

**INCLUSION OF EXTRUDED SORGHUM [*Sorghum bicolor* (L.) Moench] MEAL
AND EXOGENOUS PHYTASE ENZYME ON THE PERFORMANCE OF BROILER
CHICKEN**

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for the Master of Science Degree in Animal Nutrition of Egerton University**

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DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has not been presented in this university or any other for the award of a degree.

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Recommendation

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ABSTRACT

Maize grain is the main energy source in broiler diets. However, its production is scarce and expensive, making commercial broiler chicken diets expensive and unavailable, which makes broiler production not sustainable. Sorghum [*Sorghum bicolor* (L.) Moench] grain is identified as an alternative to maize grain in broiler diets. Sorghum is locally available, underutilized, high-value, and drought-tolerant. However, the digestibility of sorghum grain as an alternative to maize in broiler diets is constrained by anti-nutritional factors like tannins, kafirins, and phytates. This study determined the effect of extrusion cooking variables on *in vitro* dry matter digestibility (IVDMD) of extruded sorghum meal (ESM), the inclusion of ESM and exogenous phytase enzyme in broiler diets on performance. The first experiment determined the effect of feed moisture content (40, 45, and 50%), screw speed (280 and 300 rpm), and barrel temperature (70 and 90°C) on the IVDMD of ESM. The second experiment determined the effect of inclusion levels (0, 50, and 100%) of ESM as an energy source with or without (0 and 0.035%) exogenous phytase enzyme on broiler performance. The study used 108 mixed-sex Cobb 500® day-old broiler chicks. The chicks were weighed, grouped into six, assigned cages and each cage randomly assigned to one of the six dietary treatments. The treatments were: T1 (0% ESM + 0% phytase), T2 (0 % ESM + 0.035 % phytase), T3 (50 % ESM + 0 % phytase), T4 (50 % ESM + 0.035 % phytase), T5 (100 % ESM + 0 % phytase), and T6 (100 % ESM + 0.035 % phytase). The grower and finisher diets were offered from days 1-21 and 22-42, respectively. Weekly average daily feed intake (ADFI), average daily gain (ADG), and feed conversion ratio (FCR) were measured. The third experiment determined the carcass characteristics and descriptive sensory quality using three randomly sampled broilers per treatment after day 42. Data were subjected to analysis of variance in a completely randomized design using the general linear model procedure of the SAS Institute Inc. (version 9.4; 2015). Mean separation was done using Tukey's HSD tests at a 0.05 level of significance. A reduction in the feed moisture content and an increase in the screw speed and barrel temperature significantly increased the IVDMD of ESM. In the grower phase, inclusion of ESM up to 50% had no significant effect on the feed conversion ratio, while the exogenous phytase enzyme improved average daily feed intake and average daily gain. Inclusion of ESM up to 50% with exogenous phytase enzyme did not affect average daily feed intake, average daily gain, feed conversion ratio, carcass characteristics and descriptive sensory quality. From the results, it was concluded that inclusion of ESM up to 50% with exogenous phytase enzyme did not adversely affect average daily feed intake, average daily gain, feed conversion ratio, carcass characteristics and descriptive sensory quality of broiler chicken.

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LIST OF ABBREVIATIONS AND ACRONYMS

ADFI	Average Daily Feed Intake
ADG	Average Daily Gain
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
ESM	Extruded Sorghum Meal
FAO	Food and Agriculture Organization of the United Nations
FCR	Feed conversion ratio
GDP	Gross Domestic Product
GMP	Good Manufacturing Practices
IVDMD	<i>In vitro</i> Dry Matter Digestibility
NRC	National Research Council
PVP	Polyvinylpyrrolidone
RPM	Revolutions Per Minute
SDG	United Nations Sustainable Development Goal

CHAPTER ONE

INTRODUCTION

1.1 Background Information

The population of Kenya is projected to increase from 55 million in 2023 to 85 million by 2050. At the same time, the urban population will increase from 16.9 million to 44.2 million people (Food and Agriculture Organization of the United Nations [FAO], 2023). The demand for food of animal origin, especially meat, is expected to increase alongside the human population, urbanization, and disposable income (D'Amour et al., 2020).

Households in Kenya spend about 20% of their income on purchasing food and approximately 6% on poultry meat (Kenya Markets Trust [KMT], 2019). Due to the increase in consumer income and growing health concerns, preference is shifting from red meat to white meat (KMT, 2019). These consumption patterns, combined with an increased human population and urbanization, are expected to drive the demand for chicken meat.

The ability of Kenya's broiler production to meet demand is constrained by seasonal shortages and the high cost of primary feed ingredients (Njagi, 2022; Njoroge et al., 2015). Maize meal is commonly used as the main feed ingredient in commercial broiler chicken diets, yet it remains the staple food, creating severe competition between livestock feed and human food. Developed countries demand maize grain for biofuel production, which increases its global market price (Cisse et al., 2017). The availability of maize grain is constrained by biotic and abiotic factors such as drought, high temperatures, poor soil fertility, waterlogging/excess moisture, and susceptibility to new diseases and insect pests caused by global climate change (Shiferaw et al., 2011). Maize lethal necrosis disease is an emerging threat to maize-based food security in sub-Saharan Africa. It was reported in Kenya in 2011 (Wangai et al., 2012), and in the next year, it led to an estimated grain loss of 126,000 metric tonnes (Mahuku et al., 2015). These challenges limit the ability of domestic maize production to meet the growing demand, which has led to an escalating overreliance on imported maize grain (Njagi, 2022). The prices of maize grain keep fluctuating (Adolwa et al., 2021; FAO, 2022). The prices in Kenya were 25-40% higher in April 2022 due to lower domestic cereal production in the previous year (FAO, 2022). As a result, commercial broiler chicken feeds are very costly and unavailable in times of shortage of maize grain. The tendency of broiler farmers in such circumstances is to downsize the flock size, which hinders broiler production in the country (Njoroge et al., 2015). The focus has shifted to alternative energy sources in broiler diets to sustain broiler production amidst supply chain uncertainties (Cisse et al., 2017).

Sorghum [*Sorghum bicolor* (L.) Moench] grain is an alternative to maize grain in broiler chicken diets. Sorghum is more tolerant to drought than other cereal crops (Orr et al., 2016), considering that over 80% of Kenya's landmass is classified as arid and semi-arid (ASAL). The nutritional composition of sorghum grain is similar to that of maize grain. Compared to maize grain, sorghum grain has a crude protein of 9.0% vs. 8.5% and metabolizable energy of 3250 kcal/kg vs. 3330 kcal/kg, respectively (Leeson & Summers, 2005). However, the nutrient digestibility of sorghum grain is lower in comparison with maize grain. The low nutrient digestibility is due to the tannins, kafirins, and phytates in the grain (Sohail et al., 2019).

Enhancement of nutrient digestibility prevents environmental pollution by reducing the excretion of nutrients by animals (FAO, 2011). Various feed processing methods, such as extrusion cooking, are used to improve the digestibility of livestock feeds. Extrusion cooking is a high-temperature, relatively short-time method that combines moisture, pressure, temperature, and shearing impact to plasticize and cook starchy food materials (Navale et al., 2015). Moist-heat processing increases disulphide cross-linking of kafirin proteins inside the endosperm and causes conformational changes in β and γ -kafirin, which is detrimental to the digestibility of sorghum grain (Emmambux & Taylor, 2009; Selle et al., 2010b). Thus, moist-heat processing of sorghum grain is associated with suboptimal growth performance in broiler chicken. It is hypothesized that low-moisture and high-temperature extrusion cooking of sorghum flour and the subsequent addition of exogenous phytase enzyme improve the digestibility of sorghum in broiler chicken.

Therefore, this study determined the effect of feed moisture content, screw speed and barrel temperature on IVDMD of extruded sorghum meal and its potential as an energy source with or without exogenous phytase enzyme on broiler performance, carcass characteristics and descriptive sensory quality.

1.2 Statement of the Problem

Sorghum grain is identified as an alternative to maize grain in broiler diets, but its digestibility is compromised by the content of anti-nutritional factors like tannins, kafirins, and phytates. When high-tannin sorghum grain (containing 15.5 to 56.3 mg catechin equivalents g⁻¹ dry matter basis) is used in broiler diets without mitigating these anti-nutritional factors, it adversely affects broiler performance. Extrusion cooking has the potential to improve the digestibility of sorghum. However, the effect of feed moisture content, screw speed, and barrel temperature on the digestibility of extruded sorghum meal is not clear. Therefore, this study determined the effect of feed moisture content, screw speed, and barrel temperature on IVDMD

of extruded sorghum meal and the effect of inclusion levels of extruded sorghum meal with or without exogenous phytase enzyme in the diet on broiler chicken performance, carcass characteristics, and sensory quality.

1.3 Objectives

1.3.1 Broad Objective

To contribute to food and nutrition security through sustainable broiler production using extruded sorghum meal and exogenous phytase enzyme in broiler diets.

1.3.2 Specific Objective

- i. To determine the effect of feed moisture content, screw speed, and barrel temperature on the *in vitro* dry matter digestibility of extruded sorghum meal.
- ii. To determine the effect of inclusion levels of extruded sorghum meal as an energy source with or without exogenous phytase enzyme on the average daily feed intake, average daily gain and feed conversion ratio of broiler chicken.
- iii. To determine the effect of inclusion levels of extruded sorghum meal as an energy source with or without exogenous phytase enzyme on broiler carcass characteristics (internal and external parts) and descriptive sensory quality.

1.4 Hypotheses

- i. Feed moisture content, screw speed, and barrel temperature have no significant effect on the *in vitro* dry matter digestibility of extruded sorghum meal.
- ii. Inclusion levels of extruded sorghum meal as an energy source with or without exogenous phytase enzyme have no significant effect on the average daily feed intake, average daily gain and feed conversion ratio of broiler chicken.
- iii. Inclusion levels of extruded sorghum meal as an energy source with or without exogenous phytase enzyme have no significant effect on broiler carcass characteristics (internal and external parts) and descriptive sensory quality.

1.5 Justification

Maize production has remained low due to the high cost of inputs, drought, high temperatures, poor soil fertility, waterlogging/excess moisture, and new diseases and insect pests (Shiferaw et al., 2011). The accelerating rate of climate change has also worsened the situation. Therefore, the cost of maize grain as a primary feed ingredient in poultry diets is high, which increases the cost of poultry production. Sorghum grain is regarded as an alternative to maize grain in poultry diets. Sorghum does well in regions where water

availability can hinder adequate maize production. Sorghum copes well with dry conditions and is more resistant to insect and mould attacks. Its roots grow deeper in the soil than those of maize (Orr et al., 2016). Sorghum rolls its leaves to reduce leaf area, minimizing transpiration water loss. The crop stays green and competes well with most weeds (MoALFC, 2020). The nutritional composition of sorghum grain is similar to that of maize grain. The crude protein of sorghum and maize grain is 9.0% and 8.5%, while the metabolizable energy is 3250 kcal/kg and 3330 kcal/kg, respectively. However, the nutritive value of sorghum grain is considered 5% lower compared to maize grain (Leeson & Summers, 2005). The digestibility is hindered by tannins, kafirins, and phytates in the grain. Extrusion cooking is used to mitigate these anti-nutritive factors. This study determined the effect of feed moisture content, screw speed, and barrel temperature on IVDMD of extruded sorghum meal. The effect of inclusion levels of extruded sorghum meal as an energy source with or without exogenous phytase enzyme on the performance of broiler chicken was also determined. The knowledge generated from this study will guide the utilization of extruded sorghum meal and exogenous phytase enzyme in the formulation of broiler diets, thereby contributing to sustainable broiler production. This study contributed to SDG 2, which aspires to have zero hunger, achieve food security, improve nutrition, and promote sustainable agriculture.

1.6 Definition of Terms

Extrusion cooking is an advanced technology that uses a high-temperature, relatively short-time mechanism to combine moisture, pressure, temperature, and shearing impact to plasticize and cook starchy and/or proteinaceous food materials, resulting in molecular transformation and chemical reactions (Navale et al., 2015).

Extruded sorghum meal refers to the milled end-product of the extrusion cooking of sorghum flour.

Inclusion level refers to the proportion of extruded sorghum meal included in the diet as an energy source.

Kafirins are the storage proteins of sorghum and are found in protein bodies in the seed endosperm. They are reclassified into four groups (α , β , γ , and δ) based on solubility properties, electrophoretic mobility, and nucleotide sequences (Shull et al., 1992). They are discussed in detail in section 2.8.2.

CHAPTER TWO

LITERATURE REVIEW

2.1 The Poultry Industry in Kenya

The agricultural sector directly contributes an estimated 33% of Kenya's total gross domestic product (GDP) and another 27% via linkages with other sectors such as manufacturing, distribution and services (MoALFI, 2019). The poultry sub-sector contributes about 30% of the agricultural GDP. Poultry production is widespread in Kenya, and 65% of households keep poultry (Omiti & Okuthe, 2009). The number of chickens in Kenya is estimated at 43,796,477, of which 83% are indigenous chickens and 17% are exotic broilers, layers, dual-purpose hybrids, and other poultry species (MoALFC, 2019). Indigenous chicken is predominant in rural areas, while broilers and layers are found in peri-urban areas (Magothe et al., 2012). The main breeds of commercial layer chicken are Isa Brown and Ross broiler, whereas broiler chicken breeds are Arbor Acres, Hybro, Cobb, and Hypeco (Omiti & Okuthe, 2009). The poultry industry is a source of employment for an estimated two million Kenyans (Omiti & Okuthe, 2009). Poultry eggs and meat provide the high-quality animal protein required for human nutrition (Magothe et al., 2012; Omiti & Okuthe, 2009). Poultry production generates income for many households from the sale of eggs, chicken meat, and live chickens. Poultry also plays sociocultural roles, such as cockerel fighting among some communities (Omiti & Okuthe, 2009).

2.2 Poultry Production Systems in Kenya

The three production systems used in poultry rearing in Kenya are free-range (extensive), semi-intensive, and intensive commercial systems. The choice of production system depends on household land availability and the objective of the enterprise (Magothe et al., 2012).

2.2.1 Free-Range System (extensive)

In this system, poultry can scavenge freely for feed around the homestead in a low-input low-output system. Flock sizes of 5 to 30 local chickens are kept, mainly under the management of women and children. The adoption of technology is limited in this system. It is commonly practised in the Western region, Lower Eastern region, North Rift areas, and coastal areas of Kenya (FAO, 2017).

2.2.2 Semi-Intensive System

The semi-intensive system involves a larger poultry flock size of 30 to 100, usually confined using simple structures. Indigenous and exotic poultry are reared in this system. There

is a deliberate use of feed supplements in this system. Most of the poultry are sold in the market, while the rest are slaughtered for meat at the household level. This system is widespread across the country (FAO, 2017).

2.2.3 Intensive Commercial System

The intensive commercial system is common in urban and peri-urban areas of the country. The system utilizes less space with exotic chicken being predominant. The system is market-oriented and requires a high level of inputs, such as commercial feeds. Broiler chicken are raised on small-scale (50-500), medium-scale (500-10,000), and large-scale (>10,000) poultry farms (FAO, 2017).

2.3 Status of Poultry Meat Production in Kenya

Poultry meat production increased in the last decade, although there was a decline in 2019 due to the COVID-19 pandemic. The estimated per capita consumption of poultry meat in Kenya in 2020 was 1.31kg, compared to 0.64 kg in 2010 (FAO, 2023). The demand for poultry meat is expected to rise alongside other food products as the demand for food continues to increase alongside the human population (Magothe et al., 2012). Other factors that increase the demand for poultry meat are urbanization and rising incomes in developing countries. Poultry is the most popular meat among consumers worldwide due to its relatively low cost, low-fat levels, and few religious and cultural restrictions. Some challenges facing broiler production in developing countries include disease outbreaks, high feed costs, the unavailability of day-old chicks in time, poor markets, and a lack of quality vaccines (Ebsa et al., 2019).

2.4 Status of the Feed Industry in Kenya

Most feed manufacturers and suppliers are located in urban and peri-urban areas. This is influenced by high livestock numbers and good industrial infrastructure, including roads, water, and electric power. The distribution of commercial feeds from urban to rural areas is accompanied by high transportation costs, increasing the prices of commercial feeds in rural areas. The gap creates room for the establishment of small and medium millers in rural areas. Some of these millers are unregulated and produce low-quality feeds. It is estimated that 90% of feed manufacturers operate on a small scale, producing less than 1,000 tonnes/month. At the same time, 7% of feed manufacturers produce 1,000–5,000 tonnes per month, while about 2-3 % can produce above 5,000 tonnes per month. Poultry feeds account for 41% of the total feed produced, while dairy feeds and other feed types account for 39% and 4%, respectively (KMT, 2017).

There is severe competition between raw materials produced in the country and those imported from neighbouring countries, which make up at least 70% of the raw materials used by Kenya's feed industry (Köster & Köster, 2016; Njagi, 2022). The market prices of livestock feeds have risen since 2021 due to the high demand for crucial feed ingredients such as soybean and maize grain (Njagi, 2022). Kenya produces stable quantities of maize grain for human food, but importation is required to meet the country's total demand due to competition from other uses. Usually, maize grain is imported from Tanzania and Uganda (Njagi, 2022). The additional raw ingredients necessary to produce animal feed, such as wheat, rice, oil cakes, and vitamins, are also imported. Uganda is the primary source of maize germ, wheat grain, wheat pollard, rice bran, rice polish, and millet. Most soybeans, soybean meals, and groundnut cakes are imported from Tanzania. Sunflower meal, cotton meal, and *Omena* are jointly imported from Uganda and Tanzania. Prolonged droughts caused by climate change have reduced the availability of raw materials due to their high dependence on rain (Njagi, 2022). Cultivation of drought-tolerant crops, such as sorghum, is recommended.

2.5 Nutrient Requirements of Broiler Chicken

There has been no recent publication of poultry nutrient requirements by the National Research Council (NRC), although many developments in this research have taken place. Modern poultry genotypes' growth and productive potential have changed over the years, as reviewed by Applegate and Angel (2014). Broiler chickens are carefully selected for high weight gain and an efficient feed conversion ratio. Although some attention is paid to regulating feed intake to avoid accumulating excessive carcass fat, they are typically fed without restriction to facilitate faster development to market size (NRC, 1994). The nutrient requirements depend on sex, the criterion of adequacy, age, and broiler chicken strains (NRC, 1994). Table 2.1 shows the nutrient requirements of broiler chicken.

The crude protein (CP) at 0 to 3 and 3 to 6 weeks of age are 23 and 20%, respectively, while the metabolizable energy is 3,200 kcal/kg for all ages. Energy sources are the largest component of poultry feeds, followed by plant protein sources and animal protein sources. Usually, maize meal is the most commonly used energy source, while soybean meal is a popular plant protein source. Other grains, such as wheat and sorghum, and plant protein meals, such as canola meal, peas and sunflower meal are also used. The main animal protein ingredients are fishmeal and meat meal (Ravindran, 2013).

Table 2.1. Nutrient requirements of broiler chicken at two different ages

Nutrient (%)	0 to 3 weeks	3 to 6 weeks
Crude protein	23	20
Arginine	1.25	1.1
Histidine	0.35	0.32
Isoleucine	0.3	0.73
Leucine	1.2	1.09
Lysine	1.1	1
Methionine	0.5	0.38
Phenylalanine	0.72	0.65
Threonine	0.8	0.74
Tryptophan	0.2	0.18
Valine	0.9	0.82
Calcium	1.00	0.90
Non-phytate phosphorus	0.45	0.35
<i>Energy</i>		
Metabolizable energy (kcal/kg)	3200	3200

Source: NRC. (1994)

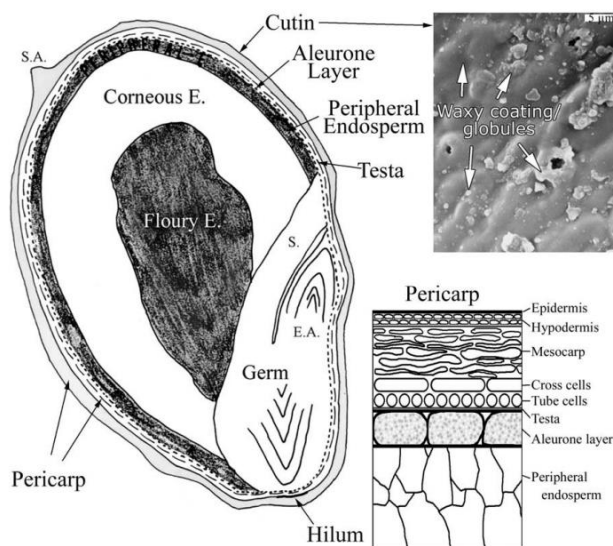
2.6 Sorghum

Sorghum [*S. bicolor* (L.) Moench] is the fifth most important food and feed cereal crop globally, after maize, wheat, rice, and barley. Sorghum takes 3 to 4 months to mature. The crop possesses a terminal grain panicle and reaches a height of 1 to 3 metres. The panicle might be short or long, tight or open, rounded or droopy (MoALFC, 2020).

Sorghum is a common crop in low-rainfall areas where maize does not do well. Apart from coping with dry conditions, sorghum is resistant to insect and mould attacks due to the presence of tannin in some varieties (Sharma, 1993). Its roots grow deeper than maize (Orr et al., 2016). Sorghum can roll its leaves and reduce leaf area, minimizing transpiration water loss. The crop is more adapted to dry conditions than other cereal crops due to its ability to stay green and compete well with most weeds (MoALFC, 2020). Sorghum thrives well in deep, fertile, well-drained loamy, clay, clay loam, or sandy loam soils with a pH of between 5.5 and 8.5. A rainfall amount of 450-900 mm annually is optimum, although the crop can also be produced at lower rainfall levels below 250-300 mm. The temperatures of 7 to 10°C for germinating seeds and 20-35°C for growth are recommended (MoALFC, 2020). In 2020, Kenya's sorghum yield was relatively lower than maize's: 1,434.1 kg/Ha vs. 1731 kg/Ha,

respectively. Sorghum yields have rapidly increased since 2016. The increasing yields can be attributed to the development of high-yielding varieties and the adoption of climate-smart agriculture, which promotes the cultivation of drought-tolerant crops such as sorghum. The total land area where sorghum and maize were harvested in the same year was 219,657 Ha and 2,188,911 Ha, respectively (FAO, 2023). Sorghum is cultivated alongside maize to spread the risk of drought in some African countries (Orr et al., 2016).

Sorghum has a wide range of uses. The grain is used to make traditional bread from fermented dough and other food products such as *ugali*, *sadza*, and *uji*. Low-tannin varieties are widely utilized for brewing compared to high-tannin varieties. Tannins bind enzymes during brewing and affect beer quality due to their bitterness and astringency (Taylor et al., 2013). Sorghum is also a source of livestock feed (Orr et al., 2016). The commercialization of sorghum generates an estimated KES 4 billion, engaging 250,000 farmers in the value chain. Research has resulted in superior varieties of the sorghum crop with high adaptability to local growing conditions and suitability to the intended end use (MoALFC, 2020). Varieties of sorghum in Kenya include Isudi, Gadam, KARI Mtama-1, Seredo, Serena, Kamani, BJ28, E1291, E6518, Sila, and E97 (MoALFC, 2020). Sorghum grain is included in poultry diets as a constituent or sometimes the only energy source (Selle et al., 2010a).



To the upper right is a view of the cuticle from the outside of the grain. S.A. ¼ stylar area; E.A. ¼ embryonic axis; S ¼ scutellum

Figure 2.1 A schematic diagram of sorghum grain showing pericarp layers

Source: Earp et al. (2004)

Sorghum grains typically have a spherical shape and vary in size and colour (Ratnavathi, 2019). The sorghum kernel comprises the pericarp or bran on the exterior, the germ or embryo, and the endosperm or storage tissue (Figure 2.1). The pericarp is a relatively minor component of the sorghum grain and contains cellulose and hemicellulose in addition to 4% protein, 11% fat, and 4% starch (Zarei et al., 2022). According to Earp et al. (2004), the pericarp is about 8 to 160 μm thick and comprises three layers: the epicarp, mesocarp, and endocarp. Beneath the pericarp is a layer of 8-40 μm thickness referred to as the seed coat or testa (Earp & Rooney, 1982). The central part of the sorghum grain is the endosperm, which contains starch granules. The germ has significant levels of protein (19%), ash (10%), and lipids (28%) (Zarei et al., 2022).

2.7 Nutrient Composition of Sorghum Grain

Sorghum grain is relatively similar to maize grain in nutrient composition, with crude

Table 2.2 Nutrient composition of sorghum, maize, wheat and barley grains

Nutrient profile (%)	Sorghum	Maize	Wheat	Barley
Dry Matter	85.00	85.00	87.00	85.00
Crude Protein	9.00	8.50	12-15	11.50
Metabolizable Energy (kcal/kg)	3250.00	3330.00	3150.00	2780.00
Calcium	0.05	0.01	0.05	0.10
Available Phosphorus	0.14	0.13	0.20	0.20
Sodium	0.05	0.05	0.09	0.08
Chlorine	0.07	0.05	0.08	0.18
Potassium	0.32	0.38	0.52	0.48
Selenium (ppm)	0.04	0.04	0.50	0.30
Fat	2.50	3.80	1.50	2.10
Linoleic acid	1.00	1.90	0.50	0.80
Crude Fibre	2.70	2.50	2.70	7.50
Methionine	0.12	0.20	0.20	0.21
Methionine + Cysteine	0.29	0.31	0.41	0.42
Lysine	0.31	0.20	0.49	0.39
Tryptophan	0.09	0.10	0.21	0.19
Threonine	0.32	0.41	0.42	0.40
Arginine	0.40	0.39	0.72	0.51

Source: Leeson and Summers (2005)

protein of 9.0% vs. 8.5%, respectively, as shown in Table 2.2. Sorghum grain's metabolizable energy (ME) is slightly lower than that of maize grain (3250 kcal/kg vs. 3330 kcal/kg).

2.8 Anti-Nutritional Factors in Sorghum Grain

The utilization of sorghum grain as an energy source is constrained by several anti-nutritional factors that adversely affect animal production (Liu et al., 2013). The anti-nutritional factors include tannins, kafirins, and phytates. These factors make sorghum grain inferior to maize grain in broiler feeds.

2.8.1 Tannins

All sorghums comprise phenolic compounds containing a benzene ring with a hydroxyl group (Dykes & Rooney, 2007). Tannin is one of the three main classes of phenolic substances, together with phenolic acid and flavonoids (Naczka & Shahidi, 2004; Tapiwa, 2019). The B₁ and B₂ genes influence the amount of tannin and other polyphenols found in the testa and pericarp. The pericarp appears brown and contains significant polyphenols in cases where the two genes and a spreader gene (S) dominate (Earp et al., 2004; Earp & Rooney, 1982). Sorghum varieties can be divided into three classes based on their genetic makeup and chemical composition (Rooney et al., 1981). Type I sorghums (b1b1B2, B1b2b2, and b1b1b2b2) lack a pigmented testa, low phenol concentrations, and tannin. The testa of types II and III are coloured and they contain tannins. The level of tannins in low-tannin, medium-tannin, and high-tannin sorghum varieties have been reported to range between 0 and 1.8, 6.4 and 15.5, and 15.5 and 56.3 mg catechin equivalents g⁻¹ dry matter, respectively (Dykes & Rooney, 2006). During the vanillin/HCl assay, the tannin in Type II sorghums (B1 B2 ss) is extracted using acidified methanol (1% HCl methanol). In contrast, the tannin in Type III sorghums (B1 B2 S) is extracted with either methanol or acidified methanol. Tannin binds to proteins, starch, and minerals, lowering the digestibility of sorghum grain in animals (Dykes & Rooney, 2006).

2.8.2 Kafirins

Kafirins are the storage proteins of sorghum and are found in protein bodies in the seed endosperm. They are reclassified into four groups (α , β , γ , and δ) based on solubility properties, electrophoretic mobility, and nucleotide sequences (Shull et al., 1992). The α -kafirin is the main group, constituting about 80%, and is encapsulated at the centre of a spherical protein body by the β , and γ -kafirin (Shull et al., 1992) and a small amount of δ -kafirin (Izquierdo & Godwin 2005). There is evidence that, although α -kafirin is relatively highly digestible compared to the other kafirins, its enzymatic digestibility is impeded by the inter- and intra-molecular disulphide bonding between β - and γ -kafirin proteins on the periphery of the protein

bodies (Duodu et al., 2003; Liu et al., 2019). The digestibility of the grain is also affected by the interaction of sorghum proteins with non-protein compounds such as polyphenols, non-starch polysaccharides, starch, phytates and lipids (Duodu et al., 2003).

During moist-heat processing of sorghum grain, there is significant disulphide cross-linking of kafirin proteins inside the endosperm. Therefore, one obstacle in the processing of sorghum is the grain's susceptibility to 'moist heat'. The β - and γ -kafirins largely cross-link and lose their digestibility during cooking (Selle et al., 2010b). Swine and poultry feed producers are concerned about the detrimental effects of moist-heat processing on sorghum protein solubility and digestibility. Various methods, including reducing compounds, may minimize the disulphide linkage of its protein. Low-moisture extrusion cooking results in higher starch gelatinization while reducing the disulphide cross-linking of the kafirins, which occurs during high-moisture extrusion cooking.

2.8.3 Phytate

Sorghum grain contains phytate (salt form) or phytic acid, just like all other feedstuffs of plant origin. Phosphorus is stored in plant seeds in the form of phytate. Phytate comprises 28.2% phosphorus (phytate phosphorus), which cannot be efficiently utilized by non-ruminant animals (McCustion et al., 2019). Additionally, it lowers the digestibility of calcium (Ca), magnesium (Mg), trace minerals (zinc and iron), and protein and lowers energy utilization in chickens (Moss et al., 2018).

Various feed processing methods are evaluated to mitigate the anti-nutritional effects and thus the digestibility of sorghum grain in chicken. Hydrothermal processing methods involve heat and moisture, and they include steam pelleting, steam flaking, and extrusion cooking. The inclusion of exogenous enzymes in poultry feeds is also widely practised. Phytate-degrading enzymes are commonly used in sorghum-based diets to liberate phosphorus (Liu et al., 2013).

2.9 Extrusion Cooking

Extrusion cooking is a high-temperature, relatively short-time method that combines moisture, pressure, temperature, and shearing impact to plasticize and cook starchy food materials (Navale et al., 2015). In this process, the food/feed material is made to flow through a particular set of mixing conditions, heat, and shear via a die to form and puff-dry the material (Riaz, 2000). The process denatures proteins and inactivates enzymes and microorganisms in food/feed materials. It also gelatinizes starch and liberates complexed phenolic compounds (Navale et al., 2015).

The major types of extruders include piston extruders, roller-type extruders, and screw extruders. Screw extruders, which are the most popular types of extruders, are further classified into single and twin screw extruders (Alam et al., 2016). A typical extruder comprises a barrel that surrounds the primary screw, a feeder that metres in raw ingredients, and a power source to run the screw. The raw material is transported via the screw into a shaped hole where a die shapes the finished product (Riaz, 2012).

The characteristics of the extruded material are influenced by several factors, including screw speed, barrel temperature, screw, and barrel arrangement, die opening, and feed rate (Navale et al., 2015). Depending on the formulation, ingredients with a wide variety of moisture contents can be processed using dry extrusion. There is no need to dry the material with a lower initial moisture content after extrusion cooking. In dry extrusion, moisture typically escapes as steam at the exit, and this moisture loss is usually influenced by the initial moisture content of the material. Water injection during extrusion is an option for dry extruders. Starchy materials often need some moisture to gelatinize. Before the material leaves the die in dry extrusion, pressure and temperature should peak. The opening and design of the die can have a significant impact on pressure development. Different dies are utilized for various materials and forms. A dry extruder has a cooking range that can reach 180–320°F under very high pressure. Pressure is immediately released from the products as soon as they emerge from the extruder dies, which allows internal moisture to vaporize and cause the product to expand (Riaz, 2009).

2.9.1 Extrusion Cooking of Sorghum Flour

This process destroys anti-nutritional factors by denaturing proteins and breaking down polymers of phenolics that bind proteins (D'Almeida et al., 2021). Earlier studies indicated that extrusion cooking of sorghum flour reduced polymeric tannins by 85%, with a 29-478% reduction in lower molecular weight tannins (Awika et al., 2003). Another study involving the extrusion cooking of polished brown sorghum grain reported a reduction ($p < 0.001$) of tannin content from 8.82 to 4.59 mg/g. The phytic acid was also reduced ($p < 0.013$) from 2.08 to 1.15 mg/g (Murtini et al., 2021).

According to Murtini et al. (2021), the extrusion cooking of brown sorghum flour increased ($p < 0.001$) digestibility (pepsin-trypsin) from 48.98 to 75.67%. In another study, the extrusion cooking of brown sorghum grain increased the fibre content, enhanced starch digestibility, and increased the availability of proteins (Campelo et al., 2020). According to the authors, the increase in fibre content was due to the formation of resistant starch and indigestible glucans. Extrusion cooking of sorghum flour increases proteins' solubility. It

breaks linkages between phenolic compounds and proteins, which improves their accessibility for proteolysis (Campelo et al., 2020; D'Almeida et al., 2021). Although minerals are resistant to thermal processes of extrusion, their bioavailability can be enhanced by the reduction of phytic acid, which binds them (Murtini et al., 2021).

2.10 Exogenous Phytase Enzyme in Broiler Chicken Diets

The use of exogenous phytase enzymes optimizes phosphorus bioavailability. In addition, it optimizes growth, feed intake, feed conversion ratio, and the cost-benefit ratio in broiler chicken fed sorghum-based diets (Ahmed & Tanveer 2021). The mechanism involves the destruction of the anti-nutritional properties of phytic acid/phytate, which frees phosphorous from plant-based feed materials. Due to the phytic acid, the proteins, vitamins, and numerous minerals are of limited bioavailability to animals (Afify et al., 2011). Phosphorus and other minerals are liberated to contribute to the crucial physiological functions of broiler chicken. Similar studies involving the supplementation of red sorghum meal with phytase reported an increased apparent metabolizable energy of the diet, feed intake, weight gain, and feed conversion ratio (Anyaegbu et al., 2021). In that study, the enzyme enhanced carbohydrate digestibility and reduced gut viscosity. The study concluded that red sorghum meal could be used up to 50% in broiler starter diets and up to 100% in broiler finisher diets when supplemented with exogenous phytase enzyme without detrimental effects. Gidado et al. (2020) concluded that sorghum meal supplemented with exogenous feed enzymes could entirely replace maize meal in finisher broiler chickens without compromising broiler growth rate or nutrient utilization.

CHAPTER THREE

EFFECT OF EXTRUSION COOKING VARIABLES ON *IN VITRO* DRY MATTER DIGESTIBILITY OF SORGHUM [*Sorghum bicolor* (L.) Moench] MEAL

Abstract

Maize is the most commonly used energy source in non-ruminant diets and is a staple food in many countries. The grain is also used for biofuel production in developed countries, thus causing severe competition and a high global market price. The search for alternative energy sources to replace maize is rising. Sorghum [*S. bicolor* (L.) Moench] grain is potentially a high-quality energy source in non-ruminant diets. However, its digestibility is constrained by tannins, kafirins and phytates, and when included in non-ruminant diets, there is sub-optimal performance. This study investigated the effect of feed moisture content, barrel temperature, and screw speed on the *in vitro* dry matter digestibility (IVDMD) of extruded sorghum meal. The study followed a (3×2×2) factorial arrangement in a completely randomized design, each with three replicates, to determine the effect of feed moisture content, barrel temperature, and screw speed on IVDMD of extruded sorghum meal using a co-rotating twin screw extruder. A preliminary trial with feed moisture content between 40 to 50% (wet basis), screw speed between 280 and 300 rpm, and barrel temperature between 70 and 90 °C was done to determine the IVDMD of extruded sorghum meal. The IVDMD of extruded sorghum meal increased as feed moisture content was reduced while screw speed and barrel temperature were increased. For the tested conditions of extrusion cooking in this study, a combination of low feed moisture content (40%), high screw speed (300 rpm), and high barrel temperature (90 °C) resulted in high IVDMD.

3.1 Introduction

In recent years, the demand for energy cereal sources for humans and animals has rapidly increased due to the rising human population and high demand for biofuel production in developed countries (Khanal et al., 2022). Thus, efforts to improve the utilization of some locally available, underutilized, high-value, and drought-tolerant crops for food, feed, and industrial use have increased (Rashwan et al., 2021; Selle et al., 2010a). Sorghum [*S. bicolor* (L.) Moench] is the fifth most important food and feed cereal crop globally, after maize, wheat, rice, and barley (Assefa et al., 2020). Compared to maize, sorghum does well in relatively hot and dry climates, amidst accelerating global climate change (Orr et al., 2016). Sorghum has a wide range of uses, like brewing and making traditional bread from fermented dough and other food products such as *ugali*, *sadza*, and *uji*. Sorghum is also a source of livestock feed (Orr et

al., 2016), and it is included in poultry diets as a constituent or sometimes the only energy source (Selle et al., 2010a). The nutritional composition of sorghum grain is similar to that of maize grain, with a crude protein of 9.0% vs. 8.5% and metabolizable energy of 3250 kcal/kg vs. 3330 kcal/kg, respectively (Leeson & Summers, 2005). However, the nutrient digestibility of sorghum grain is lower in comparison with maize grain due to endogenous anti-nutritional factors such as tannins, kafirins, and phytates (Sohail et al., 2019).

Extrusion cooking is an advanced technology that uses a high-temperature, relatively short-time mechanism to combine moisture, pressure, temperature, and shearing impact to plasticize and cook starchy and/or proteinaceous food materials, resulting in molecular transformation and chemical reactions (Navale et al., 2015). The unique advantage of extrusion cooking over the other heat processing methods is that the food/feed material undergoes intense mechanical shear, thus breaking the covalent bonds in biopolymers and causing intense structural disruption and mixing (Singh et al., 2007). Extrusion cooking changes the physical, chemical and nutritional properties of food/feed constituents. Some desirable effects of extrusion cooking on the extrudate product include the inactivation of anti-nutritional factors (trypsin inhibitors, haemagglutinins, tannins, and phytates), starch gelatinization, the formation of soluble dietary fibre and the reduction of lipid oxidation (Singh et al., 2007). Although some undesirable effects, such as Maillard reactions between protein and sugars and loss of heat-labile vitamins, may occur, the desirable effects outweigh the undesirable effects. The quality of the extruded product depends on several factors, especially those that affect the temperature of the food/feed mass within the extruder barrel. The quality of extruded products depends on several factors, which include feed composition (moisture), barrel temperature, screw speed, extruder model, feed particle size, feed rate, screw configuration, die properties, specific mechanical energy, and mass (product) temperature (Camire, 2011). Extrusion cooking has become very popular in food processing to produce breakfast cereals, ready-to-eat snack foods, and other textured foods. The technology has been recognized as the main method in the processing of food and feed, and it is attracting more attention and research (Alam et al., 2016; Rashwan et al., 2021; Riaz et al., 2009).

Many studies have been conducted on the effect of extrusion cooking variables on the nutritional quality of extruded sorghum meal. However, most of these studies focused on the physicochemical properties of the extrudates, such as expansion ratio, bulk density, and water absorption capacity, rather than their nutritional quality. Therefore, this study investigated the effects of extrusion cooking variables (feed moisture content, screw speed and barrel temperature) on *in vitro* dry matter digestibility, nutritional composition, mineral bioavailability and anti-nutritional factors of extruded sorghum meal.

3.2 Materials and Methods

3.2.1 Study Site

An *in vitro* experiment was conducted at Egerton University in the Department of Dairy and Food Science and Technology (extrusion cooking) and Animal Science laboratories (laboratory analysis). The university is located in Njoro Sub-County, Nakuru County, at 0° 23' S, 35° 55' N. The altitude of the area is 2,238 m above sea level. The average temperature of the area is 21 °C and annual rainfall ranges between 900 and 1,020 mm (Egerton University Department of Agricultural Engineering, Meteorological Station 2018, Personal Communication).

3.2.2 Materials

One indigenous variety of high-tannin sorghum grain (local name-Isudi) containing 4.83% tannin content (tannic acid equivalent) was sourced from the Solai Ward, located in Rongai Sub-county, Nakuru County, at 00°03'28"S 36°07'34"E. The altitude of the area is 1,952 metres above sea level (Solai, 2023), while the annual rainfall ranges between 800 and 1,100 mm (County Government of Nakuru, 2018). The sorghum grain was cleaned to remove glumes before milling using a hammer mill with a 2 mm sieve (9FC-22A, Shandong Gongyou Group Limited, China).

3.2.3 Proximate Analysis and Cell Wall Constituents

Moisture, ash, crude fibre and ether extract were determined by the Association of the Official Analytical Chemists (AOAC, 2006) methods 934.01, 942.05, 962.09, and 920.39, respectively. Protein content ($N \times 6.25$) was determined by the AOAC Kjeldahl method (method 984.13). Nitrogen-free extracts (NFE) were calculated using the formula shown below:

$$NFE = 100 - (ash + ether\ extracts + crude\ fibre + crude\ protein)$$

The Van Soest method was used to determine acid detergent fibre (ADF), acid detergent lignin (ADL) and neutral detergent fibre (NDF) (Van Soest et al., 1991).

3.2.4 Determination of Calcium and Phosphorus

Calcium was determined using the titrimetric method, using calcium carbonate as the standard solution (Siong, 1989). To precipitate out the calcium, an aliquot of the ash solution was mixed with the ammonium oxalate solution. Centrifugation and liquid supernatant decantation were followed by dissolving the precipitate in 4N sulphuric acid. The solution was maintained at 75-85°C for the titration of calcium in solution against 0.01N potassium

permanganate. The titre was used to calculate the amount of calcium in the test ash solutions after titrating standard solutions of calcium carbonate (Siong, 1989).

Phosphorus was determined using the ascorbic acid method (Nielsen et al., 2017). About 5g of sample was weighed in a clean crucible and heated at 550°C for 24 hours in a muffle furnace. Exactly 2 mL of ascorbic acid was added to pre-prepared 18 mL of Murphy and Riley reagent and swirled to mix. Aliquots of phosphorus were pipetted into clean test tubes, and water was added up to 4 mL and 1 mL of Murphy and Riley reagent was added to each test tube to contain 5 mL of the mixture. The mixture was then vortexed and absorbance was read at 700 nm. A phosphorus standard curve was prepared and phosphorus concentration was expressed in mg per 100 g sample (Nielsen et al., 2017).

3.2.5 Determination of Total Phenolics and Tannin Content

The total tannins were evaluated following the procedure of Makkar (2003). Tannins were determined by binding with polyvinyl polypyrrolidone (PVPP). The first step involved the measurement of the total phenolics. In the second step, tannins were precipitated together with the PVPP. The results were subtracted from the total phenols to determine the tannin as a percentage of tannic acid equivalent in dry matter.

3.2.6 Determination of Phytate Content

Phytate content was determined according to Wheeler and Ferrel (1971). Phytates were extracted with 50 ml of 3% trichloroacetic acid and precipitated as the ferric salt (ferric phytate). The phytate content in the sample was calculated using the formula:

$$\text{Phytate content in } \mu \text{ per } 100g \text{ sample} = \left(\frac{C \times E}{S \times Av} \right) \times 100$$

where: C is phytate concentration from the standard graph; E is total extraction volume; S is analytical sample taken; Av is analytical volume.

3.2.7 Extrusion Cooking of Sorghum Flour

The flour was extruded in the Department of Dairy and Food Science and Technology using a co-rotating twin-screw extruder (PSHJ-20, Jiangsu Xinda Tech Limited, China). The study followed a (3×2×2) factorial arrangement in a completely randomized design, each with three replicates, to determine the effect of feed moisture content, screw speed and barrel temperature on IVDMD of extruded sorghum meal. According to preliminary trials, the feed moisture content was between 40 and 50% (wet basis), the screw speed was between 280 and 300 rpm and the barrel temperature was between 70 and 90 °C. The experimental design, comprising 12 treatments, was summarized in Table 3.1.

Table 3.1 Factors and levels studied during extrusion cooking of sorghum flour

Factors	Levels
Feed moisture content (%)	40, 45, 50
Barrel temperature (die) (°C)	70, 90
Screw speed (rpm)	280, 300

The feed moisture content was achieved by adding a calculated amount of distilled water before the extrusion cooking process. The extruder had four barrel temperature zones: the entry barrel temperature zone (pv1), the centre barrel temperature zone (pv2), the end barrel temperature zone (pv3), and the die temperature zone (pv4). The first barrel temperature regime for pv1, pv2, pv3, and pv4 was 40, 50, 60, and 70°C, respectively. The second barrel temperature regime for pv1, pv2, pv3, and pv4 was 60, 70, 80, and 90°C, respectively. Extrudates were collected after the process stabilised and cooled at room temperature (21±4°C) and then dried in a hot air oven at 60°C for 24 h before grinding to pass through a 2 mm sieve and preserved in plastic bags for analysis of IVDMD.

3.2.8 *In vitro* Dry Matter Digestibility (IVDMD)

A two-phase *in vitro* dry matter digestibility method was used with modifications to simulate the digestive process of chicken (Boisen & Fernandez, 1997). The treatments were the 12 extrudates from the extrusion cooking process, each replicated three times. The effect of feed moisture content, screw speed and barrel temperature on IVDMD of extruded sorghum meal was determined using the following statistical model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + \varepsilon_{ijk} \quad i=1,2,3; \\ j=1,2; k=1,2$$

where: Y_{ijk} is observations made on the response variables; μ is the overall mean; α_i , β_j and γ_k is the effect of i^{th} , j^{th} , and k^{th} main factors in the experiment; $\alpha\beta_{ij} + \alpha\gamma_{ik} + \alpha\beta\gamma_{ijk}$ is the effect of the interaction of factors in the experiment; ε_{ijk} is random error effect associated with the experiment.

Phase one: simulating the stomach of a chicken

A ground feed sample (0.4 g) was weighed into a 250-ml digestion tube, followed by the addition of simulated stomach fluid. The fluid was composed of 1,550 U/mL pepsin, a porcine grade enzyme with 4x USP activity (pepsin from porcine gastric mucosa powder, ≥ 25 units/mg solid, Sigma-Aldrich Corp., St. Louis, MO, USA) to simulate the natural action of pepsin in the stomach fluid of chicken, as postulated by Sturkie (1976). The stomach buffer solution contained 16.9 mmol/L of NaCl, 9.6 mmol/L of KCl, and ten mmol/L of HCl to simulate the natural ionic concentration of stomach fluid from chicken (Sturkie, 1976). The pH was adjusted to 2.0 at 39°C by adding 200 mmol/L of HCl. Exactly 2 ml of chloramphenicol C-0378 (Sigma-Aldrich, St. Louis, MO, USA) (0.5g/100 ml ethanol) was added to each conical flask to inhibit bacterial growth. The tubes were sealed using rubber stoppers and kept at 39°C in a water bath with continuous stirring until after 2 hours.

Phase two: simulating the intestines of a chicken

The mixture from phase one was mixed with 80 ml of phosphate buffer (0.2M, pH 6.8) and 20 ml of 0.6M NaOH. The pH was adjusted to 6.8 using 1M HCl or 1M NaOH to achieve a stable environment for the activity of intestinal enzymes. Exactly 10.6 ml of artificial pancreatin P-1625 (porcine grade enzyme with 3 x USP activities) from Sigma-Aldrich, St. Louis, MO, USA, containing 100 mg/litre buffer, was added to the mixture and incubated at 39°C with continuous stirring for 4 hours. The residues were put in 15-mL centrifuge tubes and centrifuged at 1250 x g for 10 min at a temperature of 5°C. The supernatant was taken carefully, rinsed with distilled water, and washed twice with 20 ml of 95% ethanol and 20 ml of 99.5% acetone. The drying was done in the oven for 12 hours at 70°C, and the weight of the residue was recorded.

The IVDMD was computed as described by Boisen and Fernandez (1997):

$$\text{DM digestibility} = \left(\frac{\text{DM}_{\text{In}} - \text{DM}_{\text{RS}}}{\text{DM}_{\text{In}}} \right) \times 100$$

where, DM_{In} and DM_{RS} are the initial and residual DM, respectively.

3.2.9 Statistical Analysis

The results were subjected to a three-way analysis of variance using the general linear model (GLM) of the SAS Institute, Inc. (version 9.4; 2015). The mean separation was done using Tukey's HSD test at a significance level of 0.05.

3.3 Results

The *in vitro* dry matter digestibility of extruded sorghum meal as affected by feed moisture content, barrel temperature and screw speed is presented in Table 3.2. The *in vitro* dry matter digestibility of extruded sorghum meal as influenced by the main effects and the interaction effects are shown in Figure 3.1 and Table 3.3, respectively. The effect of extrusion cooking on the chemical composition of sorghum is presented in Table 3.4.

Table 3.2 Mean of squares of analysis of variance (ANOVA) of *in vitro* dry matter digestibility of extruded sorghum meal

Source of variation	IVDMD (%)
FMC ^a	279.62*
SS ^b	565.09*
BT ^c	61.03 ^{ns}
FMC×SS	223.25*
FMC×BT	187.19*
SS×BT	0.00 ^{ns}
FMC×SS×BT	73.49*

*Significant at $p < 0.05$; ns not significant at $p < 0.05$; ^afeed moisture content (%); ^bscrew speed (rpm); ^cbarrel temperature (°C)

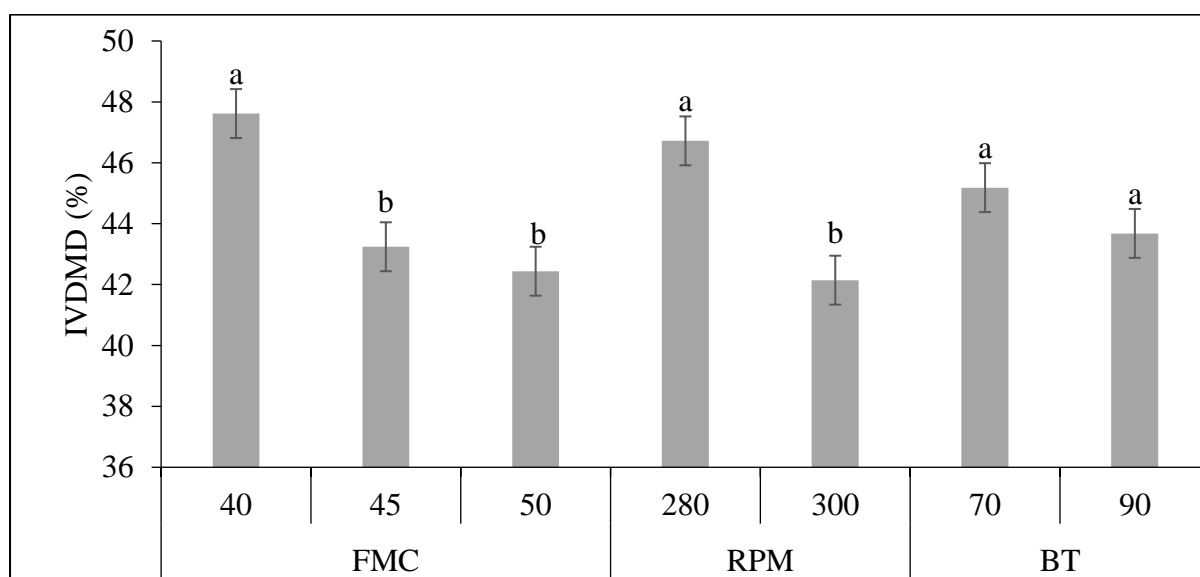


Figure 3.1 Effect of feed moisture content, screw speed, and barrel temperature on the *in vitro* dry matter digestibility of extruded sorghum meal

FMC = feed moisture content (%); RPM = revolutions per minute (screw speed); BT = barrel temperature (°C); each bar represents the mean \pm standard error. Bars with the same letter within the same process variable are not significantly different at $p < 0.05$

Table 3.3 Effect of the three-way interaction of extrusion cooking variables on *in vitro* dry matter digestibility of extruded sorghum meal

Extrusion cooking variable			IVDMD (%)	
FMC ¹	SS ²	BT ³		
40	280	70	47.51 ^{abc}	
		280	90	47.74 ^{abc}
		300	70	44.24 ^{abcd}
		300	90	50.98 ^a
45	280	70	49.85 ^{ab}	
		280	90	46.52 ^{abc}
		300	70	42.09 ^{dc}
		300	90	34.50 ^e
50	280	70	45.06 ^{abcd}	
		280	90	43.63 ^{bdc}
		300	70	42.35 ^{cd}
		300	90	38.71 ^{de}
SEM			1.51	

¹Feed moisture content (%); ²screw speed (rpm); ³barrel temperature (°C); means followed by the same superscript in the same column are not significantly different

Table 3.4 Effect of extrusion cooking on the chemical composition of sorghum (DM basis)

Parameter	Raw sorghum (g/100g)	ESM (g/100g)	t-value	p-value
Moisture content	8.62±0.14	5.68±0.08	17.77	<.0001
Ash	1.79±0.04	1.75±0.06	0.52	0.6331
Crude protein	8.72±0.40	8.89±0.36	-0.31	0.7726
Crude fibre	3.86±0.27	2.76±0.24	3.06	0.0375
Ether extract	2.60±0.19	0.90±0.03	8.71	0.0111
Nitrogen-free extracts	74.41	80.02	-	-
Acid detergent fibre	9.46±0.26	4.72±0.19	14.94	0.0001
Acid detergent lignin	1.96±0.00	0.42±0.12	12.69	0.0061
Neutral detergent fibre	30.19±1.57	13.43±0.75	9.61	0.0007
Calcium ¹	10.85±0.15	15.15±0.55	-7.61	0.0016

Phosphorus ¹	128.81± 0.50	177.62± 1.04	-42.44	<.0001
TEPH	6.82±0.01	5.62±0.03	39.85	<.0001
TET	4.83±0.03	4.23±0.06	9.43	0.0007
Phytates ¹	455±13.07	375.6±0.60	6.07	0.0258

TEPH = total extractable phenolics; TET = total extractable tannins (both in tannic acid equivalent); ESM = extruded sorghum meal; ¹value expressed in mg/100g; values are expressed as mean ± standard error

3.4 Discussion

The *in vitro* dry matter digestibility of extruded sorghum meal was significantly affected by feed moisture content and screw speed (main effects), with feed moisture content × screw speed, feed moisture content × barrel temperature, and feed moisture content × screw speed × barrel temperature interaction effects. Feed moisture content and barrel temperature influenced the gelatinization of starch, protein denaturation, barrel lubrication and the final quality of the extruded product (Mościcki & Zuilichem, 2011). On the other hand, screw speed regulated the heat flow from the mechanical energy input to the food/feed material, shearing action, and the residence time of the feed/food material in the barrel. It is well documented that these variables significantly influence the properties of the extruded product (Alam et al., 2016; Navale et al., 2015). The IVDMD values ranged from 34.50 to 50.98%, depending on the extrusion cooking variables used. The highest IVDMD was attained at a feed moisture content, screw speed, and barrel temperature of 40%, 300 rpm, and 90°C, respectively.

An increase in feed moisture content from 40 to 50% negatively influenced the IVDMD of extruded sorghum meal (Figure 3.1). This was because high feed moisture content promoted the formation of protein aggregates due to the cross-linking of disulphide bonds between starch and protein (Dalbhat et al., 2019), which lowered their accessibility for enzymatic hydrolysis. It is also possible that high moisture content increased the lubricating effect in the extruder barrel, reducing friction among the feed/food material, screw and barrel (Dalbhat et al., 2019). High moisture content decreased the viscosity and shortened the residence time of feed/food material, reducing the shearing effects on feed/food material and die pressure (Jafari et al., 2017). Kim et al. (2006) observed a similar trend upon extrusion cooking of pastry wheat flour and attributed it to resistant starch formation. The work of Fapojuwo et al. (1987) found that feed moisture content did not affect the *in vitro* protein digestibility of sorghum extrudate. This can be attributed to lower moisture contents (15 and 25%) compared to those used in the current study.

As screw speed increased from 280 to 300 rpm, there was a decrease in IVDMD of extruded sorghum meal (Figure 3.1). It is probable that, at high screw speed, the increased degree of starch gelatinization resulted in resistant starch formation (Sajilata et al., 2006). Similarly, at high screw speed, a higher degree of protein denaturation resulted in the formation of protein aggregates resistant to enzymatic hydrolysis, leading to lower IVDMD values (Singh et al., 2014). Similar findings were documented by Guha et al. (1997), who reported a decreased *in vitro* starch digestibility of rice flour when screw speed increased from 200 to 400 rpm. On the contrary, Fapojuwo et al. (1987) found that screw speed did not affect the *in vitro* protein digestibility of low-tannin sorghum varieties. In that study, barrel temperature had a higher effect on the *in vitro* protein digestibility of sorghum compared to the other extrusion cooking variables.

As the barrel temperature increased from 70 to 90°C, there was no significant effect on the IVDMD of extruded sorghum meal. This is probably because the effect of feed moisture content was higher than that of the resultant temperature increase, which may have altered the gelatinization/melting temperatures of starch (Yacu, 2012). This agrees with the work of Koa et al. (2017), who studied the effect of extrusion temperatures on the *in vitro* starch digestibility of sorghum-barley blends.

Extrusion cooking significantly decreased moisture content, crude fibre, ether extract, acid detergent fibre, acid detergent lignin and neutral detergent fibre. Extrusion cooking reduced the moisture content of the extruded sorghum meal, probably due to pressure and temperature differences, as the extruded sorghum meal exited from a high pressure and temperature zone to a low pressure and temperature zone, resulting in expansion, which promoted easy evaporation of moisture. Moisture content influences the shelf life of the extruded product. The decrease in moisture content upon extrusion cooking was within the range observed by Byaruhanga et al. (2014), who researched the properties of extruded sorghum meals in Uganda. Extrusion cooking significantly reduced crude fibre, acid detergent fibre, acid detergent lignin, and neutral detergent fibre. This is probably due to a reduction in the molecular weight of pectin and hemicellulose molecules, which increased soluble dietary fibre. During the process, the β 1,4 linkages were significantly broken. This agrees with the findings of a study on the properties of extruded sorghum meal (Byaruhanga et al., 2014) and the extrusion cooking of oats (Zhang et al., 2009). The ether extracts were reduced significantly upon extrusion cooking. This was expected since, during extrusion cooking, some oil may bind starch and proteins, which makes them less extractable with nonpolar solvents (Gulati et al., 2020). According to the researchers, the binding of lipids to starch is not desirable because it

increases the oxidative stability of the lipids. The ether extract of 0.9% after extrusion was within the range reported by Byaruhanga et al. (2014).

The bioavailability of minerals (Ca and P) increased significantly upon extrusion cooking. This was due to a reduction in tannins and phytates that bind these elements, thus reducing their bioavailability. Gulati et al. (2018) reported a 30% increase in P dialyzability upon extrusion cooking of beans.

The total extractable phenolics and total extractable tannins were reduced significantly upon extrusion cooking. Their decrease was probably due to the destruction of the phenolic structure by the high barrel temperatures and moisture content, which promoted decarboxylation, thus increasing the polymerization of phenols and tannins. This was similar to reports of other studies investigating the effect of extrusion cooking on the total phenols, tannin content, and antioxidant activity of sorghum grains (Cardoso et al., 2015; Dlamini et al., 2007; Llopart et al., 2014). Extrusion cooking significantly reduced phytate content. The underlying mechanism is probably the thermal degradation of phytate, causing hydrolyzation of inositol hexaphosphate to lower molecular weight forms (Kumar et al., 2018). This agrees with the work of Gulati et al. (2018), who reported a 46% reduction in phytates upon the extrusion cooking of Great Northern beans. Similarly, Kumar et al. (2018) found a 57% reduction in phytic acid upon extrusion cooking of sorghum-soya blends. In that study, phytic acid in the extrudates decreased with increasing barrel temperatures, confirming the thermal degradation effect of phytate. Thus, extrusion cooking of sorghum flour under 40% feed moisture content, 300 rpm screw speed, and 90°C barrel temperature conditions produced an extruded product with high *in vitro* dry matter digestibility.

3.5 Conclusion

A decrease in feed moisture content from 50 to 40%, an increase in screw speed from 280 to 300 rpm, and the barrel temperature from 70 to 90°C produced an extruded sorghum meal with the highest IVDMD.

3.6 Recommendation

The feed/food processors should use extruded sorghum meal produced under these optimum extrusion conditions in feed/food formulation.

CHAPTER FOUR

PERFORMANCE OF BROILER CHICKEN FED EXTRUDED SORGHUM [*Sorghum bicolor* (L.) Moench] MEAL AND EXOGENOUS PHYTASE-BASED DIETS

Abstract

The sustainability of the poultry sub-sector in developing countries is dependent on the supply of primary feed ingredients. Maize grain, which is commonly used as the main energy source in broiler diets, is a staple food and is also used for biofuel production in developed countries, thus increasing its global market price. This has stimulated interest in locally available, underutilized, high-value, and drought-tolerant crops such as sorghum [*S. bicolor* (L.) Moench] as alternative energy sources. However, some sorghum varieties contain high levels of anti-nutritional factors like tannins, kafirins and phytates, which adversely affect nutrient utilization in broilers, hence poor performance. Extrusion cooking of sorghum and the incorporation of exogenous phytase enzyme in sorghum-based diets have been proposed to improve feed utilization, but the effect on broiler performance is unclear. Therefore, this study investigated the effect of including extruded sorghum meal (ESM) as an energy source with or without exogenous phytase on the performance of broiler chicken. A total of 108 mixed-sex Cobb 500® day-old broiler chicks were used. The chicks were weighed and grouped in six, assigned cages, each cage randomly assigned to one of the six dietary treatments. The treatments were: T1 (0% ESM + 0% phytase), T2 (0% ESM + 0.035% phytase), T3 (50% ESM + 0% phytase), T4 (50% ESM + 0.035% phytase), T5 (100% ESM + 0% phytase), and T6 (100% ESM + 0.035% phytase). Each treatment had three replications. The grower and finisher diets were offered from days 1-21 and 22-42, respectively. The parameters measured were the weekly average daily feed intake, average daily gain and feed conversion ratio. Data were subjected to a two-way analysis of variance in a completely randomized design using the general linear model (GLM) procedure of the SAS Institute Inc. (version 9.4; 2015). Mean separation was done using Tukey's HSD test at a level of significance of 0.05. The inclusion of ESM above 50% in the diets adversely affected the average daily feed intake, average daily gain and feed conversion ratio. In the grower phase, the inclusion of exogenous phytase enzyme improved average daily feed intake and average daily gain. In conclusion, the inclusion of ESM up to 50% with exogenous phytase enzyme in diets did not affect average daily feed intake, average daily gain and feed conversion ratio. Therefore, ESM may be included up to 50% with exogenous phytase enzyme in diets without adversely affecting broiler chicken performance.

4.1 Introduction

The demand for affordable animal protein sources such as broiler meat in developing countries is high (D'Amour et al., 2020). The sustainability of the poultry subsector is dependent on the supply of primary feed ingredients. Maize grain is commonly used as the main energy source in broiler diets, yet it is the staple food, creating severe competition between livestock feed and human food. Maize is also used for biofuel production in developed countries, which increases its global market price (Cisse et al., 2017; Njoroge et al., 2015). The overreliance on maize grain makes commercial broiler feeds very costly and sometimes unavailable, which limits broiler production in Kenya (Njoroge et al., 2015). This has stimulated interest in improving the utilization of some locally available, underutilized, high-value, and drought-tolerant crops for food, feed, and industrial use.

Sorghum [*S. bicolor* (L.) Moench] is more drought-tolerant and adapted to varying soil types, and its nutrient composition is similar to maize grain (Moritz et al., 2022). Sorghum is a potential alternative cereal energy source in poultry diets. However, some sorghum varieties (those with a pigmented testa, classified as type II and III sorghum) contain anti-nutritional factors such as tannins, kafirins, and phytates (Awika & Rooney, 2004; Moritz et al., 2022), which compromise protein, carbohydrate, and mineral metabolism in poultry (Cowieson et al., 2008; Liu et al., 2015; Sohail et al., 2019). Therefore, when included in broiler diets as an energy source, they decrease feed intake, weight gain, and feed conversion efficiency. As a result, tannin-sorghum varieties are not commonly included in broiler diets or are used in smaller quantities (Kumar et al., 2005; Selle et al., 2010a).

The feed efficiency of broiler chicken fed high-tannin sorghum-based diets is increased through feed processing. The use of a particular feed processing method depends on its effectiveness in reducing anti-nutritional factors and increasing digestibility. Extrusion cooking is used to enhance the nutritional value and feed efficiency of poultry feeds. Some advantages of extrusion cooking on the feed product include the inactivation of anti-nutritional factors (trypsin inhibitors, tannins, and phytates), starch gelatinization, formation of soluble dietary fibre and the reduction of lipid oxidation (Singh et al., 2007). Based on *in vitro* studies, extrusion cooking of grain sorghum holds promise for broiler chicken diets. Some studies have suggested that improvements in nutrient digestibility through extrusion cooking do not always result in improved performance in animals (Amornthewaphat & Attamangkune, 2008; Rodrigues et al., 2016). The effect of extruded sorghum meal and exogenous phytase-based diets on broiler performance is unclear. Therefore, this study investigated the effect of inclusion levels of ESM as an energy source with or without exogenous phytase enzyme on the average daily feed intake, average daily gain and feed conversion ratio of broiler chicken.

4.2 Materials and Methods

4.2.1 Ethical Consideration

This study was approved by the Egerton University Research Ethics Committee with approval number EUISERC/APP/224/2023 (Appendix B) and the National Commission of Science and Technology of Kenya with license number NACOSTI/P/23/25493 (Appendix A).

4.2.2 Study Site

The feeding experiment was conducted at Egerton University in the Tatton Agriculture Park. The university is located in Njoro Sub-County, Nakuru County, at 0° 23' S, 35° 55' N. The climatic conditions of the area are described in Section 3.2.1 of Chapter 3.

4.2.3 Experimental Design

A (3x2) factorial arrangement was used in a completely randomized design to determine the effect of extruded sorghum meal as an energy source at three inclusion levels (0, 50, and 100%) with or without (0 and 0.035%) exogenous phytase enzyme. There were six dietary treatments: T1 (0% ESM + 0% phytase), T2 (0% ESM + 0.035% phytase), T3 (50% ESM + 0% phytase), T4 (50% ESM + 0.035% phytase), T5 (100% ESM + 0% phytase), and T6 (100% ESM + 0.035% phytase). Each treatment was replicated three times and each replicate comprised six mixed-sex Cobb 500® day-old chicks. The statistical model used was as follows:

$$Y_{ijk} = \mu + A_i + B_j + (AB)_{ij} + \varepsilon_{ijk} \quad i=1,2,3; j=1,2;$$

where: Y_{ijk} is observation k in level i of extruded sorghum meal and level j of phytase; μ is the overall mean; A_i is the effect of level i of extruded sorghum meal; B_j is the effect of level j of phytase; $(AB)_{ij}$ is the effect of the interaction of level i of the extruded sorghum meal with level j of phytase; ε_{ijk} is a random error with mean 0 and variance σ^2 .

4.2.4 Experimental Diets

The experimental diets were formulated to be isocaloric and iso-nitrogenous and meet or exceed broiler chicken requirements, according to the NRC (1994). Tables 4.1 and 4.2 show the calculated composition of the experimental diets, while Table 4.3 shows the analyzed proximate composition of the diets.

Table 4.1 Composition of the experimental grower diets

Ingredient (% in the diet)	Dietary treatments					
	T1	T2	T3	T4	T5	T6
Maize meal ²	56.4	56.4	28.2	28.2	0.0	0.0
Extruded sorghum meal	0.0	0.0	28.2	28.2	56.4	56.4
Soybean meal	33.8	33.8	33.5	33.5	32.8	32.8
Fish meal (<i>Omena</i>) ¹	3.8	3.8	3.8	3.8	4.2	4.2
Vegetable oil ³	3.5	3.5	3.8	3.8	4.1	4.1
Dicalcium phosphate	1.2	1.2	1.2	1.2	1.2	1.2
Limestone	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin and mineral premix	0.5	0.5	0.5	0.5	0.5	0.5
Common salt	0.3	0.3	0.3	0.3	0.3	0.3
Natuzyme®	0	0.035	0	0.035	0	0.035
Total	100.0	100.0	100.0	100.0	100.0	100.0
Calculated analysis						
CP	23.3	23.3	23.3	23.3	23.3	23.3
ME (kcal/kg)	3155.5	3154.6	3151.8	3150.9	3148.8	3147.9
CF	2.5	2.5	2.5	2.5	2.6	2.6

¹Scientific name: *Rastrineobola argentea*, common name; silver cyprinid, or the Lake Victoria sardine or *Mukene*, ²variety: 6213; ³fully refined palm olein; the vitamin and mineral premix per 2.5kg supplied: Vit A 10000000IU, Vit D3 2800000IU, Vit E 25000mg, Vit K₃ 2800mg, Vit B₁ 2000mg, Vit B₂ 7000mg, niacin 40000mg, pantothenic acid 12000mg, Vit B₆ 3500mg, folic acid 1000mg, Vit B₁₂ 15mg, biotin 80mg, manganese 60000mg, zinc 60000mg, iron 30000mg; Natuzyme® contains 1,500 units/g of phytase, 12,000 units/g of xylanase, 6,000 units/g of cellulase, 700 units/g of beta-glucanase, 700 units/g protease, 400 units/g of alpha-amylases

Extrusion cooking of sorghum flour was done at 40% feed moisture content, 300 rpm screw speed and 90°C barrel temperature. The extrudate was ground using a hammer mill fitted with a 2 mm sieve (9FC-22A, Shandong Gongyou Group Limited, China). The tannin and phytate content was reduced to 4.23% TA equivalent and 375.6 mg/100g, respectively, upon extrusion cooking. Natuzyme®, a commercially available multi-enzyme, was added at 0.035% and mixed thoroughly according to the manufacturer's instructions and recommendations.

Table 4.2 Composition of experimental finisher diets

Ingredient (% in the diet)	Dietary treatments					
	T1	T2	T3	T4	T5	T6
Maize meal ²	65.0	65.0	32.5	32.5	0.0	0.0
Extruded sorghum meal	0.0	0.0	32.5	32.5	65.0	65.0
Soybean meal	24.8	24.8	24.3	24.3	23.7	23.7
Fish meal (<i>Omena</i>) ¹	4.5	4.5	4.6	4.6	4.8	4.8
Vegetable oil ³	3.2	3.2	3.6	3.6	4.0	4.0
Dicalcium phosphate	1.2	1.2	1.2	1.2	1.2	1.2
Limestone	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin and mineral premix	0.5	0.5	0.5	0.5	0.5	0.5
Common salt	0.3	0.3	0.3	0.3	0.3	0.3
Natuzyne®	0	0.035	0	0.035	0	0.035
Total	100.0	100.0	100.0	100.0	100.0	100.0
Calculated analysis						
CP	20.1	20.1	20.1	20.1	20.1	20.1
ME (kcal/kg)	3204.2	3203.3	3203.6	3202.7	3203.1	3202.2
CF	2.4	2.4	2.5	2.5	2.5	2.5

¹Scientific name: *Rastrineobola argentea*, common name; silver cyprinid, or the Lake Victoria sardine or *Mukene*, ²variety: 6213; ³fully refined palm olein; the vitamin and mineral premix per 2.5kg supplied: Vit A 10000000IU, Vit D3 2800000IU, Vit E 25000mg, Vit K₃ 2800mg, Vit B₁ 2000mg, Vit B₂ 7000mg, niacin 40000mg, pantothenic acid 12000mg, Vit B₆ 3500mg, folic acid 1000mg, Vit B₁₂ 15mg, biotin 80mg, manganese 60000mg, zinc 60000mg, iron 30000mg; Natuzyne® contains 1,500 units/g of phytase, 12,000 units/g of xylanase, 6,000 units/g of cellulase, 700 units/g of beta-glucanase, 700 units/g protease, 400 units/g of alpha-amylases

Table 4.3 Analysed proximate composition of the experimental diets (g/100g DM)

Treatment	Grower diets					
	Moisture	Ash	Crude protein	Crude fibre	Ether extracts	Nitrogen-free extracts
1	8.87 ^a ±0.34	5.97±0.37	23.27±0.13	4.39 ^a ±0.33	11.85 ^a ±0.29	45.65
2	8.56 ^a ±0.13	5.68±0.26	23.48±0.53	3.82 ^{ab} ±0.47	12.07 ^a ±0.61	46.39
3	7.81 ^b ±0.03	6.82±0.48	23.58±0.12	3.44 ^{abc} ±0.19	9.92 ^b ±0.29	48.43
4	7.79 ^b ±0.15	6.48±0.63	23.61±0.13	3.15 ^{bc} ±0.15	8.80 ^b ±0.20	50.17
5	7.74 ^b ±0.14	5.63±1.10	23.07±0.24	2.43 ^c ±0.39	9.39 ^b ±0.06	51.74
6	7.91 ^b ±0.12	7.00±0.25	23.54±0.24	3.05 ^{bc} ±0.67	9.53 ^b ±0.12	48.97
Finisher diets						
1	10.75 ^a ±0.10	6.16 ^b ±0.23	21.01±0.12	5.17 ^a ±0.05	10.65 ^a ±0.19	46.26
2	10.27 ^b ±0.05	6.87 ^b ±0.23	20.77±0.13	4.17 ^b ±0.51	10.40 ^{ab} ±0.19	47.52
3	10.58 ^{ab} ±0.20	6.46 ^b ±0.41	20.80±0.31	4.48 ^{ab} ±0.11	9.69 ^b ±0.12	47.99
4	10.31 ^b ±0.08	6.63 ^b ±0.37	20.38±0.31	4.41 ^{ab} ±0.07	9.72 ^b ±0.18	48.55
5	10.31 ^b ±0.06	7.94 ^a ±0.07	21.74±1.41	4.18 ^b ±0.07	10.06 ^{ab} ±0.21	45.77
6	10.33 ^b ±0.20	6.93 ^b ±0.36	19.85±0.14	4.17 ^b ±0.47	10.01 ^{ab} ±0.35	48.71

^{a, b, c} means in a column bearing different superscripts differ significantly

4.2.5 Management of the Chicken

A total of one hundred and eight (108) healthy, mixed-sex, day-old commercial broiler chicks (Cobb 500® breed) were purchased from a local commercial hatchery (Kenchic® Limited). The chicks were vaccinated against Gumboro and Newcastle diseases at the hatchery. On the first day, the chicks were administered Lemycin® (a water-soluble chick booster containing an antibiotic, glucose, vitamins, and amino acids) and liquid paraffin (to soften and lubricate droppings). The chicks were fed a standard maize-soybean diet for the first 48 hours while acclimatizing to the experimental conditions. On the third day post-hatching, chicks were weighed in groups of six and randomly allocated into eighteen deep litter cages measuring 1 m². Chicks in each cage were randomly assigned to one of the six diets with three replicates. The grower and finisher diets were offered from days 1-21 and 22-42, respectively. No transition period was allowed between the grower and finisher diets. However, there were no digestive problems observed due to the sudden change in the diet. Water and feed were provided *ad libitum*.

4.2.6 Data Collection and Calculations

Data were collected on the following parameters:

i. Average daily feed intake

Feed was provided *ad libitum* by giving a weighed amount of the respective diets (Tables 4.1 and 4.2) once daily at 0900 hours. Leftovers were collected and weighed before the next morning's feeding. Average daily feed intake (g) per broiler per day was calculated as the difference between the amount of each diet offered and that leftover daily divided by the number of broilers in the cage.

ii. Average daily gain

The body weights of broilers were measured at the end of each week at 0900 hours before feeding. The average daily gain per broiler was calculated by dividing the difference between the initial and final weight of the broilers by the number of days.

iii. Feed conversion ratio

The feed conversion ratio was calculated by dividing the average feed intake by the average weight gain (g) of the broilers at the end of each growth phase.

4.2.7 Statistical Analysis

Data were subjected to a two-way analysis of variance in a completely randomized design using the general linear model (GLM) procedure of the SAS Institute Inc. (version 9.4; 2015). Mean separation was done using Tukey's HSD test at a level of significance of 0.05.

4.3 Results and Discussion

The average daily feed intake, average daily gain and feed conversion ratio of broilers as affected by ESM and exogenous phytase enzyme main effect are summarized in Table 4.4, while the interaction effect is summarized in Table 4.5.

Table 4.4 Average daily feed intake, average daily gain, and feed conversion ratio of broiler chicken as influenced by the dietary treatments (main effects)

Items	ADFI (g/bird)			ADG (g/bird)			FCR		
	1-21 d	22-42 d	1-42d	1-21 d	22-42 d	1-42d	1-21 d	22-42 d	1-42d
ESM main effect									
0%	25.54 ^a	45.868 ^a	36.90 ^a	10.91 ^a	15.88 ^a	13.49 ^a	2.37 ^b	2.89 ^b	2.80 ^b
50%	17.94 ^b	33.04 ^b	25.33 ^b	6.55 ^b	8.23 ^b	7.39 ^b	3.04 ^b	4.14 ^a	3.61 ^a
100%	13.22 ^c	15.70 ^c	14.85 ^c	3.33 ^c	4.21 ^c	3.77 ^b	4.10 ^a	3.88 ^a	3.97 ^a
SEM	1.05	1.83	1.48	0.69	0.55	0.84	0.24	0.20	0.18
Significance	*	*	*	*	*	*	*	*	*
Phytase main effect									
0%	17.42 ^b	30.69 ^a	25.11 ^a	5.97 ^b	9.03 ^a	7.90 ^a	3.36 ^a	3.61 ^a	3.53 ^a
0.035%	20.38 ^a	32.38 ^a	26.28 ^a	7.89 ^a	9.85 ^a	8.53 ^a	2.98 ^a	3.66 ^a	3.38 ^a
SEM	0.86	1.50	1.21	0.56	0.45	0.69	0.19	0.17	0.14
Significance	*	ns	ns	*	ns	ns	ns	ns	ns

^{a, b, c} means in a column bearing different superscripts differ significantly; *significant at $p < 0.05$; ns significant at $p < 0.05$; ADFI = average daily feed intake; ADG = average daily gain; FCR = feed conversion ratio; ESM = extruded sorghum meal; SEM = standard error of the mean

Table 4.5 Average daily feed intake, average daily gain, and feed conversion ratio of broiler chicken as influenced by the dietary treatments (interaction effects)

Items	ADFI (g/bird)			ADG (g/bird)			FCR		
	1-21 d	22-42 d	1-42d	1-21 d	22-42 d	1-42d	1-21 d	22-42 d	1-42d
ESM× phytase effect									
0% ESM without phytase	23.37 ^{ab}	44.77 ^a	36.46 ^a	9.78 ^a	15.35 ^a	13.75 ^a	2.44 ^{bc}	2.92 ^b	2.69 ^b
0% ESM with phytase	27.71 ^a	46.97 ^a	37.34 ^a	12.03 ^a	16.42 ^a	13.22 ^a	2.31 ^c	2.86 ^b	2.91 ^b
50% ESM without phytase	16.04 ^c	30.39 ^b	23.21 ^{bc}	4.87 ^{bc}	6.70 ^{bc}	5.79 ^b	3.63 ^{abc}	4.55 ^a	4.13 ^b
50% ESM with phytase	19.83 ^{bc}	35.68 ^{ab}	27.45 ^{ab}	8.23 ^{ab}	9.75 ^b	8.99 ^{ab}	2.45 ^{bc}	3.72 ^{ab}	3.09 ^{ab}
100% ESM without phytase	12.85 ^c	16.90 ^c	15.65 ^c	3.25 ^c	5.04 ^c	4.15 ^b	4.02 ^{ab}	3.37 ^{ab}	3.79 ^b
100% ESM with phytase	13.60 ^c	14.50 ^c	14.05 ^c	3.40 ^c	3.38 ^c	3.39 ^b	4.17 ^a	4.39 ^a	4.15 ^b
SEM	1.49	2.59	2.10	0.98	0.77	1.19	0.34	0.29	0.25
Significance	*	*	*	*	*	*	*	*	*

^{a, b, c} means in a column bearing different superscripts differ significantly; *significant at $p < 0.05$; ns significant at $p < 0.05$; ADFI = average daily feed intake; ADG = average daily gain; FCR = feed conversion ratio; ESM = extruded sorghum meal; SEM = standard error of the mean

The average daily feed intake, average daily gain and feed conversion ratio of broilers were significantly affected by the ESM and exogenous phytase enzyme inclusion. The adverse effect of ESM on broiler performance was attributed to the high levels of tannins and phytates. Tannins are considered detrimental to the utilization of sorghum in broiler diets because they limit the bioavailability of macromolecules such as proteins, carbohydrates, amino acids, and vitamins and lower the protein efficiency ratio (Zarei et al., 2022). According to Zarei et al. (2022), tannins lower feed intake, weight gain, and feed efficiency. Phytates, on the other hand, comprise 28.2% phosphorus (phytate-phosphorus), which cannot be efficiently utilized by non-ruminant animals (McCuiston et al., 2019). The ability of phytates to bind minerals lowers the digestibility of calcium (Ca), magnesium (Mg), trace minerals (zinc and iron), and protein and lowers energy utilization in chickens (Cowieson et al., 2008; Moss et al., 2018).

Inclusion of ESM in diets reduced the average daily feed intake of broilers by 31 to 60%. This was attributed to the bitter taste and astringency of tannins contained in the ESM (Ambula et al., 2001). Tannins bind to salivary proteins and mucins (slippery constituents of saliva), causing their aggregation or precipitation, which reduces the ability of saliva to coat and lubricate the oral tissue (Lawless & Heymann, 2010). The presence of tannins in sorghum acts as a natural defence against bird depredation in the fields. Similar results where high-tannin sorghum-based diets constrained feed intake in broiler chicken are documented (Ambula et al., 2001; Hidayat et al., 2021; Nyachoti et al., 1997).

Overall, the inclusion of ESM in broiler diets reduced the average daily gain by 40 to 69%. This is probably due to the deleterious effects of tannins, which include a reduction in both total and protein digestibility and inhibition of amylase enzyme activity by tannins, hence constraining energy utilization (Tapiwa et al., 2019; Zarei et al., 2022). Tannins bind to proteins, lipids, and carbohydrates, forming indigestible complexes and reducing the availability of nutrients for growth (Cherian et al., 2002). This confirms the results of similar studies in broilers on the use of high-tannin sorghum in diets (Ambula et al., 2001; Hidayat et al., 2021; Torres et al., 2013). On the contrary, Kumar et al. (2005) observed no negative effect of high-tannin sorghum on the body weights of broilers in the overall growth phase. This is probably due to different levels of dietary tannins. The tannin content of the sorghum used in the study by Kumar et al. (2005) was lower compared to the current study (2.3% vs. 4.23% TA equivalent). During the grower phase, the inclusion level of 50% ESM did not affect the feed conversion ratio of the broilers, although, in the overall phase, the feed conversion efficiency reduced by 28.9 to 41.8%. The mode of action of tannins that results in a depression in body weight probably involves binding proteins, carbohydrates, and minerals, thus compromising the digestibility of these nutrients (Dykes & Rooney, 2006). This constrained the utilization of

energy, protein, and specific amino acids (Treviño et al., 1992) resulting in reduced feed efficiency, hence depressed body weight gain. Ambula et al. (2001) observed a 23-42.5% reduction in feed efficiency of broilers fed a high-tannin sorghum-based diet (2.71 to 3.54% catechin equivalent). Nyachoti et al. (1998) also found that broilers fed a high-tannin sorghum-based diet (1.57% catechin equivalent) were 6.5% less efficient in feed utilization.

The exogenous phytase inclusion significantly improved average daily feed intake (17%) and average daily gain (32%) in the grower phase. There is a possibility that the phytase enzyme response was more pronounced in the grower phase than the finisher phase because of the difference in dietary crude protein levels; the grower and finisher diets contained approximately 23 and 20% crude protein, respectively. As reviewed by Moss et al. (2018), apart from liberating phytate-bound phosphorus, the “extra-phosphoric effect”, phytase may prevent the *de novo* formation of binary protein-phytate complexes in the crop, the “protein effect”, thus increasing protein digestibility in broilers. Moreover, the phytase response may have been influenced by the age of the broilers. In young poultry, the rate of feed passage in the digestive tract is relatively faster (Amerah et al., 2007), thus the lack of adequate phytate hydrolysis in the gizzard may have increased the phytase response. This was consistent with the results of the work of Li et al. (2018), who reported greater phytase responses in younger broilers (7 d) than in older broilers (21 d). Another reason for a higher exogenous phytase enzyme response in the grower phase could be due to a better feed conversion ratio in the grower phase than in the finisher phase. Feed conversion efficiency is higher in young broilers than in old ones (Reece & Lott, 1983). The somewhat depressed phytase response observed in the current study is probably because the enzyme does not address the deleterious effects of kafirins and tannins, which are present in sorghum-based diets (Selle et al., 2021). This concurs with another study in broilers fed sorghum-based diets, where the phytase responses were lower in comparison with those fed maize or wheat-based diets (Liu et al., 2014). There was a significant ESM-phytase interaction effect on the average daily gain of broilers in the finisher phase and the feed conversion ratio in the finisher phase and the overall period.

4.4 Conclusion

Inclusion of ESM up to 50% with exogenous phytase enzyme in the diets did not affect the average daily feed intake, average daily gain and feed conversion ratio of broiler chicken.

4.5 Recommendation

ESM as an energy source can be included up to 50% with exogenous phytase enzyme in diets without adversely affecting the average daily feed intake, average daily gain and feed conversion ratio of broiler chicken.

CHAPTER FIVE

CARCASS CHARACTERISTICS AND DESCRIPTIVE SENSORY QUALITY OF BROILER CHICKEN FED EXTRUDED SORGHUM [*Sorghum bicolor* (L.) Moench] MEAL AND EXOGENOUS PHYTASE-BASED DIETS

Abstract

The interest in the utilization of alternative energy sources to replace maize in poultry diets for sustainable poultry production is rising. However, there is little information on whether such alternatives affect carcass characteristics and the sensory quality of poultry meat. This study investigated the effect of extruded sorghum meal (ESM) and exogenous phytase enzyme on broiler carcass characteristics and descriptive sensory quality. A total of 108 day-old Cobb 500® chicks were weighed, grouped in six and randomly placed in deep litter cages. Each cage was randomly assigned one of the six dietary treatments, each replicated three times. The treatments were: T1 (0% ESM + 0% phytase), T2 (0% ESM + 0.035% phytase), T3 (50% ESM + 0% phytase), T4 (50% ESM + 0.035% phytase), T5 (100% ESM + 0% phytase), and T6 (100% ESM + 0.035% phytase). The grower and finisher diets were offered from days 1-21 and 22-42, respectively. After day 42, all broilers were weighed, and three randomly sampled from each treatment to determine carcass characteristics and descriptive sensory quality. The carcass weight and the weights of the internal and external carcass parts were measured and expressed as a percentage of live weight. The breast meat was used for sensory analysis. A total of 10 experienced, semi-trained panellists rated the sensory attributes of the broiler meat on both the quantitative descriptive analysis scale and the just-about-right scale. Data were subjected to a two-way analysis of variance in a completely randomized design using the general linear model (GLM) procedure of the SAS Institute Inc. (version 9.4; 2015). Mean separation was done using Tukey's HSD test at a level of significance of 0.05. Results showed that inclusion of ESM above 50% in the diets reduced slaughter, hot carcass and cold carcass weights, abdominal fat, and the weight of the gizzard. It also increased scores for aroma intensity and the sustained impression of juiciness, chewiness, and flavour while reducing scores for white colour intensity. All sensory attributes analysed using the just-about-right scale were 'just about right'. The inclusion of ESM up to 50% and exogenous phytase enzyme did not affect carcass characteristics or descriptive sensory quality. It was concluded that ESM may be included up to 50% with exogenous phytase enzyme in broiler diets without adversely affecting broiler carcass characteristics and descriptive sensory quality.

5.1 Introduction

White meat, such as chicken meat, is considered superior in health benefits compared to red meat due to its low fat and cholesterol levels and high iron levels. Additionally, it is relatively cheaper, can be sold in typically convenient portions, and its consumption lacks religious restriction (Jaturasitha et al., 2008). Chicken meat accounts for an estimated 90% of the total poultry meat consumed in the world (D'Amour et al., 2020; Erdaw & Beyene, 2022). It is necessary to increase overall chicken production to cope with the growing demand for chicken meat. The broiler meat produced must be of good quality, acceptable, and highly nutritious (Gunya et al., 2018).

The sustainability of broiler production is dependent on the supply of some limited primary feed ingredients as energy and protein sources (Njagi, 2022; Njoroge et al., 2015). Despite a lot of attention being drawn to alternative energy sources such as sorghum [*S. bicolor* (L.) Moench] grain in poultry feeds, maize is still the most commonly used energy cereal in broiler feeds. Maize, which is a staple food, is in demand for biofuel production in developed countries, creating severe competition and thus increasing its global market price (Cisse et al., 2017; Khanal et al., 2022). Some sorghum varieties contain anti-nutritional factors such as tannins, kafirins and phytates that, when included in diets without mitigation, adversely affect broiler performance (Alshelmani et al., 2021; Awika & Rooney, 2004; Moritz et al., 2022). The feed efficiency of broilers fed sorghum-based diets is increased through feed processing methods such as extrusion cooking. Extruded sorghum meal with an exogenous phytase enzyme can potentially replace maize grain in broiler diets.

The composition of diets, especially the energy source, has a direct influence on broiler carcass characteristics and sensory quality (Lyon et al., 2004). The consumer's sensory perception of a food product affects preference, acceptability, and willingness to purchase the product (Ketkaew et al., 2021). Sensory attributes such as appearance, odour, texture, tenderness, juiciness and flavour determine the sensory quality of food products (Lyon et al., 2004; Mir et al., 2017).

Although there is growing interest in the utilization of extruded sorghum meal as an alternative energy source with exogenous phytase enzyme in broiler diets, there is little information on whether this affects broiler carcass characteristics and sensory quality. This information is necessary to understand if consumers accept or reject broiler meat. Thus, this study determined the effect of inclusion levels of extruded sorghum meal with or without exogenous phytase enzyme in diets on broiler carcass characteristics and sensory quality.

5.2 Materials and Methods

5.2.1 Ethical Consideration

The ethical considerations for the study are described in Section 4.2.1 of Chapter four.

5.2.2 Study Site

The study was conducted at Egerton University in the Tatton Agriculture Park (feeding trial) and the Department of Dairy and Food Science and Technology (carcass characteristics and descriptive sensory analysis). The university is described in Section 3.2.1 of Chapter three.

5.2.3 Experimental Design and Diets

The experimental design and diets are described in Sections 4.2.3 and 4.2.4 of Chapter four.

5.2.3 Management of the Chicken

The management of chicken for the study is described in Section 4.2.5 of Chapter four.

5.2.4 Slaughter Method and Determination of Carcass Characteristics

All broilers were weighed after day 42 to obtain slaughter weight, and three per treatment were sampled randomly for carcass characteristics (internal and external parts) and descriptive sensory quality evaluation. The broilers were starved for 8-10 hours with free access to water before slaughter. The slaughter process involved stunning and severing the jugular vein and then allowing blood to drain for five minutes (Moses et al., 2022). The slaughtered chicken were scalded in hot water (about 50°C) for one minute to allow for the plucking of feathers and manual evisceration. The carcasses were dressed by removing the neck and the shank and the hot-dressed weight was recorded. They were then chilled at 4°C for 24 hours and reweighed to obtain the cold carcass weight. The dressing percentage was expressed as hot-dressed weight divided by the weight of live chicken, as described by Maidala et al. (2020).

$$\text{Dressing percentage} = \frac{\text{hot dressed weight (g)}}{\text{slaughter weight (g)}} \times 100$$

The wings, breast, drumsticks, thigh and abdominal fat were removed, weighed, and expressed as a percentage of the weight of the live chicken. The skin between the thigh and the body was cut to expose and dislocate the hip joint (articulation *coxae*). The tendons and ligaments around the joint were cut to remove the thighs. The thigh and the drumstick were separated at the stifle joint (articulation *genus*). A cut was made in the vertical side of the carcass at the shoulder joint (articulation *humeri*) to remove the wings. The breast was separated from the sternum with the tip of the knife.

Internal organs (liver, heart and gizzard) were removed and weighed in grammes (g) using a 5 kg generic digital weighing scale (SF-1924, Sonifer, China) with 1 g accuracy. The length of the small and large intestines was measured in centimetres (cm) using a tailor's flexible measuring tape. The weights of the various organs were expressed as a percentage of the live weight.

5.2.5 Descriptive Sensory Evaluation

A total of 10 (6 males, 4 females, aged 25-45 years) experienced, semi-trained panellists were recruited for quantitative descriptive analysis (QDA) of broiler meat sensory attributes for three days. The panellists were previously screened and trained for one day before being selected to participate in the descriptive sensory evaluation as recommended by ISO guidelines (ISO, 2007; 2012). Panellist screening involved affective and acuity tests on relevant sensory attributes of broiler meat. The panellists were trained to develop sensory descriptors and define sensory attributes related to the texture, flavour, juiciness and appearance of broiler meat. They agreed on definitions, references and anchor terminologies used during the evaluation. The panellists evaluated various sensory attributes on both the quantitative descriptive analysis scale (Appendix E) and the just-about-right (JAR) scale.

In the first bite, initial hardness, cohesiveness, and moisture release were determined. In the chew-down stage, hardness of mass, cohesiveness of mass, fibrousness, and chewiness were determined. The just-about-right (JAR) scale was used to evaluate the appropriateness of colour, texture, tenderness, juiciness, taste, flavour and overall acceptability (1 = much too low, 2 = too low, 3 = just about right, 4 = too high, 5 = much too high). The JAR scales were used because they indicate the reaction of consumers towards a specific attribute and can be used for diagnostics and explanation if the overall product appeal is lacking (Lawless & Heymann, 2010).

Skinless breast muscles (*Pectoralis major*) used in the determination of carcass characteristics were thoroughly washed with clean water for sensory evaluation. The breast was chosen because it yields sufficient meat and is one of the most commercially important chicken parts (Oloo, 2021). Each sample was boiled in a different pot for 45-60 minutes at 100°C without adding any spice or salt. The samples were then deboned manually, cut into cubes of approximately 2 cm³, and wrapped in aluminium foil to maintain a warm temperature. Each panellist was presented with six pieces (six dietary treatments) every day for three days on white sensory-evaluation plates labelled with 3-digit blinding codes in a completely randomized design. The panellists were provided with warm water and biscuits to cleanse their

palates between each sample evaluation. The sensory evaluation room was organized according to the recommendations of Lawless and Heymann (2010).

5.2.6 Statistical Analysis

Data were subjected to a two-way analysis of variance in a completely randomized design using the general linear model (GLM) procedure of the SAS Institute Inc. (version 9.4; 2015). The mean separation was done using Tukey's HSD test at a level of significance of 0.05. Pearson correlation coefficients were tabulated using the PROC CORR procedure to investigate any relationships between the sensory attributes.

5.3 Results and Discussion

Table 5.1 Effect of dietary treatments on carcass characteristics (internal and external parts) of broilers

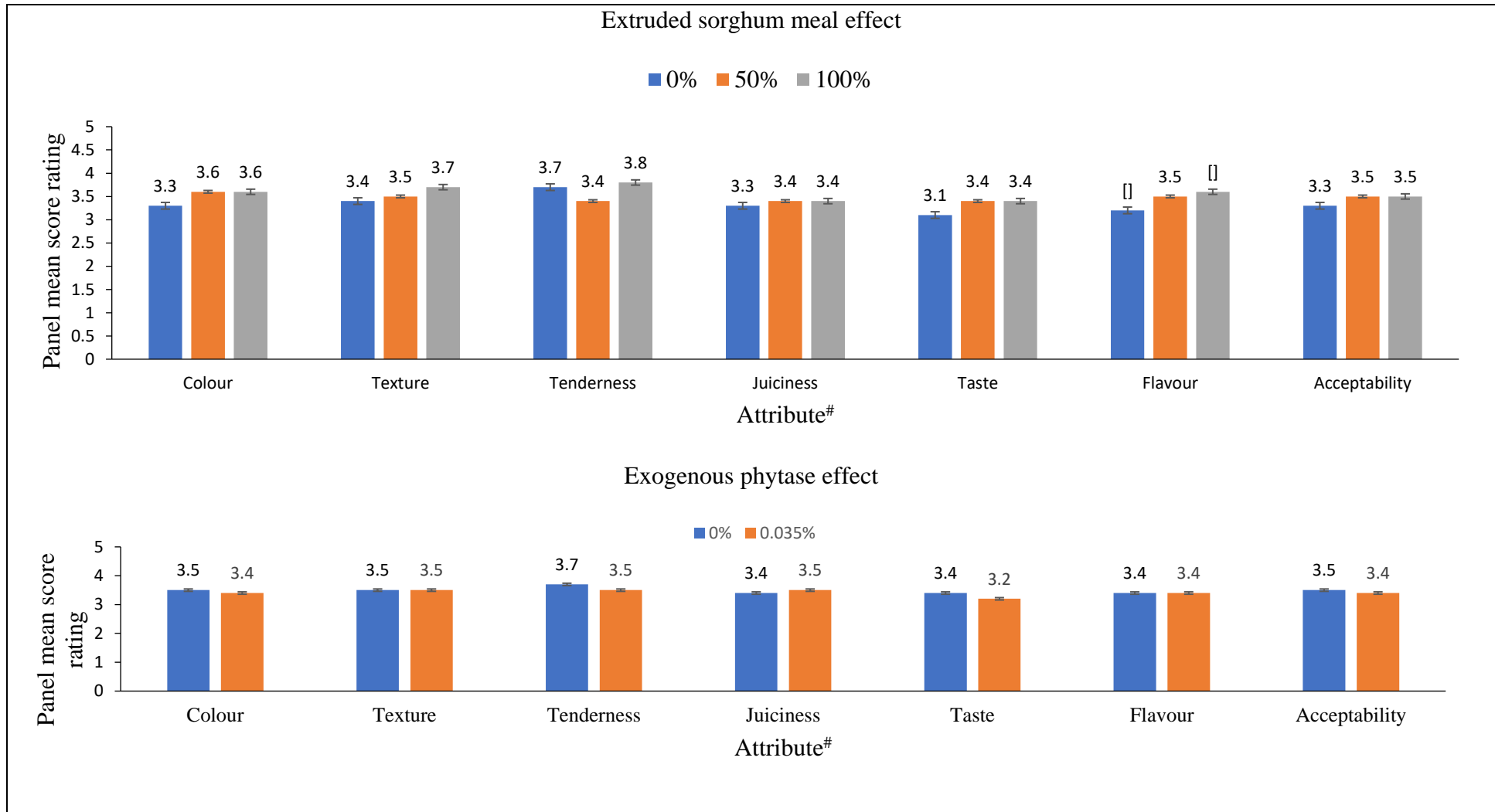
Parameter	ESM effect					phytase effect				ESM× phytase
	0%	50%	100%	SEM	<i>p</i> -value	0%	0.035%	SEM	<i>p</i> -value	<i>p</i> -value
Slaughter weight (g)	1078.2 ^a	962.0 ^a	395.2 ^b	70.3	*	776.6 ^a	847.0 ^a	57.4	ns	ns
Hot carcass weight (g)	889.7 ^a	790.3 ^a	320.5 ^b	59.1	*	633.7 ^a	700.0 ^a	48.2	ns	ns
Cold carcass weight (g)	884.2 ^a	746.2 ^a	318.8 ^b	62.2	*	630.1 ^a	669.3 ^a	50.8	ns	ns
Dressing weight (%)	82.5 ^a	81.9 ^a	81.0 ^a	0.7	ns	81.1 ^a	82.5 ^a	0.5	ns	ns
Wing weight (%)	9.4 ^a	9.9 ^a	9.2 ^a	0.4	ns	9.5 ^a	9.5 ^a	0.3	ns	ns
Breast weight (%)	22.4 ^a	20.4 ^a	19.7 ^a	0.8	ns	21.6 ^a	20.1 ^a	0.6	ns	ns
Drumstick weight (%)	11.3 ^a	11.5 ^a	9.6 ^a	0.6	ns	10.8 ^a	10.8 ^a	0.5	ns	ns
Thigh weight (%)	13.4 ^a	12.2 ^a	15.2 ^a	2.8	ns	15.3 ^a	11.9 ^a	2.3	ns	ns
Abdominal fat weight (%)	2.3 ^a	1.7 ^{ab}	1.2 ^b	0.2	*	1.6 ^a	1.8 ^a	0.1	ns	*
Liver weight (%)	2.1 ^a	2.2 ^a	2.6 ^a	0.1	ns	2.3 ^a	2.3 ^a	0.1	ns	ns
Gizzard weight (%)	2.6 ^b	3.0 ^{ab}	3.7 ^a	0.2	*	3.2 ^a	3.0 ^a	0.2	ns	ns
Heart weight (%)	0.8 ^{ab}	0.7 ^b	0.9 ^a	0.1	ns	0.9 ^a	0.8 ^a	0.0	ns	ns
Small intestine length (cm)	157.0 ^a	172.7 ^a	146.2 ^a	7.3	ns	151.8 ^a	165.4 ^a	5.9	ns	ns
Large intestine (cm)	27.8 ^a	30.0 ^a	25.2 ^a	1.3	ns	28.7 ^a	26.7 ^a	1.1	ns	ns

^{a, b} means in the same row with different superscripts are significantly different ($p < 0.05$); *significant at $p < 0.05$; ns significant at $p < 0.05$; ESM = extruded sorghum meal; SEM = the standard error of the mean.

Table 5.2 Descriptive sensory attribute scores of broiler breast meat as influenced by dietary treatment

Attribute [#]	ESM effect					phytase effect				ESM× phytase
	0%	50%	100%	SEM	<i>p</i> -value	0%	0.035%	SEM	<i>p</i> -value	<i>p</i> -value
Texture										
Initial hardness	4.5 ^a	5.1 ^a	5.2 ^a	0.229	ns	5.1 ^a	4.8 ^a	0.187	ns	ns
Chewiness	5.4 ^b	6.0 ^b	7.1 ^a	0.212	*	6.2 ^a	6.1 ^a	0.173	ns	ns
Cohesiveness	5.7 ^a	6.0 ^a	5.9 ^a	0.209	ns	5.9 ^a	5.8 ^a	0.171	ns	ns
Flavour										
Aroma intensity	5.4 ^b	5.9 ^{ab}	6.3 ^a	0.181	*	6.1 ^a	5.6 ^b	0.147	*	ns
Overall chicken flavour	5.5 ^b	5.9 ^{ab}	6.3 ^a	0.190	*	6.0 ^a	5.8 ^a	0.156	ns	ns
Juiciness										
Moisture release	5.9 ^a	6.2 ^a	6.4 ^a	0.178	ns	6.3 ^a	6.0 ^a	0.145	ns	ns
Sustained impression of juiciness	5.5 ^b	6.1 ^{ab}	6.6 ^a	0.209	*	6.0 ^a	6.1 ^a	0.170	ns	ns
Appearance										
Fibrousness	5.7 ^a	5.9 ^a	6.2 ^a	0.208	ns	6.1 ^a	5.8 ^a	0.170	ns	ns
White colour	5.9 ^a	5.5 ^{ab}	4.9 ^b	0.213	*	5.7 ^a	5.2 ^b	0.174	*	ns

^{a, b} means in the same row with different superscripts are significantly different ($p < 0.05$); [#] scale ranging from 1 to 9, where 1 is lowest intensity and 9 is highest intensity; *significant at $p < 0.05$; ns is not significant at $p < 0.05$; ESM = extruded sorghum meal; SEM = standard error of the mean.



#Scale ranging from 1 to 5 (1 = much too low, 2 = too low, 3 = just about right, 4 = too much, 5 = much too high)

Figure 5.1 Effect of extruded sorghum meal and exogenous phytase enzyme on the JAR scores for broiler sensory attributes

Table 5.3 The correlation coefficients of the sensory attributes on the quantitative descriptive analysis scale rating

	AI	MR	IH	MC	SIJ	CH	F	WC	OCF
Aroma intensity (AI)	1	0.28357*	0.12636 ^{ns}	0.30576*	0.39394*	0.2646*	0.28219*	0.19711*	0.49762*
Moisture release (MR)		1	-0.0631 ^{ns}	0.35556*	0.57661*	0.13171 ^{ns}	0.20279*	-0.0103 ^{ns}	0.16553*
Initial hardness (IH)			1	0.21013*	0.01432 ^{ns}	0.28819*	0.13022 ^{ns}	0.04138 ^{ns}	0.12615 ^{ns}
Mass cohesiveness (MC)				1	0.36529*	0.03797 ^{ns}	0.53942*	0.1329 ^{ns}	0.22175*
Sustained impression of juiciness (SIJ)					1	0.32057*	0.35568*	0.11969 ^{ns}	0.48197*
Chewiness (CH)						1	0.00216 ^{ns}	0.13382 ^{ns}	0.23083*
Fibrousness (F)							1	0.20849*	0.42861*
White colour (WC)								1	0.20375*
Overall chicken flavour (OCF)									1

*significant at $p < 0.05$; ^{ns}not significant at $p < 0.05$

Table 5.4 The correlation coefficients of the sensory attributes on the JAR scale

	Colour	Texture	Tenderness	Juiciness	Taste	Flavour	Acceptability
Colour	1	0.28233*	0.17928*	0.25634*	0.44183*	0.42610*	0.50170*
Texture		1	0.28081*	0.13863 ^{ns}	0.25416*	0.32244*	0.28593*
Tenderness			1	0.14442 ^{ns}	0.16113*	0.26086*	0.20528*
Juiciness				1	0.40055*	0.40203*	0.42987*
Taste					1	0.54344*	0.69405*
Flavour						1	0.59670*
Acceptability							1

*significant at $p < 0.05$; ^{ns}not significant at $p < 0.05$

5.3.1 Carcass Characteristics

As shown in Table 5.2, the ESM significantly affected broiler carcass characteristics, while the exogenous phytase enzyme did not affect the carcass characteristics. The inclusion of ESM above 50% as an energy source in the diet affected slaughter, hot carcass and cold carcass weights, and relative weights of abdominal fat and gizzard.

The reduction in carcass traits was attributed to higher levels of dietary anti-nutritional factors (tannins, kafirins and phytates), which hinder nutrient digestibility. This agrees with the work of Moses et al. (2022), where there was a reduction in hot carcass and cold carcass weights in broilers fed red sorghum-based diets compared to those fed white sorghum-based diets. On the contrary, carcass weights, dressing percentage, and weights of carcass parts were not affected by the partial and total replacement of maize with sorghum in broiler diets (Puntigam et al., 2020). The discrepancy may be because the study by Puntigam et al. (2020) used low-tannin sorghum, unlike this study, where high-tannin sorghum was used.

The abdominal fat of broilers fed 100% ESM-based diets was 48% lower compared to that of broilers fed 50% ESM or maize-based diets. This was attributed to either the lower crude fat or metabolizable energy in ESM compared to maize. Although the diets were isocaloric and isonitrogenous, tannins reduced carbohydrate digestibility in ESM-based diets, which reduced any extra energy that could be stored in the form of abdominal fat in the broilers (Cherian et al., 2002). This had also manifested in lower carcass traits in broilers fed 100% ESM-based diets compared to those fed 50% ESM or maize-based diets. Similarly, Adamu et al. (2012) reported a 77% reduction in abdominal fat in broilers fed sorghum-based diets. Likewise, Ciurescu et al. (2023) reported a 42% reduction in abdominal fat in broilers fed sorghum-based diets. Nevertheless, low-fat chicken meat is considered beneficial to consumers' health. There was an ESM-phytase interaction effect on the abdominal fat of the broilers.

The broilers fed 100% ESM-based diets had relatively heavier gizzards than those fed 50% ESM or maize-based diets. The development of the gizzard is stimulated by larger feed particle sizes (Nir et al., 1994; Yan et al., 2022). During milling, the particle size reduction depends on several factors, including the type of grain and the hardness of the endosperm. Although milling of the experimental diets was done using the same hammer mill under similar conditions, the particle size reduction in maize and ESM may have varied (Amerah et al., 2007). ESM particles were coarser than maize. This observation suggests that during milling, different sieve sizes may be necessary depending on grain type to achieve the desired particle size distribution. The coarseness of feed particles has been found to stimulate an increase in the relative weight of the gizzard in broilers (Idan et al., 2023; Moses et al., 2022; Puntigam et al., 2020; Yan et al., 2022).

The dressing percentage, relative weights of wings, breasts, drumsticks, thighs, liver, heart and the length of the small and large intestines were not affected by the dietary treatments. This finding was consistent with Ciurescu et al. (2023), where the breasts, legs, liver, heart, and small and large intestines were not affected by the inclusion of sorghum in the broiler diets. A similar observation was made in broilers fed reconstituted red-sorghum-based diets (Kumar et al., 2005). Likewise, Elnagar et al. (2014) observed no differences in carcass traits and relative weights of internal organs of broilers at 0, 50, and 100% replacement levels of maize by sorghum in diets. These results indicate a similarity in nutritive value between ESM and maize, although ESM contained anti-nutritional factors like tannins, kafirins and phytates.

5.3.2 Descriptive Sensory Quality

Broiler descriptive sensory attribute scores as influenced by the dietary treatments are summarized in Table 5.5. The just-about-right scores of broiler meat as influenced by the dietary treatments are presented in Figure 5.1. The correlation coefficients of sensory attributes on the quantitative descriptive analysis and JAR scales are presented in Tables 5.4 and 5.5, respectively. The average scores of the descriptive sensory attributes ranged from 4.5 to 7.1, where 1 was the lowest score and 9 was the highest score for the descriptors. The inclusion of ESM above 50% as an energy source in the diets increased the average scores for chewiness, aroma intensity, overall chicken flavour and the sustained impression of juiciness but reduced the white colour intensity of the meat. The incorporation of exogenous phytase enzyme increased average scores for aroma intensity and reduced white colour intensity.

The meat of broilers fed ESM up to 100% as an energy source was perceived as chewier than that of broilers fed ESM up to 50% or maize as an energy source. The toughness of meat is influenced by three types of muscle proteins: connective tissue protein, myofibrils, and sarcoplasmic proteins. The main structural protein in connective tissue, collagen, affects the toughness of meat (Asghar et al., 1985). The amount of collagen in the muscle increases as body fat decreases (Purwanti et al., 2019). The reduction in abdominal fat observed earlier in broilers fed ESM-based diets explains why their meat was chewier than that of broilers fed maize-based diets. This was in agreement with the work of Lyon et al. (2004), where broilers fed sorghum-based diets scored higher for chewiness than those fed maize-based diets.

The average scores for flavour were higher in the meat of broilers fed 100% ESM-based diets than in that of broilers fed 50% ESM or maize-based diets. The JAR scale scores indicated that flavour was 'just about right' but tended towards 'too high' with increased levels of ESM in diets. Coincidentally, the 100% ESM-based diets contained slightly higher levels of fish meal than the 50% ESM and maize-based diets. Fish oils contain a high amount of n-3 polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid (C_{20:5n-3}),

docosapentaenoic acid (C22:5n-3), and docosahexaenoic acid (C22:6n-3), which modify the fatty acid composition of the broiler meat, thus affecting the flavour and oxidative stability of the meat (Enser, 1999). Meat lipids are oxidized during storage, causing off-flavours (cardboard, paint-like, fishy, and warmed-over flavours) that are not liked by consumers (Lawless & Heymann, 2010). Tannins have been found to have antioxidant properties, such as the prevention of superoxide formation and free-radical scavenging activity (Cherian et al., 2002; Yokozawa et al., 2000). Tannins prevented lipid peroxidation during storage, which reduced off-flavours and retained the natural aroma and overall chicken flavour. This was in agreement with other findings (Cherian et al., 2002; Waters et al., 2018). Apart from diet, flavour in meat depends on species, age, fatness, type of tissue, locality, gender, and method of cooking (Oloo, 2021).

The white colour intensity was reduced in the breast meat of broilers fed ESM-based diets. The presence of pigments or compounds such as nitrates and nitrites in diets may reduce the white colour intensity of broiler meat (Smith et al., 2002). In the current study, the reduced white colour intensity was attributed to the presence of a red pigment, 3-deoxyanthocyanidins, in the ESM-based diets (Xiong et al., 2022). These findings agree with those of Puntigam et al. (2020), where the abdominal fat colour of chicken fed sorghum-based diets was darker than that of those fed maize-based diets. Likewise, Smith et al. (2002) observed that broilers fed sorghum-based diets had redder fillets than those fed maize or wheat-based diets. In comparison with lighter-coloured broiler meat, darker-coloured broiler meat performs better during further processing in terms of marination absorption, cook yields, water-holding capacity, and emulsification capacity. Apart from the diet, other factors that affect meat colour include age, pre-slaughter handling (feed withdrawal and stress), and genetics (Smith et al., 2002).

The effect of exogenous phytase on reducing the aroma and white colour intensity was not clear in the current study. Although the enzyme breaks down phytate to liberate bound phosphorus and other minerals, it was not clear if this hydrolysis formed sensory-active molecules. Similar results where the enzyme influenced the sensory quality of meat were found by Yasar et al. (2018).

The dietary treatments did not affect the average scores for initial hardness, cohesiveness, moisture release and fibrousness of the broiler meat. Inclusion of ESM up to 50% did not affect all sensory attributes analysed. These results indicate that the meat of broilers fed 50% ESM as an energy source had similar descriptive sensory attributes scores to that of broilers fed maize-based diets.

The just-about-right scale scores showed that all sensory attributes evaluated were ‘just about right’. Thus, the dietary treatments did not affect the panellists’ acceptability of the colour, texture, tenderness, juiciness, taste, flavour and overall acceptability of the broiler meat. The JAR scores provided more information on how panellists responded to the descriptive sensory attributes of the broiler meat (Oloo, 2021).

On the quantitative descriptive analysis, the most notable correlations were between moisture release and the sustained impression of juiciness ($r = 0.58$). The two are descriptors of juiciness. The mass cohesiveness and fibrousness were also correlated ($r = 0.54$). On the JAR scale, overall acceptability was correlated to taste ($r = 0.69$), flavour ($r = 0.60$), and colour ($r = 0.50$). Thus, taste, flavour, and colour were the most important attributes relied upon by the panellists to decide on the acceptability of the broiler meat. Flavour and taste were closely correlated (0.54). The colour was correlated with all the sensory attributes analysed, thus influencing the way the panellists perceived the other attributes (Lawless & Heymann, 2010).

5.4 Conclusion

Inclusion of ESM as an energy source of up to 50% and the incorporation of exogenous phytase enzyme did not affect broiler carcass characteristics (internal and external parts) or descriptive sensory quality.

5.5 Recommendation

ESM with exogenous phytase enzyme can conveniently replace up to 50% of maize in broiler diets without adversely affecting broiler carcass characteristics or descriptive sensory quality.

CHAPTER SIX

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

6.1 Discussion

The exploitation of locally available, underutilized, high-value and climate-smart crops has been proposed as one of the strategies to attain food and nutritional security (Orr et al., 2016; Rashwan et al., 2021; Selle et al., 2010a). This is particularly prevalent in developing countries, which are often faced with the challenges of food insecurity, malnutrition and climate change. Sorghum is considered a food or feed security crop due to its similar nutrient composition to that of maize, its ability to cope well in drier areas where maize production is not feasible, and its resistance to insect and mould attacks (Orr et al., 2016). The utilization of sorghum grain as an energy source to replace maize is identified as one approach to promoting sustainable poultry production amidst feed shortages exacerbated by climate change. The content of anti-nutritional factors in sorghum grain, such as tannins, kafirins and phytates, lowers its digestibility in broiler chicken. Extrusion cooking is one of the most effective feed processing methods for inactivating anti-nutritional factors and enhancing the digestibility of feeds (Campelo et al., 2020; D'Almeida et al., 2021). However, the effect of feed moisture content, screw speed and barrel temperature on the digestibility of extruded sorghum meal and the effect of extruded sorghum meal and exogenous phytase-based diets on broiler chicken performance, carcass characteristics and descriptive sensory quality are not clear. This study involved a two-step approach where, in the first step, the effect of different extrusion cooking variables on *in vitro* dry matter digestibility (IVDMD) of extruded sorghum meal (ESM) was studied. In the second step, sorghum flour was extruded using a combination of extrusion cooking variables that resulted in the highest IVDMD. In a nutrition study, ESM was included in broiler chicken diets with or without exogenous phytase enzyme to determine:

- i. The effect of inclusion levels of extruded sorghum meal as an energy source with or without exogenous phytase enzyme on the average daily feed intake, average daily gain and feed conversion ratio of broiler chicken
- ii. The effect of inclusion levels of extruded sorghum meal as an energy source with or without exogenous phytase enzyme on broiler chicken carcass characteristics (internal and external parts) and descriptive sensory quality

The first objective was achieved using *in vitro* experiments, while the other objectives were achieved through *in vivo* experiments. Hypothesis testing involved analysis of variance using the general linear model (GLM) of the SAS Institute Inc. (version 9.4; 2015).

6.1.1 Determining the Effect of Feed Moisture Content, Screw Speed and Barrel Temperature on the *in vitro* Dry Matter Digestibility of Extruded Sorghum Meal

Feed manufacturers are more interested in *in vitro* digestibility methods because they are quicker, more reliable and inexpensive for accurately predicting the digestibility of raw or processed feed ingredients and by-products for a specific class of livestock compared to *in vivo* digestibility methods. Moreover, *in vitro* digestibility methods offer more information on the effect of specific factors on nutrient digestibility compared to *in vivo* digestibility methods (Moyano et al., 2015). In this study, the digestive process in chicken was simulated to precisely predict the effect of extrusion cooking variables on the IVDMD of ESM in chicken. The results showed that a high IVDMD of ESM was achieved by reducing the feed moisture content and increasing screw speed and barrel temperature.

6.1.2 Determining the Effect of Inclusion Levels of Extruded Sorghum meal as an Energy Source with or without Exogenous Phytase Enzyme on the Average Daily Feed Intake, Average Daily Gain and Feed Conversion Ratio of Broiler Chicken

In Chapter four, the results indicated that in the grower phase, the inclusion of extruded sorghum meal up to 50% did not affect the feed conversion ratio of broiler chicken. On the other hand, the incorporation of exogenous phytase enzyme in diets enhanced broiler chicken performance in the grower phase. Inclusion of ESM as an energy source of up to 50% with exogenous phytase enzyme in diets did not affect the average daily feed intake, average daily gain and feed conversion ratio of broiler chicken. The implication for feed manufacturers in developing countries, such as Kenya, that are more vulnerable to food/feed insecurity and climate change challenges is to include ESM up to 50% as an energy source with exogenous phytase enzyme to replace maize grain in commercial broiler diets. This will promote sustainable broiler production and promote food and nutritional security amidst the challenges of global climate change.

6.1.3 Determining the Effect of Inclusion Levels of Extruded Sorghum Meal as an Energy Source with or without Exogenous Phytase Enzyme on Broiler Chicken Carcass Characteristics (internal and external parts) and Descriptive Sensory Quality

In chapter five, the inclusion of extruded sorghum meal as an energy source of up to 50% and the incorporation of exogenous phytase enzyme did not affect broiler chicken carcass characteristics or descriptive sensory quality. The inclusion of extruded sorghum meal at 100% reduced the slaughter weights, hot carcass weights, cold carcass weights, and abdominal fat while increasing the relative weight of the gizzard. Moreover, it increased average scores for flavour as scored by the panellists. These results present a case for the promotion of large-scale

sorghum production. This can be achieved through the provision of quality seeds, improved agronomic practices, mechanization, and provision of inputs and services that are climate-smart. The commercialization of sorghum production will create a market and consumer demand to benefit the key stakeholders in the sorghum value chain. Extrusion cooking as a form of value addition, together with other processing technologies, is envisaged to create diversified sorghum products.

6.2 Conclusions

Based on the results of this study, it was concluded that:

- i. The *in vitro* dry matter digestibility of extruded sorghum meal is highest at 40% feed moisture content, 300 rpm screw speed and 90°C barrel temperature
- ii. The inclusion of extruded sorghum meal as an energy source in broiler diets up to 50% with exogenous phytase enzyme did not affect the average daily feed intake, average daily gain and feed conversion ratio of broiler chicken
- iii. The inclusion of extruded sorghum meal as an energy source of up to 50% and incorporation of exogenous phytase enzyme in broiler diets did not adversely affect broiler chicken carcass characteristics (internal and external parts) or descriptive sensory quality.

6.3 Recommendations

Based on the results of this study, it was recommended that:

- i. During extrusion cooking of sorghum meal, the feed moisture content, screw speed and barrel temperature should be 40%, 300 rpm and 90°C, respectively, for high *in vitro* dry matter digestibility
- ii. Extruded sorghum meal should be included as an energy source up to 50% with 0.035% exogenous phytase enzyme in diets to avoid adverse effects on the average daily feed intake, average daily gain and feed conversion ratio of broiler chicken
- iii. Extruded sorghum meal should be included as an energy source of up to 50% with 0.035% exogenous phytase enzyme in broiler diets to avoid adverse effects on broiler chicken carcass characteristics (internal and external parts) and descriptive sensory quality.

6.3 Further Research

- i. To determine the effect of other extrusion cooking variables, such as die geometry, feed rate, and specific mechanical energy, on the IVDMD of extruded sorghum meal.

- ii. To determine the effect of extruded sorghum meal and exogenous phytase-based diets on the performance of commercial layer chicken.
- iii. To determine the effect of extruded sorghum meal and exogenous sources of meat flavour on the consumer acceptability of broiler chicken meat.

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APPENDICES

Appendix A. National Commission for Science Technology and Innovation Research Permit



 REPUBLIC OF KENYA



NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY & INNOVATION

RefNo: 282279
Date of Issue: 04/May/2023

RESEARCH LICENSE



This is to Certify that Mr. VICTOR Mbulu Mutinda of Egerton University, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Nakuru on the topic: EFFECT OF SORGHUM (Sorghum bicolor (L.) Moench) EXTRUDATE AND PHYTASE ENZYME ON PERFORMANCE OF BROILER CHICKEN for the period ending : 04/May/2024.

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Appendix B. Egerton University Research Ethics Permit

EGERTON

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**EGERTON UNIVERSITY INSTITUTIONAL SCIENTIFIC AND ETHICS
REVIEW COMMITTEE**

EU/RE/DIR/009

Approval No. EUISERC/APP/224/2023

10th March, 2023

Victor Mbuku Mutinda
Department of Animal Sciences
Egerton University
Telephone +254 0724461833
E-mail: victormutinda497@gmail.com

Dear Victor,

**RE: ETHICAL APPROVAL: EFFECT OF SORGHUM (*Sorghum bicolor* L.) GRAIN
EXTRUDATE AND PHYTASE ENZYME ON PERFORMANCE OF BROILER
CHICKEN**

This is to inform you that *Egerton University Institutional Scientific and Ethics Review Committee* has reviewed and approved your above research proposal. Your application approval number is EUISERC/APP/224/2023. The approval period is 10th March, 2023 – 11th March, 2024

This approval is subject to compliance with the following requirements;

- i. Only approved documents including (informed consents, study instruments, MTA) will be used.
- ii. You are required to adhere Institutional Experimental Animals use and Care policy.
- iii. All changes including (amendments, deviations, and violations) are submitted for review and approval by *Egerton University Institutional Scientific and Ethics Review Committee*.
- iv. Death and life-threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to *Egerton University Institutional Scientific and Ethics Review Committee* within 72 hours of notification
- v. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to *Egerton University Institutional Scientific and Ethics Review Committee* within 72 hours.

“Transforming Lives through Quality Education”

- vi. Clearance for Material Transfer of biological specimens must be obtained from relevant institutions.
- vii. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
- viii. Submission of an executive summary report within 90 days upon completion of the study to *Egerton University Institutional Scientific and Ethics Review Committee*.

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) <https://oris.nacosti.go.ke> and also obtain other clearances needed.

Yours sincerely,

Prof. Raphael M. Ngure
**CHAIRMAN, EGERTON UNIVERSITY INSTITUTIONAL SCIENTIFIC AND ETHICS
REVIEW CTTEE**
RMN/BK/



Appendix C. Research Pictorial



A) Milling of raw sorghum grain



B) Twin-screw extruder



C) Extruded sorghum meal



D) IVDM incubation



E) Experimental broiler chicken



F) Measurement of abdominal fat weight

Appendix D. Pre-screening questionnaire for broiler meat quality sensory analysis

1. Name: _____
2. Phone Number: _____
3. Are there any weekdays (Monday- Friday) that you will not be available regularly?

4. Do you have any of the following? Dentures _____ Diabetes _____ Oral or gum disease _____ Hypoglycaemia _____ Food allergies _____ Hypertension _____
5. Do you take any medications that affect the senses, especially taste and smell?

6. Are you currently on a restricted diet? If yes, explain.

7. How often in a month do you eat broiler meat? _____
8. What is (are) your favourite food(s)? _____
9. What is (are) your least favourite food(s)? _____
10. What foods can you not eat? _____
11. What foods do you not like to eat? _____
12. Is your ability to distinguish smell and taste: Above average _____ Average _____ Below average _____
13. If a recipe calls for vinegar and there is none available, what would you substitute?

14. Are you familiar with food products termed 'broiler meat'? _____
15. How would you describe the difference between flavour and aroma?

16. How would you describe the difference between flavour and texture?

17. What is the best one- or two-word description of cookies?

18. Describe some of the noticeable flavours in roasted nuts.

19. Describe some of the noticeable flavours in

foods _____

20. Is your sensitivity to textural characteristics in foods above average _____

average _____ or below average _____

21. Describe some of the textural properties of foods in general.

22. Describe some of the particles one finds in foods.

23. Describe some of the properties that are apparent when one chews on broiler meat.

24. Describe the differences between spongy and rubbery.

25. What are some textural properties of potato chips?


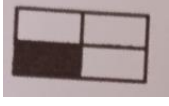



26. What are some textural properties of biscuits?

27. What are some textural properties of toffee/eclairs?

28. For what types of products is texture important?

29. Instructions: Mark the line at the right to indicate the proportion of the area that is shaded.

EXAMPLES

	None -----/-----All
	None -----/-----All
<p>3.</p> 	None-----All
<p>4.</p> 	None-----All
	None -----All

Contract

I _____ agree to be a panellist for **DESCRIPTIVE QUALITY SENSORY ANALYSIS OF BROILER MEAT** for one week.

I agree to comply with the terms and conditions.

Signature:

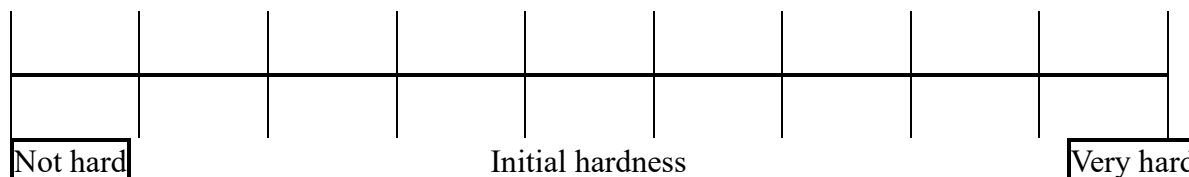
Date:

Appendix E. Quantitative descriptive analysis graphic line scale used for broiler sensory evaluation

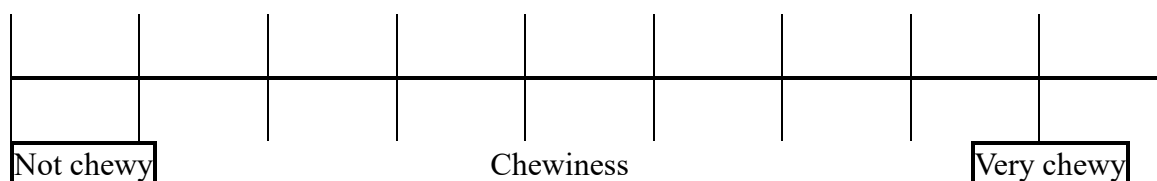
Panelist code: _____ **Name of panelist:** _____ **Date:** _____

You are provided with six coded cooked broiler meat samples (BCM, OMW, ZWK, PNK, KHX, and XYP). Kindly score your judgement of the attributes listed on the graphic line scale provided for each sample.

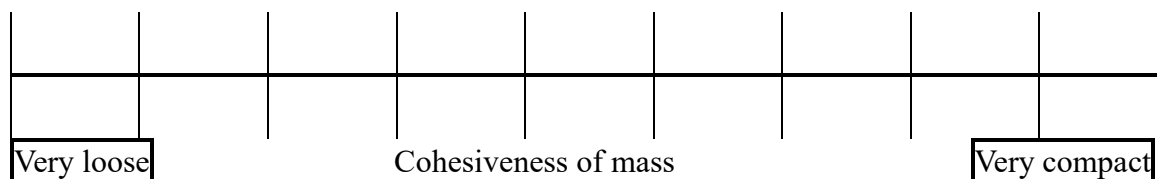
1. How much force did you use to compress the sample during the initial chewing?



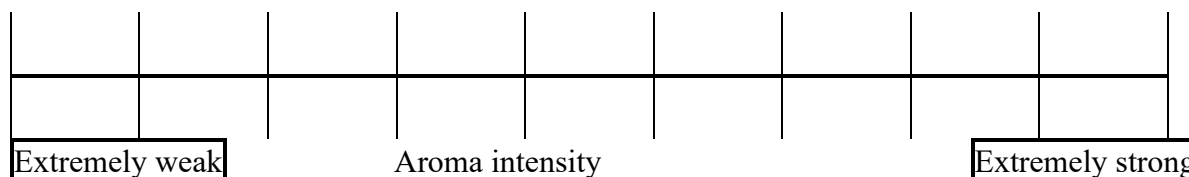
2. How much work did you require to chew the sample to the point of swallowing it?



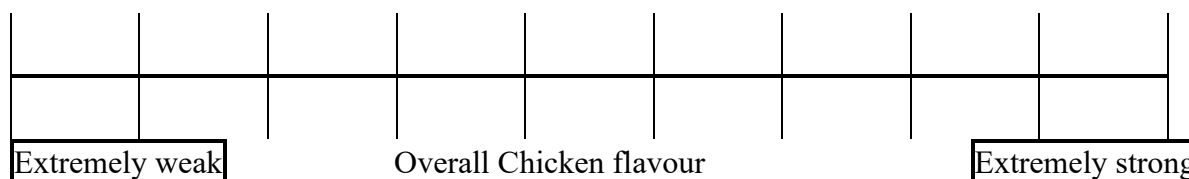
3. To what degree did the chewed sample hold together in a wad?



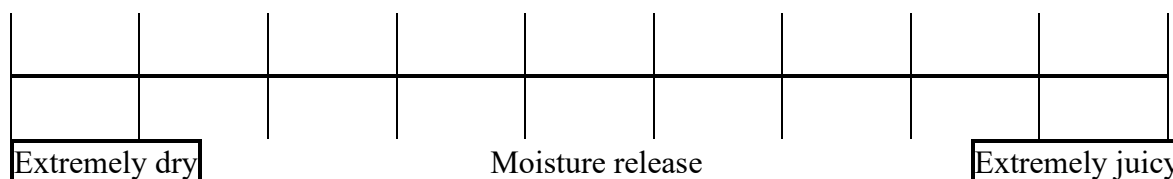
4. How much aromatic taste sensation associated with a meat stock is in the sample?



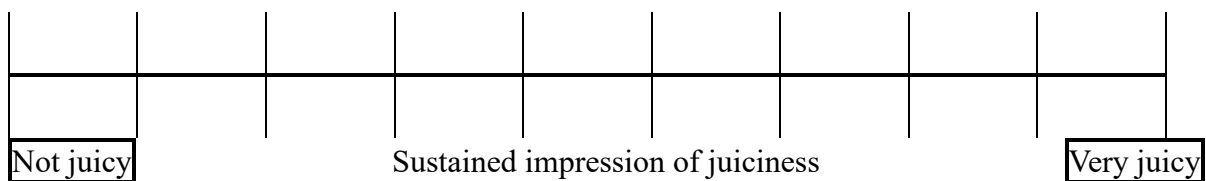
5. How much cooked chicken-meat flavour is in the sample?



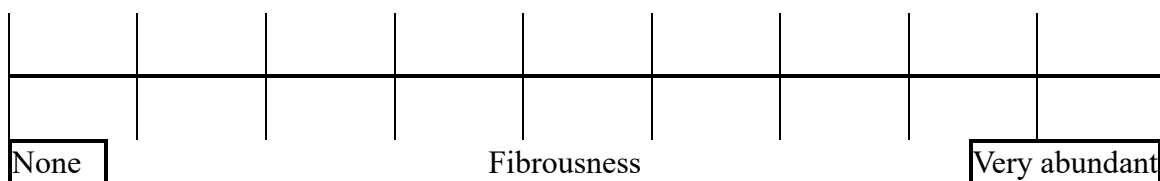
6. How much wetness did you feel in the mouth after the first two bites?



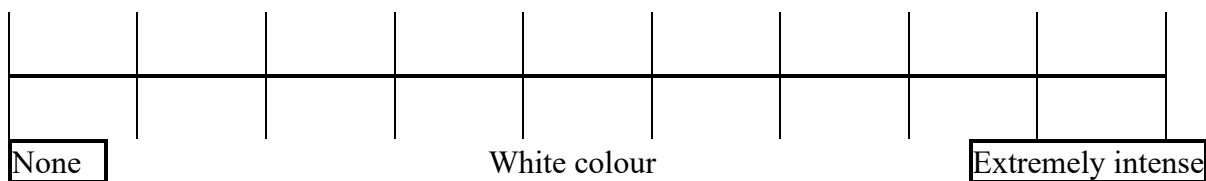
7. How much wetness did you feel in the mouth after 10-12 bites?



8. How much fibrousness appears on the cutting surface of cooked meat?



9. How intense is the white surface colour of the cooked meat?



Appendix F. Just-about-right sensory analysis scoring form used for broiler sensory evaluation

Panelist code: _____ Name of panelist: _____ Date: _____

You are provided with six coded samples (BCM, OMW, ZWK, PNK, and KHX). Kindly score and record each sample as per your judgement of the attributes listed on the left side of the table in the appropriate box. (1 much too low, 2 too low, 3 just about right, 4 too high, 5 much too high).

Attribute	Just-about-right scale	Sample codes					
		BCM	OMW	ZWK	PNK	KHX	XYP
Colour	1 = much too low, 2 = too low, 3 = just about right, 4 = too high, 5 = much too high						
Texture	1 = much too low, 2 = too low, 3 = just about right, 4 = too high, 5 = much too high						
Tenderness	1 = much too low, 2 = too low, 3 = just about right, 4 = too high, 5 = much too high						
Juiciness	1 = much too low, 2 = too low, 3 = just about right, 4 = too high, 5 = much too high						
Taste	1 = much too low, 2 = too low, 3 = just about right, 4 = too high, 5 = much too high						
Flavor	1 = much too low, 2 = too low, 3 = just about right, 4 = too high, 5 = much too high						
General acceptability	1 = much too low, 2 = too low, 3 = just about right, 4 = too high, 5 = much too high						
Comment (if any):							

Appendix G. Sensory attributes and definitions used in descriptive sensory analysis of the broiler meat

Attribute	Definition	Anchor terminology
<u>Texture</u>		
Initial hardness	The force required to compress the sample during initial chewing	Not hard to very hard
Chewiness	Amount of work to chew the sample to the point of swallow (or spit out)	Not chewy to very chewy
Cohesiveness of mass	Degree the chewed sample holds together in a wad	Very loose to very compact
<u>Flavour</u>		
Aroma intensity	Aromatic taste sensation associated with meat stock	Extremely weak to extremely strong
Overall chicken flavour	Cooked chicken meat flavour	Extremely weak to extremely strong
<u>Juiciness</u>		
Moisture release	The amount of wetness felt in the mouth after the first two bites	Extremely dry to extremely juicy
Sustained impression of juiciness	The amount of wetness felt in the mouth after 10-12 bites	Not juicy to very juicy
<u>Appearance</u>		
Fibrousness	The appearance of texture on the cutting surface of cooked meat	None to very abundant
White colour	White surface colour of cooked meat	None to extremely intense

Appendix H. Analysis of variance (ANOVA) tables of broiler performance in the overall period

Dependent Variable: ADFI

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	1491.961378	298.392276	22.62	<.0001
Error	12	158.279267	13.189939		
Corrected total	17	1650.240644			

R-square	Coeff Var	Root MSE	ADFI mean
0.90409	14.13456	3.631796	25.69444

Source	DF	Type I SS	Mean Square	F value	Pr > F
ESM	2	1460.012478	730.006239	55.35	<.0001
Phytase	1	6.148356	6.148356	0.47	0.5077
ESM*Phytase	2	25.800544	12.900272	0.98	0.4041

Source	DF	Type III SS	Mean Square	F value	Pr > F
ESM	2	1460.012478	730.006239	55.35	<.0001
Phytase	1	6.148356	6.148356	0.47	0.5077
ESM*Phytase	2	25.800544	12.900272	0.98	0.4041

Dependent Variable: ADG

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	306.2697111	61.25394222	14.42	0.0001
Error	12	50.9781333	4.2481778		
Corrected total	17	357.2478444			

R-square	Coeff Var	Root MSE	ADG mean
0.8573	25.0913	2.061111	8.214444

Source	DF	Type I SS	Mean Square	F value	Pr > F
ESM	2	289.5525778	144.7762889	34.08	<.0001
Phytase	1	1.8304222	1.8304222	0.43	0.5239
ESM*Phytase	2	14.8867111	7.4433556	1.75	0.215

Source	DF	Type III SS	Mean Square	F value	Pr > F
ESM	2	289.5525778	144.7762889	34.08	<.0001
Phytase	1	1.8304222	1.8304222	0.43	0.5239
ESM*Phytase	2	14.8867111	7.4433556	1.75	0.215

Dependent Variable: FCR

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	6.18459705	1.23691941	6.64	0.0035

Error	12	2.23399482	0.18616623		
Corrected total	17	8.41859187			
	R-square	Coeff Var	Root MSE	FCR mean	
	0.73464	12.47789	0.43147	3.457874	
Source	DF	Type I SS	Mean Square	F value	Pr > F
ESM	2	4.31447023	2.15723511	11.59	0.0016
Phytase	1	0.10265358	0.10265358	0.55	0.472
ESM*Phytase	2	1.76747324	0.88373662	4.75	0.0303
Source	DF	Type III SS	Mean Square	F value	Pr > F
ESM	2	4.31447023	2.15723511	11.59	0.0016
Phytase	1	0.10265358	0.10265358	0.55	0.472
ESM*Phytase	2	1.76747324	0.88373662	4.75	0.0303

Tukey's Studentized Range (HSD) Test for ADFI

Alpha	0.05		
Error Degrees of Freedom	12		
Error Mean Square	13.18994		
Critical Value of Studentized Range	3.77278		
Minimum Significant Difference	5.5938		
Means with the same letter are not significantly different.			
Tukey			
Grouping	Mean	N	ESM
A	36.902	6	0
B	25.332	6	50
C	14.85	6	100

Tukey's Studentized Range (HSD) Test for ADG

Alpha	0.05		
Error Degrees of Freedom	12		
Error Mean Square	4.248178		
Critical Value of Studentized Range	3.77278		
Minimum Significant Difference	3.1746		
Means with the same letter are not significantly different.			
Tukey			
Grouping	Mean	N	ESM
A	13.487	6	0
B	7.39	6	50
C	3.767	6	100

Tukey's Studentized Range (HSD) Test for FCR

Alpha	0.05		
Error Degrees of Freedom	12		
Error Mean Square	0.186166		
Critical Value of Studentized Range	3.77278		

Minimum Significant Difference			0.6646
Means with the same letter are not significantly different.			
Tukey			
Grouping	Mean	N	ESM
A	3.966	6	100
A	3.6111	6	50
B	2.7965	6	0

Tukey's Studentized Range (HSD) Test for ADFI

Alpha			0.05
Error Degrees of Freedom			12
Error Mean Square			13.18994
Critical Value of Studentized Range			3.08132
Minimum Significant Difference			3.7302
Means with the same letter are not significantly different.			
Tukey			
Grouping	Mean	N	Phytase
A	26.279	9	0.035
A	25.11	9	0

Tukey's Studentized Range (HSD) Test for ADG

Alpha			0.05
Error Degrees of Freedom			12
Error Mean Square			4.248178
Critical Value of Studentized Range			3.08132
Minimum Significant Difference			2.117
Means with the same letter are not significantly different.			
Tukey			
Grouping	Mean	N	Phytase
A	8.5333	9	0.035
A	7.8956	9	0

Tukey's Studentized Range (HSD) Test for FCR

Alpha			0.05
Error Degrees of Freedom			12
Error Mean Square			0.186166
Critical Value of Studentized Range			3.08132
Minimum Significant Difference			0.4432
Means with the same letter are not significantly different.			
Tukey			
Grouping	Mean	N	Phytase
A	3.5334	9	0
A	3.3824	9	0.035

Level of ESM	Level of Phytase	N	ADFI		ADG		FCR	
			Mean	StdDev	Mean	StdDev	Mean	StdDev
0	0	3	36.4633	6.86522	13.7533	3.57503	2.68576	0.20952
0	0.035	3	37.34	2.18995	13.22	2.77512	2.90727	0.64058
50	0	3	23.2133	3.63478	5.78667	1.72344	4.12797	0.59631
50	0.035	3	27.45	2.75303	8.99333	1.21344	3.09419	0.53962
100	0	3	15.6533	2.22019	4.14667	0.69292	3.78645	0.12354
100	0.035	3	14.0467	1.22165	3.38667	0.29006	4.1456	0.02665

Appendix I. Publication abstracts

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EFFECT OF EXTRUSION COOKING VARIABLES ON IN VITRO DRY MATTER DIGESTIBILITY OF SORGHUM [*Sorghum Bicolor* (L.) Moench] EXTRUDATE

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ABSTRACT

Sorghum grain can potentially be a high-quality energy source in non-ruminant diets. However, its digestibility is constrained by endogenous anti-nutritional factors. This study investigated the effect of extrusion cooking variables on in vitro dry matter digestibility (IVDMD) of sorghum extrudate. The study followed a (3×2×2) factorial arrangement in a completely randomized design, each with three replicates, to determine the effect of feed moisture content, barrel temperature, and screw speed on IVDMD of sorghum extrudate using a co-rotating twin screw extruder. According to preliminary trials, the feed moisture content was between 40 to 50% (wet basis), the barrel temperature was 70 and 90 °C, and the screw speed was 280 and 300 rpm. The IVDMD of sorghum extrudate increased as feed moisture content and screw speed decreased. Among the tested range of extrusion cooking variables in this study, a combination of low feed moisture content (40%), high screw speed (300 rpm) and high barrel temperature (90 °C) resulted in high IVDMD. The study demonstrated that feed moisture content and screw speed are important extrusion cooking variables that can significantly influence the IVDMD of sorghum extrudates.

Keywords: *Anti-nutritional factors, barrel temperature, high-tannin sorghum, screw speed, twin screw extruder*



PERFORMANCE OF BROILERS FED ON EXTRUDED SORGHUM (*Sorghum bicolor* (L.) Moench) MEAL AND EXOGENOUS PHYTASE-BASED DIET

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ABSTRACT

Sorghum (*Sorghum bicolor* (L.) Moench) is more drought-resilient and adapted to varying soil types, and its grain has a similar nutrient composition to maize. However, some varieties contain high levels of tannins, kafirins, and phytates which adversely affect broiler performance. This study investigated the effect of extruded sorghum meal (ESM) and exogenous phytase on the performance of broilers. In total, 108, mixed-sex, Cobb 500, day-old broiler chicks were used. The chicks were weighed in groups of six and randomly assigned to cages and each one of the six dietary treatments comprising; T1 (0% ESM + 0% phytase), T2 (0 % ESM + 0.035 % phytase), T3 (50 % ESM + 0% phytase), T4 (50 % ESM + 0.035 % phytase), T5 (100 % ESM + 0 % phytase), and T6 (100 % ESM + 0.035 % phytase). The grower diets were offered 1-21 d and finisher diets 22-42 d. The average daily feed intake, average daily gain and feed efficiency were recorded weekly. ESM at a 50% inclusion did not affect the feed efficiency at the grower phase. The exogenous phytase enzyme improved ($p < 0.05$) the average daily feed intake and average daily gain in the grower phase. In conclusion, ESM adversely affected broiler performance while the incorporation of exogenous phytase enzyme in the feed enhanced the performance of broilers in the grower phase.

Keywords- Extrusion Cooking, Feed Efficiency, High-Tannin Sorghum, Kafirin, Phytates