78 MJ/kg DM, respectively, were the apparent and true metabolic energy (AMEn and TMEn) values. Methionine had the highest digestibility and lysine had the lowest, with the mean digestibility of the AAs ranging from 72.8 to 81.0%.

Several studies have demonstrated potential for substantial inclusion of processed cowpeas as a protein source in poultry diets. In broiler and layer chicks, previous studies recommend maximum level of cowpea inclusion of 200 and 300 g/kg, respectively (Akanji *et al.,* 2016). Defang *et al.* (2008) evaluated boiled-cowpea broiler diets. Broiler starter intake and weight gain were significantly higher compared to broilers fed on the control diet, which no effect on feed conversion. Carcass yield was significantly higher for birds finished on the boiled cowpea diet. Eljack *et al.* (2010) reported higher weight gain and better feed conversion ratio, as the levels of cowpeas were increased to 20% in broiler chicken feed. Feeding de-hulled cooked cowpea at 20% and de-hulled roasted cowpea at 20% diets did not affect weight gain, feed efficiency and Protein Efficiency Ratio (PER) (Akanji *et al.,* 2016). Feed intake, weight gain, live weight, carcass weight, and dressing percentage were not affected by the inclusion of cowpeas at 5-15% levels in broiler chicken diets (Abdelgani *et al*, (2013). Chakam *et al.* (2010) reported that up to 20% cooked cowpeas could be included in broiler finisher diets without having a harmful impact on feed intake, live weight, weight gain, feed conversion ratio, cost of producing a kg of meat, carcass yield, some carcass parts, survivability and serum creatinine.

2.6. Effects of sprouting on the nutritive value of cowpeas

Economical ways to enhance cowpea nutrient availability are highly desirable. One of the feasible methods that have been recommended is sprouting (Modu *et al.,* 2010). Hübner and Arendt (2012) reported that the germination process increases the nutritional value of legumes by enhancing their digestibility, raising their protein efficiency ratio, and lowering their levels of anti-nutritional components including lectins and proteolytic inhibitors. These processes cause hydrolysis of oligosaccharides (raffinose and stachyose) present in soybeans, which produce flatulence. Germination also provides higher levels of methionine, which is the first limiting amino acid in soy protein. The breakdown of complex molecules into simpler forms, their transformation into vital constituents, and the breakdown of nutritionally undesirable elements are what primarily cause the beneficial nutritional changes that take place during sprouting (Mohammadi *et al.,* 2007; Inyang and Zakari, 2008). Grains that have been sprouted exhibit increased lipase activity, improved total protein, fat, essential amino acid, total sugar, B-group vitamin, and starch digestibility, as well as decreased phytates and protease inhibitor levels. During sprouting, an increase in proteolytic activity results in the hydrolysis of prolamins and a rise in the amino acid lysine (Abbas and Ahmad. 2018).

Sprouting grain increases enzyme activity, alters the proximate composition and other nutrients, though with loss of total DM (Dikshit *et al.*, 2003). Inyang and Zakari, (2008) stated that during sprouting, there is loss of dry matter caused by the energy reserve in the endosperm fueling the growth process. Protein, which is not used for growth, increases in percentage, though in absolute terms, remains fairly static; this also generally applies to the other nutrients. However, fibre as the major constituent of cell walls, increases both in percentage and real terms, with the synthesis of structural carbohydrates, such as cellulose and hemicelluloses. The benefit of improved nutrients and detoxification of antinutritional factors can therefore be offset by marked loss of digestible carbohydrates and the accumulation of indigestible fibre, which factors should be considered in determining the extent of sprouting. From day four of sprouting, Shah *et al.* (2011) noted rapid increases in ash and protein, which corresponded with the radicle's (root's) elongation and encouraged the intake of minerals. The intake of nitrates enhances the metabolism of nitrogenous molecules from carbohydrate reserves, therefore raising the amounts of crude protein (CP).

2.7. Role of exogenous enzymes in poultry diets

Fibre contains significant amounts of cell wall components including lignin (14% w/w) and non-starch polysaccharides (NSPs) mainly hemicellulose (11%, w/w); cellulose (35%, w/w) and pectin (6%, w/w) (Francis *et al.,* 2009). Monogastric livestock such as poultry and pigs do not produce enzymes to use these polysaccharides, which increase gut viscosity, with a resultant adverse effect on animal growth and performance (Kocher *et al.,* 2000). Enzyme supplementation is well documented as effective in assisting the breaking of polymeric compounds, which improves feed nutritive value (Giraldo *et al.,* 2008; Zhu *et al.,* 2014). Exogenous enzymes can enhance nutrient digestibility directly or indirectly by reducing the anti-nutrient effect of specific components in the diet by breaking down the anti-nutritional substances, e.g., arabinoxylans, trypsin inhibitors, and phytate (Barletta., 2011). Therefore, exogenous enzymes present the opportunity to expand the feed base to include nonconventional ingredients, affording flexibility in feed formulation to improve profitability of poultry production (Alabi *et al.,* 2019**).**

2.8. The use of exogenous dietary enzymes in maize-legume based diets.

Previous research (Segobola, 2016) on the efficacy of exogenous enzymes focused on wheat and barley-based diets, with emphasis on mitigating the anti-nutrition properties of soluble high molecule weight pentosans. Enzyme supplementation to maize-based diets had been ignored due to low concentrations of soluble NSPs (<1g/kg), compared to up to 25 g/kg for wheat (Choct, 2006). The effectiveness of exogenous enzyme provides the potential to strategically formulate maize-soya diets by considering the relative concentration of ingredients. With the supplementation of exogenous carbohydrates and phytases to maize-soya diets, the digestibility of Ca, P, energy and amino acids improved (Cowieson *et al.,* 2006).

2.9. Mode of action of exogenous dietary enzymes

The mode of action of supplemental exogenous enzymes to improve the profitability of poultry production was outlined by Cowieson *et al.* (2010). Cowieson *et al.* (2010) described it as enhancing the apparent digestibility of dietary nutrients. Successful application of exogenous enzyme to dry diets presupposes that the enzyme will be active in the digestive tract of an animal, and it must, therefore, fulfill some criteria (Attia *et al.,* 2012). The enzyme needs to function in the animal's digestive system under physiological conditions, which means it must be able to withstand proteolysis by endogenous proteases rather than competing with the animal's digestive enzymes. Exogenous enzyme activities are likely to be impacted by variations in the anatomy and physiology of various digestive tract species. Çiftci *et al*. (2003) indicated that differences in enzyme efficacy between poultry and pigs reflect anatomical (in poultry, feed passes into the crops, where any added enzymes can act for several hours at a pH of approximately 6.0 before giving into the acid environment of gizzard, whereas in the pig, feed passes directly into the acid environment of the stomach immediately after ingestion), digestive capacity (poultry have a shorter small intestine and thus, reduced possibilities for enzyme inactivation by microflora) and physiological (shorter mean retention times in the small intestine (1-2 hours) in poultry versus 4-5 hours in the pigs) and lower water content in the upper part of the gastrointestinal tract, bacterial activity (the importance of the microflora in the gut of poultry is much less than in pigs) and fibre fermentation (there is less fermentation of fibre in poultry than pigs, due to the much smaller hindgut in poultry).

2.10. Factors affecting broiler meat quality.

Meat is a source of numerous nutrients and is regarded as a nutrient-dense food. Consumer satisfaction is influenced by the quality of meat, which depends on the diet (Selaledi *et al.,* 2021). Poultry meat quality is defined by several factors such as colour, pH, texture and water holding capacity.

2.10.1. Colour and pH

Meat colour has a greater impact on retail purchasing decisions than any other quality element because consumers use colour as a solitary visual indicator of deterioration and shelf life (Castigliego *et al.,*2010; Grujić *et al.,*2017). Due to its low abundance, hemoglobin has a negligible impact on meat colour. Instead, red myoglobin molecules found in muscle tissue, muscle fiber orientation, space between the muscle fibers, and pH are largely responsible (Guidi and Castigliego, 2010; Barbut, 2015; Hughes *et al.,* 2017).

However, additional elements like breed, nutrition, muscle type, changes after death, processing techniques, and packaging might impact meat colour (Barbut, 2015). Myoglobin concentration varies between muscles, which directly influences colour and colour stability. The breast meat of chicken is primarily made of white muscle fibers, which is low in myoglobin while the thigh meat is composed of red fibers, which is higher in myoglobin making them appear darker (Barbut, 2015). The molecular state of the heme affects the meat colour differently, depending on whether the iron molecule of myoglobin is oxidized or reduced and what compounds are attached to the heme ring of myoglobin. When oxygen is attached and the iron molecule is reduced, the colour of the meat is bright red. When the iron molecule is oxidized, the meat has a brown colour from the lack of oxygen inside the muscle (Barbut, 2002). The arrangement and spacing of the sarcomeres in muscle tissue also affects the colour of the meat. Light absorption and reflection off the structure of the meat are influenced by the sarcomere structure. As an illustration, pale, soft, and exudative (PSE) meat has a more open sarcomere structure that permits more light reflection, giving the flesh a paler appearance (Swatland, 2008; Barbut, 2015).

Muscle pH and meat colour are highly correlated (Ruedt et *al.,* 2022). Several other quality characteristics, including tenderness, water-holding capacity, heat loss, juiciness, and shelf-life, have all been linked to muscle pH (Fletcher, 1999). The accumulation of lactic acid and the onset of glycolysis cause the pH of the muscle to decrease once the resolution of stiffness has begun. This drop in pH to 5.6-5.8 can be caused by the normal development of meat colour (Braden, 2013). Muscle from chickens is typically post-mortem pH 6.0-6.2 after 24 hours (Keeton and Osburn, 2010). It's crucial to pay attention to how quickly the muscle pH drops once rigor is complete. Muscle pH levels that are close to the isoelectric point cause a rapid pH decrease, which affects the characteristics of meat quality. The ability of proteins to bind water and therefore determine meat quality traits like juiciness and tenderness depends on the isoelectric point, which is a balance between positive and negative charges on protein side-groups with a pH level of 5.1 (Maxwell, 2017). The PSE condition is defined as watery and pale in colour in muscle with a fast pH decrease and a pH value close to the isoelectric point (Braden, 2013). However, the pH levels will be significantly higher than the isoelectric point if the pH decline is restricted in rate and/or extent, resulting in dry meat that is dark in colour. This condition is referred as the dark, firm, and dry (DFD) condition (Braden, 2013). For producers of fresh and further processed goods, the effect that pH has on the colour and functioning of the meat has a significant bearing on the product's profitability and shelf life (Barbut, 2015).

2.10.2. Texture

Texture is a sensory interpretation and expression of a product's internal architecture or structure in relation to how that product will react under stress and its haptic qualities (Coppes *et al*., 2002; Lepetit 2007). The performance of hardness/firmness, gumminess, resilience, cohesiveness, springiness, adhesiveness, and viscosity are some mechanical properties that are frequently measured and presented as texture by the vision, hearing, somesthesis, and kinesthesis of human sense in the muscle based on the hand, finger, tongue, jaw, or lips (Hagen *et al*., 2007).

When a product exhibits significant resistance to deformation or the "first bite," as determined by human sensory analysis, it is said to be hard (Bourne, 2002). Human subject sensory studies often refer to products on a scale of soft-firm-hard (Szczesniak, 2002).

Due to vagueness of Szczesniak’s (2002) definition, further attempts to define the texture parameter were made. Most explanatory the definition by Munoz (1986) was “the amount of deformation undergone by the material before rupture when biting completely through sample using the molar”. Gumminess, which is correlated with the basic characteristics of hardness and cohesiveness, is the energy needed to break down a semisolid food into a state that is ready for swallowing (Szczesniak, 2002). Cohesiveness can be categorized as mealy-pasty-gummy on a scale of opinion. According to Munoz (1986), springiness can be described as the product's elasticity or the force at which the sample expands to its original size following compression. The acceptance and desirability of the food product are influenced by all its characteristics. These characteristics can also be a great way to assess how well the protein matrix forms during mixing and heating. The texture profile study is one of several further initiatives to employ mechanical tools to gauge a product's protein matrix strength (Caine *et al*., 2003).

Some measurements of the binding components of a meat system have completely ignored the textural component of a protein's binding ability and instead concentrated on the protein's emulsion capacity (Lanier and Labudde, 1995). This system is called the “bind value” system designed by Santhi *et al*., (2017). The bind value system, which is widely employed in the meat business, largely concentrates on a protein's ability to from an emulsion (Santhi *et al*., 2017). The system has been criticized for not addressing the aspect of texture in a meat system (Liu, *et al*., 2016).

2.10.3. Water-holding capacity

One of the crucial features of the meat's quality is its ability to hold water (Jiang *et al.,* 2021) and it contributes to the sensation of juiciness and tenderness (Birhanu, 2019). The ability of meat to hold onto naturally occurring or added water when subjected to external forces like cutting, heating, grinding, or pressing is known as water-holding capacity (Aberle *et al.* 2001; Noraldin and Sabow, 2022). Water in the muscle is usually bound to a certain region (Barbut, 2002; Keeton and Osburn, 2010). Water that is bound cannot simply transfer to other compartments. Water that is directly affixed as an inner layer to thick and thin filament formations is known as bound water, and it cannot be altered through processing techniques. Bound water is driven off by a normal hearth and is unaffected by freezing (Apple and Yancey, 2013).

Weakly held bonds are produced as a result of surface forces in the protein holding the free water primarily. Because that free water can be easily removed from the meat system by forces imposed by processing, this category is crucial in further processed meats because the objective is to keep it in the meat product (Keeton and Osburn, 2010). Water-holding capacity is manipulated in two occurrences: the ionic effect and the steric effect. The ionic effect and the steric impact both affect the water-holding capacity. The capacity of water to connect to actin and myosin is diminished when the post-mortem pH of muscle is at the isoelectric point and there are equal amounts of positive and negative charges, leading to a drip loss (Apple and Yancey, 2013). Actin and myosin's capacity to tightly bind water will rise as meat pH varies from the isoelectric point as a result of the ratio of positive to negative charges (Murray, 2020).

The distance between the myofibrillar proteins determines how much of an impact the steric effect has on the ability to store water. During contraction the space between the myofibrillar protein structures becomes shorter restricting the space for water to bind to actin and myosin. The quantity of interstitial space that can contain water can change depending on the muscle's pH and state of contraction. Water is instead ejected into the muscle's extracellular space when the sarcomeres are compressed and there is little interstitial space (Puolanne, 2022). When the sarcomeres are compressed and there is limited interstitial space, water is instead discharged into the extracellular space of the muscle (Miller *et al.,* 2001).

2.11. Summary

Overall, the available evidence suggests that cowpeas have a good nutritional profile and can be included at poultry diets. They are rich in protein, energy, and amino acids. Information on the use of sprouted cowpea and exogenous enzymes in broiler diets is limited, with inconsistent results. Generally, it is assumed that the use of exogenous enzymes is of benefit to both young chicks and not older birds. The use of exogenous enzymes in broiler diets that are high in β-glucans and other NSP's may, therefore, be part of improving the nutritive value of certain grains. In South Africa, the use of sprouted cowpea together with exogenous enzymes in broiler diets may provide an inexpensive strategy to replace soybean. There is, therefore, a need for researchers to evaluate the optimum dietary inclusion of sprouted cowpeas in broiler diets, and the benefit of supplementary exogenous enzymes.

CHAPTER 3: MATERIAL AND METHODS

3.1. Introduction

In recent years, broiler genetics and nutrition have undergone vast improvement, thereby markedly increasing productivity and efficiency (Adeola *et al*, 2016 and Soomro *et al*, 2018). To support the high productivity, precision broiler feeding is employed whereby diets are strictly constituted from the highest quality processed raw materials to guarantee balanced nutrient provision, efficient digestion and assimilation. Increasingly however, poultry productivity is constrained by competition for high quality conventional feeds from human demand for food (Chisoro *et al.,* 2018) and biofuels (Younis *et al.,* 2019), and by climate-change disruption (Soomro *et al*., 2018) of feed grain production and supply chains, which is escalating the cost of stock feed (Akanji *et al*., 2016, Hejdysz *et al*., 2019).

To mitigate the nutritional risks so as to remain profitable, broiler producers need innovative, sustainable feeding solutions (Alagawany *et al.,* 2018). Options include climatically adaptable, traditional grain crops which currently trade outside commercial feed chains. The shift to nutritionally non-descript traditional legume grains as main dietary protein sources presents risks which include highly variable, inferior nutrient content and prevalence of anti-nutrients (Anjos *et al.,* 2012; Akanji *et al.,* 2016). Successful integration of traditional legume grains into precision feeding systems requires effective, low cost, energetically efficient customised bio-processing, with potential for tailored exogenous enzymes (Bedford, 2000). In low-cost production scenarios, energy and eco-friendly bioprocessing methods are considered ideal (Shah *et al.,* 2011; Mouneshwari *et al.,* 2019), which include germination/sprouting (Naik *et al.,* 2015; Mouneshwari *et al.,* 2019). The associated metabolism improves legume grain quality through biosynthesis of amino acids, vitamin and other organic nutrients (Alinaitwe *et al.,* 2019*)*. It also depolymerizes complex macromolecules (Ola and Oboh, 2000), including the antinutrients (Iyabo *et al.,* 2018; Kimberly *et al.,* 2019).

The cowpea *(Vigna unguiculata)* is among the nutritionally and ecologically versatile traditional pulses, with 23–25 % crude protein (Naik *et al.,* 2016),1.45 MJ ME energy, and rich in water–soluble vitamins (Fateema *et al* 2019, Foti *et al.*, 2019). For poultry diets, relative to soybean, the cowpea amino acids are less complimentary to maize (Embaye *et al*., 2018), with potential for cysteine and methionine deficiency (Frota *et al.,* 2017).

The objectives of the study were to determine the effects of the impact of including graded levels of the sprouts in diets fortified with Rononzyme ® ProAct on broiler performance.

3.2. Study site

The study was conducted at the University of Venda, School of Agriculture Experimental Farm (22°58'32" S, 30°26'45" E) 596 m above sea level. The university of Venda is in Vhembe district located in the far north of Limpopo Province under Thulamela local Municipality in Thohoyandou town.

3.3. Processing of cowpeas

Cowpea grain (*Vigna unguiculata cv. Southern pea)* was purchased from the local market and sprouted following procedures described by Mohammadi *et al.* (2007). Grain was cleaned and screened for viable seed, sterilized by 30-minute treatment in 2% sodium hypochlorite, soaked overnight (12 hours) in tap water, spread evenly on open plastic sheets and irrigated manually to maintain adequate moisture to support sprouting over 4 days, and sun-dried on a concrete slab.

3.4. Supplementary exogenous enzyme

An exogenous commercial enzyme product, Ronozyme® ProAct (EC 3.4.21; 75000 PROT/kg^{-1} serine protease) was supplied by DMS product, South Africa. Ronozyme® ProAct is a preparation of serine protease produced by a genetically modified strain of *Bacillus licheniformis*. It is produced by fermentation of a sporulation-deficient *Bacillus licheniformis* strain which expresses a synthetic gene encoding a serine protease.

3.5. Formulation and preparation of diets

The composition of maize meal, the raw and sprouted cowpeas used in the diet formulation are presented in Table 3.1.

Table 3.1: Analysed composition of sprouted and raw cowpeas (DM basis)

Components	Maize meal	[1]Raw cowpeas	[2]Sprouted cowpeas
Dry Matter *(g/kg)*	854.0	982.0	964.0
Ash *(g/kg)*	15.8	46.7	47.2
Crude Protein *(g/kg)*	82.3	235.6	261.1
Fat (ether extract) *(g/kg)*	34.8	13.4	19.30
Crude Fibre *(g/kg)*	19.0	22.1	20.0
Neutral Detergent Fibre *(g/kg)*	103.4	105.4	423.7
Acid Detergent Fibre *(g/kg)*	28.8	128.0	156.8
Ca *(g/kg)*	0.01	0.8	1.2
P *(g/kg)*	2.4	5.3	4.9
Zn *(mg/kg)*	18.0	35.3	44.7
Cu *(mg/kg)*	1.0	5.3	3.0
Mn *(mg/kg)*	7.0	15.3	19.0
Fe *(mg/kg)*	115.0	74.0	119.3

[1]Manually cleaned and screened for viable seed. [2]Sterilized by 30-minute treatment in 2% aqueous sodium hypochlorite, soaked overnight (12 hours) in tap water, spread evenly on open plastic sheets and irrigated manually to maintain adequate moisture to support sprouting over 4 days, and sun-dried on a concrete slab.

Chicks were started on Meadow classic (Meadow Feeds (Pty) Ltd, Delmas, South Africa Select Broiler range, Product V 10757). Experimental diets (Table 3.2) were formulated in two steps; step 1) constitution of complete grower and finisher broiler maize-sprouted cowpea diets which were iso-nutrient to standard commercial grower (G) (Meadow Feeds (Pty) Ltd, Delmas, South Africa Select Broiler ranget, Product V 10754)) and finisher (F) ((Meadow Feeds (Pty) Ltd, Delmas, South Africa Select Broiler ranget, Product V10761)) (controls); step 2) blending of the sprouted cowpea (SC) and control diets at 0, 50 and 100%, to generate respective grower (SCG0, SCG50, SCG100) and finisher (SCF0, SCF50 and SCF100) experimental diets. The experimental diets were prepared with (+) and without (-) Ronozyme® ProAct at 200g/tonne. Grains were milled through a 5-mm screen using an electric motor hammer mill (Name, Model CF158, South Affrica) and mixed along with the mineral, vitamin and salt additives into mash diets for 20 minutes using a vertical mixer (MORHLANG VERTA MIX 1200VM, South Africa).

Table 3.2: Ingredient composition of experimental grower and finisher diets

Ingredients (% DM)	Grower	Finisher
[1]Sprouted Cowpeas	48.5	41.5
Maize	46.5	53.0
[2]Broiler Mineral & Vitamin mix	3.0	3.5
*-Limestone	1.0	0.7
Mono-Di-calcium	1.0	1.2
Salt	0.0	0.5
Total	100	100

[1]Cleaned, screened for viable seed, sterilized by 30-minute treatment in 2% sodium hypochlorite, soaked overnight (12 hours) in tap water, spread evenly on open plastic sheets and irrigated manually to maintain adequate moisture to support sprouting over 4 days, and sun-dried on a concrete slab. [2] Trouw feeds- Grower-provides per kilogram of diet /Grower Macro: Vitamin A, 294117.700 UI; Vitamin D3, 58823.530 UI; Vitamin E, 882.353 UI; Vitamin K, 58.824mg; Vitamin B1, 58.824 mg; Vitamin B2, 161.765 mg; Vitamin B6, 117.647; Niacin 1029.412 Calcium Pantothenate, 323.529 mg; Biotin, 2941.177 mcg; Folic acid, 23.529mg; Vitamin B12, 588.235 mcg; Choline Chloride, 7639.412 mg; 6-Phytase (FTU) ,1 29411.770 FTU; Lasalocid, 2647.059 mg; Zinc bacitracin, 661.765 mg; Limestone (as carrier) 485.294 g; Salt g 117.647g; Mono-dicalcium phosphate, 294.118 mg; Cobalt from cobalt sulphate, 14.706 mg; C, 220.588 mg; Iron from Ferrous sulphate, 588.235 mg; Iodine from Potassium iodide, 1.029.412 mg; Manganese from Manganese sulphate, 2352.941mg; Zinc from Zi 1470.588 mg; Selenium from Sodium selenite, 8.824 mg; Lysine 5.882g, Methionine 38.235 g. Finisher- provides per kilogram of diet /finisher Macro: Vitamin A, 303030.300 UI; Vitamin D3, 60606.060 UI; Vitamin E, 757.576 UI; Vitamin E, 51.515 UI; Vitamin K, 60.606 mg; Vitamin B1, 60.606mg; Vitamin B2, 166.667mg; Vitamin B6, 121.212 mg; Niacin, 1060.606mg, Calcium Pantothenate, 333.333mg; Biotin, 3030.303mcg; Folic acid, 24.242mg; Vitamin B12, 606.061 mcg; Choline Chloride, 7870.909 mg; 6-Phytase (FTU), 30303.030 FTU; Lasalocid, 2727.273mg; Zinc bacitracin, 681.818mg; Limestone, 544.182g;Salt, 121.212g; Mono-dicalcium phosphate, 272.727g; Cobalt from cobalt sulphate, 15.152mg; Cu, 227.273mg; Fe, 606.061mg; I, 30.303mg; Manganese from Manganese sulphate mg 80.000 2424.242mg; Zinc from Zinc sulphate mg 50.000 1515.151 Selenium from Sodium selenite mg 0.300 9.091 Methionine, 3.030g; Enzyme Natuphos E, 3.030g.

Table 3.3: T: Chemical composition of experimental grower and finisher diets

[3]Experimental diets	Dietary Treatments					
	Grower			Finisher		
	SCG0	SCG50	SCG100	SCF0	SCF50	SCF100
[1]Commercial diet (%)	100	50	0	100	50	0
[2]Sprouted cowpea diet (%)	0	50	100	0	50	100
Total (%)	100	100	100	100	100	100
Calculated chemical composition (g/ kg $^{-1}$)						
Crude Protein	180	180.0	179.6	160.0	161.9	163.9
Neutral detergent fibre	126	74.3	22.60	35.0	27.5	20.6
Acid detergent fibre	29.4	17.0	2.7	13.0	11.2	9.3
Ca	7.0	7.2	7.5	6.0	6.0	6.1
P	5.5	5.6	5.7	5.0	5.3	5.5

[1]Complete grower (G) and finisher (F) [4]Meadow Feeds (PTY) LTD) Budget grower and finisher diets (controls), diluted with [5]sprouted cowpea (SC) diets iso-nutrient at 0, 50 and 100 % into the respective grower (SCG0, SCG50, SCG100) and finisher (SCF0, SCF50 and SCF100) experimental diets. [2]Cowpeas cleaned, screened for viable seed, sterilized by 30-minute treatment in 2% sodium hypochlorite, soaked overnight (12 hours) in tap water, spread evenly on open plastic sheets and irrigated manually to maintain adequate moisture to support sprouting over 4 days, and sun-dried on a concrete slab.

3.6. Experimental design and broiler management

The trial was conducted in a 7m (East-West) x 4m (North-South), deep litter, naturally ventilated, artificially supplementary lit open production house in which the Northern half was partitioned into thirty 1m x 1 m mesh-wire pens, each equipped with one 0.42 m x 0.39mm Launch republic Poltex tube feeders, one 0.4m x 0.36 m Poltek poultry water fountain, thereby giving a 0.21 m^2 floor space per bird. Each pen was equipped with a 175 W infra-red brooder lamp (PAR 38). Nine hundred mixed-sex, day-old Ross 308 broiler chicks were randomly allotted to 30 birds/pen in a balanced 3 (diet) x 2 (enzyme) factorial experiment replicated five times. Chicks were fed *ad libitum* on uniform starter (days 1-21) diets, followed by the experimental grower (days 22-35) and finisher (days (days 36-45) diets. Prophylaxis included standard vaccination for Newcastle (Clone 30), Infectious Bronchitis (IBH 120), Gumboro (D78), and Tylo tad (product, G2423 (Act 36/1947) in the drinking water.

3.7. Chemical analysis

The dry matter content was determined according to the AOAC (1990) method, 930.15. Ash contents were determined by drying the sample at 550°C overnight (AOAC, 1990; method 942.05). The Nitrogen (N) content were evaluated using a Kjeldahl procedure (AOAC, 1990; method 984.13). Fat content was determined by soxhlet fat extraction (AOAC, 1990; method 930.15).

3.8. Measurements

Feed intake, the mean live weight of random 8 birds/pen were evaluated for each feeding phase, from which the feed conversion ratio (FCR) (intake/gain) was calculated. After 42 days, broilers were fasted overnight for sanitary, humane slaughter using the Kosher method (Abe *et al*.,1996). Upon slaughter, 2 birds randomly selected per pen were used to evaluate the hot-carcass and abdominal visceral organ (heart, liver, gizzard, and intestines) weights. The dressed carcasses were stored at 4 ºC to allow dripping over 24 hours, after which the breast, wing, thigh, drumsticks yield (% live weight) were determined. Samples of the breast meat were used to determine pH, colour, water holding capacity and texture.

3.8.1. Meat colour

The colour L* (lightness), a* (redness), and b* (yellowness) values of raw broiler meat (breast) were measured using Hunterlab (ColourFlex; Hunter Associates Laboratory Inc., Reston, VA). All the measurements were conducted in triplicate. Total colour difference (ΔE), which indicates the magnitude of change in colour parameters between the initial and final colour values was calculated using the following equation.

$$\Delta E *= \sqrt{(\Delta a\hat{}(*2) + \Delta b\hat{}(*2) + \Delta L\hat{}(*2))}$$

3.8.2. Meat texture

Breast meat Warner-Bratzler shear force was measured using a TA-XT Texture analyser (Stable Micro System Ltd, Surrey England). Each sample was immobilized between stainless steel plates and then compressed perpendicular to muscle fibre orientation, in two consecutive cycles of 30% compression with 5s between cycles using cylinder probe of 4 cm diameter. The cross- head was programmed to move at a constant speed of 1 mm/s to determine sample hardness. The parameter was obtained by using the computer software whereby: Hardness (toughness) is the maximum force reached during the first compressive cycle.

3.8.3. Meat pH

Breast meat pH was measured using a Basic 20 pH meter, CRISON INSTRUMENT, SA, EU, following the method of Jiang *et al.* (2012).

3.8.4. Meat water holding capacity.

Water holding capacity was conducted according to the centrifugal method (Updikea *et al.*, 2005), with modification. A 5 g sample was added in a 12 ml 0.6 M NaCl solution in the test tube and was centrifuged at 5ºc for 30 min at the 1500 rpm in centrifuge (Universal 320R, BABOTEC South Africa). The supernatant was decanted and measured. Water holding capacity (WCH) was measured as ml of 0.6 M NaCl per 5 g of sample.

3.9. Statistical analysis

Data were subjected to analyses of variance for a 3 x 2 factorial experiment using the General Linear Model (GLM) procedures of Minitab Statistical package version 18 (Minitab, 2017).

$$Yijkl = \mu + Si + Ej + (SE)ij + \varepsilon ijkl$$

Y_{ijkl} - the l^{th} observed value

μ - the overall mean

S_i - effect of i^{h} diet

E_j - effect of level of the j^{th} enzyme level

$(SE)_{ij}$ - interaction of the factors

ε_{ijkl} - random error

Different (P<0.05) means were separated using Tukey's test.

Where performance parameters (feed intake, weight gain and the feed conversion ratio) were not (P>0.05) subject to diet * enzyme interaction, means of responses to the inclusion level of the sprouted cowpea diet were fitted into linear regression model to estimate the quantum of treatment effects, at statistical significance P<0.05;

$$Y = a + \beta X$$

where:

Y	-	response variable
X	-	treatment level
β	-	regression coefficient
a	-	intercept

CHAPTER 4: RESULTS

4.1. Growth performance

Broiler growth responses to treatments during the grower, finisher phases, and the cumulative effects are presented in Table 4.1. In the grower phase, broilers on the SCG100 diet had low ($P<0.05$) 35-day live weight gain (LGW_{35}) and consequently, attained low ($P<0.05$) live weight (LW_{35}), with a high FCR ($P<0.05$). In the finisher phase, diet x enzyme interaction was significant (p=0.013) for live weight on day 42 (LW_{42}). Lowest ($P<0.05$) LW_{42} was recorded for broilers on SCF100 (-) and SCF100 (+) diets, both similar ($P>0.05$) to the SCF50 (+) diet. The LW_{42} was highest ($P<0.05$) on the SCF0 (-), SCF0 (+) and SCF50(-) diets, while SCF100 (+) diets had the lowest. Diet x enzyme interaction was significant (p=0.013) for the 36-42 feed intake (FI_{36-42}). Lowest FI_{36-42} was recorded on the SCF0 (+), followed by the SCF0 (-), with high ($P<0.05$) FI_{36-42} on SCF100 (+), SCF100 (-), SCF50 (+), SCF50 (-) diets. Diet x enzyme interaction was significant (P=0.03) for the 36-42-day feed conversion ratio (FCR_{36-42}). Highest ($P<0.05$) FCR_{36-42} was recorded on the SCF100(+) diets, which was similar ($P>0.05$) to the SCF50 (+) diet. The least ($P<0.05$) FCR_{36-42} was recorded on the SCF0 (-) diet, which was similar to the SCF0 (+) diet. Intermediate FCR_{36-42} were recorded for SCF50 (-) and SCF100 (-) diets. Cumulatively, grower-finisher (day 22-42) live weight gain (LWG_{22-42}) was in the dietary order SCG0> SCG50>SCG100 ($P<0.05$), with a strong (Figure 4.1.a), significant linear effect ($Y = 1231.3 - 2.776x$, P = 0.043 and R^2 = 99.48). Feed intake (FI_{22-42}) was low ($P<0.05$) on the SCG0 diet, and was reduced ($P<0.05$) by enzyme supplementation. The feed conversion ratio (FCR_{22-42}) was in the dietary order SCG0< SCG50<SCG100 ($P<0.05$), with a strong, significant linear effect ($Y=1.6532 + 0.009x$, P = 0.017, R^2 = 99.93). Only the significant linear parameter regressions on the maize-sprouted cowpea dietary inclusion levels are depicted in Figure 4.1.

Table 4.1: Effects of grower-finisher phase dietary inclusion of sprouted cowpeas and supplementary enzymes on the performance of Ross 308 broilers

Treatments		Grower phase (days 22-35)					Finisher phase (days (36-42)				Grower-finisher days (22-42)		
		LW_{22} (g)	LW_{35} (g)	LWG (g/b/d)	FC (g/b/d)	FCR	LW_{42} (g)	LWG (g/b/d)	FC (g/b/d)	FCR	LWG (g/b/d)	FC (g/b/d)	FCR
Diet													
[1]SC0		792.8	1622.8^{a}	59.3^{a}	85.1	1.4^{b}	2018.1^{a}	56.5	116.8^{b}	2.2^{b}	58.4^{a}	69.3	1.6^{c}
[2]SC50		797.8	1572.1^{a}	55.3^{a}	89.8	1.6^{b}	1901.8^{a}	47.1	155.0^{a}	3.4^{a}	52.6^{b}	67.2	2.1^{b}
[3]SC100		801.8	1431.1^{b}	45.0^{b}	92.6	2.1^{a}	1749.6^{b}	45.5	156.5^{a}	3.8^{a}	45.1^{c}	69.3	2.6^{a}
SEM		14.10	26.90	1.94	2.91	0.09	104.39	4.70	4.99	0.85	1.59	2.00	0.23
[2]Enzyme													
-		799.3	1556.6	54.1	92.3	1.8	1910.8	50.6	1040.9	3.1	52.9	68.6	2.1
+		795.5	1527.4	52.3	86.0	1.7	1868.9	48.8	971.8	3.2	51.1	63.9	2.1
SEM		11.50	21.90	1.58	2.38	0.08	151.62	3.83	4.07	1.09	1.30	1.63	0.45
Diet	[2]Enzyme												
[1]SC0	-	789.2	1651.2	61.6	85.8	1.4	2015.3^{a}	52.0	132.2^{b}	2.7^{bc}	58.4	63.5	1.7
[2]SC50	-	815.0	1597.5	55.9	95.2	1.7	1950.3^{ab}	50.4	161.6^{a}	3.3^{b}	54.1	71.2	2.2
[3]SC100	-	793.5	1421.0	44.8	95.8	2.2	1766.8^{c}	49.4	152.3 ^{ab}	3.3^{b}	43.9	71.1	2.5
[1]SC0	+	796.0	1594.3	57.0	84.4	1.5	2021.0^{a}	61.1	101.4^{c}	1.7^{c}	58.3	61.1	1.6
[2]SC50	+	780.5	1546.7	54.7	84.3	1.6	1853.5^{bc}	43.8	148.8^{ab}	3.5^{ab}	51.1	63.3	2.1
[3]SC100	+	810.0	1441.2	45.1	89.3	2.0	1732.52^{c}	41.6	166.7^{a}	4.3^{a}	43.9	67.5	2.6
SEM		20.00	38.00	2.74	4.12	0.13	105.61	6.64	7.05	0.78	2.25	2.83	0.23
P values													
Diet		0.901	0.000	0.000	0.208	0.000	0.000	0.223	0.000	0.000	0.000	0.056	0.000
Enzyme		0.820	0.356	0.424	0.072	0.551	0.100	0.742	0.100	0.714	0.334	0.054	0.534
Diet*Enzyme		0.413	0.538	0.669	0.526	0.580	0.013	0.386	0.013	0.030	0.794	0.603	0.366

^{ab} For each factor and interactions, parameter means with different superscripts differ significantly at $P < 0.05$. [1]Sterilized by 30-minute treatment in 2% sodium hypochlorite, soaked overnight (12 hours) in tap water, 4-day open sprouting, and sun-dried. cowpea (SC)-maize grower (G) diets diluted at 0% (SGC0), 50% (SCG50) and 100% (SCG100) into iso-nutrient respective Meadow Feeds (PTY) LTD Budget Grower (G) Feed (control). [2]Duplicate diets supplemented with (+) or without (-) 200 g tonne^{-1} of Rononzyme ® ProAct (75 000 PROT g^{-1} serine protease). LW- live weight, LWG- live weight gain, FC- Feed consumption, FCR- Feed conversion ratio. SEM: Standard error of the mean).

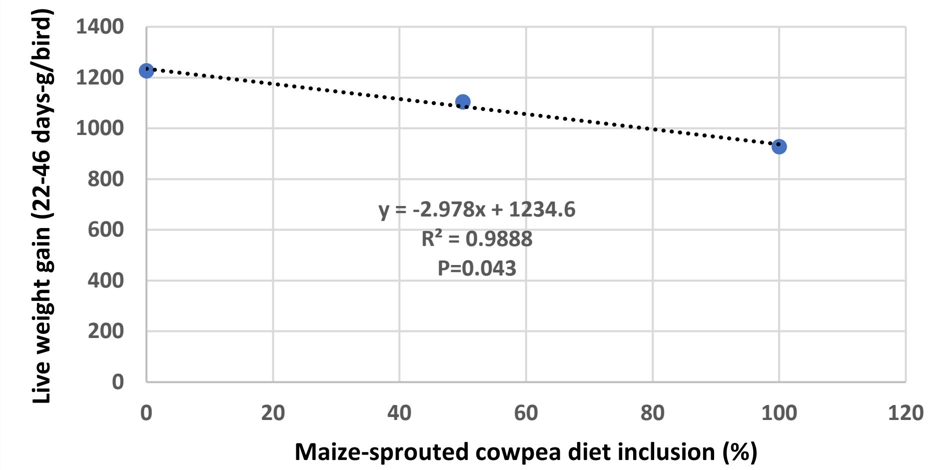

Figure 4.1a:Regression of the cumulative (day 22-42) live weight gain on maize-sprouted cowpea diet dilution into a maize soybean diet

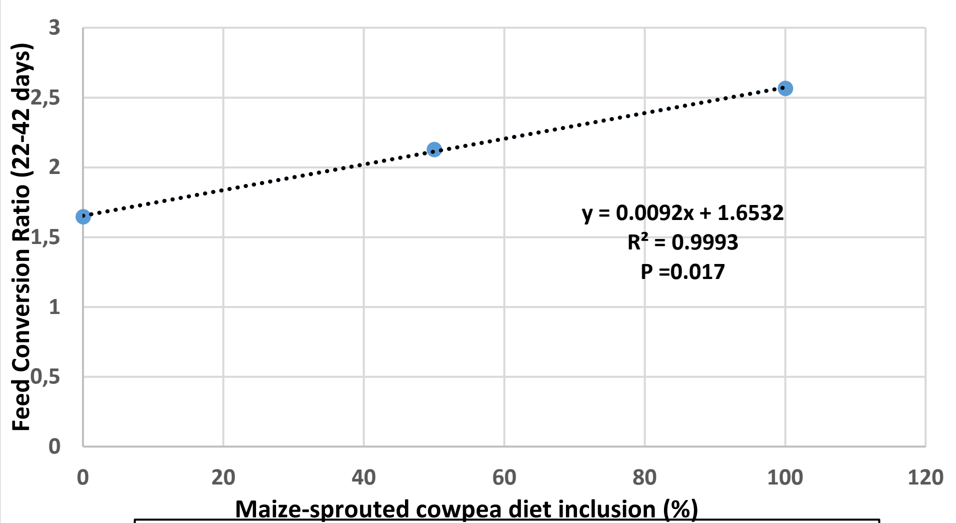

Figure 4.1b Regression of the cumulative (day 22-42) feed feed conversion ratio on maize-sprouted cowpea diet dilution into a maize soybean diet

Figure 4.1a-b: Grower + Finisher phase broiler growth performance

Figure 4.1a-b: Grower+ Finisher phase broiler growth performance. [1]Sprouted Cowpea-maize grower-finisher diets diluted at 0%, 50% and 100% (SCG0-SCF0, (SCG50-SCF50) and SCG100-SCF100 feeding regimens, respectively) into iso-nutrient respective Meadow Feeds (PTY) LTD Budget Grower and Finisher Feeds (controls), duplicates of experimental diets supplemented with (+) or without (-) 200 g tonne^{-1} of Rononzyme ® ProAct (75 000 PROT g^{-1} serine protease

4.2. Slaughter performance

Treatment effects on broiler carcass parameters and abdominal viscera are presented in Table 4.2. There were no diet * enzyme interactions across all parameters (P>0.05). The 42-day slaughter live weight (LW_{42}) was in the dietary order SCG0-SCF0> SCG50-SCF50>SCG100-SCF100 (P<0.05). Broilers on the SCG0-SCF0 dietary regime had larger carcasses and the proportionate breast (P<0.05). Broilers on the SCG100-SCF100 dietary regime had smallest proportions of wings and thighs (P<0.05) compared to other dietary regimes. The treatments did not (P>0.05) affect the abdominal viscera. Broilers on diets with enzyme fortification had smaller wings and heart effect (P<0.05) while there was no effect on all other slaughter parameters.

The SCG100-SCF100 feeding regime with or without enzyme inclusion, had smallest slaughter weight and proportionate slaughter weight (P<0,05) compared with SCG0-SCF0 (with or without enzyme fortification). The experimental treatments did not have an effect on the dressing percentage (P>0.05). Broilers on diets without cowpeas had significantly higher wings and breast proportions (P<0.05) compared to broilers on 100% maize-cowpea diets. There was no significant difference in thigh weight within the maize-cowpea diets (P>0.05). The experimental treatment combinations did not affect the abdominal viscera of the broilers.

Table 4.2: Effects of grower and finisher dietary inclusion of maize-sprouted cowpeas and supplementary enzymes on slaughter performance of Ross 308 chickens

Treatments		Live Weight (g)	Carcass Weight (g)	Dressing (%)	Dressed carcass components. (% dressed weight)			Abdominal viscera (% live weight)				
					Wings	Breast	Thighs	Heart	Liver	Gizzard	Spleen	Abdominal fat
Diet												
SCG0-SCF0		2105.7^{a}	1535.3^{a}	73.0	8.2^{a}	58.4^{a}	22.1^{a}	0.8	3.7	4.1	0.3	0.3
SCG50-SCF50		1953.7^{b}	1443.7^{b}	74.0	8.1^{b}	51.7^{b}	20.6^{a}	0.8	3.7	4.2	0.3	0.3
SCG100-SCF100		1794.3^{c}	1342.3^{c}	75.2	7.6^{c}	49.2^{b}	18. 8^{b}	0.8	3.7	3.9	0.2	0.3
SEM		189.65	147.10	6.35	1.52	6.72	2.52	0.16	0.52	0.62	0.07	1.33
Enzyme												
-		1967.3	1435.8	73.3	8.1^{a}	53.4	20.8	0.85^{a}	3.7	4.1	0.2	0.3
+		1935.1	1445.1	74.9	7.8^{b}	52.8	20.1	0.77^{b}	3.6	4.0	0.2	0.3
SEM		227.63	166.51	6.33	0.88	7.74	2.84	0.159	0.51	0.62	0.08	0.13
Diet-Enzyme treatments												
Diet	Enzyme											
SCG0-SCF0	-	2105.3^{a}	1507.3^{a}	71.5	8.2^{a}	57.4^{a}	21.6^{ab}	0.9	3.7	4.0	0.2	0.3
SCG50-SCF50	-	2010.7^{ab}	1463.3^{ab}	72.6	8.3^{a}	54.0^{ab}	21.5^{ab}	0.9	3.8	4.4	0.3	0.3
SCG100-SCF100	-	1786.0^{c}	1336.7^{b}	75.6	8.0^{ab}	48.5^{b}	19.4^{bc}	0.8	3.8	4.0	0.2	0.4
SCG0-SCF0	+	2106.0^{a}	1563.3^{a}	74.5	8.3^{a}	59.3^{a}	22.6^{a}	0.8	3.7	4.1	0.3	0.3
SCG50-SCF50	+	1896.7^{bc}	1424.0^{ab}	75.4	7.9^{ab}	49.5^{b}	19.7^{bc}	0.8	3.5	4.0	0.2	0.3
SCG100-SCF100	+	1802.7^{c}	1348.0^{b}	74.8	7.2^{b}	49.5^{b}	18.1^{c}	0.8	3.6	3.9	0.2	0.3
SEM		189.91	148.26	6.35	0.83	6.67	2.45	0.16	0.52	0.62	0.073	0.13
P Values												
Diet regime		0.000	0.000	0.411	0.011	0.000	0.000	0.385	0.938	0.413	0.110	0.720
Enzyme		0.504	0.791	0.221	0.044	0.684	0.237	0.019	0.244	0.272	0.607	0.489
Diet-Enzyme treatments		0.000	0.000	0.420	0.004	0.000	0.000	0.145	0.828	0.289	0.185	0.144
Diet * Enzyme		0.352	0.463	0.300	0.136	0.147	0.064	0.686	0.710	0.204	0.239	0.300

^{abc} For each factor and interactions, parameter means with different superscripts differ significantly at $P < 0.05$.[1]Sprouted (sterilized by 30-minute treatment in 2% sodium hypochlorite, soaked overnight (12 hours) in tap water, 4-day open sprouting, and sun-dried, sprouted cowpea -maize grower-finisher diets diluted at 0%, 50% and 100% (SCG0-SCF0, (SCG50-SCF50) and SCG100-SCF100 feeding regimens, respectively) into iso-nutrient respective Meadow Feeds (PTY) LTD Budget Grower and Finisher Feeds (controls). [2]Duplicates of experimental diets supplemented with (+) or without (-) 200 g tonne^{-1} of Rononzyme ® ProAct (75 000 PROT g^{-1} serine protease)

4.3. Meat quality

Treatment effects on parameters of breast meat quality are presented in Table 4.3. The diet did not affect the lightness coordinate (L) of the meat. The SCG50 - SCF50 and SCG100-SCF100 feeding regime resulted in a low value for the meat redness coordinate (a) ($P < 0.05$). The yellowness coordinate (b) was in the order SCG0-SCF0>SCG50-SCF50>SCG100-SCF100 ($P < 0.05$). Meat water holding capacity and the shear force were higher on the SCG0-SCF0 compared to the SCG100-SCF100 feeding regime ($P<0.05$). The diet did not affect the meat pH ($P>0.05$).

The enzyme fortification resulted in higher ($P<0.05$) value of meat redness coordinate (a) while it did not affect the lightness and yellowness coordinates. The meat pH, water holding capacity and shear force were not affected by the enzyme fortification. The SCG100-SCF100 with enzyme fortification had the least redness and yellowness coordinates. There was a significant ($P<0.05$) diet*enzyme interaction on the shear force parameter of the carcass. SCG0-SCF0 had a higher ($P<0.05$) shear force than SCG50-SCF50 when there was enzyme fortification but they were not different without enzyme inclusion.

Table 4.3: Effects of grower and finisher dietary inclusion of sprouted cowpeas and supplementary enzymes on meat quality of Ross 308 chicken

Treatments		Colour				pH	Water holding capacity. (%)	Shear force (kg/cm)
		L	a	b	ΔE			
Diet								
SCG0-SCF0		56.7	8.4^{a}	17.4^{a}	30.4	6.0	7.8^{a}	75.3^{a}
SCG50-SCF50		56.4	7.8^{b}	16.4^{b}	30.0	6.0	6.7^{ab}	60.5^{b}
SCG100-SCF100		56.4	7.7^{b}	15.2^{c}	32.1	6.0	5.3^{b}	49.5^{b}
SEM		4.21	1.70	1.66	7.49	0.30	6.55	4.00
Enzyme								
+		56.0	7.6^{a}	16.4	29.2^{b}	6.0	7.0	63.4
-		57.0	8.3^{b}	16.3	32.5^{a}	6.0	6.2	60.1
SEM		4.17	1.70	1.88	7.35	0.30	6.61	6.41
Diet-Enzyme treatments								
Diet	Enzyme							
SCG0-SCF0	-	56.2	8.7^{a}	17.3^{ab}	31.9^{ab}	6.0	6.2^{ab}	63.6^{b}
SCG50-SCF50	-	55.7	8.1^{ab}	16.4^{b}	31.8^{ab}	6.0	6.9^{ab}	68.6^{ab}
SCG100-SCF100	-	56.0	8.0^{ab}	15.3^{c}	33.6^{a}	6.0	5.5^{ab}	48.5^{b}
SCG0-SCF0	+	57.1	8.1^{ab}	17.5^{a}	28.9^{b}	6.0	9.4^{a}	87.2^{a}
SCG50-SCF50	+	57.1	7.5^{b}	16.4^{b}	28.3^{b}	5.9	6.4^{ab}	52.5^{b}
SCG100-SCF100	+	56.8	7.3^{b}	15.1^{c}	30.5^{ab}	6.0	5.1^{b}	50.5^{b}
SEM		4.20	1.68	1.67	7.35	0.30	6.51	11.10
P Values								
Diet		0.895	0.010	0.000	0.149	0.931	0.036	0.000
Enzyme		0.055	0.003	0.815	0.000	0.348	0.362	0.501
Diet-Enzyme treatments		0.530	0.002	0.000	0.006	0.854	0.030	0.000
Diet*Enzyme		0.876	0.942	0.748	0.965	0.625	0.086	0.002

[abc] For each factor and interactions, parameter means with different superscripts differ significantly at $P < 0.05$. [1]Sprouted (sterilized by 30-minute treatment in 2% sodium hypochlorite, soaked overnight (12 hours) in tap water, 4-day open sprouting, and sun-dried) cowpea (SC)-maize diets diluted at 0% (SC0), 50% (SC50) and 100% (SC100) into iso-nutrient respective Meadow Feeds (PTY) LTD Budget Grower and Finisher Feeds (controls). [2]Duplicates of experimental diets supplemented with (+) or without 200 g tonne^{-1} of Rononzyme ® ProAct (75 000 PROT g^{-1} serine protease). L: luminosity, a: red, b: yellow, ΔE: change in colour. SEM: Standard Error of the Mean

CHAPTER 5: DISCUSSION

In this study, dilution of the standard diets with the maize-cowpea diet negatively impacted on broiler growth. Unlike findings by Adino *et al*., (2018), who recommended cowpea inclusion rate of up to 75%, growth was most depressed above the 50 % inclusion level. The reduction in growth was accompanied by a corresponding increase in feed intake, resulting in poor feed conversion ratio. The reduction in growth was consistent with previous reports in broilers on cowpea diets (Defang *et al*., 2008). The observed weight gain on the cowpea diets was lower than reported by Embaye *et al.* (2018), consistent with the higher inclusion levels of cowpeas in the current study. Dal Bosco *et al*., (2013) suggested cowpea anti-nutritional factors such as vicine and convicine could limit performance, even in low concentrations. Previous studies indicated that dietary tannins affect palatability, with consequent decrease in feed intake (Carew, *et al*., 2003; Tuleun, *et al*.,2009). However, in the present study, feeding broilers sprouted cowpeas cumulatively increased their feed intake, which implied efficient tannin destruction through sprouting. The increased intake with dietary inclusion of sprouted cowpea did not increase live weight gain. Consequently, birds fed the maize-sprouted cowpea diets showed the worst feed conversion during the grower, suggesting inefficient nutrient, particularly protein digestion and metabolism, which can result in more fat, relative to lean accretion. Díaz, *et al*. (2006) similarly observed higher feed conversion ratio during the grower period when extruded faba bean replaced soybean.

Cumulatively, live weight gain through the grower-finisher phases decreased with sprouted cowpea dietary dilution in strong, significant linear fashion. The feed conversion ratio was in the reverse dietary order to the weight gain, with a similarly strong, significant linear effect. Feed intake was reduced by enzyme supplementation. Despite limited overall enzyme inefficacy, diet * enzyme interactions on growth parameters were observed which implied some level of enzyme action. In the finisher phase, diet x enzyme interaction occurred for 42-day live weight, with low weight recorded for broilers on the SCF100 regardless of enzyme supplementation, and similar low weight on the SCF50 (+) diet. Feeding the SCF0 diets achieved the heaviest live weights regardless of the enzyme, similar to the SCF50 diets. Diet x enzyme interaction also occurred for the feed intake, with low intake of the SCF0 (+), slightly more than that of the SCF0 (-) diet, compared to the high intake of the SCF100 and SCF50 diets regardless of enzyme supplement. FCR was low on the SCF0 diet regardless of enzyme supplementation. Intermediate FCR were recorded for SCF50 (-) and SCF100 (-) diets. The biochemical effects which could explain these diet-enzyme interactions need further investigation. There are no reports on the effects of dietary interactions on broiler growth parameters when cowpea diets are supplemented with protease enzymes. Similar effects on weight gain and feed conversion ratios among treatment groups were similar to those reported by Goodarzi Boroojeni *et al*. (2017). Improvement in FCR in diets supplemented with an exogenous enzyme may be due to enhanced feed digestibility, metabolism and subsequent growth.

Limited, diet dependent overall efficacy of Ronozyme® ProAct is in agreement with a previous study by Adeoye, *et al*. (2016). However, the results contradicted the findings of Naela, *et al*. (2017) who reported that serine-protease enzyme (Ronozyme® ProAct) increased growth performance on a cultured Oreochromis niloticus fed diets. O'Shea, *et al.* (2015) reported that pigs offered a standard balanced diet with protease enzymes had reduced average daily weight gain. In contrast, Aswar *et al.,* (2018) reported significant improvement in live weight of birds fed 5%, 10% and 15% moong dal waste with enzyme supplementation. The cumulative enzyme benefit on feed intake observed in the current study was inconsistent with findings of several researchers (Ivarsson & Wall, 2017, Aswar, *et al.,* 2018., Metwally, *et al*., 2020, and Park, *et al.,* 2020), who, in different livestock species, reported no effects on feed intake with exogenous enzymes. Aswar, *et al*. (2018) observed contrasting results to this study when they fed birds with 10% toor dal waste with enzyme and found significantly better feed conversion ratio as compared to control.

Kaankuka, *et al.,* (2000) reported that processing improves the utilization of proteins and energy contained in legumes for growth. In this study, slaughter live weight decreased with the dilution with the sprouted cowpea diet. The disparity is likely due different efficiencies of nutrient utilization. Broilers on the sprouted cowpea diets had smaller carcasses and proportionate breast meat. High level dilution with the sprouted cowpea diets also reduced the proportionate weight of wings and thighs. Similar findings were reported by Abdel-Monein (2013). They reported a decrease in the total edible parts with increase in the inclusion level of green bean, despite similar percentage dressed weight. Akintunde, *et al.* (2013) reported reduced variation in carcass characteristics with supplementary enzymes. In the present study, the enzyme had no effect on any of the slaughter parameters.

In the present study, internal organ weights of broiler chickens were not affected by the inclusion of sprouted cowpea in the diets. The observation is supported by the findings obtained by Abdelgani *et al*., (2013). Feeding broiler chicks with cowpea inclusion rate of up to 5% did not affect the liver and pancreas weights. Ravindran, *et al*., (2010) and Nalle *et al*.,

(2011) also reported that the liver, gizzard, and pancreas weights were not affected when chickens were fed graded levels of field pea diets. However, Defang, *et al.* (2008), noticed an increase in relative weight of the liver and the gizzard in birds fed cowpea-based diets and attributed it to the intense activity undertaken by these organs to counteract the toxic effect of dietary anti-nutritional factors. Observations in this study could be due to the effect of sprouting on reducing the anti-nutrient activity in cowpea seeds. Devi *et al.*, (2015) reported an improvement in nutritional quality and a significant reduction in trypsin inhibitor activity and other toxic elements in sprouted cowpeas.
While in previous studies (Eljack *et al.*, 2010), cowpea inclusion was reported to improve the dressing percentage and relative carcass cuts, an opposite was noticed in the current study. This could be linked to the poor FCE observed with an increase in cowpea inclusion level, suggesting a lower bioavailability of cowpea nutrients compared to the control.

Meat colour is among the first quality characteristics to be noticed by customers, more so in boneless products. Chicken breast meat is generally ideally characterised by a pink colour, which is considered most desirable to the consumer (Choo, *et al.,* 2014). In the present study, broilers on the SCG50 - SCF50 feeding regime had low meat redness coordinate (a). The yellowness coordinate (b) decreased with dietary dilution with the sprouted cowpea diet. The overall effect of feeding cowpeas to broiler was therefore a decline in meat colour. Differences in lightness (L*) and redness (a*) were observed, and not for yellowness (b*) and the total colour difference (∆E) when control diet was replaced by maize-sprouted cowpea diet. This is supported by Dotas, *et al.* (2014), who reported no differences in meat colour among the dietary treatments. However, in a study by Laudadio and Tufarelli, (2010) chickens fed sprouted cowpeas, had similar yellowness (b*), higher lightness (L*) coordinates, and lower redness (a*) in breast muscle compared to birds fed a standard soybean diet. The different observations may be attributed to the differences in the broader nutrient compositions of the total mixed rations.

Water-holding capacity is an important attribute of meat quality, which if poor, whole meat and further-processed products will lack juiciness (Gentry, et *al.* 2004). In this study, both water holding capacity and the shear force were higher on the SCG0-SCF0 compared to the SCG100-SCF100 feeding regime (P<0.05). The effects of cowpeas on WHC were similar to findings by Laudadio and Tufarelli (2010). Water-holding capacity was higher in birds fed a pea diet, with higher WHC in drumstick, than in breast muscle (Laudadio and Tufarelli, 2010).

The shear force value is an indication of the degree of toughness or tenderness. The higher the value obtained the tougher the meat. In the present study, variable (49 and 75 kg/cm) shear force values were obtained for the breast muscles of birds across the treatments, which decreased at the high level of dietary cowpeas inclusion.

Meat pH has been associated with the carcass water holding capacity which influences the cook-loss, shelf life and meat tenderness (Mir *et al*., 2017). In this study, meat pH 24-hours post-mortem was not influenced by the dietary treatment, indicating similar acidification.

In the present study, enzyme action was only evident in the diet x enzyme interaction which occurred for the shear force. According to Werner, *et al*. (2009), enzymes do not generally affect quality parameters. For example, in broilers on corn and soybean meal, Zakaria, *et al*. (2010) did not observe any enzyme effects on pH, water holding capacity, colour and luminosity at 42 days of age.

5.1. Conclusion

Dilution of the control diet with the sprouted cowpea diets linearly reduced the live weight gain, feed efficiency ratio, carcass and prime meat cut yields, and caused adverse effect on meat quality, with more adverse carcass and meat quality effects at the 100%, compared to the 50% dilution level. Adverse metabolic and physiological effects were, however, not indicated by the enlargement of the liver and gizzard at the high inclusion of sprouted cowpea in broiler diets. The better performance in birds fed the control, compared to the cowpea diets may have been due to its superior protein quality, and adverse effects of residual cowpea anti-nutrients in the latter. Despite limited overall enzyme inefficacy, diet * enzyme interactions on growth parameters were observed which implied diet dependent enzyme action. Diet dependent negative effects of Ronozyme® ProAct were attributed to dietary characteristic such as the fibre content. Other potential biochemical effects which could further explain these interactions need further investigation. Further research is recommended to determine the most economical methods to improve cowpea processing to enhance the quality, combined with cost-benefit analyses to determine viable inclusion levels in broilers diets.

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