Effect of Processed Cowpea-based Diets (*Vigna unguiculata*) on Intake and Digestibility, Growth Performance, Meat Quality and Sensory Characteristics of Broiler Chickens

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A Dissertation Submitted in Fulfilment of the Requirements for the Master of Philosophy Degree in Animal Production and Technology

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June 2024

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Declaration

I, Edmore Bumhira, hereby certify that this Dissertation is the product of my own research and has not been turned in for evaluation at any University. Citations and references have been used to acknowledge information gleaned from the work of other researchers.

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ACKNOWLEGEMENTS

I would like to express my heartfelt gratitude to my research supervisor Professor James Madzimure for his guidance, encouragement and tireless support that enabled me to complete this research. Professor Madzimure stood with me during times of emotional distress and he motivated me to work very hard since there is no substitute for hard work. Moreover, I extend my gratitude to my co-supervisor Professor Irvin Mpofu for his resolute assistance during my academic career. I also wholeheartedly appreciate the help I got from Dr Tonderai Mutibvu in enlightening me on how to use SAS in analysing statistical data.

I also do not forget Thomas Musiiwa and Tanaka Mutsindiko who successfully looked after the experimental birds during the six-week experimental period. They intervened with their practical experience of poultry rearing to make the research a beacon of success. I thank them for their invaluable time they devoted to aid in the completion of the research experiment.

I am also thankful to Linda Nhandara, Chinhoyi University of Technology's Animal Production and Technology Laboratory Technician who helped me to carry out the sensory evaluation test of broiler meat samples that were collected from my experimental birds. She benevolently shared her laboratory skills to me for free without any monetary or material benefit

A lot of gratitudes are given to Cold Storage Company's Corporate Rescue Practioner, Mr Crispen Mwete and his two advisors viz; Mr Maxen Kasore and Mr Clifford Mushambadzi who assisted me financially by offering me an education loan which I used to pay the tuition fees and meet all research expenses without any financial hassles.

ABSTRACT

Effect of Processed Cowpea-based Diets (*Vigna unguiculata*) on Intake and Digestibility, Growth Performance, Meat Quality and Sensory Characteristics

The objective of this study was to increase knowledge on the effectiveness of processed cowpeas as a soybean supplement in broiler diets. The main goal was to improve domestic self-sufficiency in supplementary protein in poultry diets and lowering imports of soybeans. Three experiments (I – III) were conducted to determine the effects of processed cowpea-based diets on feed intake and growth performance (I), protein digestibility and ammonia emissions (II) and meat quality and sensory characteristics of broiler chickens (III). A total of 210 unsexed day-old broiler chicks were randomly allocated to seven dietary treatments in a completely randomized design. The dietary treatments included maize and cowpeas in partial substitution of soybean namely: maize-soybean-based (control), raw cowpea-based, boiled cowpea-based, roasted cowpea-based, dehulled cowpea-based, dehulled boiled cowpea-based and dehulled roasted cowpea-based diets. Diets were formulated to be iso-energetic and iso-nitrogenous. Dietary treatment had no effect (P>0.05) on feed intake nor live weight gain (LWG). Diets containing boiled, roasted and dehulled-roasted cowpea had similar (P>0.05) true protein digestibilities as the basal diet (64.9%). True protein digestibility in raw, dehulled-boiled, and dehulled cowpea-based diets were lower (P<0.05) ranging from 51.0 to 55.2%. Dietary treatment had no effect (P>0.05) on the feed conversion ratio (FCR) and ammonia emission but influenced meat chemical composition (P<0.05). The average weekly FCR per bird ranged from 1.70 to 1.80. The average weekly ammonia emission ranged from 1.2 ppm in broilers fed diets containing dehulled cowpea meal to 1.5 ppm in broilers fed diets containing raw cowpea meal. Dietary treatments influenced the proximate composition of broiler meat (P<0.05). Treatment significantly affected (P<0.05) broiler meat pH and water holding capacity. Meat from broilers that were fed with diets containing roasted cowpeas had the highest water holding capacity of 74.1% and the meat from broilers that were fed with diets containing raw cowpea meal had the lowest water holding capacity (61.5%). The diets containing boiled cowpea meal produced broiler meat with the highest pH of 6.6 and the raw cowpea meal treatment group had the lowest (6.0). Meat from broilers that were fed with diets containing raw cowpea meal and boiled cowpea meal had the highest crude fiber of 1%. Meat from broilers fed diets containing roasted, dehulled-roasted and dehulled-boiled cowpea meals had the lowest crude fibre (0.8% each). Dietary treatments had an influence (P<0.05) on the color, texture, taste, and tenderness of broiler meat. Meat from broilers fed diets containing raw cowpeas had the lowest colour and texture scores, while the rest of treatments were similar in the two parameters. There were no differences in the aroma scores of all treatments. The meat from dehulled-boiled, dehulled and boiled cowpea-based groups had the highest taste scores (5.0). Meat from raw cowpea-based treatments had the lowest taste score (2.0), while the rest of the treatments were similar. The meat from the control, dehulled-boiled cowpea and boiled cowpea-based diets had the highest tenderness scores and raw cowpea-based diet had the lowest. The remaining dietary treatment groups were not different. Therefore, dehulled, dehulled-roasted, dehulled-boiled, boiled and roasted cowpeas can be included in broiler starter and broiler finisher diets at 10 and 15% inclusion levels respectively without negatively affecting broiler feed intake and protein digestibility, growth performance, carcass quality and sensory characteristics of broiler meat.

Keywords: Alternative plant protein, anti-nutrients, nutrient utilisation, body weight gain, environmental pollution, meat's organoleptic attributes

LIST OF ABBREVIATIONS

AA	Amino Acids
AME	Apparent Metabolisable Energy
ANF	Anti-Nutritional Factors
AOAC	Association of Official Analytical Chemists
CF	Crude Fibre
CP	Crude Protein
DFD	Dark Firm Dry
DM	Dry Matter
EE	Ether Extract
FCR	Feed Conversion Ratio
HAB	Harmful Algal Blooms
HCI	Hydrochloric Acid
MT	Metric tons
Na^+	Sodium
NSP	Non Starch Polysaccharides
$PM_{2.5}$	Particulate Matter 2.5
PSE	Pale Soft Exudative

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CHAPTER 1

INTRODUCTION

1.1 Background

Cowpeas are a very important, carbohydrate, dietary fiber and protein-rich crop commonly grown in Africa (Wakibia, 2015). Both human food and animal feed can be made from it. Antinutrients, such as phytates, protease inhibitors, lectins and tannins, however, limit its use in livestock feed manufacture, particularly for broiler feed (Abbas and Ahmad, 2018). The problem is further compounded by technical limitations including the lack of research and the need for processing technologies aimed at improving their utilisation as a broiler feed ingredient (Omenna*et al*., 2016).

According to Koivunen *et al*. (2016), the majority of the protein in broiler feeds now comes from soybean meal, with minor percentages coming from animal sources such blood meal, meal derived from flesh and bones, and meal derived from fish. The popularity and use of the latter has been reduced and, in some countries, abolished in livestock feed formulations due to their association with zoonotic diseases e.g salmonellosis. In developing and underdeveloped countries, their use is limited by high cost and unavailability (Omenna *et al*., 2016).

Direct competition between humans and livestock for soybeans (Katumo, 2018) has driven increased demand for the commodity, leading to increased prices on the market. Due to low productivity brought on by recurrent droughts, inadequate irrigation systems, costly supplies, a lack of funding, price controls, unfavourable contract systems, unbackable land tenure, and low technical farmer skill, there is a scarcity of soybeans in Zimbabwe (Nyoni, 2021). As a major ingredient in feed making, high prices of soybean meal (US$780 per ton) mean high feed costs, which reduce the viability of poultry production. The high cost of soybeans makes it important to look for alternatives as well as practical ways to combine other protein sources for making chicken feed (Gadzirayi *et al*., 2021). In Zimbabwe, cowpeas are a versatile and locally accessible source of plant protein. Cowpeas have greater drought tolerance compared to the conventional soybeans. Cowpeas are an underutilized legume and are not utilized in commercial poultry feed formulation, yet they have good nutritional profiles for potential combination with conventional protein sources for poultry feeds (Katumo, 2018).

Besides its beneficial effects in terms of drought tolerance and good nutritional profile, cowpeas contain some anti-nutritional factors (Abbas and Ahmad, 2018) that are known to have the substantial effect of reducing the digestion of proteins in the gut of monogastric animals. Tannins precipitate with proteins to form insoluble complexes which make proteins indigestible to enzymes (Omenna *et al*., 2016). Phytates precipitate with minerals and make them indigestible and not absorbable in small intestines (Koivunen, 2016). Katumo (2018) reported that Protease inhibitors inactivate chymotrypsin, trypsin, and α-amylase and render them unable to digest proteins.

The undigested protein is excreted from the avian digestive tract as uric acid in urine and protein in faeces (Ritz *et al*., 2004). Because of its volatility, uric acid can be readily hydrolyzed, mineralized, and volatilized to produce ammonia (Ritz *et al*., 2004; Gerber *et al*., 2010; Ershadi, 2020). Ammonia is produced when bacteria, specifically *Bacillus pastures*, break down undigested protein and uric acid in poultry litter (Such *et al*., 2021). According to published data, ammonia concentrations above 25 ppm reduce broiler feed intake, feed efficiency, and immunity to disease as well as cause conjunctivitis in the eyes (Ali, 2021). According to Ali (2021), ammonia contaminates the land, water, and air. When ammonia is released into the atmosphere, it combines with acidic substances like nitric acid and sulfuric acid to create ammonium nitrate and sulphate, which can be left behind on soil, vegetation, and water surfaces (Gerber *et al*., 2010). These acidic deposits can cause eutrophication in open water bodies and abundant vegetation growth leading to the replacement of slow-growing species with fast - species thereby affecting both aquatic and terrestrial ecosystems (Ritz *et al*., 2004).

It is well established that when poultry are fed on raw legume grain, several nutritional disorders ensue. Therefore, to lessen the anti-nutritional elements in legume grains, a variety of processing techniques have been employed, including roasting, dehulling, soaking, and boiling (Nalle, 2010; Koivunen, 2016). Dehulling removes tannins and structural fibers that are located in the seed hulls but do not alter the composition of protease inhibitors, lectins, and phytates which are located in the seed cotyledon (Cho *et al*., 2019). Boiling can reduce or completely eradicate all anti-nutrients in legume grains but inadvertently reduce nutrient concentrations through leaching (Chisowa, 2022). Roasting removes anti-nutritional factors in the grains but over-heating and underheating can negatively affect the chemical composition of grain legumes (Bumhira and Madzimure, 2022). Under–heating fails to remove anti-nutrients and overheating can cause soluble sugars to react with an amino – group to form insoluble nutrient complexes (Maillard reactions) that are not digestible.

There hasn't been much research done on the effects of feeding raw and processed cowpea grain on nutritional digestibility and broiler performance (Usubharatana and Phungrassani, 2016), and the findings of the few studies that have been done have been inconsistent. Furthermore, not much is known regarding the impact of feeding cowpea meal to broiler diets on the atmospheric ammonia output. Therefore, more research is needed to understand how partially substituting cowpea meal for soybean meal in broiler diets affects feed intake, nutritional digestibility, and ammonia emission.

Nowadays, health concerns are top of mind for consumers who eat chicken, along with the overall look and quality of chicken flesh and other poultry items. The quality of chicken meat is influenced by various factors such as pH, color, cooking loss, water holding capacity, drip loss, and more (Bennato *et al*., 2021; Bosque *et al*., 2022; Kyakma *et al*., 2022). Numerous aspects, such as hybrid and sex, length of fattening, handling before slaughter, freezing of carcass, storage duration, and feed composition, influence the flesh and sensory qualities of broiler meat (Bogosavlievic-Boskovic *et al*., 2010). It is claimed that alteration of chicken feed through the combination of several feedstuffs with varying nutritional profiles affects the composition of chicken meat but there is a dearth of information on the impact of feeding broilers with processed cowpea grains on meat and the sensory qualities of broiler meat.

1.2 Problem Statement

There is a shortage of soybeans to use in the manufacture of livestock feed in Zimbabwe. Chigumira *et al*. (2017) reported that the area planted under soybeans in Zimbabwe declined from 61000ha in the year 2001 to 42000ha in the year 2016 and as a result, the national soybean yield declined from 140763 metric tonnes (MT) to 47755 MT in the year 2001 and year 2016, respectively. During the 2021–2022 growing season, Zimbabwe harvested 71300 MT of soybeans, significantly less than the country's needs of 240000 MT for industrial oil pressing and production of animal feed (Nyoni, 2021; Gatsi, 2024). Prices for both soybeans and chicken feed have increased as a result of the local soybean shortage.

Cowpeas have a lot of potential to replace soybean meal in broiler diets. According to Chiduku (2022), cowpeas are grown on roughly 14.5 million hectares worldwide and 13 million hectares in Africa, respectively. A yearly harvest of 6 million tonnes of cowpeas is made worldwide. Africa produces 83.4% of the world's total output (Kebede *et al*., 2020). According to Chiduku (2022), despite Zimbabwe's favorable climate and suitable soils for cowpea production, not much is known about the country's trends in cowpea production. To ensure that farmers can harvest enough grains for their own use and the feed of their livestock, several awareness programs are needed to educate farmers about the advantages of producing drought-tolerant crops, such as cowpeas. However, cowpea usage is limited by the presence of anti-nutritional factors (ANF), primarily lectins, phytates, tannins, and enzyme inhibitors (Manole *et al*., 2024). These products are anti-physiological, anti-metabolic, and unpalatable to monogastric animals (Abbas and Ahmad, 2018). In addition to increasing the quantity of ammonia released into the atmosphere from broiler houses and having a detrimental impact on the productivity, meat quality, and carcass characteristics of broiler chickens, the ANF also reduces the nutritional quality, digestibility, absorption, and utilization of nutrients from diets based on cowpeas.

1.3 Justification of the Study

Zimbabwean smallholder farmers who produce two-thirds of broiler meat (Zimbabwe Poultry Association, 2021) might benefit from this research because they will be able to tap on the underutilized cowpea grain which is locally available and cheap to make broiler feed. Cowpea grain costs US$$390 per MT and soybean costs US$620 per MT (Gatsi, 2024; Shambare, 2024) This might improve nutrition at the household level and the nation at large since most people will be able to consume meat protein from cheaply produced broiler meat. Farmers might be able to use cheap and effective processing methods to remove anti-nutrients from cowpeas to formulate their broiler feed. The use of processed cowpea grains in broiler diets might directly empower women and improve their economic status since 60% of poultry producers in Zimbabwe are women (Mupanedemo, 2021). Utilization of cowpea grains in poultry diet formulation might also provide small-scale farmers with a good market for this dry land crop.

Considerable research has not been carried out to quantify ammonia concentrations emitted from livestock facilities including poultry facilities in Sub-Saharan African countries (Omotoso *et al*., 2024). Determining the amount of ammonia emitted from excreta of broilers fed with processed cowpea-based diets may help poultry farmers to know whether cowpeas will contaminate or not contaminate the environment with excessive ammonia emissions and enable Zimbabwe to come up with national regulations that stipulate the permissible amount of ammonia to be emitted from poultry facilities.

1.4 Objectives of the study

1.4.1 General objective

The general objective of this study was to increase knowledge on the effectiveness of processed cowpeas as a soybean supplement in broiler diets. The main goal was to improve domestic self-sufficiency in supplementary protein in poultry diets and lowering imports of soybeans.

1.4.2 Specific objectives

The specific objectives of this study were to:

i) Determine the effects of processed cowpea grain on feed intake and broiler chicken growth performance.

ii) Determine the effect of cowpea grain processing techniques on the broiler chicken's nutrient digestibility and ammonia emissions.

iii) Determine the effect of cowpea grain processing techniques on broiler meat quality and sensory attributes.

1.5 Alternate hypotheses

The following hypotheses were tested:

i) Processing cowpea grains improves feed intake and growth performance of broilers.

ii) Processing cowpea grains improves digestibility of nutrients in poultry cowpea-based diets and reduces ammonia emissions from the excreta.

iii) The quality and sensory attributes of broiler meat are improved by the processing of cowpea grain.

1.6 Conceptual Framework

The conceptual framework shown on Figure 1.1 below is trying to depict the relationship between the independent variable (Processed Cowpea-based Diets) with the dependent variables namely; Feed Intake, Growth Performance, True Protein Digestibility, Ammonia Emission, and Meat Quality and Sensory Characteristics.

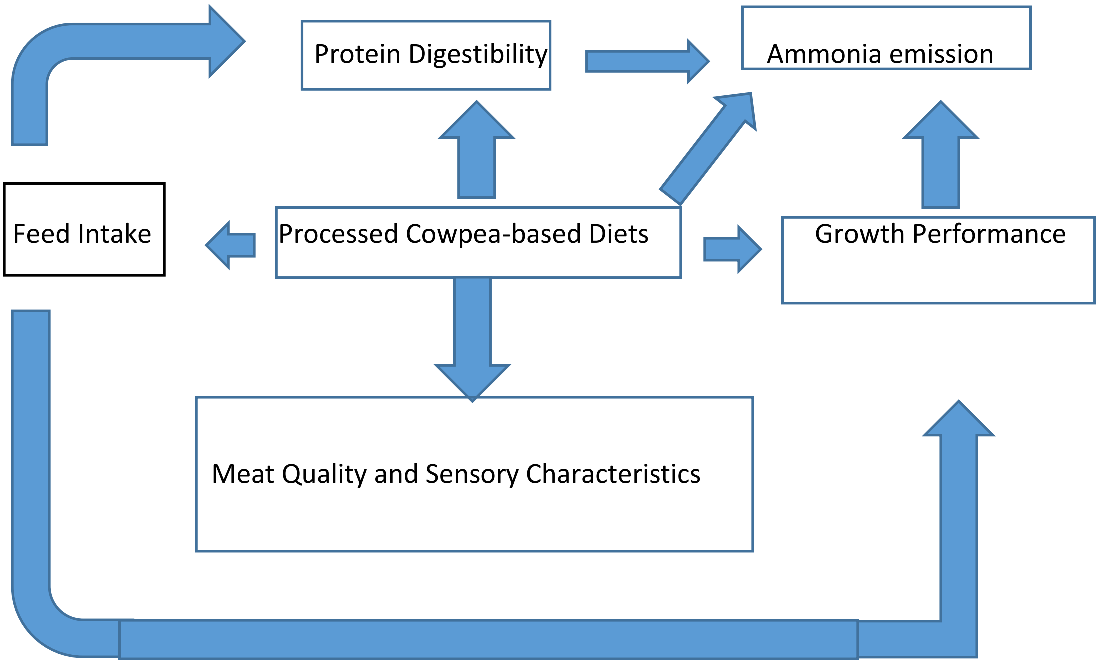

Figure 1.1 Conceptual Framework

1.7 Outline of Dissertation

There are six chapters in this dissertation. A review of poultry nutrition, the research gap, and alternative farming techniques for broiler farmers to utilize in order to address existing issues are provided in Chapter 1. A comprehensive analysis of the literature on the possible substitution of cowpeas for soybeans in the composition of broiler diets was provided in Chapter 2. The impact of processing cowpea grains on broiler feed intake and growth performance was ascertained and reported in Chapter 3. The impact of processing cowpeas on protein digestibility and ammonia emissions during broiler production was examined in Chapter 4. Chapter 5 assessed the impact of processing cowpea grains on the sensory qualities and quality of broiler meat. Chapter 6 presents general discussion, conclusions and recommendations.

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CHAPTER 2

2. Literature Review

2.1 Broiler Industry in Zimbabwe

The raising of chickens (*Gallus gallus domesticus*) for meat production is known as broiler farming. The exact period when broiler farming started in Zimbabwe is not documented, but it is assumed that commercial broiler farming began in the 1950s when broiler franchisers like Irvine' Day Old Chicks Private Limited were born (Sukume, 2016). Broiler farming in Zimbabwe is done at smallholder scale, large-scale commercial, and contract farming levels (Sukume, 2016). Smallholder farmers rear 25 to 5000 broilers, purchase inputs such as feeds, medicines, vaccines, and day-old chicks, rear them on their farms, and sell them to retailers and wholesalers. Large-scale commercial broiler farmers own more than 5000 broilers, and get most of their inputs at much-discounted rates because of the volume of purchase. Gororo and Kashangura (2016) stated that large-scale broiler farmers own their feed-mixing plants and produce broilers at lower costs. Contract farming refers to a system whereby feed manufacturers, hatchery men, and wholesale broiler merchants supply broiler chicks, feed, vaccines and medicines to farmers with farmers being expected to provide labour, electricity, water, litter, poultry equipment, and housing facilities until broilers reach market age. The contractor looks for a market to sell the birds (Zimunya and Dube, 2021).

The large-scale broiler industry in Zimbabwe features intensive management, mechanization, and specialization. The production landscape is dominated by large companies as opposed to small-scale broiler production where poor broiler farming skills and lack of mechanization are common (Sukume, 2016). The broiler industry in Zimbabwe grew phenomenally from a production of 4 785 to 26000 metric tonnes of meat between the years 1971 and 2009, respectively. Total annual production reached a staggering 143000 metric tonnes of meat per annum in the year 2020 (Livestock and Meat Advisory Council, 2020). Annual chick production grew from 18 million chicks in year 2009 to 91 million chicks in year 2020 (Livestock and Meat Advisory Council, 2020). Small and medium producers who produce two-thirds of the country's broiler meat supplied chicken to retail butcheries and supermarkets in low-income markets (Zimbabwe Poultry Association, 2020).

According to the Stockfeed Manufacturers Association (2020), 68% of all stockfeed purchased by weight and 75% by monetary value is still chicken feed, which continues to rule the stockfeed market. In the year 2019, Zimbabwe produced 615862 metric tonnes of stockfeed and in the year 2020, it produced 554831 metric tonnes of stockfeed (Stockfeed Manufacturers Association, 2020).

2.2 Broiler Nutrition in Zimbabwe

Broiler diets, like other livestock diets, are formulated in tandem with broiler nutrient requirements (Dale, 1994). For the birds to grow as healthily as possible, a broiler diet needs to provide all the essential nutrients, including water, carbohydrates, proteins, vitamins, lipids, and minerals (Ravindran and Abdollahi, 2021). Rostago *et al*. (2019) claimed that cereal grains which supply the bulk of energy in broiler feeds must be included up to 50% - 60%, and vegetable protein and animal protein should comprise 20%-25% and 10%-12% of the diet, respectively. Agricultural by-products and mineral-vitamin premix must be added at 15%-25% and 2%-3% respectively. No single feedstuff is more important than the other but the ultimate aim is to mix different feed ingredients to produce a feed with balanced nutrients (Cobb500, 2018). While soybeans and animal by-products like meat and bone meal, blood meal, and fish meal are the traditional protein sources frequently used in the production of broiler feed in Zimbabwe, maize, white sorghum, and pearl millet are the primary energy feedstuffs (Zimbabwe Poultry Association, 2018).

According to Cobb500 (2018), broilers should be fed starter, grower, and finisher diets for the first eight, nineteen, and nineteenth days of life to market age, respectively. Broiler starter feed must contain 21-22% crude protein and 2975 kilocalories per kg of energy, broiler grower feed must contain 19-20% CP and 3025 kcal/Kg of energy, and broiler finisher diet must contain 18-19% CP and 3100 kcal/kg of energy (Cobb500, 2018).

2.3 Soybean (*Glycine max*) Production in Zimbabwe

Soybean is a leguminous crop that adapts well in areas that receive an average annual rainfall of 700 mm and soils that are deep, fertile and well drained hence in Zimbabwe it can be grown in Agro-Ecological Region 1, 2a, 2b and 3 only. Foundation of Netherlands Volunteers (SNV)-Zimbabwe (2016) stated that few farmers grow it in Masvingo and Matabeleland Provinces under supplementary irrigation. However, yields decrease with unpredictable rainfall distribution, suggesting that it cannot be farmed in the nation's semi-arid regions (Chifamba, 2012). This crop can only be successfully grown under irrigation in arid, hot climates like Agro-Ecological regions (AER) IV and V (SeedCo, 2018).

The Zimbabwe Economic Policy Analysis and Research Unit (2017) reported that the area planted under soybean in Zimbabwe declined from 61000ha in year 2001 to 42000ha in year 2016 and as a result the national soybean yield declined from 140763 metric tonnes (MT) to 47755 MT in year 2001 and year 2016, respectively. During the 2021–2022 growing season, Zimbabwe is predicted to harvest 71300 MT of soybeans, significantly less than the country's needs of 600000 MT for industrial oil expression and 240000 MT for the production of animal feed (Nyoni, 2021).

2.4 Cowpea (*Vigna unguiculata*) Production in Zimbabwe

Cowpea is a drought tolerant annual leguminous crop that can utilise soil moisture more efficiently than other crops including soybean and groundnuts (SNV, 2009). In cowpeas, the mitigation of moisture stress involves restricting growth and decreasing leaf area through adjustments to leaf orientation and stomata closure (Government of Zimbabwe, 2010). Additionally, the cowpea plant also lowers flower and pod abscission which enhances prevents moisture stress (Ngumbi, 2019). Smallholder farmers in Zimbabwe are affected by droughts because they do not have irrigation technology and as a result entirely rely on rainfall for their crops. According to Ngumbi (2019), climate change has rendered smallholder farmers incapable of organizing their farming operations. Farmers in tropical and subtropical countries are therefore encouraged to increase cowpea production which adapts well during periods of low rainfall and can be intercropped (cover crop) with other crops such as maize in conservation agriculture (Ngumbi, 2019).

According to Chiduku (2022), cowpeas are grown on roughly 14.5 million hectares worldwide and 13 million hectares in Africa, respectively. A yearly harvest of 6 million tonnes of cowpeas is made worldwide. Africa produces 83.4% of the world's total output (Kebede *et al*., 2020). According to Chiduku (2022), despite Zimbabwe's favorable climate and suitable soils for cowpea production, not much is known about the country's trends in cowpea production. To ensure that farmers can harvest enough grains for their own use and the feed of their livestock, several awareness programs are needed to educate farmers about the advantages of producing drought-tolerant crops, such as cowpeas.

Cowpea grain contains an average of 25% crude protein (Table 2.1) and, just like soybean, is rich in lysine but has low methionine and cysteine-sulphur-containing amino acids (Balail, 2014; Katumo, 2018). Its amino acid profile is good and compares well with that of soybean (Wiryanwan, 1999; Akanji *et al*., 2016). Consequently, in broiler diets, cowpea grain can be utilized to partially replace soybeans (Embaye et al., 2018). However, its use in broiler diet formulations is hampered by the presence of appreciable levels of ANF in its cotyledons and seed-hulls (Abbas and Ahmad, 2018). Cowpeas' nutritional makeup and anti-nutritional content vary depending on the type, region, agronomic techniques used, and storage circumstances (Embayeet al., 2018). The results of research conducted in various nations on the nutritional makeup and anti-nutrient content of processed cowpeas varied, possibly as a result of variations in agronomic techniques, soil types, climates, and varieties (Abbas and Ahmad, 2018).

Table 2.1: Approximate Raw Cowpea Grain Composition

	DM (%)	CP (%)	EE (%)	CF (%)	Ash (%)
	87 – 94	24 – 33	1 – 2	2 – 5	2 – 5
References	1, 2, 3, 4, 5	1, 2, 3, 4, 5	1, 2, 3, 4, 5	1, 2, 3 , 4, 5	1, 2, 3 , 4, 5

The following are some references: 1-Akanji *et al*. (2016); 2-Adegunwa *et al*. (2012); 3-Tshovhote *et al*. (2003); 4-Bumhira *et al*. (2023); 5-Aletan (2018).

Table 2.2: Estimated Constituents of Processed Cowpea Grain

	DM (%)	CP (%)	EE (%)	CF (%)	Ash (%)	References
RC	90 -97	20 – 31	1 -2	2 – 10	3 – 6	1, 2, 3
BC	90 – 97	20 – 31	1 – 2	2 – 10	3 – 6	1, 2, 3 , 4
DC	90 – 97	20 – 31	1 – 2	2 – 10	3 – 6	1, 2, 3

The following sources are cited: 1- Akanji *et al*. (2016); 2- Adegunwa *et al*. (2012); 3-Tshovhote *et al*. (2003); 4- Bumhira *et al*. (2023).

2.5 Cowpea Grain's Anti-nutritional Components

Plants naturally create metabolites, which are anti-nutritional agents. They interfere with nutrient digestion, absorption and utilisation in monogastric animals (Nalle, 2009; Abbas and Ahmad, 2018). According to Embaye *et al*. (2018), tannins, phytates, non-starch polysaccharides, trypsin inhibitors, and lectins are the main anti-nutritional components of cowpea grains. In monogastric animals, antinutrients can cause anything from mild growth retardation to death.

In broiler diets containing grain legumes, these characteristics of cowpeas decrease nutrient digestibility and anabolism, resulting in increased excretion of uric acid and undigested protein in faeces (Such *et al.*, 2021). As such *et al*. (2021) noted, uric acid and nitrogen in faeces excreted by broilers are further lost as ammonia into the atmosphere resulting in environmental pollution through eutrophication of open water bodies, acidification of soil and negative alteration of the natural ecosystem.

2.5.1 Biology of Anti-Nutritional Factors (ANF)

Samtiya *et al*. (2020) stated that plants develop certain defence mechanisms to protect themselves against herbivores and various microorganisms. In addition, plants also compete with other plants for light, water and nutrients. The seedling is the most vulnerable in the plants' lifecycle and hence, this is the reason why they need to develop defence mechanisms in their seeds (Bumhira and Madzimure, 2022). The adverse effects of ANF include toxicity (lectins and cyanogen glycosides), reduced palatability (tannins and saponins), "anti-nutritive" through reduced growth and vitality of its consumer (phytates) or reduce nutrient digestibility (enzyme inhibitors, non-starch polysacharrides and lectins). Besides being antimicrobial and, antiherbivore, ANF have been selected and utilised in agriculture as phytochemicals that protect crops against pests and pathogens (Samtiya *et al.*, 2020).

2.5.1.1 Inhibitors of Enzymes

Enzyme inhibitors are located within the seed cotyledon and inhibit enzyme activity through the catalytic mode by blocking the enzyme active sites (Samtiya *et al.*, 2020). Mikic *et al*. 2009 claimed that legumes contain protease inhibitors and α-amylase that reduce digestion, absorption and bioavailability of nutrients.

2.5.1.2 Tannins

Tannins are polyphenols that are located within the seed coat (Hodges, 2022). They have an astringent taste and can affect animals that consume them through reduced palatability, impaired digestion and decreased bioavailability of nutrients to the animal. Mohammadi *et al*. (2023) reported that tannins precipitate with proteins to form complexes that are indigestible to enzymes.

2.5.1.3 Lectins

Lectins are resistant to destruction by dry heat and are eliminated by wet heat (Samtiya et al., 2020). They bind to intestinal epithelial cell surfaces thereby preventing protein digestion by proteolytic enzymes (Mikic *et al*. 2009). These toxic substances can combine with glycoproteins of red blood cells causing agglutination of the cells (Kumar *et al*., 2021). Lectins can pierce the gut's wall lining, resulting in internal bleeding and microbial blood infiltration that might lead to health issues (Kumar *et al*., 2021).

2.5.1.4 Phytate

Phytate, also known as phytic acid, is the primary phosphorus storage form in plants, accounting for the bulk of phosphorous in the plant (Kumar *et al*., 2021). All pulse crops include phytotic acid, which is found as inositol phosphate. Gupta *et al*. (2015) observed that phytates occur in the outer-most layers of the endosperm. Kumar *et al*. (2021) and Samtiya et al. (2020) claimed that phytates precipitate with minerals forming insoluble complexes with zinc, calcium, iron and magnesium, to mention a few, and impeding the absorption of minerals leading to mineral deficiencies in animals. Kumar *et al*. (2021) stated that phytic acid can also form insoluble complexes with proteins and starch resulting in reduced digestion of proteins and starch, respectively.

Since grain legumes contain some anti-nutritional factors that reduce nutrient digestibility and limit their use in poultry feeds, various traditional processing techniques have been used to eliminate them to improve the nutritional content of legume seeds (Bumhira and Madzimure, 2022).

2.5.2 Processing Methods Aimed at Reducing Anti-nutrients in Grain Legumes

Various methods are used to remove anti-nutrients in grain legumes (Akande and Fabiyi, 2010). According to studies by Samtiya *et al*. (2020), the processing technique for grain legumes is determined by factors such as chemical structure, seed fraction distribution, biological impacts, heat sensitivity, and water solubility. Dehulling, soaking, fermentation, roasting, boiling, autoclaving, extrusion and addition of enzymes are some of the methods that are being currently employed to reduce anti-nutrients in grain legumes.

2.5.2.1 Dehulling

Dehulling is the process of removing the grain legumes' seed coverings, according to Kumar et al. (2021). Grain legume seed coatings contain both tannins and saponins (Diouf *et al*., 2019). Removal of tannins and saponins improves the palatability of grain legumes since tannins are reported to cause a bitter taste to the grain legumes (Mande *et al*., 2024). Dehulling greatly increased the nutritional value of cowpea grains, as shown by Belal *et al*. (2011). Raw cowpeas had 29.81% CP, 6.22% crude fiber, 2.30% crude fat, and 4.60% ash, while dehulling produced 31.80% CP, 3.61% crude fiber, 2.31% crude fat, and 4.71% ash. Akanji *et al*. (2020) reported that crude protein of dehulled cowpea was lower than the CP of raw cowpea grain (22.54% vs. 23.78%) but the concentrations of crude fiber, ether extracts and ash improved from 2.45%, 1.27% and 3.21% in raw cowpeas to 2.34%, 1.20% and 3.16% in dehulled cowpea grains, respectively.

2.5.2.2 Thermal Processing

Thermal processing, as defined by Bumhira and Madzimure (2022), is the method of applying heat to grain legumes to lessen or eliminate anti-nutritious elements and increase the nutritional content of the grain legumes. Dry and wet heat treatment are the two categories of thermal processing techniques (Abbas and Ahmad; David *et al*., 2024). The purpose of dry heating is to improve the flavour, palatability and nutritional content of feed ingredients (David *et al*., 2024). Wet heat treatment inactivates heat-labile antinutrients and increases nutrient digestibility.

Roasting, popping and micronizing are some of the examples of dry heat treatments (Akande and Fabiyi, 2010). David *et al*. (2024) listed boiling, pelleting, expansion, extrusion, steam flaking and compacting as typical examples of wet heat treatments. The efficacy of thermal treatment depends on the temperature and duration of heating (Bumhira and Madzimure, 2022). David et al. (2024) reported that both overheating and underheating have negative effects on the nutrient content of grain legumes. Under heating leaves residual anti-nutrients in grain legumes which affects protein digestibility (Kur *et al*., 2013). According to Soetan and

Oyewole (2019) over heating leads to Maillard reactions. Maillard reaction refers to the binding of free carbonyl groups of reducing sugars with free lysine particles of an amino group to form completely indigestible compounds that renders lysine unavailable to poultry (David et al., 2024). Excessive heat also leads to aggregation of proteins that reduces digestibility of legume seeds (Kur *et al*., 2013; David *et al*., 2024).

Extrusion is the process of passing feed through a die, shearing, heating, and mixing under high pressure to create the end product (David *et al*., 2024). The same authors went on to say that the shear force, temperature, moisture content, residence duration, and pH of the feed components being treated are some of the variables that affect how effective the extrusion process is. David *et al*. (2024) claimed that extrusion causes the gelatinisation of starch, denaturation of proteins, disruption of the grain structure and modification of liquids. The benefits of extrusion are reduction of ANFs, improving digestibility of grain legumes, destruction of pathogens and extending the storage time of feed (Soetan and Oyewole, 2019).

Roasting is practiced by exposing a mixture of grain legumes and sand to an elevated temperature of 100 – 120ºC for a duration of 15 to 45 minutes (Adeoye and Oyetayo, 2021; David *et al*., 2024). Sand is added in order to achieve an even roasting. A 2.0mm sieve was used to separate the roasted legume seeds from the sand. The beneficial effects of dry heat treatment that have been earlier alluded to, however are reversed by roasting at elevated temperatures that cause the unintended volatilisation of lysine and cysteine which are heat-sensitive amino acids (Soetan and Oyewole, 2019).

Boiling has been described by Adeoye and Oyetayo (2021) as the exposure of grain legumes to hot water having temperatures that ranges from 100–120 ºC for duration of 15 – 45 minutes. Heat-labile anti-nutrients found in seed cotyledons, such as lectins, phytic acid, and protease inhibitors, are destroyed by boiling. It also reduces the level of tannins through leaching since tannins and saponins are water soluble (David *et al*., 2024). However, boiling decreases the nutritional content of feed ingredients due to the loss of soluble nutrients by leaching into the treatment water (Bumhira and Madzimure, 2022).

2.6 Effect of Processing Grain Legumes on Production Traits and Nutrient Digestibility

Offering chickens meals that include raw legume seeds has been linked to a number of nutritional problems (Akanji *et al*., 2016). According to Viera *et al*. (2023), birds that were fed diets based solely on cowpeas performed poorly. According to studies by Kur *et al*. (2013), Macelline *et al*. (2022), Bumhira *et al*. (2023), and others, broilers fed diets including raw cowpeas showed low feed intake, reduced live weight, decreased weight increase, and poor feed conversion ratio. Bumhira and Madzimure (2022) ascribed the subpar results to the unfavorable impact of raw cowpeas' anti-nutrients. In order to find out how feeding roasted cowpeas to cecectomized broilers affected their chemical composition, real metabolisable energy, and amino acid digestibility, Dos Anjos *et al*. (2016) conducted a study. They found that roasted cowpeas had better amino acid digestibility ($P<0.05$). The addition of cowpeas to the broiler diet did not significantly affect the digestibility of proteins (72.8% - 81.0%) in another feed trial conducted by Tshovhote *et al*. (2003). Kur *et al*. (2013) studied the effects of feeding treated cowpeas to broiler chickens. The results showed that the broilers fed treated cowpeas had significantly higher feed intake, weekly live weight, weekly weight gain, overall weight gain, and FCR than the birds fed the control diet.

2.7 Nutrient Digestibility

Livestock diets are made up of different nutrients that include proteins, vitamins, minerals, fats, carbohydrates and water (Abbas and Ahmad, 2018). Pomar et al. (2021) reported that not all the nutrients that are taken in by an animal are digested and utilised by that animal but only 35% - 50% of the nutrients are used for tissue building and the rest is excreted. All nutrients are equally important in formulating livestock diets. Bumhira and Madzimure (2022) noted that even though all nutrients are crucial, great alertness should be exerted on dietary protein since approximately 25 - 30% of poultry rations can be accounted for by proteins.

2.7.1 Proteins

Along with carbon, oxygen, and hydrogen, proteins also contain nitrogen and, in certain situations, sulfur (Green *et al*., 1987). Proteins are similar to dietary fats and carbohydrates in this regard. Peptide bonds bind amino acids together to make proteins (Tahmasebi *et al*., 2019). Di-, tri-, and tetra-peptides are the terms for simple peptides with two, three, or four amino acid residues, respectively. Proteins can contain one or more polypeptide chains, which are chains made up of many amino acid residues. Greek word "proteios," which means first and most important, is where the word "protein" originated, according to Dutch chemist G. J. Mulder (Green *et al*., 1987).

2.7.1.1 Functions of Proteins

Proteins are very important in life and play various roles within animals' bodies (Table 3). They are the major structural components (e.g. collagen, sclerotin, α-keratin, elastin, mucoproteins). Protiens are crucial in biochemical reactions (enzymes), transportation activities (e.g. haemoglobin, myoglobin, and serum albumin), regulatory activities (hormones), protective activities (antibodies, fibrinogen, and thrombin) and storage purposes (casein, ovalbumin) (Green *et al*., 1987). They also provide energy when carbohydrates and fats are in short supply within an animal's body.

A protein deficiency will result in retardation in growth performance (Bumhira and Madzimure, 2023). Furthermore, the surplus protein in broiler diets is discarded, contributing to pollution and exacerbating problems with animal welfare. The latter include hock burns, breast blisters and carcass defects (Bumhira and Madzimure, 2023). In order to avoid the above-mentioned problems, dietary protein must be quantified and qualified by conducting digestibility and growth performance trials involving birds (Fassinou *et al*., 2023).

Digestion and metabolism are the two main processes that determine the bioavailability of proteins (Figure 2.1) (Borin et al., 2002). Chemical analysis can be used to estimate the potential value of a given nutrient, but the only way to quantify the nutrient's actual value to an animal is to account for losses that occur during digestion, absorption, and metabolism (Borin et al., 2002; Bumhira and Madzimure, 2023). A nutrient's bioavailability ought to be a property of the feedstock it is contained in, not the animal consuming it (Bumhira and Madzimure, 2023).

2.7.2 A Chicken's Digestive Tract

The digestive tract of a chicken begins at the beak and goes through the upper and lower oesophagus, crop, true stomach, also called proventriculus, gizzard, small intestines, two blind terminals called the caeca, colon, and, lastly, the cloaca, as shown in Figure 2.1 by Jacob and Pescatore (2024).

2.7.3 Process of Protein Digestion in Poultry

2.7.3.1 Beak

The beak picks up the feed and puts it into the mouth (Hussein *et al*., 2023). The mouth of a chicken does not have teeth to grind feed (Huang *et al*., 2022). The mouth has some glands that secrete salivary amylase enzymes which are responsible for the digestion of starch (Hussein *et al*., 2023). There is no digestion of protein that takes place in the mouth. The saliva moistens the feed before it is swallowed into the crop (Huang *et al*., 2022). The mouth uses its tongue to swallow the feed and deposit it into the oesophageal portion (Hussein *et al*., 2023).

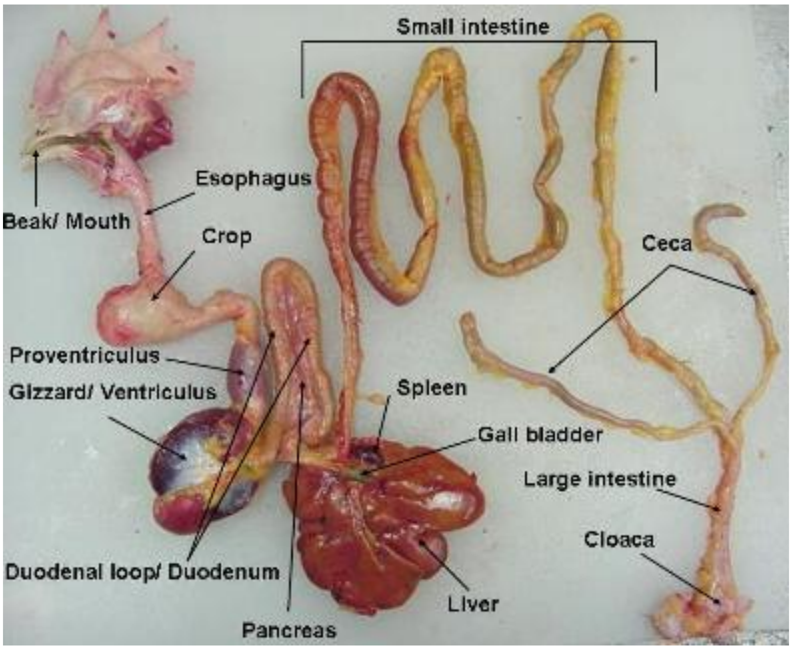


Figure 2.1. A chicken's digestive tract (Created from Jacob and Pescatore, 2024)

2.7.3.2 Oesophagus

The oesophagus, also called the throat, is a tube that connects the mouth to the small intestine, according to Hussein *et al*. (2023). In the case of the upper oesophagus, the tube is extended to create the crop, and in the case of the lower oesophagus, it is in charge of transporting food from the mouth to the proventriculus.

2.7.3.3 Crop

Located in the neck region (Hussein *et al*., 2023) the crop serves as a temporary storage site for feed. When acidonic bacteria are introduced into a crop, the pH is lowered to 2.5, which is the lowest temperature that protease enzymes—which break down proteins in the stomach—prefer (Huang *et al*., 2022; Hussein *et al*., 2023).

2.7.3.4 Stomach

The proventriculus and the ventriculus, sometimes known as the gizzard, make up the two sections of a broiler chicken's stomach (Huang *et al*., 2022). The proventriculus is sometimes referred to as the true stomach. The parietal cells in the gastric glands of the proventriculus mix sodium chloride and carbonic acid to produce hydrochloric acid (HCI) which is released into the stomach and it lowers the pH of the proventriculus to 4.5. The HCI activates pepsinogen into pepsin which initiates the digestion of proteins in the proventriculus (Huang *et al*., 2022). Due to its ability to denature dietary proteins and cause them to lose their naturally folded

Table 2.3 Functions of Proteins

Type of Protein	Examples	Function
Structural	Collagen	Tendons, bone, cartilage
	Sclerotin	Exoskeleton of insects
	α-keratin	Skin, nails hooves, horn
	mucoproteins	Mucous secretions
Enzymes	Trypsin	Catalyses hydrolyses of proteins
Hormones	Insulin	Regulate glucose
	Glucagon	Metabolism
Transport	Haemoglobin	Transport O_2 in blood
	Serum albumin	Transport in blood, fats, lipids etc
Protective	Myosin	Moving filaments in myofibril of sarcomere
	Actin	Stationary filaments in myofibril of sarcomere
Storage	Ovalbumin	Egg white protein
	Casein	Milk protein

Source: Green *et al*. (1987)

shapes, which exposes their peptide linkages to proteolysis by protease enzymes, the HCI is also crucial to the process of protein digestion (Huang *et al*., 2022).

The digesta (small peptides, large polypeptides and free amino acids from pepsin digestion) from the proventriculus enters the gizzard, which is defined as the mechanical stomach responsible for the grinding of food using its two sets of muscular walls that acts as teeth. In the ventriculus, the feed is ground into fine particles and mixed with digestive enzymes from the proventriculus (Huang *et al*., 2022).

2.7.3.5 Small Intestines

The ileum, jejunum, and duodenum make up the small intestine. The duodenum is the active site of protein digestion (Huang *et al*., 2022). The duodenum receives zymogens (inactivated protein digestive enzymes) from the pancreas (Hussein *et al*., 2023). These zymogens are trypsinogen, chymotrypsinogen, proelastase and procarboxypeptidase. Duodenal enterocytes release enterokinase into the intestinal lumen, where it activates the following enzymes: procarboxypeptidase to carboxypeptidase, trypsinogen to trypsin, proelastase to elastase, and chymotrypsinogen to chymotrypsin (Huang *et al*., 2022). The duodenum has a pH between 6.0 and 6.5. However, the short length and transit time of food particles through this intestinal segment (7 minutes) limits the amount of protein that can be digested in this organ (He *et al*., 2021).

The digesta from the duodenum moves to the jejunum (pH = 6.5 – 7.0) where much proteolysis takes place due to its extended length and high protease activity (Svihus, 2014). The digesta transit time through the jejunum is 25 minutes. The digestion of peptides continues in the ileum segment (pH = 7.0 – 7.4) and the digesta transit time through the ileum is 60 minutes (He *et al*., 2021).

According to He *et al*. (2021), enterocytes absorb free amino acids in the intestinal lumen through two different mechanisms: (a) a sodium (NA+)-dependent system (active transport, e.g., acidic amino acids) and (b) a sodium (NA+)-independent system (facilitated system, e.g., for basic amino acids). Amino acid transporters that are NA+-dependent or NA+-independent account for 60% and 40%, respectively, of the free amino acids that are absorbed by the enterocytes from the intestinal lumen of the small intestines (He *et al*., 2021).

2.7.3.6 Caeca

At the meeting point of the small and large intestines are two blind tubes called the caeca (Hussein *et al*., 2023). In addition to absorbing any leftover water from the meal, it ferments nutrients that have not yet been fully digested to produce fatty acids and some B vitamins.

Since the caeca are situated near the end of the digestive tract, not much of the nutrients produced there are absorbed, providing little benefit (Svihus, 2014). Through the cloaca, the unabsorbed nutrients are expelled, potentially contaminating the surrounding area (Huang *et al*., 2022).

2.7.3.7 Colon

The colon (shown in Figure 2.2 below) are also called the large intestines and is responsible for water reabsorption (Huang *et al.,* 2022) from the watery digesta coming from the small intestines and expels the waste material through the cloaca.

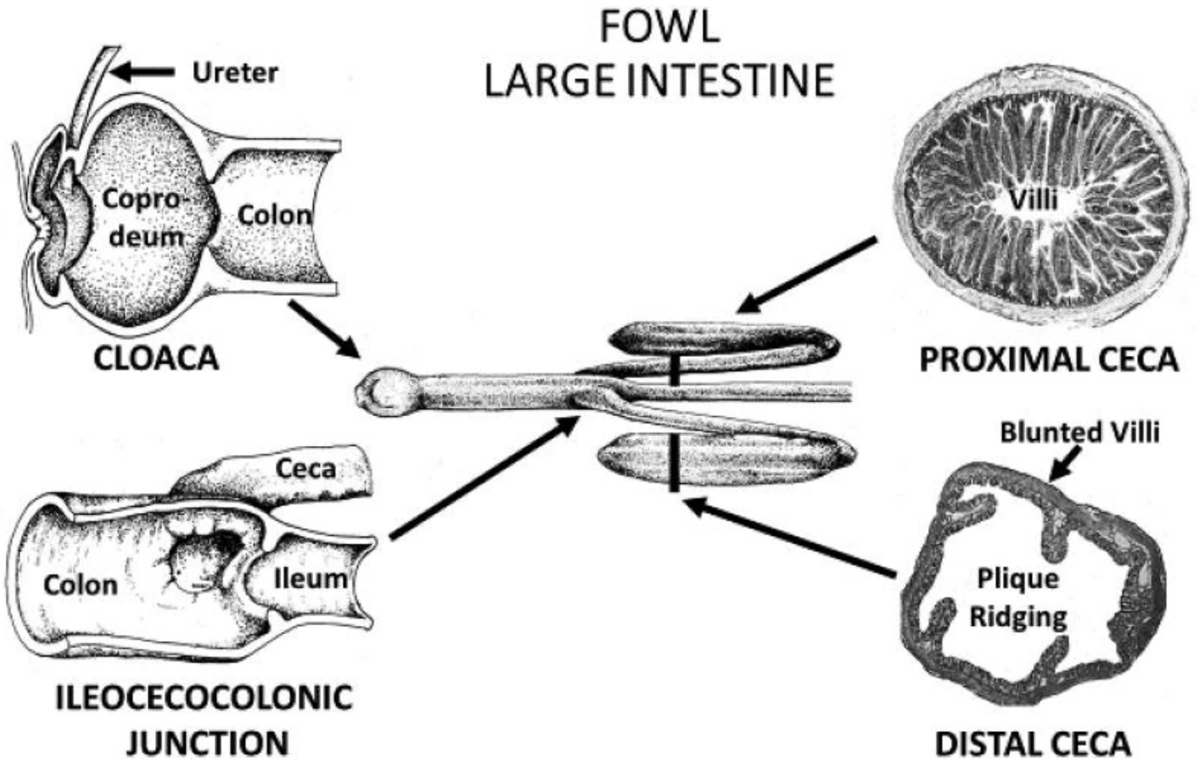


Figure 2.2. The large intestine of the fowl (Adapted from: Moran and Bedford, 2022)

2.7.3.8 Cloaca

The cloaca is the exit passage of digestive wastes (Huang *et al*., 2022). It is a place where the digestive waste material mixes with the urinary waste material (urates). Chickens are reported to void excreta sprinkled with uric acid crystals (Hussein *et al*., 2023).

The bioavailability of proteins and amino acids from feed ingredients to several chicken species has been estimated in previous research using a variety of methodologies (Macelline *et al*., 2022; Llaboya and Omatsuli, 2023; Viera *et al*., 2023). Numerous researchers have employed direct in-vivo techniques, such as ileal digesta analysis and excreta analysis, to estimate the amount of bioavailable proteins and amino acids (Viera *et al*., 2023). Conventional feeds were used in numerous studies on the digestibility of feed nutrients (Macelline *et al*., 2022; Llaboya and Omatsuli, 2023). Consequently, research on the digestibility of diets based on cowpeas must be done.

2.8 Ammonia

Colorless and strongly scented, ammonia (NH3) is a gas (Bumhira and Madzimure, 2023). According to Liu *et al,* (2021), ammonia can be detected at concentrations between 5 and 18 parts per million and poses a risk to animal wellbeing. According to Sheikh *et al*. (2018) and Malomo *et al*. (2018), ammonia can be produced naturally or as a result of human activity (anthropogenic). According to Bumhira and Madzimure (2023), ammonia can be created by industrial processes such as the production of fuel, fertilizer, and refrigerant, as well as biological processes involving the breakdown of organic materials (Figure 2.4).

2.8.1 Ammonia Formation in Poultry Facilities

Chickens need protein and other kinds of nitrogen to create meat and eggs, but they only use about half of this, and between 50 and 80 percent of the nitrogen they ingest ends up in their excreta (Zhao *et al*., 2014; Bumhira and Madzimure, 2023). According to Bumhira and Madzimure (2023), uric acid (C5H4N4O3), which can be mineralized into ammonia, makes up roughly 50% of the nitrogen (N) in poultry manure.

$$C_5H_4N_4O_3 + 1.5\ O_2 + 4H_2O \longrightarrow 4NH_3 + 5CO_2$$ **Equation 2.1**

Mineralisation involves a series of biological and chemical processes which are influenced by dietary composition and environmental conditions as shown in Figure 2.5. Uric acid is converted to urea by uricase enzymes, and ammonia is produced by urease enzymes (Sheikh et al., 2018). According to Senyondo (2013), as seen in Figure 2.5 below, microorganisms biologically break down faecal nitrogen to make ammonia. Burt *et al*. (2018) reported that uricolytic bacteria releases uricase enzymes that decompose uric acid to produce allantoic acid.

The enzyme allantoinase hydrolyses allantoic acid to urea (Burt *et al*., 2018). Carlile (1984) and Chai (2023) asserted that ureolytic bacteria releases urease enzymes that hydrolyse urea to form ammonia and carbon dioxide. A rise in temperature, pH, moisture content, and nitrogen concentration are some of the factors that speed up the process of ammonia production (Sheikh *et al.*, 2018). Poultry manure decomposes more quickly due to the growth of microorganisms that are encouraged by high temperatures and excessive moisture content in the litter. Sheikh *et al*. (2018) asserted that ammonia-causing microbes have been shown to be adversely affected by acidic environments, but their development and multiplication are favoured by pH values greater than 8.5.

2.8.2 Chemistry of Ammonia

Senyondo (2013) reported that ammonia exists in three forms viz; salt (NH_4^+), gas - $NH_{3\,(g)}$ and in aqueous solution - NH_3 (aq). The ammonia in poultry manure exists in two forms namely NH_4^+ and NH_3 (aq). Senyondo (2013) claimed that the three above–mentioned forms of ammonia can exist in equilibrium as depicted in equations 2.2 and 2.3 below:

$$NH_4^+ (aq) \rightleftharpoons NH_3 (aq) + H^+ (aq) \qquad \textbf{Equation 2.2}$$

$$NH_3 (aq) \rightleftharpoons NH_3 (g) \qquad \textbf{Equation 2.3}$$

When manure pH is low (high concentration of H^+), the chemical reaction in equation 2.2 proceeds to the left to maintain the equilibrium, and when litter pH increases, the reaction in equation 2.3 proceeds to the right releasing gaseous ammonia from the litter (Senyondo, 2013).

2.8.3 Effect of Ammonia on Poultry, Humans, and the Environment

According to Sheikh *et al*. (2018), ammonia concentrations above 25 ppm have a negative impact on broiler productivity and overall poultry health, which raises bird mortality. According to Bumhira and Madzimure (2023) research, broilers that are exposed to high ammonia concentrations risk respiratory infections such non-communicable diseases (NCD) because the mucous membranes of their respiratory tracts are harmed.

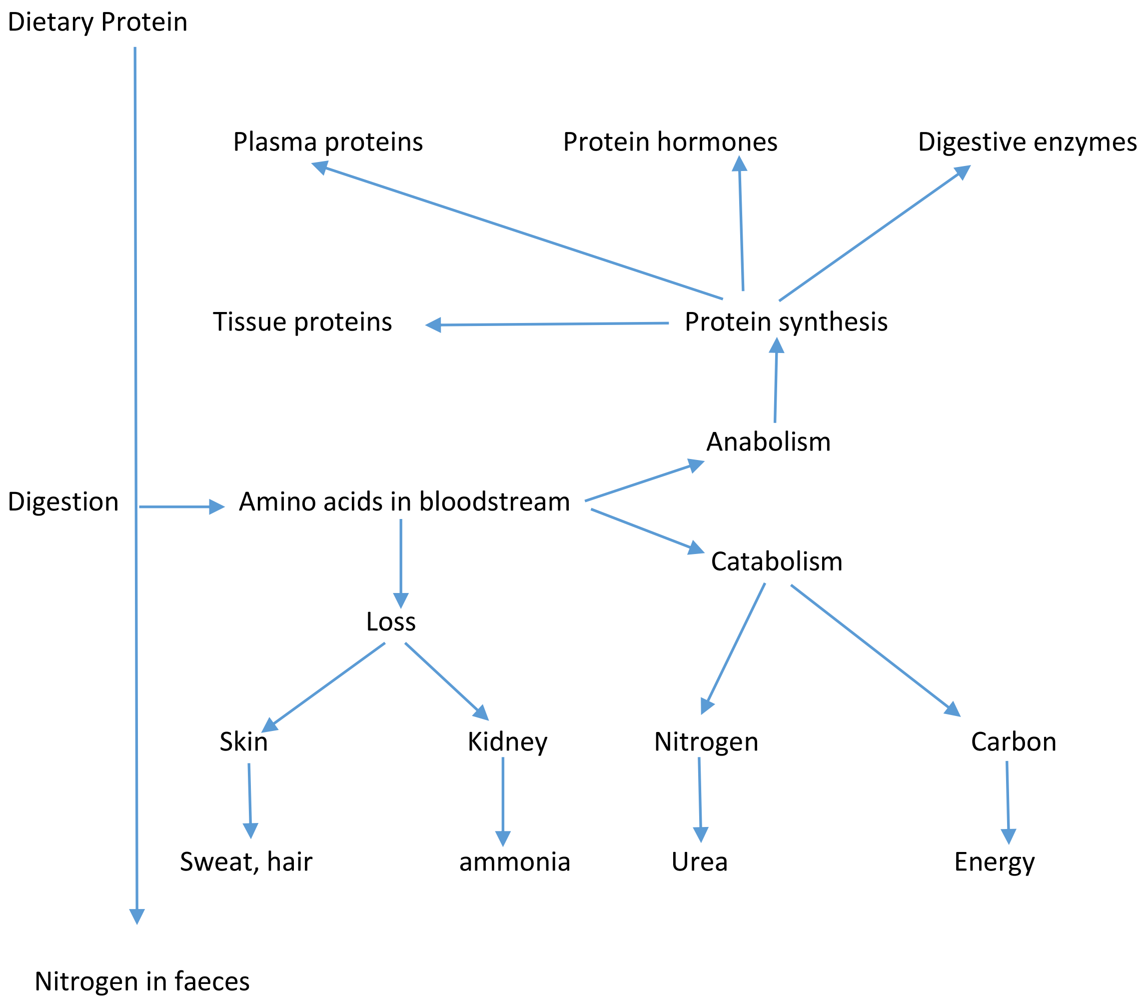


Figure 2.3. Summary of mammalian protein metabolism

Source: Green *et al*. (1987)

According to Chai (2023), broiler house ammonia levels beyond the minimum allowable level of 25 ppm might result in ascites, breast blisters, hock burn, and ultimately death in the afflicted birds. Following slaughter, ammonia can potentially lead to carcass condemnation (Bumhira and Madzimure, 2023).

In human beings, the threshold limit value (TLV) of 25ppm for an 8-hour exposure or 35ppm for exposure of 15 minutes is permitted even though humans can withstand a high of 100ppm for 8 hrs (Carlile, 1984; Chai, 2023). According to Bumhira and Madzimure (2023), ammonia combines with acidic substances in the environment to generate extremely hazardous particulate matter (PM2.5), which can cause respiratory illnesses in people.

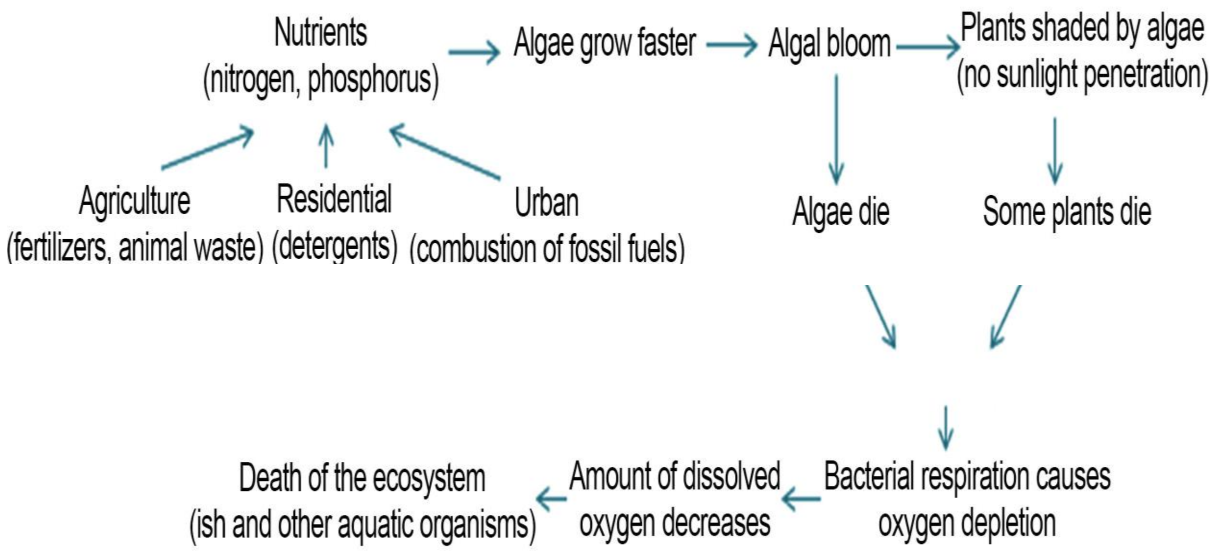


Figure 2.4 Eutrophication and its effects to fish
Source: Knockaert (2024).

Ammonia released into the atmosphere combines with rain to produce acid rain or with acidic materials to produce particulate matter (PM2.5), which includes ammonium chloride, sulphate, and nitrate, as shown by equations 2.4, 2.5, and 2.6, according to research by Sheikh *et al*. (2018).

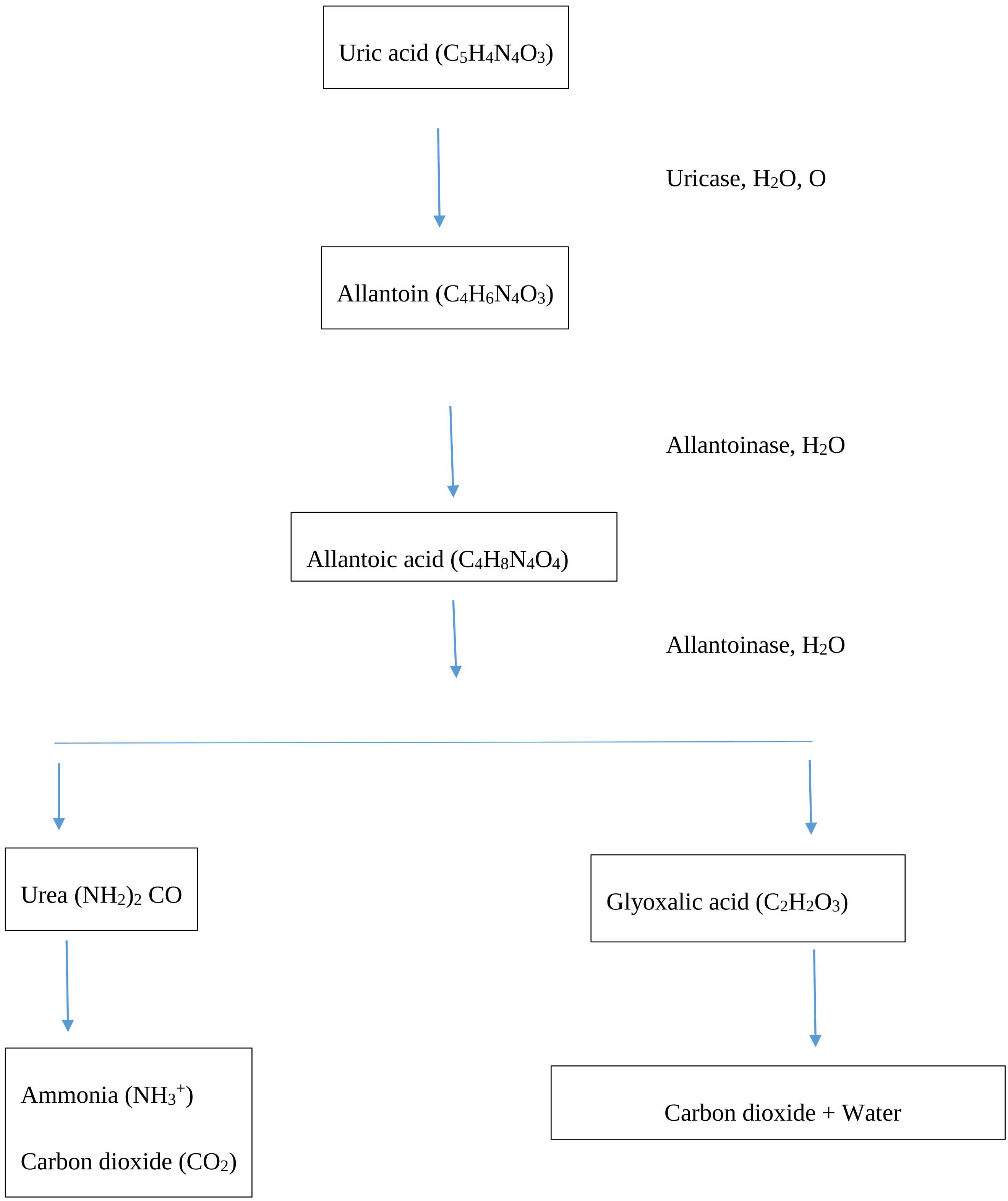


Figure 2.5 Mineralization of uric acid to produce ammonia (adapted from Senyondo, 2013)

$NH_3 + HNO_3 \rightarrow NH_4NO_3$ **Equation 2.4**

$2NH_3 + H_2SO_4 \rightarrow (NH_4)_2SO_4$ **Equation 2.5**

$NH3 + CHI \rightarrow NH_4CI$ **Equation 2.6**

In the end, soil, open water bodies, and plants may absorb PM2.5 (Bumhira and Madzimure, 2023). Soil acidification and eutrophication are caused by ammonium nitrate deposits on open water bodies and soil, respectively (Carlile, 1984; Chai, 2023; Knockaert, 2024). According to Kies (2024), eutrophication is the excessive nitrate and phosphate enrichment of open water bodies that causes an overabundance of harmful algal blooms (HABs). According to Kies (2024), the decomposition of hazardous algal blooms causes a decrease in the amount of dissolved oxygen in the water, which causes a large number of fish to suffocate to death. When ammonia is converted to nitrite and nitrate by ammonia-forming bacteria (Knockaert, 2024), dissolved oxygen is also used. Massive fish fatalities and a decline in species biodiversity are caused by dissolved oxygen deficiency (Chislock *et al*., 2013).

2.8.4 Quantification of Ammonia Emissions

Ammonia gas analyzers are modern equipment currently being used to measure ammonia gas concentrations in poultry houses (Bumhira and Madzimure, 2023). The digital ammonia test meters directly measure ammonia from the source and are not negatively affected by the ventilation rate which disperses ammonia into the atmosphere (Senyondo, 2013; Hidayat, 2021).

2.8.5 Mitigation of Ammonia Emission from Broiler Litter

It is realistically difficult to maintain ammonia concentrations below the minimum threshold levels of 25 ppm in broiler houses because smallholder farmers are mainly concerned about reducing heating costs at the expense of ventilation during periods when heat is seriously needed for example in winter and during the brooding period (Bumhira and Madzimure, 2023). According to Senyondo (2013) and Chai (2023), there are two main strategies for reducing ammonia emissions in chicken facilities: pre-release (which involves reducing ammonia volatilization through management, house design, and litter additives) and pre-excretion (which involves reducing ammonia before excretion through dietary interventions) as shown in Figure 2.6 below:

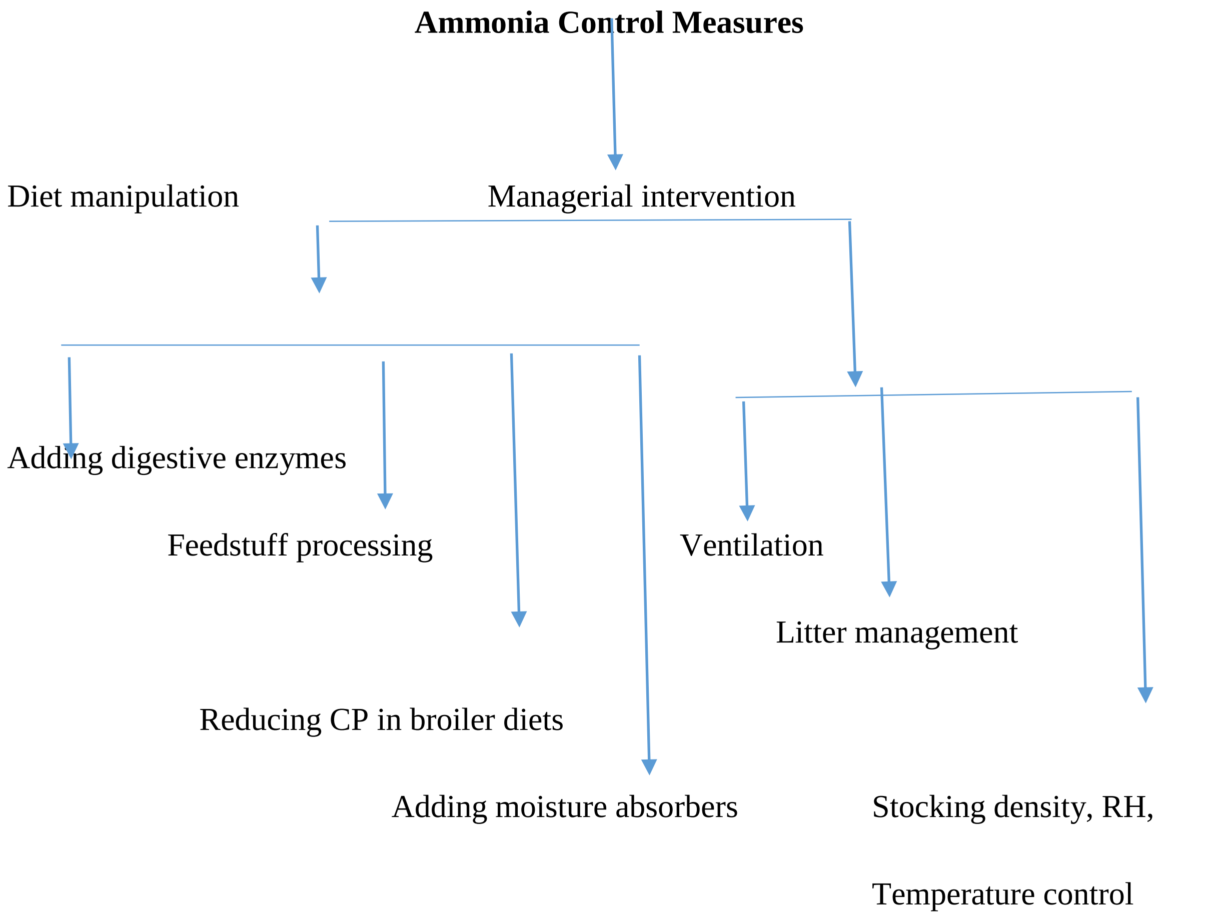


Figure 2. 6 Mitigation Strategies to Control Ammonia Emission in Poultry Facilities
Source: Chai (2023).

2.8.5.1 Litter Management

Chai (2023) revealed that ammonia emission from poultry litter is increased when litter temperature, pH, and moisture content are very high. The process of ammonia formation is reportedly (Senyondo, 2013) speeded up at temperatures between 30^{o}C and 40^{o}C. Ammonia generated at 40.6^{o}C was seven times greater than that produced at 18.3^{o}C (Miles *et al*., 2011; Senyondo, 2013; Chai, 2023). Bumhira and Madzimure (2023) reported that maintaining air temperature in broiler facilities in line with temperatures that are recommended for broilers at different stages of growth will help reduce the amount of ammonia emitted from broiler facilities.

Bumhira and Madzimure (2023) claimed that ammonia emission increases with a corresponding rise in litter pH. Equation 2.2 states that NH_3 (aq) combines with H+ at low pH (acidic circumstances) to create NH_4^+, which is not volatile. At pH greater than 7, NH4+ dissociates to NH_3 (aq) and finally volatilizes to gaseous NH_3 (g) (Equations 2.2 and 2.3, respectively). The addition of acidifying agents to poultry litter such as sulfuric acid, phosphoric acid, hydrochloric acid, and nitrate salts of magnesium and calcium reduces volatilization of ammonia which is also beneficial to crops when the treated litter is applied to croplands since the above-named acidic compounds can be absorbed by crops for growth (Zolnowski *et al*., 2022).

According to Hidayat *et al*. (2021), ammonia-forming microbes thrive and breed well in litter with a high moisture content. Bumhira and Madzimure (2023) claimed that litter moisture content can be reduced by providing adequate ventilation in fowl runs and adding moisture adsorbents to litter (zeolites). Hidayat *et al*. (2021) noted that the inclusion of $5kg/m^2$ of zeolite to litter reduced ammonia concentrations by up to 35%. The addition of zinc and copper (uricase inhibitors) to broiler litter can reduce the volatilization of ammonia (Hidayat *et al*., 2023).

2.8.5.2 Diet Manipulation

It is cost-effective and environmentally friendly to control ammonia emissions by manipulating diet formulations (Chai, 2023). Feeding low-protein diets to broilers reduces the loss of CP in poultry faeces (Mezolaki *et al*., 2021). Hidayat *et al*. (2021) reported that processing grain legumes and adding digestive enzymes to poultry diets lower the feed's antinutrients and enhance the digestion of proteins and resultantly decreases the amount of CP lost in broiler feces. Korsheva and Chaunina (2021) claimed that mixing a variety of grain legumes in poultry feeds enhances the diet's amino acid profile, increases the digestibility of CP, and reduces the quantity of CP lost to feces. According to Gao *et al*. (2021), adding organic acids (citric, formic, fumaric, and lactic acids) and inorganic acids (such as hydrochloric, phosphoric, and sulphuric acids) to poultry diets can improve feed intake, nutrient digestibility, controls pathogenic bacteria and meat quality in broilers. Oliveira *et al*. (2021) claimed that organic acidifiers can cause no pollution, no residue, rapid absorption in the body, participation in metabolism and is an important energy carrier. On the other hand, inorganic acidifiers are cheaper than organic acids. However, the use of both organic and inorganic acids in chicken feeds is limited due to the higher cost of organic acids and the significant dissociation of inorganic acids, which significantly reduces the pH value of the digesta in the stomach (Gao *et al*., 2021). The steep reduction of pH can cause a reduction in gastric acid secretion, burn the oesophagus and stomach, and negatively affects gastric physiological systems (Gao *et al*., 2021). When the acidifying agents exceeds 3% in poultry feeds, they can cause a consequential loss of vitamins due to high acidity (Gao *et al*., 2021). The resultant negative effects of mixing broiler feeds with acidifying agents are known, hence the need to explore the impact of processing cowpea grains on protein digestion by broiler chickens and ammonia is warranted.

2.9 Broiler Meat Quality

The qualities of meat that influence its cost and consumer acceptance are referred to as meat quality (Bogosavljevic-Boskovic *et al*., 2010). Meat quality can be classified into two groups: (i) compliance quality and (ii) functional quality. The desired characteristics of a product, such as yield, color, texture, flavor, water holding capacity, and meat taste, are referred to as its functional quality. The essence of conformance quality is to provide a product that precisely satisfies the needs of the customer. e.g. producing chickens with a certain weight as per consumers' request. Both types of quality are very crucial to meat producers and consumers because no consumer will accept broiler chickens having the exact dressing-out percentage but with a poor flavour, texture and colour (Bogosavljevic-Boskovic *et al*., 2010; Nusairat *et al*., 2022).

According to Northcutt (1997) and Nusairat *et al*. (2022) there are three levels of meat quality namely wholesomeness, functional and eating quality. Wholesomeness describes microbiological, chemical and nutritional qualities of meat. Eating quality describes flavour, juiciness, taste, texture, and tenderness of meat. Functional quality refers to the yield and carcass composition, water-retention capacity, pH and colour. Sakowski *et al*. (2022) reviewed the effects of genetic and non-genetic factors on the quality of meat. Age, sex, and genotype are genetic determinants; feeding technique, nutrition, and rearing system are non-genetic factors (Toplu, 2014; Nusairat *et al*., 2022). According to Sakowski *et al*. (2010), primary non-genetic factor that substantially affects certain meat quality attributes is broiler feeding. The same authors claimed that nutritional strategies can be used to modify the chemical composition of muscle tissue, for example, they observed that feeding beef steers with diets containing Lucerne silage and legume – cereal silage caused a great rise in the content of desirable polyunsaturated fatty acids (PUFA) in meat compared to their counterparts fed maize silage. Choice of raw materials for feed formulation, their chemical composition, the formulated rations' variable protein and energy values, the extent of nutrient utilisation, and the different interactive or associative effects (positive or negative) of feed components are some of the factors that affect the chemical and nutritional quality of broiler meat (Sakowski *et al*., 2010). Animal nutritionists are busy searching for alternative sources of protein to soybean meal that is scarce and expensive. Therefore, an investigation on the effects of incorporating cowpeas, which is locally available and cheap, in broiler diets on the quality of broiler meat should be explored.

2.9.1 Wholesomeness of Meat

Wholesomeness is very important to the broiler farmer, processor, wholesaler, retailer and consumer because all of them are mainly concerned about public health safety (Gellynck *et al*., 2006). Meat for human consumption must be free from parasites, pathogens, residual antibiotics and growth promoters (Sohaib *et al*., 2016). Consumers do not want to be poisoned from eating meat with high levels of disease causing organisms and residues from previous veterinary medication of the broilers. Food poisoning can result from broiler meat contaminated with diseases like *Escherichia coli* bacterium and Clostridium spp. Meat contaminated by pathogens has a reduced shelf life (Manyi-Loh and Lues, 2023). Additionally, people desire to gain nutrients from meat consumption, including vitamins, minerals, vital fatty acids, carbohydrates, proteins and water in their adequate amounts. The production parameters and constituents of broiler meat can be impacted by the nutritional value of feeds, according to Bogosavljevic-Boskovic *et al*. (2010). The same authors asserted that diets richer in protein cause meat to have a higher protein content while simultaneously lowering muscular fat percentage. Meat's fat content rises and its protein content falls when broilers are fed diets rich in energy and low in protein (Sakowski *et al*., 2022). According to Betti *et al*. (2009), feeding broiler diets with varying fat sources can have a major impact on how much polyunsaturated fatty acid is deposited in the abdomen and other muscles. According to Chobanova et al. (2019), broiler meat has a low calorie value and is high in protein (Table 2.4).

Table 2.4: Broiler Meat Chemical Composition

Moisture (%)	Crude Protein (%)	Lipids (%)	Minerals (%)	References
71 – 76	19 – 23	1.5 – 6.5	1 – 1.3	1,2,3

1 – Winiarska-Mieczan *et al*. (2016), 2 – Bennato *et al*. (2021), 3 – Kartikasari *et al*. (2023).

2.9.2 Functional Quality of Meat

The yield, carcass composition, color, pH, and water retention capacity of broiler meat are the primary determinants of its functional quality (Mir *et al*., 2017). The different aspects of functional quality of meat are discussed in the sections below:

2.9.2.1 Yield of Meat

The yield refers to the quantity of chicken meat that can be sold. Higher yields give the seller more profit. The poultry farmer and the wholesaler are mostly concerned about the yield. The quantity, size, and length of the muscle fibers that make up an animal's skeletal muscles impact its meat yield (Szulc *et al*., 2022). According to Tumova and Teimouri (2009), the total number, total area, and length of fibers determine weight of muscles. Nonetheless, it is well known that a chicken's muscle mass is predetermined prior to hatching, meaning that any post-hatching increase in muscle mass depends on the muscle fibers' increased length and diameter (Weng *et al*., 2022). Tumova and Teimouri (2009) stated that the average size in *pectoralis major*, *biceps femoris*, *extensor halluces* and *gastrocnemius* muscle in broiler chicken are 60%, 51.6%, 59.8% and 60.45% µm, respectively. In poultry, glycolytic fibres constitute the largest cross-sectional area, meaning that for a given number of fibres, muscle weight must rise in response to an increase in the percentage of glycolytic fibers (Weng *et al*., 2022). Huo *et al*. (2022) reported that muscle fibres increase with age in broilers. Besides age, sex breed, nutrition is undoubtedly a key for normal skeletal muscle growth (Szulc *et al*., 2022). Feed restriction in either quantity or quality or both decreases muscle fibre growth. It is reported that undernutrition negatively impacts fiber hypertrophy by lowering the build-up of nuclei and proteins. Ismail and Joo (2017) claimed that high-density diets improved the growth of the pectoralis muscle and were accompanied by hypertrophy of type IIB fibres. The proportion of lean to fat to bone constitutes the composition of broiler meat, it should have a higher yield of saleable lean to either fat or waste bone material. The majority of consumers prefer lean meat to fatty meat due to health reasons. Much of the fat can consequently be trimmed and thrown away resulting in financial loss to the consumer. However, a small amount of fat (marbling fat) is needed since it is associated with juiciness and the development of flavour.

2.9.2.2 Colour of Broiler Meat

Consumers only consider meat's colour when determining whether or not to purchase it (Kralik *et al*., 2018). Broiler meat should be white or bright and not brown in colour ((Petracci *et al*., 2005). To extend the meat's shelf life, the color should remain consistent (Qamar *et al*, 2019). Myoglobin and haemoglobin are the two proteins related to the colour of broiler meat (Qamar *et al*, 2019). Myoglobin is fixed in tissue cells rather than circulating in blood while on the other hand haemoglobin flows in blood (Barbut, 2015). Reddish-purple in color, myoglobin is composed of an iron element (Qamar *et al*, 2019). When the myoglobin is mixed with oxygen and carbon dioxide it is converted to oxymyoglobin and carboxymyoglobin, respectively. In both oxymyoglobin and carboxymyoglobin, the iron is reduced to ferrous state and the colour is bright red.

Age, food, and exercise levels have been reported to have an influence on the colour of meat ((Mir *et al*., 2017). Older animals have meat that is deeper in color because myoglobin levels are thought to rise with age. However, Nusairat *et al*. (2022) reviewed that colour of broiler meat is not affected by age. According to Barbut (2015), plant pigments that are absorbed from the diet and deposited in the skin affect the color of broiler meat. For example, xanthophyll from maize can give the skin a yellow appearance.

2.9.2.3 Water-holding Capacity of Broiler Meat

Water-holding capacity is the term which describes the ability of meat to retain water when it is cut, heated, pressed, and stored (Mir *et al*., 2017). Broiler meat typically has a water-holding capacity (WHC) of roughly 75%. WHC affects the appearance of raw meat, its cooking process, and its chewability. This is especially noticeable in processed meats like mince and sausages since the proteins can no longer hold water and the muscle structure has been disrupted (Mir *et al*., 2017). Water loss is indicated by the exudation of a proteinous fluid from the flesh, which is referred to as "weep," "purge," or "drip" in raw meat and "shrink" in cooked meat (Kralik *et al*., 2018). Drip is claimed to emanate from spaces within myofibrils, spaces between myofibrils, and spaces between myofibrils and cell membranes (Kralik *et al*. (2017) and Hussain *et al*. (2017). Water is drawn to charged objects like proteins because of its dipolar nature (Kralik *et al*., 2018). Muscles contain three different types of water namely bound water, immobilized water, and free water (Selemat *et al*., 2021). Protein and bound water have a tight bond and limited movement. It is difficult to relocate to other locations. Water that is immobilized or entrapped, according to Hussain *et al*. (2017), is found in specific muscle regions, including the extracellular space and the space between myofibrils. This is the water not bound to the protein itself but held within the structure itself. This is the water that is impacted by the pH drop (Saelin *et al*., 2017; Kralik *et al*., 2017) which takes place when a rigor process transforms muscle into meat.

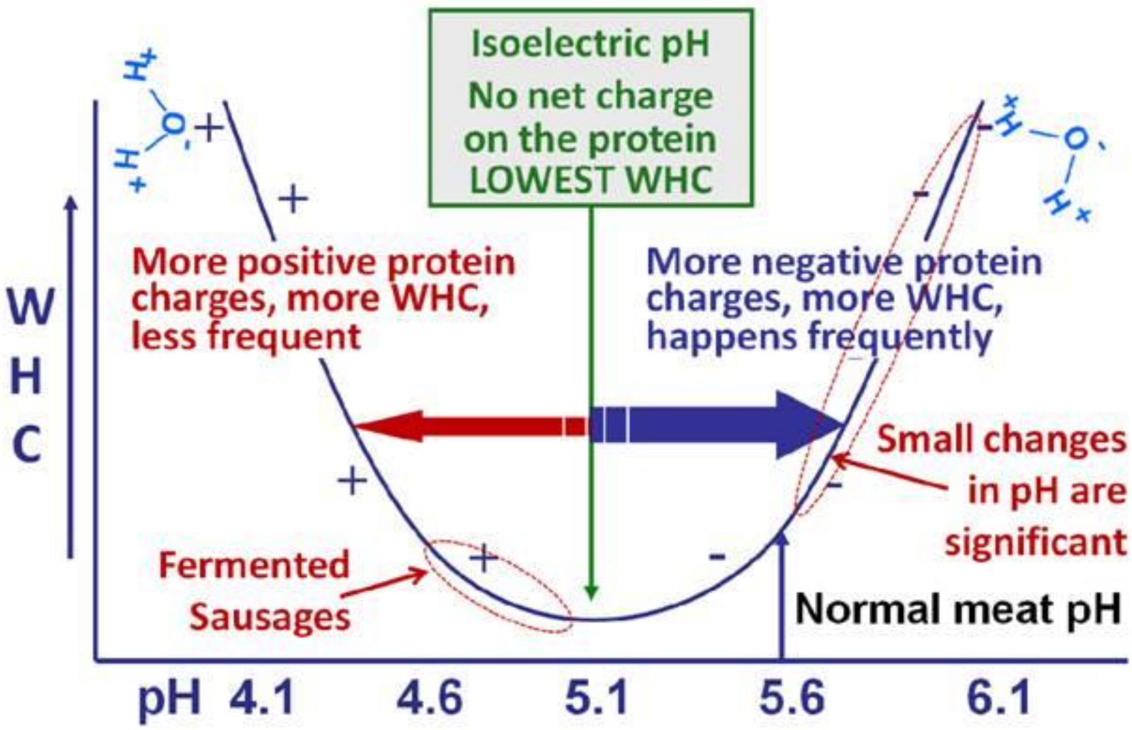


Figure 2.7 The connection between broiler meat's water-holding capacity and pH. From Kralik *et al*. (2018).

2.9.2.4 Meat pH

The pH refers to the degree of acidity or alkalinity of a solution (Ristic and Damme, 2013; Kralik *et al*., 2018). Chicken meat has a normal pH range of about 5.3 to 6.5 as shown on Figure 2.6 (Baldi *et al*., 2020). The ability of meat to store water is increased with every gradual increase in pH and vice versa. At high pH, meat protein has more negative charges (Figure 2.6) relative to positive charges which increases the affinity of proteins to water (Kralik *et al*., 2018). According to Saelin *et al*. (2017) during the conversion of muscle to meat, post-rigor, glycolysis (glycogen to glucose conversion) lowers meat pH which causes negative charges and positive charges to be equal (isoelectric point). At this point, the net charge is zero which means that proteins will not be able to attract and bind water (Kralik *et al*., 2018). Additionally, proteins that have been denatured by low pH might compress the myofibrillar spacing, which increases drip loss and eventually lowers the potential of meat to retain water (Kralik *et al*., 2018).

2.9.3 Meat Palatability

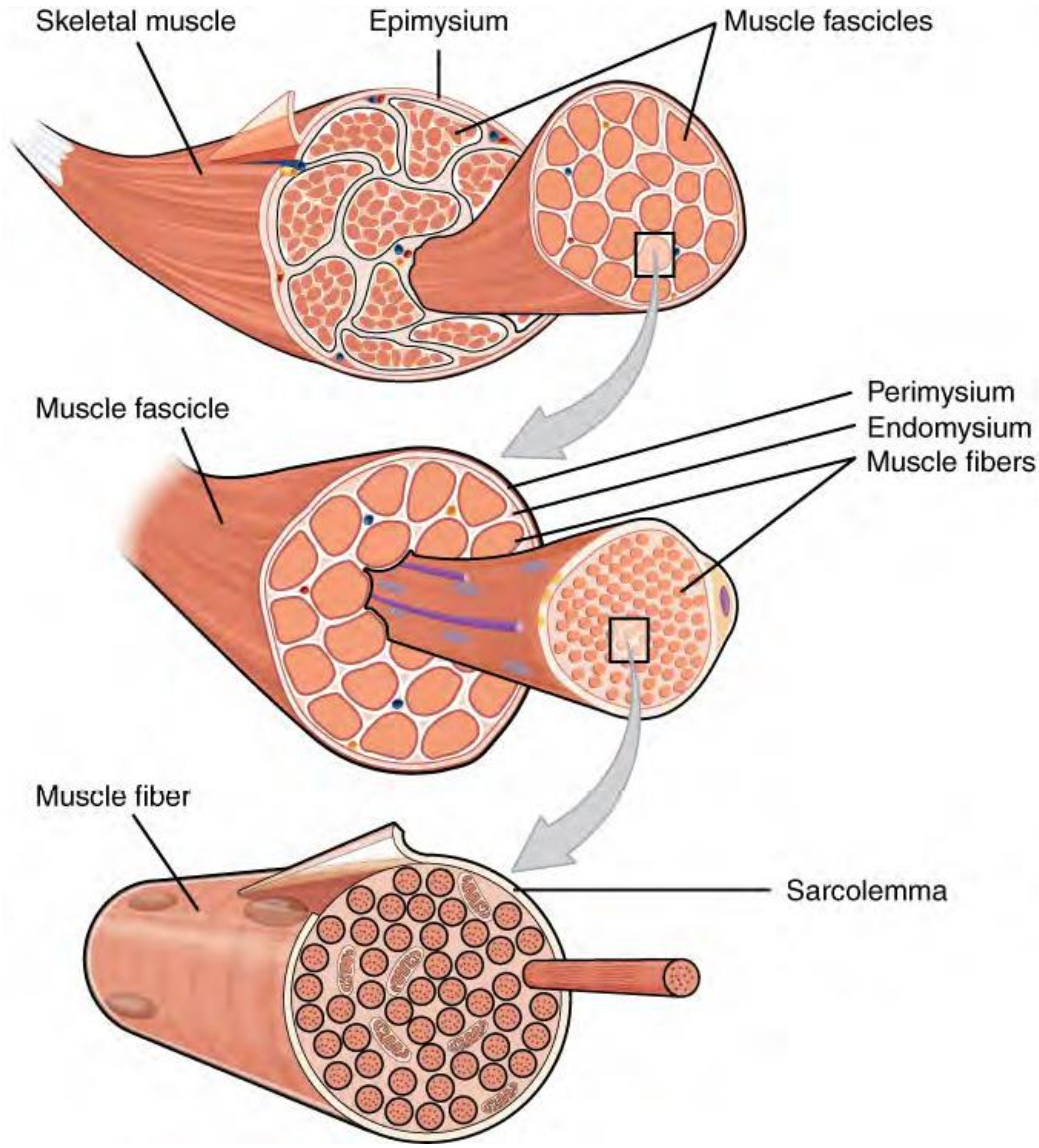
The eating quality is defined as the value of satisfaction the consumer gets from eating meat and is determined by the texture, tenderness, flavour, juiciness, chewiness and taste (Nusairat *et al*., 2022). The eating quality of meat varies between people, for example, consumers in developed countries prefer tender meat, Africans prefer chewy meat and Indians prefer bone-in meat (Nusairat *et al*., 2022).

2.9.3.1 Texture of Meat

Sarcomere length, the intensity of connective tissue linkage, pH, and cooking time all influence the texture of meat (Nusairat *et al*., 2022). High pH (> 6.8) can cause broiler meat to be tender and low pH (< 5.8) causes meat to be tough (Leonid *et al*., 2023). High pH causes meat to become dark, firm, and dry (DFD) and increases its ability to store water (Adzitey and Huda, 2011). According to Kralik *et al*. (2018), low pH causes meat to become soft (PSE) and decreases its ability to hold water. High temperatures when cooking cause the meat's dense connective tissue to denature and turn into gelatine (Barbut, 2015; Leonid *et al*., 2023). Long cooking duration causes meat to be very tender (Barbut, 2015; Leonid *et al*., 2023).

Broiler meat's texture and tenderness are also influenced by the quantity, size, and cross-linking of its muscle fibers (Barbut, 2015; Kralik *et al*., 2018). Broiler muscles are made up of muscle bundles that are bundled together and exteriorly covered by epimysium connective tissue (Barbut, 2015). The perimysium connective tissue divides the muscle bundle from adjacent bundles (Figure 2.5). Muscle fibers make up the muscle bundle, and endomysium connective tissue covers each muscle fiber (Barbut, 2015). Muscle fibers are the basic building blocks of a muscle (Nusairat *et al*., 2022). Myofibrils are the building blocks of a muscle fiber. Meat texture is greatly influenced by the endomysium, perimysium, and epimysium connective tissues, which are primarily composed of collagen (Huo *et al*., 2022). Collagen appears in large proportions in all animal tissues including flesh and bone (Weston *et al*., 2002). Numerous amino acids and imino acids make up the structural components of collagen; proline accounts for around one-third of the imino acids and glycine for approximately one-third of the amino acids (Nusairat *et al*., 2022). Tropo-collagen are the building blocks of collagen and each tropo-collagen is made up of three polypeptide chains which are coiled around each other to form a compound with high strength (Weston *et al*., 2002). Collagen cross-linkages is the contributory factor to the toughness of meat (Zhang et al., 2023).

Lee *et al*. (2023) reported that modifying the nutrient composition of poultry feed with various feed additives such as probiotics, prebiotics and organic acids, to mention a few, can have some beneficial effects on poultry meat quality but Owosibo *et al*. (2017) claimed that feeding broilers with feed containing different feedstuffs do not cause significant effects on meat texture. Owosibo *et al*. (2017) fed broilers with sesame oil seed meal at inclusion levels of 0%, 25%, 50% and 75% but found that sesame oil seeds meal caused no discernible change in the broiler meat's texture. Therefore, these contradictory results from earlier researchers need to be verified by conducting an independent research by partially replacing soybean meal with cowpea meal in broiler diets.



2.8 Illustration of a muscle, connective tissues, muscle bundle and muscle fibre. From Barbut (2002).

2.9.3.2 Broiler Meat Flavour

The aroma or smell of broiler meat is detected by the nasal passageways' posteriorly situated olfactory receptors (Warriss, 2000). Because heat causes the Maillard reaction, which is the result of carbonyl groups (CO) from reducing sugars reacting with the amino groups (nitrogen dioxide) from free amino acids, meat has very little flavor when it is raw. However, as the meat cooks, its flavor appears (Wang *et al*., 2023). The product formed out of Maillard reaction is dicarbonyl compounds, furanones, and furfurals that have been dehydrated and deaminated

(Celia Resconi *et al*., 2012). The latter may then combine with cysteine to produce an aldehyde (Warriss, 2000). Kayim *et al*. (2011) emphasized that the resulting aldehydes, ammonia (NH2), and hydrogen sulfide (H2S) could be engaged in other processes to come up with a variety of compounds that produce aromas such as pyrazines, thiazoles and thiazolines. Pyrazines are composed of nitrogen and carbon, thiazoles composing sulphur, carbon and nitrogen while oxazolines are made up of carbon, nitrogen and oxygen (Warriss, 2000). Maillard reactions are referred to as non-species-specific flavours common to all types of meat. In addition, we also have species-specific flavours that are caused by heating the fats in meat, especially phospholipids. When heated, the lipids undergo oxidative degradation thereby changing unsaturated fatty acids into different aldehydes thereby giving rise to different flavour notes (Jayasena *et al*., 2013).

Warriss (2000) claimed that lambs and beef cattle fed on clover and other legumes produced meat with a better flavour than meat from animals fed with corn diets and he attributed this to the fact that meat from animals fed legume-based diets contained more carbohydrates needed for the Maillard reaction to take place.

Different processing methods of grain legumes have been studied extensively (Abbas and Ahmad, 2018) to reduce anti-nutrients in grain legumes and these include soaking, fermentation, germination, roasting, boiling, dehulling, and autoclaving. Research conducted to look into how processing affects the nutritional makeup of cowpeas, protein digestibility, nitrogen content in litter, and ammonia emission in broilers fed cowpea-based diets show contradictory results (Balail, 2014, Akanji *et al*., 2016) and hence, there is a need to carry out further studies to come up with more reliable information that can enhance knowledge in this area of study.

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CHAPTER 3

Effect of Processed Cowpea-based Diets (*V. unguiculata*) on Intake and Growth Performance

3.1 Abstract

This study aimed to determine the effect of processing cowpea grains on feed intake and growth performance of broiler chickens. Two hundred and ten (210) broilers were randomly allocated into seven dietary treatments replicated three times with ten birds per replicate. The control diet was maize-soybean meal (MS). The other diets were based on maize-soybean-processed cowpeas as: maize-soybean-dehulled-boiled cowpea meal (MSDBC), maize-soybean-dehulled-roasted cowpea meal (MSDRC), maize-soybean-dehulled cowpea meal (MSDC), maize-soybean-boiled cowpea meal (MSBC), maize-soybean-roasted cowpea meal (MSRC), and maize-soybean-untreated or raw cowpea meal (MSUC). The diets were formulated to be isonitrogenous and isocaloric. Processing improved the nutrient composition of cowpeas. Processing increased crude protein but crude fibre and crude fat were reduced compared to the untreated cowpeas ($P<0.05$). Feed intake decreased in the MSBC treatment diet, although feed intake from the other treatments was similar to the control diet. There was no difference across all the dietary treatments on body weight gain and feed conversion ratio. The economic efficiency for broilers fed processed cowpea-based diets was higher and ranged from 0.80 to 0.86% and the economic efficiency of broilers fed the control diet was 0.76%. The broilers fed the raw cowpea-based diet had the lowest profit margins (US$0.70 per US$1.00) invested. Cowpea grains processed by dehulling-boiling, dehulling-roasting, dehulling, boiling, or roasting can be added up to 10% and 15% to broiler starter and finisher diets respectively, without compromising feed intake and growth performance of broilers.

Keywords: Alternative plant protein, anti-nutrients, chickens, body weight gain, feed conversion, economic efficiency

3.2 Introduction

Due to rising demand for white meat as people become more health conscious, Zimbabwe's broiler sector has expanded over the past few years (Bumhira and Madzimure, 2023). There are various elements that contribute to the expansion of the broiler sector that include; spiralling population growth, urbanization, changes in consumer preferences, and an increase in unemployment rate (Chikwengo, 2023). The annual production of day-old broiler chicks in Zimbabwe rose from 91.6 million in the year 2021 to 113.9 million in the year 2022. The production of broiler meat reached a record high in 2022, rising by 34% from 143500 million tonnes in 2021 to 191813 million tonnes (Chikwengo, 2023).

However, the sustainability and profitability of rearing broilers in Zimbabwe is being disturbed by the scarcity of soybeans, the primary source of proteins in chicken feeds (Nyoni, 2012). Soybean shortage in Zimbabwe is being caused by recurrent droughts that are negatively affecting crop yields, lack of irrigation infrastructure, lack of bankable loans, and high input costs (Bumhira and Madzimure, 2023). The lack of readily available and affordable conventional sources of protein for use in chicken feed formulations has made the prices of poultry meat and other poultry by-products to skyrocket (Kur *et al*, 2013). Cowpeas (*Vigna unguiculata*) is the possible source of protein that, due to its tolerance to regional climates, can either completely or partly substitute soybeans in broiler nutrition (Manole *et al*., 2024). Cowpeas have a have an amino acid profile that is comparable to soybeans but lower in amino acids that include sulfur (Embaye *et al.*, 2018).

Cowpeas are limited in their use in chicken diets because they contain anti-nutritional components like lectins, phytic acid, protease inhibitors, and tannins that lower feed intake and growth performance (Akanji *et al*, 2016; Cardoso *et al*., 2021). When broiler chickens were fed diets containing raw, dehulled, dehulled-boiled, and dehulled-roasted cowpeas at 20% inclusion levels, Akanji *et al*. (2016) found that raw cowpeas had a negative impact on feed intake (234g per week), weight gain (103g per week), feed efficiency (440g weight gain per kg feed eaten), and mortality rate (15% per week) compared to broiler chickens fed diets containing dehulled-roasted and dehulled-boiled cowpeas, which saw 158 to 170g of weight gain, 572 to 592g of weight gain per kg of feed consumed, and 5% mortality of broiler chickens fed diets containing these three types of cowpeas. Cowpeas were processed using a variety of techniques to lessen anti-nutrients, including boiling, roasting, dehulling, germination, and extrusion (Gutema and Tolesa., 2024). When dehulling, roasting, and boiling are used singly, they leave residual anti-nutrients in feedstuffs causing broilers to be less able to digest, absorb, and use nutrients (Malik *et al*., 2020). Therefore, combining dehulling with either boiling or roasting to process cowpea grains to entirely remove anti-nutrients, increase its nutrient profile, and improve broiler production performance should be explored.

3.3 Materials and Methods

The experimental procedures were approved by the National Animal Research Ethics Committee (NAREC) SI 246 of 2021.

Study site

This experiment was conducted at the Kadoma premises of the Cold Storage Company, which is situated in the Kadoma District of Mashonaland West Province at 18^{o} 20' 24" S 29^{o} 54' 00" E. It receives 739 mm of rain on average per year, with minimum and highest temperatures of 14°C and 28°C, respectively. The study site is 1183 meters above sea level.

3.3.1 Cowpeas Grain and Preparation

The cowpea grains (CBC5 variety) utilized in this experiment were bought in a Kadoma neighbourhood market. To get rid of bad grains, the cowpea grains were sorted. While some of the sorted cowpea grains were left untreated, others were roasted in a metal box roaster for 15 minutes at 120°C. Cowpea grains were mixed with an equal amount of sand and then roasted. The sand was used to evenly distribute the heat and ensure even roasting of cowpea grains. The roasted cowpea grains were, afterwards, separated from the cowpeas-sand mixture using a 2mm sieve. Another sample of cowpea grains was boiled at 120^{o}C for 15 minutes, others were soaked in sodium chloride solution for 12 hours, physically dehulled by hand, and air dried for 5 days. One sample was dehulled and roasted at 120^{o}C for 15 minutes. Another sample was dehulled and boiled at 120^{o}C for 15 minutes. The last sample consisted of dehulled cowpea grains which were boiled at 120^{o}C for 15 minutes. The cowpeas in the six groups were ground so they could fit through a 1.5 mm sieve.

3.3.2 Chemical analyses of samples from differently processed cowpea grain

Representative 500g samples of the above-mentioned differently processed cowpea grain were extracted using official standard techniques recommended by Afolabi *et al*. (2021) and sent to the laboratory of Agri-analysis Centre Pvt Ltd (Aglabs) in Harare to ascertain their chemical compositions and gross energy.

3.3.2.1 Moisture Content

The AOAC method proposed by Vakili *et al*. (2023) was employed to ascertain the moisture content. The moisture content was determined using the five-gram samples. Feed samples weighing five-gram each were oven-dried at 105°C and cooled for seventy-two hours with desiccators. The amount of moisture in each sample was determined using the formula below:

$$\text{Moisture (\%)} = \frac{(W_1 - W_2)100}{W_1}$$

Where;

W_1 = weight (g) of wet sample

W_2 = weight (g) of dry sample

3.3.2.2 Content of Proteins

The AOAC (2023) recommended feed analytical methodologies were used to calculate the protein content. Ten milliliters of pure H_2SO_4 and one Kjeldahl catalyst tablet were added to the micro-Kjeldahl flask along with five-gram samples. The digestion process was carried out for a duration of four hours. The resultant digest was then transferred and contained within a 100- milliliter volumetric flask. For the Kjeldahl analysis, a 5 milliliter aliquot of the digest was pipetted into the Kjeldahl apparatus. Then, 5 milliliters of a 40% (w/v) sodium hydroxide (NaOH) solution was added to the sample. The mixture was then steam distilled, and the resulting ammonia collected into a 50 milliliter conical flask containing 10 milliliters of boric acid and a methyl red-methylene blue indicator mixture. The green-colored solution was subsequently titrated using a 0.01 N hydrochloric acid (HCl) solution.

The %N is equal to (Titre × nitrogen atomic mass × HCl utilized normalcy × 4) To calculate the protein, the percentage of nitrogen was multiplied by a constant 6.25.

3.3.2.3 Ether Extract

According to the AOAC feed analytical methodology described by Vakili *et al*. (2015), the fat content was determined using the Soxhlet extraction system. For this analysis, five-gram samples were placed into a fat-free extraction thimble, which was then gently packed with cotton wool. The pre-dried, cooled, and weighed 250 ml Soxhlet flask and the reflux condenser were then connected to the thimble, which was placed inside the Soxhlet extractor. Petroleum ether was added to the soxhlet flask until it reached a quarter of its capacity, and the extractor and condenser were ready to be mounted on the heater. The heater was left running with the water running from the tap for six hours in order to promote ether vapor condensation. The percentage fat content was computed using the formula below:

$$\text{Fat (\%)} = \frac{(W_1 - W_0)100}{\text{Weight of sample}}$$

Where; W_1 is the weight of fat plus empty flask

W_0 is the weight of empty flask

3.3.2.4 Percentage Ash content

The ash content was determined using the standard Association of Official Analytical Chemists Method (2023). Five-gram samples were weighed and put into a ceramic crucible with a known weight. A muffle furnace was used to heat the crucible containing a 5 g feed sample to 550$^oC$for eight hours. It was cold, in a desiccator. The following formula was used to calculate ash content:

$$\text{Ash (\%)} = \frac{(P_2 - P_1)100}{P_0}$$

Where,

P_0 is the sample weight before drying, P_1 is the empty weight of the porcelain, and P_2 is the combined weight of the sample and porcelain after drying for eight hours at 550^oC in the furnace.

3.3.2.5 Unprocessed Fiber

The samples of differently treated cowpea grains were analysed for crude fibre using the method described by Igwemmar *et al*. (2022). Five grams (5g) cowpeas sample without lipid (W) was placed into a conical flask which contained 200 cm^3 of 0.3188M H_2SO_4 for 30 minutes and was then washed until all the acid was removed. The remaining product was then mixed with 0.32M NaOH solution and washed again to remove the base. The residue was subjected to elevated oven temperature of 105^oC and weighed (X). The dried material was incinerated at a temperature of 550^oC for 2 hours and the residue was allowed to cool and then weighed (Z).

$$\text{\% Crude fibre} = \frac{(X - Z)\ 100}{W}$$

Where W = weight of the sample (g), X = weight of dry residue (g), Z= weight ash sample (g).

3.4 Treatment diets

Broiler diets were produced using IDT Feed Formulation Software to be iso-caloric and iso-nitrogenous. In order to test the effects of protein source and processing techniques rather than protein level, the dietary treatments were designed to include the same amounts of energy and protein. Changing the components will introduce an additional variable into the experiment and alter the diets' quantities of protein and energy.

The feeds for broilers were designed to fulfil their nutritional needs in accordance with Cobb500 (2022). The broiler starter and finisher diets were divided into seven distinct dietary treatments, as shown in Tables 3.1 and 3.2, respectively.

3.5 Experimental Design

Two hundred and ten one-day-old unsexed Cobb 500 broiler chicks were randomly allocated into seven dietary treatments using a completely randomised design in a completely randomised (CRD) design. Each dietary treatment was replicated three times with 30 broilers per treatment and 10 birds per replicate.

3.6 Animal Management and data collection

Irvine's Day Old Chicks (Pvt) Ltd supplied two hundred and ten (210) one-day-old, unsexed Cobb 500 broiler chicks. The chicks were housed in an open-sided deep litter housing. After being cleaned and disinfected, the broiler house was allowed to rest for two weeks. The floor was covered with 10mm of fresh litter. The birds were raised for two weeks in accordance with Cobb's (2017) recommendations for the best practices for raising chicks. Weekly weight increase and feed intake records were measured. Mortality was recorded as it occurred.

Table 3.1 Cowpea-Based Starter Diets for Broilers

Ingredient	Treatment						
	MS (%)	**MSUC** (%)	**MSBC** (%)	**MSDC** (%)	**MSDBC** (%)	**MSDRC** (%)	**MSRC** (%)
Maize	62	57	57	58	58	58	57
SBM	35	30	30	29	29	29	30
CWP	0	10	10	10	10	10	10
Vit-min mix	3	3	3	3	3	3	3
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**
CP %	21	21	21	21	21	21	21
ME (MJ/Kg)	12.23	12.16	12.16	12.23	12.22	12.22	12.18

MS = Maize-Soybean, **MSUC** = Maize-Soybean Untreated Cowpeas, **MSBC** = Maize-Soybean-Boiled Cowpeas, **MSDC** = Maize-Soybean-Dehulled Cowpeas, **MSDBC** = Maize-Soybean-Dehulled Boiled Cowpeas, **MSDRC** = Maize-Soybean-Dehulled Roasted Cowpeas, **MSRC** = Maize-Soybean-Roasted Cowpeas.

Table 3.2 Cowpea-Based Finisher Diets for Broilers

Ingredient	Treatment						
	MS (%)	MSUC (%)	MSBC (%)	MSDC (%)	MSDBC (%)	MSDRC (%)	MSRC (%)
Maize	67	60	61	61	61	61	60
SBM	30	22	22	21	21	21	22
CWP	0	15	15	15	15	15	15
Vit-min mix	3	3	3	3	3	3	3
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**
CP %	19	19	19	19	19	19	19
ME (MJ/Kg)	12.32	12.32	12.32	12.39	12.38	12.38	12.33

MS = Maize-Soybean, **MSUC** = Maize-Soybean Untreated Cowpeas, **MSBC** = Maize-Soybean-Boiled Cowpeas, **MSDC** = Maize-Soybean-Dehulled Cowpeas, **MSDBC** = Maize-Soybean-Dehulled Boiled Cowpeas, **MSDRC** = Maize-Soybean-Dehulled Roasted Cowpeas, **MSRC** = Maize-Soybean-Roasted Cowpeas. **ME (MJ/Kg)** = Metabolisable energy in Megajoules per Kilogram.

Measurement of Production traits (Average weight gain and FCR)

At six weeks, nine birds were randomly selected from each treatment group and weighed to determine the live weight per bird, average weight gain, and FCR;

$$Voluntary\ feed\ intake\ (VFI) = Feed\ offered - feed\ refused$$

$$Weekly\ weight\ gain\ (WWG) = Weight_{week\ (n)} - \ weight_{week\ (n-1)}$$

$$Week\ 6\ weight\ gain\ (WWG) = Weight_{week\ (6)} - \ weight_{week\ (1)}$$

$$Feed\ Conversion\ Ratio\ (FCR) = \frac{Voluntary\ feed\ intake\ (g)}{Average\ weight\ gain\ (g)}$$

The following approach was used to calculate **Economic Efficiency** in accordance with Abou El-Fadel et al. (2019) guideline;

Economic Efficiency = Net revenue per bird divided by total fee cost per bird

3.7 Statistical analyses

For the data analysis, the researchers utilized the General Linear Model Procedure (PROC GLM) in the Statistical Analysis System (SAS) ver. 9.3 (SAS Institute Inc., 2010). Significant differences between the means were determined using the adjusted Tukey's method for mean comparisons, with differences considered significant at the $P<0.05$ level.

The following model was used in the study:

$Y_{ijkl} = \mu + R_i + T_j + Rep_i \times T_j + W_k + e_{ijkl}$

Where:

Y_{ijkl} = response variable (AWFI, AWWG, AWFCR)

μ = the overall mean

R_i = effect of the ^{ith} replicate

T_j = the effect of the j^{th} treatment

$Rep_i \times T_j$ = Effect of replication by treatment interaction

W_k = the effect of the k^{th} week (K = I, 2, 3, 4, 5, 6)

e_{ijkl} = the experimental random error

3.8 Results

3.8.1 Chemical Composition of Cowpea Grains

The proximate composition results of differently processed cowpea grains (unanalysed) are shown in Table 3.3 below. Removal of seed coats (dehulling) from cowpea grains increased both the crude protein content and metabolisable energy but reduced the crude fibre content of cowpea grains. Dehulled-boiled cowpeas had the highest CP content of 30.2% and raw cowpeas had the lowest CP content of 28.1%. Dehulled cowpeas had the highest metabolisable energy (ME) of 11.79MJ/Kg and raw cowpeas had the lowest ME of 11.50MJ/Kg.

3.8.2 Average Feed Intake

The average weekly feed consumption (AWFI) results per bird across all the seven dietary treatments are presented in Table 3.4 below. The broilers that were fed with Maize-Soybean-Boiled Cowpea (MSBC) meal consumed the least (P<0.05) amount of feed (643.3g) but the feed consumed by broilers from the remaining dietary treatments did not differ (P>0.05). Replication had no effect on average weekly feed intake (P=0.06). Only week had a significant effect on AWFI (P<0.05). Replication × Treatment had no effect on average feed intake.

3.3. Chemical Composition of Differently Processed Cowpea Grains

Parameter	Dry Matter (%)	Crude Protein (%)	Fat (%)	Crude Fibre (%)	Ash (%)	ME (MJ/kg)
UC	89.3	28.1	2.15	8.44	5.7	11.50
BC	90.8	28.2	0.46	6.12	5.6	11.51
DC	93.6	29.8	1.55	3.62	5.4	11.79
DBC	84.5	30.2	1.62	4.07	4.8	11.74
DRC	96.6	29.9	1.71	4.19	5.8	11.73
RC	93.6	28.2	1.72	5.21	5.7	11.61

UC = Untreated Cowpeas, **BC** = Boiled Cowpeas, **DC** = Dehulled Cowpeas, **DBC** = Dehulled Boiled Cowpeas, **DRC** = Dehulled Roasted Cowpeas, **RC** = Roasted Cowpeas.

Table 3.4 Feed Intake (g DM/Bird) of Broilers Fed on Processed Cowpea-Based Diets

Treatment	Average Weekly Feed Intake (g / Bird)
MSUC	699.2 ± 20.85^{a}
MS	$691 .8 \pm 20.43^{a}$
MSDBC	675.3 ± 20.43^{a}
MSDRC	672.5 ± 20.43^{a}
MSRC	668.9 ± 20.43^{a}
MSDC	663.3 ± 20.43^{a}
MSBC	643.0 ± 20.43^{b}

MS = Maize-Soybean, **MSUC** = Maize-Soybean Untreated Cowpeas, **MSBC** = Maize-Soybean-Boiled Cowpeas, **MSDC** = Maize-Soybean-Dehulled Cowpeas, **MSDBC** = Maize-Soybean-Dehulled Boiled Cowpeas, **MSDRC** = Maize-Soybean-Dehulled Roasted Cowpeas, **MSRC** = Maize-Soybean-Roasted Cowpeas.

$^{a-b}$**Means** with different superscripts in the same column are significantly different (P<0.05)

3.8.3 Average Weight Gain (g/bird) per Week

The average weekly weight gains (AWWG) for all the seven experimental groups are shown in Table 3.5. The dietary groups did not show any difference (P=0.66) in terms of weight gain. Replication had no significant effect on AWWG (P=012). Only week significantly influenced AWWG (P<0.0001). Replication × Treatment had no effect on average weight gain per week.

3.8.4 Average Feed Conversion Ratio (FCR)

The Average feed conversion ratios (AFCRs) of broilers fed diets containing differently processed cowpeas are presented in Table 3.6. Feed conversion ratio was not affected (P=0.36) by dietary treatments. The FCR ranged from 1.70 in birds fed the Maize-Soybean diet to 1.80 in broilers fed the Maize-Soybean-Boiled Cowpeas diet. Replication had no significant effect on AWFCR (P=0.15). Only week significantly influenced AWFCR (P<0.0001). Replication × Treatment had no effect on FCR.

Table 3.5: Average Weight Gain (g/bird) per Week of Broilers Fed on Processed Cowpea-Based Diets

Treatment	Average Weekly Weight Gain (g /Bird)
MS	375.6 ± 34.98
MSDC	373.6 ± 34.61
MSDRC	364.2 ± 34.98
MSRC	363.1 ± 34.98
MSBC	356.9 ± 34.98
MSUC	353.4 ± 34.98
MSDBC	343.1 ± 34.98

MS = Maize-Soybean, **MSUC** = Maize-Soybean Untreated Cowpeas, **MSBC** = Maize-Soybean-Boiled Cowpeas, **MSDC** = Maize-Soybean-Dehulled Cowpeas, **MSDBC** = Maize-Soybean-Dehulled Boiled Cowpeas, **MSDRC** = Maize-Soybean-Dehulled Roasted Cowpeas, **MSRC** = Maize-Soybean-Roasted Cowpeas.

3.8.5 Dietary Intervention's Impact on Economic Efficiency

The economic efficiency values of feeding broilers with diets containing differently processed cowpeas are shown in Table 3.7. Feeding broilers with diets containing untreated cowpea meal decreased the economic efficiency (US$0.70 per every US$1.00 invested) compared to an economic efficiency of U$0.76 per every US$1.00 invested in broilers fed the Maize-Soybean (control) diet. Feeding broilers with diets containing processed cowpeas improved ($P<0.05$) the economic efficiency values (US$0.80 to US$0.86 per every dollar invested).

Table 3.6 Average Weekly Feed Conversion Ratio (FCR)/Bird of Broilers Fed on Cowpea-Based Diets

Treatment	FCR
MSBC	1.80 ± 0.15
MSDRC	1.80 ± 0.15
MSDBC	1.80 ± 0.15
MSUC	1.80 ± 0.15
MSDC	1.70 ± 0.15
MSRC	1.70 ± 0.15
MS	1.70 ± 0.15

MS = Maize-Soybean, **MSUC** = Maize-Soybean Untreated Cowpeas, **MSBC** = Maize-Soybean-Boiled Cowpeas, **MSDC** = Maize-Soybean-Dehulled Cowpeas, **MSDBC** = Maize-Soybean-Dehulled Boiled Cowpeas, **MSDRC** = Maize-Soybean-Dehulled Roasted Cowpeas, **MSRC** = Maize-Soybean-Roasted Cowpeas.

Table 3.7: Impact of Cowpea-based diets on Economic Efficiency of Broilers

	MS	MSDBC	MSDRC	MSDC	MSBC	MSRC	MSUC
Price (US$) / kg diet	0.49	0.47	0.47	0.47	0.47	0.47	0.47
Total feed intake/broiler (g)	4151	3829	4035	4052	4009	3858	4195
Total feed cost (US$) / broiler	2.05	1.81	1.9	1.89	1.87	1.81	1.99
Chick cost (US$)	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Labor cost (US$) / bird	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Other costs (US$) / bird	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Total variable costs (US$)/ bird	3.35	3.11	3.2	3.19	3.17	3.11	3.29
Total weight (g) / bird	2360	2290	2350	2305	2284	2315	2235
Feed cost (US$) / kg gain	1.41	1.36	1.36	1.38	1.39	1.34	1.47
Total revenue (US$) / broiler	5.9	5.73	5.88	5.76	5.71	5.79	5.59
Net revenue (US$) / broiler	2.55	2.62	2.68	2.57	2.54	2.68	2.3
Economic efficiency	**0.76^{b}**	**0.84^{a}**	**0.84^{a}**	**0.81^{a}**	**0.8^{a}**	**0.86^{a}**	**0.7^{b}**

MS = Maize-Soybean, MSUC = Maize-Soybean Untreated Cowpeas, MSBC = Maize-Soybean-Boiled Cowpeas, MSDC = Maize-Soybean-Dehulled Cowpeas, MSDBC = Maize-Soybean-Dehulled Boiled Cowpeas, MSDRC = Maize-Soybean-Dehulled Roasted Cowpeas, MSRC = Maize-Soybean-Roasted Cowpeas.

3.9 Discussion

3.9.1 The Proximate Composition

The findings of this study indicated that processing positively affected the proximate composition of cowpea grains. All processing techniques that involved removal of the cowpea seed hulls caused a reduction of crude fibre (CF) in processed cowpeas. The results of the study align with Bumhira and Madzimure (2022) and Choi *et al*. (2023) who reported that dehulling increased the protein and fat contents but reduced the crude fibre in faba beans. The reduction in crude fibre in cowpea grains whose seed hulls had been removed can be attributed to the elimination of cellulolytic material and lignin that are generally found in the seed coats of legume seeds. However, roasting and boiling caused a slight increase in the CP content of cowpeas in the current research, which, is consistent with the findings of Anjos *et al*. (2016), who reported that roasting had a minimal impact on the CP content of cowpeas. Rafiee-Yarandi *et al*. (2016) claimed that roasting caused an inevitable loss of heat-sensitive amino acids such as lysine to volatilisation.

Processing resulted in a considerable increase in metabolizable energy of cowpea grains. These results are in support of the findings of Nalle *et al*. (2010) who observed that dehulling resulted in a significant increase in metabolisable energy of cowpeas. This can be due to the removal of non-starch polysaccharides and tannins from the hulls of the cowpea grains. The metabolisable energy (ME) of feed ingredients used in the design of chicken diet is crucial since it affects feed intake (Noblet *et al*., 2022). According to Massuquetto *et al*. (2020), broilers on a low-energy diet eat more feed in order to make up for any energy deficit. High dietary energy allows proteins to synthesize muscular tissues rather than focusing on generating energy (Jiang, 2007; Abdollahi et al., 2021). Formulating broiler feeds basing on book value ME without taking into consideration the actual ME values of the ingredients used, results in variations in ME of complete feeds produced (Abdollahi *et al*., 2021). Jiang (2007) claimed that a deviation in ME of feed by more than 50kcal/kg affects the cost of feed by US$2.20 to US$2.90 per tonne. Variations in metabolisable energy, according to the same author, have an impact on growth performance, carcass quality, and eventually profitability. However, in the current study, diets were made to be iso-energetic.

3.9.2 Average Weekly Feed Intake

The results of this study showed that both thermal treatment and dehulling caused no differences on feed intake with the exception of a reduced feed intake by broilers fed the boiled cowpea diet. These results are consistent with the findings of Belal *et al*. (2011) and Ciurescu *et al*. (2023) who observed that feeding broilers with diets containing dehulled cowpeas did not cause any significant effect on feed consumption. However, Akanji *et al*. (2016) claimed that giving broiler chicks meals that included 20% dehulled cowpeas reduced the growth performance indices (feed consumption, growth rate, protein efficiency ratio, and feed conversion ratio). These contradicting results can be attributed to the assumption that Akanji *et al*. (2016) might have used a particular cowpea cultivar that had significant concentrations of protease inhibitors and lectins since dehulling only removes tannins that are contained in seed coats leaving lectins and protease inhibitors unaffected. Bumhira and Madzimure (2022) reviewed that thermal treatment of grain legumes at 120^{o}C for 30 minutes cannot cause any significant effect on feed intake. Similar results were reported by Balaiel (2011), who found no difference in the amount of feed consumed by broilers fed finisher diets containing 20% boiled velvet (*Mucuna utilis*).

3.9.3 Average Weekly Weight Gain

Dietary treatments did not affect the weekly weight gains of the broiler chickens. The findings are consistent with those of Ciurescu *et al*. (2022) who reported that partial replacement of soybean meal with processed cowpeas in broiler feeds at inclusion levels not exceeding 20% did not affect broiler weight gain. Embaye *et al*. (2018) and Akure *et al*. (2021) obtained similar results by feeding broilers with 20% boiled cowpeas and 20% *Mucuna utilis* beans, respectively. Abdullahi *et al.* (2023) also observed that broiler growth performance was not affected by feeding them with diets containing 25% roasted Kidney beans (*Phaseolus vulgaris*). These outcomes might stem from the presumption that cowpea processing successfully eliminated anti-nutrients. This might have enabled the broilers to fully utilise the nutrients for optimal performance.

3.9.4 Average Weekly Feed Conversion Ratio (FCR)

This research shows that dietary treatments did not affect the amount of feed converted into meat (FCR) of the broilers). The FCR results of this investigation are similar to those of Embaye *et al*. (2018) and Belal *et al*. (2011) who found that broilers fed diets containing 20%

dehulled, dehulled-roasted, and raw cowpeas had similar FCR results. In addition, Mussa (1998) also reported that the inclusion of dehulled cowpeas in broiler diets did not result in any significant difference in the feed conversion ratio between the broilers fed the maize-soy diet and those fed diets containing varying levels of decorticated (dehulled) cowpeas. The similar FCR results obtained might be attributed to the fact that cowpeas' processing effectively eliminated anti-nutritional factors in cowpeas allowing optimal performance.

3.9.5 Economic Efficiency

In this study, the processing of cowpeas greatly improved the economic efficiency (profit obtained per every dollar invested) of feeding broilers. Birds fed with diets that include roasted cowpeas had the greatest economic efficiency followed by birds fed with dehulled-roasted, dehulled-boiled, and dehulled cowpea-based diets. These outcomes concur with those of Abou El-Fadel *et al*. (2019). Adino *et al*. (2018) also emphasised that the cost of rearing broilers can be reduced by incorporating processed cowpeas into diets. The diet consisting solely of raw cowpeas exhibited the least economic efficiency, which is likely caused by the broilers' inability to absorb and utilise nutrients due to the presence of anti-nutritional factors (tannins, protease inhibitors, lectins and non-starch polysaccharides) in unprocessed cowpeas (Korant *et al*., 2023).

3.10 Conclusion

The nutritional value of processed cowpeas can be enhanced through roasting, boiling, dehulling, or a combination of dehulling with either roasting or boiling. The incorporation of processed cowpeas in broiler starter and finisher diets improves growth performance, feed intake, FCR and feed efficiency of broilers.

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Chapter 4

Effect of Processed Cowpea-based Diets (*V. unguiculata*) on Protein Digestibility and Ammonia Emission in Broiler Production

4.1 Abstract

The objective of this study was to determine the effects of processing cowpea grains on the amount of protein digestible and ammonia released during the broiler production process. Dehulling, roasting, boiling, dehulling-roasting and dehulling-boiling techniques were used to process the cowpea grains. Broiler diets were formulated using soybean, white maize and cowpea meal as ingredients for broiler starter and finisher diets at 10 and 15% inclusion levels, respectively. The diets plans were designed to be iso-caloric and iso-energetic. Two hundred and ten (210) day-old Cobb 500 broiler chicks were randomly assigned into seven dietary groups using a completely randomized design. Each dietary treatment was replicated three times with ten birds per replicate. The dietary treatments included maize-soybean meal (MS), maize-soybean-dehulled-boiled cowpea meal (MSDBC), maize-soybean-dehulled-roasted cowpea meal (MSDRC), maize-soybean-dehulled-cowpea meal (MSDC), maize-soybean-boiled cowpea meal (MSBC), maize-soybean-roasted cowpea meal (MSRC) and maize-soybean-raw cowpea meal (MSUC). At 21 days of age, broilers from all seven dietary treatments underwent a 24-hour fast during which they were allowed to drink only water mixed with 50g of glucose solution. After 24-hr fasting, nine birds were isolated from each dietary treatment and were further deprived of feed for another 24 hrs in order to correct for endogenous protein losses. The remaining birds from every dietary group were fed with a known amount of feed for the next 24 hrs. Samples of poultry excrement from every experimental group were gathered over the course of 48 hours, measured, freeze-dried, and refrigerated at -20°C. Feed and freeze-dried excreta samples were analysed for their respective protein contents. The difference method was used to calculate digestibility of proteins. MSUC had the least digestibility coefficient (51%). However, there were no differences among other treatments (range 54.6 to 65.2%). Processing caused no differences on the amount of ammonia emitted from broiler excreta across all the dietary treatments. Therefore, processing of cowpeas using dehulling, roasting, boiling, and a combination of dehulling and roasting or dehulling and boiling can be used to improve protein digestibility, prevent environmental pollution, and promote climate-smart farming through the reduction of ammonia emissions from poultry facilities.

Keywords: Alternative grain legume, Processing, Chickens, Protein utilisation, Nitrogen excretion

4.2 Introduction

For maintenance, growth, and reproduction, poultry need diets that are well-balanced and provide enough nutrients (Bumhira and Madzimure, 2022). Since proteins are the most expensive feed ingredient and make up 20–35% of all ingredients used to formulate broiler feed, they are crucial to the health of broiler chicks (Ritz *et al*., 2004; Beski *et al*. 2015). Dietary proteins are essential for tissue formation, rejuvenation, and growth in chicken production (Beski *et al*., 2015; Kim *et al*., 2022). In the manufacture of hormones, enzymes, and antibodies, they are also extremely important (Hossain *et al*., 2017). All chicken diets are designed with the age-appropriate nutritional requirements of the birds in mind, as well as the temperature of the raising environment. This means that the birds are fed more nutrients than they need because the diets are not based on actual digestible nutrients, so they excrete the nutrients they do not use as waste through their feces (Qui *et al*., 2023). The anti-nutritional forces present in the vegetable protein sources used to produce broiler diets also result in decreased protein digestibility in broiler chickens (Bumhira and Madzimure, 2022).

Worldwide, the main source of protein for monogastric animal diets, like broiler rations, is soybean meal (Janocha *et al*., 2022). The price of soybean meal is rising due to its decreasing supply in developing and impoverished nations. Due to severe local shortages brought on by livestock and human competition for soybeans, Zimbabwe is forced to import soybeans from neighboring nations to supplement the 71 300 MT of soybeans that are currently being harvested (Nyoni, 2021). This helps the nation meet its needs for 240 000 MT for industrial oil expression and stock feed manufacture. Due to the high cost of importing soybeans, the price of broiler feeds rises at the same time as the cost of raising broilers eventually rises (Bumhira et al., 2023). The broiler production industry is less profitable due to low producer prices and high input costs (Phiri *et al*., 2023). Therefore, it's essential to look for less priced, locally obtained soybean substitutes.

The cowpea (*Vigna unguiculata*), which can be grown throughout Zimbabwe's agro-ecological zone and requires little input, is drought-tolerant and has been selected for this study (Bumhira *et al*., 2023). However, its use is restricted due to anti-nutritional properties that reduce protein digestibility when given to broiler diets (Samtiya *et al*., 2020). Like all grain legumes, cowpeas include anti-nutrients such as lectins, tannins, protease inhibitors, phytates, and non-starch polysaccharides (Sigh and Basu, 2012). Protein digestibility is decreased by tannins when they form insoluble complexes with proteins (Sigh and Basu, 2012). Certain cross-links that non-starch polysaccharides create with proteins prevent digestive enzymes from accessing those proteins (Koivunen, 2016). Protease inhibitors prevent the small intestine from breaking down proteins by deactivating proteinase enzymes (Koivunen, 2016).

Numerous studies have shown that although broilers obtain protein from their diet, they only use about one-third of it for the synthesis of tissue, hormones, enzymes, and antibodies, with the remaining portion being lost as waste in urine and faeces (Bumhira and Madzimure, 2022; Kim *et al*., 2022; Cerrate *et al*., 2019). According to Strifler *et al*. (2023), ejected protein contaminates the environment and is not cost-effective for broiler farmers. According to Kim *et al*. (2022), nitrogen found in undigested protein can be released into the atmosphere as ammonia gas through hydrolysis, mineralization, and volatilization. Ammonium nitrate and ammonium sulfate are produced when ammonia gas combines with acidic elements (SO_2 and NO_2) in air. (Wang *et al*., 2015). These compounds are then deposited on soil, open water bodies, and vegetation. These substances can acidify soil, induce eutrophication, and naturally replace slow-growing trees with fast-growing ones, respectively (Bumhira and Madzimure, 2022). Broiler growth, immunity to disease, and feed intake can all be adversely affected by ammonia (NH3) gas, which can even result in the birds' death (Sheikh *et al*., 2018).

The treatment of legume seeds proved to lower anti-nutrients and increase protein digestibility when added to broiler diets (Bumhira and Madzimure, 2022). According to Manole *et al*. (2024), thermal treatment, dehulling, and the inclusion of exogenous enzymes can decrease anti-nutrients in grain legumes and increase nutrient digestibility. However, dehulling cannot get rid of the lectins and protease inhibitors that are present in the seed cotyledons; it can only get rid of tannins and some non-starch polysaccharides from the seed coats (Bumhira and Madzimure, 2022). The same scientists found that tannins and non-starch polysaccharides are unaffected by dry heat or wet heat processing techniques, which solely eliminate the anti-nutrients found in the seed cotyledons. From research conducted, many processing techniques were scruitinised to ascertain their impacts on nutritional and anti-nutrients content of cowpeas, however, their impact on ammonia emission and protein digestibility has not been widely evaluated. Therefore, research is required to ascertain the impact of dehulling, roasting, boiling, and other adjustments to the aforementioned methods of cowpea treatment on ammonia emission and protein digestibility in broiler raising.

4.3 Materials and Methods

Study site

The experimental broilers were raised in an open-sided broiler house owned by Cold Storage Company in the Kadoma District of Mashonaland West Province. The Agri-analysis (Aglabs) laboratory in Harare performed the digestibility tests and chemical analysis of the excreta samples obtained from the seven dietary treatments.

4.3.1 Cowpea Grain and Preparation

The cowpea seeds (CBC5 variety) utilized in this experiment were bought from a Kadoma neighbourhood market. The identical techniques outlined in subsection 3.3.1 above were used to prepare the cowpeas.

4.3.2 Formulation of Experimental Diets

White maize, soybean, and cowpea meal were used to produce the starter and finisher diets for broilers, as shown in Tables 3.2 and 3.3 in the earlier chapter. Cowpeas that had undergone different processing were added to the broiler starter and finisher mashes at inclusion levels of 10% and 15%, respectively. The diets were designed with the same proportions of calories and protein.

4.3.3 Experimental Design

Description of the experimental design has been discussed in Chapter 3 subsection 3.5.

4.3.4 Animal Management and Data Collection

Irvine's Day-Old Chicks (Pvt) Limited supplied 210-day-old, unsexed broiler chicks of the Cobb 500 breed. The chicks were put in a deep litter housing unit with open sides. The broiler house was cleaned, disinfected, and left to rest for a period of two weeks. The floor was covered with wheat straw that was dry and dust-free. Two weeks were spent brooding the chicks. On days 12 and 16, the birds were vaccinated against infectious bursal disease (IBD) using the Gumboro D78 vaccine. On day 21, they underwent a second dose of the ND Lasota vaccine, which protects against Newcastle disease. Starting on day one and continuing through day fourteen, and day fifteen through day forty-two, the chicks were fed broiler starter and finisher mashes. The experiment was terminated six weeks following the placement of the birds.

Following the recommendations of Tshovhote *et al*. (2003), all birds from the seven treatment groups were fasted for twenty-four hours at the age of twenty-one. The broilers survived on 50g glucose solution during this fasting period. After fasting, twenty one birds from each dietary treatment were fed with ad libitum feed for a period of 24 hrs. However, to make some corrections for endogenous protein losses, 9 birds from each dietary treatment were fasted for another 24 hrs but were given 50g glucose solution. Following a 24-hour period, 100g excreta samples were obtained from every dietary group, freeze-dried at -20^{o}C, and refrigerated.
Ammonia emitted by faecal matter from the seven dietary treatments was measured using a digital Ammonia Test Meter (NH3 Gas Detector, Model FD-90A, range 0 – 100 ppm, designed in CA, USA and made in China)) every week until the broilers reached 6 weeks of age. Ammonia was measured in parts per million (ppm) units at birds' height level.

4.3.5 Chemical Analyses of Broiler Excreta Samples

The freeze-dried excreta samples weighing 100g each collected from the 7 dietary treatments were sent to the Agri-analysis (Aglabs) laboratory in Harare for chemical analysis. The faecal samples were ground so they could pass through a 1.5 mm filter after being dried in an oven at 90^{o}C. The AOAC (2006) techniques of analysis, as stated by Vakili *et al*. (2015) and illustrated in Chapter 3 subsection 3.3.2, were used to calculate the moisture content, crude protein, ether extracts, crude fiber, and ash in poultry excreta samples.

4.3.6 True Protein Digestibility

The following formula described by Borin *et al*. (2002) was used to calculate protein digestibility coefficients of the 7 dietary groups:

%True Protein Digestibility (%TPD) = $\frac{(FC \times DP) - (EC \times ECP - (EL \times ELP))}{FC \times DP}$

Where **FC** represents total feed consumed, **DP** represents crude protein of diet, **EC** represents excreta, **ECP** represents crude protein of excreta, **EL** represents endogenous losses, and **ELP** represents crude protein in endogenous losses.

4.3.7 Statistical analyses

For data analysis, the Statistical Analysis System (SAS) Version 9.3 (SAS, 2010) General Linear Model Procedure (GLM Proc) was used. Significant variations between means were identified using the adjusted Tukey's method for mean comparisons, with differences deemed significant at (P<0.05). The investigation used the following models:

$Y_{ijk} = \mu + R_i + T_j + Rep_i \times T_j + e_{ijk}$

Where;

Y_{ijk} = response variable (TPD)

μ = mean common to all observations

R_i = effect of the ^{ith} replicate

T_j = the effect of j^{th} treatment (j = MS, MSDBC, MSDRC, MSDC, MSBC, MSRC, MSUC)

$Rep_i \times T_j$ = Effect of replication by treatment interaction

e_{ijk} = the random error term.

The week effect was not included since TPD was only done when the birds were 21-days-old, meaning that it was not conducted on a weekly basis.

$Y_{ijkl} = \mu + R_i + T_j + Rep_i \times T_j + W_k + e_{ijkl}$

Where;

Y_{ijkl} = response variable (Ammonia emission)

R_i = effect of the ^{ith} replicate

T_{ji} = effect of the jth treatment (j = MS, MSDBC, MSDRC, MSDC, MSBC, MSRC, MSUC)

$Rep_i \times T_j$ = Effect of replication by treatment interaction

e_{ijkl} = the random error term

4.4 Results

4.4.1 True Protein Digestibility (TPD) Coefficients

The digestibility coefficients of proteins in broiler starter diets made with variously processed cowpeas at 10% inclusion levels are shown in Table 4.1 below. The protein digestibility (PD) coefficients of diets containing variously processed cowpeas were not different and ranged from 54.6 to 65.2% which, was higher than 51.0% ($P<0.05$) of the diet which contained raw cowpea meal. Processing cowpeas, therefore, improved the digestion of proteins in starter diets containing cowpea meal. Replication had no effect on TPD ($P=0.68$). Replication × Treatment had no effect on TPD.

4.4.2 Amount of Ammonia Emitted from Faecal Material

Table 4.2 displays the weekly average ammonia (ppm) levels released by the excreta of broilers fed diets formulated using processed and raw cowpeas. It was found that processing had no effect on ammonia emission across all dietary treatments. The amount of ammonia emitted from the dietary treatments ranged from 1.2 to 1.5 ppm. Replication did not affect ammonia emission ($P=0.75$) also. Only week significantly influenced ammonia emission ($P<0.0001$). Replication × Treatment did not affect ammonia emission.

Table 4.1 True Protein Digestibility (TPD) Coefficients of Broilers fed on Cowpea-based Diets

Treatment	TPD (%)
MSBC	65.2 ± 0.74^{a}
MS	64.9 ± 0.74^{a}
MSDRC	62.7 ± 0.74^{a}
MSRC	61.7 ± 0.74^{a}
MSDC	55.2 ± 0.74^{a}
MSDBC	54.6 ± 0.74^{a}
MSUC	51.0 ± 0.74^{b}

MS = Maize-Soybean, **MSUC** = Maize-Soybean Untreated Cowpeas, **MSBC** = Maize-Soybean-Boiled Cowpeas, **MSDC** = Maize-Soybean-Dehulled Cowpeas, **MSDBC** = Maize-Soybean-Dehulled Boiled Cowpeas, **MSDRC** = Maize-Soybean-Dehulled Roasted Cowpeas, **MSRC** = Maize-Soybean-Roasted Cowpeas.

$^{a-b}$**Means** with different superscripts in the same column are significantly different ($P<0.05$)

Table 4.2 Amount of Ammonia Emitted by Faecal Material Excreted by Broilers Fed Diets Containing Differently Processed Cowpea Grains

Treatment	WAE (ppm)
MSUC	1.5 ± 0.12[a]
MSDBC	1.3 ± 0.12[a]
MS	1.3 ± 0.12[a]
MSRC	1.3 ± 0.12[a]
MSBC	1.2 ± 0.12[a]
MSDRC	1.2 ± 0.12[a]
MSDC	1.2 ± 0.12[a]

MS = Maize-Soybean, **MSUC** = Maize-Soybean Untreated Cowpeas, **MSBC** = Maize-Soybean-Boiled Cowpeas, **MSDC** = Maize-Soybean-Dehulled Cowpeas, **MSDBC** = Maize-Soybean-Dehulled Boiled Cowpeas, **MSDRC** = Maize-Soybean-Dehulled Roasted Cowpeas, **MSRC** = Maize-Soybean-Roasted Cowpeas.

4.5 Discussion

4.5.1 True Protein Digestibility (TPD) Coefficients

Processing increased cowpea proteins' digestibility and this is consistent with data published by Abu El-Fadel *et al*. (2019) and Lewis *et al*. (2019). However, the current digestibility coefficients are not as high as results obtained by other authors (Kalpanadevi and Mohan 2013; Oghbaei *et al*., 2016; Dos Anjos *et al*., 2016; Oba *et al*., 2023). Differences in the age and sex of the birds employed as well as variances in the digestibility studies' methodologies may account for the discrepancy between the current study's findings and those of previous investigations. Adult birds, that are better able to digest feed nutrients than the younger birds employed in this experiment, have been used in the majority of nutrient digestibility investigations.

Furthermore, cecectomized cockels were employed by Kim *et al*. (2011) and Oba *et al*. (2023) to evaluate the digestibility of protein. To eliminate hind gut microbial fermentation, which breaks down protein that escapes the proximal gut undigested and converts it into ammonia and other toxic compounds, two modern techniques are used: cecectomy and ileostomy. This procedure causes an underestimation of protein lost through faecal material and an overestimation of protein retained (Borin *et al*., 2002). The *in-vivo* method of total excreta measurement also overestimates the quantity of protein lost through feces because feathers, scales, and spilled meal can occasionally adulterate the faecal material. *In vitro* digestibility trials such as the pepsin nitrogen digestibility assay are ineffective because they typically use a single proteolytic enzyme (pepsin) in an unnatural setting, whereas the gastro-intestinal tract of intact birds contains a wide variety of digestive enzymes (Lewis *et al*., 2019).

The improved TPDs' obtained from feeding broilers with processed cowpea-based diets are very important in broiler production because proteins in feed are not wasted through excretion but are effectively utilised for building of muscles. Efficient utilisation of proteins is very economical and environmentally friendly since it reduces contamination of the air, water and soil with nitrogen (Sheikh *et al*., 2018).

4.5.2 Ammonia Emission

The quantity of ammonia released from the faeces of broilers fed diets comprising differently processed cowpeas was not significantly affected by the method of processing cowpeas. These results are consistent with published literature which indicated that supplementing soybeans with processed vegetable proteins reduces the amount of ammonia emitted from poultry litter (Lokaewmanee, 2020; Sugiharto, 2022). Processing promotes protein retention in chickens, lowers anti-nutrients (tannins, protease inhibitors, and non-soluble polysaccharides) from grains and legumes, and eventually lowers the quantity of nitrogen or ammonia released through chicken excrement (Mattila *et al*., 2018). According to Jimenez-Moreno *et al*. (2019), insoluble fibers found in vegetable proteins can alter gut architecture and increase the microbiota in the small intestine, both of which enhance nutrient digestion and retention. According to Sitiya *et al*. (2019), insoluble fibre from vegetable protein sources aids in reducing the pH of the small intestine, which is advantageous for microbes and proteolytic enzymes involved in food digestion.

Poultry litter produced by broilers fed processed cowpea-based diets emits low levels of ammonia, which is environmentally beneficial as it helps prevent soil, water, and air pollution caused by ammonia from poultry production. The presence of excess ammonia can lead to eutrophication of open water bodies and a decrease in dissolved oxygen as dead aquatic plants decompose (Bumhira and Madzimure, 2023). Suffocation and mass fish deaths can occur as a result of this (Sheikh *et al*., 2018). When ammonia reacts with acidic compounds in the air, it produces particulate matter 2.5 (PM2.5), which can lead to respiratory illnesses in humans. Utilizing processed cowpea-based diets aids in safeguarding the environment from ammonia contamination, thus supporting Sustainable Development Goals 3 (Good Health and Well-being), 14 (Life below water), and 15 (Life on Land).

4.5.3 Conclusion

The processing of cowpea grains considerably improved protein digestibility and lowered the quantity of ammonia released into the environment when cowpeas are added to broiler diets. To guarantee sustainable chicken farming, it is advised that cowpeas that are used to partially replace soybeans in broiler diets be processed using either roasting, boiling, dehulling, or a combination of dehulling and roasting techniques.

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CHAPTER 5

Effect of Processed Cowpea-based Diets (*V. unguiculata*) on Meat and Sensory Characteristics of Broilers

5.1 Abstract

Nowadays meat consumers are now mindful of health, meaning that they buy meat that has a balanced nutritional profile, free from adulteration, and is not contaminated by microorganisms. The objectives of this study were to determine the effect of processing cowpeas on meat and sensory characteristics of broilers. A total of 210-day-old broiler chicks were randomly allocated into seven different feeding groups in a completely randomised design. Each dietary treatment was replicated three times with ten birds in each replicate. The control group contained maize-soybean meal (MS). The cowpea-based groups were: maize-soybean-dehulled boiled cowpea meal (MSDBC), maize- soybean-dehulled roasted cowpea meal (MSDRC), maize-soybean-dehulled cowpea meal (MSDC), maize-soybean-boiled cowpea meal (MSBC), maize-soybean-roasted cowpea meal (MSRC) and maize-soybean-untreated cowpea meal (MSUC). A total of fifteen birds (five per replicate) were randomly chosen and killed at the 42-day mark of the experiment. Meat samples were selected from the breasts to analyse meat quality and sensory characteristics. Forty trained panellists were randomly selected to conduct the organoleptic quality test of broiler meat. Meat from MS diet had the highest protein content of 22.5%, while meat from MSDBC treatment had the lowest (18.6%). Meat from raw cowpea-based diet had the highest fat content (1.3%) while meat from MSDBC had the lowest fat (0.9%) and other treatments were not different (1.2%). Meat from MSDBC, MSDRC, and MSRC had similar crude fiber content (0.8%), whereas meat from MSBC and MSUC had the highest (1.0%) compared to the control diet (0.9%). The amount of crude fiber in meat from the MSDC group was similar to that of the control. Meat form MSBC had the highest water holding capacity of 74.1%, MSUC had the lowest (61.5%) and other treatments were similar. Meat from MSBC had the highest pH of 6.6. Meat from MSUC had the lowest pH of 6.0, while pH from the remaining dietary groups was not different. Meat from broilers fed diets containing raw cowpeas had the lowest colour and texture scores, while the rest of treatments were similar in the two parameters. There were no differences in the aroma scores of all treatments. Meat from MSDBC, MSDC and MSBC had the highest taste scores, while MSUC had the lowest ($P<0.05$) and the remaining treatments were similar. Tenderness scores of meat were highest in MS, MSDBC and MSBC groups and lowest in the MSUC treatment. Tenderness of meat from the remaining treatments was not different in this parameter. In conclusion, processed cowpeas can be added to broiler diets up to 15% inclusion levels without compromising the quality and sensory attributes of broiler meat.

Keywords: Alternative grain legume, Processing, Chickens, pH, Water holding capacity, Organoleptic Attributes

5.2 Introduction

There has been a larger demand for chicken meat due to factors such as the recent increase in the global population, urbanization, shifting consumer preferences, rising per capita income, and its affordability relative to other meat varieties. (Beatriz Alvarado Soares and de Oliveira Silva, 2022). Broiler meat is also not affected by religious and cultural beliefs (Petracci *et al*., 2015). Broiler meat is highly digestible (contains less collagen) and is very nutritious (Ahmad *et al*., 2018). It has low levels of cholesterol and fats but is rich in proteins (Al-Baadani *et al*., 2023). Al-Baadani *et al*. (2023) found that chicken meat is composed of 22.9 % crude protein (CP), 1.9% fats, and 113 kilocalories (kcal) of energy when compared to 20.9% CP, 3.2% fats, and 115 kcal of energy in beef and 21.9% CP, 4.9% fats and 134% kcal of energy in pork (Ahmad *et al*., 2018). Nowadays meat consumers are now mindful of health, meaning that they buy meat that has a balanced nutritional profile, free from adulteration, and is not contaminated by microorganisms (Beatriz Alvarado Soares and de Oliveira Silva, 2022). The meat should be of high quality for it to be acceptable to the consumers.

According to Bogosavljevic-Boskovic *et al*. (2010), the characteristics of meat that affect its price and acceptability by consumers are referred to as meat quality. Meat quality can be classified into two groups: (i) conformance quality and (ii) functional quality. The desirable qualities of a product, such as yield, color, texture, flavor, water-holding capacity, and meat taste, are referred to as functional quality. Conformance quality is mainly concerned with creating a product that precisely satisfies the needs of the customer e.g. producing chickens with a certain weight as per consumers' request. Both types of quality are very crucial to meat producers and consumers because no consumer will accept broiler chickens having the exact dressing-out percentage but with a poor flavor, texture, and color (Bogosavljevic-Boskovic *et al*., 2010; Nusairat et al., 2022).

According to Northcutt (1997) and Nusairat *et al*. (2022), there are three levels of meat quality namely wholesomeness, functional, and eating quality. The term "wholesomeness" describes the nutritional, chemical, and microbiological characteristics of meat. Meat's texture, flavor, juiciness, taste, and tenderness are all considered aspects of its eating quality. The term "functional quality" describes the meat's color, pH, water-holding capacity, yield, and carcass composition.

Al-Baadani *et al*. (2023) reviewed the influence of genetic and non-genetic factors on meat quality. Genetic determinants include genotype; non-genetic factors include nutrition, feeding strategy, and rearing system (Nusairat *et al*., 2022). Bogosavljevic-Boskovic *et al*. (2010) claimed that broiler nutrition is the most important non-genetic factor that significantly influences certain meat quality traits. The same authors claimed that the chemical makeup of muscle tissue can be somewhat influenced by feed consumption and diet composition. The choice of raw materials for feed formulation, their chemical composition, the varying protein and energy values of the formulated rations, the extent of nutrient utilization, and the various interactive or associative effects (synergistic or antagonistic) of feed components are some of the factors that affect the quality of broiler meat (Bogosavljevic-Boskovic *et al*., 2010).

The use of organic acids and other feed additives, such as antibiotics, has increased broiler growth rates, decreased the incidence of sickness, and improved the grade of meat produced by broiler chickens. However, because health hazards (such as cancer and coronary heart disease) are connected with the antibiotics' residual effects in meat, the use of antibiotics in chickens is banned in all EU member states (Castanon, 2007). Probiotics, prebiotics, and exogenous digestive enzymes are increasingly being used in place of antibiotics in modern broiler production to improve meat quality, growth rate, and nutrient digestibility (Alloui *et al*., 2013). Probiotic dosage rates and modes of action, however, are still unknown (Jha *et al*., 2020). Moreover, probiotic supplementation in human diets may carry certain health hazards, according to current research on probiotics in humans (Jha *et al*., 2020).

Soybeans, a conventional source of protein used to prepare poultry feeds, is scarce in Zimbabwe and the shortage of this commodity is causing a proportional increase in the price of poultry feeds. The shortage of soybeans has raised awareness for stakeholders in the poultry industry to search for alternative plant sources of protein that can partially substitute soybeans in poultry feed manufacture. Grain legumes such as faba beans (*Vicia faba*), chickpeas (*Cicer* *arietinum*), pigeon pea (*Cajanas cajan*) and velvet bean (*Mucuna pruriens*) have been evaluated in several studies to analyse their suitability in replacing soybeans in broiler feeds. However, all the above-mentioned grain legumes are not widely distributed in Zimbabwe (Odeny, 2007) and are not sufficient to be used as a supplement to soybeans in broiler feed formulation. It is necessary to investigate whether cowpeas, which are inexpensive, readily accessible, resistant to drought, and able to adapt to Zimbabwe's semi-arid climate, may take the place of soybeans in the production of chicken feed. Since consumers of meat are now health-conscious, adding cowpeas to broiler diets shouldn't have an impact on the sensory qualities and quality features of the meat (Mir *et al*., 2017). They do not prefer meat that has high levels of fat and cholesterol (Wideman *et al*., 2016). Consumers like meat which is tender and light in colour. Meat with a high content of saturated fatty acids and cholesterol predisposes human beings to coronary heart diseases and obesity (Ponte *et al*., 2004). On the other hand, meat processors are mainly concerned about water holding capacity and pH of broiler meat since they determine the yield and shelf life of meat (Mir, *et al*., 2017)

A lot of studies (Kur *et al*., 2014; Akanji *et al*., 2016) were carried out to investigate the effect of partial replacement of soybeans with cowpeas in broiler diets on production parameters and carcass characteristics but there is dearth of information on the influence of cowpea-based diets on sensory attributes and meat quality parameters of broiler meat. Therefore, research is needed to investigate if cowpeas can be incorporated into broiler diets without negatively affecting sensory characteristics and quality of broiler meat.

5.3 Materials and Methods

Study Site

Two hundred and ten day-old broiler chicks were purchased from Irvine’s Day Old Chicks Pvt Ltd and were raised in fowl runs owned by Cold Storage Company in Kadoma District of Mashonaland West Province. The sensory evaluation of broiler meat was conducted at Chinhoyi University of Technology Food Science Laboratory, located in Mashonaland West Province, Zimbabwe. It is on a Latitude of 17^{o} 20’ 59” South and a Longitude of 30^{o} 12’ 31”. The area has a minimum and maximum temperature of 15.07°C and 27.94°C, respectively. The area has an elevation of 1140m above sea level.

5.3.1 Cowpea Grain and Preparation

The cowpea grains utilized in this experiment were bought in a Kadoma neighbourhood market. The cowpeas were prepared using the same methods that are described in subsection 3.3.1 above.

5.3.2 Experimental Design

A detailed description of the experimental designed used to conduct this study has been done in Chapter 3 subsection 3.5. Breast meat samples weighing 50g were collected from 15 broiler carcasses per each treatment and were used to measure the nutritional composition, pH and water holding capacity of meat. A sample of fifteen broiler carcasses per treatment (five per replicate) were used to measure the colour, taste, aroma, texture and tenderness of broiler meat.

5.3.3 Animal Management and Data Collection

As mentioned in the previous Chapter's subsection 4.3.3, the two hundred and ten experimental birds were reared in accordance with Cobb (2023) broiler production parameters. Upon completion of the experiment, measurements were made of the broiler meat's water holding capacity, pH, nutritional composition, and sensory attributes (42 days post-hatch). Fifteen birds from each food group (five per replicate) were randomly chosen on day 42 of the experiment, killed, defeathered, eviscerated, and refrigerated at -20^{o}C for a period of two days. On the third day, Breast meat samples weighing 50g were selected from each feeding group and were sent to the University of Zimbabwe in Harare, where tests were done on the meat's pH, water-holding capacity, and nutritional makeup. Fifteen birds per dietary group were brought to Chinhoyi University of Technology, where students with training in food science, animal production and technology performed the sensory evaluation.

5.3.3. 1 Water Holding Capacity of Broiler Meat

The water holding capacity (WHC) of the fresh breast meat samples was determined using the method described and authorized by Hussain *et al*. (2016). Each dietary treatment's 8g of meat samples were put in a centrifuge tube along with 12ml of 0.6M NaCl solution. After centrifuging the tube for 15 minutes at 4^{o}C and 10,000 rpm, the following computation was used to quantify the supernatant after it was decanted;

WHC (%) = $\frac{\text{before centrifuge weight – After centrifuge weight *100}}{\text{Before Centrifuge weight}}$

5.3.3.2 pH of Broiler Meat

Samples of broiler meat were analyzed using the protocol that Hussain *et al*. (2016) advised. Two birds per replicate provided 10g of breast meat, which was then crushed in a blender and homogenized for one minute with 100ml of distilled water. The pH was determined using a digital pH test meter.

5.3.3.3 Meat Sensory Characteristics

Fifteen broiler carcasses were used for each dietary treatment. Samples of breast meat were taken out, sliced into 3 cm by 3 cm pieces, homogenized, and then cooked for 30 minutes. A hedonic scale ranging from 1 to 5 was used by forty trained students from Chinhoyi University of Technology to undertake the sensory evaluation of the boiled meat samples. Table 5.1 lists the characteristics that were evaluated: color, aroma, texture, tenderness, and taste.

5.3.3.4 Statistical analyses

For the data analysis, the General Linear Model Procedure (Proc PRINCOMP) in the Statistical Analysis System (SAS) ver. 9.3 (SAS Institute Inc., 2010) was used. Significant differences between the means were determined using the adjusted Tukey's method for mean comparisons, with differences considered significant at the $p<0.05$ level.

The following was used to model the data;

$Y_{ijk} = \mu + R_i + T_j + Rep_i \times T_j + e_{ijk}$

Where;

Y_{ijk} = Response variable (Prox Comp, WHC, pH, colour, tenderness, texture, aroma, taste)

μ = The overall mean,

R_i = Effect of the i^{th} Replicate

T_j = Effect of the j^{th} treatment

$Rep_i \times T_j$ = Effect of replication by treatment interaction

e_{ij} = The experimental random error.

Table 5.1 Broiler Meat Assessment Score Guide

Score	Color	Texture	Aroma	Taste	Tenderness	General Acceptability
1	Brown	E. rough	V. dislike	V. dislike	V. N. soft	3 – 5
2	Brownish	Rough	D. not like	D. N. like	N. soft	3 – 5
3	M. White	R. rough	Q. like	Q like	S. enough	3 - 5
4	White	Smooth	L	Like	Soft	3 – 5
5	V. White	E Smooth	R. like	R. like	V. soft	3 – 5

Whereas V. smooth means extremely smooth, R. rough means rather rough, V. dislike means very dislike, D. N. like means do not like, V. N. soft means very not soft, N. soft means not soft, and V. soft means very soft. And where M. white means medium white, V. white means very white, E. rough means extremely rough, R. rough means rather rough, and E. smooth means extremely smooth.

5.4 Results

5.4.1 The Proximate Composition of Broiler Meat

The nutritional composition values of meat from broilers fed with diets containing differently processed cowpeas are presented in Table 5.2 below. The crude protein and fat of meat ranged from 18.6% to 22.5% and 0.9% to 1.3%, respectively. Dehulled-boiled cowpea-based diets produced meat with the lowest crude protein of 18.6% and the control diet produced broilers with meat containing 22.5% CP. The meat of broilers that were fed with dehulled-boiled cowpea-based diets had the lowest fat content (0.9%) and the meat of broilers from the MSUC and control diet had the highest fat content (1.3) %. The fiber content in meat varied from 0.8% to 1.0%. MSRC, MSDRC and MSDBC produced meat with the least fibre and MSUC and MSBC had the highest. Meat from MSDBC and MSDRC had the least ash content and ash content of meat from the remaining groups were similar. Replication × Treatment had no effect on proximate composition of meat.

5.4.2 Moisture Content, Water Holding Capacity and pH of Meat

The results of moisture content, water holding capacity and pH of broiler meat from broilers fed cowpea-based diets are indicated in Table 5.3 below. The highest moisture content (77.8%) was found in the meat of broilers fed diets containing dehulled-roasted cowpeas (MSDRC), while the meat from MSUC, MSBC, MSDC and MS had the lowest moisture content. Meat from the remaining dietary groups had similar amounts of moisture content. Meat from broilers given raw cowpea-based diets had the lowest water-holding capacity (61.5%), while meat from broilers fed dehulled-roasted cowpea-based diets had the best water-holding capacity (74.1%). The meat from broiler chickens fed diets based on raw cowpeas had the lowest pH (6.0), whereas the greatest pH (6.6) was found in the boiled cowpeas treatment group. Meat from MSRC had an average pH of 6.4. The pH of meat from the remaining treatments did not differ. Replication × Treatment had no effect on moisture content, WHC and pH of broiler meat.

Table 5.2 Proximate Composition of Broiler Meat

Nutrient Composition (%)

	Crude Protein	**Crude Fat**	**Crude Fiber**	**Ash**
MS	22.5 ± 0.07^{a}	1.3 ± 0.03^{a}	0.9 ± 0.01^{b}	1.2 ± 0.03^{b}
MSUC	22.1 ± 0.07^{b}	1.3 ± 0.03^{a}	1.0 ± 0.01^{a}	1.3 ± 0.03^{ab}
MSBC	21.9 ± 0.07^{b}	1.2 ± 0.03^{b}	1.0 ± 0.01^{a}	1.3 ± 0.03^{ab}
MSDC	19.4 ± 0.07^{d}	1.2 ± 0.03^{b}	0.9 ± 0.01^{b}	1.2 ± 0.03^{b}
MSRC	19.1 ± 0.07^{d}	1.2 ± 0.03^{b}	0.8 ± 0.01^{c}	1.4 ± 0.03^{a}
MSDRC	19.1 ± 0.07^{d}	0.9 ± 0.03^{c}	0.8 ± 0.01^{c}	1.1 ± 0.03^{c}
MSDBC	18.6 ± 0.07^{c}	0.9 ± 0.03^{c}	0.8 ± 0.01^{c}	0.9 ± 0.03^{d}

[a-d]**Means** with different superscripts within the same column denotes that they are significantly different (P<0.05).

MS = Maize-Soybean diet (control), **MSUC** = Maize-Soybean-Untreated Cowpea diet, **MSBC** = Maize-Soybean-Boiled Cowpea diet, **MSDC** = Maize-Soybean-Dehulled Cowpea diet, **MSRC** = Maize-Soybean-Roasted Cowpea diet, **MSDRC** = Maize-Soybean-Dehulled-Roasted Cowpea diet, **MSDBC** = Maize-Soybean-Dehulled-Boiled Cowpea diets.

Table 5.3 Moisture Content, Water Holding Capacity and pH of Broiler Meat

Treatment	Moisture (%)	Water Holding Capacity (%)	pH
MS	75.0 ± 0.16^{d}	66.5 ± 0.01^{b}	6.3 ± 0.54^{b}
MSDBC	76.3 ± 0.16^{c}	67.1 ± 0.01^{b}	6.4 ± 0.54^{b}
MSDRC	77.8 ± 0.16^{a}	70.4 ± 0.01^{b}	6.4 ± 0.54^{b}
MSDC	75.0 ± 0.16^{d}	66.7± 0.01^{b}	6.3 ± 0.54^{b}
MSBC	75.0 ± 0.16^{d}	74.1 ± 0.01^{a}	6.6 ± 0.54^{a}
MSRC	76.9 ± 0.16^{b}	71.0 ± 0.01^{b}	6.4 ± 0.54^{b}
MSUC	74.6 ± 0.16^{d}	61.5 ± 0.01^{c}	6.0 ± 0.54^{c}

$^{a-d}$**Means** with different superscripts within the same column denotes that they are significantly different (P<0.05).

MS = Maize-Soybean diet (control), **MSUC** = Maize-Soybean-Untreated Cowpea diet, **MSBC** = Maize-Soybean-Boiled Cowpea diet, **MSDC** = Maize-Soybean-Dehulled Cowpea diet, **MSRC** = Maize-Soybean-Roasted Cowpea diet, **MSDRC** = Maize-Soybean-Dehulled-Roasted Cowpea diet, **MSDBC** = Maize-Soybean-Dehulled-Boiled Cowpea diets.

5.4.3 Sensory Characteristics of Broiler Meat

The sensory evaluation results of meat from broilers fed diets containing differently processed cowpeas are shown in Table 5.4. Meat from broilers fed diets containing raw cowpeas had the lowest colour and texture scores, while the rest of treatments were similar in the two parameters. There were no differences in the aroma scores of all treatments. The meat from broilers fed diets containing raw cowpeas had the lowest taste score of 3, and the highest taste scores (5.0) came from broilers fed diets containing dehulled, boiled, and dehulled-boiled cowpeas. The meat from broilers fed with the MSUC diet had the lowest tenderness score of 2.0. The meat from broilers fed the MS, MSBC and MSDBC diets had the highest tenderness scores of 4.0, while the meat from broilers fed MSDC, MSDRC and MSRC had average tenderness scores of 3.0. Replication × Treatment had no effect on sensory characteristics.

Table 5.4 Organoleptic Quality Characteristics of Meat from Broilers Fed Cowpea-Based Diets

	Colour	Texture	Aroma	Taste	Tenderness
MS	$4.0±0.13^a$	$4.0±0.32^b$	$4.0±0.06^a$	$4.0±0.20^b$	$4.0±0.27^a$
MSDBC	$4.0±0.13^a$	$4.0±0.32^b$	$4.0±0.06^a$	$5.0±0.21^a$	$4.0±0.27^a$
MSDRC	$4.0±0.13^a$	$4.0±0.32^b$	$4.0±0.06^a$	$4.0±0.20^b$	$3.0±0.26^b$
MSDC	$4.0±0.13^a$	$4.0±0.32^b$	$4.0±0.06^a$	$5.0±0.21^a$	$3.0±0.26^b$
MSBC	$4.0±0.13^a$	$4.0±0.32^b$	$4.0±0.06^a$	$5.0±0.21^a$	$4.0±0.27^a$
MSRC	$4.0±0.13^a$	$4.0±0.32^b$	$4.0±0.06^a$	$4.0±0.20^b$	$3.0±0.26^b$
MSUC	$2.0±0.11^b$	$5.0±0.32^a$	$4.0±0.06^a$	$3.0±0.19^c$	$2.0±0.25^c$

$^{a-c}$**Means** with different superscripts within the same column denotes that they are significantly different (P<0.05).

Where; **MS** stands for basal diet, **MSDBC** stands for dehulled-boiled cowpeas, **MSDRC** stands for dehulled-roasted cowpeas, **MSDC** stands for dehulled cowpeas, **MSBC** stands for boiled cowpeas, **MSRC** stands for roasted cowpeas, **MSUC** stands for raw cowpeas.

5.5 Discussion

5.5.1 Proximate Composition of Breast Meat

In this study, it has been observed that feeding broiler diets including processed cowpea meal reduced the crude protein content of breast meat. The results were in line with the findings of several authors (Bogosavljevic-Boskovic, 2010; de Oliveira *et al*., 2016; Biesek *et al*., 2020) who reported that the crude protein content of broiler meat ranges from 15.8 to 23.5% depending on diet composition and sex of the experimental birds among other factors. The crude fat content and crude fiber content of broiler meat were different among the dietary treatments. The same results were observed by Souza *et al*. (2011). The findings of this study are in tandem with the results obtained from researches conducted using other types of grain legumes (Janocha *et al*., 2022). The same writers observed the crude protein, ether extract (EE), and ash content of broiler meat ranges from 15 to 25% CP, 1.5 to 5.3% EE, and 1.00 to 1.26% Ash.

5.5.2 The Moisture Content of Broiler Meat

In this study, feeding broilers with feed containing roasted, dehulled-roasted and dehulled-boiled cowpeas increased the moisture content of broiler meat. These findings are supported by Kucukyilmaz *et al*. (2012) who reported that the moisture content of broiler meat is influenced by feed composition. However, the moisture content of breast meat observed across all the dietary treatments in this current study was in line with the acceptable moisture content of broiler meat which ranges from 65 to 80% (Qiao *et al*., 2001; Rokonuzzaman, 2018; Kyakma *et al*., 2022). High moisture content in broiler meat is not recommended because it provides a conducive breeding environment for spoilage microorganisms that reduce the shelf life of meat (Mir *et al*., 2017).

5.5.3 The pH of Broiler Meat

A decrease in the pH of breast meat from broilers fed the MSUC diet was observed. Fernandes *et al*. (2016) claimed that oxygen becomes deficient when an animal is slaughtered and the glycogen in meat is anaerobically converted to lactic acid that lowers the pH of meat during rigor mortis. The pH of meat from broilers fed the MSBC diet was higher and it might have been caused by a lower concentration of glycogen in the pectoralis major muscle that consequently ended up releasing low amounts of lactic acid during glycolysis (Mir *et al*., 2017). The pH is very important in meat science because it influences the colour, tenderness, water holding capacity and overall, the shelf life of meat (Jankowiak *et al*., 2021). The same authors claimed that water holding capacity below 5.7 reduces the WHC (increases drip loss) and negatively affects the yield and juiciness of meat. The yield determines the profit and juiciness is preferred by those who are into the business of meat processing (high WHC increases the juiciness of minced meat). High drip loss in packaged meat products results in discolouration of meat, predisposes it to spoilage and pathogenic microorganisms and loss of essential nutrients (Kaic *et al*., 2020).The pH results obtained in this feeding trial are consistent with studies by Janocha *et al*. (2020) which showed that feeding broiler chicks diets containing *Pisum sativa* meal resulted in pH meat falling within a range of 6.21 to 6.29 and by Dinesh *et al*. (2013) who found that breast meat had a pH range of 5.7 to 6.6. Tavaniello *et al* (2022) also confirmed that the pH of broiler meat fell within acceptable levels of 5.7 to 6.8 when broilers were fed diets containing soybeans (control) and *Pisum sativum*.

5.5.4 The Water Holding Capacity of Broiler Meat

The observed increased water holding capacity from the MSBC treatment can be attributed to an elevated pH of broiler meat since information from the previous literature state that the proteins of meat with a high pH have more negative charges that have a greater propensity to bind water, and this is evident when the pH of meat is higher than the isoelectric point (Saelin *et al*., 2017). The lowest water holding capacity results obtained from the MSUC group can be attributed to the low pH of meat from this dietary group (Table 5.3) Kaic *et al*. (2020) emphasised that the WHC is generally decreased when the positive and negative charges of proteins in meat are equalled (isoelectric point) due to glycolysis. In proximity to the isoelectric point, meat loses its ability to bind water (Kralik *et al*., 2018). The current trial's WHC results for meat from broilers fed diets including processed cowpeas support the findings of Agbetuyi *et al*. (2024), who found that the meat's WHC values ranged from 75 to 86%. Garcia *et al*. (2010) recorded lower WHC values ranging from 64 to 69%. Lower WHC readings in meat are typically the result of pre-slaughter variables like stress, which lowers the meat's pH. Meat from diets containing processed cowpea had good water-holding capacity (WHC), an important factor for consumer perception of meat quality, weight loss during cooking, and sensory attributes when consumed (Nusairat *et al*., 2022). The yield of meat is negatively impacted by drip loss, leading to reduced profits from the sale of broiler meat (Agbetuyi *et al*., 2024). Additionally, purge loss can influence the colour, juiciness, tenderness, and taste of the meat (Saelin *et a*l., 2017).

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5.5.5 Sensory Analysis

It has been observed that the colour, texture and taste of broiler meat are influenced by the incorporation of cowpeas in broiler diets. The poor colour score of meat from broilers fed the MSUC diet can be due partly to the presence of carotenoids and xanthophylls in untreated cowpeas (Mir *et al*., 2017). The inferior taste of meat from broilers fed with diets containing raw cowpeas might have emanated from the bitter taste of anti-nutrients that are contained in raw cowpeas (Akanji *et al*., 2016). The best colour, taste, texture and tenderness scores obtained from the remaining treatments might be credited to the high pH and water holding capacity values that were associated with these feeding groups. WHC and pH are responsible for good sensory attributes of meat when they are high and the vice-versa is also true (Li *et al*., 2021). The good color, taste, texture, tenderness, and aroma scores obtained from the meat of broilers fed with diets containing processed cowpeas are very important to meat consumers since consumer acceptance of meat is determined by its freshness, which is determined by its colour, and repeat purchases are prompted by the eating quality (taste, texture, aroma, and tenderness) of meat (Nusairat *et al*., 2022).

5.6 Conclusions

Cowpeas that have been either dehulled, roasted, boiled, dehulled-roasted, or dehulled-boiled can be substituted for soybeans in broiler diets at inclusion levels no more than 15% without affecting the meat's pH, ability to hold water, or sensory qualities.

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CHAPTER 6

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

6.1 General Discussion

Making cowpea-based (*Vigna unguiculata*) broiler feeds that are economically viable for livestock farmers was the study's main goal. Zimbabwe suffers from a shortage of soybeans, the traditional protein source utilized in the production of broiler feed, as a result of the damaging impacts of frequent droughts exacerbated by a lack of irrigation infrastructure (Nyoni, 2021). With comparable amino acid profiles to soybeans, cowpeas, a cheap and readily accessible grain legume, was investigated as a partial soybean substitute in broiler diets in this study.

Reducing greenhouse gas emissions into the environment is now the main objective of any farming operation to lessen the detrimental effects of climate change that are being felt worldwide (Copley and Wiedemann, 2023). Methane and ammonia are among the gases released into the environment by poultry rearing (Broucek and Cermak, 2015). Ammonia contaminates open water, the air, and the soil (Sheikh *et al,* 2018). Massive fish kills and oxygen depletion in bodies of water are the outcomes of eutrophication, which is caused by excess ammonia deposited in open water bodies (Knockaert, 2024). As a result, changing the diets of the chickens should lower the amount of ammonia emissions from poultry enterprises.

In order to encourage repeat purchases, broilers produced using feed made by mixing different feed ingredients should have meat with high pH, water holding capacity and excellent nutritional quality (Bisschoff, 2017). Broiler consumers are now health conscious and would prefer to purchase broiler meat that has good appearance.

The hypothesis tested in Chapter 3 was that feeding processed cowpeas improves broiler feed intake and growth performance. Dehulled, dehulled-boiled, dehulled-roasted and roasted cowpeas were incorporated into broiler starter and finisher diets at inclusion levels of 10% and 15%, respectively, but did not affect feed intake, average body weight gain and FCR when compared to the control diet and MSUC based diet. However, broilers from the MSBC based diet consumed the least amount of feed compared to the control diet and the raw cowpea-based diet.

The hypothesis tested in Chapter 4 was that processing cowpea grains improves nutrient digestibility and ammonia emission in broiler chickens. Dehulled, dehulled-boiled, dehulled-roasted, boiled and roasted cowpeas-based diets protein digestibility coefficients obtained in this study were higher than the digestibility coefficient of protein obtained in the MSUC diet.

Chapter 5 tested the hypothesis that the quality and sensory attributes of broiler meat are improved by processing of cowpeas. In this study, broiler starter and finisher diets were fed with processed cowpeas in lieu of soybeans at 10% and 15% inclusion levels, respectively. The results demonstrated that feeding broiler diets including processed cowpeas enhanced the meat's pH and ability to hold water compared to the meat from the raw cowpea-based diet. Cowpeas that have been dehulled, boiled, roasted, and dehulled-roasted did not affect the texture, flavor, color, or scent of the meat of broilers.

6.2 Conclusions

Similar amounts of feed can be consumed by broilers fed roasted, dehulled, dehulled-roasted and dehulled-boiled compared to the commercial diet. When broilers are fed diets containing processed cowpeas, their body weight gain and feed conversion ratio (FCR) are all equivalent to those of the broilers fed a control diet. Broilers fed diets containing processed cowpeas had better economic efficiency in comparison to the broilers fed the raw cowpea based and control diets. When processed cowpeas are substituted for soybeans in broiler diets at inclusion levels no higher than 15%, neither ammonia emission nor protein digestibility are impacted in comparison with broilers fed either the control diet or diets containing untreated cowpeas. The meat from broilers fed cowpea-based diets is not different from the meat from broilers fed the control diet in terms of pH, water-holding capacity, or sensory qualities.

6.3 Recommendations

It is advised that the Zimbabwean government launch awareness campaigns to inform farmers about cowpeas, a crop that can withstand drought and be grown in semi-arid regions of Zimbabwe at low cost (Bumhira and Madzimure, 2022). Cowpeas can also be used to supplement soybeans in broiler diets without negatively impacting the environment or the quality or sensory qualities of the meat produced from them.

The areas that need further research are;

1) To identify cowpea varieties that have less anti-nutritional factors to be used in the formulation of broiler diets.

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2) To conduct additional studies to determine the effects of adding cowpeas to broiler diets on the fatty acid profiles of meat.

6.4 References

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APPENDICES

Appendix I: Broiler Breast Meat Assessment Score Sheet

Parameter	Hedonic Score	Information	T1	T2	T3	T4	T5	T6	T7
	1	Brown							
	2	Brownish							
Colour	3	Pink							
	4	Medium							
	5	white							
		White							
		Very white							
Texture	1	Extremely							
	2	rough							
	3	Rough							
	4	Rather Rough							
	5	Smooth							
		Extreme							
		smooth							
Aroma	1	Very dislike							
	2	Do not like							
	3	Quite like it							
	4	Like it							
	5	Really like it							
Taste	1	Very dislike							
	2	Do not like							
	3	Quite like it							
	4	Like it							
	5	Really like it							
Tenderness	1	Very not soft							
	2	Not soft							
	3	Soft enough							
	4	Soft							
	5	Very soft							

Source: Hafid et al. (2020).

Hafid, H., Patriani, P., Nuraini, Norma, Ananda, S. H. and Inderawati. (2020). Organoleptic Characteristics of Broiler Chicken Meat using Juice of Starfruit (*Averrhoa bilimbi*). IOP Conf. Series: Earth and Environmental Science 454 (2020) 012057

Appendix II: List of Research Publications

This thesis is based on the following journal articles, which are referred to by the Roman numerals I – III.

I Bumhira and Madzimure (2022). Effect of Processing on Nutritional Content and Protein Digestibility of Cowpea Grain in Broilers. *International Journal of Natural Sciences Research*. 10 (1): 81-87. https:/doi:10.18488/63.v10i1.3157.

II Bumhira and Madzimure (2023).Effect of Incorporating Cowpea (*Vigna unguiculata*) Meal into Broiler Diets on Environmental Pollution by Nitrogen from Poultry Excreta: A Review. *Journal of Agricultural Chemistry and Environment*. 12**:** 84-92. https://doi.org/10.4236/jacen.2023.122007.

III Bumhira, E., Chikwanda, D., Washaya, S. and Nyamushamba, G. (2023).Biological and Economic Efficiency of Partial Dietary Substitution of Soybean (Glycine max) Meal with Cowpea (*Vigna unguiculata*) Meal in Broiler Production. *Journal of Agricultural Chemistry and Environment*, 12: 296-305. https://doi.org/10.4236/jacen.2023.123022.

CONTRIBUTIONS

	I	**II**	**III**
Planning the experiment	EB, JM,	EB, JM,	EB, DC, SW, GN
Conducting the experiment	EB, JM,	EB, JM,	EB, DC, SW
Conducting the autopsies	EB	EB	EB
Data analysis	EB, JM,	EB, JM,	EB, TM
Calculating and interpreting the results	EB, JM,	EB, JM,	EB, TM, DC
Manuscript	EB, JM, IM	EB, JM,	EB, TM, DC, GM

EB = Edmore Bumhira

JM = James Madzimure

DC = Denice Chikwanda

SW = Soul Washaya

GN = Godfrey Nyamushamba

TM = Tonderai Mutibvu

Appendix III: Plagiarism Report for Edmore Bumhira's Thesis

PAPER NAME	AUTHOR
CHAPTER 1 Bumhira.docx	Edmore Bumhira
WORD COUNT	CHARACTER COUNT
11685 Words	64948 Characters
PAGE COUNT	FILE SIZE
42 Pages	1.3MB
SUBMISSION DATE	REPORT DATE
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Appendix IV: Average Weekly Weight Gain and Weekly FCR Analysed Data

The SAS System

The GLM Procedure

Class Level Information

Class	Levels	Values
TRNT	7	BASAL BOIL DBC DEHUL DRC RAW ROAST
WEEK	6	1 2 3 4 5 6

Number of Observations Read	126
Number of Observations Used	126

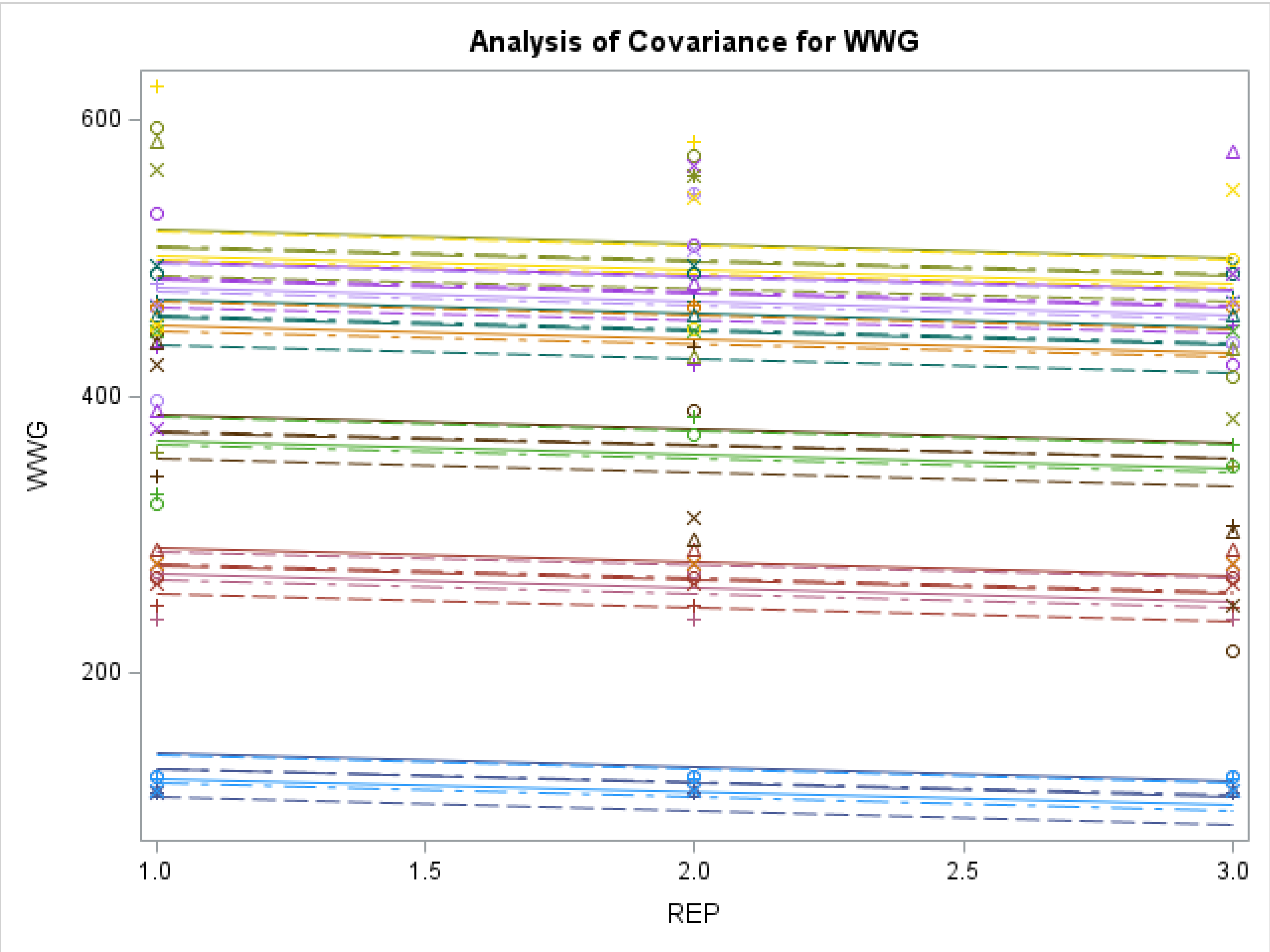
The GLM Procedure

Dependent Variable: WWG

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	12	2246971.563	187247.630	54.46	<.0001
Error	113	388499.807	3438.051		
Corrected Total	125	2635471.370			

R-Square	Coeff Var	Root MSE	WWG Mean
0.852588	16.22441	58.63490	361.3992

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRNT	6	14056.325	2342.721	0.68	0.6649
WEEK	5	2224620.903	444924.181	129.41	<.0001
REP	1	8294.334	8294.334	2.41	0.1232



The SAS System

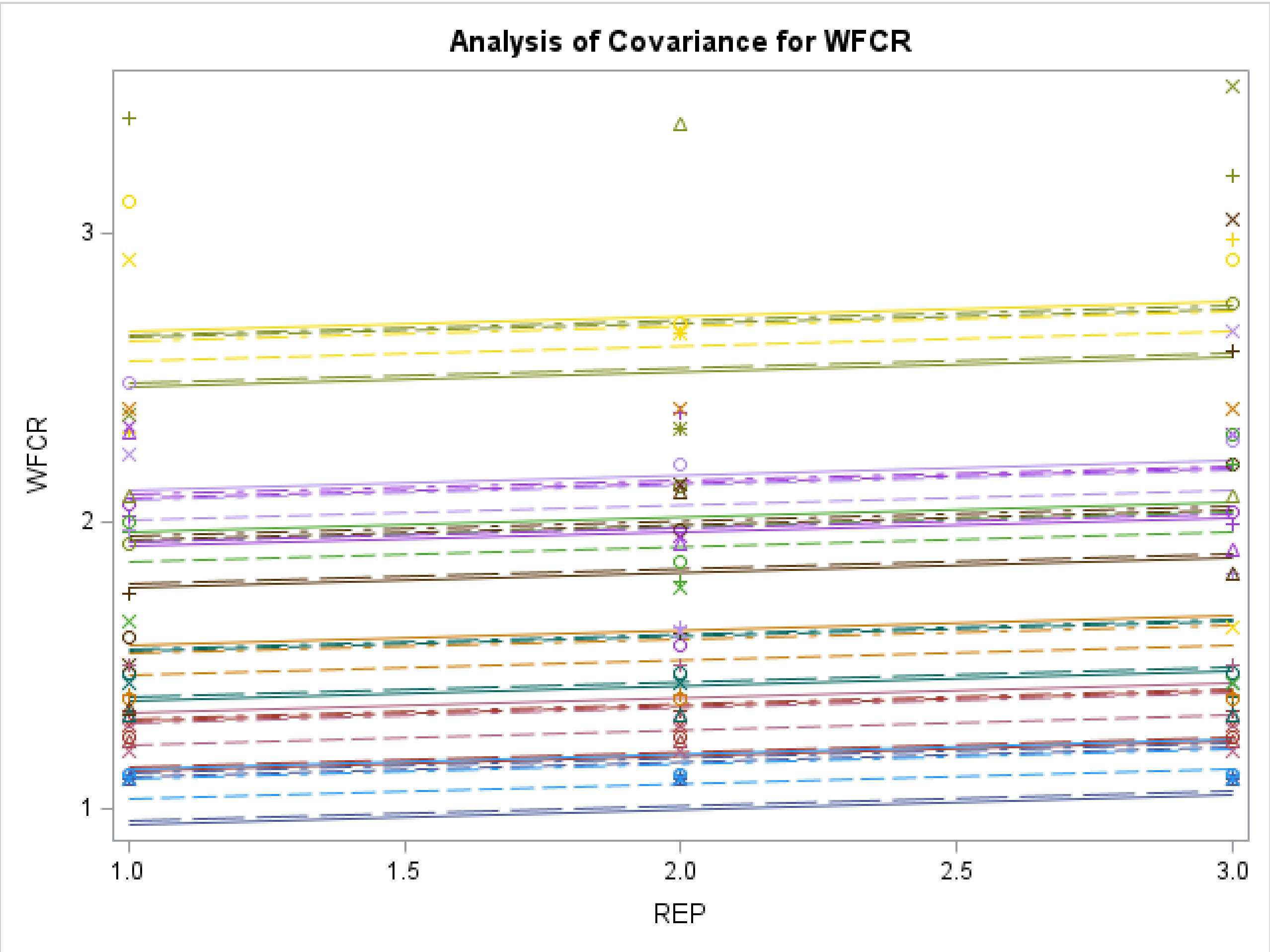
The GLM Procedure

Dependent Variable: WFCR

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	12	33.85664722	2.82138727	26.27	<.0001
Error	113	12.13583532	0.10739677		
Corrected Total	125	45.99248254			

R-Square	Coeff Var	Root MSE	WFCR Mean
0.736134	18.53156	0.327714	1.768413

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRNT	6	0.71578254	0.11929709	1.11	0.3605
WEEK	5	32.91766349	6.58353270	61.30	<.0001
REP	1	0.22320119	0.22320119	2.08	0.1522

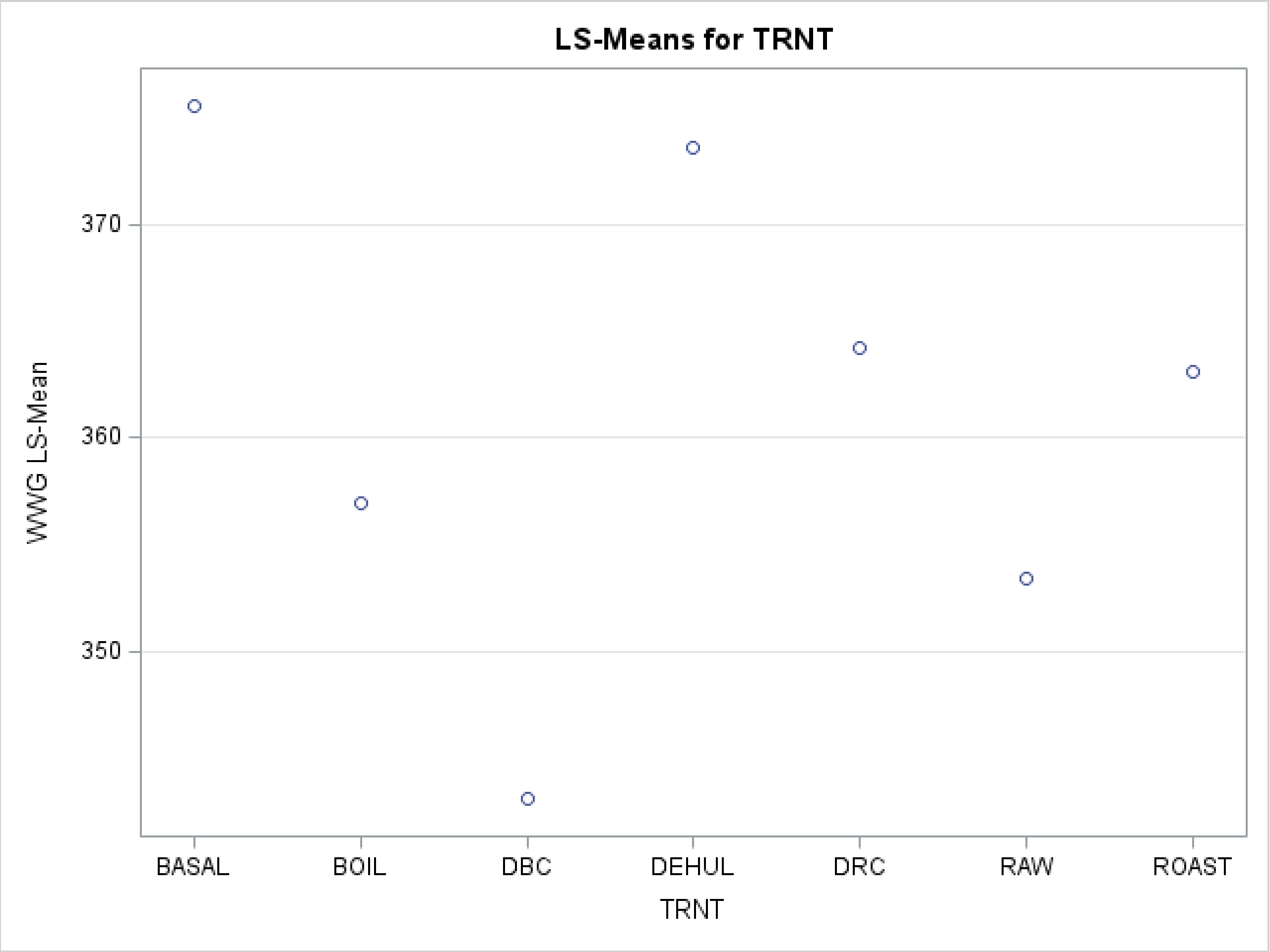


The GLM Procedure
Least Squares Means

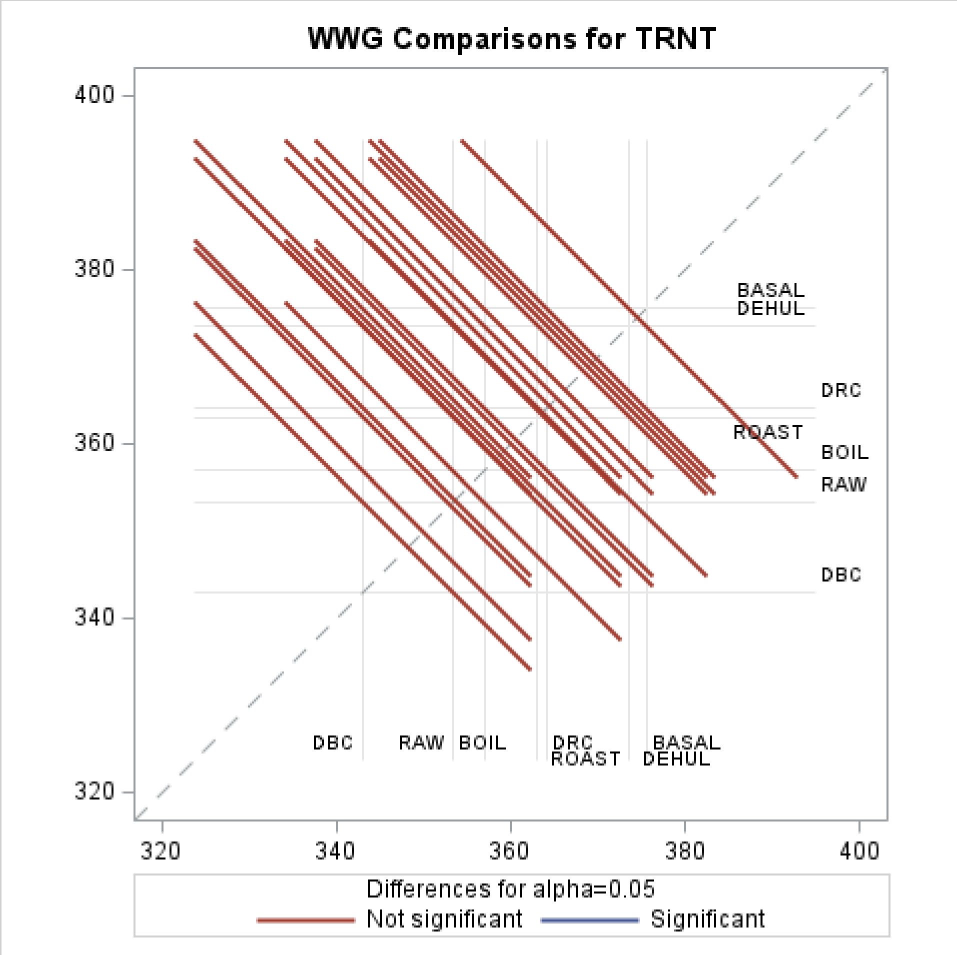
TRNT	WWG LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
BASAL	375.572222	13.820378	<.0001	1
BOIL	356.944444	13.820378	<.0001	2
DBC	343.055556	13.820378	<.0001	3
DEHUL	373.611111	13.820378	<.0001	4
DRC	364.166667	13.820378	<.0001	5
RAW	353.388889	13.820378	<.0001	6
ROAST	363.055556	13.820378	<.0001	7

Least Squares Means for effect TRNT
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: WWG

i/j	1	2	3	4	5	6	7
1		0.3426	0.0989	0.9203	0.5607	0.2588	0.5232
2	0.3426		0.4788	0.3956	0.7124	0.8560	0.7551
3	0.0989	0.4788		0.1208	0.2824	0.5981	0.3084
4	0.9203	0.3956	0.1208		0.6299	0.3030	0.5902
5	0.5607	0.7124	0.2824	0.6299		0.5824	0.9548
6	0.2588	0.8560	0.5981	0.3030	0.5824		0.6219
7	0.5232	0.7551	0.3084	0.5902	0.9548	0.6219	



LS-Means for TRNT
WWG LS-Mean
370
360
350
BASAL
BOIL
DBC
DEHUL
DRC
RAW
ROAST
TRNT



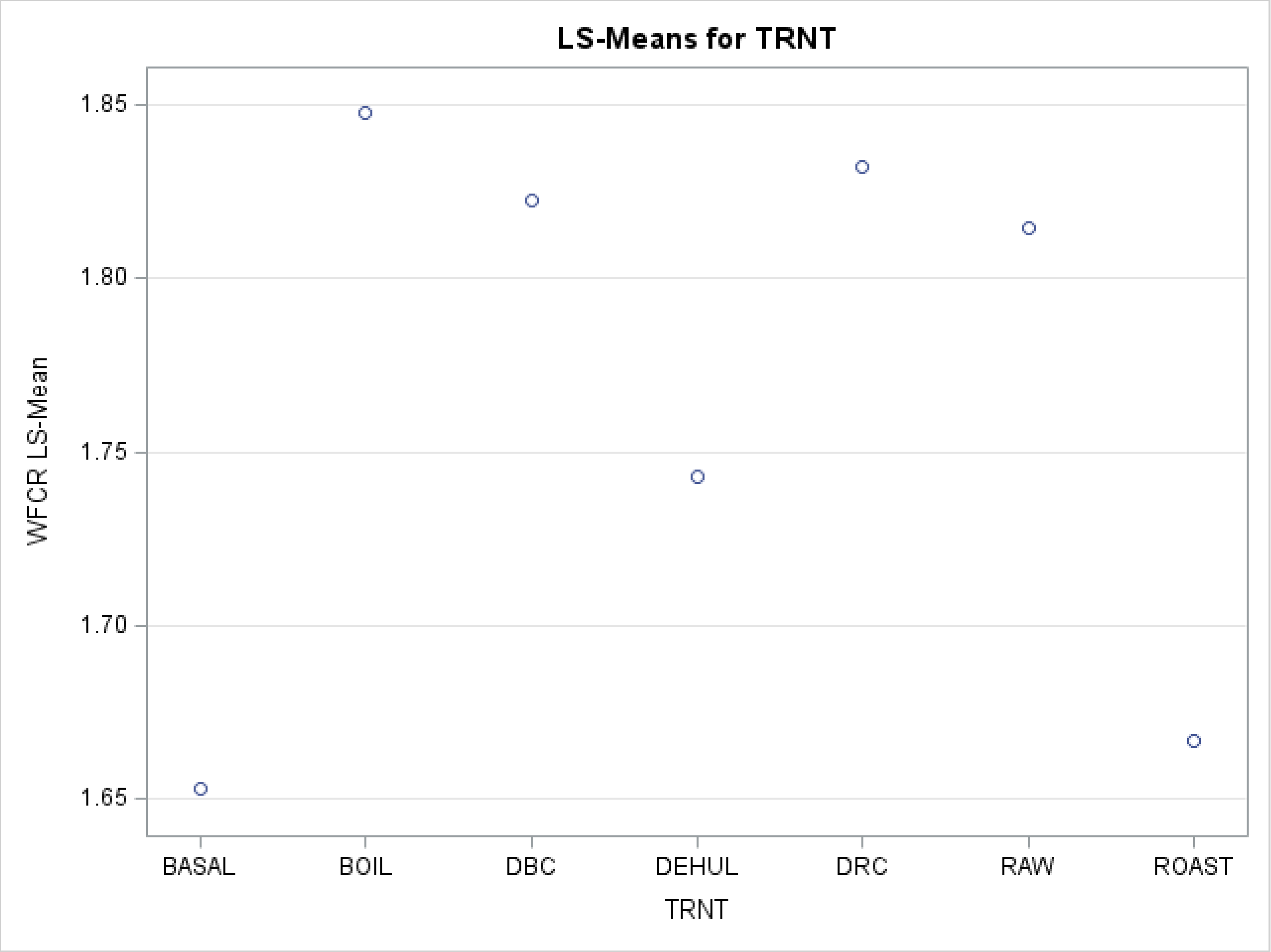
TRNT	WFCR LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
BASAL	1.65277778	0.07724304	<.0001	1
BOIL	1.84777778	0.07724304	<.0001	2
DBC	1.82222222	0.07724304	<.0001	3
DEHUL	1.74277778	0.07724304	<.0001	4
DRC	1.83222222	0.07724304	<.0001	5
RAW	1.81444444	0.07724304	<.0001	6
ROAST	1.66666667	0.07724304	<.0001	7

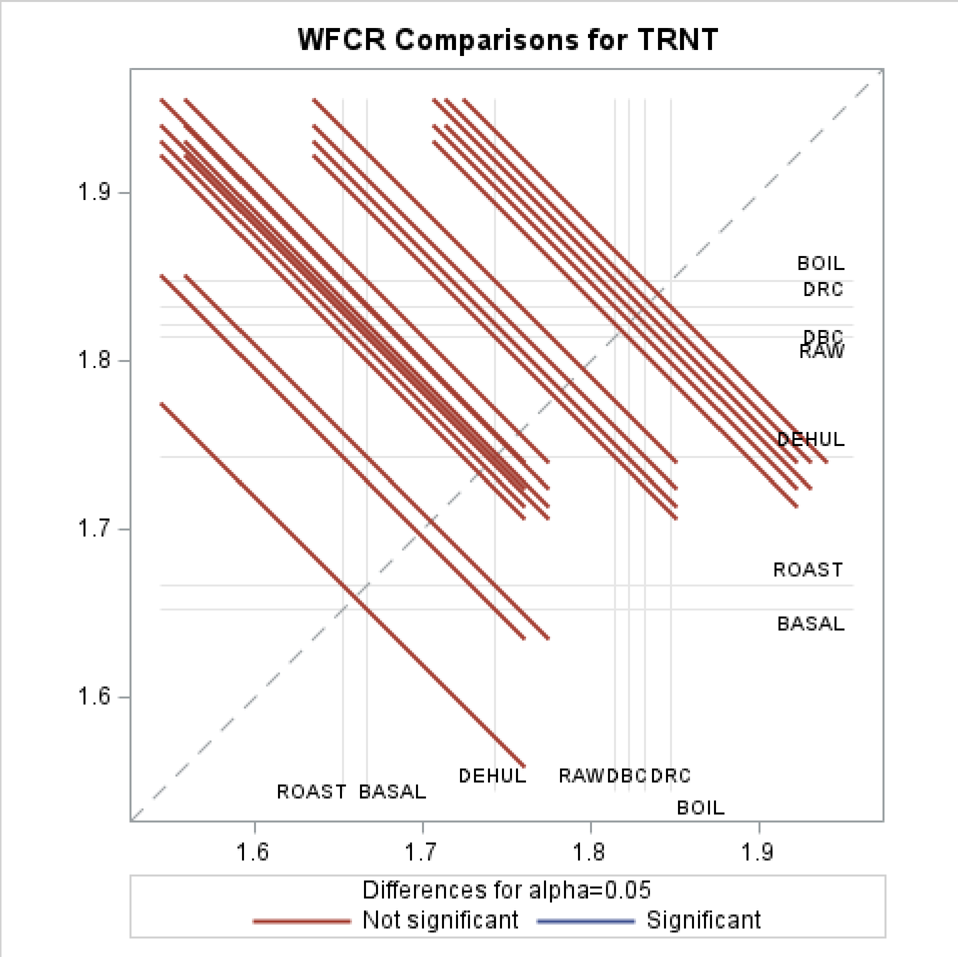
Least Squares Means for effect TRNT
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: WFCR

i/j	1	2	3	4	5	6	7
1		0.0769	0.1237	0.4117	0.1032	0.1417	0.8991
2	0.0769		0.8155	0.3385	0.8870	0.7608	0.1001

Least Squares Means for effect TRNT
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: WFCR

i/j	1	2	3	4	5	6	7
3	0.1237	0.8155		0.4686	0.9272	0.9434	0.1572
4	0.4117	0.3385	0.4686		0.4146	0.5131	0.4874
5	0.1032	0.8870	0.9272	0.4146		0.8710	0.1324
6	0.1417	0.7608	0.9434	0.5131	0.8710		0.1788
7	0.8991	0.1001	0.1572	0.4874	0.1324	0.1788	





Note: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

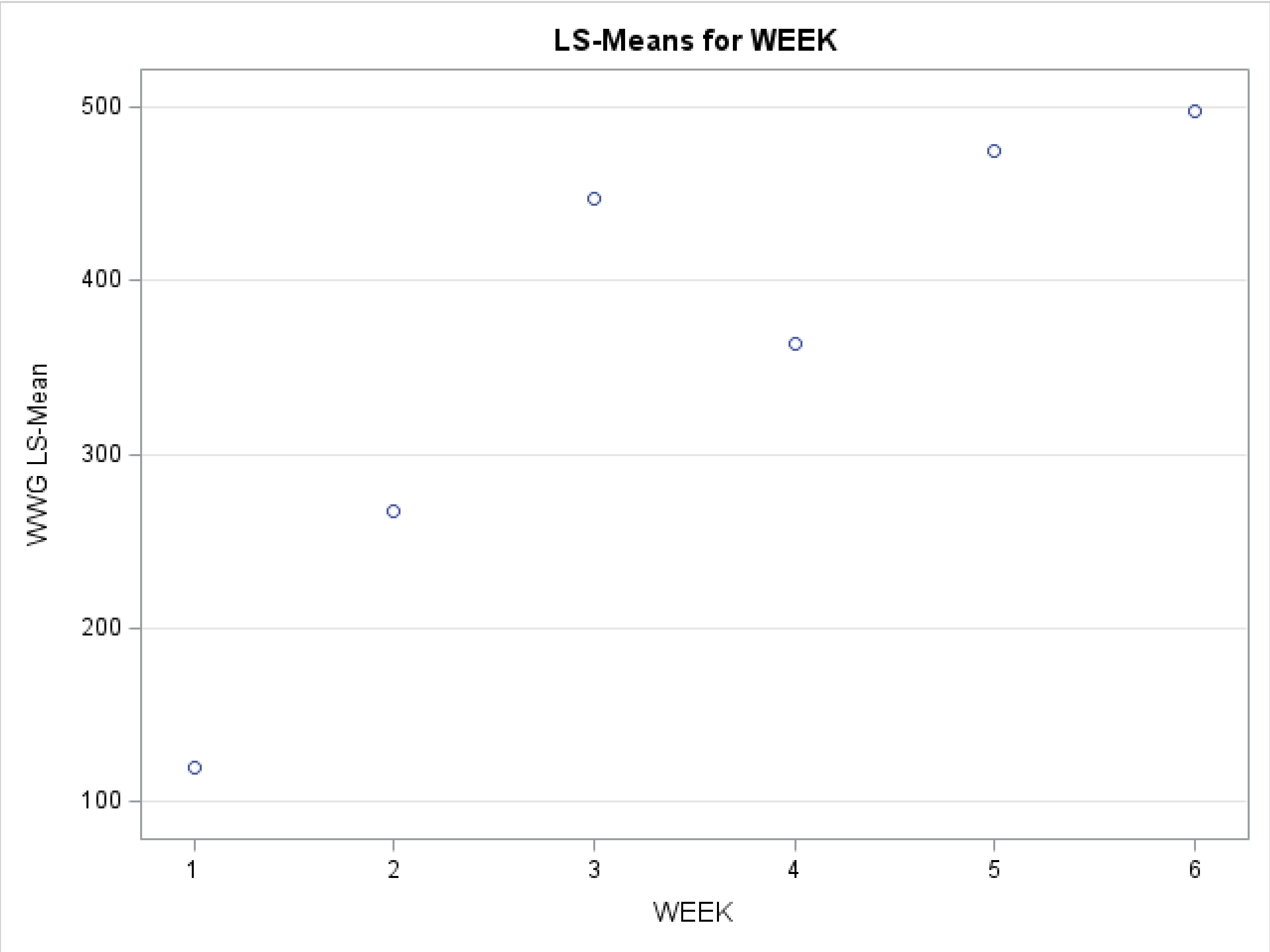
WEEK	WWG LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
1	119.528571	12.795184	<.0001	1
2	266.819048	12.795184	<.0001	2
3	446.809524	12.795184	<.0001	3
4	363.761905	12.795184	<.0001	4
5	474.190476	12.795184	<.0001	5
6	497.285714	12.795184	<.0001	6

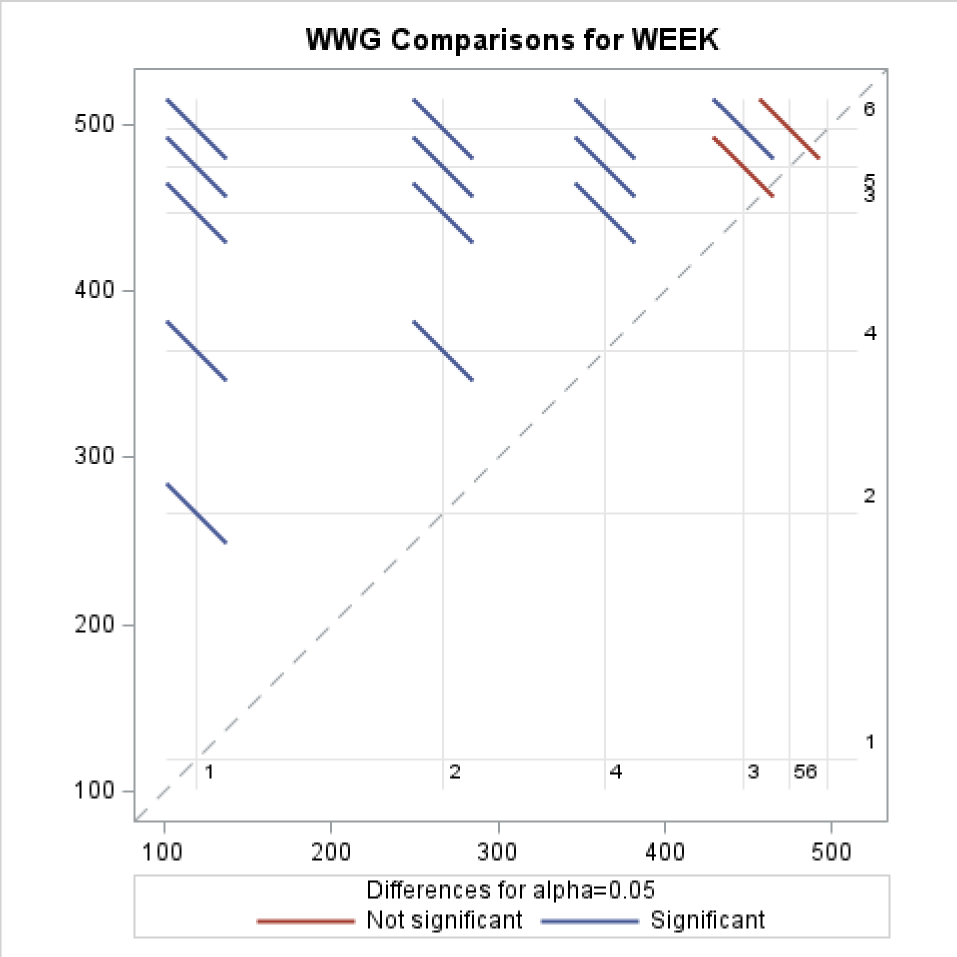
Least Squares Means for effect WEEK
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: WWG

i/j	1	2	3	4	5	6
1		<.0001	<.0001	<.0001	<.0001	<.0001

Least Squares Means for effect WEEK
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: WWG

i/j	1	2	3	4	5	6
2	<.0001		<.0001	<.0001	<.0001	<.0001
3	<.0001	<.0001		<.0001	0.1330	0.0062
4	<.0001	<.0001	<.0001		<.0001	<.0001
5	<.0001	<.0001	0.1330	<.0001		0.2045
6	<.0001	<.0001	0.0062	<.0001	0.2045	





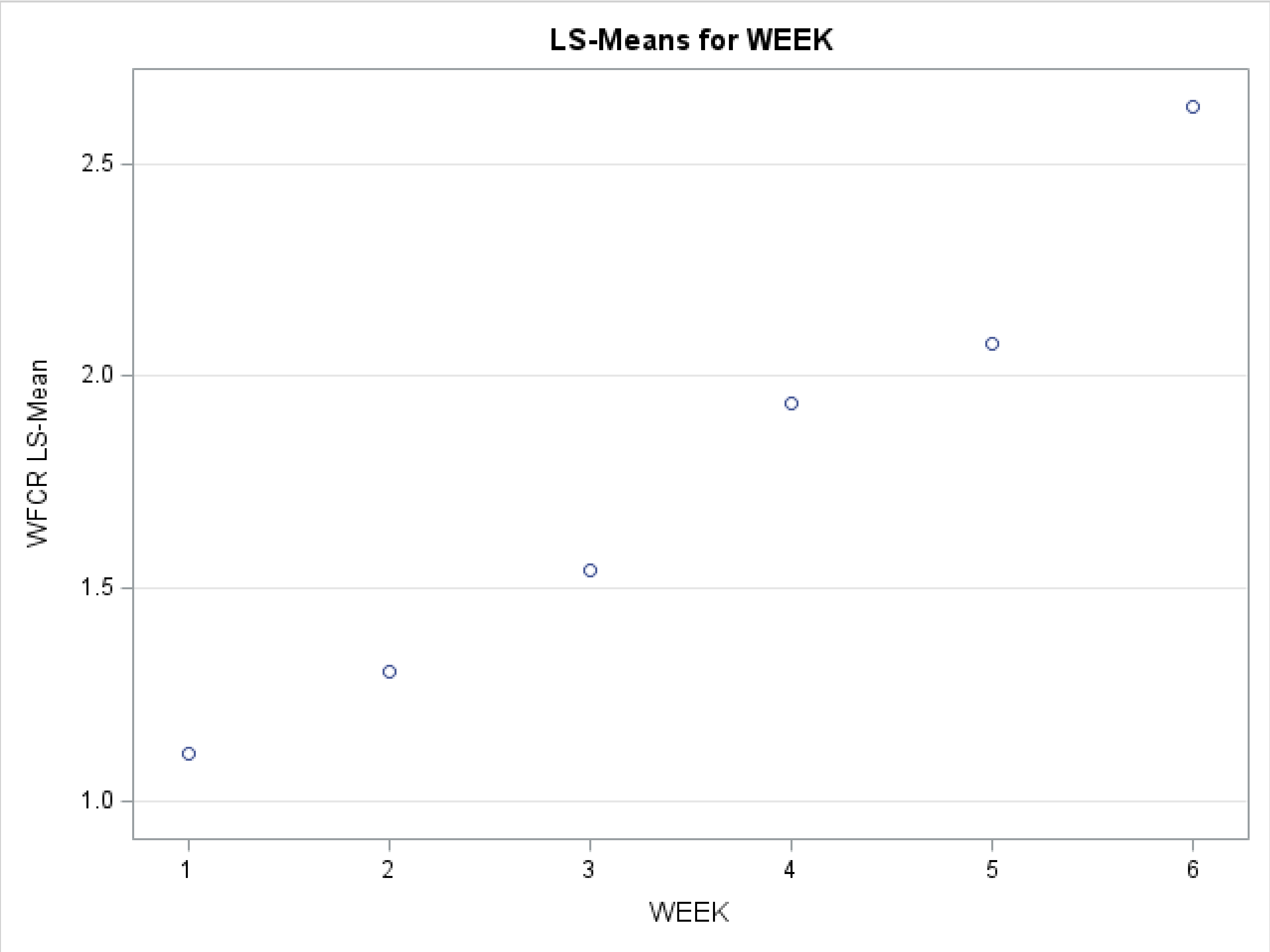
WEEK	WFCR LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
1	1.11428571	0.07151316	<.0001	1
2	1.30428571	0.07151316	<.0001	2
3	1.54380952	0.07151316	<.0001	3
4	1.93523810	0.07151316	<.0001	4
5	2.07857143	0.07151316	<.0001	5
6	2.63428571	0.07151316	<.0001	6

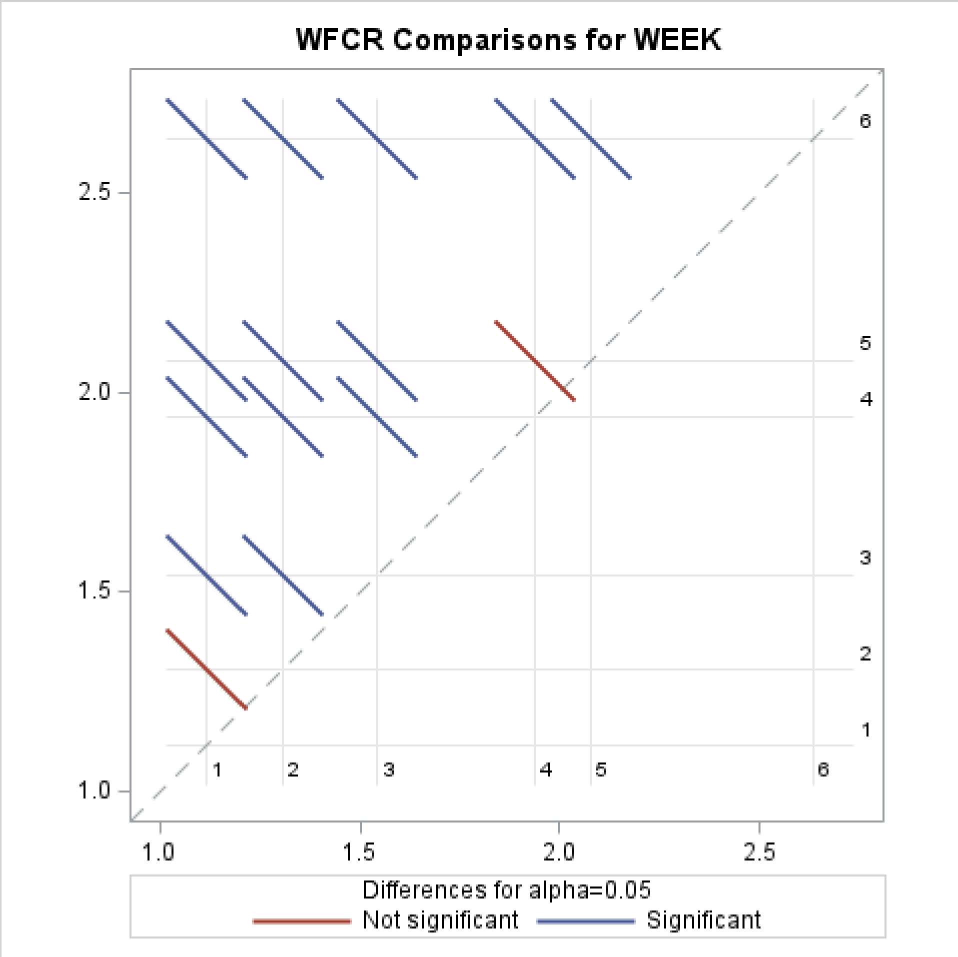
Least Squares Means for effect WEEK
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: WFCR

i/j	1	2	3	4	5	6
1		0.0629	<.0001	<.0001	<.0001	<.0001
2	0.0629		0.0196	<.0001	<.0001	<.0001
3	<.0001	0.0196		0.0002	<.0001	<.0001

Least Squares Means for effect WEEK
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: WFCR

i/j	1	2	3	4	5	6
4	<.0001	<.0001	0.0002		0.1592	<.0001
5	<.0001	<.0001	<.0001	0.1592		<.0001
6	<.0001	<.0001	<.0001	<.0001	<.0001	





Note: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

Appendix V: TPD Analysed Data

The SAS System

The GLM Procedure

Dependent Variable: TPD

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	62.30652381	7.78831548	4.34	0.0115
Error	12	21.52457143	1.79371429		
Corrected Total	20	83.83109524			

R-Square	Coeff Var	Root MSE	TPD Mean
0.743239	1.538722	1.339296	87.03952

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRNT	6	60.87402857	10.14567143	5.66	0.0054
REP	2	1.43249524	0.71624762	0.40	0.6794

Appendix VI: Meat Quality and Sensory Attributes Analysed Data

Genstat Release 18.1 (PC/Windows 8) 20 September 2014 20:26:19
Copyright 2015, VSN International Ltd.
Registered to: ICARDA

Genstat Eighteenth Edition
Genstat Procedure Library Release PL26.1

```
1  SET [WORKINGDIRECTORY='C:/Users/G-8/Documents']
2  "Data taken from file: 'C:/Users/G-8/Desktop/E Bumhira Data.xlsx'"
3  DELETE [REDEFINE=yes] _stitle_: TEXT _stitle_
4  READ [PRINT=*; SETNVALUES=yes] _stitle_
8  PRINT [IPRINT=*] _stitle_; JUST=left
```

Data imported from Excel file: C:\Users\G-8\Desktop\E Bumhira Data.xlsx
on: 20-Sep-2014 20:26:59
taken from sheet "Meat Quality", cells A2:J22

```
   9  DELETE [REDEFINE=yes]
Treatment,Replicate,WHC,pH,Prox_Composition,Colour,\
  10  Texture,Aroma,Tenderness,Taste
  11  UNITS [NVALUES=*]
  12  FACTOR [MODIFY=no; NVALUES=21; LEVELS=7;
LABELS=!t('MS','MSBC','MSDBC',\
  13  'MSDc','MSDRC','MSRC','MSUC'); REFERENCE=1] Treatment
  14  READ Treatment; FREPRESENTATION=ordinal
```

Identifier	Values	Missing	Levels
Treatment	21	0	7

```
  16  FACTOR [MODIFY=no; NVALUES=21; LEVELS=3; LABELS=*; REFERENCE=1]
Replicate
  17  READ Replicate; FREPRESENTATION=ordinal
```

Identifier	Values	Missing	Levels
Replicate	21	0	3

```
  19  VARIATE [NVALUES=21] WHC
  20  READ WHC
```

Identifier	Minimum	Mean	Maximum	Values	Missing
WHC	61.50	68.20	74.10	21	0

```
  23  VARIATE [NVALUES=21] pH
  24  READ pH
```

Identifier	Minimum	Mean	Maximum	Values	Missing	
pH	5.100	8.519	53.00	21	0	Skew

```
  27  VARIATE [NVALUES=21] Prox_Composition
  28  READ Prox_Composition
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Prox_Composition	73.60	75.81	77.80	21	0

```
  31  VARIATE [NVALUES=21] Colour
  32  READ Colour
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Colour	2.000	3.524	5.000	21	0

```
  34  VARIATE [NVALUES=21] Texture
  35  READ Texture
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Texture	2.000	3.571	5.000	21	0

```
  37  VARIATE [NVALUES=21] Aroma
  38  READ Aroma
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Aroma	2.000	3.762	5.000	21	0

```
  40  VARIATE [NVALUES=21] Tenderness
  41  READ Tenderness
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Tenderness	2.000	3.571	5.000	21	0

```
  43  VARIATE [NVALUES=21] Taste
  44  READ Taste
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Taste	3.000	4.190	5.000	21	0

```
  46
  47  %PostMessage 1129; 0; 100001 "Sheet Update Completed"
  48  "General Analysis of Variance"
  49  BLOCK Replicate
  50  TREATMENTS Treatment
  51  COVARIATE "No Covariate"
  52  ANOVA [PRINT=aovtable,information,means,%cv; FACT=32; CONTRASTS=7;
PCONTRASTS=7; FPROB=yes;\
  53   PSE=diff,lsd,means; LSDLEVEL=5] Aroma
```

Analysis of variance

Variate: Aroma

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	6	9.1429	1.5238	2.78	0.062
Residual	12	6.5714	0.5476		
Total	20	15.8095			

Tables of means

Variate: Aroma

Grand mean 3.76

Treatment	MS	MSBC	MSDBC	MSDc	MSDRC	MSRC	MSUC
	3.00	4.67	4.67	4.00	3.67	3.33	3.00

Standard errors of means

Table	Treatment
d.f.	12
e.s.e.	0.427

Standard errors of differences of means

Table	Treatment
d.f.	12
s.e.d.	0.604

Least significant differences of means (5% level)

Table	Treatment
d.f.	12
l.s.d.	1.316

Stratum standard errors and coefficients of variation

Variate: Aroma

Stratum	d.f.	s.e.	cv%
Replicate	2	0.082	2.2
Replicate.*Units*	12	0.740	19.7

```
  54  ADISPLAY [PRINT=aovtable,mean,%cv; FPROB=yes; PSE=lsd,means;
LSDLEVEL=5]
```

Analysis of variance

Variate: Aroma

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	6	9.1429	1.5238	2.78	0.062
Residual	12	6.5714	0.5476		
Total	20	15.8095			

Tables of means

Variate: Aroma

Grand mean 3.76

Treatment	MS	MSBC	MSDBC	MSDc	MSDRC	MSRC	MSUC
	3.00	4.67	4.67	4.00	3.67	3.33	3.00

Standard errors of means

Table	Treatment
d.f.	12
e.s.e.	0.427

Least significant differences of means (5% level)

Table	Treatment
d.f.	12
l.s.d.	1.316

Stratum standard errors and coefficients of variation

Variate: Aroma

Stratum	d.f.	s.e.	cv%
Replicate	2	0.082	2.2
.*Units*	12	0.740	19.7

```
"Data taken from file: 'C:/Users/G-8/Desktop/E Bumhira Data.xlsx'"
DELETE [REDEFINE=yes] _stitle_: TEXT _stitle_
READ [PRINT=*; SETNVALUES=yes] _stitle_
PRINT [IPRINT=*] _stitle_; JUST=left
```

Data imported from Excel file: C:\Users\G-8\Desktop\E Bumhira Data.xlsx
on: 20-Sep-2014 20:29:57
taken from sheet "Weekly feed C and FCR", cells A2:E127

```
DELETE [REDEFINE=yes] Treatment,Replicate,Week,Weekly_Weight_Gain_g,\
Weekly_FCR
UNITS [NVALUES=*]
TEXT [NVALUES=126] Treatment
READ Treatment
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Treatment				126	119

```
VARIATE [NVALUES=126] Replicate
READ Replicate
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Replicate	1.000	2.000	3.000	126	0

```
VARIATE [NVALUES=126] Week
READ Week
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Week	1.000	3.500	6.000	126	0

```
VARIATE [NVALUES=126] Weekly_Weight_Gain_g
READ Weekly_Weight_Gain_g
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Weekly_Weight_Gain_g	115.0	361.4	625.0	126	0

```
VARIATE [NVALUES=126] Weekly_FCR
READ Weekly_FCR
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Weekly_FCR	1.110	1.768	3.510	126	0

```

%PostMessage 1129; 0; 100002 "Sheet Update Completed"
"Data taken from file: 'C:/Users/G-8/Desktop/E Bumhira Data.xlsx'"
DELETE [REDEFINE=yes] _stitle_: TEXT _stitle_
READ [PRINT=*; SETNVALUES=yes] _stitle_
PRINT [IPRINT=*] _stitle_; JUST=left
```

Data imported from Excel file: C:\Users\G-8\Desktop\E Bumhira Data.xlsx
on: 20-Sep-2014 20:30:39
taken from sheet "Meat Quality", cells A2:J22

```
DELETE [REDEFINE=yes] Treatment,Replicate
UNITS [NVALUES=*]
FACTOR [MODIFY=no; NVALUES=21; LEVELS=7;
LABELS=!t('MS','MSBC','MSDBC',\
'MSDc','MSDRC','MSRC','MSUC'); REFERENCE=1] Treatment
READ Treatment; FREPRESENTATION=ordinal
```

Identifier	Values	Missing	Levels
Treatment	21	0	7

```
FACTOR [MODIFY=no; NVALUES=21; LEVELS=3; LABELS=*; REFERENCE=1]
Replicate
READ Replicate; FREPRESENTATION=ordinal
```

Identifier	Values	Missing	Levels
Replicate	21	0	3

```
124  VARIATE [NVALUES=21] WHC
125  READ WHC
```

Identifier	Minimum	Mean	Maximum	Values	Missing
WHC	61.50	68.20	74.10	21	0

```
128  VARIATE [NVALUES=21] pH
129  READ pH
```

Identifier	Minimum	Mean	Maximum	Values	Missing	
pH	5.100	8.519	53.00	21	0	Skew

```
132  VARIATE [NVALUES=21] Prox_Composition
133  READ Prox_Composition
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Prox_Composition	73.60	75.81	77.80	21	0

```
136  VARIATE [NVALUES=21] Colour
137  READ Colour
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Colour	2.000	3.524	5.000	21	0

```
139  VARIATE [NVALUES=21] Texture
140  READ Texture
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Texture	2.000	3.571	5.000	21	0

```
142  VARIATE [NVALUES=21] Aroma
143  READ Aroma
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Aroma	2.000	3.762	5.000	21	0

```
145  VARIATE [NVALUES=21] Tenderness
146  READ Tenderness
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Tenderness	2.000	3.571	5.000	21	0

```
148  VARIATE [NVALUES=21] Taste
149  READ Taste
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Taste	3.000	4.190	5.000	21	0

```
151
152  %PostMessage 1129; 0; 100003 "Sheet Update Completed"
153  ADISPLAY [PRINT=aovtable,mean,%cv; FPROB=yes; PSE=lsd,means;
LSDLEVEL=5]
```

Analysis of variance

Variate: Aroma

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	6	9.1429	1.5238	2.78	0.062
Residual	12	6.5714	0.5476		
Total	20	15.8095			

Tables of means

Variate: Aroma

Grand mean 3.76

Treatment	MS	MSBC	MSDBC	MSDc	MSDRC	MSRC	MSUC
	3.00	4.67	4.67	4.00	3.67	3.33	3.00

Standard errors of means

Table	Treatment
d.f.	12
e.s.e.	0.427

Least significant differences of means (5% level)

Table	Treatment
d.f.	12
l.s.d.	1.316

Stratum standard errors and coefficients of variation

Variate: Aroma

Stratum	d.f.	s.e.	cv%
Replicate	2	0.082	2.2
Replicate.*Units*	12	0.740	19.7

```
"General Analysis of Variance"
BLOCK Replicate
TREATMENTS Treatment
COVARIATE "No Covariate"
ANOVA [PRINT=aovtable,information,means,%cv; FACT=32; CONTRASTS=7;
PCONTRASTS=7; FPROB=yes;\
 PSE=diff,lsd,means; LSDLEVEL=5] Colour
```

Analysis of variance

Variate: Colour

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	6	11.9048	1.9841	8.93	<.001
Residual	12	2.6667	0.2222		
Total	20	15.2381			

Message: the following units have large residuals.

Replicate 1 *units* 3	-0.86	s.e.	0.36

Tables of means

Variate: Colour

Grand mean 3.52

Treatment	MS	MSBC	MSDBC	MSDc	MSDRC	MSRC	MSUC
	3.33	4.67	3.67	4.00	3.67	3.33	2.00

Standard errors of means

Table	Treatment
d.f.	12
e.s.e.	0.272

Standard errors of differences of means

Table	Treatment
d.f.	12
s.e.d.	0.385

Least significant differences of means (5% level)

Table	Treatment
d.f.	12
l.s.d.	0.839

Stratum standard errors and coefficients of variation

Variate: Colour

Stratum	d.f.	s.e.	cv%
Replicate	2	0.218	6.2

Replicate.*Units*	12	0.471	13.4

```
"General Analysis of Variance"
BLOCK Replicate
TREATMENTS Treatment
COVARIATE "No Covariate"
ANOVA [PRINT=aovtable,information,means,%cv; FACT=32; CONTRASTS=7;
PCONTRASTS=7; FPROB=yes;\
 PSE=diff,lsd,means; LSDLEVEL=5] Prox_Composition
```

Analysis of variance

Variate: Prox_Composition

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	6	28.1924	4.6987	22.67	<.001
Residual	12	2.4876	0.2073		
Total	20	30.9457			

Message: the following units have large residuals.

Replicate 2 *units* 1	0.790	s.e.	0.344

Tables of means

Variate: Prox_Composition

Grand mean 75.814

Treatment	MS	MSBC	MSDBC	MSDc	MSDRC	MSRC	MSUC
	75.367	75.400	76.300	76.900	77.800	74.333	74.600

Standard errors of means

Table	Treatment
d.f.	12
e.s.e.	0.2629

Standard errors of differences of means

Table	Treatment
d.f.	12
s.e.d.	0.3718

Least significant differences of means (5% level)

Table	Treatment
d.f.	12
l.s.d.	0.8100

Stratum standard errors and coefficients of variation

Variate: Prox_Composition

Stratum	d.f.	s.e.	cv%
Replicate	2	0.1378	0.2

Replicate.*Units*	12	0.4553	0.6

```
"General Analysis of Variance"
BLOCK Replicate
TREATMENTS Treatment
COVARIATE "No Covariate"
ANOVA [PRINT=aovtable,information,means,%cv; FACT=32; CONTRASTS=7;
PCONTRASTS=7; FPROB=yes;\
 PSE=diff,lsd,means; LSDLEVEL=5] Taste
```

Analysis of variance

Variate: Taste

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	6	11.90476	1.98413	25.00	<.001
Residual	12	0.95238	0.07937		
Total	20	13.23810			

Message: the following units have large residuals.

Replicate 2 *units* 1	-0.476	s.e.	0.213
Replicate 2 *units* 3	-0.476	s.e.	0.213

Tables of means

Variate: Taste

Grand mean 4.190

Treatment	MS	MSBC	MSDBC	MSDc	MSDRC	MSRC	MSUC
	3.667	5.000	5.000	5.000	3.667	4.000	3.000

Standard errors of means

Table	Treatment
d.f.	12
e.s.e.	0.1627

Standard errors of differences of means

Table	Treatment
d.f.	12
s.e.d.	0.2300

Least significant differences of means (5% level)

Table	Treatment
d.f.	12
l.s.d.	0.5012

Stratum standard errors and coefficients of variation

Variate: Taste

Stratum	d.f.	s.e.	cv%
Replicate	2	0.1650	3.9
Replicate.*Units*	12	0.2817	6.7

```
"General Analysis of Variance"
BLOCK Replicate
TREATMENTS Treatment
COVARIATE "No Covariate"
ANOVA [PRINT=aovtable,information,means,%cv; FACT=32; CONTRASTS=7;
PCONTRASTS=7; FPROB=yes;\
 PSE=diff,lsd,means; LSDLEVEL=5] Tenderness
```

Analysis of variance

Variate: Tenderness

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	6	17.14286	2.85714		0.045
Residual	12	11.45	9.45		
Total	20	17.14286			

Tables of means

Variate: Tenderness

Grand mean 3.57

Treatment	MS	MSBC	MSDBC	MSDc	MSDRC	MSRC	MSUC
	4.00	4.00	4.00	5.00	3.00	3.00	2.00

Standard errors of means

Table	Treatment
d.f.	2
e.s.e.	0.000

Standard errors of differences of means

Table	Treatment
d.f.	2
s.e.d.	0.000

Least significant differences of means (5% level)

Table	Treatment
d.f.	2
l.s.d.	0.6

Stratum standard errors and coefficients of variation

Variate: Tenderness

Stratum	d.f.	s.e.	cv%
Replicate	2	0.34	14.0
Replicate.*Units*	12	0.23	15.0

```
"General Analysis of Variance"
BLOCK Replicate
TREATMENTS Treatment
COVARIATE "No Covariate"
ANOVA [PRINT=aovtable,information,means,%cv; FACT=32; CONTRASTS=7;
PCONTRASTS=7; FPROB=yes;\
 PSE=diff,lsd,means; LSDLEVEL=5] Texture
```

Analysis of variance

Variate: Texture

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	6	10.4762	1.7460	4.78	0.010
Residual	12	4.3810	0.3651		
Total	20	15.1429			

Tables of means

Variate: Texture

Grand mean 3.57

Treatment	MS	MSBC	MSDBC	MSDc	MSDRC	MSRC	MSUC
	3.33	4.67	4.33	3.67	3.33	3.33	2.33

Standard errors of means

Table	Treatment
d.f.	12
e.s.e.	0.349

Standard errors of differences of means

Table	Treatment
d.f.	12
s.e.d.	0.493

Least significant differences of means (5% level)

Table	Treatment
d.f.	12
l.s.d.	1.075

Stratum standard errors and coefficients of variation

Variate: Texture

Stratum	d.f.	s.e.	cv%
Replicate	2	0.143	4.0
Replicate.*Units*	12	0.604	16.9

```
"General Analysis of Variance"
BLOCK Replicate
TREATMENTS Treatment
COVARIATE "No Covariate"
ANOVA [PRINT=aovtable,information,means,%cv; FACT=32; CONTRASTS=7;
PCONTRASTS=7; FPROB=yes;\
 PSE=diff,lsd,means; LSDLEVEL=5] WHC
```

Analysis of variance

Variate: WHC

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	6	297.900	49.650		0.034
Residual	12	4.3810	0.3651		
Total	20	297.900			

Tables of means

Variate: WHC

Grand mean 68.20

Treatment	MS	MSBC	MSDBC	MSDc	MSDRC	MSRC	MSUC
	66.50	66.70	67.10	71.10	70.40	74.10	61.50

Standard errors of means

Table	Treatment
d.f.	2
e.s.e.	0.2

Standard errors of differences of means

Table	Treatment
d.f.	2
s.e.d.	0.23

Least significant differences of means (5% level)

Table	Treatment
d.f.	2
l.s.d.	0.3

Stratum standard errors and coefficients of variation

Variate: WHC

Stratum	d.f.	s.e.	cv%
Replicate	2	2	13.0
Replicate.*Units*	12	2	18.0

```
"General Analysis of Variance"
BLOCK Replicate
TREATMENTS Treatment
COVARIATE "No Covariate"
ANOVA [PRINT=aovtable,information,means,%cv; FACT=32; CONTRASTS=7;
PCONTRASTS=7; FPROB=yes;\
 PSE=diff,lsd,means; LSDLEVEL=5] pH
```

Analysis of variance

Variate: pH

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	6	566.0	94.3	0.87	0.542
Residual	12	1297.5	108.1		
Total	20	2079.8			

Message: the following units have large residuals.

Replicate 2 *units* 7	27.2	s.e.	7.9

Tables of means

Variate: pH

Grand mean 8.5

Treatment	MS	MSBC	MSDBC	MSDc	MSDRC	MSRC	MSUC
	6.3	6.3	6.4	6.4	6.4	6.6	21.2

Standard errors of means

Table	Treatment
d.f.	12
e.s.e.	6.00

Standard errors of differences of means

Table	Treatment
d.f.	12
s.e.d.	8.49

Least significant differences of means (5% level)

Table	Treatment
d.f.	12
l.s.d.	18.50

Stratum standard errors and coefficients of variation

Variate: pH

Stratum	d.f.	s.e.	cv%
Replicate	2	3.93	46.1
Replicate.*Units*	12	10.40	122.1