



GROWTH PERFORMANCE, HAEMO-BIOCHEMICAL PARAMETERS,
CARCASS CHARACTERISTICS AND MEAT QUALITY IN BROILER
CHICKENS FED MALTED SORGHUM-BASED DIETS

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BY

CHANDAPIWA MOSES

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**GROWTH PERFORMANCE, HAEMO-BIOCHEMICAL PARAMETERS, CARCASS
CHARACTERISTICS AND MEAT QUALITY IN BROILER CHICKENS FED
MALTED SORGHUM-BASED DIETS**

Chandapiwa Moses

Main Supervisor Dr. M. H. D. Marcko
Co - Supervisor Professor O. R. Madibela
Co - Supervisor Dr. F. Manyeula


Faculty of Animal and Veterinary Sciences
Department of Animal Sciences

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requirements for the degree of Master of Science (MSc) in Animal Science (Animal
Management Systems)**

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DECLARATION

I, Chandapiwa Moses declare that this thesis submitted by me for the degree of Master of Science in Animal Science (Management Systems) at the Botswana University of Agriculture and Natural Resources is my own original and independent research work. The thesis was carried out under the supervision of Dr. M. H. D. Mareko, Professor O. R. Madibela and Dr. F. Manyeula. This thesis or any part of it has not been previously submitted by me for any degree or examination to another faculty or University. The research work reported in this thesis does not contain any person's data, pictures, graphs or other information unless specifically acknowledged as being sourced from those persons.

Signature: -----

Date: 22/06/2021

Chandapiwa Moses (Student)



APPROVAL

Supervisor's name

MHD Murekiro

Date

29/06/21

Signature

[Signature]

Co-Supervisor's name

Prof OR Madibela
Dr. F. Mamegella

Date

22/06/21

22/06/21

Signature

[Signature]
F Mamegella

Head of Department's name

P.M. Kgwarekware

Date

28/6/21

Signature

[Signature]

GENERAL ABSTRACT

This study was conducted to investigate the effect of feeding malted sorghum grains in place of maize grains on growth performance, blood parameters and meat quality of Ross 308 broiler chickens (R308BC). A pilot work (Study 1) was conducted to investigate the effects of malted sorghum grains on their chemical composition before the feeding trials commenced. Unscreened grains of Mr Buster (red sorghum) and *Segaolane* (white sorghum) were malted and analysed for crude protein (CP), crude fibre (CF), organic matter (OM), ash, energy, crude fat, and condensed tannin levels and their interaction effects. Condensed tannin content of malted and unmalted Mr Buster (0.08% versus 0.09%) was significantly higher than that of malted and unmalted *Segaolane* grains (0.05% versus 0.04%). Grain type had effects on dry matter (DM), OM, ash, CP and condensed tannin while the effects were not observed in energy and crude fat contents. Malting affected ($P < 0.05$) OM, ash, CF and condensed tannins but did not affect DM, CP and energy. Interaction effects of grain type and malting were observed on CF and condensed tannin content of sorghum. It was concluded that although malting does not alter chemical composition of the grains, it however, reduces CF and condensed tannin levels. Based on these initial findings a feeding trial was conducted to determine growth performance of broiler chickens as reported below.

In the second study (Study 2), 150 day old R308BC were randomly allotted to the following dietary starter treatment diets: Control diet = Broiler commercial diet containing 100% maize; *Segaolane* diet = Broiler commercial diet containing 100% malted *Segaolane* in place of maize; and Mr Buster diet = Broiler commercial diet containing 100% malted Mr Buster in place of maize. The results for growth performance, in weeks 3 and 4 showed rapid increase in average mean feed intake (AWFI) across all experimental diets. There was an increase in average

weight gain (AWG) and mean weekly body weights of R308BC across the diets. No significant differences in AWG were observed at weeks 3 and 6 on R308BC fed malted Mr Buster and *Segaolane* based diets. In week 2, low feed conversion ratio (FCR) was recorded in R308BC fed Control diet followed by those fed malted *Segaolane*-based diet. Chickens fed malted Mr Buster diet had highest FCR in weeks 3, 4, 5 and 6. However, no significant difference was observed between the FCR from R308BC on Control or malted *Segaolane*-based diets at weeks 3, 4 and 5. This means that malted *Segaolane* and maize caused similar response suggesting that *Segaolane* can be a suitable replacement of maize grains. The R308BC fed malted Mr Buster diet had poor FCR at weeks 3, 4, 5 and 6 compared to other experimental diets. The protein intake (PI) of birds fed Control diet was lower than those fed other experimental diets. In week 5, birds fed on Control diet had the highest protein efficiency ratio (PER) compared to those on malted Mr Buster-based diet. Birds fed on Control diet had similar PER as those fed malted *Segaolane*-based diet.

The results for blood parameters were within the normal range. Birds fed Control diet had significantly higher ($P < 0.05$) white blood cells count ($46.8 \times 10^3/\mu\text{L}$) compared to those fed malted sorghum-based diets. The highest ($P < 0.05$) basophil count was observed on birds fed malted *Segaolane*-based diet (0.57%) and malted Mr Buster-based diet (0.56%). Glutamic oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) enzymes were lowest ($P < 0.05$) in birds fed malted *Segaolane*-based diets. No significant differences were observed in lymphocytes, neutrophils, monocytes, eosinophils, red blood cells, haemoglobin, haematocrit, and blood platelets of birds across experimental diets. Experimental diets did not have any significant effect on albumin, total protein, triglycerides, urea and bilta of Ross 308 chickens. Therefore, it can be postulated that malted sorghum grains did not have a negative impact on the blood biochemistry and physiological function of birds. This implies that malted

sorghum grains can be used as substitute to maize in broiler diets without having any adverse effects on the health status of chickens. Positive growth response and good health due to manipulation of diets of food animals should also result in acceptable quality of the food product. Therefore, the third study was designed to test the quality of the meat derived from chickens fed malted sorghum-based diets.

In the carcass characteristics and meat quality study (Study 3), birds fed Control diet had the highest ($P<0.05$) slaughter, hot carcass and cold carcass weights. On the other hand, birds fed Control diet had the lowest ($P<0.05$) drumstick-thigh and vertebrae. The highest ($P<0.05$) vertebrae weight was recorded in birds fed malted *Segaolane*-based diet. This implies that malted sorghum-based diets performed similar to Control in these portions regardless of lower carcass weights. The fact that malted sorghum-based diets improved drumstick-thigh weight portions, which are popular with consumers indicates that these diets can be considered as energy ingredients in formulating chicken diets.

Regarding viscera macromorphometry, birds fed malted Mr Buster-based diet had the longest ($P<0.05$) large intestine, heavy gizzard, heart and liver implying that the diet affected the bird's internal organs negatively. The initial pH (pHi) of the meat of birds fed malted Mr Buster were significantly higher ($P<0.05$) than that of the birds fed Control or malted *Segaolane*-based diets. Experimental diets did not significantly affect DM, ash, OM and energy content of breast muscle from R308BC. Regarding the mineral composition of the breast, birds fed malted sorghum-based diets had lower ($P<0.005$) phosphorus content compared to breast muscle of birds fed Control diet. Potassium and magnesium contents in the breast muscle from birds fed malted sorghum-based diets were the highest ($P<0.05$). The findings of this study suggest that malted *Segaolane* grains can be used as an energy alternative to maize in broiler diets. This can

reduce the competition of maize grains between livestock feed processing and human consumption and possibly reduce costs of livestock feeds.

Keywords: *Segaolane*, Mr Buster, malted, growth performance, meat quality, R308BC

DEDICATION

This work is dedicated to my daughter Zawadi Moses. She has been my motivation and reason to focus on my studies. I love you my baby girl. My Mother Ms. Nyaladzi Moses thank you for taking care of my daughter when the journey was getting tough and Zanele Moses for being a great Aunt to my girl at difficult moments. All this is for you family. God bless you.

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ABBREVIATIONS AND ACRONYMS

%	Percentage
µL	Microlitre
µmol/L	micromole/litre
ANFs	Antinutritional factors
ANOVA	One-way analysis of variance
AOAC	Association of Official Analytical Chemists
AWFI	Average weekly feed intake
AWG	Average weekly gain
BAMB	Botswana Agricultural Marketing Board
BDG	Brewers dried grain
BOBS	Botswana Bureau of Standards
BOS	Botswana Standard
Ca	Calcium
CF	Crude fibre
CT	Condensed tannin
DDGS	Distillers dried grains
DM	Dry matter
EE	Ether extract
FCR	Feed conversion ratio
fl	Femtolitres
g	gram
g/Dl	gram/decilitre
g/L	gram/litre
GIT	Gastro-intestinal tract

GLM	General linear model
GPT	Glutamic Oxaloacetic Transaminase
HCW	Hot carcass weight
hr	Hour
IU/L	International units per litre
Kg	Kilogramme
MCH	Mean Corpuscular Haemoglobin
MCHC	Mean Corpuscular Haemoglobin Concentration
MCV	Mean Corpuscular Volume
ME	Metabolisable energy
MJ	Mega Joules
MJ/Kg	Mega Joule/kilogramme
mmol/L	millimoles/litre
MSP	Malted sorghum sprout
NFE	Nitrogen free extract
°C	Degrees Celsius
OM	Organic matter
PER	Protein Efficiency Ratio
Pg	Pictograms
pH	The power of hydrogen ions
PI	Protein intake
SAS	Statistical Analysis System
SE	Standard error
SSM	Salseed Meal

CHAPTER 1

GENERAL INTRODUCTION

1.1 Background

The poultry industry in the past two decades, has been one of the most dynamic and constantly growing animal production sectors in the world (Alkhalaf *et al.*, 2010). In Botswana, the poultry industry has two distinct sub-sectors namely; the small-scale and commercial. There are two common poultry production systems in Botswana. These are commercial and poultry (also known as traditional sector) production systems.

A broiler is any chicken that is bred and raised specifically for meat production. Most commercial broilers reach slaughter weight between four and seven weeks of age. This high growth performance and high meat yield is realised because of genetic selection and improved nutrition (Barbosa-Filho *et al.*, 2017). However, selection for fast growth and high yield may have negatively impacted the sensory and functional qualities of the meat (Fanatico *et al.*, 2007). Meat and eggs from chickens are good sources of protein, vitamins and minerals for humans, but production by birds to supply these important nutrients may be compromised by poor quality of feeds. These poor quality feeds can be improved by incorporating cheaper yet good quality protein and energy ingredients from local producers to enhance their utilisation.

It is well known that protein and energy are amongst the two expensive nutrients used when formulating poultry rations, hence they are of great concern to poultry nutritionists (Rose, 1997). As a result, there is a need to search for alternative cheaper energy and protein sources

and associated feed processing methods in order to improve protein and energy utilisation by poultry.

Over the years there has been an increase in poultry feed prices leading to resource-poor farmers failing to supply adequate nutrition to their chickens, thus resulting in low productivity. Maize (*Zea mays*) is the predominant cereal for human and animal nutrition, as a source of energy, as well as, in the production of biofuels. In Botswana, this scenario creates a competition for maize between animal and humans, leading to an increase in the price of poultry feed and further influencing maize shortage. One of the potential and suitable replacements of maize grains in poultry diets is sorghum grain.

Sorghum (*Sorghum bicolor*) is readily available in Botswana, and the crop is tolerant to harsh climatic conditions compared to maize. However, it is known that sorghum grains contain anti-nutritional factors (ANFs) which make sorghum less digestible (Taylor *et al.*, 2007). The ANFs make feed not to be efficiently utilized, thus lowering its feeding value (Etuk *et al.*, 2012). These anti-nutritional factors include tannins and phytic acid (Osman and Gasseem, 2013), which lower palatability and protein utilisation in non-ruminants (Gualtieri and Rapaccini, 1990). In an earlier study by Legodimo and Madibela (2013), it was noted that malted red sorghums (Mahube and BSH1) tended to have higher organic matter (OM) digestibility in simulated rumen environment *in vitro*. Recent preliminary data from our laboratory have shown that malted sorghum varieties; Mr Buster and *Segaolane* grains, have crude protein (CP), ash, gross energy and fat of 11.64% and 12.40%; 15.33% and 10.44%; 16.58% and 16.22%; 9.34% and 8.51%, respectively. Therefore, the general aim of this research is to investigate possible inclusion of malted sorghum varieties in poultry diets with anticipation to improve digestibility and utilisation by chickens, consequently improving chicken production.

1.2 Problem Statement

Yellow maize grain which is the main energy source in poultry diets is in short supply in Botswana, hence it is usually imported. This is because Botswana has inadequate rainfall levels and lacks irrigation resources to support large-scale maize production for both human and livestock consumption. Compared to sorghum, maize needs a lot of water for irrigation. With the advent of climate change and increase in aridity, the cultivation of maize will become more difficult. Unlike maize, the sorghum crop can resuscitate after a dry spell since the roots are deep and can withstand local harsh climatic conditions. Therefore, farmers should be encouraged to grow more sorghum and channel surplus into livestock feed production. Grain and grain by-products are traditionally used in poultry feeding, the challenge is the deficit in the supply of the grains and the high costs associated with the purchase of commercially produced feeds. Therefore, it is ideal to innovate around alternative energy feed sources for poultry, based on sorghum in order to reduce competition between human and livestock needs for maize. Malting sorghum grains is a processing technique that can help improve nutritive value of sorghum in non-ruminant feeding

1.3 Justification

Poultry meat and eggs are high quality protein sources for human daily requirements, and are good for embryonic and brain development and for provision of essential amino acids (Northcutt, 1997; Gracey *et al.*, 1999; Warriss, 2000). Increased urbanization leads to more consumption of poultry proteins as they are cheaper and more accessible. Poultry need less rearing space compared to other livestock species, hence farmers can rear poultry in the backyards to improve household food security and capital investments since poultry are also more efficient in feed conversion.

Feed cost is the largest single challenge in poultry production (Mosinyi, 1999). The basic idea in the present research work is to replace maize grains with malted sorghum grains in poultry feeds so as to make maize available for human consumption. Maize grain prices have remained high and will probably increase in the future due to climate change and increasing human population. Therefore, alternative, accessible and cost-effective ingredients are needed that can provide the same nutrients as maize. As indicated by Medugu *et al.* (2010), sorghum (*Sorghum bicolor*) has been identified as a suitable replacement for maize. Sorghum is abundant in most parts of Africa and Asia, and is among the main cereal crops that are used as food and feed. The adaptive agronomic characteristics of sorghum make it suitable for cultivation in different environmental conditions. However, inclusion of sorghum in animal feeds, especially monogastrics has been found to reduce intake and affect growth. This is attributed to anti-nutritional compounds contained in sorghum grains.

In order to have effective utilisation of cereal or legume grains in poultry, it is important that anti-nutritional factors (ANFs) be reduced (Akande and Fabiyi, 2010). Therefore, before using sorghum grains as feed ingredients in both poultry and pig diets, some form of processing is needed. The principle of malting has been practised in the country from pre-independence days to produce beer but not for inclusion in animal feed. Legodimo and Madibela (2014) used malting to treat sorghum grains and found it to improve digestibility of red than white sorghums. Therefore, utilizing this indigenous knowledge to formulate chicken feeds will be of help to resource resource-poor farmers and possibly increase profitability to small-scale producers who intend to produce for the poultry market and the commercial feed millers which struggle in sourcing maize grains to formulate poultry feeds.

1.4 General Objective

The broad objective of this study was to investigate the growth performance, haemo-biochemical parameters, carcass characteristics and meat quality in broiler chickens fed malted sorghum-based diets.

1.4.1 Specific Objectives

The specific objectives of this study were to:

1. determine chemical characterization of malted sorghum grains;
2. evaluate growth performance and haemo-biochemical parameters of broiler chickens fed malted sorghum-based diets; and
3. evaluate the effects of malted sorghum-based diets on carcass characteristics and meat quality of broiler chickens.

1.5 Research Question

Does feeding malted sorghum-based diets influence growth performance, blood chemistry, carcass characteristics and meat quality of broiler chickens?

Ha (Alternative Hypothesis) of the first objective

There is difference in chemical characterization between malted (Mr Buster and *Segaolane*) and unmalted grains as feed to broiler chickens reared under intensive system.

Ho (Null Hypothesis)

There is no difference in chemical characterization between malted (Mr Buster and *Segaolane*) and unmalted grains as feed to broiler chickens reared under intensive system.

Ha (Alternative Hypothesis) of the second objective

There is a difference in growth performance and blood parameters of broiler chickens fed malted (Mr Buster and *Segaolane*) grains compared to maize-based diets.

Ho (Null Hypothesis)

There is a no difference in growth performance and blood parameters of broiler chickens fed malted (Mr Buster and *Segaolane*) grains compared to maize-based diets.

Ha (Alternative Hypothesis) of the third objective

There is difference in carcass characteristics and meat quality of broiler chicken fed malted (Mr Buster and *Segaolane*) grains compared to maize-based diets.

Ho (Null Hypothesis)

There is no difference in carcass characteristics and meat quality of broiler chicken fed different malted (Mr Buster and *Segaolane*) grains compared to maize-based diets.

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CHAPTER 2

LITERATURE REVIEW

2.1 Poultry Production in Botswana

The consumption of chicken meat is growing worldwide, mainly because it is inexpensive compared to meats from other species and its nutritional attributes are suitable for human health (Jaturasitha *et al.*, 2008). The study by Grynberg and Motswapong (2011) reported that there were 9-10 relatively large producers of poultry in Botswana who are members of the Botswana Poultry Association. Lekobane and Seleka (2011) reported that the minimum efficient scale in the broiler industry in Botswana is achieved when a facility is producing between 30,000 and 50,000 units per week. There are a large number of small-scale and contract producers who are producing well below this scale level. These small-scale contract producers supply the large firms on a contract basis and also supply government institutions, such as schools and the army through tender processes (Grynberg and Motswapong, 2011).

Poultry production system entails rearing day-old chicks to slaughter age and then slaughtering them in an abattoir. One of the major constraints to the poultry business is the rising costs of inputs, mostly feed costs as feed is imported from South Africa. Reddy (1991) stresses that there are no strict and compulsory quality control measures either in the hatchery or on feed millers hence the industry is not regulated, resulting in frequent low production episodes.

2.2 Poultry Nutrition

There are many factors that influence the selection of feedstuffs with regard to poultry, and these include but not limited to nutrient availability which is affected by the fibre content, fat

content, and amino acid balance (Kumar *et al.*, 2005). Poultry convert feed into food products quickly, efficiently, and with relatively low environmental impact compared with other livestock. The high rate of productivity of poultry results in relatively high nutrient needs of animals. Poultry require the presence of at least 38 dietary nutrients in appropriate concentrations and balance (Dozier *et al.*, 2010). Carbohydrates are an important source of energy for poultry (Kumar *et al.*, 2005). Maize, wheat and other cereal grains are the main sources of carbohydrates in poultry diets. Certain dietary carbohydrates are not easily digested by poultry; therefore, it is always ideal to add appropriate enzymes and to ferment the ingredient to enhance nutrient absorption (Rostagno *et al.*, 2007).

In non-ruminant animals, dietary requirements for protein are in fact requirements for amino acids for lean tissue and immune function. Amino acids are also used for structural and protective tissues such as skin, feathers, bones and ligaments (Rostagno *et al.*, 2007). Minerals are required for various functions such as formation of the skeleton and stabilising pH levels throughout the body (Dozier *et al.*, 2010) and immune function (Cowieson and Ravindran, 2008) Water must always be provided as it softens the feed and carries it through the digestive tract and transport nutrients from the digestive tract to cells. Broiler chickens have different nutritional requirements during their three phases of growth (Table 2.1).

2.2.1 Maize grains

Maize is a major source of dietary energy in poultry nutrition. This is because maize is high in energy compared to other cereal grains. The total procurement of maize by the Botswana Agricultural Marketing Board (BAMB), which is the only significant buyer, was approximately 4,500 tonnes in 2009, and almost all of it went largely to the two largest milling (Bokomo and Bolux) firms in Botswana (World Bank, 2015). The domestically produced

maize available through BAMB was used by these firms in the maize milling sector to produce maize meal for human consumption and not for animal feed (World Bank, 2015). Although maize is produced throughout the world, there is stiff competition for maize grains among humans, livestock and the feed manufacturing industry. However, with rapidly changing environment from suitable to the one not conducive for maize production, there is need to explore other environmentally resilient crops such as sorghum as a source of energy for poultry.

Table 2.1 Nutritional requirements of broilers

Nutrient	Phase		
	Starter	Grower	Finisher
Metabolisable energy MJ/kg	12.14-12.98	12.56-13.81	12.98-13.81
Crude protein %	21-24	18-23	17-22
Crude fibre %	5	8	8
Calcium %	0.85-1.05	0.80-1.00	0.3-0.5
Methionine %	0.37	0.33	0.3
Lysine %	1.10	0.85	0.9
Sodium %	0.01-0.03	0.01-0.3	0.01-0.3

Source: Rostagno *et al.* (2007).

2.2.2 Sorghum grains

Sorghum bicolor (L.), is the fifth leading crop in the world following rice, maize, wheat and barley. Its protein content is higher than that of maize although its nutritional protein quality is lower (Dowling *et al.*, 2002). Sorghum has been a staple food for most of the African countries for ages. It can grow in soils where the pH ranges from 5.0-8.5 and is more tolerant to salinity than maize. Its adaptation and performance in poor soil allows it to produce grain under

conditions where many other crops would fail (Martin, 1970). The nutritive significance ($p < 0.05$), cost and availability make sorghum the closest alternative feed ingredient to maize in poultry diets (Maunder, 2002). One of the constraints on utilisation of sorghum grains as feed is the presence of condensed tannins in some cultivars. According to Chen *et al.* (1994) and Etuk *et al.* (2012), sorghum grain has ANFs that make it less digestible compared to maize. The ANFs include tannins and phytic acid (Khattab and Arntfield, 2009), and these lower the palatability and protein utilisation in non-ruminants (Gualtieri and Rapaccini, 1990). The possibility to remove or greatly reduce the tannin component of sorghum has caused much interest to producers, growers and nutritionists. To overcome the constraints of ANFs in sorghum grain-based diets, Legodimo and Madibela (2013) have tested effects of malting in improving digestibility of sorghum grain. These authors found out that, malts from white grain sorghum varieties had higher neutral detergent fibre (52.0%) than those from red coloured varieties (42.3%). While malts from red coloured grains had higher percentage of acid detergent fibre (5.6 %) than those white grains (4.5 %).

2.3 Factors Affecting Energy Digestibility and Utilisation

Energy and protein are the two most expensive nutrients that need to be supplied in practical poultry rations and are of greatest concern to poultry nutritionists (Rose, 1997). Olukosi *et al.* (2008) reported that improvements in broiler performance are often associated with increased nutrient digestibility and energy utilisation. Concentration of energy in the diet greatly influences intake of other nutrients and utilisation of metabolisable energy (Ferket and Gernat, 2006). Energy supply has an important impact on performance of animals. Some of the factors affecting energy digestibility and utilisation are discussed below.

2.3.1 Diet composition

Diet composition has an impact on energy digestibility. It is well established that when birds are fed energy in excess of their metabolic needs, fat deposition increases largely in the area of abdomen and viscera (Ravindran, 2014). Several nutritional approaches are possible to restrict energy intake, and these include feed restriction and narrower energy to protein ratio.

The addition of phosphate or carbonates has been found to reduce the digestibility of energy, OM or crude protein (CP) (Noblet and van Milgen, 2013). Variation of digestibility of energy is related to the presence of dietary fibre (Noblet and van Milgen, 2013) and also reduces the apparent faecal digestibility of other dietary nutrients such as crude protein (CP) and fat. In an experiment by Ball *et al.* (2010), high digestibility coefficients for the cereal-based diets were found to be higher than product-based diets plus oil in growing pigs. The above authors noted higher live weight gains in pigs fed cereal-based diets compared to those fed by-product-based diets plus oil. It was concluded that cereal-based diets resulted in higher levels of digestible energy intake and live weight gains.

2.3.2 Animal factor

Different livestock species and their production states affect energy digestibility and utilisation. According to Noblet (2013), digestibility coefficient of energy (DCE) increases with increasing body weight in pigs. In a study by Olorunnisomo *et al.* (2006) the effect of nitrogen level on the utilisation of maize offal and sorghum brewer's grain in sheep indicated a slightly improved digestibility of dry matter (75.84% vs. 74.26%), CP (71.80% vs. 70.38%) and neutral detergent fibre (NDF) (77.90% vs. 76.34%) of sorghum brewer's grain over maize offal. However, the authors observed that energy digestibility was higher for sheep fed maize offal than sorghum brewers' grain (78.38% vs 75.65%). Metabolisable energy values tend to increase with age

(Noblet, 2013). Mandal *et al.* (2006) noted no difference in the metabolisable energy values of white (low tannin), brown (medium tannin) and red (high tannin) sorghum varieties fed to cockerel (chickens), guinea fowl and. To improve digestibility and utilisation of feeds, it is sometimes necessary to treat or process feeds before feeding.

2.3.3 Feed processing

Feed processing has an impact on energy digestibility and utilisation by an animal. Noblet (2013) stated that digestibility can be modified by technological treatments which can neither be less complex or more sophisticated. Pelleting of feeds increases the energy digestibility of feeds by about 1% (Le Gall *et al.*, 2009). Germination and malting increase activity of α -amylase (Traore *et al.*, 2004), and consequently increase the digestibility of starch. Therefore, it is important that any processing of livestock feeds, like malting, be tested for its effects on growth, carcass characteristics, meat quality and health of animals. In addition to improving feed quality, processing can be used to remove and/or reduce ANFs.

2.3.4 Anti-nutritional factors

According to Bora (2014), ANFs also referred to as anti-nutritive factors, or plant secondary metabolites, are constituents which may be used either by themselves or through their metabolic products. Hariprasanna *et al.* (2015) defines ANFs as deleterious compounds that are found in the grain which interfere with the absorption of biomolecules and hamper their bioavailability to the human beings and monogastric animals. The ANFs interfere with animal feed utilisation and affect the health and production of animals. Anti-nutritional factors may produce several adverse effects and reduce nutrient digestibility by binding with proteinous material (Hamid *et al.*, 2017). Therefore, if sorghum is to be used in livestock diets, especially

monogastrics, it is important to find out which ANFs it contains and how these can be mitigated.

2.3.4.1. Anti-nutritional factors in sorghum

Sorghum is a rich source of phytochemicals including tannins and phenolic acids. The ANFs present in sorghum grain are mainly polyphenols and phytic acid. The polyphenols (tannins) and phenolic acids present in sorghum are generally associated with grain pigmentation and they interfere with bioavailability of other major nutrients (Hariprasanna *et al.*, 2015). Nutrient digestibility of sorghum is influenced by the level of tannin concentration in the grain (Rostagno *et al.*, 1973). According to Proietti *et al.* (2015), tannins impact negatively on the digestibility of proteins and carbohydrates by forming complexes with these molecules and the enzymes involved in their digestion thus inhibiting their activities.

The negative impact of tannins on feed intake, weight gain, feed conversion ratio (FCR) and nutrient digestibility in poultry has been reported in many studies. For instance, Sannama (2002) reported reduced weight gain in broiler chickens on high tannin sorghum-based diet compared to maize based diet. Ibrahim *et al.* (1988) also reported that, feeding high tannin sorghum to broiler chickens impaired feed intake compared to the low tannin sorghum. In addition, Mitaru *et al.* (1983) reported that feeding high tannin sorghum lowered feed conversion efficiency of broiler chickens compared to low tannin sorghum.

A feeding trial was carried out by Melingasuk *et al.* (2012) to study the effects of sorghum tannins on the performance and carcass characteristics of broiler chickens. In contrast to the findings highlighted above, it was found that broiler performance and carcass characteristics showed inconsistent trend as the level of tannins increased, since the birds were consuming

sorghums with high tannin content gave better performance than their counterparts fed medium tannin content. The strain of bird used was the unsexed Hubbard commercial broilers and a justification to their findings was that tannin level may not be the sole factor affecting the nutritional quality of sorghum grains other unknown factors may be involved.

Studies (Waghorn *et al.*, 1987; Hoste *et al.*, 2012; Naumann *et al.*, 2014; Tedeschi *et al.*, 2014) in ruminants fed forages or diets containing condensed tannins of <5g/kg DM of total ratio noted improved gains and immune response due to tannins initially binding to protein in the rumen and subsequently releasing them in the acidic abomasum environment and increasing amino acids supply in the small intestine. It is possible that small amounts of condensed tannin in poultry diets elicit similar physiological and biochemical trends, thus resulting in inconsistent results in different studies. Perhaps a comparative study on graded levels of condensed tannins in poultry diets is needed to unpack this issue. This is because according to Reyes-Sanchez *et al.* (2000), slaughter weights and carcass weights in poultry are expected to be negatively affected by increased tannin concentration. So far, there is strong evidence from literature that high tannin sorghum-based diets in poultry have negative effects on performance of chickens. Therefore, there is need to innovate around ways of reducing tannins and other phytochemicals in sorghum destined for poultry diets.

2.4 Reducing the effects of anti-nutritional factors

There are various processing methods that can be used to reduce ANFs in grains to improve their utilisation by livestock. The choice of method of reducing ANFs depends on its effectiveness in reducing tannin and the cost involved (Medugu *et al.*, 2012). These techniques include but are not limited to malting, addition of enzymes, fermentation and some chemical methods as described below.

4.1 Malting, fermentation and germination

The malting process converts raw grain into malt (Dewar *et al.*, 1997) through first germinating the grains and then drying the sprout which is ground to make a meal. This meal is traditionally used to make beer. However, when used to process poultry diets malting has been found to improve digestibility, utilisation and efficiency of nutrients (Ogbonna *et al.*, 2012). In their study, Ogbonna *et al.* (2012) tested the effects of malting of sorghum grains and reported that malting reduced the amount of oxalate by 34.13%, tannins by 8.45%, and trypsin inhibitor activity by 36.5% and phytate by 66%. The authors concluded that malting as a processing technique can be used to enhance nutritional status of sorghum with reduction in some of its anti-nutritional factors. Osuntogun *et al.* (1989) reported that the condensed tannin content of high tannin sorghum decreased from 2.92% to about 1.3% after 48 hours of germination.

Another study by Elmaki *et al.* (1999) observed that 10 hour soaking and 48 hour germination of Gadamelhamam and cross 35:18 (low and high tannin content sorghums) reduced the tannin content determined by vanillin-HCl method (CE) from 0.34% to 0.20% and from 1.44% to 0.31%, respectively. Kyarisiima *et al.* (2004) also reported that germination of high tannin sorghum for 28 hours reduced tannin content from 8.27 to 6.51 mg catechin/100 g. The reduction of tannin was attributed to its leaching out in the water during steeping phase (Ogbonna *et al.*, 2012). Osman and Gasseem (2013) studied the effect of 72 hour germination on tannin content of three varieties of sorghum (baidha, shahla and hamra) in South-west Saudi Arabia and observed that tannin content of baidha increased from 0.058 to 0.084% and from 0.866 to 1.174% for hamra variety while it decreased from 0.392 to 0.364% in shahla. The increase in tannin content could be attributed to hydrolysis of condensed tannins such as proanthocyanidin (Osman and Gasseem, 2013) while the decrease may be due to their binding

with cotyledon endosperm that is usually undetected by routine method because of their insolubility in a solvent.

Osman (2004) also investigated the effects of fermenting sorghum for 24 hours on ANFs and found that phytic acid, trypsin inhibitors and tannins were reduced, whereas *in vitro* protein digestibility increased. In another study, Yousif and El-Tinayi (2001) observed that fermenting sorghum for 36 hours resulted in an increase in titratable acidity, CP, protein digestibility, and total solids. El-Hag *et al.* (2002) also tested the effects of fermentation on pearl millet and reported a reduction in trypsin inhibitors and increased protein digestibility.

Fermentation has also been found to be beneficial to other nutrients. For instance, Sripriya *et al.* (1997) investigated the effects of fermentation of finger millet and found improved bioavailability of calcium, phosphorus, and iron. Similarly, Ojha *et al.* (2018) noted that malting and fermentation on ANFs increased crude fibre, minerals, protein digestibility, α -amylase activity but resulted in a decrease in oxalates, tannins, and phytates in the resultant sorghum flour. Another study by Ongol *et al.* (2013) using maize found that germination and fermentation increased crude fibre, total protein, free and conjugated phenolics, and niacin but decreased fat content. From these results, it appears that malting or germination can reduce tannin content in variety of cereals. Information on nutritional effects of malted sorghum-based diets fed to Ross 308 chickens and their performance is scarce. In addition to fermentation and malting, feed additives can be used to improve feed utilisation by poultry.

2.4.2 Addition of enzymes

As more agricultural by-products are included in poultry diets, there are greater opportunities to maximise nutrient availability by the use of feed enzymes. Cowieson and Ravindran (2008)

investigated the growth and digestibility assay of male Cobb broiler chickens to assess the effects of an enzyme cocktail of xylanase, amylase and protease in maize-based diets. The researchers concluded that the energy and amino acid values of maize-based diets for broilers can be enhanced by supplementation with an enzyme cocktail of xylanase, amylase and protease, which offers potential economic benefits to producers. Chimote *et al.* (2009) studied the effect of adding enzyme on growth performance of quail and found that inclusion of multi-enzyme improves body weight gain significantly. Also, Raza *et al.* (2009) reported that the use of enzyme in broiler diets containing 6% crude fibre significantly increased body weight compared to a diet without enzymes. In contrast to these studies, Rabie and Abo El-Maaty (2015) observed that final live body weight and body weight gain of growing Japanese quails fed diet supplemented with Bio-Feed[®] Pro enzyme were significantly depressed and this could be attributed to the fact that maybe the enzymes did not breakdown the feed particles for easy utilisation by the quails. Mikhail *et al.* (2013) demonstrated that the dietary enzyme supplementation with distiller's dried grains (DDGS) of quail diet did not affect body weight and body weight gain.

In a study that evaluated the efficacy of exogenous enzymes supplementation on performance, carcass traits and some blood parameters of broilers chickens Goli and Shahryar (2015) found that the lowest feed consumption and weight gain were in the control group while the highest was obtained in groups that were fed with multi enzyme supplementation. The researchers also noted a poor FCR in broilers fed control diet while the best FCR (lowest) was observed in broilers fed multi enzymes supplemented diets. The discrepancy between the above studies maybe due to the different strain of bird used, components of diets and environmental conditions. In addition to enzymes, chemical methods may be used in poultry diets to enhance their quality.

2.4.3 Chemical methods

Mahmood *et al.* (2006) investigated the effects of chemical treatments of salseed meal (SSM) on nutrient digestibility and digestive enzymes in colostomized hens and intact broilers. The authors used wheat-based diet (control) whereas the other four diets were SSM-based diets (untreated SSM or SSM treated with water, acetic acid, and sodium bicarbonate). The results of the study indicated that chemical treatments of SSM improved the protein and starch digestibility in colostomized hens and broilers and it was concluded that bicarbonate was the most effective treatment to improve nutrient digestibility and mitigate, to some extent, the poor digestion of SSM. Ibraihm *et al.* (2002) found that soaking cowpea for 16 hours in bicarbonate solution caused remarkable reduction in ANFs of the bean.

According to Mohan *et al.* (2016), legumes contain ANFs such as protease inhibitors, lectins, cyanogens, total free phenolics, tannins, phytic acid, saponins, toxic amino acids, antivitamins, and oxalate. Legumes have complex sugars such as raffinose, stachyose, and verbascose, which are responsible for flatulence (Hall *et al.*, 2017). These compounds reduce protein digestibility and availability, and are considered ANFs. Ultimately, the objective of enhancing quality of livestock is to be able to supply specific nutrients like protein and energy in required amounts for growth, meat quality and health.

2.5 Effects of Dietary Energy on Growth, Meat Quality and Health of Chickens

Dietary energy has an impact on growth parameters, carcass characteristics and health status of the animal as discussed below.

Growth

Beneficial effect of grain fermentation on nutrient digestibility and energy utilisation has been shown previously in broilers (Sharif *et al.*, 2012). In a study by Fanimu and Akinola (2015) using raw and ground malted sorghum sprout as source of energy, no significant difference was observed on the growth indices while weight gain and protein efficiency ratio value decreased significantly with increased level of processed malted sorghum sprout in the diets.

Medina *et al.* (2015) conducted an experiment to evaluate replacing different levels of maize with sorghum as source of energy on growth rate and FCR, on Hubbard classic broiler chickens. The authors found that there were no significant differences in daily, total DM intake, final weight, average daily body weight gain and DM conversion ratio between treatments and control group for starter, finisher and entire period. The authors concluded that replacement of maize with sorghum up to 45% appeared to be biologically better and not having adverse effect on broiler performance.

Alta *et al.* (1985) conducted a graded replacement experiment of maize grains with sorghum at a rate of 20, 30 or 40%. The results showed no differences between the final average weights gain, feed intake and FCR. This indicates that sorghum grain has a potential to replace part of maize in poultry diets and this would have effects of reducing costs and sparing land for human consumption. Therefore, it is important to investigate processing techniques that would reduce ANFs such as tannins in sorghum. Reduction of tannin content in sorghum will increase the availability of proteins, carbohydrates and minerals for digestion and absorption, thus improving performance. Torki and Pour (2007) reported that germinated sorghum-based diet increased body weight gain and FCR of broiler chicks compared to non-germinated sorghum. The inclusion of germinated sorghum at 0.8% in maize-based diet was

observed by Bohoua and Yelakan (2007) to improve 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 treating of high tannin sorghum will reduce the effect of tannin on performance of broilers and laying hens.

Kovalik *et al.* (2018) studied the effect of feeding 5% pre-fermented cereal-based bio-product as a source of energy on production indicators, chemical composition, fatty acid profile and lipid oxidation of broiler meat. The researchers found that broilers fed fermented cereal-based bio-product when compared to those fed commercial diet reached higher final weight, and showed lower average daily feed intake, FCR, and feed intake compared to the control group. In another study, Emami *et al.* (2012) investigated the effect of feeding untreated sorghum grain (*Sorghum bicolor L.*) as a source of energy on growth performance of Japanese quails and reported low body weights and feed intake compared to those fed maize. As much growth is important to measure performance, the value chain of livestock production end up at the table of customers (fork end of value chain) and within this value chain carcass quality is critical.

2.5.2 Carcass characteristics

The main indicator of the quality of poultry meat is carcass characterization, which determines its status, considering the degree of fat and muscle tissue (Tyasi and Gxasheka, 2015) and this is important for consumers. There are several characteristics that determine the overall quality of meat. In poultry these are mainly appearance and meat tenderness. According to Qiao *et al.* (2001), appearance (*e.g.*, colour of skin and meat, presence of muscular white striations) is critical for both consumers' initial selection of the product and final product satisfaction. El-Hack *et al.* (2019) investigated the effect of dietary brewers dried grain (BDG) as a source of

energy on carcass characteristics of Arbor Acres broilers and reported that gizzard size increased, which was likely due to the more grinding activities needed for the increased fibre content of the diet resulting in increased musculature. Similarly, Denstadli *et al.* (2010) reported that relative gizzard weight significantly increased with increasing levels of BDG in diet up to 30%.

A study by Rosa *et al.* (2007) investigated the effects of energy intake and broiler genotype on performance, carcass yield, and fat deposition on broiler chicken and found that male chicks of AgRoss 308 (commercial line) broilers compared to PCLC (Embrapa non-improved line) had better performance and carcass yield, and presented lower abdominal fat deposition. The authors also found that the highest dietary energy level increased weight gain, heart relative weight, and fat deposition. In a study by Infante-Rodriguez *et al.* (2017), diets with different energy levels had no influence on total carcass weight or carcass cuts. Therefore, it would be worthwhile to investigate carcass characteristics of broiler chickens fed malted sorghum-based diets. Within the carcass, the components that end-up as meat are important for economic reasons and customers' preference.

1.5.3 Meat quality

Meat quality is defined as a measurement of attributes or characteristics that determine the suitability of meat to be eaten as fresh or stored for reasonable period of time (Fletcher, 2002). These attributes include chemical, physical, sensory, microbial, hygiene and nutritional properties. Meat quality is a function of tenderness, pH, colour, juiciness, flavour (Muchenje *et al.*, 2009). According to Deiss *et al.* (2009), different chicken breeds perform differently in these attributes. However, nutrition play an important role meat quality which is under the farmer's control under short term.

2.5.3.1 Meat pH

Due to post-mortem glycolytic activity that results in lactic acid accumulation in muscle, meat pH tends to decline significantly after slaughter (Immonen and Puolanne, 2000). The increase or decrease in pH in chicken meat depends on levels of glycogen and lactic acid in the muscle (Zhang *et al.*, 2012). Muscles are converted to meat as soon as the animal is slaughtered due to a number of metabolic and structural processes that occur immediately post-mortem (Zhang *et al.*, 2012). According to Deiss *et al.* (2009), the muscle conversion into meat can be measured by the level of pH and temperature.

Muscle glycogen is metabolised through anaerobic glycolysis and generate lactate which accumulates in the muscles and in turn lowers intracellular pH and this occurs until it reaches ultimate pH (pH_u) of about 5.4-5.7 (Dyubele *et al.*, 2010). According to Mushi *et al.* (2009), a high ultimate pH (pH_u) in meat can be linked to a lower glycogen reserve due to insufficient energy. Meat pH usually ranges from 5.2 to 7.0 with the highest quality meat product falling between a pH range of 5.2 and 6.0. In a study by Li *et al.* (2015), finishing pigs fed low starch and high fibre and fat diet had greater initial pH without affecting the ultimate pH. The authors concluded that rapid glycolysis rate at the early stage of *post-mortem* resulted in lower initial pH but not in ultimate pH.

2.5.3.2 Mineral, protein and proximate composition of meat

A recent study of Kovalik *et al.* (2018) investigated the effect of feeding pre-fermented cereal-based bio-product as a source of energy on chemical composition, fatty acid profile and lipid oxidation of broiler meat. The researchers found that meat from broilers fed fermented cereal had lower amounts of total protein and fat, and concluded that replacing 5% of the commercial feed by pre-fermented cereal-based bio-product could not only improve performance

parameters of chickens, but also affect positively chemical composition and fatty acid content of meat. Kim and Kang (2016) investigated the effects of diets containing fermented barley or wheat as source of energy on proximate analysis of breast meat of broilers and reported lack of significant difference in the concentration of moisture, ash, and CP of breast muscle across experimental diets including the basal treatment.

In another study, Infante-Rodriguez *et al.* (2017) studied the effects of diets containing sorghum with different energy concentrations on chemical composition of meat of broiler chickens in dry tropics. The authors found that meat from breast muscle had similar CP percentages among treatments; ether extract was higher in treatment which had 12.38 MJ/kg and 12.72 MJ/kg ME in starter and finisher phase, respectively than treatment which contained 12.89 MJ/kg and 13.22 MJ/kg of ME. The percentages of water, ether extract, ash and CP in thigh meat were not significantly different among treatments. Energy is used for various biochemical and physiological processes in the body of animals. To monitor status of such processes, it is imperative to measure blood chemistry and metabolites.

2.5.4 Dietary energy on blood parameters of chickens

Blood parameters are important tools that help provide information on the health and nutritional status of the animal (Orawan and Aengwanich, 2007; Khawaja *et al.*, 2012). As stated by Etim *et al.* (2014), the nutritional status of an animal depends on its dietary intake and the effectiveness of its metabolic processes. In a study by El-Katcha *et al.* (2014), the inclusion of wheat as source of energy had no significant effect on blood serum total protein, albumin, globulin and glucose concentrations, while it reduced blood serum triglycerides and cholesterol concentrations compared to maize. Inclusion of wheat in the broiler chicken diets had a

detrimental effect on liver function (El-Katcha *et al.*, 2014), through elevation of some blood serum enzymes.

Regarding immune response, it was observed that inclusion of wheat as source of energy instead of maize reduced phagocytic activity index and antibody production of broiler chickens (El-Katcha *et al.*, 2014). In a study by Burlikowska and Szymeczko (2011) that investigated feeding mixed maize and wheat based diets as source of energy found a significant relationship between age and concentration of serum calcium, magnesium and iron. Total protein, albumins and total calcium (Ca) levels showed a constant increase between the 14th and 42nd (Burlikowska and Szymeczko, 2011). The authors concluded that the obtained results may be helpful in the evaluation of changes in the metabolic profile, health condition and production patterns in growing broiler chickens.

Yang *et al.* (2016) studied the effects of metabolisable energy (ME) and CP levels on laying performance, egg quality and serum biochemical indices of Fengda-1 layers and found that dietary metabolisable energy and CP level had no effect on the serum concentrations of total proteins and albumin. However, the authors noted that the serum concentrations of uric acid and triglyceride in layers fed with 11.51 MJ/kg ME diets were higher than those fed with 10.60 MJ/kg ME diets implying that diets with high energy provided that energy for protein and lipids metabolism.

In a study conducted by Fafiolu *et al.* (2006) on malted sorghum sprout (MSP) as source of energy in the diets of pullets and laying hens, it was found that albumin and serum globulin were significantly reduced by feeding MSP diets while the serum and uric acid levels were elevated. The authors also noted a significant reduction in egg cholesterol content with

inclusion of MSP. It was then concluded that MSP can be included in the diets of laying hens and pullets at 300 g/kg.

2.6 Summary

Broiler production plays a vital role in low income families and provide a cheap source of protein. In addition, small-scale broiler production provides a source of income and employment opportunities to resource-poor households. Due to its short production cycle, income turnover and meat supply to market broiler production provides a quick cash flow to disadvantaged families. Despite its importance, the broiler production in Botswana is limited due to expensive feed ingredients which is due to competition with human needs for major energy source needed in the diet. Maize grain is currently the major energy source in all livestock feeds including poultry, and it is used as well for human consumption. It is, therefore, ideal to search for cheaper readily available energy sources to substitute for maize grains in poultry feed manufacturing. Sorghum grains are possible energy sources that can be used to replace maize grains. However, their limit for utilisation by broilers is due to anti-nutritional factors such as tannins. Reduction of tannin content of sorghum would increase the availability of proteins, carbohydrates and minerals for digestion and absorption, thus improving performance. The tannin content of sorghum can be reduced through biological methods such as malting or germination. There are limited studies on feeding malted sorghum diets on the growth performance, carcass characteristics and meat quality of broilers. It is therefore ideal to assess the effects of feeding malted sorghum-based diets on the performance of broilers in place of maize-based diets.

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CHAPTER 3

CHEMICAL CHARACTERIZATION OF MALTED AND UNMALTED SORGHUM GRAINS

Abstract

This study was a pilot work to investigate the effects of malting sorghum grains on their chemical composition prior to commencement of the feeding trials. Unscreened grains of Mr Buster (red sorghum) and *Segaolane* (white sorghum) were malted and analysed for crude protein, crude fibre, organic matter, ash, energy, fat, and condensed tannin levels. Malting did not alter chemical composition within a variety on dry matter and condensed tannin content, but statistically significant differences were observed across varieties ($P < 0.05$). Mr Buster (Malted (94.81%) and unmalted (95.84%)) had the highest ($P < 0.05$) dry matter content while the lowest was found in *Segaolane* (Malted (94.05%) and unmalted (94.29%)) but there were no significant difference between the malted and unmalted sorghum type. Unmalted Mr Buster grains (80.99%) had the lowest ($P < 0.05$) organic matter while the highest was in malted *Segaolane* (89.56%). There were no significant difference observed in ash contents between Mr Buster (Malted and unmalted) and unmalted *Segaolane*. Unmalted *Segaolane* (48.67%) had the highest ($P < 0.05$) crude fibre content while malted *Segaolane* (28.90%) and Mr Buster (28.40%) had the lowest crude fibre content. Condensed tannin content of malted and unmalted Mr Buster (0.08% versus 0.09%) was significantly higher than that of malted and unmalted *Segaolane* grains (0.05% versus 0.04%). Grain type had effects on DM, OM, ash, CP and condensed tannin while the effects were not observed in energy and crude fat contents. Malting affected ($P < 0.05$) OM, ash, crude fibre and condensed tannin but did not affect DM, CP and energy. Interaction effects of grain type and malting were observed on crude fibre and

condensed tannin content of sorghum. It can be concluded that although malting does not alter chemical composition of the grains, it however reduces crude fibre level which can lead to an increase in feed intake and consequently better livestock performance by availing nutrients for utilisation. It also reduced condensed tannin level in red sorghum variety which already has high condensed tannins which may benefit digestibility of sorghum-based poultry diets.

Key words: Malted, Unmalted, Proximate analysis, *Segolane*, Mr Buster

3.1 Introduction

The use of maize as the main energy source in poultry diets is subjected to the age-old competition for food between animals and humans. Many countries in the developing world, especially in sub-Saharan Africa are not self-sufficient in grain production to meet both human and animal needs and they resort to importation of cereal grains. Maize grain prices have therefore remained high and will probably increase in the future. Therefore, alternative, accessible and cost-effective ingredients are needed that can provide the same nutrients as maize for livestock feeding. Sorghum (*Sorghum bicolor*) has been identified as a suitable replacement for maize (Medugu *et al.*, 2010) in livestock diets. It is abundant in most parts of Africa and is among the main cereal crops that are used as food and feed.

The adaptive agronomic characteristics of sorghum make it suitable for cultivation in different environmental conditions (Muui *et al.*, 2013). The limiting factor that hinders efficient utilisation of sorghum grains in monogastric feed is its anti-nutritional factors (ANFs). Some authors (Gous *et al.*, 1982; Boren and Waniska, 1992) have reported ANFs in sorghum grain having adverse effects on the utilisation of sorghum protein and metabolisable energy by poultry, such as tannins. Others such as Nyachoti *et al.* (1997) have reported that these ANFs do not always reduce the performance of poultry. This indicates the possibility of other factors contributing to the poor feeding value of certain sorghum varieties.

The Department of Agricultural Research in the Ministry of Agricultural Development and Food Security has over the years developed several sorghum varieties for both food and beer making in Botswana. Making malt using sorghum for brewing traditional beer is an established tradition in Botswana, and even other countries. According to Madibela and Lekgari (2005), there are opportunities to utilize surplus sorghum grain in livestock diets if national sorghum

production could be increased. However, some form of processing may be needed to overcome ANFs contained in sorghum grains. One such intervention is malting of the sorghum grains. Vast literature has shown that malting may overcome the problem of ANFs (Ogbonna *et al.*, 2012; Ojha *et al.*, 2018; Hassan *et al.*, 2020). It is important to determine the chemical characterization of malted sorghum grain as an alternative to maize grains before formulating poultry diets and undertaking feeding studies. Therefore, this pilot study was designed to determine chemical characterization of malted Mr Buster (Red type) and *Segaolane* (White type) grains. It was hypothesised that there was no significant difference in chemical characterization of malted and unmalted sorghum grains.

3.2 Materials and Methods

3.2.1 Study site

The study was carried out at the Botswana University of Agriculture and Natural Resources (BUAN), Content Farm, Sebele, Gaborone in the Southern part of Botswana. The University is located 24°36' 40.90'' S and 25° 56' 13.35'' E at 994 m above sea level (Mojeremane *et al.*, 2014). Laboratory work was done at the Biochemistry Laboratory in the Department of Animal Sciences. The study was conducted during the summer months of February to April with temperatures varying from ~12–15 °C during the early morning, to ~30–40 °C in the afternoons and an average annual rainfall of 550 mm.

3.2.2 Source and preparation of sorghum malt

Unscreened *Segaolane* and Mr Buster sorghum grains were purchased from Botswana Agricultural Marketing Board (BAMB) in Gaborone, Botswana. The grains were prepared by soaking in a plastic container covered with a jute bag for 48 hours at room temperature, and

then spread on hessian bag and allowed to germinate for 7 days under dark conditions. After germination, the malted grains were sun dried and stored in bags prior to diet formulations.

3.2.3 Proximate analysis

Raw sorghum grains of malted and unmalted Mr Buster and *Segaolane* were sampled from stored bags and milled to pass through a 1 mm sieve prior to chemical characterization. The grains were analysed using standard AOAC (2005) procedures to determine dry matter (DM; method 930.15), Ash (method 942.05), crude protein (CP; method 954.01) while organic matter percentage was calculated as 100% - % Ash. Crude fibre was determined by boiling 1.00 g of samples with 1.25% dilute H₂SO₄ washed with water and further boiled with 1.25% dilute sodium hydroxide and remaining residue after digestion was taken as crude fibre. Crude Fat content was estimated by Soxhlet method. Energy content was determined by combustion of about 1.00 g of each sample in a bomb calorimeter (Model-IKA C2000, IKA[®]-Werke GmbH & Company, Hamburg, Germany). Condensed tannins were determined using the Butanol-HCL method and expressed as leucocyanidin equivalent (% DM) according to Markkar (2000).

3.2.4 Statistical analysis

The General Linear Model (GLM) procedure (SAS 2010) was used to test the chemical characterization of the sorghum grains using the following model.

$$Y_{ijk} = \mu + GT + M + GTM + E_{ijk}$$

GT = Grain type; M = malting; GTM = GT x M interactions

Where Y_{ijk} = response variable (parameters), μ = general mean, di = the fixed effects (malted and unmalted sorghum grain), E_{ij} = random error associated with observation ijk = assumed to be normally and independently distributed. When the analysis of variance revealed significant effect, probability of difference (PDIFF) option in the LSMEANS statement of the GLM

procedure of SAS (2010) was used to separate means. The level of significance was set at $P < 0.05$.

3.3 Results

3.3.1 Chemical composition of grains

The average chemical composition of Mr Buster and *Segaolane* grains is shown in Table 3.1. *Segaolane* grains had significantly higher concentration of organic matter ($P = 0.020$), crude protein ($P = 0.029$), energy, crude fat and crude fibre between *Segaolane* and Mr Buster grains. Dry matter ($P = 0.001$), Ash ($P = 0.020$), and condensed tannin content ($P < 0.001$) were high in Mr Buster than *Segaolane* grains.

3.3.2 Malt effect on chemical composition

The effects of malt on chemical composition is shown in Table 3.2. Malt had a significant effect on organic matter, ash, crude fibre and condensed tannin content. Malt did not affect ($P > 0.05$) dry matter, crude protein, energy and crude fat.

Table 3.1 Chemical composition of Mr Buster and *Segaolane* grains in percentages (unless stated otherwise)

Parameter	Mr Buster	<i>Segaolane</i>	SE	P-value
Dry matter	95.12	94.17	0.16	0.001
Organic matter	82.83	85.92	0.82	0.020
Ash	17.17	14.08	0.82	0.020
Crude protein	11.48	12.45	0.28	0.029
Energy MJ/kg	16.28	16.70	0.80	0.711
Crude fat	8.72	9.92	0.97	0.396
Crude fibre	32.49	38.78	1.59	0.016
Condensed Tannin	0.08	0.06	0.00	0.0002

Table 3.2 The effects of malt on chemical composition of sorghum grains in percentages (unless stated otherwise)

Parameter	Malt	Unmalted	SE	P-value
Dry matter	94.43	94.87	0.16	0.072
Organic matter	87.11	81.64	0.82	0.001
Ash	12.89	18.36	0.82	0.001
Crude protein	12.02	11.91	0.28	0.791
Energy (MJ/kg)	16.40	16.58	0.80	0.874
Crude fat	8.93	9.71	0.97	0.576
Crude fibre	28.65	42.62	1.59	<.0001
Condensed Tannin	0.06	0.07	0.00	0.039

3.3.3 Interaction effects of malted and unmalted grains

The effects of grain type, malting, and their interaction in chemical composition is presented in (Table 3.3). Mr Buster (Malted (94.81%) and unmalted (95.84%)) had the highest ($P<0.05$) DM content while the lowest was found in *Segaolane* (Malted (94.05%) and unmalted (94.29%)) but there were no significant differences between the malted and unmalted sorghum type. Regarding organic matter, unmalted Mr Buster grains (80.99%) had the lowest ($P<0.05$) while the highest was in malted *Segaolane* (89.56%). However, no significant differences were observed between malted Mr Buster and Unmalted *Segaolane*.

Unmalted Mr Buster had the highest ash contents whilst malted *Segaolane* had the lowest. However, no significant differences were observed in ash contents on Mr Buster (Malted and unmalted) and unmalted *Segaolane*. Unmalted *Segaolane* (48.67%) had the highest ($P<0.05$) crude fibre content while malted *Segaolane* (28.90%) and Mr Buster (28.40%) had the lowest crude fibre contents. Mr Buster (Malted (0.08%) and unmalted (0.09%)) had the highest ($P<0.05$) tannin content while the lowest was found in *Segaolane* (Malted (0.05%) and unmalted (0.06%)). No significant differences were observed between the malted and unmalted sorghum types. Grain type had effects on DM, OM, ash, CP and tannin while the effects were not observed in energy and crude fat contents. Malting affected ($P<0.05$) OM, ash, crude fibre and tannin but did not affect DM, CP and energy. interaction effects (grain type and malting), were noted in crude fibre and tannin content of the grains.

Table 3.3 Chemical composition of malted and unmalted *Segaolane* and Mr Buster sorghum grains (% , unless stated otherwise) and their interaction effects

Parameters	Mr Buster		<i>Segaolane</i>		SE	Effects		
	Malted	Unmalted	Malted	Unmalted		G	M	G x M
Dry matter	94.81 ^{ab}	95.84 ^a	94.05 ^c	94.29 ^{bc}	0.22	0.001	0.072	0.393
Organic matter	84.67 ^b	80.99 ^c	89.56 ^a	82.29 ^b	1.15	0.020	0.001	0.145
Ash	15.33 ^b	19.01 ^{ab}	10.44 ^c	17.72 ^b	1.15	0.020	0.001	0.145
Crude protein	11.64	11.32	12.40	12.51	0.39	0.029	0.791	0.596
Energy MJ/kg	16.22	16.34	16.58	16.83	1.13	0.711	0.874	0.958
Crude fat	9.34	8.09	8.51	11.33	1.37	0.396	0.576	0.162
Crude fibre	28.40 ^c	36.57 ^b	28.90 ^c	48.67 ^a	2.25	0.016	<.0001	0.024
Tannin	0.08 ^a	0.09 ^a	0.05 ^b	0.06 ^b	0.00	0.000	0.039	0.048

3.4 Discussion

From Table 3.1 it was noted that there was a difference in chemical composition of *Segaolane* (white type grain) and Mr Buster (red type grains). The observation that unmalted Mr Buster grains had higher ash content than *Segaolane* grains maybe due to the fact that red sorghum contains pigments that are polyphenolic compounds in nature (Taylor, 2005). It was observed that malt affected chemical composition of the grains (Table 3.3). Malting did not alter DM content of each sorghum variety, but it was observed that there was a significant difference across varieties and the high DM content observed on malted and unmalted Mr Buster grains may have resulted from enzyme hydrolysis of starch and microbial breakdown of cellular materials as reported by Chinma *et al.* (2009).

Dry matter content of the white sorghum grains both malted and unmalted was observed to be the lowest than that of malted and unmalted red sorghum grains, and this could be due to the fact that red sorghum grains have a complex structure because of high content of anti-nutritional factors and malting did not breakdown all (Hariprasanna *et al.*, 2015). Although there was variation in DM content, the values were relatively high implying that nutrient composition in the grains was still high, suggesting that energy would be availed for utilisation by an animal. The current study revealed a significant increase on OM contents after malting Mr Buster and *Segaolane* grains. This is in line with the findings by Otutu *et al.* (2014) who observed an increase in OM content after fermenting sorghum grains for 7 days.

Crude fibre, consists of cellulose, lignin, and hemicelluloses (Kim *et al.*, 2012). The current study revealed that malting reduced crude fibre content on each variety. The current finding is in disagreement with that of Laxmi *et al.* (2015) who found that crude fibre increases significantly during germination process as the plant cells synthesize different cellular

components. As the grains germinate, there is an increase in cellular structure of the plants (Kim *et al.*, 2012). The discrepancies between these studies could be attributable to the different processing methods used (germination vs malting) and the sorghum varieties.

Malted and Unmalted Mr Buster grains were observed to have high tannin content compared to malted and unmalted *Segaolane* grains. This finding is in line with Myer *et al.* (1983), who reported high tannin contents in red sorghum compared to white sorghum grain varieties. The observation that malting did not reduce tannin content on each sorghum variety contradicts the findings by Ogbonna *et al.* (2012) who investigated the effect of malting conditions on the nutritional and anti-nutritional factors of sorghum and concluded that malting white sorghum grains reduced its tannin content by 8.45 %.

The current study also indicates that there was a significant difference in tannin content of malted and unmalted grains of *Segaolane* (White type) and Mr Buster (Red type). This contradicts the findings by Badubi (2012) in Botswana who found lack of significant differences in tannin content in both red and white sorghum. The discrepancy could be due to the different varieties of grains used. According to Taylor (2005), the red colour of sorghums is due to the presence of pigments (anthocyanins and anthocyanidins) which are polyphenolic compounds. Therefore, it is obvious that red sorghums will contain more phenolic compounds than white sorghums. The superiority of white over red sorghum is in terms of its starch digestibility (Perez-Maldonado and Rodrigues, 2009). It is interesting that in the pig and poultry industries, there is the debate that energy utilisation of white sorghum is better than that of red sorghum and the majority of energy is derived from the starch component of the sorghum. According to Selle *et al.* (2013), the energy or starch component of white sorghum is better utilized than that of red sorghums. A decrease in tannin content in cereals as stated by

Emambux and Taylor (2003) may be due to their binding with cotyledon endosperm that is usually undetected by routine method due to its insolubility in solvent or microbial phenyl oxidase action.

The observation that malting did not alter energy, CP and fat content of the grains in this study is in line with finding of Moongngarm and Saetung (2010), who did not find changes in fat content when rice grains were germinated. Similarly, Kazanas and Fields (1981) did not observe any significant change in crude fat content of sorghum after natural lactic acid fermentation for 4 days. This may be due to the fact that cereal grains are in general low in fat content. There was an interaction effect of grain and malting on crude fibre and condensed tannin content of the sorghum grains, whereas no interaction effect were noted on DM, OM, ash, CP, energy and crude fibre content of the grains.

3.5 Conclusion

It was concluded that malting has an effect on chemical composition of the sorghum grains, and it also reduced crude fibre content of the grains. However, malting did not reduce their condensed tannin content. Condensed Tannin content of red sorghum grain was found to be higher than that of white sorghum grains before and after malting. However, chemical analysis data is not enough to support the facts that sorghum variety or malting enhances utilisation sorghum, and as such it should be followed by feed trials. It is, therefore, prudent to formulate broiler diets using malted sorghum grains to test their effects on growth performance and haematological parameters of broilers.

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CHAPTER 4

GROWTH PERFORMANCE AND HAEMO-BIOCHEMICAL PARAMETERS OF BROILER CHICKEN FED MALTED SORGHUM-BASED DIETS

Abstract

This study was conducted to evaluate the growth performance and haemo-biochemical parameters of broiler chickens fed malted sorghum-based diets (Mr Buster and *Segaolane*). A total of 150 day old Ross 308 broiler chickens (R308BC) with homogeneous body mass were allocated to iso-caloric and iso-nitrogenous groups. The group was randomly allocated to treatment diets being Control = Broiler commercial diet containing 100% maize; malted *Segaolane*-based diet = Broiler commercial diet containing 100% malted *Segaolane* and malted Mr Buster-based diet = Broiler commercial diet containing 100% malted Mr Buster. From day 1 to 14, birds were offered a starter mash, day 14 to 28 a grower mash and day 28 to 42 a finisher mash from the above diets respectively. Each treatment had 5 replicated pens holding 10 broiler chickens in a completely randomised design, and data were analysed using Statistical Analysis System (SAS). The day 7 weight was balanced among the treatment groups and was used as the initial weight. Thereafter, chickens were weighed weekly for 5 consecutive weeks. Average daily feed intake (AWFI), average weight gain (AWG), feed conversion ratio (FCR), protein intake (PI) and protein efficiency ratio (PER) were calculated weekly. Blood samples were collected on the 6th week for haematological and serum biochemistry analyses. In weeks 3 and 4 there was a rapid increase in AWFI across all experimental diets. However, there was no significant difference in AWFI in R308BC between experimental diets at the final week (160.86 g for Control; 164.40 g for malted *Segaolane* and 161.97 g for malted Mr Buster-based diet). The R308BC fed malted Mr Buster-based diets had lower AWFI hence lower

AWG leading to lower body weights compared to those fed on malted *Segaolane*-based diets and Control diets. Time effects caused linear, quadratic and cubic trends in feed intake, AWG and body weight patterns ($P < 0.001$). There was an increase in AWG and mean weekly body weights of R308BC across experimental diets. AWG of R308BC fed malted *Segaolane*-based diet were significantly similar to R308BC on Control diet. No significant differences were observed in body weights of R308BC fed malted *Segaolane*-based diet and Control diet throughout the experimental period making malted *Segaolane* grains a suitable replacement for maize grains as they yielded similar body weights.

The R308BC fed malted Mr Buster-based diet had poor feed conversion ratio (FCR) in week 3, 4, 5 and 6 compared to other experimental diets. No significant difference were observed between the FCR from R308BC on Control or malted *Segaolane*-based diets at week 3, 4 and 5 meaning that malted *Segaolane* and maize caused similar response hence *Segaolane* can be a suitable replacement of maize grains. Protein intake (PI) on R308BC fed Control diet was lower than those fed other experimental diets.

The R308BC fed Control diet had significantly higher ($P < 0.05$) white blood cells count ($46.8 \times 10^3/\mu\text{L}$) compared to those fed malted sorghum-based diets. The highest ($P < 0.05$) basophils count was observed on R308BC fed malted *Segaolane* (0.57%) or malted Mr Buster (0.56%) diets, whilst those fed Control diet (0.33%) had the lowest. The R308BC fed Control diet had significantly higher ($P < 0.05$) mean corpuscular haemoglobin concentration (40.54 g/L) compared to those fed malted *Segaolane* (39.86 g/L) or Mr Buster (39.01 g/L) diets. R308BC fed malted *Segaolane* or Mr Buster based diets had higher ($P < 0.05$) creatinine counts compared to those fed the Control diet. Glutamic oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) enzymes were lowest ($P < 0.05$) in R308BC fed malted *Segaolane*-based

but highest in R308BC fed Control diet or malted Mr Buster-based diet. No significant differences were observed in lymphocytes, neutrophils, monocytes, eosinophils, red blood haemoglobin, haematocrit, and blood platelets of R308BC across experimental diets indicating that malted sorghum-based diets did not affect much in terms of the blood parameters used to assess health in chickens. Overall, the results from the study suggest that maize grains can be replaced with malted sorghum grains, especially *Segaolane* in poultry diets without any adverse effects on the R308BC growth's performance and health status. *Segaolane* is a white sorghum variety and its low anti-nutritional compounds making it a candidate for supplying energy in poultry diets in place of maize.

Keywords: *Segaolane*, malted sorghum, Mr Buster, Ross 308 chicken, haematology, serum chemistry

4.1 Introduction

The poultry industry produces high-quality proteins for human nutrition and is a source of income for communities in many countries; hence it is important in economic development of any country (Tarhyel *et al.*, 2012). Globally, broiler production has grown dramatically in the past two decades due to improvements in nutrition and breeding programmes which further enhanced feed utilisation and growth rate (Grace *et al.*, 2020). Most rapid growth or weight gains are made when the chick is young (Mignon-Grasteaus *et al.*, 2001). Birds usually consume just enough feed to meet their energy requirements since the regulation of feed intake is believed to be based primarily on the amount of energy in the diet (Nahashon *et al.*, 2006). Increasing the dietary energy concentration leads to a decrease in feed intake and vice versa (Veldkamp *et al.*, 2005), thus affecting growth. In contrast a study by Mbajjorgu (2010), observed that Venda chickens increased their feed intake with an increase in feed energy level and an increase in feed protein.

Haematological parameters are used as indicators for physiological health and nutritional status of animals (Aro *et al.*, 2013; Ewuola *et al.*, 2004). According to Karesh *et al.* (1997), these parameters provide a clearer diagnosis and clinical monitoring of diseases affecting animals. Haematological profiles are affected by age, gender, breed, nutrition, stress, season, bacterial and viral infection and growth development stage (Puspamitra *et al.*, 2014). Therefore, in the present study it would be of interest to monitor effects of the formulated diets on these parameters.

Maize (*Zea mays*) is the major feed ingredient in broiler rations and its inclusion level can be as high as 60% of whole diet. Its main function in the livestock industry is to supply energy but also small amounts of proteins. It also has competing functions such as source of bio-fuel, used

Bravo (1998), total phenols in sorghums range from 2 to 103 g/kg, while they are negligible in corn and wheat and 14 g/kg in barley. Tannins are part of total phenols and due to their hydroxyl groups, tannins may interact with and form complexes with proteins which may lead to precipitation because of the large size of the tannins (Duodua *et al.*, 2003). Dietary fibre is a feed component that has major influence in feed intake (Duodua *et al.*, 2003). According to Mohammed *et al.* (2019), proteins are able to bind to dietary fibre or more specifically, cell wall components. Dietary fibre components are not digested by endogenous digestive enzymes, and consequently are the main substrates for bacterial fermentation in the distal part of the gut.

Different studies (Emami *et al.*, 2012; Osman and Gaseem 2013; Torres *et al.*, 2013; Mohamed *et al.*, 2015) have been carried to evaluate the feeding value of raw sorghum grains in poultry diets. These authors found that body weight decreased significantly in chickens fed sorghum-based diet. Therefore, it is important to employ processing techniques that can reduce its anti-nutritional factors (ANFs) to improve feed quality and value. It has been reported by Makhoka *et al.* (2002) that malting improves digestibility of sorghum grains *in vitro* using the porcine pepsin method. A study by Legodimo and Madibela (2013) that employed ruminant rumen fluid as an inoculum, observed improved dry matter (DM) digestibility of malted sorghum grains. The pilot study reported earlier have shown that malting does not affect protein and energy content of grains but reduces their condensed tannin and crude fibre contents. However, grain variety (red vs. white type) affected condensed tannins content (CT) with red type having high CT content.

Based on the foregoing, the present study was conducted to evaluate the growth performance and haemo-biochemical parameters of broiler chickens fed malted sorghum-based diets (Mr Buster (red type) and *Segaolane* (white type). It was hypothesised that feeding malted

sorghum-based diets would promote similar growth performance and haemo-biochemical parameters of broiler chickens as maize-based diet.

4.2 Materials and Methods

4.2.1 Ethical Considerations

The management and care of the chickens were in accordance with Botswana University of Agriculture and Natural Resources' Animal Ethics Committee accepted standards for the welfare and ethics of animals (Ethics Number BUAN-2020-08).

4.2.2 Study Site

The study was carried out at the Botswana University of Agriculture and Natural Resources (BUAN), whose location and climatic conditions are described in detail in Chapter 3, Section 3.2.1.

4.2.3 Experimental design

A total of 150 R308BC chicks were individually weighed and allocated to pens which were balanced and blocked by weight. The 15 pens (each measuring 2.3 x 1.24 m) were then randomly assigned to the 3 experimental diets. Thus, each dietary treatment had 5 replications (pens) with 10 chickens per pen. The study was arranged in a completely randomised design (CRD) with the pen as the experimental unit.

4.2.4 Preparation of the house

Before the arrival of experimental chicks, the poultry house was cleaned. All dust was removed exposing all surfaces to a detergent. Equipment was washed with virkon (virucidal disinfectant).

4.2.5 Source of feed and malt preparation

Unscreened *Segaolane* and Mr Buster sorghum grains were purchased from Botswana Agricultural Marketing Board (BAMB) in Gaborone, Botswana and were prepared as described in detail in Chapter 3, Section 3.2.2.

4.2.6 Experimental diets

The study consisted of 3 diets; a Control diet (that contained maize) and two tests diets were formulated using malted Mr Buster and malted *Segaolane* sorghum grains. The dietary treatment using malted Mr Buster and *Segaolane* sorghum grains were formulated to meet nutritional requirements of commercial broiler diets according to BOS 225:2014 Animal feeding stuffs – Broiler feeds -Specification (Botswana Bureau of Standards (BOBS)). A premix containing soya oil cake, full fat soya and amino acids and minerals was obtained from Optifeeds (PTY) LTD, Gaborone, Botswana and it was mixed with malted sorghum grains during the formulation of the diets for the 3 phases of broiler chicken production. Starter mash was mixed for day 1 to 14 feeding, grower mash for day 14 to 28 feeding and finisher mash for day 28 to 42 feeding as shown in Table 4.1.

Table 4.1 Ingredients composition of experimental diets fed to Ross 308 broiler chicken in g/kg

Ingredient	Experimental diets		
	Control	Malted <i>Segaolane</i>	Malted Mr Buster
Starter			
Soya oil cake	293.80	293.80	293.80
Full fat soya	40.00	40.00	40.00
Malted Sorghum grains	0.00	619.60	619.60
Maize grains	619.60	0.00	0.00
Amino acids and mineral premix	46.60	46.60	46.60
Total	1000.00	1000.00	1000.00
Grower			
Soya oil cake	104.7	104.70	104.70
Full fat soya	180.00	180.00	180.00
Malted sorghum grains	0.00	642.70	642.70
Maize grains	642.70	0.00	0.00
Amino acids and mineral premix	39.30	39.30	39.30
Total	1000.00	1000.00	1000.00
Finisher			
Soya oil cake	145.60	145.60	145.60
Full fat soya	90.40	90.40	90.40
Malted sorghum grains	0.00	730.02	730.20
Maize grains	730.20	0.00	0.00
Amino acids and mineral premix	33.80	33.80	33.80
Total	1000.00	1000.00	1000.00

Table 4.2 Nutrient Composition of experimental diets provided to Ross 308 from 2 – 6 weeks of age on air dry basis (%)

Nutrient	Experimental diets		
	Control	Malted <i>Segaolane</i>	Malted Mr Buster
Starter			
Dry matter	93.40	93.67	93.36
Ash	9.74	10.92	10.14
Organic matter	90.26	89.08	89.86
Crude protein	24.94	24.64	25.09
Energy (MJ/Kg)	17.45	18.33	18.44
crude fat	6.62	7.29	6.62
Crude fibre	10.41	10.16	11.27
Condensed Tannin	0.036	0.051	0.071
Grower			
Dry matter	93.81	93.61	93.41
Ash	7.94	7.17	9.92
Organic matter	92.06	92.83	91.08
Crude protein	22.79	22.40	23.02
Energy (MJ/Kg)	18.11	18.33	18.32
Crude fat	7.52	8.78	7.79
Crude fibre	13.31	13.23	14.36
Condensed Tannin	0.045	0.056	0.057
Finisher			
Dry matter	93.47	92.81	93.20
Ash	6.96	9.68	8.46
Organic matter	93.04	90.32	91.54
Crude protein	21.49	21.62	22.11
Energy (MJ/Kg)	18.50	18.45	18.58
Crude fat	7.85	8.06	7.35
Crude fibre	13.62	13.74	14.87
Condensed Tannin	0.049	0.053	0.071

4.2.7 Experimental chickens and their management

A total of 150 one day old Ross 308 (R308BC) broiler chicks were purchased from Ross Breeders hatchery at Notwane, south-east of Gaborone. The chicks were weighed on arrival and balanced according to weights and left for a period of a week on experimental pens for acclimatization. The chicks were fed on commercial starter mash from Opti Feeds Pty Ltd. for the first week. The experimental diets were fed from week 2 and the chicks were weighed at the beginning of week 2 and subsequently on weekly basis. 10 chicks were allocated to three groups which were balanced for weight. Each group was randomly assigned to each of the experimental diets (Malted *Segaolane*, Malted Mr Buster and the Control diet). From day 1 to 14, chicks were offered a starter mash, day 14 to 28 a grower mash and day 28 to 42 a finisher mash formulated from test ingredients. The birds were raised on a deep litter system bedded with wood shavings. The chicks were provided with feed and water at *ad libitum* for the whole feeding period (6 weeks). General flock management was carried out according to routine commercial poultry standards. Feeding troughs and water troughs were cleaned regularly to avoid contamination and transmission of diseases.

4.2.8 Proximate analysis

Samples of formulated malted Mr Buster, *Segaolane* and the Control diets were milled to pass through a 1 mm sieve for chemical characterization and analysed as described in detail in Chapter 3, Section 3.2.3.

4.2.9 Mineral analysis

Phosphorus (P), Calcium (C), Magnesium (Mg), Potassium (K), and Sodium (Na) were determined using the procedure by Sahrawat *et al.* (2002) by digesting 1.25 g of feed sample in sulphuric acid-selenium (Se). After digestion the minerals were read using coupled plasma

optical emission spectrometer (ICP-OES) from Perkin. Phosphorus was read using a UV-Vis spectrophotometer (Model UV-1601) from Japan and absorbance was determined at 670 nm wavelength following Molybdenum blue method of Dickman and Bray (1940).

4.2.10 Feed intake

Feed offered to the birds and feed refusal were weighed daily. Average weekly feed intake was calculated by subtracting feed refusals from the feed offered and divided by a week (7 days) as follows:

$$AWFI = \frac{\text{Feed offered} - \text{Feed refusal}}{7 \text{ days}}$$

4.2.11 Growth performance

4.2.11.1 Body weights and weight gain

The birds were weighed at the beginning of the experiment and subsequently on a weekly basis using an electronic scale of sensitivity 0.001 g (Adam 6020 model, Adam, Gauteng Province, South Africa). Average weekly body weight gain was determined as the difference between the final weight and initial weight divided by number of days (7days).

$$AWG = \frac{\text{Final weight} - \text{Initial weight}}{7 \text{ days}}$$

4.2.11.2 Feed Conversion ratio

Feed conversion ratio was calculated by dividing AWFI by AWG according to Manyeula *et al.* (2019):

$$FCR = \frac{AWFI}{AWG}$$

4.2.11 Protein utilisation and efficiency

Weekly protein intake (PI g/bird) was calculated by multiplying the concentration (g/kg DM) of crude protein (CP_d) in the diet consumed by weekly mean feed intake (FI kg/bird).

$$PI = CP_d \times FI$$

The protein efficiency ratio (PER, g/g) was calculated by dividing AWG by the mean PI.

$$PER = \frac{AWG}{PI}$$

4.2.12 Blood biochemistry

4.2.12.1 Determination of haematology and serum biochemistry parameters

At 6 weeks of age, blood was collected from 2 birds which were randomly selected from each pen in the morning before feeding. Blood was drawn from the wing vein into the sample bottle without anticoagulant for serum biochemical analysis and purple capped tubes with anti-coagulant (Ethylene diamine tetra acetic acid) for haematology. The blood samples were taken to Clinipath Laboratory (PTY) LTD in Molepolole for analysis. The automated humanstar 100 Biochemistry analyser was used to determine the haematocrit and the full blood count according to the methods outlined by Doumas *et al.* (1981). Serum biochemical indices included the total protein, albumin, chloride, sodium, potassium, creatinine total bilirubin, and urea.

4.2.13 Statistical analysis

Time series data on live weights, AWFI, AWG, PI, PER and FCR was subjected to restricted maximum likelihood (REML) analyses to estimate repeated measures (Model 'a' below) by the use of statement repeated within Proc Mixed procedure of SAS (2002-2012) to estimate

variances and covariance (Holland 2006). The compound symmetry (CS) structure was used for within samples variance and covariance structure for these data. The model included diets, diets x time interaction and initial weight as co-variate. Individual Animal number (ID) and Replication (pens) were used in the random effect statement. To tease out the nature of trends in live weight, AWFI and AWG over time, the effect of time was broken down into polynomial contrasts. Time was dropped from the class statement, making it a continuous variable and then it was included three times on the model statement to construct non-orthogonal polynomial items (Wolfinger and Chang, 1998). Live weight, AWFI, AWG and PI means were then plotted against time to give graphic trends over time. The overall haematology and serum biochemistry data were analysed using one-way analysis of variance using SAS (SAS, 2002-2012) according to the general linear model 'b' below to test effects of diet.

$$Y_{ijt} = \mu + d_i + T_j + (T \times d)_{ij} + E_{ijt} \quad (a)$$

Where Y_{ijt} = response variable (feed intake, body weights, weight gain, feed conversion ratio, protein efficiency ratio and protein intake), μ = general mean, d_i = the fixed effects of diets, T_j = effect of week, $(T \times d)_{ij}$ = effects of interaction between diets and week, E_{ijt} = random error associated with observation ijt = assumed to be normally and independently distributed.

$$Y_{ijt} = \mu + d_i + E_{ijt} \quad (b)$$

Where Y_{ijt} = response variable (haematology and serum biochemistry), μ = general mean, d_i = the fixed effects of diets, E_{ijt} = random error associated with observation ijt = assumed to be normally and independently distributed. The level of significance will be set at $p < 0.05$.

4.3 Results

4.3.1 Feed intake

No mortalities were recorded throughout the experimental period. There were significant dietary effects ($P < 0.05$) on AWFI of R308BC whereby R308BC fed malted *Segaolane*-based diets were eating more than those fed malted Mr Buster based and control diet. But there were no diet x time effects ($P > 0.05$). Time effects caused linear, quadratic and cubic feed intake patterns ($P < 0.001$) (Table 4.3). In weeks 3 and 4 there was a rapid increase in AWFI across all dietary treatments (Figure 4.1). The AWFI was significantly lower in R308BC fed malted Mr Buster-based diets in weeks 2, 3, 4 and 5 compared to those fed malted *Segaolane*-based diets. There was no significant difference in AWFI of R308BC across all experimental diets at the final week.

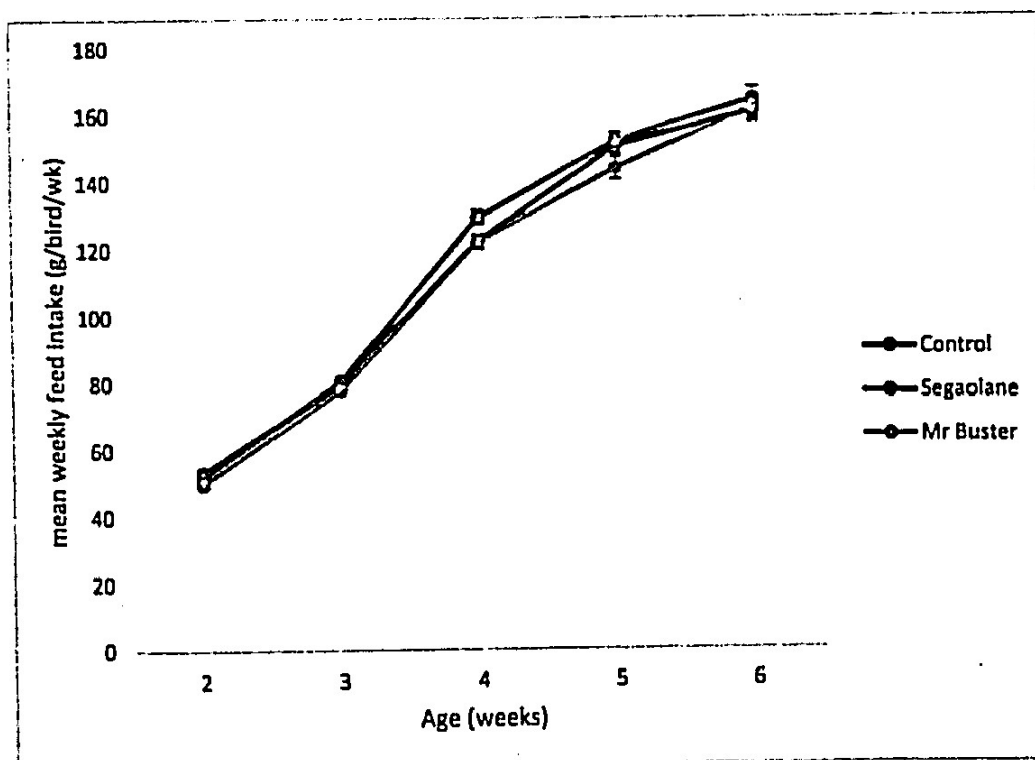


Figure 4.1 Average weekly feed intake of Ross 308 broiler chickens fed diets containing malted sorghum grains

4.3.2 Growth performance

4.3.2.1 Weight gain

Diet influenced ($P < 0.001$) AWG of R308BC whereby control diet resulted in higher gain than malted Mr Buster diet. There was also diet x time interaction ($P < 0.05$) on weight gain (Table 4.3). Polynomial contrasts showed AWG had significant high linear, quadratic and cubic components ($P < 0.001$). The quadratic effects are indicative of a rise in gain from weeks 2 to 4 and then subsequent decrease between weeks 5 and 6 (Figure 4.2). The magnitude of increase in R308BC fed malted Mr Buster-based diets in AWG was significantly lower than those fed control diet. No significant differences in AWG were observed at weeks 3 and 6 on R308BC fed malted Mr Buster and *Segaolane* based diets.

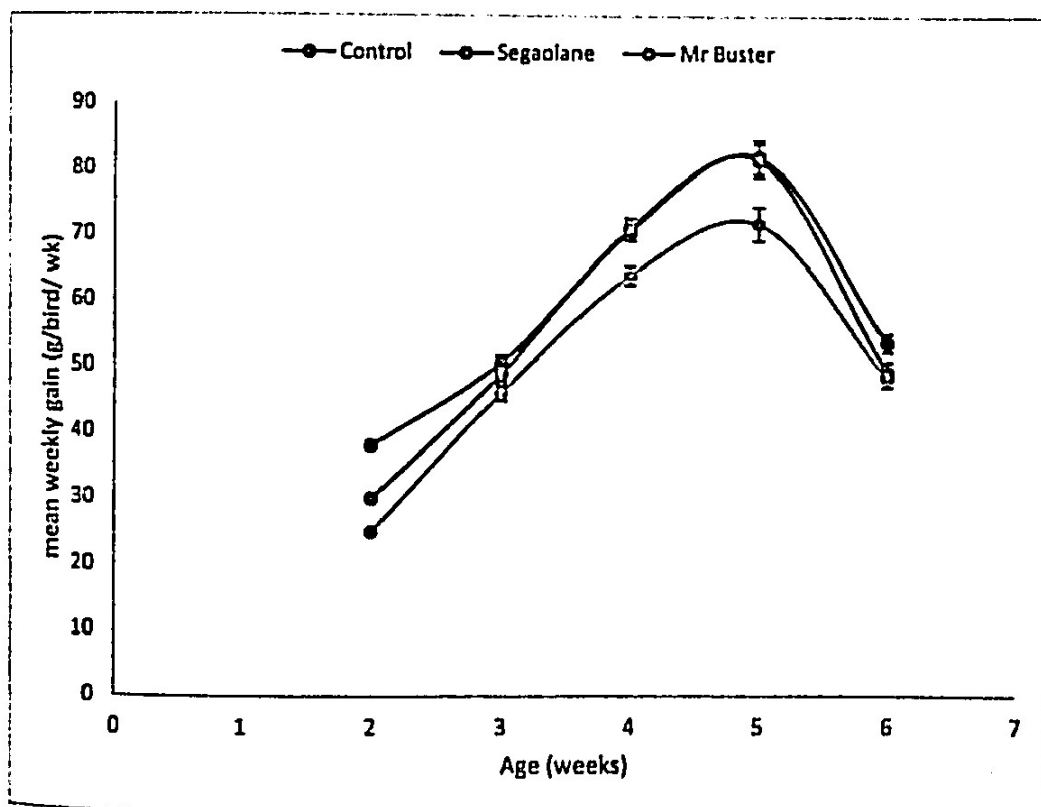


Figure 4.2 Average weekly weight gain (g) of Ross 308 broiler chickens fed malted sorghum-based diets

Table 4.3. Statistical influence of the main effects and their interactions on live weight, average weekly feed intake (AWFI), average weight gain (AWG), feed conversion ratio (FCR), protein intake (PI) and protein efficiency ratio (PER)

Parameter	Treatments			Treatment	Main Effects			Interaction effects
	Control	Malted <i>Segaolane</i>	Malted Mr Buster		Linear	Quadratic	Cubic	Treatment x Time
Live weight	1112.55±18.74	1045.28±19.99	949.22±19.00	***	***	***	***	***
AWFI	113.34±1.61	115.85±1.61	111.21±1.61	NS	***	***	***	NS
AWG	58.99±1.41	56.20±1.41	50.99±1.41	***	***	***	***	*
FCR	1.94±0.030	2.18±0.030	2.23±0.030	***	***	***	***	*
PI	2073.99±42.11	2415.07±42.11	2373.06±42.11	***	***	***	**	**
PER	0.0288±0.002	0.0244±0.002	0.0216±0.002	***	***	***	**	NS

NS- not significant, * P<.005, ** P<.001, ***P<.0001

4.3.1.2 Body weights

As illustrated in Figure 4.3, there was both linear, quadratic and cubic effects ($P < 0.001$) in mean weekly body weights of R308BC in all experimental diets. Although there was an increase in body weights of R308BC, those fed malted Mr Buster-based diet were lighter throughout the experimental period. Polynomial contrasts showed that body weights had significant linear, quadratic and cubic components ($P < 0.001$) (Table 4.3) and a significant diet x time effects ($P < 0.0001$) was observed across all experimental diets. At week 2, R308BC fed control and malted *Segaolane* based diets yielded a significant and high weights but no differences ($P = 0.2276$) were observed between R308BC fed malted Mr Buster and *Segaolane* based diets. At week 3, a significant, yet lower weights were observed on R308BC fed malted Mr Buster and *Segaolane* based diets. At weeks 4, 5 and 6 R308BC fed malted Mr Buster diets recorded the least significant weights compared to those fed control and malted *Segaolane* based diets. Interestingly, no significant differences were observed in body weights of R308BC fed malted *Segaolane*-based diet and Control diet throughout the experimental period.

4.3.1.3 Feed conversion ratio

Diet had an effect ($P < 0.05$) on weekly FCR of the birds across the 6 weeks (Table 4.4). In week 2, the best FCR was recorded in R308BC fed Control diet followed by those fed *Segaolane* diet. Birds fed malted Mr Buster diet had highest FCR in weeks 3, 4, 5 and 6. However, no significant difference were observed between the FCR from R308BC on Control or *Segaolane* diets at week 3, 4 and 5. In Week 6, R308BC fed on Control diets had lower FCR (more efficient) compared to those fed malted *Segaolane* or malted Mr Buster diets.

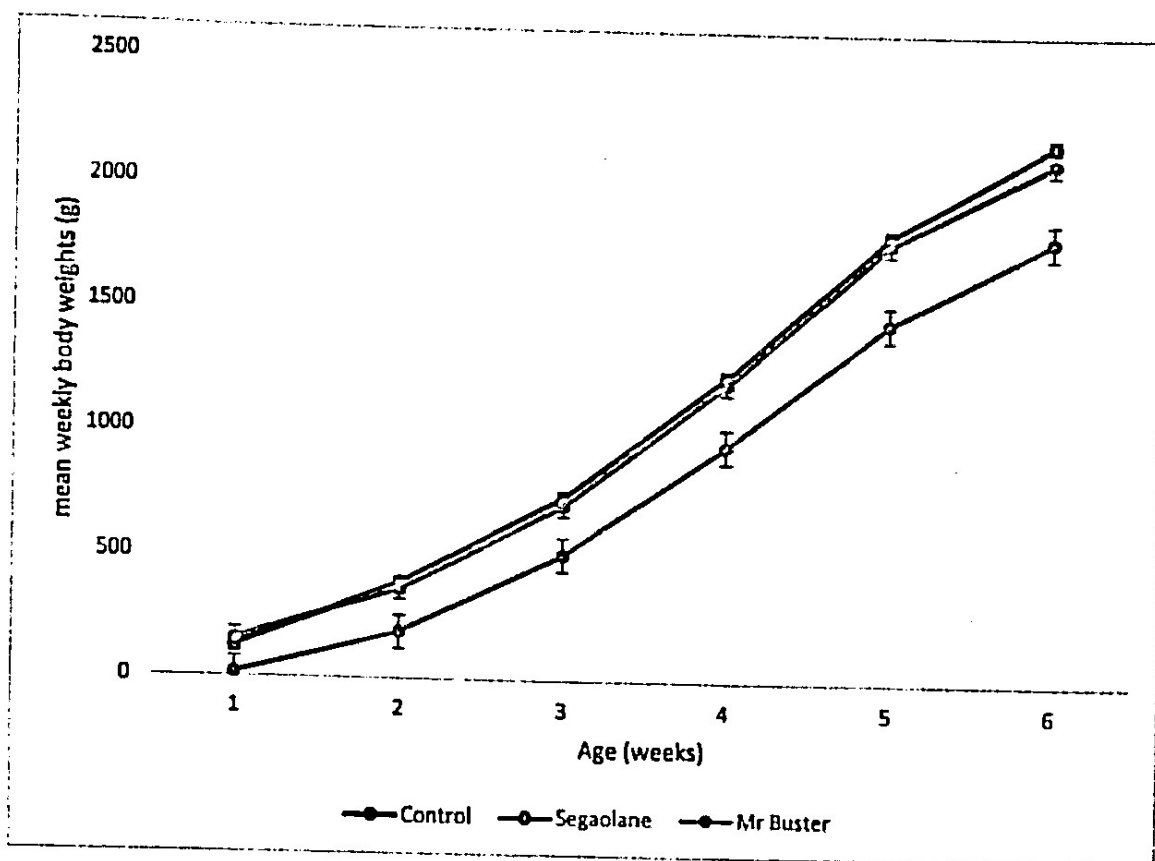


Figure 4.3 Mean weekly body weights of Ross 308 chicken fed diets contain malted sorghum grains as replacement to maize

Table 4.4 Feed conversion ratio of Ross 308 broiler chickens fed diets contain malted sorghum grains as replacement for maize grains

Age (Week)	Experimental diets			SE	P-value
	Control	<i>Segalane</i>	Mr Buster		
2	1.40 ^c	1.75 ^b	2.01 ^a	0.04	<0.0001
3	1.59 ^b	1.66 ^{ab}	1.70 ^a	0.04	0.0858
4	1.74 ^b	1.82 ^{ab}	1.91 ^a	0.04	0.0456
5	1.84 ^b	1.87 ^{ab}	2.00 ^a	0.04	0.0665
6	3.00 ^c	3.48 ^a	3.35 ^{ab}	0.05	<0.0001

^{abc} Means within a row that do not share a common superscript differ significantly (P<0.05)

4.3.2 Protein utilisation efficiency

Diets had a significant effect on PER at week 2 ($P = 0.0005$), 5 ($P = 0.0005$), and 6 ($P = 0.0011$), only (Table 4.5). In weeks 2 and 6, R308BC fed Control diets had the highest PER whilst those fed malted sorghum-based diets had the lowest. In week 5, R308BC on Control diet had the highest PER compared to those on malted Mr Buster-based diet but had similar PER as those fed malted *Segaolane*-based diet. Nonetheless, no significant dietary differences were detected for PER across diets at weeks 3 and 4. Diets had a significant effect on protein intake from week 1 to 5 (Figure 4.4). Polynomial contrasts showed PI had significant high linear, quadratic and cubic components ($P < 0.001$) from week 2 to 6 (Table 4.3). In weeks 1, 2 and 4, R308BC fed *Segaolane* diet had the highest ($P < 0.05$) PI, whereas at weeks 3 and 5 R308BC fed malted Mr Buster and *Segaolane* based diets had a higher PI compared to control diet (Figure 4.4).

Table 4.5 Protein efficiency ratio of Ross 308 fed diets containing malted sorghum grains as substitute to maize grains

Age (Week)	Experimental diets			SE	P-value
	Control	<i>Seguolane</i>	Mr Buster		
2	0.032 ^a	0.022 ^b	0.020 ^b	0.002	0.0005
3	0.030	0.030	0.028	0.001	0.3966
4	0.030	0.030	0.020	0.000	<.00001
5	0.032 ^a	0.030 ^{ab}	0.026 ^b	0.002	0.1005
6	0.020 ^a	0.010 ^b	0.014 ^b	0.001	<.00011

^{abc} Means within a row that do not share a common superscript differ significantly (P<0.05)

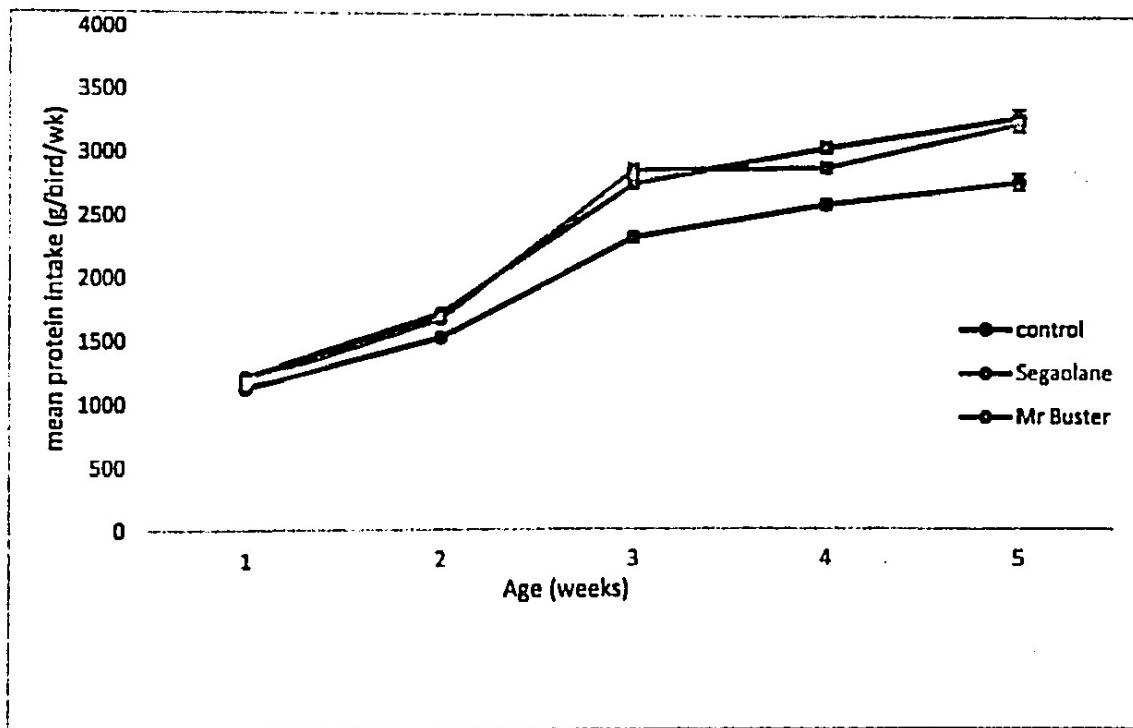


Figure 4.4 Average weekly protein intake (g) of Ross 308 broiler chickens fed malted sorghum-based diets

4.3.3 Haematology

Experimental diets had statistical effects ($P < 0.05$) on haematological parameters except lymphocytes, neutrophils, monocytes, eosinophils, red blood cells, haemoglobin, haematocrit and blood platelets (Table 4.6). The R308BC fed on Control diet ($46.8 \times 10^3/\mu\text{L}$) had significantly higher ($P < 0.05$) white blood cells counts compared to those fed malted sorghum-based diets. However, no significant effect on white blood cell counts was observed between R308BC fed malted sorghum-based diets. The highest ($P < 0.05$) basophil counts were observed on R308BC fed malted *Segaolane*-based diet (0.57%) or malted Mr Buster-based diet (0.56%), whilst those fed the Control diet (0.33%) had the lowest.

The R308BC fed malted Mr Buster-based diet (137 femtolitres (fL)) or *Segaolane*-based diet (135.40 fL) had the highest ($P < 0.05$) mean corpuscular volume, whilst those fed the Control diet (128.79 fL) had the lowest. Mean corpuscular haemoglobin was the highest ($P < 0.05$) on R308BC fed malted *Segaolane*-based diet (53.94 Pg) or malted Mr Buster-based diet (53.52 Pg). The mean corpuscular haemoglobin concentration was highest ($P < 0.05$) for R308BC fed Control diet (40.54 g/L) followed by those fed *Segaolane*-based diet (39.86 g/L) and lastly those fed Mr Buster-based diet (39.01 g/L). No significant differences were observed in lymphocytes, neutrophils, monocytes, eosinophils, red blood cells, haemoglobin, haematocrit, and blood platelets of R308BC across experimental diets (Table 4.6).

Table 4.6 Effects of substituting maize with malted sorghum grains on haematological parameters of Ross 308 broiler chickens.

Parameters	Experimental diets			SE	P-value
	Control	<i>Segaolane</i>	Mr Buster		
White blood cells [$10e^3/\mu\text{L}$]	46.8 ^a	39.2 ^c	41.8 ^{bc}	1.44	0.0031
Lymphocytes %	47.3	45.9	42.8	2.30	0.3857
Neutrophils %	44.8	46.4	50.4	2.21	0.2013
Monocytes %	0.17	0.35	0.00	0.16	0.2952
Eosinophils %	7.45	6.06	6.39	0.69	0.3456
Basophils %	0.33 ^c	0.57 ^a	0.56 ^{ab}	0.06	0.0137
Red blood cell [$10e^6/\mu\text{L}$]	2.61	2.48	2.56	0.06	0.2998
Haemoglobin g/Dl	13.65	13.40	13.67	0.29	0.7671
Haematocrit %	33.74	33.62	35.02	0.72	0.3242
MCV Fl	128.79 ^c	135.40 ^b	137.00 ^{ab}	1.29	0.0003
MCH Pg	52.66 ^c	53.94 ^a	53.52 ^{abc}	0.36	0.0502
MCHC g/dL	40.54 ^a	39.86 ^b	39.01 ^c	0.24	0.0004
Platelets [$10e^3/\mu\text{L}$]	3.10	3.00	3.60	0.52	0.6870

^{abc} Means within a row that do not share a common superscript differ significantly ($P < 0.05$)

μL = microliter; % = percentage; g/Dl = gram/deciliter; Fl = femtolitres; Pg = pictograms;

Mean corpuscular volume (MCV); Mean corpuscular haemoglobin (MCH);

Mean corpuscular haemoglobin concentration (MCHC)

4.3.4 Serum biochemistry

Experimental diets did not affect ($P>0.05$) serum biochemistry parameters except creatinine, bilta, glutamic-pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT) (Table 4.7). The R308BC fed diets containing malted *Segaolane* or Mr Buster had higher ($P<0.05$) creatinine counts compared to those fed the Control diet. However, no statistical difference was observed between R308BC fed malted sorghum-based-diets. Bilda counts was observed to be highest ($P<0.05$) for R308BC fed malted Mr Buster-based diet. The GOT and GPT were observed to be lowest ($P>0.05$) in R308BC fed malted *Segaolane*-based diets and highest in R308BC fed on the Control diet or malted Mr Buster-based diets. Experimental diets did not have any significant effect on albumin, total protein, triglycerides, urea and bilta of Ross 308 chickens.

4.3.5 Blood minerals

Diet significantly affected ($P<0.05$) blood minerals except potassium and sodium (Table 4.8). Blood of R308BC fed the Control diet (2.16 mmol/L) or malted Mr Buster-based diet (1.89 mmol/L) diet contained the highest ($P<0.05$) amount of calcium compared to those fed malted *Segaolane*-based diet (1.60 mmol/L). However, there was no significant difference in blood calcium between R308BC fed malted sorghum-based diets. Magnesium (0.73 mmol/L) and chloride (104.68 mmol/L) were significantly low in the blood of R308BC fed malted *Segaolane*-based diet, whilst the highest values were observed in the blood of R308BC fed the Control diet or malted Mr Buster-based diet.

Table 4.7 Effects of substituting maize with malted sorghum grains on serum biochemistry parameters of Ross 308 broiler chicken (Mean \pm SE)

Parameter	Experimental diets			P-value
	Control	<i>Segaolane</i>	Mr Buster	
Albumin (g/L)	6.91 \pm 0.95	6.81 \pm 0.95	8.01 \pm 0.95	0.6155
Creatinine (μ mol/L)	14.34 \pm 1.59 ^c	23.13 \pm 1.59 ^a	22.61 \pm 1.59 ^{ab}	0.0007
Total protein (g/L)	25.95 \pm 2.99	23.57 \pm 2.99	28.60 \pm 2.99	0.5019
Triglycerides (mmol/L)	0.41 \pm 0.08	0.43 \pm 0.08	0.63 \pm 0.08	0.1369
Bilda (μ l)	1.17 \pm 0.40 ^b	1.57 \pm 3.60 ^b	2.56 \pm 3.60 ^{ab}	0.0379
Urea (mmol/L)	0.62 \pm 0.29	0.37 \pm 0.26	0.59 \pm 0.26	0.7691
Bilta (μ l)	4.54 \pm 1.70	7.70 \pm 1.61	6.42 \pm 1.61	0.4134
GPT (IU/L)	167.89 \pm 18.00 ^a	84.80 \pm 17.08 ^b	138.60 \pm 17.08 ^a	0.0080
GOT (IU/L)	329.00 \pm 35.08 ^a	165.70 \pm 33.28 ^b	267.60 \pm 33.28 ^a	0.0078

^{abc} Means within a row that do not share a common superscript differ significantly (P<0.05)

μ mol/L= micromole/litre; g/L= gram/litre; mmol/L = millimoles/litre; μ l = microliter; IU/L = international units per litre; GPT= glutamic-pyruvic transaminase; GOT= glutamic oxaloacetic transaminase (GOT)

Table 4.8 Effects of substituting maize with malted sorghum grains on blood minerals of Ross 308 broiler chicken in mmol/L

Parameters	Experimental diets			SE	P-value
	Control	<i>Segaolane</i>	Mr Buster		
Potassium	5.35	5.10	5.46	0.28	0.6621
Sodium	147.63	144.12	145.92	1.63	0.3281
Calcium	2.16 ^a	1.60 ^b	1.89 ^{ab}	0.18	0.1027
Magnesium	1.03 ^a	0.73 ^b	0.97 ^a	0.08	0.0294
Chloride	113.21 ^a	104.68 ^b	113.05 ^a	2.16	0.0131

^{abc} Means within a row that do not share a common superscript differ significantly (P<0.05)

Mmol/L= millimoles/litre; SE = standard error

4.4 Discussion

4.4.1 Feed intake

There are limited studies conducted to evaluate the effects of malted grains on the performance of broiler chickens. The higher AWFI on R308BC fed malted *Segaolane*-based diet could be attributed to the diet being highly palatable whereas the low AWFI on R308BC fed malted Mr Buster-based diet could be attributed to high tannin content on the diets (Table 4.2). High tannin content in cereals negatively affects AWFI through reduced voluntary feed intake (Tapiwa, 2019). The low AWFI in R308BC fed malted Mr Buster-based diet may be attributed to the presence of condensed tannin that imparted unpalatable taste resulting in low feed intake.

In the current study, the lower AWFI observed on R308BC fed malted Mr Buster-based diet compared well with Demeke (2007) and Onyimba (2020) who fed broiler chickens on fermented grains, as well as, Ambula *et al.* (2001) and Hassan *et al.* (2003) who fed broiler chickens on high tannin sorghum and reported decreased feed intake. Hassan *et al.* (2003) reported that tannin imparted unpalatable taste to the diets resulting in reduced feed intake in chickens. The presence of CT in sorghum affects its protein digestibility and have high affinity for the protein proline hence lowering voluntary feed intake.

A study by Taylor *et al.* (2007) showed that the bond between kafirins and CT resulted in 50% reduction in protein digestibility. On the contrary, Torki and Pour (2007) did not observe any significant effects on feed intake when germinated high tannin sorghum was fed to broiler chickens compared to those fed ungerminated sorghum. The authors further concluded that germinated sorghum is a better alternative for tannin treatment. Ingredients used in the formulation of diets influences physicochemical properties of the diets (Disetlhe *et al.*, 2018). In the current study, similar AWFI at week 6 suggests that the broilers had fully developed

digestive system to deal with physiochemical properties and these results compare well with those of Hassan *et al.* (2003) and Sharif *et al.* (2012) who reported an increase in feed intake as the broiler chicken ages.

4.4.2 Growth performance

4.4.2.1 Body weight and Weight gain

The lower live body weight observed in R308BC fed malted Mr Buster-based diet could be attributed to low AWFI. This finding is in line with Demeke (2007) who reported that the inclusion of high levels of fermented agricultural residues into chicken feed led to decrease in weight gains. The higher body weight observed on R308BC fed Control diet or *Segaolane*-based diet suggest that the substitution of maize with malted *Segaolane* did not suppress weight gain due to low tannin content (Table 4.2) compared to Mr Buster-based diet, therefore being much comparable to the Control diet. Similarly, Akinola *et al.* (2015) reported improved final weights in broiler chickens fed dry, wet and fermented-wet feed.

The low AWG on R308BC fed malted Mr Buster-based diet in this study can be explained by low AWFI indicating that diet was rich in tannin content (Table 4.2) suggesting that malting the grains did not alter the physicochemical property (presence of condensed tannins) of this sorghum variety. Interestingly, it was observed that in the final week, there was a decline in AWG in all R308BC fed experimental diets. The decline could be due to the fact that broiler chickens reach their maturity at approximately 5 weeks (35 days); hence the gain in weight at a decreasing rate. It is also known that high fibre diets reduce AWG since they increase the passage rate of feed in the gastrointestinal tract leading to low utilisation (Bach-Knudsen, 2001). Therefore, it is not surprising that in the current study R308BC fed malted Mr Buster-

based yielded lower average weekly gains, which may be linked to tannin and fibre contents of the diets compared to birds fed on malted *Segaolane*-based diet and Control diet.

Mandal and Ghosh (2010) reported that tannin interferes with protein and carbohydrate digestion through formation of complexes with enzymes. In the current study, the lower AWG observed in R308BC fed Mr Buster-based diet compared well with Demeke (2007) and Onyimba (2020) who found that broiler chickens fed on fermented grains recorded lower AWG compared to the Control diet. The increase in AWF1 with age of chicken in this study is in line with Fafiolu *et al.* (2016) who found that chickens increase feed intake with increase in body weight (age). This could be due to the fact that broilers were feeding more to compensate for large body size (Sohail *et al.*, 2013) without addition of a significant weight gain in the last two final weeks of rearing. In the present study, malted *Segaolane*-based diet was found to have positive effects on weight gain and body weight compared to malted Mr Buster-based diets; hence it could be considered a candidate for replacing maize in poultry diets.

4.4.2.2 Feed conversion ratio

The FCR in this study was high in R308BC fed malted Mr Buster-based diet for the entire feeding period compared to those fed the malted *Segaolane*-based diet and Control diet, which suggests that R308BC fed malted Mr Buster-based diet utilised more feed to convert it into 1 kg of muscle. These findings are in line with Fanimu and Akinola (2006) who observed that an increased level of malted sorghum sprout resulted in higher value of FCR. However, the current results contradict the finding of Toriki and Pour (2007) who reported lack of significant differences in FCR when germinated sorghum was fed to broilers as partial replacement to maize. The differences between other reports and the current results may be due to differences in the processing methods.

4.4.3 Protein Utilisation efficiency

The higher protein intake in R308BC fed *Segaolane*-based diets contradict the study by Onyimba (2020), who observed a decrease in protein intake and protein efficiency in broiler chickens fed fermented spent sorghum. The reason for higher protein intake in R308BC fed *Segaolane*-based diet could be ascribed by high feed intake. The findings of this study imply that malted sorghum-based diets can be used in broiler chickens without having any negative effects on protein intake and PER. Malted *Segaolane* grains, when compared to Mr Buster has less tannin content hence the impact of ANFs on broiler chickens was minimal.

4.4.4 Haematology

Sorghum bicolor grains are rich in phytochemicals known to considerably impact health (Olawole *et al.*, 2018). Currently, there is very limited information on the effects of malted sorghum diets on blood haematology of broilers and this makes it difficult to compare the current results with those of other studies. However, the process of malting of foods has numerous nutritional and health benefits such as improvement in flavour, appearance, texture, nutritional value and palatability through increased bioavailability of nutrients and increased carbohydrate digestibility (Olawole *et al.*, 2018). According to Lowe *et al.* (2005), lactic acid bacteria produced during malting reduces the growth of undesirable microorganisms in the gastrointestinal tract of broiler chickens.

The normal ranges of haematological parameters of poultry are as follows: white blood cells $9.00-32.00 \times 10^3/\mu\text{L}$ / μL , lymphocytes 20.0-50.00 %, neutrophils 40.0- 75.00 %, monocytes 5.0-10.0 %, eosinophils 1.0-6.0%, basophils 0.02-1.0%, red blood cells $2.5-3.5 \times 10^6/\mu\text{L}$, haemoglobin 7.0-13.0 g/dl, mean corpuscular volume 90.0-143.0 Fl, mean corpuscular haemoglobin 33.0-47.0 Pg, mean corpuscular haemoglobin concentration 32.41-35 g/dl and

platelets $1.50-3.60 \times 10^3/\mu\text{L}$ (Jain, 1993). Values obtained in the present study fell within these normal ranges and compare well with reports by Kim and Kang (2016) who reported lack of significant differences in leukocytes when fermented barley and wheat were used in broiler diets. Therefore, it can be postulated that malted sorghum grains did not impact negatively on the biochemistry and physiological function of birds suggesting that sorghum can be used as a substitute to maize in broiler diets without having any adverse effects on the health status of the chickens.

Changes in haematological indices can be used to elucidate the impact of nutritional factors and additives supplied in the feeds (Kim and Kang, 2016). In the current study, no effect of diet was observed on lymphocytes, monocytes, neutrophils, eosinophils, red blood cells, haemoglobin, haematocrit and blood platelets, suggesting that there was enough dietary energy and vitamins to carry out all physiological activities by the animal. All experimental diets promoted higher white blood cell counts than the normal range, implying that all the birds were protected against infectious and foreigner pathogen invasion. Indeed, Abdi-Hachesoo *et al.* (2013) reported that increase in white blood cell counts reflect strong immune system response due to anti-oxidants found in sorghum grains.

Basophils play a role in preventing blood clotting and these blood cells contain a substance called heparin which is a naturally occurring blood-thinning substance (Jain, 1993). Basophils participate in phagocytosis, which is the process of destroying invading organisms in the animal body (Pandian *et al.*, 2012). In the present study, high basophil counts were observed in the blood of chickens fed malted sorghum-based diets, implying that malted sorghums may contain nutrients or compounds that promote formation of bone marrow (Jain, 1993), which assists in production of basophils.

4.4.5 Serum biochemistry

Blood parameters are good indicators of physiological, pathological and nutritional status of an animal (Kim and Kang, 2016). The current results showed no effect on serum albumin, total protein, triglycerides, urea and bilta, suggesting that malted sorghum grains provided sufficient dietary energy for transportation of protein for steroids and thyroid hormones in the blood (Kim and Kang, 2016). Creatinine as reported by Iyayi and Tewe (1998), is a chemical waste product found in the blood that passes through the kidneys to be filtered and eliminated in urine. The higher creatinine on R308BC fed malted sorghum-based diets indicates production of more waste products.

It is known that high level of liver enzyme above the normal range symbolize hepatocellular degeneration (Mohammed *et al.*, 2014). Interestingly, R308BC fed Mr Buster-based diet or the Control diet had similar and high GOT and GPT values, suggesting hepatoprotective abilities of those diets in reducing free radicals that could have caused liver damage (Hernández *et al.*, 2006). Significantly low GOT and GPT values on R308BC fed *Segaolane*-based diet is indicative of less liver cell damage. Lee *et al.* (2010) and El-Katcha *et al.* (2014) indicated lack of significant differences in the activities of serum GOT and GPT among Control and enzyme of treated groups of broiler chickens fed graded levels of wheat. Despite lack of significant differences in albumin and total protein in blood of R308BC in the present study, the values for total protein content fell below the normal range of 30-50 g/L as reported for the majority of birds species (Lloyd and Gibson, 2006; Nazifi *et al.*, 2012).

According to Esubonteng *et al.* (2011), lower concentration of albumin and total protein is associated with malnutrition and infections. However, during the entire study no R308BC in this study showed any form of illness associated with the experimental diets and no mortalities

were recorded. Therefore, malted *Segaolane* can be safely used to replace maize grains in broiler diets without any adverse effect on serum biochemistry of broiler chickens. And thus malting, a process known by local communities in beer making (indigenous knowledge) can be employed by local poultry industry to formulate diets with partial or complete replacement of maize grain.

4.4.6 Blood Minerals

The findings of the current study, that blood values for magnesium and chloride were high in R308BC fed the Control or malted Mr Buster-based diets is in agreement with several published reports (Szymeczko *et al.*, 2010; Payvastegan, *et al.*, 2013; Saki *et al.*, 2017). These researchers reported similar range of blood minerals in different poultry species despite differences in diets. The mineral content in the blood of birds is considerably dependent on its mineral concentration in feeds, as well as, factors influencing the degree of their absorption in the digestive tract. Although there were differences in blood calcium concentrations of the R308BC, there was no hypocalcaemia observed in the chickens throughout the study. Results of the present study showed no effect of diet on blood potassium and sodium.

There were no observed signs of rickets and all birds looked apparently healthy throughout the experimental period. This implies that experimental diets provided enough dietary minerals which were absorbed in the blood of chickens for efficient utilisation hence maintenance of bone integrity. However, further studies on mineralogy of the bones of birds fed malted sorghum-based diets are needed to ascertain this fact. This lack of an effect indicates that maize grains can be replaced with malted sorghum grains in broiler diets without any effect on blood minerals of the chickens.

4.5 Conclusions

Results of this study indicate that malting can be used as a feed processing tool to positively influence the utilisation of sorghum by birds. Total replacement of maize with malted *Segaolane* and Mr Buster did not negatively affect the growth and blood biochemistry of the birds. Compared to the Control diet, R308BC fed *Segaolane*-based diet had similar average weekly feed intake, average weight gains, good body weights and better FCR. These lack of an effect in the above parameters, taken together with similarities in blood metabolites and chemistry between birds fed malted *Segaolane*-based diet and birds fed Maize-based diets indicate that malted *Segaolane* grains can be used as an alternative dietary energy source in broiler diets without any adverse effects on health and production of the chickens. The effect of replacing maize with malted sorghum grains in broiler diets on growth performance may affect meat quality. It is thus important to evaluate the effect of replacing maize with malted sorghum grains on carcass characteristics and meat quality of R308BC.

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CHAPTER 5

THE EFFECTS OF MALTED SORGHUM-BASED DIETS (*SEGAOLANE* AND MR BUSTER) ON CARCASS CHARACTERISTICS AND MEAT QUALITY OF BROILER CHICKEN.

Abstract

The present study was designed to evaluate the effects of feeding malted sorghum-based diets (Mr Buster and *Segaolane*) on carcass characteristics and meat quality of broiler chickens. A total of 150 day-old Ross 308 broiler chickens (R308BC) were randomly allocated to three iso-caloric and iso-nitrogenous diets. Malted sorghum replaced maize grain in conventional broiler diets as follows; Control with 0% Sorghum and 100% Maize Commercial diet, 100% Malted *Segaolane*-based diet and 100% Malted Mr Buster-based diet. Allocation of the treatments to the pens was in a completely randomised design (CRD), with pens replicated five times with 10 birds in each pen. After feeding for 6 weeks, birds were humanely sacrificed. Internal organs and weight of external organs were measured on day of slaughter and samples of breast muscle were analysed for chemical composition and pH. Data were analysed using the Statistical Analysis System (SAS). Treatment means were separated using least square means. The R308BC fed Control diet had the highest ($P<0.05$) slaughter, hot carcass weights and cold carcass weights. The R308BC fed Control diet had the lowest ($P<0.05$) drumstick-thigh and vertebrae weight. The R308BC fed malted Mr Buster based diet had the longest ($P<0.05$) small intestine length (91.5mm), gizzard percentage (1.85%) and liver percentage (2.20%). The initial pH (pHi) of the meat of R308BC fed malted Mr Buster based diets was significantly higher ($P<0.05$) than that of the R308BC fed Control or malted *Segaolane* based diets. Breast muscle from R308BC fed malted Mr Buster based diet had the lowest ($P<0.005$) crude fat

content (1.76%) compared to those fed Control diet. Experimental diets did not significantly affect dry matter, ash, organic matter and energy content of breast muscle. Breast muscle of R308BC fed malted sorghum-based diets had lower ($P<0.005$) phosphorus content compared to those fed Control diet. The breast muscle of R308BC fed malted sorghum-based diets had the highest ($P<0.05$) concentration of potassium and magnesium compared to those fed Control diet.

Carcass characteristics, viscera macromorphometry and meat quality traits from carcasses of R308BC fed malted sorghum-based diets compared well to those fed Control diets. Therefore, malted sorghum grains can be used to replace maize in broiler diets without having adverse effects on carcass characteristics, viscera macromorphometry parameters and meat quality traits.

Keywords: *Segaolane*, Mr Buster, malted, carcass characteristics, R308BC

5.1 Introduction

Feeding and management of meat-type poultry have a great impact on poultry carcass characteristics and meat quality (Grashorn, 2010). The dietary energy supply of birds via carbohydrates directly affects fatness of carcasses (Waldroup *et al.*, 2001). It is a common practice to use maize as a major source of dietary energy in poultry and human nutrition, as well as, for industrial uses (Kumaravel *et al.*, 2014) compared to other cereal grains. The multiple uses of maize create stiff competition among humans, livestock and in industry, leading to its price in the market becoming unpredictably high. Its price keeps on rising to levels that are unsustainable for small- and medium-scale poultry farmers. However, to save the collapse of the poultry sector, there is a need to search for and evaluate alternative energy sources such as sorghum.

In comparison to maize, sorghum can be grown successfully on relatively poor soils and with lower moisture condition in Botswana. According to Dei (2017), the metabolised energy (ME) and percent crude protein (CP) content of sorghum are 13.4 MJ/Kg and 9.5% CP, respectively, which is comparable with 14.2 MJ/Kg ME and 10.1% CP, respectively of maize. The major constraint on the use of sorghum in poultry diets is the presence of high amounts of anti-nutritional factors (ANFs), like tannins that depress poultry performance (Hassan *et al.*, 2003). Effects of tannins in chicken diets have been reported to reduce feed intake due to reduced palatability, resulting in low live weight gain, low digestibility and poor feed conversion efficiency (Oke *et al.*, 2015). However, physical, chemical and biological treatments have been used to upgrade cereals for different uses including its use as animal feed. Medugu *et al.* (2012) reported that fermentation process improves feed utilisation in poultry. A study by Fafiolu *et al.* (2006) concluded that up to 300 g/kg of malted sorghum sprout can be fed to grow pullets without any adverse effect. It is for these reasons that treated sorghum which at present has

limited alternative uses, is being considered by the present study for its feeding value in broiler chickens. Therefore, it is imperative that when fed to poultry malted sorghum diets are not assessed not only for feed intake or conversion efficiency but also for carcass and meat quality of animals. This is because the ultimate test for food animal products is their acceptability by consumers.

Traditionally, the term meat quality covers inherent properties of meat that influence its suitability for eating, further processing and storage, including retail display. The main attributes of meat quality include but are not limited to safety, nutritional value, flavour, texture, water-holding capacity, colour, lipid content, lipid composition, oxidative stability and uniformity (Andersen *et al.*, 2005).

Management system, breed, genotype, feeding, and pre-slaughter handling and stunning, slaughter method, chilling and storage conditions are known to affect meat quality (Vestergaard *et al.*, 2000). However, feeding strategy is the management factor which is most actively used as a quality control tool in the production of meat and in relation to improvement and/or control of performance, animal welfare, safety, nutritional value, and eating and technological quality (Andersen *et al.*, 2005) and it is under the control of a farmer. The utility of malted sorghum as dietary energy on carcass characteristics and meat quality of broiler chicken strains is limited, and thus worth researching. This will open up opportunity to utilize sorghum in livestock feeding, increase its value as a crop and hence its production by farmers (Madibela and Lekgari, 2005).

Therefore, the present study was undertaken to evaluate the effects of feeding malted sorghum-based diets containing malted Mr Buster or malted *Segaolane* grains on carcass characteristics

and meat quality of broiler chickens. It was hypothesised that feeding malted sorghum-based diets would not have deleterious effects on carcass characteristics and meat quality of broiler chickens.

5.2 Materials and Methods

5.2.1 Study site, diet formulation, management of chickens and experimental design

Study site, management of chickens, diet formulation and experimental design of the feeding trials were described in detail in Chapter 3 (Section 3.3.1) and Chapter 4 (Sections 4.2.7, 4.2.6 and 4.2.3).

5.2.2 Ethical Consideration

The management and care of the chickens were in accordance with Botswana University of Agriculture and Natural Resources' Research Ethics Committee accepted standards for animal welfare and ethics (Ethics Number BUAN-2020-08).

5.2.2 Slaughtering process

All R308BC were slaughtered according to standard abattoir procedures. Feed and water were withdrawn 12 hours prior to slaughter to empty the digestive tract. Chickens were weighed before slaughter to obtain slaughter weights (final body weight). The birds were sacrificed humanely by electrical stunning and then killing by cutting jugular vein for bleeding. The carcasses were scalded at 55-60 °C in water bath for 30 sec before de-feathering. The dressed chicks were then eviscerated to assess the carcass characteristics, size of internal organs and meat quality.

5.2.3 Carcass characteristics

After slaughter, the feathers were plucked and gastrointestinal tract (GIT) was removed. The carcass was weighed immediately after dressing to obtain the hot carcass weights (HCW). The final live weight and the carcass weight were used to determine dressing out percentage (%) of the birds, expressed as $((\text{carcass weight}/\text{final weight}) \times 100)$. Hot carcass yield percentage was calculated by dividing hot carcass weight by pre-slaughter weight, then factored by 100 as shown in formula (b). The carcasses were then chilled at 4 °C for 24 hr. and cold carcass weights (CCW) (a) were thereafter recorded. After 24 hr. post-mortem the breast muscle portion was obtained, kept frozen at -20°C in polythene bags pending use for proximate analysis of the meat.

$$\text{Dressing out percentage} = \frac{\text{Carcass weight}}{\text{Body weight}} \times 100 \quad (\text{a})$$

$$\text{Hot Carcass Yield} = \frac{\text{Hot carcass yield.}}{\text{Slaughter weight}} \times 100 \quad (\text{b})$$

Three (3) birds per replicate were randomly selected for determination of carcass characteristics and meat quality. For the measurement of carcass cuts, head and shanks were removed close to the skull and at hock joint, respectively. Wings were removed by cutting at the humeroscapular joint, the cuts were made through the rib head to the shoulder girdle and the vertebrae was then removed intact by pulling interiorly (Manyeula *et al.*, 2020). The breast muscle, wings, thighs-drumstick and vertebrae (back) were each weighed separately and expressed as percentage weight. The weights of the GIT and other viscera including the liver, gizzard and heart were recorded. The following equation was used to compute breast muscle and thigh-drumstick percentage relative to the carcass:

$$\text{Breast muscle percentage} = \frac{\text{Breast muscle (g)}}{\text{Hot carcass weight (g)}} \times 100$$

$$\text{Drumstick percentage} = \frac{\text{Drumstick (g)}}{\text{Hot carcass weight (g)}} \times 100$$

5.2.4 Meat pH

A portable digital pH meter (CRISON pH25, CRISON Instruments SA, Spain) with a piercing electrode was used to measure the pH of the breast muscle at 45 minutes for initial pH (pHi) and 24 hours post slaughter to obtain the ultimate pH (pHu) (Sanka and Mbagu, 2014).

5.2.5 Proximate analysis of meat

Proximate analysis of meat was done as described in detail in Chapter 3.2.3 similar to feeds samples.

5.2.6 Mineral and protein content of meat

Phosphorus (P), Calcium (C), Magnesium (Mg), Potassium (K) and Sodium (Na) were determined using the procedure by Sahrawat *et al.* (2002) by digesting 1.25 g of meat sample in sulphuric acid-selenium (Se). After digestion the minerals were read using coupled plasma optical emission spectrometer (ICP-OES) (Perkin, Japan). Phosphorus was read using a UV-Vis spectrophotometer (Model UV-1601; Japan) and absorbance was determined at 670nm wavelength following Molybdenum blue method of Dickman and Bray (1940). Kjeldahl method (CP; method 954.01) was used to determine crude protein of breast meat.

5.2.7 Statistical analysis

Data on carcass characteristics, size of internal organs and meat quality parameters were analysed using one-way analysis of variance (ANOVA) using general linear model (GLM) procedures of SAS (2002-2012), according to the following model:

$$Y_{ijk} = \mu + d_i + E_{ijk}$$

Where Y_{ijk} = response variable, μ = general mean, d_i the diets fixed effects. E_{ijk} is the random error associated with observation ijk , assumed to be normally and independently distributed. Treatment means were separated using least square means. The level of significance was set at $p < 0.05$.

5.4 Results

5.4.1 Carcass traits and weight of external components

Results on effects of diet on carcass traits and weights of external organs are shown in Table 5.1. Diet had a significant effect on carcass traits and weight of external organs of Ross 308 broiler chicken (R308BC) (Table 5.1). The R308BC fed malted Mr Buster-based diet had the lowest ($P < 0.05$) slaughter, hot carcass and cold carcass weights. Higher ($P < 0.05$) hot carcass yield and dressing percentage were observed in the carcass of R308BC fed the Control diet compared to those fed malted sorghum-based diets. Nonetheless, carcass from R308BC fed either of the two malted sorghum-based diets had the same ($P > 0.05$) hot carcass yield and dressing percentage. The R308BC fed malted Mr Buster or malted *Segaolane*-based diets had the highest ($P < 0.05$) wing weight compared to those fed the Control diet. The carcass of R308BC fed the Control diet had the highest breast weight and breast weight ratio while those fed *Segaolane*-based diet had the lowest. However, no significant difference was detected between the breast weights of R308BC fed malted Mr Buster based diet and the other two diets. Thigh-drumstick weight and the thigh drumstick ratio were significantly high ($P < 0.05$) in

carcass of R308BC fed malted sorghum-based diets. The highest ($P < 0.05$) vertebrae weight was recorded in R308BC fed malted *Segaolane* based diet while the lowest was in those fed Control diet. However, no significant difference was observed in the vertebrae weight of R308BC fed malted Mr Buster based diet and the other experimental diets. The lowest pH_i was recorded in the meat of R308BC fed malted Mr Buster based diet while the highest was of meat of those fed the other two diets. Diet had no significant effect ($P > 0.05$) on the ultimate pH (pH_u).

Table 5.1 Effects of substituting maize with 100% malted sorghum grains on carcass traits and weight of external organs of Ross 308 broiler chickens (mean±SE)

Carcass traits	Experimental diets			P-value
	Control	<i>Segaolane</i>	Mr Buster	
Slaughter weight (g)	2212.44±33.16 ^a	2101.43±33.54 ^b	1933.75±33.54 ^c	<.0001
Hot carcass weight (g)	1719.51±26.49 ^a	1594.52±26.79 ^b	1450.45±26.79 ^c	<.0001
Cold carcass weight (g)	1694.11±26.47 ^a	1570.80±26.77 ^b	1421.55±26.77 ^c	<.0001
Hot carcass yield %	76.17±0.69 ^a	74.09±0.69 ^b	73.31±0.69 ^{bc}	0.0160
Dressing percentage (%)	75.38±0.68 ^a	73.43±0.68 ^b	72.26±0.68 ^{bc}	0.0079
Wing weight (%)	5.45±0.09 ^c	5.74±0.09 ^{ab}	5.83±0.09 ^a	0.1042
Breast weight (%)	33.50±0.72 ^a	31.10±0.72 ^b	31.80±0.72 ^{ab}	0.0260
Breast weight ratio	33.48±1.04 ^a	31.13±1.04 ^b	31.76±1.04 ^{ab}	0.0260
Thigh-drumstick weight (%)	13.67±0.24 ^b	14.72±0.24 ^a	14.69±0.24 ^a	0.0570
Thigh-drumstick weight ratio	13.66±0.34 ^b	14.72±0.34 ^a	14.69±0.34 ^a	0.0570
Vertebrae (back) weight (%)	9.61±0.27 ^b	10.78±0.27 ^a	10.58±0.27 ^{ab}	0.0816
pH _i	5.84±0.05 ^a	5.92±0.05 ^a	5.61±0.05 ^b	0.0002
pH _u	5.51±0.04	5.47±0.04	5.58±0.04	0.3767

^{abc} Means within a row that do not share a common superscript differ significantly (P<0.05)

5.4.2 Viscera macromorphometry

Diet significantly affected size of internal organs and the length of intestine of R308BC (Table 5.2). The R308BC fed malted Mr Buster-based diet had longer ($P<0.05$) large intestine and heavier heart weight (91.5 mm and 0.64%, respectively) compared to those fed malted *Segaolane* based diet (82.86 mm and 0.56%) and commercial diet (80.46 mm and 0.53%). The heaviest liver ($P<0.05$) was from R308BC fed malted sorghum-based diets; *Segaolane* (2.21%) and malted Mr Buster (2.20%). The R308BC fed malted Mr Buster based diet had the highest ($P<0.05$) gizzard percentage (1.85) followed by those fed malted *Segaolane* based diet (1.73%) and the Control diet (1.53%). Experimental diets did not significantly affect the length of the small intestine and caeca.

5.4.3 Chemical composition of breast muscle

The results of chemical composition of breast muscle are shown in Table 5.3. Diet did not affect chemical composition of breast muscle from R308BC fed experimental diets except crude fat content (Table 5.3). Breast muscle from R308BC fed malted Mr Buster-based diet had the lowest ($P<0.05$) crude fat content (1.76%) compared to those fed Control diet (3.56%). However, there were no significant differences observed in crude fat content of breast muscle of R308BC fed malted *Segaolane*-based diet (2.82%) when compared to Control diet (3.56%) and malted Mr Buster-based diet (1.76%). Experimental diets did not significantly affect dry matter, ash, organic matter, energy content, crude fibre and crude protein content of breast muscle from R308BC.

Table 5.2 Effects of substituting maize with 100% malted sorghum grains on size of internal organs (% of HCW, unless stated otherwise) of Ross 308 broiler chickens at 6 weeks old

Internal Organ	Experimental diets			SE	P-value
	Control	<i>Segaolane</i>	Mr Buster		
Large Intestine length (mm)	80.46 ^b	82.86 ^{ab}	91.5 ^a	2.64	0.0197
Small Intestine length (mm)	1585.00	1181.60	1670.80	46.89	0.5749
Caeca (mm)	263.03	176.43	165.20	38.01	0.4122
Liver	2.05 ^b	2.21 ^a	2.20 ^a	0.03	0.0445
Gizzard	1.53 ^c	1.73 ^b	1.85 ^a	0.03	<.0001
Heart	0.53 ^b	0.56 ^b	0.64 ^a	0.02	0.0071

^{abc} Means within a row that do not share a common superscript differ significantly (P<0.05)

Table 5.3 Effects of substituting maize with 100% malted sorghum grains on chemical composition (% , unless stated otherwise) of breast meat of Ross 308 chickens at 6 weeks of age

Parameter	Experimental diets			SE	P-v
	Control	<i>Segaolane</i>	Mr Buster		
Dry matter	97.81	98.25	97.36	0.49	0.21
Ash	7.16	6.93	7.29	0.88	0.65
Organic matter	92.84	93.07	92.27	0.88	0.65
Energy (MJ/Kg)	22.40	22.42	22.34	0.14	0.75
Crude fat	3.56 ^a	2.82 ^{ab}	1.76 ^b	0.33	<0.01
Crude fibre	6.88	7.11	7.31	0.74	0.91
Crude protein	21.58	23.22	23.36	0.97	0.35

^{abc} Means within a row that do not share a common superscript differ significantly ($p < 0.05$)

5.4.4 Macro mineral content of breast meat

Table 5.4 presents the results of mineral content in the breast muscle. It was found that diet significantly affected mineral concentration of breast muscle (Table 5.4). Breast meat of R308BC fed malted sorghum based diets (*Segaolane* (36.07 mg/L) or Mr Buster diets (34.23 mg/L) recorded lower ($P < 0.05$) phosphorus content compared to Control diet. However, there was no difference between birds fed malted *Segaolane*-based diet and control diet. Breast meat from R308BC fed malted *Segaolane*-based diet (723.22 mg/L) had the lowest ($P < 0.05$) calcium concentration compared to R308BC fed Control (834.94 mg/L) or malted Mr Buster diet (832.80 mg/L). Sodium concentration of breast meat from R308BC fed malted Mr Buster-based diet (1591 mg/L) was significantly high ($P < 0.05$) followed by that of R308BC fed malted *Segaolane* based diet (1333.01 mg/L) and lastly the R308BC fed Control diet (923.42 mg/L). Breast muscle of R308BC fed malted sorghum-based (*Segaolane* (6372.66 mg/L) or Mr Buster (6588.10 mg/L) diets had the highest ($P < 0.05$) concentration of potassium compared to those fed Control diet (4357.11 mg/L). Highest concentration ($P < 0.05$) of magnesium was recorded in breast of chickens fed malted sorghum-based diets, with those fed Control diet having the lowest concentration values.

Table 5.4 Effects of substituting maize with 100% malted sorghum grains on macro mineral content of breast meat of Ross 308 chickens at 6 weeks old (mg/L)

Mineral	Experimental diets			SE	P-value
	Control	<i>Segaolane</i>	Mr Buster		
Phosphorus	37.63 ^a	36.07 ^{ab}	34.23 ^b	0.84	0.0373
Calcium	834.94 ^a	723.22 ^b	832.80 ^a	31.57	0.0381
Sodium	923.42 ^c	1333.01 ^b	1591.70 ^a	69.54	<.0001
Potassium	4357.11 ^b	6372.66 ^a	6588.10 ^a	138.66	<.0001
Magnesium	273.07 ^c	421.26 ^a	374.93 ^{ab}	26.71	0.0042

^{abc} Means within a row that do not share a common superscript differ significantly (p<0.05)

5.5 Discussion

5.5.1 Carcass traits and weight of external components

Reports explaining the effects of malted sorghum on carcass traits of broilers are limited, making it difficult to compare the present results with any studies carried elsewhere. The observation that hot carcass weights, cold carcass weight, hot carcass yield and dressing percentage were low in R308BC fed malted *Segaolane* or malted Mr Buster based diets could be due to slaughter weight. However, these results contradict the findings by Yaşar *et al.* (2016), who observed that broiler birds fed fermented cereals (barley, wheat and oats) produced higher carcass yields than those fed unfermented cereals (70.2% vs. 68.8%). The differences between the two studies may have been influenced by the types of grains (sorghum versus barley, wheat and oats) used and processing employed (fermentation vs malting). It is important to note that different cereals contain different anti-nutritional factors (ANFs) which are substances that when present in animal feed either by themselves or through their metabolic products reduce the availability of one or more nutrients (Yacout, 2016).

It is expected that slaughter weights should be correlated with external components (Vasdal *et al.*, 2019) but in the present study it was not the case. The observation in this study was that the wing, thigh-drumstick and vertebrae (back) were heavy in R308BC fed malted sorghum-based diets compared to those fed control diet and the reason is unknown.

Wing weights were observed to be heavy in R308BC fed malted sorghum-based diets, which is indicative of increased locomotion in the birds. This finding is in line with findings by Fasuyi and Olumuyiwa (2012) who reported that birds fed on rice husk which was fermented for 3 weeks had the highest mean weight of wing compared to those fed on maize based diet. Heavier thigh- drumstick in R308BC fed malted sorghum-based diets is in line with findings by Zhai *et al.* (2020), who found a higher percentage of thigh muscle on ducks fed fermented liquor

distiller's grains. These portions; wing, thigh- drumstick and vertebrae are the most popular meat cuts with poultry meat consumers in Botswana and this places malted sorghum grains in a good position to earn a decent profit margins for producers or retailers.

The observation that breast weight was the same among R308BC fed malted sorghum diets is in agreement with findings by Zhai *et al.* (2020), who found no significant differences in ducks fed fermented liquor distiller's grains on breast weight compared to those fed unfermented liquor distiller's grains. The low breast weight of R308BC fed malted sorghum diets may suggest a limitation on either nutrient supply or utilisation by malted sorghum-based diets. The breast is the heaviest cut in a broiler chicken, therefore it contributes more in final body weight. Malted sorghum based diets may have not supplied or availed sufficient energy or protein critical for muscle tissue deposition. As indicated by Rosa *et al.* (2007) breast yield increases with time, and genetically selected broilers deposit more muscular mass in the breast than the non-selected ones. Breast muscle with skin is also considered a healthy meat portion, however, it is not popular with consumers in Botswana, perhaps due to the way its prepared in household kitchens. Therefore, improved product development of this meat cut is needed to boost its palatability.

The strong relationship between pH_i and pH_u *post-mortem* is a result of the decline in pH from the time of slaughter to when there is *post-mortem* aging (24 hrs *post-mortem*). The R308BC fed malted Mr Buster-based diets had the least initial pH implying that more glycogen in the muscles was converted to lactic acid. Lactic acid is necessary to produce meat, which is tasteful and tender (Zhai *et al.*, 2020). According to Dyubele *et al.* (2010), the pH range is determined by how much glycogen is in breast muscle prior to slaughter, and how rapidly the remaining glycogen is converted to lactic acid after slaughter. Nevertheless, the pH values of R308BC

fed experimental diets obtained in this study were within the 5.5 to 6.5 range that reported for simple non-ruminants by Ao *et al.* (2008).

The observation that significant differences were recorded in breast muscle ratio of R308BC fed experimental diets contradicts with studies by Zhai *et al.* (2020). The above authors investigated the effects of fermented liquor distiller's grains on carcass characteristics of cherry valley ducks, and found no significant difference in breast muscle ratio when compared to those fed unfermented grains. Choi *et al.* (2014) also who studied the effects of dietary fermented seaweed and seaweed fusiforme on growth performance, carcass parameters and immunoglobulin concentration in broiler chickens and found no effect on breast muscle ratio. The lack of significant difference in breast muscle ratio is indicative of a better meat: bone ratio. Hence all experimental diets promoted the storage of lysine and methionine which are essential amino acids needed for growth and breast meat yield in broilers (Straková, *et al.*, 2006).

5.5.2 Viscera macromorphometry

The R308BC fed malted Mr Buster based diet had longer large intestines and heavier heart which could be due to the presence of high fibre content in malted Mr Buster based diet (Table 4.2). Recently, Mnisi *et al.* (2019) and Manyela *et al.* (2020) reported enlarged and thickening of intestines when chickens were fed diets with high fibre. Studies by Jorgensen *et al.* (1996) and Ahamed and Olorede (2003) reported that monogastric animals enlarge and thicken their intestines as an adaptation mechanism. On the contrary, the reports by Manyelo *et al.* (2019) found no differences in the large intestine of R308BC fed up to 100% sorghum-based diets compared to maize based diets. The discrepancy in the current study and that of the other

studies may be due to differences in the diets (malted versus unmalted) and that condensed tannins may also create variation on morphology of GIT.

The function of heart is to pump and supply blood to body organs in an animal. In the present study, the heavier heart reflects that more energy was needed to pump blood in R308BC fed malted Mr Buster-based diets due to their high fibre content in the diet (Table 4.2) compared to other diets, leading to formation of more muscles in the hearts hence heavy weights. The R308BC fed malted sorghum-based diet had heavier liver as compared to control diet. This may be due to the ANFs found in sorghum-based diets. Malting in (Chapter 3) did not totally remove condensed tannin level and hence the liver was over worked to detoxify those phytochemicals (Manyeula *et al.*, 2020). Contrary to other studies, Silva *et al.* (2015) using broiler chickens of 14, 21, 28, 35, and 42 days of age, observed higher relative liver weights when ground maize was fed compared to ground sorghum.

In another study, Ahmed *et al.* (2013) observed that replacement of maize by 100%, 75%, 50%, 25%, and 0% sorghum in broiler diets did not show any significant effects on liver weights. Generally, an increase in liver and heart sizes could be indicative of the need to deal with toxic substances in the feed. It is known that feeding high fibrous diets increases the volume of the GIT and organ development in chickens, ducks and geese (Deniz *et al.*, 2007). Heavier liver can also be due to high intake, when high nutrients pass through the liver. Heavy gizzard of R308BC fed high fibre malted Mr Buster-based diets could be explained by structural wall of Mr Buster (red sorghum grains) being tougher to grind and causing the gizzard muscle to enlarge as an adaptive measure from constantly grinding the grains that would have been smoothed for digestion in the stomach. Similar results were reported by Manyelo *et al.* (2019) who found heavy gizzard weights on broiler chickens fed 75% and 100% sorghum-based diets.

There were no significant effects observed on the small intestine and caeca length of all R308BC across the experimental diets in the present study implying that the crude fibre contents of the experimental diets were not high enough to influence the development of the caeca and small intestine length (Chapter 4, Table 4.2). This finding is in line with a study by Silva, *et al.* (2015) who compared ground maize, sorghum, (50:50), or whole-grain sorghum on broilers, and did not find any differences in small intestine lengths. Garcia *et al.* (2005) also found no influence of diets based on maize or low- and high tannin sorghum on small intestine or cecum length of broilers. Longer small intestines in broiler chickens are associated with ability to provide greater surface area for nutrient absorption (Manyelo *et al.*, 2019).

5.5.3 Chemical composition of meat

Excess supply of carbohydrate and protein from the diets are stored as crude fats in the muscle (Attia, *et al.*, 2000). It is therefore not surprising in the present study that lower crude fat was found in the breast meat of R308BC fed malted Mr Buster-based diet. This could be due to limited supply of energy due to binding of carbohydrates with tannins found in the diet (Table 4.2). Similarly, Marcinčák *et al.* (2018) found that breast meat of Cobb 500 fed with cornmeal fermented with filamentous fungi *Umbelopsis isabellina* CCF 2412 had lower water and fat contents when compared to the commercial diet. Results from the current study imply that malted sorghum-based diets reduce the fat content in chicken breast muscle, which is a good meat attribute since too much fat consumption is associated with nutritional diseases like high blood pressure and obesity in consumers. Contrary to the current study, Kim and Kang (2016) found no difference in crude fat content of breast meat from broilers fed fermented barley when compared to birds fed unfermented diet. The discrepancy between these two studies may be due to the different cereal grains used and processing (fermented vs malting). The observation that experimental diets did not affect dry matter, ash, organic matter, energy, crude fibre and

crude protein content of breast meat is in line with a study by Kim and Kang (2016) who investigated the effects of diets containing fermented barley or wheat on proximate analysis of breast meat of broilers. The authors reported lack of significant difference in the concentration of moisture, crude ash, and crude protein of breast muscle across experimental diets. Malted sorghum-based diets did not alter chemical composition of the breast meat compared to those fed control diet suggesting that malted diets did not alter the biochemical property of the breast muscle.

5.5.4 Macro mineral content of meat

There is limited research on the effects of malted sorghum diets on macro mineral content of breast meat. The observation that malted sorghum-based diet had lower concentration of phosphorus compared to control diet could be due to lower concentration and/or supply of this mineral from the experimental diets. Anti-nutritional factors like phytic acid bind with phosphorus to limit its supply to the birds. Phosphorus is required for the utilisation of energy in an animal (Fung *et al.*, 2019).

The breast meat of R308BC fed malted *Segaolane*-based diet had low calcium content compared to those fed Control and malted Mr Buster-based diets. This may have resulted from antagonistic relation with other minerals leading to poor absorption of minerals from the diet. Al-Yasiry *et al.* (2017) reported that there is a relation between fat and calcium level in the muscle. High calcium level is usually accompanied by a reduced fat content in the meat. As stated by Cormick and Belizán (2019), calcium from meat products is beneficial to consumers' health and good functioning of nerves and muscle tissue, as well as, for maintaining bone integrity.

The R308BC fed malted Mr Buster based diets had breast meat with higher concentration of sodium implying that the diet was rich in sodium; hence the deposition of the mineral in the breast muscle. Sodium concentration in diet has an influence in the bird's faecal moisture content, therefore minimizing excessive levels in the diet is important (Murakami *et al.*, 1997).

The observation that R308BC fed malted sorghum-based diets had higher concentration of potassium and magnesium could be an indication of availability of these minerals in the malted sorghum grains and the ability of the chickens to absorb and utilize them from malted sorghum-based diets. Mohammed *et al.* (2019) reported that generally sorghum grains contain higher concentration of potassium. Magnesium plays a vital role in the absorption or accumulation of calcium in the blood and breast muscle (Estevez and Petracci, 2019). Previous study by Herke *et al.* (2016) investigated the effects of a phytogenic additive on nutritional composition of turkey meat and found lack of significant effect of the additive on breast meat on magnesium, phosphorus, potassium, sodium and calcium.

5.6 Conclusion

Results from this study revealed that carcass characteristics, viscera macromorphometry and meat quality from carcasses of R308BC fed malted *Segaolane* or malted Mr Buster diets compared well to those fed the Control diet. It can be concluded that malted sorghum grains can be used to replace maize in broiler diets without having adverse effects on carcass characteristics, viscera macromorphometry parameters and meat quality traits of chickens. This was more pronounced in meat cuts which happen to be popular with customers. Further research on graded level in substitution of maize with malted sorghum meal to determine performance of birds and economic analysis of such substitution is needed.

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CHAPTER 6

GENERAL DISCUSSION AND CONCLUSION

6.1 General Discussion

It is important to provide alternative energy sources for broiler diets as there is stiff competition of maize grains between human and livestock consumption. The main objective of the study was to investigate the performance of broiler chickens fed malted sorghum-based diets. There was an observation that malting did not alter chemical composition within a variety of grains (*Segaolane* or Mr Buster), but significant differences were observed across varieties. Red Sorghum grain (Mr Buster) was found to have more tannin content compared to white sorghum grains (*Segaolane*). Malting reduced condensed tannin content of both varieties.

In the feeding Study 2) it was observed that there was an increase in average mean feed intake (AWFI) across all experimental diets which led to a polynomial linear, quadratic and cubic patterns and increase in average weight gain and mean weekly body weights of R308BC across experimental diets. However, it was observed that R308BC fed malted Mr Buster based diets had lower body weight than those fed *Segaolane* based diets and Control diets. The R308BC fed malted Mr Buster based diet had poor feed conversion ratio (FCR); this could be attributable to high tannin content in the diets compared to other diets, implying inefficiency in feed utilisation.

Haematological parameters were influenced by experimental diets except lymphocytes, neutrophils, monocytes, eosinophils, red blood cells, haemoglobin, haematocrit and blood platelets. However, serum biochemistry such as creatinine, bilda, glutamic-pyruvic

transaminase (GPT) and glutamic oxaloacetic transaminase (GOT), were significantly influenced by experimental diets, except for bilta, urea, total protein, albumin and triglycerides. Diet significantly affected blood minerals except potassium and sodium. The results, therefore, suggest that inclusion of malted *Segaolane* grains can not cause any barriers to the digestion dynamics and utilisation of energy by broiler chickens. Therefore, malted sorghum grains can be used to replace maize grains without major effects on blood metabolites.

The results of Study 3 suggested that substitution of maize grains by malted *Segaolane* grains can effectively improve some carcass cuts weights and meat quality traits of broilers for the benefit of consumers. Experimental diets did not significantly affect dry matter, ash, organic matter and energy content of breast muscle from R308BC. Carcass characteristics, viscera macromorphometry and meat quality traits from carcasses of R308BC fed malted sorghum-based diets compared well to those fed control diets. Therefore, malted sorghum grains can be used to replace maize in broiler diets without having adverse effects on carcass characteristics, viscera macromorphometry parameters and meat quality traits. Overall, malted sorghum grains have shown to have great potential as an alternative of maize grains in broiler diets. Current findings can be helpful in designing low-cost feed formulations that will benefit farmers and improve growth performance, health status, carcass characteristics, and meat quality in poultry farming systems in future.

6.2 Recommendations

Sorghum grains can be used to replace maize grains as energy source in broiler diets. With its good agronomic properties, local farmers can be advised to plough sorghum in large quantities so that it can be used in poultry feed processing. It would be advisable for the small-scale farmer to use malted sorghum grains to replace maize grains as it will reduce the cost of

production. This is because sorghum can be plough even in this changing harsh climatic conditions. It is clearly evident from the current results that malted sorghum grains did not have any negative effects on all measured parameters in broiler chickens. Moreover, malting sorghum grains can be a source of income on its own as farmers may opt to malt in large quantities and sell to other farmers and brewing companies.

6.3 Further research recommendations

1. Bone and immune development in broilers fed malted sorghum based diets
2. Effects of feeding graded levels of malted sorghum based diet on performance of broiler chickens
3. Nutrient digestibility of broiler chickens fed malted sorghum based diets
4. Effects of feeding malted sorghum based diets on gut health of broiler chickens
5. Economic analysis of feeding malted sorghum in comparison with maize