

**EFFECT OF FEEDING GERMINATED BROWN SORGHUM AND METHIONINE  
SUPPLEMENTATION ON PERFORMANCE OF BROILER CHICKEN**

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A thesis submitted in partial fulfillment for the requirement of the degree of master science in animal nutrition and feed Science in the Faculty of Veterinary Medicine, Department of Animal

Production

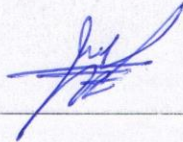
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## DECLARATION

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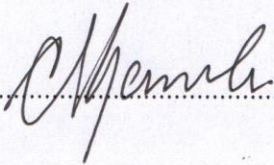
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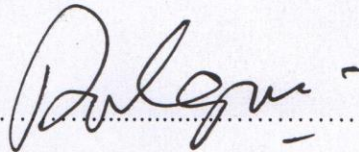
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## **DEDICATION**

I dedicate this work to my late father Mamur Bako and my mother Christina Kani, who has been my supporter and spiritual pillar during my study. To my children Anita and Emmanuel.

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First of all I would like to give all the glory, honor and praise to almighty God who has enabled me to complete my studies. I would like to give countless thanks to my spiritual mentor T. B. Joshua for his teaching that strengthens my desire to God and determination in life.

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## **LIST OF ABBREVIATIONS**

ANF	Anti-nutritional factor
ANOVA	Analysis of variance
AOAC	Association of official analytical chemists
ASALS	Arid and semi arid lands
CRD	Completely randomized design
DNA	Deoxyribonucleic acid
FAO	Food and Agriculture of the United States
GIT	Gastro intestinal tract
GDP	Gross domestic product
GOK	Government of Kenya
KEPOFA	Kenya poultry farmers association
KIPPRA	Kenya institute for public policy research and analysis
TBAs	Tannin biding agents
Masl	Meters above sea level
MDG's	Millennium development goals
MOALF	Ministry of agriculture livestock and fisheries

mRNA	Messenger ribo nucleic Acid
NRC	National research council
O.E.C.D	Organization for Economic Co-operation and Development
OIE	World organization for animal health
ROS	Reactive oxygen species
UCP	Uncoupling protein

## **ABSTRACT**

The study investigated the effects of germination on chemical composition and tannin content of brown sorghum (*var. Serena*) as well as feeding germinated and ungerminated Serena sorghum based diets to broiler chickens for a period of six weeks. The grains were soaked in water for 12 h and thereafter germinated for 48 h. One hundred and sixty day old Arbor Acres chicks were allocated to four isocaloric and isonitrogenous diets containing: ungerminated sorghum + 0.46% Methionine (Met) (UG46), germinated sorghum + 0.46% Met (G46), ungerminated sorghum + 0.69% Met (UG69) and ungerminated sorghum + 0.92% methionine (UG92). Germination slightly increased the crude protein content of Serena sorghum (9.9 to 10.3%). Germination reduced ( $p > 0.05$ ) the tannin content of the Serena variety from 0.47 to 0.32% TA. Birds fed the G46 diet had lower ( $P < 0.05$ ) feed intake during starter phase. Germination had no effect on weight gain, but improved ( $P < 0.05$ ) feed conversion ratio (FCR). Birds fed the UG69 diet decreased feed intake only at starter phase but had the highest ( $P < 0.05$ ) weight gain during starter and whole growth period (785.1 and 2258 g/chick). Increased methionine in the diet UG92 improved weight gain only during starter phase but decreased ( $p < 0.05$ ) feed intake in starter and finisher phases. Birds fed the diets UG69 and UG92 had lower ( $p < 0.05$ ) FCR. In conclusion, germination of the Serena sorghum had no effect on performance of broiler chickens. Supplementation of Serena sorghum based diets with 50% methionine above the recommended improved the performance of broilers with no beneficial effect beyond level.

**Keywords:** - Sorghum, Tannin, Germination, Methionine, Broiler chickens

## CHAPTER ONE: INTRODUCTION

### 1.1 Background information

Poultry meat accounts for 87% of global meat production (FAO, 2010) and has become the second highest source of animal protein consumed worldwide after pork (Valceschini, 2006). It is rich in proteins, phosphorus and other minerals as well as vitamin B-complex (Omiti and Okuthe, 2008). In addition, the poultry sector plays a very important role in the economy of East African countries where it employs two to three million people (USAID, 2010).

Agriculture in Kenya contributes 25 to 26% of the Gross Domestic Product (GDP) with the poultry sector contributing 1.6% of this (Nyaga, 2007; Njoroge *et al.*, 2015). According to USAID (2010), Kenya has a poultry population of 37 million birds of which 84% (31million) are indigenous chicken, 8.3% (3.1million) are layers, 5.7% (2.1 million) are broiler chicken while other species such as ducks, quails, turkeys, pigeons, ostriches, and guinea fowls accounted for 1.8% (0.7 million). Kenya is known to have a fast population growth rate and rapid urbanization. In 2010, the country's population and urbanization percentage were 40.65 million people and 9.0% respectively (USAID, 2010). These figures increased to 46.17 million and 11.1% respectively in 2015, and are projected to reach 51.69 million and 13.7% in that order by 2020. Coupled with this rapid urbanization is an increase in the middle class (BLGG Group, 2013). As a result of increased employment opportunities and per capita income for middle income consumers, there is and will be increased demand for higher-value food of animal origin such as milk, meat and eggs. Consequently, there is need to identify ways of increased production of inexpensive poultry food products.



Maize currently is the main energy source in most of the manufactured poultry feeds in Kenya (Kumaravel *et al.*, 2014) and yet remains the main staple food creating great competition between animal feed and human food. As a result of this competition, manufactured poultry feeds are expensive and at times of maize shortage, they become unavailable. As such, there is need to use alternative cereal based energy sources. Approximately 84% of the Kenyan land mass is either arid or semi-arid and is characterized by erratic rainfall (Mwadalu and Mwangi, 2013) and high ambient temperatures of more than 38°C for most part of the year ( Medugu *et al.*, 2010). These conditions are suitable for sorghum production due to its high drought tolerance compared to other cereals and its ability to withstand periods of high temperature.

Sorghum can grow well in areas where the annual rainfall is in the range of 500-700mm per year (Olembo *et al.*, 2010). The only constraint limiting the use of sorghum in poultry feeds is presence of tannins which affect its utilization thus performance. Effects of tannin in chicken diets have been reported as reduction in feed intake due to reduced palatability, reduced live weight gain, low digestibility and poor feed conversion efficiency (Hassan *et al.*, 2003; Oke *et al.*, 2015). Reduction in tannin content in sorghum can lead to its increased usage in poultry feeds without adverse effects on performance.

Tannin levels in sorghum vary among varieties. Melingasuk *et al.* (2012) reported tannin content of 0.15% as Catechin Equivalent (CE) in white variety of sorghum while Abbas and Musharaf (2008) reported 0.34% (CE) in white sorghum. Etuk and Ukaejiofo, (2007) indicated that brown coat colored sorghum contains 0.42% tannin while Osuntogun *et al.* (1989) reported 2.92% (CE) tannin in red coat colored sorghum. Processing techniques to reduce tannin content in sorghum based poultry rations to increase efficiency of feed utilization have been reported. These strategies are commonly classified as physical methods which involve cooking, dehulling,

autoclaving, toasting/roasting (Medugu *et al.*, 2012) and biochemical methods such as soaking and germination (Chavan *et al.*, 1989). Other strategies are classified as chemical methods such as the use of enzymes, wood ash, addition of fat (tallow), urea treatment and use of Tannin Biding Agents (TBAs) (Douglas *et al.*, 1990; Ambula *et al.*, (2001); Kyarisiima *et al.*, 2004; Elnagar and Abdel-Wareth, 2014).

However, the use of a particular method will depend upon the cost involved and effectiveness to reduce the negative effects of tannin in poultry feeds. Germination of sorghum grain has been reported to be an effective treatment to reduce its tannin content. According to Elmaki *et al.* (1999), the tannin content of sorghum grain reduced from 1.44 to 0.31% and from 0.32 to 0.20% CE (Catechin Equivalent) in high and low tannin sorghum after 48 h germination. Ogbonna *et al.* (2012) observed that tannin content reduced by 8.45%, while (Badi, 2004) reported reduction in tannin content by 73.69%.

Methionine, when used as dietary supplement, has alleviated the adverse effect of tannin in sorghum–soybean meal based diet (Elkin *et al.*, 1978). Though the first limiting amino acid in sorghum is methionine (Gomez and Angeles, 2016), other suggested mechanisms are via either being an essential nutrient, playing a big role as sulfur containing amino acid in methyl donor metabolism (Brosnan and Brosnan, 2006) or both. Therefore this study aims to determine the impact of germination on tannin content of local sorghum variety (Serena) and the effects of feeding germinated sorghum (high tannin content) and supplementation with methionine on performance of broiler chicken.

## 1.2 Problem statement

Only one-third of the total land mass of Kenya receives adequate rain, the other two-thirds being semi-arid to arid, which cannot favour maize production due to its high moisture requirement during the period of flowering to beginning of grain filling (Omoyo *et al.*, 2015). The national maize production ranges between 2.8 million metric tons per annum and does not match the consumption level of 4 million metric tons (estimated in 2014), due to population growth estimated at 2.7% per annum which has put pressure on land (Kariuki, 2015). Production is also constrained by drought, low soil fertility and pests (Gitonga, 2016). These factors result in high price of maize grain in Kenya compared to other countries in eastern and southern Africa region (Ariga *et al.*, 2010; Kang'ethe, 2011). The huge deficit in maize has encouraged importation from neighboring and other countries (Kariuki, 2015) to meet needs for both human food and animal feed industries. In Kenya, maize is the main staple food accounting for 65% of total staple food caloric intake (Ariga *et al.*, 2010), with an annual per capita consumption rate of 89kg (Drive *et al.*, 2009).

Maize is also a major source of energy in poultry production (Kumaravel *et al.*, 2014), which creates the serious competition for maize between human food and animal feed (Mohamed *et al.*, 2015). This competition has resulted in high price of poultry feeds and since feed accounts for 60-70% (Dolberg, 2008), 70-80% (Etuk *et al.*, 2012) of total production cost, the overall cost of poultry products is high.

There is therefore need to search for alternative energy sources to reduce the cost of poultry production. Sorghum is the next alternative to maize in poultry feed in terms of nutritive value, adequateness, and cost (Etuk *et al.*, 2012) and can grow in drier areas where maize cannot(Oyier

*et al.*, 2016). But the use of sorghum is limited by presence of tannins, therefore there is need to identify inexpensive methods of minimizing the adverse effects of tannins.

### **1.3 Justification**

Competition for maize between human consumption and animal feed industries and its high cost, increases need for animal nutritionists to search for locally available and inexpensive alternative feed ingredient to supply energy in poultry feed and reduce production cost. In Kenya, sorghum is grown in most parts of the country, even in areas of low agricultural potential (Kilambya and Witwer, 2013) where maize will not do well. Sorghum is drought tolerant compared to other cereals crops and can withstand periods of high temperature and water logging as well as soil toxicities (Etuk *et al.*, 2012). It can thrive well in poor soil with lower moisture content (Mohamed *et al.*, 2015). For these reasons, sorghum can be an alternative cereal crop grown in the vast ASALs.

Sorghum has higher value of protein while the energy content of sorghum is slightly lower than that of maize. When compared to maize, crude protein and ME content of sorghum are 12% and 3270kcal/kg respectively, comparable with 10.1% CP, and 3319kcal/kg of maize (Mohamed *et al.*, 2015). The amino acid profile of sorghums is similar to maize, although the lysine content of sorghum is slightly lower (0.26% in sorghum versus 0.30% lysine in maize) (Kriegshauser *et al.*, 2006).

However, use of sorghum is constrained by presence of anti-nutritive factors (tannins) (Medugu *et al.*, 2006). Nevertheless, the negative effects of tannin in poultry feeds, especially in sorghum, can be neutralized by several methods including germination and dietary supplementation with methionine (Sell and Rogler, 1984). Past studies have used germination of different varieties of sorghum, dietary supplements (such as methionine) to reduce either tannin content or its effect

hence improves the performance of birds. The aim of this study is to test the effectiveness of these methods on performance of the birds fed grain from local high tannin sorghum varieties. .

## **1.4 Objectives**

### **1.4.1 Broad objective**

To determine the effect of germination of sorghum grains and supplementation with methionine on performance of broiler chickens

### **1.4.2 Specific objectives**

1. To determine the effect of germination on nutrient composition and tannin content of brown sorghum grains (*var. Serena*)
2. To determine the effect of feeding germinated sorghum (*Serena*) based diets on performance of broiler chicken
3. To determine the effect of feeding sorghum (*Serena*) based diets supplemented with methionine on performance of broiler chicken

## **1.5 Research Hypotheses**

The Null hypotheses that have to be tested are:

- a) Germination of sorghum grain has no effect on its chemical composition and tannin content
- b) Germination of sorghum grain has no effect on performance of broiler chicken
- c) Supplementation of sorghum grain with methionine has no effect on performance of broiler chicken

## CHAPTER TWO: LITERATURE REVIEW

### 2.1 Background of poultry sector in Kenya

Poultry sector plays a significant role in the economy of Kenya; apart from its direct contribution to the agriculture GDP, it contributes an additional 27% of the country's GDP via links with other sectors (Njoroge *et al.*, 2015). Poultry sector contributes to other farming activities via manure as fertilizer, food industries through eggs and poultry meat, tourism, sports and culture (Omiti and Okuthe, 2008). Moreover, the poultry industry employs approximately two to three million individuals' directly in production and marketing and indirectly via link with provider of inputs such as animal feeds, day-old- chicks and animal health services (USAID, 2010).

Poultry production is important source of livelihood for many Kenyans where they contribute a lot to human nutrition as high value protein and also as a source of cash income directly from selling of eggs and live birds, indirectly through sale of manure to crop farmers (Behnke and Muthami, 2011).

Poultry production has the advantages of small space requirement, simple management practices, quick returns to investment and ready market outlets for their products (Kingori *et al.*, 2010). The Kenya Economic Report (KIPPRA, 2009) identified poultry as one of the lead livestock enterprises that can contribute the most towards the attainment of Millennium Development Goals (MDG's). The mean annual poultry meat production was about 20,000 metric tons, while egg production is 1,255 million eggs (ROK, 2008). Indigenous chicken (IC) are mostly kept in rural areas under free range system by about 75% of Kenyan households (Okello *et al.*, 2010). Approximately 65–75% of poultry meat (Bergevoet and Engelen, 2014),

55% and 47% of meat and eggs respectively (Kyule *et al.*, 2014) in Kenya come from IC. The IC are attractive to poor households due to the advantages of good adaptation to the rural environment, survival on low inputs and require less start-up capital (Kyule *et al.*, 2014). The IC production is characterized by roaming birds that scavenge around the farmhouse and usually interact with other birds' species and receive some grain occasionally in the process (Aila *et al.*, 2012; Magothe *et al.*, 2012).

Commercial production system on the other hand is concentrated in urban and peri-urban areas of major cities such as Nairobi, Mombasa, Nakuru, and Kisumu due to ease of access to inputs such as feeds, medicine and ready markets for the eggs and meat (Omiti and Okuthe, 2009; Okello *et al.*, 2010). High population growth in these areas has led to the shrinking of the land sizes favoring poultry production compared to other livestock. The production of poultry has been classified in four main systems according to the FAO/OIE which are divided into 4 sectors. Sector 1 consists of the integrated industrial producers (big companies), distinguished by high bio security, uses high external input, feeding and processing. Sector 2 is made up of commercial hatcheries with moderate to high bio security, and Sector 3 is dominated by smallholder semi-commercial farmers, characterized by low to minimal bio security and birds often sold in live bird market, while Sector 4 constitutes the village or "backyard" (traditional) poultry production system (Omiti and Okuthe, 2009). The production systems for hybrids (broilers and layers) vary from large scale totally integrated systems (>3,000) to medium and small-scale systems (500 – 3000 birds) (Njoroge *et al.*, 2015).

## **2.2 The poultry feed industry in Kenya**

Animal feed industry in Kenya has been facing challenges due to unavailability and costs of raw materials (BLGG Group, 2013). BLGG Group, (2016) reported that the major challenges facing the animal feeds industry in Kenya were high cost of some of the ingredients, erratic supply of raw materials, lack of standardization and low quality of ingredients. To ameliorate this, the government in its policy to improve availability of feed ingredients, intends to encourage the cultivation of these ingredients through provision of cheap inputs (Gitonga, 2014). Kenya has about 150 animal feeds manufacturers with approximately 70% of the feeds produced being poultry feeds (Gitonga, 2014). However, poultry feeds prices fluctuate with seasons whereby the prices are inversely proportional to national grain production level (Macharia *et al.*, 2016; Njarui *et al.*, 2016).

In Kenya, the main energy source in poultry feeds is usually maize, which at the same time is also the staple food for most Kenyans. This competition for maize increases its price and at times it is unavailable for animal feed (Jacob *et al.*, 1996). This results in increased cost of poultry production thus decreasing the profit margin.

The increased demand for poultry products had led to an increased demand for poultry feeds. At the same time there is an increased demand for maize by the increasing human population. During times of inadequate rain (frequency becoming more due to climate change), maize production falls. However, other cereals that are more tolerant to low water availability thrive. There is therefore need to use these alternative cereals as energy source, sorghum being one of them.



### 2.3 Sorghum

Sorghum (*Sorghum bicolor* L. Moench) belongs to the grass family Poaceae of the tribe Andropogonae and sugarcane is close relative to sorghum (Jambunathan *et al.*, 1995; Etuk *et al.*, 2012). It is considered as the fifth most important grain crop after wheat, maize, rice and barley worldwide in terms of both production and area planted (Popescu and Condei, 2014). The name Sorghum was derived from Italian word Sorgo which in turn was derived from Latin word Syricum meaning grain of Syria (Aamir Iqbal and Iqbal, 2015). It is believed to have been domesticated in Ethiopia and surrounding countries around 4000-3000 BC (Olembo *et al.*, 2010). Sorghum has several names differing from one country to another, the most common ones encountered are: durra, milo, kafir corn, hegari, feterita, kaoliang, guinea corn, mtama, or shallu (NRC, 1996). Sorghum is C4 crop with efficient photosynthesis which is highly adapted to wide diversity of climate.

Sorghum can be grown at an altitude range of 500 metres to 1700masl requiring minimum rainfall of 300mm/year (Muui *et al.*, 2013) and an optimum rainfall of 550 to 800 mm (Rao and Kumar, 2012) with minimum temperature of 7-10 and optimum temperature of 27-32°C (Tirfesa and Ayele, 2014). Sorghum can thrive well in all types of soil such as Inceptisols, alfisols, vertisols, Chernozems, Cambisols, Loess and sandy Oxisols and can endure salinity with pH of 6.0 to 8.5 (Krishna, 2013).

The uniqueness of sorghum in tolerating drought is due to several factors (Vanderlip, 1998; Du Plessis, 2008). Its ability to remain dormant during the drought and resume growth when the condition is favorable; small leaf area per plant which limits transpiration; the epidermis of the leaf is corky and covered with a waxy layer which protects the plant from drought; the stomata close rapidly to limit water loss; extracting water from a deep depth within the root zone and

even at level of lower percentage of soil water without yield loss when the water is limited in the upper root zone.

According to Dicko *et al.* (2006) the total sorghum production per annum is about 60 million tons from cultivated area of 46 million ha worldwide. It is planted in 98 countries of Asia, Americas, Africa and Oceania. The top producers are United States of America, China, Mexico, Nigeria, Sudan, India and Argentina (Mohapatra *et al.*, 2017). Sorghum is an important staple food for more than 500 million people in more than 30 countries worldwide (Dahlberg *et al.*, 2011) with India and china being the highest consumers in Asia.

Kenya has a diversity of climate and soils that favor sorghum growing. A large proportion of Kenya land mass 80% (Bergevoet and Engelen, 2014) and 84% (Omiti and Okuthe, 2008) has been classified as arid and semi-arid areas which is not suitable for rain fed agriculture. Sorghum has been reported to thrive in these areas where drought causes regular failures of other crops (Olembo *et al.*, 2010). The sorghum varieties grown in Kenya differ in color, from white such as Sila, Kari mtama 1, Kari mtama 3 and Andiwo/Igumba and Gadam, brown such as Serena, Seredo, Gopari and Kiambere to red such as Gatururu and Muceru and deep red such as Migogo nyuol or edero/kisudi and Ochuti/Andiva (Oyier *et al.*, 2016). The sorghum's color may not be a good indicator of tannin content. Taylor *et al.* (2013) reported that, some varieties of sorghum with white coated color contain high tannin and vice versa in brown and red or even black coated-color sorghum varieties. In Kenya sorghum is grown in Nyanza, Eastern and western provinces for subsistence and used in different traditional recipes in form of thick (ugali), thin (uji) porridge fermented or unfermented, flat bread, boiled grain, non alcoholic drink, malted grains for brewing and beverages (FAO, 1999; Oyier *et al.*, 2016). Sorghum grain is also used as animal feed and the whole plant is a very crucial source of livestock fodder during

the dry season, the stover as fuel for cooking and also used as construction and fencing materials (Lim, 2013).

## **2.4 Tannins in sorghum**

All sorghums have phenolic compounds which are defined as any compound containing a benzene ring with one or more hydroxyl group (Dykes and Rooney, 2007). These phenols are divided into three class; phenolic acid, tannins and flavonoid (Tsao, 2010). Tannins are widely spread in almost all plant kingdoms where they carry out different biological and biochemical tasks such as protection against microbial pathogen attack, pests, insects and predation by herbivorous animals (Dixon *et al.*, 2005) in addition, contributing to their color, taste, flavor, texture and oxidative stability (Kumari and Jain (2015).

Grain color varies according to pericarp thickness & color, presence or absence of testa, endosperm color & texture and genetic-controlled characters (Sedghi *et al.*, 2012). According to Selle *et al.* (2006), red sorghum contains more tannin than white thus making white varieties to be superior to the red for pig and poultry feeding. In contrast, Boren and Waniska (1992) reported that seed color is not an accurate indicator of tannin content of the sorghum grain. The grain may be darker in color but contain very low tannin content depending on the dominant gene (Cheng *et al.*, 2009).

Sorghums are classified into three categories according to genotype and tannin content. Type I sorghum have no pigmented testa and no tannins, type II sorghum tannin are deposited in vesicles of the testa layers while in type III sorghum, tannins are present in the testa and pericarp (Dykes and Rooney, 2006) with the tannin content in type III being more than the type II. Type II tannin sorghum are invariably lighter in color while type III tannin sorghum are brown or red in color (Rooney and Miller, 1982). Sorghum varieties with white, cream or yellow pericarp may

contain little or no tannin while red/brown varieties contain more tannin. However, the intensity or lightness of the pericarp color is not a reliable indicator of tannins content (Boren and Waniska, 1992; Taylor *et al.*, 2013). Tannins are also present in other grains such as millet and barley (Dykes and Rooney, 2007).

According to Dykes and Rooney (2006), tannins are classified into two categories: hydrolyzable and non hydrolyzable (condensed tannins). Sorghum contains the condensed tannins which are commonly known as proanthocyanidins or procyanidins with high-molecular weight polyphenols and comprise of polymerized product of flavan-3-ols and flavan- 3-4-diols units usually linked by C4-C8 interflavan bonds. Some varieties of sorghum have pigmented testa that contain condensed tannin; these compounds are mainly located in pericarp of grains which give the bitter taste to the seed (O.E.C.D, 2010). According to Bravo (1998), tannins occur primarily in conjugated form with one or more sugar residues linked to hydroxyl groups. These sugars are monosaccharides, disaccharides, or even oligosaccharides but glucose is the most common. Other compounds such as carboxylic and organic acids, amines, lipids, and other phenols also conjugate with tannins (Kumar *et al.*, 2014). Condensed tannins have been associated with good health through acting as anticancer, cardioprotective, UV-protective, anti-inflammatory, immunomodulatory, lowering cholesterol properties, promote urinary tract health, antioxidant and radical scavenging functions (Sharma *et al.*, 2007). Sorghum cultivars with lighter seed coat color have less tannin with better nutrient composition when compared to darker ones (Kumar *et al.*, 2007). Measured tannin content for a certain food or feed from different laboratory has been reported to be quite variable. This variability are most probably due to processing, post-harvesting conservation and/or due to different assay methods used (Prior and Gu, 2005).

Boren and Waniska (1992), reported that low tannin sorghum varieties normally contains less than 0.69% CE (Catechin Equivalent), medium varieties contains 0.5 to 1.5% CE whereas high varieties contains 1.5 to 6% CE. Osuntogun *et al.* (1989) analyzed 15 sorghum varieties and reported that the tannin content vary between 0.25 to 0.46% (Catechin Equivalent) in white, cream yellow coat color varieties which are considered as low tannin while 2.9% CE (Catechin Equivalent) in red color variety considered as high tannin. Ahmed *et al.* (1996) reported condensed tannin content of 0.41% CE in white sorghum (low tannin) whereas Tulasi *et al.* 2004 reported tannin content range between 0.0023 to 0.0045% CE in low tannin varieties. Hassan *et al.* (2003) reported tannin content of 0.28% in low tannin sorghum and 1.36% CE in high tannin sorghum.

## **2.5 Nutritional composition of the sorghum**

The nutrient composition of sorghum has been well documented and the composition is very similar to that of maize (Léder, (2004) and Etuk *et al.*, 2012). The nutrient composition may vary due to cultivars and varieties, environmental conditions and cultural practices (Raihanatu *et al.*, 2011). The main component of sorghum grain is starch, followed by protein, non-starch polysaccharides and fat. Several studies have reported the entire sorghum grain to contain about 87 - 92% dry matter (DM), 8.9 – 15% crude protein (CP), 2.8-5.3% ether extract (EE), 1 – 2.5 % ash, 1.5 –4% crude fiber (CF) and 70 – 83% nitrogen free extract (NFE) on as fed basis (Tulasi *et al.*, 2004; Robertson and Perez-Maldonado, 2006; Shakouri *et al.*, 2009; Raihanatu *et al.*, 2011). A summary of the nutrient composition of whole grain of sorghum is presented in Table 2.1 below.

Sorghum starch is the main energy supplier, account for 70% of dry grain weight and consists of amylopectin being around 75% and amylose at 25% (Liu *et al.*, 2015). The amylose:

amylopectin ratio has been reported to affect starch digestion of cereal grains. Svihus *et al.*, (2005) found that the higher the amylose content in cereal grains the lower the starch digestibility. This could be associated with interaction of amylose with fatty acid which forms complexes, and reduce the rate of starch degradation (Tufvesson *et al.*, 2001). Sang *et al.* (2008) observed that in vitro starch digestion of none amylose sorghum (waxy sorghum) was higher than other varieties with 14% and 23% amylose.

**Table 2.1: Composition of sorghum grain**

Components (g/kg DM)	Percentage %
Dry matter	87- 92
Carbohydrates	65-80
Starch	60-75
Non starch polysaccharides	2-7
Protein	7-15
Fat	1.5-6
Ash	1-4

*Source: Duodu et al. (2003), Dicko et al. (2006) Robertson and Perez-Maldonado (2006); Medugu et al., 2010). DM = dry matter*

Sorghum proteins are classified into five types based on solubility, namely; albumins, kafirine, globulins, cross-link kafirines and the glutelins (Hamaker and Bugusu, 2003; Awadelkareem *et al.*, 2015). Kafirine the main storage protein of the sorghum, accounts up to 70 to 80% of whole sorghum protein (Salinas *et al.*, 2006) depending on genetic and agronomic factors. Kafirine is further categorized as  $\alpha$  (alpha-),  $\beta$  (beta-) and  $\gamma$  (gamma-) kafirine depending on their molecular weight, structure and extractability (Shull *et al.*, 1991). Arrangement in protein bodies,  $\beta$  -and  $\gamma$ -kafirins are positioned in the periphery while  $\alpha$ -kafirins located in the central core (Selle, 2011). Mesa-Stonestreet *et al.* (2010), in a review observed that  $\alpha$ -kafirins,  $\beta$ -kafirins and  $\gamma$ -kafirins content were 66–84, 8–13% and 9–21% respectively. Kafirins contain high levels of amino

acids glutamine, proline, glycine and asparagines which makes it to be considered as proline-rich proteins (PRP), but has very little of amino acid lysine (Issa, 2009).

Kafirines are located in protein bodies in sorghum endosperm, which surround the starch granules and both embedded in glutelin protein matrix (Duodu *et al.*, 2003; Mesa-stonestreet *et al.*, 2010) thus blocking the access of enzyme amylases from reaching starch during the process of digestion. Salinas *et al.* (2006) observed that the kafirine concentration in sorghum was negatively correlated to apparent metabolizable energy (AME) and total metabolizable energy (TME). As the concentration of the kafirine in sorghum increases, the digestibility of starch is reduced. Giuberti *et al.* (2012) observed that starch digestion in sorghum is relatively low in contrast to other cereal grains.

Presence of tannins in sorghum affects its protein digestibility since condensed tannins have high affinity for the protein proline. A study by Taylor *et al.* (2007) showed that the bond between kafirins and condensed tannin resulted in 50% reduction in protein digestibility. Furthermore, they found that tannins in sorghum bind mainly to  $\gamma$ -kafirine which has a high content of the amino acid proline than to either  $\alpha$ - or  $\beta$ -kafirins. Albumin and globulin protein fraction of sorghum grain are of superior nutritional value but are present in small quantities. Wu and Wall (1980) observed that glutelin and albumin have higher lysine while globulin and glutelin have higher methionine plus cystine. Sorghum proteins have been reported to be deficient in the essential amino acid lysine as well as in the sulfur-containing amino acids (FAO, 1995; Etuk and Ukaejiofo, 2007).

The word fibre is related to indigestible material such as cellulose, hemicelluloses, pectin, gums oligosaccharides and several lignified complexes. The main dominant fibre component in

sorghum is cellulose which varies from 1.19 to 5.23% (Hamad, 2007) depending on the variety and is found in cell wall and pericarp.

The fat fraction of sorghum grain is located in the germ and is rich in polyunsaturated fatty acids. Mehmood *et al.* (2008) reported that the total content of unsaturated fatty acid in sorghum range between 74 to 87%. Abugri *et al.* (2015) reported that the dominant unsaturated fatty acid in sorghum is linoleic comprising of 39.3% of overall fatty acids. Sorghum is also a good source of vitamins, notably thiamin, riboflavin, pyridoxine and the liposoluble vitamins A, D, E and K (Dicko, 2005). Vitamin content of sorghum is similar to that of maize but the mineral content is superior (Gualtieri and Rapaccini, 1990; Balota, 2012). The feeding value of sorghum varies due to genotype and processing methods as well as water availability, temperature, soil fertility and environmental conditions during grain development (Balota, 2012; Gholizadeh *et al.*, 2014).

## **2.6 Effect of tannins on poultry performance**

According to Mahmood *et al.* (1997) and Proietti *et al.* (2015) tannins impact negatively on the digestibility of proteins and carbohydrates by forming complexes with these molecules and enzymes involved in their digestion thus inhibiting their activities. Tannins also interact negatively with the bioavailability of essential minerals in the digestive tract of monogastrics, specifically, iron and zinc by chelating the metals (Hassan *et al.*, 2003; Bravo, 1998). Additionally the astringent taste of condensed tannins reduces feed intake. The negative impact of tannin on feed intake, weight gain, feed conversion ratio and nutrient digestibility in poultry has been reported in many studies (Emami *et al.*, 2012; Osman and Gasseem, 2013; Torres *et al.*, 2013). Reduction of tannin content of sorghum would increase the availability of proteins, carbohydrates and minerals for digestion and absorption, improving performance. The tannin content of sorghum can be reduced through physical methods such as germination.



## 2.7 Impact of germination on chemical composition and tannin content

Germination is a process which moisturizes the grain's embryo and endosperm in order to kick start metabolism of the embryo, triggering the synthesis of the essential enzymes (Bewley, 2001). It stimulates the synthesis of hydrolytic enzymes, such as amylases, proteases, lipases, phytases (Dicko, 2005; Klose *et al.*, 2009). The carbohydrate content of germinated sorghum decreases as it is readily degraded by enzymes such as  $\alpha$  and  $\beta$  amylases into simple sugars which are required as energy source for germinating embryo (Elmaky, 1994; Elkhier and Hamid, 2008; Ogbonna *et al.*, 2012). The  $\alpha$ -amylases are considered as endoenzymes that randomly split  $\alpha$ -1 $\rightarrow$ 4-linkages in starch while  $\beta$ -amylases are exoglucosidases which release maltose component from starch (Dicko *et al.*, 2006).

Germination decreases cross-linked kafirin fraction and increases protein content mostly albumin and globulin (Okoh *et al.*, 1989). Amino acids content was enhanced during sprouting of seeds due to protease enzyme activity which degrade peptide component to amino acids. Narsih *et al.* (2012) also reported that the fat content of germinated sorghum was reduced due to lipolytic enzyme activated during germination and hydrolyze fat into fatty acid and glycerol. Chavan *et al.* (1989) reported that phytic enzyme is also activated during seed germination hydrolyses phytic acid increasing availability of minerals such as Phosphorus, Calcium Magnesium, calcium Iron and Zinc bound by the acid.

A study by Elmaki *et al.* (1999) observed that 10 h soaking and 48 h germination of Gadamelhamam and Cross 35:18 (low and high tannin sorghum) reduced the tannin content determined by vanillin-HCl method (CE) from 0.34 to 0.20% and from 1.44 to 0.31%. Osuntogun *et al.*, (1989) reported that the condensed tannin content of high tannin sorghum SRN484 decreased from 2.92% to about 1.3% CE after 48 h germination. Kyarisiima *et al.*

(2004) also reported that germination of high tannin sorghum for 28 h reduced tannin content from 8.27 to 6.51 mg catechin/100g. The reduction of tannin was attributed to its leaching out in the water during steeping phase (Ogbonna *et al.*, 2012) or may be due to their binding with cotyledon endosperm that are undetectable by normal method due to their insolubility in solvent (Osman and Gassem, 2013). In contrast, Yang *et al.* (2016) reported increased tannin content of low tannin sorghum from 0.1 to 0.3% after 72 h germination. Eltayeb *et al.*, (2007) reported a similar observation. Osman and Gassem (2013) studied the effect of 72 h germination on tannin content of three varieties of sorghum namely; Baidha, Shahla and Hamra. They observed that tannin content in Baidha increased from 0.058 to 0.084% and from 0.866 to 1.174% for Hamra variety while Shahla showed decrease in tannin content from 0.392 to 0.364% CE. From these contradicting results, it appears that effect of germination on tannin is not conclusive and may depend on variety.

## **2.8 Methionine supplementation**

Dietary supplementation of methionine has been used to reduce the effect of tannin and effectively improve sorghum utilization by chicken. In diets containing tannic acid, methionine was reported in many studies to reverse its deleterious effect on weight gain. Booth *et al.* (1959) found that as the tannic acid or gallic acid was fed to rats, 4-o-methyl gallate was the main metabolite in the urine. This was confirmed in studies by Potter & Fuller (1968) and Kadirvel (1969) who reported that methionine acts as methyl donor in alleviating the negative effects of tannic acid via hydrolysis into gallic acid then 0-methylated which was excreted in the urine as 4-0-methyl gallic acid. The tannin in sorghum is the condensed form which resists hydrolysis due to high degree of polymerization of phenols. The idea of using methionine for detoxification of tannic acid was based on the results of Salunkhe and Chavem (1989) who reported 4-0-methyl

gallic acid (monomethyl ether) in the urine of rats after tannin acid metabolism. Since condensed tannin cannot be hydrolyzed, supplementation of methionine to sorghum based diet plays specific role as supplement to improve performance of birds. Condensed tannin affects availability of methionine more than other amino acids (Wareham *et al.*, 1991) thus supplementation of methionine alleviates deficiency. On sorghum based diets methionine is the first limiting amino acid required in DNA and protein synthesis and the synthesis of spermine and spermidine (polyamines) which necessary in nucleus and cell division events (Sikka and Johari, 1979; Bouyeh, 2012).

Methionine is a glutathione precursor and tripeptide which is involved in reduction of reactive oxygen species (ROS) that protects cells from oxidative stress (Rubin *et al.*, 2007), required for numerous metabolic reactions such as the synthesis of creatine and carnithine (Schutte *et al.*, 1997) and plays a big role for optimum muscle accretion (Vieira *et al.*, 2004). Chang and Fuller (1964) observed that birds fed on high tannin sorghum diets supplemented with methionine had similar growth performance and feed efficiency as low tannin sorghum. Sell and Rogler (1984) reported that supplementation of low and high tannin sorghum with 0.2% methionine increased feed intake of laying hens. Younis (2014) reported no significant differences in performance of quail strains on low tannin sorghum-soybean based diet with 0.2% and 0.4% methionine. Supplementation of sorghum containing tannin with methionine provides sufficient sulfur amino acid for biological activities rather than detoxification.

## **2.9 Utilization of sorghum and germinated sorghum in poultry diet**

Grain sorghum has been commonly used in poultry diets as a source of energy. Liu *et al.* (2014) observed that broiler chickens fed on low tannin sorghum based diet had a weight gain and feed

conversion ratio similar to maize based diet and both were superior to wheat based diet. Nyannor *et al.* (2007) made similar observations. Tulasi *et al.* (2004) and Medugu *et al.* (2010) reported no negative effect of feeding high tannin sorghum on feed intake, weight gain and feed conversion ratio to broiler chickens compared to maize based diet. However, Ibrahim *et al.* (1988) reported that, feeding high tannin sorghum to broiler chicks' impaired feed intake compared to the low tannin sorghum. Douglas *et al.* (1990) and Sannamani (2002) reported reduced weight gain in broiler chicken on high tannin sorghum based diet compared to maize based diet. Mitaru *et al.* (1983) reported that feeding high tannin sorghum lowered feed conversion efficiency of broiler chickens compare to low tannin sorghum. It can be concluded that high tannin sorghum based diets have negative effects on performance of chicken.

Hamid (2001) and Oke *et al.* (2015) reported that inclusion of germinated low tannin sorghum in broiler diet lowered feed intake thus growth performance. Sharif *et al.* (2012) observed that the feed intake and weight gain was improved as time of germination of the sorghum was increased. The author concluded that germination reduced the sorghum's tannin content, thus improved weight gain. In another study, Torki and Pour (2007) reported that germinated sorghum based diet increased body weight gain and feed conversion ratio of broiler chicks compared to ungerminated sorghum. Bohoua and Yelakan (2007) observed that inclusion of germinated sorghum at 0.8% in maize based diet improved the laying hens' weight gain, egg productivity, and feed efficiency index than inclusion at 1.2 and 1.6%.

From the above studies, it can be concluded that germination of high tannin sorghum reduce the effect of tannin on performance of broilers and laying hens.

## CHAPTER THREE: MATERIALS AND METHODS

### 3.1 Experimental diets

#### 3.1.1 Preparation of Sorghum

The study was carried out at the poultry unit of the Department of Animal Production, University of Nairobi Kabete campus. One variety of sorghum, Serena (brown), was obtained from Makueni County in Kenya, hand sorted to remove foreign materials, soaked in fresh water for 12 hours at a ratio of 1:3 w/v. The soak water was changed twice. After decanting the soak water, the sorghum was allowed to germinate for 48 hours by spreading the grains on trays covered with sack at room temperature. Water was sprinkled using a sprinkle can twice a day to keep the grains moist. After germination (Plate 1), the sorghum was sun-dried for of 4 to 5 days to a moisture content of about 9-10% and a sample milled to determine nutrients and tannin content.



**Plate 1:** Serena sorghum after germination

### **3. 1.2 Diets**

Four diets were formulated based on the Serena sorghum designated as UG46, G46, UG69 and UG92. UG46 (control diet) contained ungerminated sorghum with the recommended methionine content (0.46%), G46 contained germinated sorghum with the recommended methionine, UG69 contained ungerminated sorghum with addition of methionine at 0.69% while UG92 contained ungerminated sorghum with addition of methionine at 0.92 % (Table 3.1 and 3.2). The diets were formulated to be isocaloric (containing 3000 Kcal/kg for starter and finisher diets) and isonitrogenous with CP content of 22% and 18% for starter and finisher diets respectively. The feed ingredients used in the study were all purchased from a local commercial ingredient supplier in Nairobi and mixed in poultry Unit University of Nairobi Kabete campus.

### **3.2 Management of experimental birds and experimental design**

A hundred and sixty day old Abor acres broiler chicks were purchased from a commercial hatchery (Kenchic limited) in Nairobi. Prior to arrival of chicks; the poultry house, pens and equipment were cleaned with water and detergent and then disinfected using Omnicide (trade name for disinfectant containing Glutaraldehyde and cocobenzyl dimethyl ammonium chloride). The pens were metallic cages measuring 1x1m. Prior to placement of chicks, drinkers and feeders also were thoroughly cleaned and placed in each pen. Infra-red bulbs were used as a heat source for brooding during the first two weeks. Thereafter the number of infra-red bulbs was reduced gradually till removal to obtain room temperature by three weeks. Wood shavings was spread in each pen at a thickness of 4-5 cm as deep litter to absorb the moisture of droppings and keep birds dry and warm. The house was well lit and ventilated to ensure the comfort of birds. Multi-vitamin (anti-stress agent), biotrim (Anti-Bactericide and coccidiostat) and liquid paraffin (oil softens and lubricates droppings) were administered to the chicks on the day of arrival.



**Plate 2:** Metal cages housing the birds

The chicks were fed on a mixture of the experimental diets during the first two days while acclimatizing to the experimental conditions was to unify the experimental diets from the start to the end. On the third day chicks were weighed in groups of ten and randomly allocated into 16 metal pens each 1×1m in size (length and width) and 1m height. Chicks in each of the pens were randomly assigned to one of the four dietary treatments in a completely randomized design with 4 replicates for each diet and 10 chicks per replicate. The starter diet was fed to the chicks from 1 to 21days and the finisher diet from 22 to 42 days of age. Water and feed were provided *ad libitum*. The chicks were vaccinated against Newcastle disease on 10<sup>th</sup> day and Gumboro disease at 14<sup>th</sup> day of age.

**Table 3.1: Composition (%) of the starter diet used in the experiment**

Energy= 3000Kcal.ME/kg; Protein= 22%

Ingredients	UG46	G46	UG69	UG92
Serena Sorghum	50	50	50	50
Pollard	12.42	12.42	12.49	12.86
Vegetable oil	1.25	1.25	1.44	1.60
Soya been meal	30.39	30.39	30.00	29.03
Fish meal (Omena) <sup>1</sup>	2.91	2.91	2.75	3.01
Dicalcium phospate	0.50	0.50	0.53	0.50
Limestone	1.51	1.51	1.51	1.50
HCl-lysine	0.01	0.01	0.02	0.05
DL-methionine	0.06	0.06	0.30	0.51
Vitamin-mineral Premix <sup>2</sup>	0.25	0.25	0.25	0.25
Enzyme	0.1	0.1	0.1	0.1
Common Salt	0.5	0.5	0.5	0.5
Biomos (toxin binder)	0.1	0.1	0.1	0.1
Total (%)	100	100	100	100

*UG46= ungerminated sorghum based diet; G46= germinated sorghum based diet; UG69= ungerminated sorghum supplemented with 0.69% methionine based diet; UG92= ungerminated sorghum supplemented with 92% methionine based diet.*

<sup>1</sup>*Fish called Rastrineobola argentea was whole dried, ground and incorporated in broiler chickens diet; <sup>2</sup>Vitamin mineral premix- The composition of the premix was: vitamin A, 10,000,000 IU; vitamin D3 2,000,000 IU; vitamin E, 24,000 IU; vitamin K3, 3,200 mg; choline chloride, 350,000 mg; thiamine, 1,600 mg; riboflavin, 5,600 mg; Nicotinic acid, 32,000mg; panthothenic acid, 8,000 mg; pyridoxine, 4,000 mg; Biotin, 96 mg; folic acid, 960 mg; vitamin B12, 24 mg; Copper, 5,000 mg; Iron, 40,000 mg; Manganese, 150,000 mg; Zinc, 45,000 mg; Cobalt, 200 mg; Iodine, 1,400 mg; and Selenium, 120 mg.*



**Table 3.2: Composition (%) of the finisher diet used in the experiment**

Energy= 3000Kcal.ME/kg; Protein=18%

Ingredients	UG46	G46	UG69	UG92
Serena Sorghum	50	50	50	50
Pollard	23.89	23.89	24.21	24.44
Vegetable oil	3.31	3.31	3.56	3.74
Soya been meal	16.18	16.18	15.20	14.49
Fish meal (Omena) <sup>1</sup>	3.37	3.37	3.44	3.48
Dicalcium phosphate	0.50	0.50	0.50	0.50
Limestone	1.51	1.51	1.51	1.51
HCl-lysine	0.22	0.22	0.26	0.30
DL-methionine	0.06	0.06	0.37	0.60
Vitamin-mineral Premix <sup>2</sup>	0.25	0.25	0.25	0.25
Enzyme	0.1	0.1	0.1	0.1
Common Salt	0.5	0.5	0.5	0.5
Biomos (toxin binder)	0.1	0.1	0.1	0.1
Total (%)	100	100	100	100

*UG46= ungerminated sorghum based diet; G46= germinated sorghum based diet; UG69= ungerminated sorghum supplemented with 0.69% methionine based diet; UG92= ungerminated sorghum supplemented with 0.92% methionine based diet.*

<sup>1</sup>*Fish called Rastrineobola argentea was whole dried, ground and incorporated in broiler chickens diet; <sup>2</sup>Vitamin mineral premix- The composition of the premix was: vitamin A, 10,000,000 IU; vitamin D3 2,000,000 IU; vitamin E, 24,000 IU; vitamin K3, 3,200 mg; choline chloride, 350,000 mg; thiamine, 1,600 mg; riboflavin, 5,600 mg; Nicotinic acid, 32,000mg; panthothenic acid, 8,000 mg; pyridoxine, 4,000 mg; Biotin, 96 mg; folic acid, 960 mg; vitamin B12, 24 mg; Copper, 5,000 mg; Iron, 40,000 mg; Manganese, 150,000 mg; Zinc, 45,000 mg; Cobalt, 200 mg; Iodine, 1,400 mg; and Selenium, 120 mg.*

### 3.3 Data collection

Data on feed intake and weight gain were collected weekly up to 42 days of age. This was to reduce the cost of the labor and minimize the time used for collecting data. Weekly feed intake was obtained by weighing a known amount of feed into a bucket for each replicate pen, scooping and feeding from the bucket during the week and weighing the remainder. The feed intake (gram) per bird per week was calculated as differences between feed offered and the remainder. To determine weight gain, the 10 birds were placed into a bucket and weighed using a Salter dial scale. The weekly weight gain was calculated as differences in current and the previous weight

of the birds. Feed conversion ratio was obtained as the ratio of feed intake to body weight gain. Mortality was recorded when it occurred.

### 3.4 Laboratory analysis

Dry Matter (DM), Ash, Ether Extracts (EE), Crude Fibre (CF) and Crude Protein (CP) content were determined according to procedures described by Association of Official Analytical Chemists AOAC (1998). Nitrogen-free extracts (digestible carbohydrates) was calculated using Formula 1 and Metabolizable Energy was calculated as shown in Formula 2.

$$100 - (\text{Ash} + \text{EE} + \text{CF} + \text{CP}) \dots\dots\dots (\text{Formula 1})$$

$$\text{ME} = 37 \times \% \text{CP} + 81 \times \% \text{EE} + 35.5 \times \% \text{NFE} \dots\dots\dots (\text{Formula 2})$$

Tannin (polyphenols) content was determined by AOAC (1990) method (which determines the total tannins) as follows:

#### Reagents

##### a) Folin-Denis reagent

To 75mls, H<sub>2</sub>O add 10g sodium tungstate, 2g phosphomolybdic acid, 5ml H<sub>3</sub>PO<sub>4</sub> (or phosphoric acid) reflux for 2 hours, cool and dilute to 100mls.

##### b) Sodium carbonate saturated solution.

To each 100mls H<sub>2</sub>O add 35g anhydrous Na<sub>2</sub>CO<sub>3</sub> dissolve at 70-80<sup>0</sup>C and let it cool overnight. Seed supersaturated solution with crystal of Na<sub>2</sub>CO<sub>3</sub>, 10H<sub>2</sub>O, and after crystallization filter through glass wool.

##### C) Tannic acid standard solution

Dissolve 100mg tannic acid in 1 liter H<sub>2</sub>O prepare fresh solution for each determination.

### **Preparation of standard curve**

Add 2 mls Folin Denis reagent to 100 mls volumetric flask containing 50 -75 mls H<sub>2</sub>O and Pipet 0→5mls aliquot of standard tannic acid into respective flasks. Then on a uniform schedule add 5mls Na<sub>2</sub>CO<sub>4</sub> solution and dilute to volume with H<sub>2</sub>O. Mix well and after 40 minutes determine absorbance at 725nanometre plot OD against tannic acid.

### **Determination**

One gram (1g) wet material was ground completely with H<sub>2</sub>O and transferred to 100 mls volumetric flask and left to stand. 2mls extract was drawn from supernatant solution and used to determine Absorbance as for standard.

### **3.5 Statistical analysis**

Data collected on feed intake, weight gain and feed conversion ratio were entered in Microsoft excel 2007 and analyzed using a one way Analysis of Variance (ANOVA) in Genstat software Discovery 13<sup>th</sup> edition. The differences between means was determined by a least significant difference method at significance level  $P \leq 0.05$  using Fisher's protected least significant difference test.

## CHAPTER FOUR: RESULT AND DISCUSSION

### 4.1 Chemical composition of germinated sorghum

The chemical composition of the Serena sorghum variety before and after 48 hrs germination is presented in Table 4.1. The dry matter content (DM) of ungerminated Serena sorghum was 90.6%. This is in agreement with Shem *et al.* (1990) and Badi *et al.* (2004) who reported DM of 90.5 and 90.2% for high tannin sorghum. These observed DM values were slightly lower than 92.71 and 93.33% for low tannin varieties (Awadelkareem *et al.*, 2015) and 95.25% for the low tannin sorghum and 95.80% for the high tannin sorghum (Medugu *et al.*, 2010). These slight differences can be attributed to the drying conditions of the cereal post harvest but it is above 90% required for safe storage of cereals. After germination, the DM content of the sorghum reduced slightly (0.6%) which could be due to the length of the drying period post germination. Small reduction in DM of 1.6% and 0.09% after 12 h soaking and 48 h germination were reported by Shem *et al.* (1990) and Nour *et al.* 2016.

Crude protein content in ungerminated Serena sorghum was 9.9%, which was within 7 to 15% and 9 to 18% range reported by Dicko *et al.* (2006) and Hamad (2007) for whole sorghum grain (white, brown, red sorghum). Kiprotich *et al.* (2014) reported crude protein of 6.5-10.9 % in white varieties, 5.8-12.6% in cream varieties, 4.8-12.1 % in brown varieties and 5.1-10.9% in red varieties. Afify *et al.* (2012a) reported that crude protein of three white varieties sorghum ranged from 10- 12% and Elkhier and Hamid (2008) reported crude protein of 12.69% for grayish variety and 10.1% for brown in color variety.

**Table 4.1: Effect of germination on chemical composition and tannin content of Serena sorghum variety**

sorghum	Serena		
	Ungerminated	Germinated	SEM
Ingredients			
Dry matter	90.6	90.1	0.1251
Crude protein	9.9	10.3	0.552
Crude fiber	4.7	4.7	0.0247
Ether extract	2.6	2.5	0.207
Ash	1.4	1.3	0.01413
Tannin (TA) %	0.47	0.32	0.0226
<u>Calculated analysis (DM basis)</u>			
<b>NFE %</b>	<b>71.8</b>	<b>71.1</b>	
ME (Kcal/kg)	3142	3123	

*Means within a row with no superscripts do not differ significantly ( $p < 0.05$ ); ME = Metabolizable Energy  
 Calculated according to the formula of Ponzenga (1985):  $ME = 37 \times \% CP + 81 \times \% EE + 35.5 \times \% NFE$*

The crude protein content of Serena sorghum increased ( $p > 0.05$ ) after germination from 9.9 to 10.3% (by 4%) for the Serena sorghum variety. This is in agreement with Wu and Wall (1980); Kyarisiima *et al.* (2004); Oduguwa *et al.* (2007); Abbas and Musharaf. (2008); Legodimo and Madibela (2014) and Marete (2015) who reported increased protein of sorghum after germination. The increase of protein content of germinated Serena sorghum variety could not be explained as there were no other sources of nitrogen during the germination. Narish *et al.* (2012) observed increase in non-protein nitrogen coupling with formation of nucleic acid during germination of sorghum from 12 to 36 h. In contrast, El-Beltagi *et al.* (2012) and Yang *et al.* (2016) reported a decrease in crude protein after 72 h germination of low tannin sorghum varieties by 8% and 11% for Giza-15 and Butanna respectively.

The duration of soaking grain may also determine the state of enhancement or reduction of protein in grains. Elmaki *et al.* (1999) reported that protein content declined when sorghum grain was soaked in water for 30 hours compared to 10 hours of soaking. The reduction in protein content was attributed to leakage of water soluble nitrogen during the soaking or some utilization of the protein fraction for the growth of the young embryo (Wu and Wall, 1980). Elkhier & Hamid (2008) and Phattanakulkeawmorie *et al.* (2011) did not observe any changes in protein content of sorghum after germination.

The crude fibre (CF) content of Serena sorghum was 4.7%. The CF value obtained in this study is within the range reported by Medugu *et al.* (2010) of 5.8% in low tannin sorghum and 7.8% in high tannin sorghum. However, this value is higher than findings of other authors who reported 2.02% (white coat color) and 1.72 % (creamy coat color) in low tannin varieties (Awadelkareem *et al.*, 2015) while Chung *et al.* (2011) reported CF of 1.83% and 2.62% in white and red sorghum variety respectively.

Germination had no effect on CF of Serena sorghum variety. Similarly, Elkhier and Hamid (2008) and El-Beltagi *at al.* (2012) observed insignificant effect of 72 h germination on CF of white sorghum varieties (Feterita & Tabat and Dorado, Shandaweel and Giza-15 respectively). In contrast, Elmaki *et al.* (1999) and Singh *et al.* (2015) who reported that after 72 h germination of sorghum varieties, the CF decreased from 3.26 to 1% (Gadamelhamam) and from 1.7 to 1.5% (unknown variety) respectively. Baba *et al.* (2012) also reported significant reduction in CF after 72 h germination of high tannin sorghum. The reduction in CF could be explained by degradation of water-soluble dietary fiber by fiber degrading enzyme during germination (Hübner and Arendt, 2013). However, Nour *et al.* (2016) reported significant increase in CF of Tabat variety (low tannin sorghum) from 2.34 to 4.84% after overnight soaking and 48 h

germination. The length of soaking and germination period could affect the crude fiber content of the last product. The non effect of germination on CF of high tannin sorghum might be related to presence of tannin which suppresses the activity of B-glucanase enzyme responsible for hydrolysis water-soluble fiber. Salunkhe *et al.* (1983) reported that, condensed tannin is capable of inactivating several hydrolytic enzymes essential for metabolic processes during germination. Ash content of ungerminated Serena sorghum was 1.4%. The ash content obtained in this study is within the range. In agreement, Hamid (2007) and Chung *et al.* (2011) reported that, the ash content in different varieties of sorghum varied from 1.51 to 2.06 (*var.* Edo, Engaz & safra) and from 1.43 to 1.92% (*var.* Hwanggeumchal, Heuin & Chal) respectively. Awadelkareem *et al.* (2015) reported ash content of 1.78% and 1.28% in Feterita and Dabar (low tannin sorghum varieties). Elkhier and Hamid (2008) reported 1.45 and 1.75% ash in Feterita and Tabat varieties. However, this value is less than that obtained by Kinyua *et al.* (2016) of 2% and 2.2 % for Gadam and Seredo (low and high tannin sorghum varieties) but higher than that obtained by Raihanatu *et al.*(2011) of 1% in red sorghum variety.

The ash content was reduced ( $p>0.05$ ) after germination by 7.1%. Similarly, Shem *et al.* (1990); Phattanakulkeawmorie *et al.* (2011) and Baba *et al.* (2012) Reported reduction in ash content after 48 and 72h after germination of red sorghum variety from 4.01 to 2.21%, 1.34 to 1.01% and 4 to 1% respectively. This declined in ash content could be ascribed to the leaching out during soaking and germination. A study by El-Beltagi *et al.* (2012) reported that both free macro and micro elements of sorghum were reduced after 72 hours germination. Furthermore, Baba *et al.* (2012) reported that only Mg, Cu and Fe were reduced after 3 days germination. However, the results differ with those of Oduguwa *et al.* (2007) who reported an increase in ash content after germination. The increase in ash content of germinated sorghum could be apparent due to loss of

starch during germination leading to concentration of minerals in the germinating seed. Elmaki *et al.* (1999) and Nour *et al.* (2016) did not observe any change in ash content after 72 h and 48 h germination of low and high tannin sorghum.

Fat (ether extract) content of Serena sorghum was 2.6%. The value of fat content of this study is within the range of 2.2 to 5.4% reported by Phattanakulkaewmorie *et al.* (2011). Medugu *et al.* (2010) reported fat content of 4.3% in low tannin sorghum variety whereas 3.5% in high tannin variety. The observed level is however lower than 7.1% reported by Mohamed *et al.* (2015). The fat content of Serena sorghum reduced ( $p>0.05$ ) after germination by 3.8%. Reduction in fat content after germination has been reported by Elmaki *et al.* (1999); El-Beltagi *et al.* (2012) and Yang *et al.* (2016) and was attributed to its utilization as energy source by sprouting seed. The enhancement or reduction of fat content of sorghum depends on length of germination. Elkhier and Hamid (2008) observed that at 72 h of germination the fat content was reduced from 3.6 to 2.6% and 2.6 to 2.5% for the two low tannin sorghum varieties Feterita and Tabat respectively. But at seven days of germination the fat content was increased up to 6.4 and 3.7% for both varieties Feterita and Tabat respectively. This could be attributed to the synthesis of fats due to changes of vanishing starch (Chavan *et al.*, 1989). Variations in the nutrients content of the sorghum varieties could be due to genotype differences, irrigation, environmental factors, soil fertility and even farming practices (Gualtieri and Rapaccini, 1990). All these factors can affect the nutrient composition of sorghum negatively or positively.

Tannin (tannic acid) content of Serena sorghum variety was 0.47%. Germination for 48 hours resulted in a reduction of tannin content by 31.9%. The reduction in tannin content after 48 h germination of sorghum was reported by Chavan *et al.* (1989); Badi, (2015) and Maidala *et al.* (2016). They reported a reduction of up to 63.7%, 34.3% and 96.33% respectively. Baba *et al.*



(2012) reported that 72 h germinating of high tannin (dark blue in color) sorghum reduced tannin content from 2.07 to 1.54% while Venegas *et al.* (1997) found that 24 h germination of brown and white sorghums reduced tannin content by 60 and 40% for both varieties respectively.

There have been considerable studies showing reduction in tannin content of sorghum due to germination process (Nwasike, 1989; Kyarisiima *et al.*, 2004). Tannin is located in the pericarp-testa of sorghum grain which is responsible for limiting digestibility of protein and starch due to either interference with digestive enzymes (proteolytic and amylolytic enzymes) or formation of complexes with them (Chavan *et al.*, 1981; Mohammed *et al.*, 2011). According to Chavan *et al.* (1981) the reduction of tannin after germination can be due to leaching of tannin in growth medium. Glennie *et al.* (1983); Butler *et al.* (1984) and Badi (2015) suggested that the reduction of tannin is not due to genuine degradation or loss of tannin but should be attributed to the formation of tannin hydrophobic union with enzymes and grain protein during germination.

Nitrogen free Extract (NFE) of ungerminated Serena sorghum was 71.8%. This value is in agreement with those reported by Shem *et al.* (1990), Badi *et al.* (2004) and Medugu *et al.* (2010) of 71.35% for high tannin sorghum, 71.4% for high tannin sorghum and 71.45 and 69.80% for low and high tannin sorghum varieties respectively. This NFE is higher than values reported by Raihanatu *et al.* (2011) of 58.4% for white sorghum and 56.74% for red sorghum variety but less than those reported by Ahmed *et al.* (2013) and Awadelkareem *et al.* (2015) of 73.22% and 77.28% for low tannin sorghum varieties.

There was slight reduction in NFE of the sorghum after 48 h germination by 1%. In agreement, Elkhier and Hamid (2008) reported decreased NFE of low tannin sorghum Tabat by 0.5% after 12 h soaking and 72 h germination. The reduction in NFE after germination could be as result of sugars being digested to obtain energy for the growing embryo. In contrast, El-Beltagi *et al.*

(2012) reported increase in NFE of low tannin sorghum between ranges of 3-5% after 20 h soaking and 72h germination. Shem *et al.* (1990) reported no change in NFE after 12 h soaking and 48 h germination of high tannin sorghum. The change in NFE depends on long or short period of soaking and germination.

Estimated metabolizable energy of ungerminated Serena sorghum was 3142 Kcal/kg. This value is close to what was reported by Medugu *et al.* (2010) of 3300 for low tannin sorghum and 3230 Kcal/kg for high tannin sorghum. In contrast, Douglas *et al.* (1990) and Jacob *et al.* (1996) reported ME of 3838 and 4196 Kcal/kg for the white sorghum varieties and 3200, 3518 Kcal/kg for the brown or red sorghum varieties respectively. Germination reduced the ME content of Serena sorghum by 0.6%.

## **4.2 Chemical composition of diets**

### **4.2.1 Starter diets**

Chemical composition of starter diets is shown in Table 4.2. The slight differences in nutrients content of the diets was attributed to slight variation in uniformity of the raw materials. The analyzed CP of starter diets was 22.72, 22.87, 24 and 23.72% for the 4 experimental rations respectively and was all within the recommended level. The crude fibre content in starter diets were 6.66, 7.31, 7.13 and 7.35% for ungerminated, germinated, and the two methionine supplemented-diets respectively. The tannin content of starter diets was 0.28, 0.24, 0.26 and 0.27 for ungerminated sorghum, germinated sorghum and methionine supplemented-diets respectively.

**Table 4.2: Chemical composition of starter diet (% DM)**

Treatments	UG46	G46	UG69	UG92
Dry matter	90.44	91.14	90.08	90.76
Crude protein	22.72	22.87	24.00	23.72
Crude fibre	7.66	7.31	7.13	7.35
Crude fat	6.25	6.68	6.65	6.93
Ash	6.82	7.43	6.47	6.35
Tannin (As tannic acid)	0.28	0.24	0.23	0.27
<u>Calculated analysis</u>				
NFE	47.99	46.85	45.83	46.41
ME (kcal/kg)	3050	3050	3053	3086

*UG46- ungerminated sorghum + 0.46% methionine (met) based diet; G46. germinated sorghum + 0.46% met based diet; UG69- ungerminated sorghum + 0.69% methionine; UG92- ungerminated sorghum + 0.92% methionine*

#### **4.2.2 Finisher diets**

Chemical composition of finisher diets is shown in Table 4.3. The crude protein in finisher diets was within the recommended level. Crude fibre of finisher diets were 7.1, 6.08, 6.76 and 6.31% for the experimental rations respectively and were all within the recommended level (Melingasuk *et al.*, 2012).

**Table 4.3: Chemical composition of finisher diet (% DM)**

Treatments	UG46	G46	UG69	UG92
Dry matter	89.58	90.68	89.78	89.12
Crude protein	17.99	18.45	18.24	19.26
Crude fibre	7.1	6.08	6.76	6.31
Crude fat	6.44	6.43	6.54	6.86
Ash	6.26	6.34	6.59	6.80
Tannin	0.28	0.23	0.26	0.26
<u>Calculated analysis (DM basis)</u>				
Nitrogen free extract	51.79	53.38	51.65	49.89
ME (Kcal/kg)	3025	3098	3038	3039

*UG46- ungerminated sorghum + 0.46% methionine (met) based diet; G46. germinated sorghum + 0.46% met based diet; UG69- ungerminated sorghum + 0.69% methionine; UG92- ungerminated sorghum + 0.92% methionine*

The tannin content of finisher diets was 0.28, 0.23, 0.26 and 0.26% for ungerminated sorghum, germinated sorghum, ungerminated sorghum supplemented with 0.69% methionine and ungerminated sorghum supplemented with 0.92% methionine. The estimated ME was 3025, 3098, 3038 and 3039 Kcal/kg for the experimental diets respectively. The ME was slightly above the 3000kcal/kg recommended and could be due to accrued errors in the estimation formula.

#### **4.3 Broiler chicken performance during starter phase (1-21 days)**

The effects of germination and methionine supplementation of the sorghum based diets on feed intake; weight gain and feed conversion ratio during the starter phase are shown in Table 4.4. The effects of tannin and methionine intake on weight gain are shown in figures 1 and 2.

**Table 4.4: Effects of germination and methionine supplementation of sorghum based diets on performance of broiler chicken during starter phase**

Diets	UG46	G46	UG69	UG92	SEM
Parameters					
Feed intake (g/chick)	1415 <sup>b</sup>	1264 <sup>a</sup>	1318 <sup>a</sup>	1302 <sup>a</sup>	29.5
Weight gain (g/chick)	707.3 <sup>a</sup>	690.7 <sup>a</sup>	785.1 <sup>b</sup>	762.2 <sup>b</sup>	16.76
FCR	2.00 <sup>c</sup>	1.83 <sup>b</sup>	1.68 <sup>a</sup>	1.71 <sup>ab</sup>	0.0447

*Means within a row with different superscripts differ significantly (P<0.05); UG46- Ungerminated sorghum based diet + 0.46% methionine (Met); G46. germinated sorghum based diet + 0.46% Met; UG69- ungerminated sorghum based diet +0.69% met; UG92- ungerminated sorghum based diet + 0.92% met; SEM – Standard error of mean*

#### 4.3.1 Effect of germination of sorghum on feed intake during starter phase

The feed intake was lower (P<0.05) in chicks fed on germinated sorghum (G46) (1264g/chick) than those on ungerminated sorghum diet (UG46) at 1415g/ chick. The tannin intake was higher in chicks fed on UG46 diet compare to G46 diet (Figure 1). An increase of feed intake on feeding sorghum containing tannin to broiler chickens had been reported by Jacob *et al.* (1996) and Nyachoti *et al.* (1996). They reported that birds fed on high tannin sorghum diets had the highest feed intake due to the low TME (total metabolizable energy) of the sorghum thus the birds attempted to consume more feed to meet their energy requirement. In contrast, ungerminated sorghum with high tannin content has been reported to depress feed consumption in poultry (Mitaru *et al.*, 1983). This reduced intake was attributed to unpleasant taste of the diet due to presence of tannin (Becker and Makkar, 1999) as well as its interfering with protein and carbohydrate digestion through formation of complexes with enzymes while digesting them and reduces the digestibility and feed intake (Chung *et al.*, 1998; Mandal and Ghosh, 2010). Ambula *et al.* (2001) and Hassan *et al.* (2003) reported reduced feed intake when high tannin sorghum

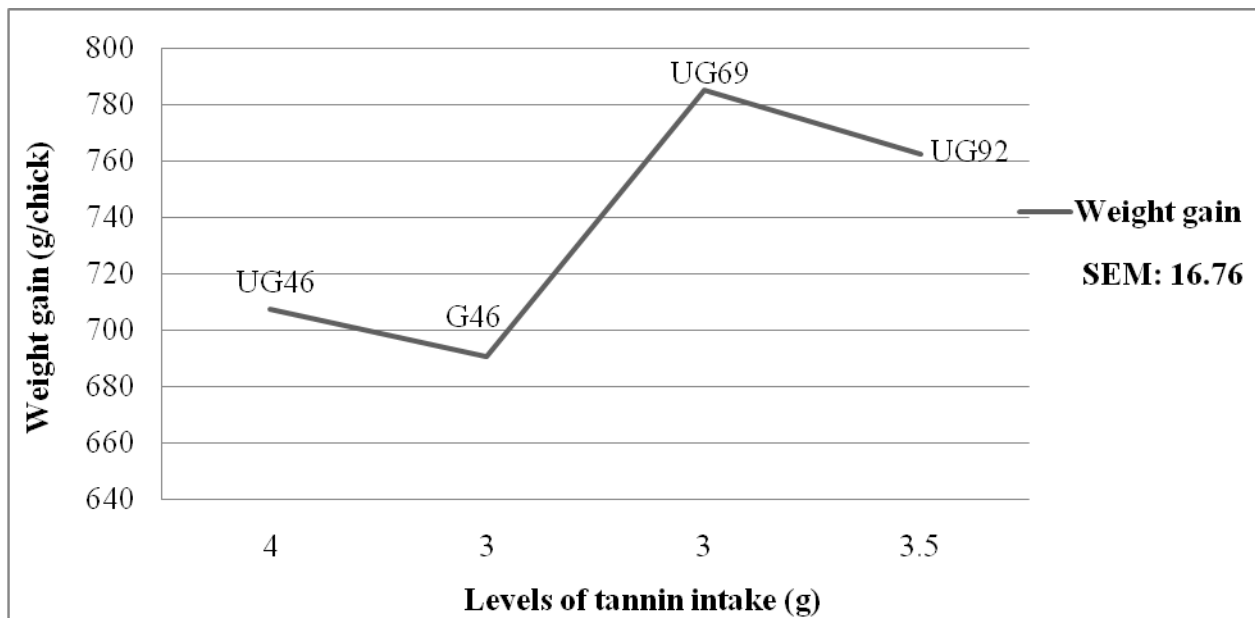
based diet was fed to chicks during starter phase compared to maize based diet. They attributed the reduction in feed intake due to presence of tannin which affects the feed's palatability thus affecting the voluntary feed intake. Santos *et al.* (2006) observed insignificant differences in feed intake when they replaced maize with unknown tannin content sorghum.

Responses of feeding germinated sorghum to broiler chickens have been variable. In this study, it was observed that birds fed on G46 diet consumed less feed compared to diet UG46 (Table 4.4). The reduction in intake of G46 diet could be attributed to accumulation of soluble/free sugar in germinated sorghum. Taylor (2001) reported that germinated sorghum contain higher glucose compared to other reducing sugars due to enzyme  $\alpha$ -glucosidase which degrades maltose and maltotriose to glucose. Glucose is a monosaccharide known to be readily absorbed into the bloodstream. Ferket and Gernat (2006) opined that blood sugar is one of the mechanisms that control feed intake (glucostatic theory) which ascribe to regulate the blood sugar and the quantity of glucose entering the liver after taking meal. However, Kyarisiima *et al.* (2004) and Turki and Pour (2007) did not observe any effect on feed intake when 28 h germinated high tannin sorghum was fed to broiler chickens compared to those on ungerminated sorghum. Okoh *et al.* (1989) and Abbas & Musharaf (2008) did not observe significant effect of feeding 72 h germinated low tannin sorghum on feed intake of chicks during starter phase. In conclusion, germination of high tannin sorghum for 48 h reduced feed intake of broiler chicks.

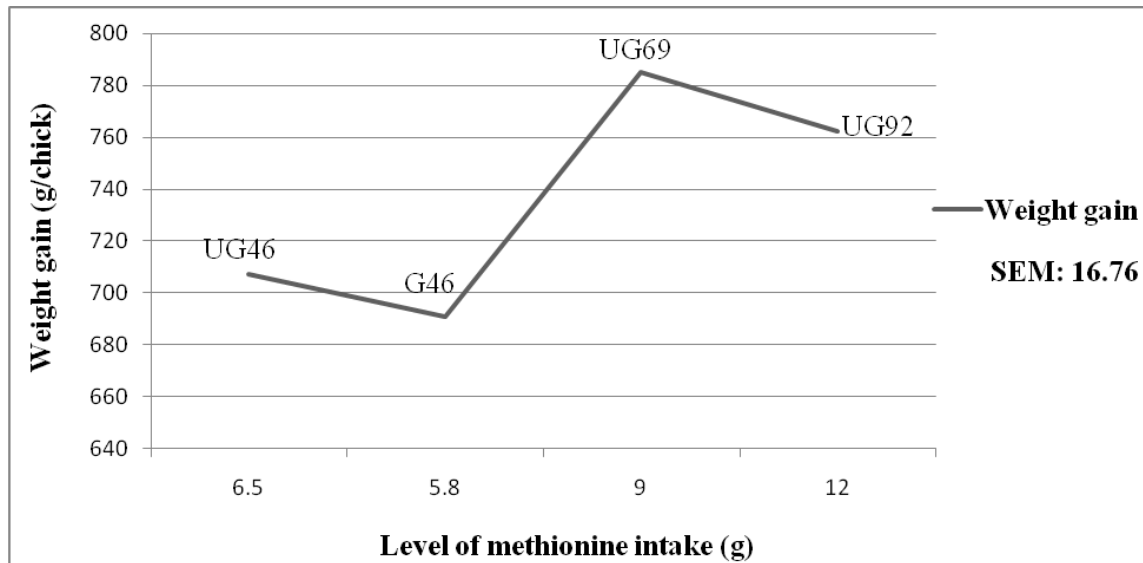
#### **4.3.2 Effect of methionine supplementation of sorghum based diets on feed intake during starter phase**

Feed intake of birds fed on diets UG69 and UG92 were significantly lower ( $p < 0.05$ ) compare to the group of bird on UG46 (Table 4.4). The estimated tannin intake was less in diets

supplemented with methionine UG69 and UG92 at 3 and 3.5g/chick compare to the diet UG46 (4g/chick) (Figure 1). The reduced feed intake of birds fed diets UG69 and UG92 could be due to high methionine level of diets at 9 and 12g respectively (Figure 2) which led to its high concentration in blood of birds. Ferket and Gernat (2006) reported that circulation of specific amino acid imbalance in blood and gut distension and motility are part of the mechanisms that regulates feed intake. They further added that when feed is provided *ad libitum*, birds regulate their voluntary feed consumption to meet requirement of both energy and daily amino acid required. This finding is in contrast with that of Jacob *et al.* (1996) and Houshmand *et al.* (2015) who also reported that supplementation of sorghum and oak acorn containing tannin based diet with 100% more methionine than NRC recommendation had no effect on feed consumption of chicks during starter phase.



**Figure 1:** Effect of tannin levels on weight gain during starter phase



**Figure 2:** Effect of methionine levels on weight gain during starter phase

#### 4.3.3 Effect of sorghum germination on weight gain during starter phase

The weight gain of chicks fed on UG46 based diet (707.3g) was not significantly different ( $P>0.05$ ) from birds fed on the G46 (690.7g) (Table 4.4). Broiler chicken fed on UG46 consumed higher amount of tannin compare to those fed on G46 diet (figure 3). Birds fed on G46 consumed less feed but attained body weight gain that was similar to those fed ungerminated sorghum diet (Table 4.4). This can be attributed to germination reducing tannin content and improving the nutritional quality of the seed which increases their bioavailability to birds (Yang *et al.*, 2016). Germination increases the protein content (albumin and globulin), minerals, vitamins, free amino acid (lysine, methionine) due to protease activity and decreases the kafirine content of sorghum grain that is responsible for impaired degradation of starch and protein (Wu and Wall, 1980; Inyang and Zakari, 2008; Afify *et al.*, 2012b; Hübner and Arendt, 2013; Yi *et al.*, 2016). Germination was also reported to improve minerals and vitamins of cereal grains and



converts starch into simple sugar which is readily taken by chicks (Asiedu, 1991). Although the weight gain was not significantly affected by germination, the FCR improved.

The insignificant effect of germination on weight gain was due to reduced feed intake. In agreement, Kyarisiima *et al.* (2004) reported that weight gain of chicks was not affected when 48 h germinated high tannin sorghum based diet was fed compared to ungerminated sorghum. Okoh *et al.* (1989) reported that chicks fed on 96 h germinated sorghum containing tannin recorded the same growth performance as those on ungerminated sorghum based diet. In contrast, Hamid (2001) observed that 72 h germinated low tannin sorghum improved weight gain of broiler chickens. Abbas and Musharaf (2008) determined the effects of germination of low tannin sorghum for different lengths of time. They reported that chicks fed on 72 h germinated sorghum gave the same weight gain as ungerminated sorghum based diet but germinating the sorghum for 120 h and 168 h resulted in lower weight gain. Sharif *et al.* (2013) observed that performance of broiler chicken fed germinated sorghum depended on the length of germination time. From the results of the current study it can be concluded that; germination of high tannin sorghum reduced tannin content but had no significant effect on weight gain of broiler chicken.

#### **4.3.4 Effect of methionine supplementation of sorghum based diets on weight gain during starter phase**

Weight gain of chicks fed on UG69 and UG92 diets were higher compared to those on the other two diets. The chicks on UG69 and UG92 diets recorded significantly higher ( $p < 0.05$ ) weight gain than those on diets not supplemented with methionine (UG46 and G46). The tannin intakes in diets supplemented with methionine (UG69 and UG92) was lower (3.0 and 3.5g) compared to diet UG46 (4.0g) (Figure 1). The results suggest that methionine supplementation has specific

benefits in sorghum based diets. The results of this study are in agreement with Armstrong *et al.* (1973) and Elkin *et al.* (1978) who reported higher ( $P < 0.05$ ) weight gain of broiler chickens fed on high tannin sorghum supplemented with 0.31 and 0.15% methionine. Gholizadeh *et al.* (2014) reported that broiler chickens fed 30% sorghum of unknown tannin content substituting maize, supplemented with 0.1% methionine had a significantly higher weight gain compared to those without addition of methionine. Armanious *et al.* (1973) also reported increased weight gain of laying hens when low and high tannin sorghum (Pioneer 828 and DeKalb BR-64) based diets were supplemented with 0.4% methionine and 0.4% choline.

Methionine supplementation has been reported in many studies to alleviate the effect of tannic acid on weight gain of broilers through its role in detoxification. Booth *et al.* (1959) found that as the tannic acid or gallic acid was fed to rats, 4-o-methyl gallate was the main metabolite in the urine. This was confirmed in studies by Potter and Fuller (1968) and Kadirvel (1969) who reported that when tannic acid was fed to chicks it hydrolyzed to gallic acid and thereafter o-methylated and excreted in the urine as 4-o-methyl gallic acid. This detoxification applies only to hydrolysable tannin which is not found in sorghum but not condensed tannin type.

According to Dykes and Rooney (2006), tannic acid is not found in sorghum but rather condensed tannin which is un-degradable in the GIT of the birds. As such, the positive effect of methionine supplementation in sorghum based diets may be due to elimination of the negative effects of tannin on methionine absorption. Condensed tannin lowers the absorption of limiting amino acids mostly lysine and methionine. Rostagno *et al.* (1973) observed that methionine availability to chicks was affected in high tannin sorghum when compared to low tannin sorghum. Methionine plays an important role in skeletal muscle development and is a precursor of S-adenosylmethionine, which serves as methyl donor for DNA methylation. High methionine

intake increases DNA methylation and changes gene expression which leads to an increase in muscle development (Waterland, 2006; Wen *et al.*, 2014). It can be concluded that methionine supplementation up to 50% above the NRC recommendation is useful in increasing weight gain of broilers fed on high tannin sorghum.

#### **4.3.5 Effect of sorghum germination and methionine supplementation of high tannin sorghum based diets on feed conversion ratio during starter phase**

Feed conversion ratio was poor for the chicks fed UG46 diet (2.00) and was poorer ( $p < 0.05$ ) than for diets G46, UG69 and UG92 (1.83, 1.68 and 1.71). The FCR of birds on diet UG69 was significantly lower than the group on G46 diet but was similar to diet UG92 (Table 4.4). The lowered FCR of birds on diet G46 could possibly be due to the reduced tannin level of the sorghum germination as well as less feed intake. Birds fed on diet G46 attained high weight gain which may mean efficient utilization of the feed. Methionine plays major roles in body as main component of protein and becomes a methyl donor which is utilized in biological activities such as DNA methylation for efficient gene expression. In high tannin sorghum diet, tannin preferentially interferes with methionine utilization (Elkin *et al.*, 1978). Methionine was reported in many studies to play important role in reversing the deleterious effect of tannin sorghum on weight gain and feed conversion efficiency. This is due to important role of methionine as first limiting amino acid in sorghum-soybean diet.

From the results, methionine supplementation of the diets improved feed utilization efficiently. Del Vesco *et al.* (2013) attributed the improvement of feed conversion efficiency in birds fed on diet supplemented with methionine to the role of dietary methionine in reducing uncoupling protein (mRNA avUCP) expression in the muscle. UCPs are proteins that regulate proton

channel present in the inner membrane of mitochondria. UCPs dissipates proton that is generated from NADH via pumping it from the mitochondrial matrix to the mitochondrial intermembrane space thus switches energy for ATP synthesis to heat production (Ledesma *et al.*, 2002; Cannon *et al.*, 2006).

On the other hand, the higher FCR of chicks fed ungerminated sorghum based diet was expected due to the anti-nutrient factors (tannin) that affects the digestibility and absorption of nutrient and thereafter bioavailability of these nutrients to birds. This is in accordance with Armstrong *et al.* (1973), (1974) and Elkin *et al.* (1978) who all reported that the FCR of birds fed on sorghum containing tannin was poor compared to sorghum based diet supplemented with methionine. Feeding sorghum containing tannin based diet has been reported to depress FCR compared to maize based diets (Nyachoti *et al.*, 1997 and Ambula *et al.*, 2001). The difference in FCR between germinated and non germinated sorghum was not significant. This is in line with the finding of Kyarisiima *et al.* (2004) who reported that use of germinated sorghum in broilers feed did not affect FCR compared to ungerminated sorghum. On the other hand, Toriki and Pour (2007) found that inclusion of germinated sorghum improved the FCR. This could have been due to inclusion of different energy sources such as maize and wheat in the experimental diet. It can be concluded from the results of the current study that; supplementation of high tannin sorghum with methionine improved the feed conversion ratio of broilers fed on both diets (UG69 and UG92).

#### 4.4 Broiler chickens performance during finisher phase (22-42 days)

The effects of germination and methionine supplementation of sorghum on feed intake, weight gain and feed conversion ratio of broiler chickens during finisher phase are shown in Table 4.5.

The effects of tannin and methionine intake on weight gain are shown in figures 3 and 4.

**Table 4.5: Effects of germination and methionine supplementation of sorghum based diets on performance of broiler chicken during finisher phase**

Parameter	UG46	G46	UG69	UG92	SEM
Feed intake (g/chick)	3278 <sup>b</sup>	3300 <sup>b</sup>	3515 <sup>b</sup>	2854 <sup>a</sup>	88.3
Weight gain (g/chick)	1299	1426	1473	1297	52.0
FCR	2.53 <sup>b</sup>	2.32 <sup>a</sup>	2.39 <sup>ab</sup>	2.20 <sup>a</sup>	0.0614

*Means within a row with different superscripts differ significantly (P<0.05); UG46- Ungerminated sorghum based diet + 0.46% methionine (Met); G46. germinated sorghum based diet + 0.46% Met; UG69- ungerminated sorghum based diet +0.69% met; UG92- ungerminated sorghum based diet + 0.92% met; SEM – Standard error of mean*

##### 4.4.1 Effect of germination of sorghum on feed intake during finisher phase

Feed intake of birds fed on G46 diet was similar ( $p > 0.05$ ) to UG46 diet. The feed intake in broiler chicken fed diet G46 was not affected by reduction of tannin as a result of germination.

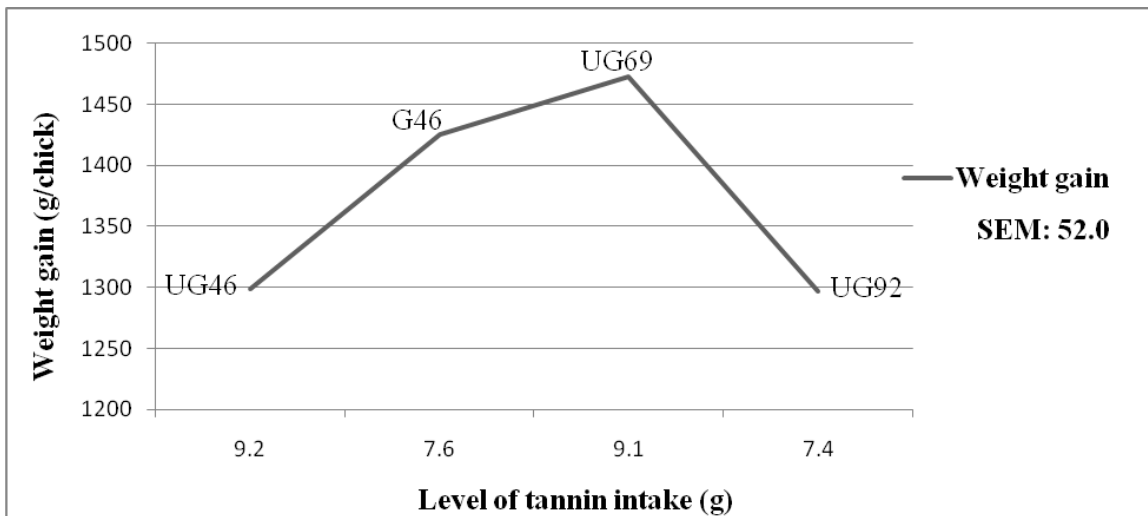
The non significance of germination on feed intake in this study could be due to low rate of tannin reduction in diet G46 (0.23%TA) compared to diet UG46 of 0.28% TA which may mean similar rate of digestion in both diets. In agreement, Torki & Pour (2007); Abbas & Musharaf (2008) and Sharif *et al.* (2013) also observed insignificant effect of sorghum germination on feed intake of broiler chicken during the finisher phase. Shem *et al.* (1990) also reported that growing

pigs fed on 48 h germinated high tannin sorghum had similar feed intake to those on ungerminated sorghum based diet. In contrast, Fafiolu *et al.* (2005); Oke *et al.* (2015) and Fafiolu *et al.* (2016) reported lower feed intake when malted sorghum sprout (MSP) was included in the diet of laying hens. This could be due to the attributes of MSP a byproduct after removing the malt for brew production in brewery industries which was reported to have bitter taste and high fibre content (Oduguwa and Farolu, 2004). It can be concluded from the current study that; germination of high tannin sorghum had no effect on feed intake of broiler chickens in the finisher phase.

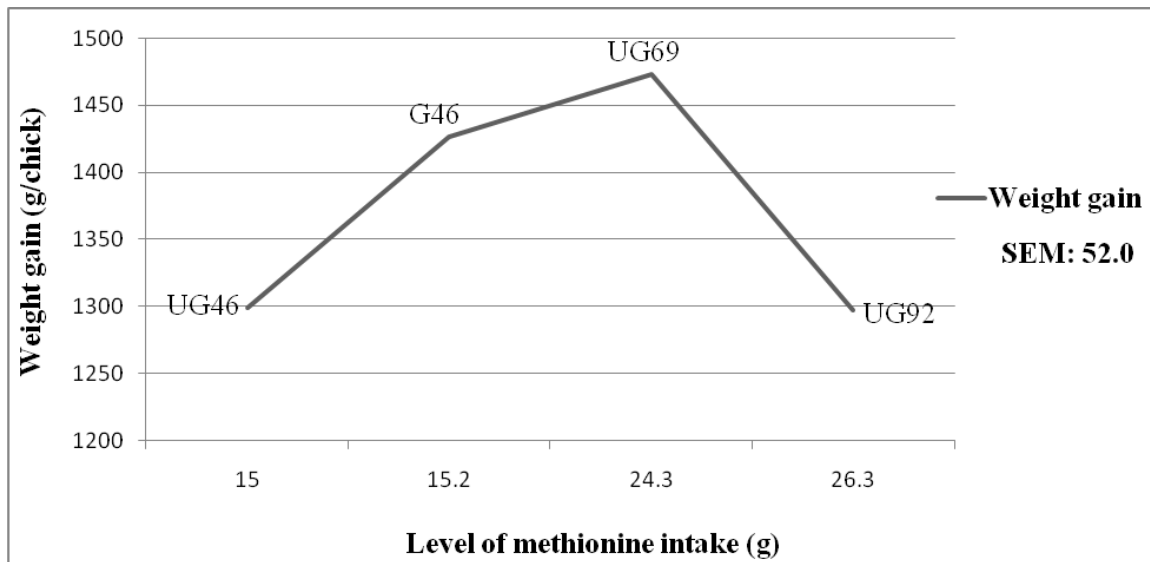
#### **4.4.2 Effect of methionine supplementation of sorghum on feed intake during finisher phase**

The feed intake was lower ( $p < 0.05$ ) for broiler chicken fed on UG92 diet (2854) compared with those on the other two diets containing ungerminated sorghum UG69 and UG46 at 3515 and 3278 g/chick. The higher intake noted for broilers fed diet UG69 could be due to role of supplemental methionine in increasing digestive enzymes such as trypsin, lipase and amylase (Wu *et al.*, 2017). The reduction in feed intake of birds fed the UG92 diet may be attributed to excess amino acid (methionine) which led to its high concentration in the blood of the birds. Li & Anderson (1983) and Han & Baker (1993) reported that excessive individual amino acid in the blood and tissues causes homeostatic mechanisms to fail thus animal reduce its feed intake. Tobin and Boorman (1979) reported similar results of reduced feed intake when amino acid lysine was injected into both jugular vein and carotid artery of cockerels. The possible reason for the depressed feed intake could be related to methionine toxicity. Harter & Baker (1978) and Annongu *et al.* (2014) reported that chicks fed excess methionine caused toxicity which was

associated with pancreatic damage. Pancreas plays a very important role in digestion, pancreatic damage can cause endocrine and exocrine system to fail which leads to reduce feed intake. In contrast, Houshmand *et al.* (2015) did not observe reduction in feed intake when oak acorn diet containing tannin was supplemented with 100% methionine more than NRC recommendation. This could be due to lower amounts of methionine supplement at 0.72% compared to 0.92% in current study. In conclusion, supplementation of high tannin sorghum with 100% methionine above recommended level lowered feed intake of broiler chickens.



**Figure 3:** Effect of tannin levels on weight gain during finisher phase



**Figure 4:** Effect of methionine levels on weight gain during finisher phase

#### 4.4.3 Effect of sorghum germination on weight gain during finisher phase

Weight gain of broiler chickens fed on G46 diet (1426g) was similar ( $P>0.05$ ) to those fed on UG46 (1299g). This lack of effect of sorghum germination could be explained by small difference of tannin intake between the both diets (UG46 9.2 g and G46 7.6 g tannin intake). This may mean that rate of digestion of both germinated and ungerminated diets were similar. In agreement, Abbas and Musharaf (2008) also reported that birds fed on 48 and 72 h germinated sorghum containing tannin, gave similar weight gain as those fed on ungerminated sorghum diet. In contrast, Torki and Pour (2007) reported that inclusion of germinated sorghum in broiler chickens diet improved the weight gain during finisher phase. However, these authors did not indicate the length of the germination period which may have been different from that used by Abbas and Musharaf (2008).



#### **4.4.4 Effect of methionine supplementation of ungerminated sorghum on weight gain at finisher phase**

Weight gain of birds fed on UG46 and diets supplemented with methionine (UG69 and UG92) were similar ( $p>0.05$ ). The methionine intake of broilers fed on diet UG92 was not much different from group of birds fed on diet UG69 (Figure 4). This was because of the effect of excess methionine on feed intake. Birds fed on diet UG69 consumed high amount of tannin as well as group fed on UG46 but had the highest (though not significant) (1473g) weight gain compared to UG46 and UG92 diets (1299g and 1297g). The lack of significance of methionine supplementation in diet UG69 on weight gain could be due to the low methionine requirement during the finisher phase. This may mean that addition of methionine during finisher phase can increase the weight gain but not necessarily to be significant. In agreement, Adbalqdadir and Arabi (2014) observed that supplementation of Fetarita sorghum based diet with 0.70% methionine had no effect on weight gain of broiler chickens during finisher phase. Similarly, Houshmand *et al.* (2015) observed that addition of 0.72% (100% more than NRC) methionine to the oak acorn containing high tannin sorghum had no effect on weight gain of broiler chickens during finisher phase. Golshahi *et al.* (2013) made similar observations. In contrast, Chang and Fuller (1964) reported that weight gain increased when high tannin sorghum diet was supplemented with 0.1% choline and 0.2% methionine. Del Vesco *et al.* (2013) observed increased weight gain of broiler chickens when low tannin sorghum was partially substituted with maize at range of 30.5% and supplemented with 0.75% (44%) methionine compare to control. Zhan *et al.* (2006) made similar observations. It can be concluded that methionine supplementation (0.69%) of high tannin sorghum based diets had beneficial effect on weight gain.

#### **4.4.5 Effect of feeding germinated sorghum and methionine supplementation of ungerminated sorghum on feed conversion ratio during finisher phase**

Feed conversion ratio was highest for broiler chickens fed on UG46 diet (2.5) significantly higher than diet G46 and UG92 (2.2) but similar to UG69 diets (2.3). The improved FCR for diet G46 was similar to that of starter phase which could be explain by reduced tannin level of the diet due to germination (figure 3). The lower FCR for diet UG92 can be explained by presence of sufficient methionine for protein synthesis and muscle accretion, although absorption of some of methionine is impaired in the GIT of the birds due to condense tannin. The poor feed conversion ratio of birds fed on UG46 diet was attributed to presence of tannin which forms complexes with protein, starch and digestive enzymes, resulting in poor utilization of feed. Armstrong *et al.* (1973) reported poor feed efficiency ratio when high tannin sorghum was fed to broiler chickens compare to diet supplemented with methionine. Crisol-Martínez *et al.* (2017) reported higher feed conversion efficiency when sorghum based diet (unknown tannin content) was fed to broiler chickens compared to wheat based diet. In contrast, Houshmand *et al.* (2015) reported no effect of feeding oak acorn diet containing high tannin on feed conversion ratio of broiler chickens compare to the diet supplemented with 0.72% methionine (Representing 100% above requirement). Adbalqadir and Arabi (2014) made similar observation. Medugu *et al.* (2010) observed insignificant effect on feed efficiency ratio of broiler chickens when high tannin sorghum was fed compare to low tannin sorghum or maize based diet. In conclusion, supplementation of the high tannin sorghum with 100% methionine above requirement improved the FCR of broiler chicken.

#### 4.5 Broiler chicken performance during entire phase (1-42 days)

The effect of germination and methionine supplementation of sorghum on feed intake, weight gain and feed conversion ratio to the recommended slaughter age of 42 days is shown in Table 4.6. The effects of tannin and methionine intake on weight gain are shown in figures 5 and 6.

**Table 4.6: Effects of germinated sorghum and methionine supplementation of ungerminated sorghum on performance of broiler chickens during entire phase 1-42 days**

Parameters	UG46	G46	UG69	UG92	SEM
Feed intake (g/chick)	4693 <sup>b</sup>	4563 <sup>b</sup>	4833 <sup>b</sup>	4156 <sup>a</sup>	103.0
Weight gain (g/chick)	2007 <sup>a</sup>	2116 <sup>ab</sup>	2258 <sup>b</sup>	2059 <sup>a</sup>	52.0
FCR	2.34 <sup>c</sup>	2.16 <sup>b</sup>	2.14 <sup>b</sup>	2.02 <sup>a</sup>	0.0374

*Means within a row with different superscripts differ significantly (P<0.05); UG46- Ungerminated sorghum based diet + 0.46% methionine (Met); G46. germinated sorghum based diet + 0.46% Met; UG69- ungerminated sorghum based diet +0.69% met; UG92- ungerminated sorghum based diet + 0.92% met; SEM – Standard error of mean*

##### 4.5.1 Effect of germination of sorghum on feed intake during entire phase

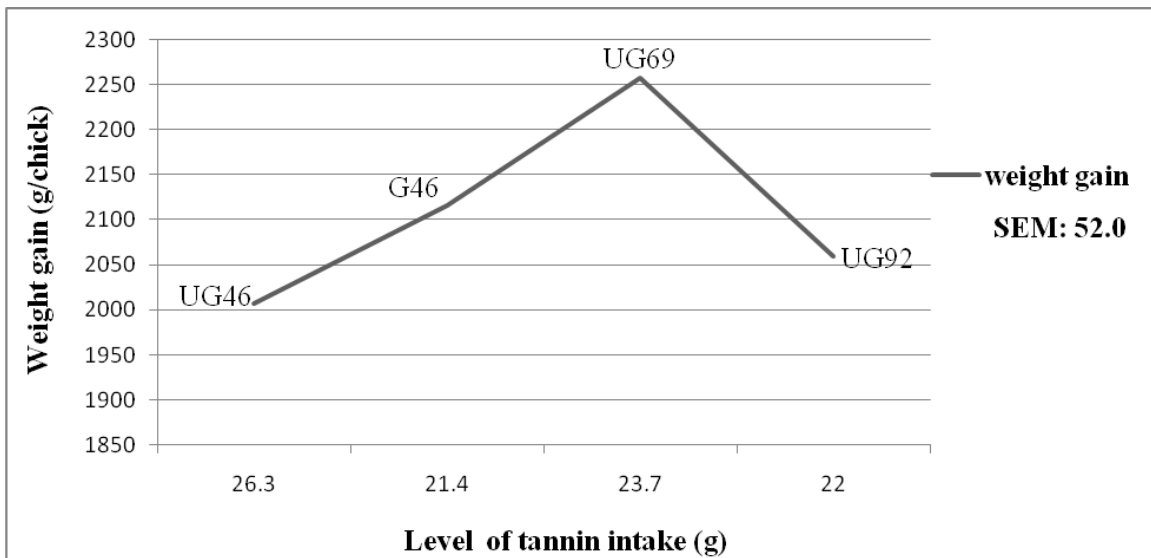
Feed intake of broiler chicken fed on G46 and UG46 diets was (P>0.05) similar, which was the case for the finisher phase. The reduced feed intake in broilers fed on diet G46 during starter phase was due to sorghum germination accumulating glucose in the blood which was known to reduce feed intake. This trend was changed at finisher phase which may mean that the broilers tried to compensate for their metabolizable energy requirement through increase of feed intake (though numerically was less). This led to lack of significant in feed intake at entire phase which support the theory by Leeson *et al.* (1996) that birds adjust their feed intake according to their energy requirement. Another possible reason could be attributed to low tannin intake in both broilers fed on diets UG46 and G46 (2.6 and 2.1g) which could not be enough to reduce feed

intake (Figure 9). The insignificant effect of germinated sorghum on feed intake was also reported by Abbas and Musharaf (2008) who observed that feeding 72h germinated low tannin sorghum did not affect the feed intake of broiler chickens during entire phase. It can be concluded that germination of high tannin sorghum have no effect on feed intake of broiler chickens at entire phase.

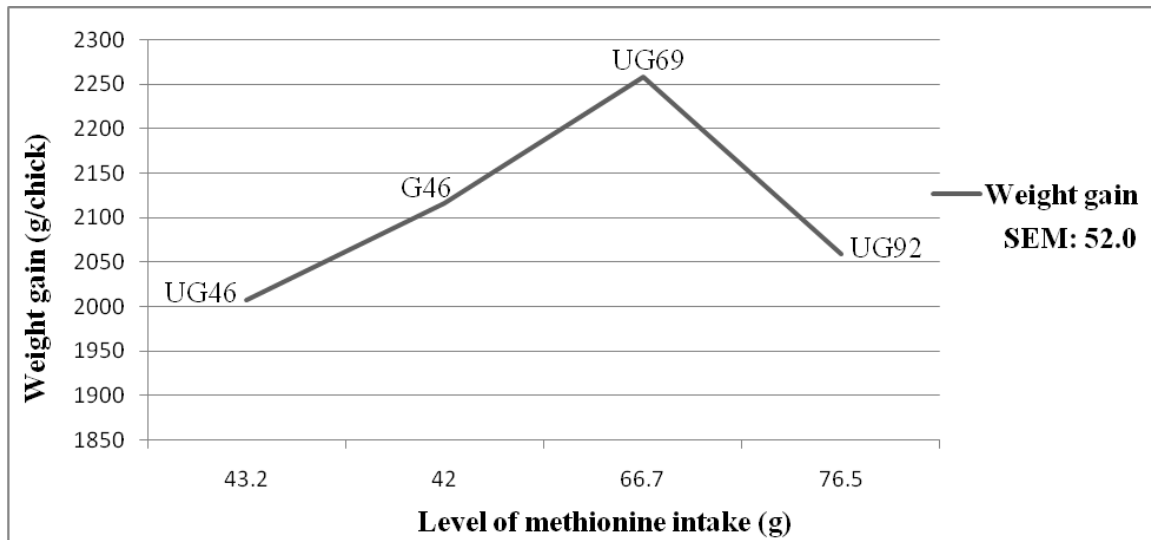
#### **4.5.2 Effect of methionine supplementation of sorghum on feed intake at entire phase**

Feed intake of broiler chickens fed on diet UG92 (4156) was lower ( $P < 0.05$ ) than those on diet UG69 and UG46 (4833, 4693). This observation was similar to that of the finisher phase. Tannin intake was higher in broilers fed on diet UG46 than in those on diets UG46 and UG69 (Figure 5). Supplementation of the methionine at 50% more than the recommendation had no effect on feed intake. The lowered feed intake in birds fed on UG92 was not due to presence of tannin but was associated with high methionine content of the diet. Reduced feed intakes have been reported in many studies when maize based diet supplemented with methionine above the requirement. In agreement, Han and Baker (1993) observed that excess methionine of 75% (Representing 1% compare to control 0.57%) above the requirement in the diet reduced voluntary feed intake of the broiler chickens. However, Kim *et al.* (2006) reviewed two theories concerning the deleterious consequences of excess limiting amino acid supplementation. First was the anabolic status saying surplus limiting amino acid stimulates the protein synthesis and instantaneously lessens the breakdown of protein in the liver which led to its reduction in the plasma, thus depresses the feed intake. Second is the catabolic status, surplus methionine may lead to a major loss of amino acid causing changes in amino acid pattern of the plasma, tissue and serum subsequently reduces the feed intake. Ueda and Tasaki (1977) observed that excess methionine in maize-soybean based diet of 1.87% methionine (Representing 225% methionine compare to control 0.83%)

more than NRC reduced the feed intake of the birds. Harter and Baker (1978) reported the same when maize soybean diet was supplemented with 1% methionine (Representing 285% compare to 0.35%) above NRC requirement. They attributed the reduced feed intake to methionine toxicity which caused by methionine buildup in plasma or tissue of common catabolic pathway such as homocysteine. In conclusion, feeding extra methionine up to 100% above the requirement lowered the feed intake in birds fed on diet UG92. This was possibly due to accumulation of methionine to plasma or tissue of bird.



**Figure 5:** Effect of tannin levels on weight gain for entire growth phase



**Figure 6:** Effect of methionine levels on weight gain for entire growth phase

#### 4.5.3 Effect of germinated sorghum on weight gain during entire feeding phase

Effect of germinated sorghum on weight gain of broiler chickens for overall feeding phase is shown in table 4:6. Weight gain of broiler chicken fed on UG46 and G46 was similar (2007g and 2116g). The lack of differences in weight gain was expected as both starter and finisher phases had similar outcome. This may be due to low tannin content of the diets which led to similar rate of digestion in both diets (UG46 and G46). In agreement, Abbas and Musharaf (2008) observed no effect of feeding 72 h germinated low tannin sorghum on weight gain of broiler chicken compare to ungerminated sorghum. Torki and Pour (2007) reported the same when germinated unknown tannin content sorghum was fed to broiler chicken. Bohoua and Yelakan (2007) reported improvement in laying hens' weight gain when 72 h germinated sorghum (unknown tannin content) was substituted with maize. This could be attributed to the partial replacement of germinated sorghum with maize. It can be concluded that germination had no effect on weight gain of broiler chicken.

#### **4.5.4 Effect of methionine supplementation on weight gain during entire phase**

The weight gain of birds fed on UG46, UG69 and UG92 is shown in Table 4.6. Weight gains of broiler chickens fed the diet UG69 was significantly higher compared to those on diets UG46 and UG92. The highest weight gain was attained by birds fed on diet UG69 when the quantity of methionine reached 66.7g grams, but the weight gain decreased when the amount of methionine was increased to 76.5g (Figure 2). This may mean that addition of methionine beyond 50% of NRC recommendation does not have additional benefits on weight gain of broiler chicks during the entire phase. This was confirmed by Houshmand *et al.* (2015). Supplementation at 50% more than requirement was beneficial to decrease the deleterious effect of tannin on feed intake and weight gain. The reduced weight gain of birds fed on diet UG92 was attributed to high methionine level of the diet other than the existence of tannin. The decreased weight gain of broiler chicken fed on diet UG46 was likely due to presence of tannin in sorghum which form complexes with protein and interfere with digestible enzymes, consequences is reduction in nutrients digestibility and their bioavailability to birds. In accordance, Chang & Fuller (1964) and Armstrong *et al.* (1973) observed that weight gain of birds fed on high tannin sorghum containing 2 and 1.57% T.A was poor compare to broilers fed on sorghum supplemented with 0.1 & 0.42% choline and 0.2 & 0.31% methionine. Armanious *et al.* (1973) reported poor weight gain when low and high tannin sorghum Pioneer 828 and DeKalb BR-64 containing tannin content of 0.20 and 1.24% T.A were fed to laying hens compare to diets supplemented with 0.4% methionine and 0.4% choline. Ahmed and Abass (2011) reported reduced growth performance when low tannin sorghum was fed to broiler chickens compare to diets supplemented with .55% (Representing10%) and 65% (30%) methionine. Kaur *et al.* (2013) observed that, excess methionine in the diet improves weight gain of birds due to amino acid balance. In contrast,

Armanious *et al.* (1973) observed high weight gain when high tannin sorghum GA 615 (1.20% TA) was fed to laying hens compare to diet supplemented with 0.4% methionine and 0.4% choline. Hind *et al.* (2012) reported that, feeding low tannin sorghum to broiler chickens had no effects on weight gain compare to diet supplemented with 0.70 (40%) and 0.90% (80% above NRC requirement) methionine. It can be concluded, feeding high tannin sorghum to broiler chickens as energy source reduced weight gain, but supplementation of the diet at 50% beyond the recommendation improved weight gain of the birds.

#### **4.5.5 Effect of germination and methionine supplementation of sorghum on feed conversion ratio during overall phase**

Feed conversion ratio of birds fed on diet UG46 (2.3) was poor and was different ( $P < 0.05$ ) from those fed diets G46, UG69 and UG92 (2.1, 2.1 and 2.0 respectively), the case is vice versa in group fed the diet UG92. Birds fed diets UG69 had similar FCR as the other group on G46 and both are significantly different from group on UG46. The lowered feed conversion ratio in birds fed on methionine supplemental diet UG69 and UG92 could be explained by beneficial impact of extra methionine on deleterious effect of tannin on methionine absorption/utilization although the feed intake was impaired in those fed on diet UG92. The improved FCR of birds on diet G46 was expected as outcome for the starter and finisher phases was similar. The poor FCR of broilers fed on diet UG46 was similar to that of starter and finisher phase which implying that presence of tannin interfered with bioavailability of protein, energy and specific amino acid (Douglas *et al.*, 1990 and Elkin *et al.*, 1996). In agreement, Melingasuk *et al.* (2012) reported poorer feed efficiency when four varieties of sorghum containing tannin range from 0.68 to 1.1% CE was fed to broiler chickens compared to maize based diet. Ibitoye *et al.* (2012) reported same when red sorghum unknown tannin content was fed to broiler chickens compare to low tannin



sorghum. Ahmed & Abass (2011) and Hind *et al.* (2012) observed that feeding low tannin sorghum resulted in poor feed conversion efficiency of broiler chickens compare to diet supplemented 40% and 30% methionine above requirement. In contrast, Chang and Fuller (1964) observed insignificant effect of feeding high tannin sorghum (tannin level 2% TA) on feed conversion efficiency of broiler chickens compared to sorghum supplemented with methionine 0.1% choline and 0.2% methionine. This could be due to low level of methionine supplementation compare to the methionine supplementation of the current study of 0.69 and 0.92%. Abbas and Musharaf (2008) reported similar feed conversion ratio when 72 h germinated sorghum containing tannin level of 0.34% CE was fed to broiler chicken compare to ungerminated sorghum. This could possibly be attributed to the similar outcome in starter and finisher phases. Shem *et al.* (1990) observed similar result when sorghum containing 0.87% tannin was fed to pigs compared to 48 h germinated sorghum. In conclusion, feeding high tannin sorghum increased the feed conversion ratio of broiler chickens.

## **CHAPTER FIVE: CONCLUSIONS AND RECOMMENDATIONS**

### **5.1 Conclusions**

The effect of germinating brown (*var Serena*) sorghum on chemical composition was determined as well as the effect of sorghum germination and methionine supplementation on broiler chickens performance was studied. This was based on the hypothesis that germination of *Serena* sorghum would reduce the tannin content and improve their nutritional composition. The other hypothesis was that, feeding germinated *Serena* sorghum or supplemented ungerminated sorghum with methionine would improve the performance of broiler chickens.

The results from the study led to the following conclusions:

1. Germination reduced the tannin content of *Serena* sorghum variety from 0.47 to 0.32% TA.
2. Germination of *Serena* sorghum had no beneficial effect on performance of broiler chickens.
3. Supplementation of high tannin diets with methionine upto 50% more than the recommended level improved performance of broiler chickens.

### **5.2 Recommendation**

1. From the study, it is recommended that high tannin sorghum should be supplemented with methionine but not more than 50% above the recommendation for better performance of broiler chickens.

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## CHAPTER SEVEN: APPENDICES

### APPENDIX 1: ANALYSIS OF VARIANCE FOR BROILER CHICKS FEED INTAKE FROM 1-21 DAYS OF AGE

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	3	49551	16517	4.76	0.021
Residual	12	41636	3470		
Total	15	91187			

### APPENDIX 2: ANALYSIS OF VARIANCE FOR BROILERS FEED INTAKE FROM 22-42 DAYS OF AGE

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	3	920016	306672	9.84	0.001
Residual	12	373838	31153		
Total	15	1293854			

**APPENDIX 3: ANALYSIS OF VARIANCE FOR BROILER CHICKENS FEED INTAKE DURING OVERALL PHASE (1-42 DAYS)**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	1022014	340671	8.03	0.003
Residual	12	509067	42422		
Total	15	1531081			

**APPENDIX 4: ANALYSIS OF VARIANCE FOR BROILER CHICKENS BODY WEIGHT GAIN DURING STARTER PHASE (1-21 DAYS)**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	3	23892	7964	7.09	0.005
Residual	12	13476	1123		
Total	15	37367			

**APPENDIX 5: ANALYSIS OF VARIANCE FOR BROILER CHICKENS BODY  
WEIGHT GAIN DURING FINISHER PHASE (22-42 DAYS)**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	3	95968	31989	2.96	0.075
Residual	12	129797	10816		
Total	15	225765			

**APPENDIX 6: ANALYSIS OF VARIANCE FOR BROILER CHICKENS OVERALL  
BODY WEIGHT GAIN (1-42 DAYS)**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	140964.	46988.	4.35	0.027
Residual	12	129599	10800		
Total	15	270563			



## **APPENDIX 7: ANALYSIS OF VARIANCE FOR BROILER STARTER FEED**

### **CONVERSION RATIO FED DURING 1-21 DAYS**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	3	0.257744	0.085915	10.77	0.001
Residual	12	0.095756	0.007980		
Total	15	0.353500			

## **APPENDIX 8: ANALYSIS OF VARIANCE FOR BROILER CHICKENS FEED**

### **CONVERSION RATIO FED DURING FINISHER PHASE (22 - 42 DAYS)**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	3	0.21884	0.07295	4.84	0.020
Residual	12	0.18095	0.01508		
Total	15	0.39980			

**APPENDIX 9: ANALYSIS OF VARIANCE FOR BROILER CHICKENS FEED  
CONVERSION RATIO FED DURING ENTIRE GROWTH PHASE (1- 42 DAYS)**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
treatment	3	0.207962	0.069321	12.40	<.001
Residual	12	0.067089	0.005591		
Total	15	0.275051			

**APPENDIX 10: PROXIMATE COMPOSITION OF MATERIALS USED IN DIETS  
FORMULATION**

<u>Composition (%)</u>	Pollard	Soybean meal	Fish meal
Dry matter	92.07	90.38	91.95
Crude protein	18.12	55.65	67.13
Crude fiber	13.84	8.42	1.13
Ether extracts	5.1	1.07	11.90
Ash	5.71	6.95	21.47