THE NUTRITIONAL USE OF MILLET GRAIN FOR FOOD AND FEED

by

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ABSTRACT

Worldwide, millets are regarded as significant grains; however, they are the least exploited. Millet grain is abundant in nutrients and health-beneficial phenolic compounds, making it suitable as food and feed. The diverse contents of nutrients and phenolic compounds present in finger and pearl millet are good indicators that the variety of millet available is important when selecting it for use as food or feed. The phenolic properties found in millets comprise of phenolic acids, flavonoids, and tannins, which are beneficial to human health. Research has shown that millet phenolic properties have high antioxidant activity. Phytochemicals present in millet grains have positive effects on human health by lowering the cholesterol and phytates in the body. The frantic demands on maize and its uses in multiple industries have merit the search for alternative grains, to ease the pressure. Substitution of maize with pearl and finger millets in the diets of different animals resulted in positive impact on the performance. Of late, millet grain has been incorporated in other foods and used to make traditional beverages. In Chapter 1, the topic of the research was introduced, stating the importance of the study and to motivate on the significance of millet grains. Aims and objectives were also listed. Chapter 2 presented extensive literature review on millet and their uses in human and livestock nutrition. In addition, the use of tannin as alternative feed was also reviewed.

Different studies have been conducted to investigate the suitability of millet grain as an energy source, in the animal industry. However, studies on the Southern African types of millet are limited; this might be due to lack of information on their nutritional composition and their suitability as animal feed. Overall, the aim of this study was to profile the nutritional characteristics of selected millet grains in South Africa and Zimbabwe, their suitability as energy source for human and livestock and to study the effect of pearl millet type on the

performance indices of Ross 308 broiler chickens. In Chapter 3, the general materials and methods used to reach the scientific conclusion for this study was summarised. In Chapter 4, physical and chemical analysis were conducted on millet grains obtained from South Africa and Zimbabwe, the results revealed that the physiochemical characteristics of the millet grain qualify it as a suitable candidate in replacing maize as an energy source. To further understand the characteristics of the millet grain, Chapter 5 analysed the phenolic compounds available in the millet grain obtained from South Africa and Zimbabwe. The results showed that the grain is endued with valuable phenolic compounds beneficial in the nutrition of both human and animals and aid as health benefit. Chapter 6 investigated the various inclusion levels of pearl millet in a bid to establish the most suitable combination for the poultry sector. Pearl millet grain used in this experiment went through different breeding improvements and selections conducted at the Grain Crop Institute in Potchefstroom, South Africa. Pearl millet grain was used as the main source of energy for Ross 308 broiler chicks for a period of 42 days with performance indices investigated. The results revealed that pearl millet can be incorporated in the diets of broiler chickens, in replacement of maize, without adversely affecting the performance. In addition, the economic justification of replacing maize with pearl millet was studied. The cost of the grains was determined and the cost per weight gain was determined. The results showed that indeed it is economically sound to replace maize with pearl millet in the diet of poultry. The quadratic function best fitting optimum treatment combination in relation to performance parameters such as body weight, body weight gain, feed conversion ratio and internal organs was also examined. In Chapter 7, the research was generally discussed to tie up the conclusions of the experiments conducted. General recommendations were also given on the compounds of millet varieties and their health benefits to both humans and animals.

ISIFINYEZO ESIQUKETHE UMONGO WOCWANINGO

Kuwo wonke umhlaba, amabele athathwa njengezinhlamvu ezibalulekile, kodwa awasetshenziswa kakhulu. Uhlamvu lwebele lunemisoco eminingi kanye nenzuzo yempilo ngama-phenolic compound, okwenza ukuthi afaneleke kakhulu njengokudla kwabantu kanye nemfuyo. Imisoco eminingi equkethwe kanye nama-phenolic compound atholakala eminweni kanye nezinhlamvu zebele ayizinkomba ezinhle zokuthi izinhlobo zamabele ezitholakalayo zibaluleke kakhulu ekukhethweni njengokudla kanye nokudla kwabantu noma imfuyo. Ama-phenolic properties atholakala kumabele aqukethe ama-phenolic acids, ama-flavonids, kanye nama-tannins, ayinzuzo kakhulu empilweni yabantu. Ucwaningo luthole ukuthi ama-phenolic properties amabele anomsebenzi wezinga eliphezulu lama-antioxidant. Ama-phytochemicals atholakala kwizinhlamvu zamabele anenzuzu enhle kakhulu kwimpilo yabantu ngokwehlisa izinga le-cholesterol kanye nama-phytate emzimbeni. Ukudingeka kakhulu kombila kanye nokusetshenziswa kwawo kwizimboni eziningi, kubangele ukuthi kwenziwe ucwaningo ngezinye izinhlamvu ukwehlisa ingcindezi. Ukuthatha isikhundla sombila, sithathwa amabele kwidayethi yezilwane ezehlukene kwaba nomphumela omuhle kakhulu ngokusebenza. Kamuva nje, izinhlamvu zamabele zifakelwe kwezinye izidlo ezisetshenziswa ukwenza iziphuzo zomdabu. Kwisahluko 1, isihloko socwaningo sethulwa khona, ukuchaza ukubaluleka kocwaningo kanye nokuqikelela ngokubaluleka kwezinhlamvu zamabele. Izinhloso nezinjongo nazo zifakelwe kuhla. Isahluko 2, sethula ukubuyekezwa kwemibhalo ngamabele kanye nokusetshenziswa kwawo kubantu kanye nokudla kwemfuyo. Nangaphezu kwalokho, ukusetshenziswa kwe-tannin njengokunye ukudla kwemfuyo kuye kwabuyekezwa.

Kwenziwe ucwaningo olwehlukene ukuphenyisisa ngokufaneleka kwezinhlamvu zamabele njengomthombo wamandla (we-eneji) kwimboni yezilwane. Kodwa, ucwaningo ngezinhlobo zamabele eNingizimu ne-Afrika alukenziwa ngokwanele; lokhu kungenzeka kungenxa

yokuswelakala kolwazi ngemisoco equkethwe kanye nokufaneleka njengokudla kwezilwane. Kanti ngokwengamele, inhloso yalolu cwaningo bekuwukwenza iprofayili yemisoco ngezinhlamvu zamabele athile eNingizimu Afrika kanye naseZimbabwe, ukufaneleka kwawo njengomthombo wamandla (we-eneji) kubantu kanye nemfuyo, kanye nokwenza ucwaningo ngemiphumela yenhlobo yamabele ngama-performance indices wamachwane e-ROss 308. Kwisahluko 3, kusetshenziswe imetheriyali kanye namamethodi asetshenzisiwe ukufinyelela isiphetho sezesayense kulolu cwaningo, kwafinyezwa. Kwisahluko 4 kwenziwe uhlaziyo lokubambekayo kanye namakhemikhali ngezinhlamvu zamabele ngokutholakale eNingizimu Afrika kanye naseZimbabwe, imiphumela ikhombise ukugqama kwe-physiochemical kwezinhlamvu zamabele ukufaneleka kwazo njengekhandideti ekuthatheni isikhundla sombila njengomthombo wamandla. Ukuqhubeka nokuqondisisa ukuphawuleka kwezinhlamvu zamabele, iSahluko 5 sihlaziye ama-phenolic compound kwizinhlamvu zamabele aseNingizimu Afrika neZimbabwe. Imiphumela ikhombisa ukuthi uhlamvu lwamabele lunama-phenolic compound ayinzuzo ekudleni okunomsoco kubantu kanye nezilwane, kanye nokuba wusizo lwenzuzo kwimpilo. Isahluko 6 siphenyisise ngamazinga okubandakanywa kwamabele ukwenzela ukuthola ukufaneleka kwawo kumkhakha wezinkukhu. Izinhlamvu zamabele e-pearl zisetshenziswe kule ekspirimenti, eyenziwa ezigabeni ezehlukene zokuthuthukisa ukuzalisa kanye nokhetho olwenziwe ngabe-Grain Crop Institute ePotchefstroom, eNingizimu Afrika. Amabele e-pearl asetshenziswe njengomthombo omkhulu we-eneji kumachwane eRoss 308 isikhathi sezinsuku ezingu 42 kanti kwaphenyisiswa ngokusebenza kwama-indices. Imiphumela iveze ukuthi amabele e-pearl angafakelwa kwidayethi yamachwane, ukuthatha isikhundla sombila, ngaphandle kokuphazamisa ukusebenza. Nangaphezu kwalokho, ukufaneleka kwezomnotho ngokuthatha isikhundla zombila sithathwa ngamabele e-pearl kuye kwacwaningwa. Izindleko zezinhlamvu ziye zabekwa kanti futhi nesisindo ngezinhlamvu kuye kwabekwa. Imiphumela ikhombisa ukuthi kuyinto enhle kwezomnotho ukuthatha isikhundla sombila sithathwa ngamabele e-pearl kwidayethi yezinkukhu. Ukusebenza kwe-quadratic function kufaneleke kakhulu kwi-optimum treatment combination mayelana nama-parameter okusebenza afana nokwenyuka kwesisindo somzimba, ukuguqula i-feed conversion ratio kanye nezitho zangaphakathi nazo ziye zahlolwa. KwiSahluko 7, ucwaningo kuye kwaxoxwa ngalo ukuhlanganisa iziphetho zama-ekspirimenti enziwe. Izincomo ezinabile, ziye zanikezwa ngama-compound ezinhlobo zamabele kanye nezinzuzo zawo kwezempilo kubantu kanye nezilwane.

SETSOPOLWA

Lefaseng ka bophara, leotša le bonwa bjalo ka mabele a bohlokwa kudu; le ge go le bjale, ke dibjalo tšeo di sa bjalwego kudu. Dithoro tša leotša di tletše ka phepo ye ntši le ditswaki tša fenoliki tšeo di nago le mohola maphelong, gomme se sa dira gore di be maleba bjalo ka dijo le phepo. Dikagare tša lona tšeo di fapafapanego le ditswaki tša fenoliki tšeo di whetšagalago ka gare ga leotša la *finger* le la pearl ke dilaetši tše kaone tša gore mehutahuta ya leotša yeo e hwetšagalago e bohlokwa ge e kgethwa bjalo ka sejo le phepo. Diteng tša fenoliki tšeo di hwetšwago ka agre ga leotša di na le diesiti tša fenoliki, difolabanoite, le dithaninse, tšeo di lego mohola go maphelo a batho. Dinyakišišo di laeditše gore diteng tša fenoliki tša leotša di na le mošomo wa godimo wa dihlwekišammele tšeo di bitšwago dianthioksitente. Difaethokhemikhale tšeo di lego gona ka gare ga dithoro tša leotša di na le diabe tše kaone go maphelo a batho ka go fokotša kholesterole le difaetheite mmeleng. Dinyakwa tša ka pela go lefela le mešomo ya lona ka diintastering tše ntši di dirile gore go be le nyakego ye kgolo ya dithoro tše dingwe tšeo di ka emelago lefela legato, go nolofatša kgatelelo yeo e beilwego go lefela. Go tšeela lefela legato ka leotša la *pearl* le la *finger* ka dijong tša diphoofolo tšeo di fapafapanego go feleleditše ka seabe se sekaone ka ga go šoma ga lona. Go fihla mo lebakeng le, dithoro tša leotša di tsentšwe ka dijong tše dingwe gomme tša šomišwa go dira dino tša setšo. Ka go Kgaolo ya 1, hlogotaba ya dinyakišišo e tsebagaditšwe, ya fa bohlokwa bja dinyakišišo tše le lebaka mabapi le bohlokwa bja dithoro tša leotša. Maike

mišetšo le dinepo le tšona di filwe. Kgaolo ya 2 e hlagišitše tekodišišo ya dingwalwa ye e tseneletšego ka ga leotša le mešomo ya lona go phepo ya batho le ya diphoofolo. Godimo ga fao, tšhomišo ya *tannin* bjalo ka phepo ya boikgethelo le yona e lekodišišitšwe.

Dinyakišišo tše di fapafapanego di dirilwe go nyakišiša go ba maleba ga thoro ya leotša bjalo ka methopo wa enetši, ka intastering ya diphoofolo. Le ge go le bjale, dinyakišišo tš0e di dirilwego mabapi le mehuta ya leotša ka Borwa bja Afrika ke tše nnyane; se se ka ba se

bakwa ke tlhoklego ya Tshedimošo mabapi le sebiopego sa phepo ka hare ha leotša le go ba maleba ga lona bjalo ka phepo ya diphoofolo. Ka kakaretšo, maikemišetšo a dinyakišišo tše e bile go lebeledišiša dikokwane tša phepo tša dithoro tša leotša tšeo di kgethilwego ka Afrika Borwa le ka Zimbabwe, go ba maleba ga lona bjalo ka methopo wa enetši go batho le go diruiwa le go nyakišiša ka ga seabe ka ga mohuta wa leotša wa *pearl* go go šoma ga dipalopalo go dikgogo tša nama tša *Ross 308*. Ka go Kgaolo ya 3, ditlabelo le mekgwa ka kakaretšo yeo e šomišitšwego go fihlelela sephetho sa tša mahlale sa dinyakišišo tše di filwe kakaretšo. Ka go Kgaolo ya 4, tshekatsheko ya naga le ya dikhemikhale e dirilwe mabapi le dithoro tša leotša tšeo di hweditšwego ka Afrika Borwa le ka Zimbabwe, dipoelo di utollotše gore dikagare tša dikhemikhale tša thoro ya leotša di le dira le be lebele leo le loketšego go tšeela legato lefela bjalo ka methopo wa enetši. Go kwešiša go tšwela pele dikagare tša thoro ya leotša, Kgaoilo ya 5 e sekasekile diteng tša fenoliki tšeo di hwetšagalago ka gare ga thoro ya leotša leo le hwetšago ka Afrika Borwa le ka Zimbabwe. Dipoelo di laeditše gore thro ya leotša le tletše ka dikagare tša fenoliki tšeo di lego mohola go phepo ya bobedi batho le diphoofolo le gore le thuša bjalo ka kholego ya tša phepo. Kgaolo ya 6 e nyakišišitše maemo a mehutahuta a kakaretšo a leotša la *pearl* ka nepo ya go hwetša motswako wa maleba kudu ka lekaleng la dikgogo. Dithoro tša leotša la *pearl* tšeo di šomišitšwego ka mo tekodišišong ye di sepedišitšwe ka go dikaonafatšo tše di fapanego tša monontšha gomme dikgetho di dirilwe ka go Sehlongwa sa Dibjalo tša Dithoro ka Potchefstroom, ka Afrika Borwa. Dithoro tša leotša la *pearl* di šomišitšwe bjalo ka mothopo wa enetši go matswiana a nama a *Ross 308* mo matšatšing a 42 fao go dirilwego dinyakišišo ka ga dipalopalo tša go gola ga dikgogo. Dipoelo di laeditše gore leotša la pearl le ka tsenywa ka dijong tša dikgogo tša nama, go tšeela legato lefela, ka ntle le go ama gampe go gola ga dikgogo. Godimo ga fao, lebaka la tša ekonomi la go tšeela lefela legato ka leotša la *pearl* le nyakišišitšwe. Theko ya dithoro e hweditšwe gomme theko ka boima bjo itšego le yona e hweditšwe. Dipoelo di laeditše gore ka nnete go tloga go kwagalago kudu go tša ekonomi go tšeela lefela legato ka leotša la *pearl*. Mošomo wa tekanelo wa wo o loketšego bokaone motswako wa tlhokomelo ya godimo mabapi le mahlakore a kgodišo ya dikgogo a go swana le boima bja mmele, go nona, rešio ya go fetošetša dijo le ditho tša ka gare le ona o lekodišišitšwe. Ka go Kgaolo ya 7, dinyakišišo di hlalošitšwe ka kakaretšo gore go fihlelelwe sephetho ka ga ditekodišišo tšeo di dirilwego. Ditšhišinyo ka kakaretšo le tšona di filwe mabapi le dikagare tša mehutahuta tša leotša le dikholego tša ona go tša maphelo go bobedi batho le diphoofolo.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
DM	Dry matter
G	Gram
Kg	Kilo grams
Mg	Milligrams
GE	Gross energy
NDF	Neutral detergent fibre
ADF	Acid detergent fibre
Min	Minutes
N	Nitrogen
ADFI	Average Daily Feed Intake
ADG	Average Daily Gain
AOAC	Association of Official Analytical Chemists
CF	Crude Fibre
CP	Crude Protein
CRC	Cooperative Research Centre
CRD	Completely Randomized Design
FCR	Feed Conversion Ratio
LSD	Least Significant Difference
PMZA	Pearl Millet South Africa
PMZM	Pearl Millet Zimbabwe
FMZA	Finger Millet South Africa
FMZM	Finger Millet Zimbabwe

CHAPTER 1

GENERAL INTRODUCTION

The increasing population in Africa and around the globe requires a smart way of diversifying the means of attaining different crops and natural resources to meet the demands for food. Several countries rely on importation of food and feed resources to satisfy their needs and meet future demands. However, there are many neglected crops and naturally occurring compounds that can be exploited to meet some of these demands. Millets and naturally occurring compounds such as tannins are some of the potential alternatives that can be studied and improved to substitute the conventional ingredients.

The availability of cereal grains for human and animal consumption is challenged by many factors, particularly the change in the climate. Human population is challenged to not only look for substitute grains to diversify the production but to also look for smart grains that can withstand challenging climatic conditions. Feeding poultry with balanced nutrition has presented a challenge to the industry over the years, as it has become costly due to competition on maize which is the common cereal used for nutrition. In South Africa, the poultry industry consists of the broiler, layer and a small proportion of the indigenous chickens kept by the rural farmers.

Despite Africa's abundant natural resources, as well as its human resources, poverty, hunger, and starvation are rife. Pearl millet and other alternative cereals have been around for a long period; however, they did not gather much attention from consumers.

Non-ruminant animal production, particularly the poultry production sector, is growing continuously, mostly due to demands for its by-products such as meat and eggs. However, this growth and the increasing demand for poultry feeds have led to high prices for poultry feed. The gap between demand and supply of balanced feed is expected to increase, and consequently increase the cost of production. On the other hand, the conventional feed ingredients such as maize, wheat and rice can no longer meet the poultry industry's demand for feed.

Maize is traditionally included in poultry diets (AgriSA, 2017), but recently the competition among its uses in different industries has led to increase in its demand. This increase in demand has resulted in stiff completion and antagonism over resources such as water and soil, which can affect the sustainability and continuous supply. This has warranted a search for alternative unconventional feed ingredients with similar nutrients (Medugu et al., 2010). This has provided an opportunity for farmers, animal nutritionists and feed manufacturers to find ingredients that are cost-effective and readily available. Pearl millet crop is grown in Central, Eastern and Southern Africa, Western Africa, India, Pakistan; and along the southern coast of the Arabian Peninsula (International Crops Research Institute for the Semi-Arid Tropics, 1996). Pearl millet is believed to be a perfect choice due to its valuable nutrients and its ability to thrive in harsh climatic conditions. It is considered to be an important crop in the strategy to ensure food security in regions of Africa and India (Passot et al., 2016). Its added advantage is that the agronomic characteristics, such as drought tolerance, allow it to be grown in diverse geographical regions (Sedghi et al., 2011). The use of pearl millet in broiler feed resulted in comparable (Davis et al., 2003; Hidalgo et al., 2004) or improved feed intake, body weight and feed efficiency. In South Africa, pearl millet varieties are undergoing different genetic selections processes in order to improve its productivity. However, pearl millet-based diets have not yet been tested on broiler chicken's production.

Since energy is the major component in a diet, it then becomes necessary to assess which level of energy that can be contributed by different cereals to identify the most profitable combination. Thus, the aim of this study was to determine the effect of replacing maize as source of energy with pearl millet in the diets for broiler chickens and to identify different properties of millet types as food and feed.

1.1. Problem statement

The use of conventional grains such maize for both human and livestock can no longer sustain the demands. In addition, unfavourable climatic conditions demand search for cereal grains which can flourish in less favourable environment. Maize is the most used feed ingredients in commercial poultry diets *(*Jacob, 2015)*.* The demand of maize has increased with its use in many industries such as the ethanol industries, and as a result, the price of maize has risen (Rattray, 2012). In addition, the drought in South Africa has also resulted in increased prices for maize. For instance, the combined effects of a severe drought and a strong El Niño events which hit South Africa in 2015-16, was believed to have had negative effects on the agro-economic system (Baudoina et al., 2017). This has increased the interest in alternative feed ingredients for use in poultry diets. Poultry production in South Africa contribute greatly to the meat and egg production chain, however the cost of production is skyrocketing, mostly due to high feed cost. Maize which is the most convenient energy source is extremely stretched leading to increase in its prices. Looking for alternative energy source in poultry sector has become an imperative. Hence, the purpose of this study is to determine the nutritional composition of millet, to determine its suitability as an alternative to maize and to study the effect of pearl millet type on the performance of broiler chickens.

1.2. Motivation of the study

Feeding the human population and its livestock has been proven to be challenging over the years. Because of the tight competition on the consumption of the conventional grains like maize, the price has increased and most likely will continue to increase in the future. For example, the cost of feed alone accounts for up to 70-80% of the production inputs in the intensive monogastric animal production, which makes the production very expensive (Ravindran and Blair, 1992; Fasuyi, 2005, Aduku, 1992; Aduku, 2004). The high cost of maize as energy source, has generated a lot of concern as to its economic justification (Fasuyi, 2005). In addition, Dube et al. (2018) believes that the urge to route for millet and sorghum instead of maize and other major crops in recent years is derived from the fact that they are believed to be more ecologically well-matched with semi–arid areas, because of their drought tolerance. They are considered tough crops in terms of growth requirements as they withstand harsh climatic factors such as, unpredictable climate and nutrient-depleted soils (Sharma et al., 2000). Finding a suitable local source of energy which might be cheaper and readily available, is justified. Therefore, this study will investigate the suitability of millet grains as candidate to replace maize grain in human and animal nutrition.

1.3. Aim of the study

The aim of this study was to determine the utilisation of millets available in Zimbabwe and South Africa as source of food and feed.

1.3.1. Objectives

This study seeks to achieve the following:

1.3.1.1 To identify and determine the nutritional composition of millet available in Southern Africa and their suitability for human and livestock consumption.

1.3.1.2 To examine the phenolic compounds found in the millet varieties and their health benefit as food and feed.

1.3.1.3. To determine the influence of various inclusion levels of pearl millet on the performance of broiler chickens and to explore the economic importance of replacing maize with pearl millet in the broiler chicken diets.

1.3.1.4. To determine the optimum level of inclusion of pearl millet on the performance parameters of broiler chickens.

1.4. Hypotheses

1.4.1 There is no significance difference in physical and chemical composition of millet types available in South Africa and Zimbabwe.

1.4.2 There is no significant difference in the phenolic compounds available in the millet types available in South Africa and Zimbabwe.

1.4.3 There is no significant difference among varying inclusion levels of pearl millet in replacement of maize on the performance of broiler chickens. There is no significant economic difference in replacing maize with pearl millet in broiler chicken diets.

1.4.4 There is no significant difference between the optimization of different treatments on the performance of broiler chickens.

1.5. Significance of the study

The outcome from this research will enrich the knowledge and understanding of alternative animal feeds. The research will be of benefit to cereal grain production in Southern Africa. Furthermore, the research will allow the identification of different millet varieties, which are available in Southern Africa, as potential food, and feed sources. In addition, the determination of the phytochemicals available in the grain will contribute to the optimization of the general health of human and its livestock; consequently, this will result in low production cost, especially in poultry industry.

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REVIEW

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The nutritional use of millet grain for food and feed: a review

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Abstract

Worldwide, millets are regarded as a significant grain, however, they are the least exploited. Millet grain is abundant in nutrients and health-beneficial phenolic compounds, making it suitable as food and feed. The diverse content of nutrients and phenolic compounds present in finger and pearl millet are good indicators that the variety of millet available is important when selecting it for use as food or feed. The phenolic properties found in millets compromise phenolic acids, flavonoids, and tannins, which are beneficial to human health. Moreover, finger millet has an exceptionally unique, more abundant, and diverse phenolic profile compared to pearl millet. Research has shown that millet phenolic properties have high antioxidant activity. The presence of phytochemicals in millet grains has positive effect on human health by lowering the cholesterol and phytates in the body. The frantic demands on maize and its uses in multiple industries have merited the search for alternative grains, to ease the pressure. Substitution of maize with pearl and finger millets in the diets of different animals resulted in positive impact on the performance. Including these grains in the diet may improve health and decrease the risks of diseases. Pearl millet of 50% or more can be used in broiler diets without adversely affecting broiler performance or egg production. Of late, millet grain has been incorporated in other foods and used to make traditional beverages. Thus, the core aim of this review is to provide insight and comprehension about the nutritional and phenolic status of millets and their impact on human and livestock.

Keywords: Health, Feeds, Finger millet, Foods, Nutritional composition, Pearl millet, Phenolic profile

Background

Millets are cereals from the Poaceae grass family and are considered one of the oldest cultivated crops. Generally, pearl millet (*Pennisetum glaucum*) and finger millet (*Eleusine coracana*) are known as the two major millets used for food and feed. Pearl millet is believed to have originated from sub-Saharan Africa, and finger millet from the sub-humid uplands of East Africa [1]. The two account for most of the world's millet production and trade [2]. The majority of the recent research and agricultural programmes, which are routed towards the development of millets, have been dedicated to pearl and finger millets. Dube et al. [3] believe that the urge to route for millet and sorghum instead of maize and other major crops in recent years is derived from the fact that these grains are ecologically well-matched with semi-arid areas because of their ability to tolerate drought. They are considered tough crops in terms of growth requirements as they withstand harsh climatic factors such as unpredictable climate and nutrient-depleted soils [4].

Globally, pearl millet is an important grain and is considered the sixth highest producing crop, after maize, wheat, rice, barley, and sorghum [5]. It is also considered one of the crops that can provide good nutrition and income to small-scale farmers [6] and thus, contributes to livelihoods and the availability of food. Despite its value and contribution, pearl millet does not receive the attention it deserves as a crop that has an important

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CHAPTER 2

LITERATURE REVIEW

2.1. THE PROBABLE USE OF MILLETS AS FEED AND THEIR CONTRIBUTION TO FOOD SECURITY

2.1.1. Introduction

Maize, wheat and rice are the most significant human food sources, accounting for 94% of all cereal consumption (FAO, 2012). In South Africa, maize is the most important grain crop produced under diverse environments (du Plessis, 2003). Maize production uses 450 to 600 mm of water per season, mainly acquired from the soil moisture reserves (du Plessis, 2003). Maize is used in different industries to produce different by-products. Starch extracted from maize grain is used in confectioneries; the corn syrup produced from maize contains high fructose and act as sweetener and retains moisture when added to certain foods. Edible oil, margarine and salad dressings are also extracted from maize seeds (du Plessis, 2003). The proteins and hulls are used for livestock feed. The recent high demand for ethanol production has resulted in increased maize prices which led to increase to the size of land used for maize production (Ranum et al., 2014). This is considered an environmental concern which could lead to biofuel carbon debt (Dixon et al., 2010). Figure 2.1.1 shows different uses of corn across different industries.

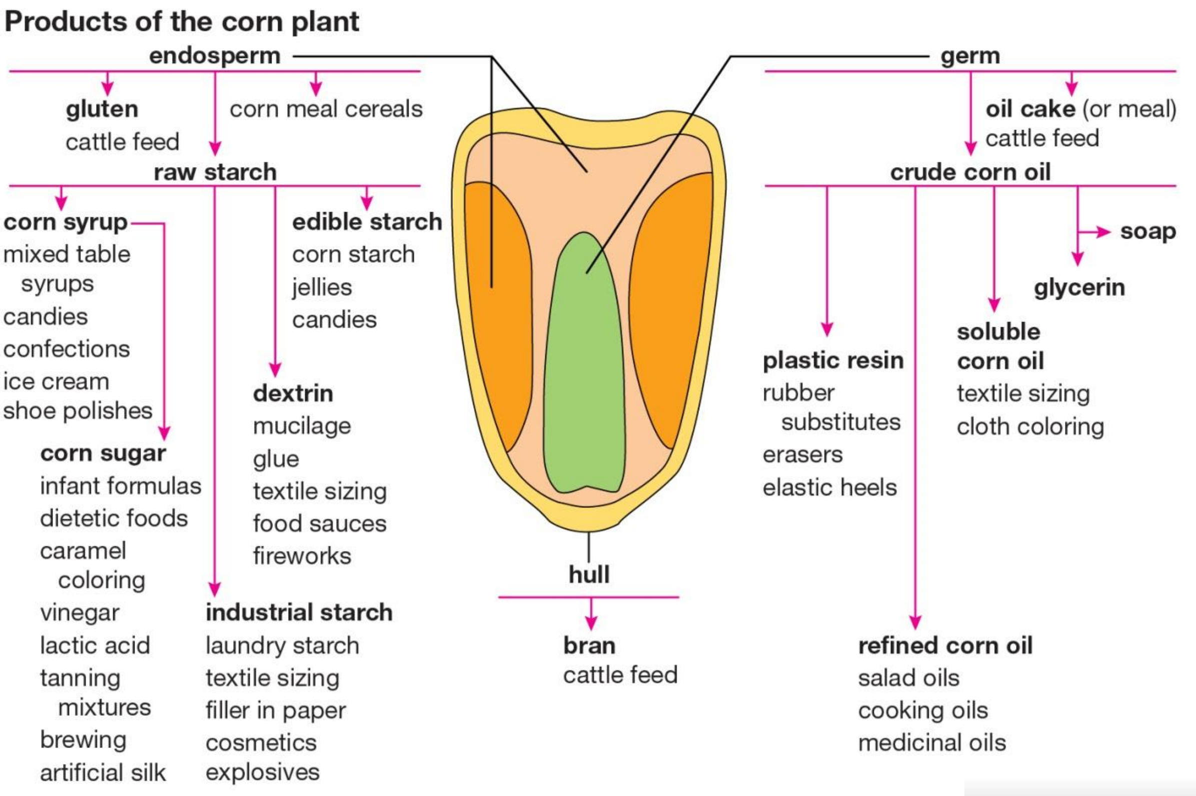

Figure 2.1.1. Products of the corn plant (Source: Encyclopidia Britannica, 2020)

Millets are cereal grains from the *Poaceae* grass family and considered as one of the oldest cultivated crops. Generally, pearl millet (*Pennisetum glaucum*) and finger millet (*Eleusine coracana*) are known as the two major millets used for food and feed. Pearl millet is believed to have originated from Sub-Saharan Africa, and the sub humid uplands of East Africa as the origin of finger millet (Dwivedi et al., 2012). The two account for most of the world's millet production and trade (FAO, 2017). Bulks of recent research and agricultural programme which are routed towards the development of millets have been dedicated to pearl and finger millets.

Pearl millet is one of the important cereal crops globally and is considered the sixth highest production of the cereal crops, following maize, wheat, rice, barley, and sorghum (Kochhar, 2016). It is considered one of the crops which can provide good nutrition and income to small-scale farmers (Patel, 2015), and thus, contributes to livelihood and food security. Despite its value and contribution, pearl millet does not receive the attention it deserves as a crop of such global importance and food security. Perhaps the neglect can largely be owed to it being termed a crop for poor farmers in marginal agricultural areas due to socio-ecological conditions (Gari, 2002). According to several researchers, millets can be an important source of essential nutrients such as amino acids, mineral and trace elements (Anitha et al., 2019; FAO, 2017). Obviously, wide variations should be evident in the nutritional composition of pearl and finger millets (FAO, 2017). Shweta, (2015) reported that pearl millet contains higher energy compared to cereal grains like rice and wheat, and are important source of thiamine, niacin, and riboflavin (Taylor, 2004). Moreover, the content of minerals such as calcium, iron and phosphorus in pearl millet is like those found in other cereals (Adeola & Orban, 1995).

Additionally, finger millet is more adapted to cooler and wetter climates than pearl millet (Tadele, 2016). In many rural communities of East and Central Africa, finger millet is known as an important cereal as it contributes significantly to the nutritional well-being. In spite of its valuable role in the food security and nutrition of many poor farmers in Africa, it is also neglected (Lost crop of Africa, 1996). The protein of finger millet is considered superior, as it contains more lysine, threonine, and valine than other millets (Ravindran, 1991).

Millets grains are also believed to have nutraceutical health benefits, which includes but not limited to, digestive system wellbeing, reduction of cholesterol in the body, prevention against heart diseases, protects from diabetes, lowers the risk of cancer, increases energy levels and improves muscular systems (Manach et al., 2005; Shobana et al., 2009; Amadou et al., 2013; Chandra et al., 2018).

These characteristics ought to put such grains at the right position in terms of alternative crops; however, due to lack of attention millet was termed the lost crop (Lost crop of Africa, 1996). Given the current challenges of sustainable food production, climatic changes, and water scarcity, coupled with overpopulation, interests have been developed towards millet. This has provided an opportunity for farmers, nutritionists, food, and feed manufacturers to engage in research in quest of the nutritional and functional characterization of millet grains. Although reviews in this area have been previously published (Reddy et al., 2006; Shahidi & Chandrasekara, 2012; Cisse et al., 2017; Liang & Liang, 2019; Maidala et al., 2020), this review provides important updates on the utilization of pearl and finger millets in diets for humans and animals. Specifically, this review (1) provides detailed nutritional composition and its benefits to humans and livestock; (2) summarizes the phenolic properties found in pearl and finger millet grains, as well as their contributions to health or as anti-nutritive factors in animal feeding and (3) discusses millets in feed and food applications. Thus, the overview and core objective of this review is to provide insights into the selection of millets for different purpose to maximize their potential as food and feeds.

2.1.2. Physical characteristics of millets and maize

Generally, the kernel structure of different millets is like that of sorghum. It consists of the pericarp, germ and endosperm (Table 2.1.1 and Figure 2.2.2). Just like in sorghum, the kernel of pearl millet is of the caryopsis type, where the pericarp is fused to the endosperm entirely. However, sack like pericarps which are loosely connected to the endosperm at only one point are found in finger millets. These types of kernels in finger millets are known to be utricle whereby their pericarp easily breaks away leaving the testa to protect the endosperm (McDonough et al., 1989).

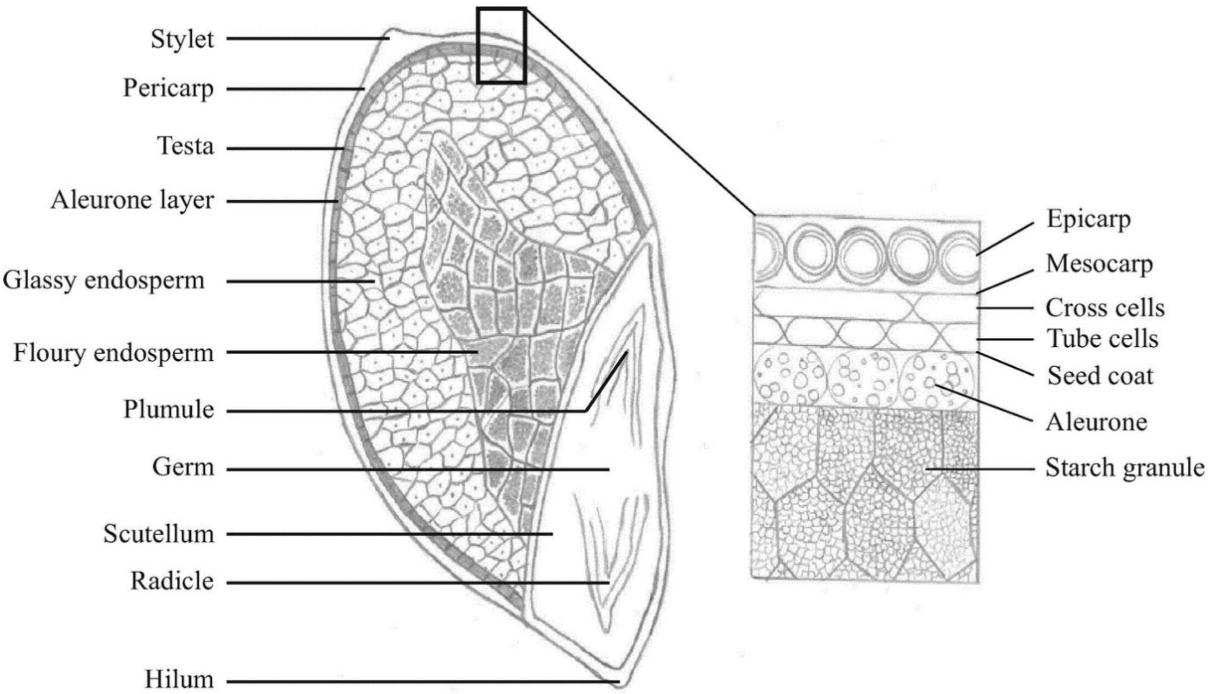

Figure 2.1.2. Grain structure of pearl millet.

As indicated by (Abdelrahman, Hoseney & Varriano-Marston, 1984), the relative distribution of the pearl millet is 8.4 % of the pericarp, 75 % endosperm and 16.5 % of the germ (Abdelrahman et al., 1984). Thus, the ratio of endosperm to germ is 4.5:1 in pearl millet and 8.4:1 in sorghum kernel. The ratio is smaller in finger millet because of its small germ; the endosperm to germ ratio is 11:1 to 12:1, which is much higher than in both sorghum and pearl millet (Abdelrahman et al., 1984). As indicated in Table 2.1.1, variations exist between the visual colour of pearl and finger millets with the 1000 kernel weight being very small for the finger millets (FAO, 2007). An endosperm is regarded the largest part of the cereal grain and it acts as a major storage tissue. It is composed of an aleurone layer and peripheral corneous and floury zones (McDonough & Rooney, 1989). In millets, "the aleurone layer is a single layer of cells which lies just below the testa" (Abdelrahman et al., 1984). The texture of the millet kernel is controlled by the size of floury and corneous endosperm. More floury than corneous endosperm is found in soft-textured kernels however, hard-textured kernels, on the other hand, have more densely packed corneous endosperm. The endosperm of the finger millet is evenly divided between the corneous and floury areas. These types of endosperm are known to have an intermediate texture. In pearl millet the kernel texture differs widely, from all floury, very soft endosperm to all corneous, very hard or vitreous endosperm.

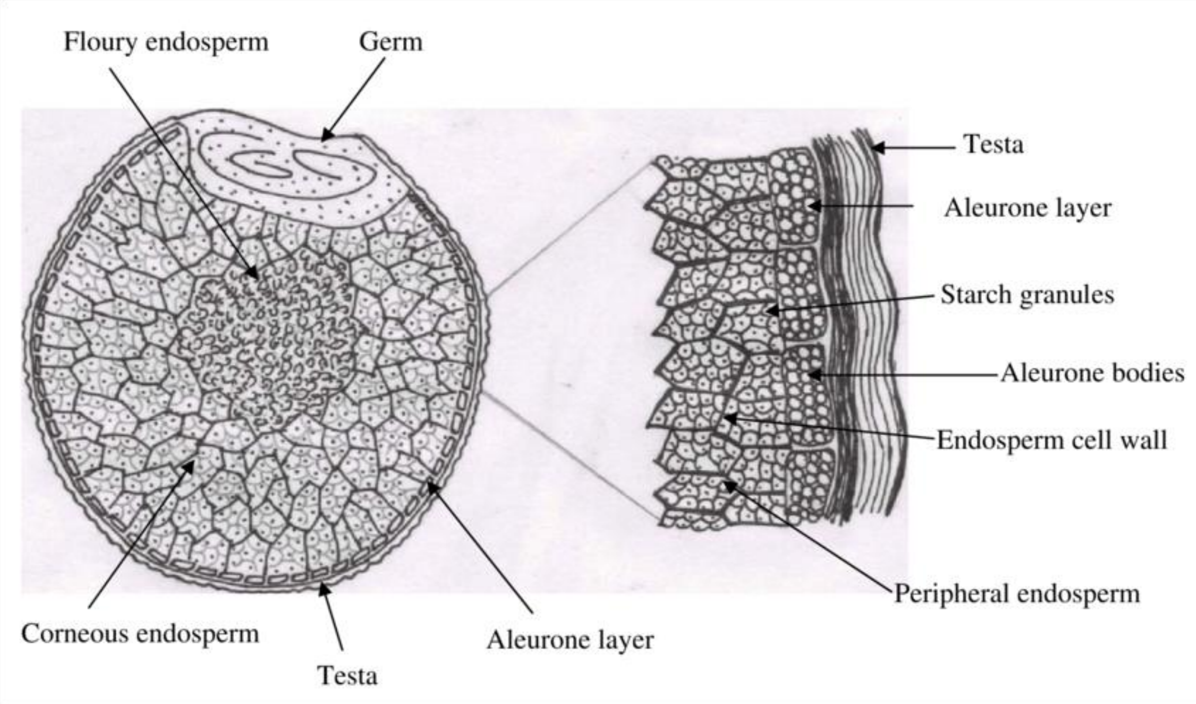

Figure 2.1.2. Schematic diagram of finger millet section.
Source: Shobana, (2009).

The embryo of finger millet is located in a depression surrounded by a characteristic ridge and the hilum is located adjacent to the germ (Shobana, 2009). Whilst the protein bodies can be seen as small spheres below the cell walls (Shobana, 2009) (Figure 2.1.2). When it comes to the processing and food quality of millets, grain texture is one of the most important determinants to consider (Rooney et al., 1986). There are higher flour yields when dry milling corneous than soft floury kernel types. Cultivars with higher amount of corneous endosperm are preferred when making thick porridge. In contrast, the flour of soft endosperm is highly favoured Rooney et al. (1986) when making bread either fermented or unfermented.

Although, in animal feeding intermediate texture is preferred over the corneous and floury texture as it increase the starch digestion.

Table 2.1.1. Anatomic characteristics of millet, maize and sorghum grains

Grain	Type	Shape	Colour	1 000-kenel Weight (g)
Sorghum	Caryopsis	Spherical	White, yellow, red, brown	25-30
Pearl millet	Caryopsis	Ovoid, hexagonal, globose	Grey, white, yellow, brown, purple	2.5-14
Finger millet	Utricle	Globose	Yellow, white, red, brown, violet	2.6
Maize	Caryopsis	Flat, round	Yellow, white, red, brown	25- 38

Source; FAO, (2007); Watson, (2003); Agri-facts, (2018).

Zea mays L is a cheap form of starch and is a major energy source for animal feed (Macrae et al., 1993). The maize kernel has four main parts – the germ, the endosperm, the pericarp and the tip cap (Figure 2.1.4). Maize contains about 72% starch, 10% protein, and 4% fat (Nuss & Tanumihardjo, 2010), it also provides many of the B vitamins and essential minerals along with fiber, but lacks vitamins B12 and C, and is, considered a poor source of calcium, folate, and iron (Ranum et al., 2014).

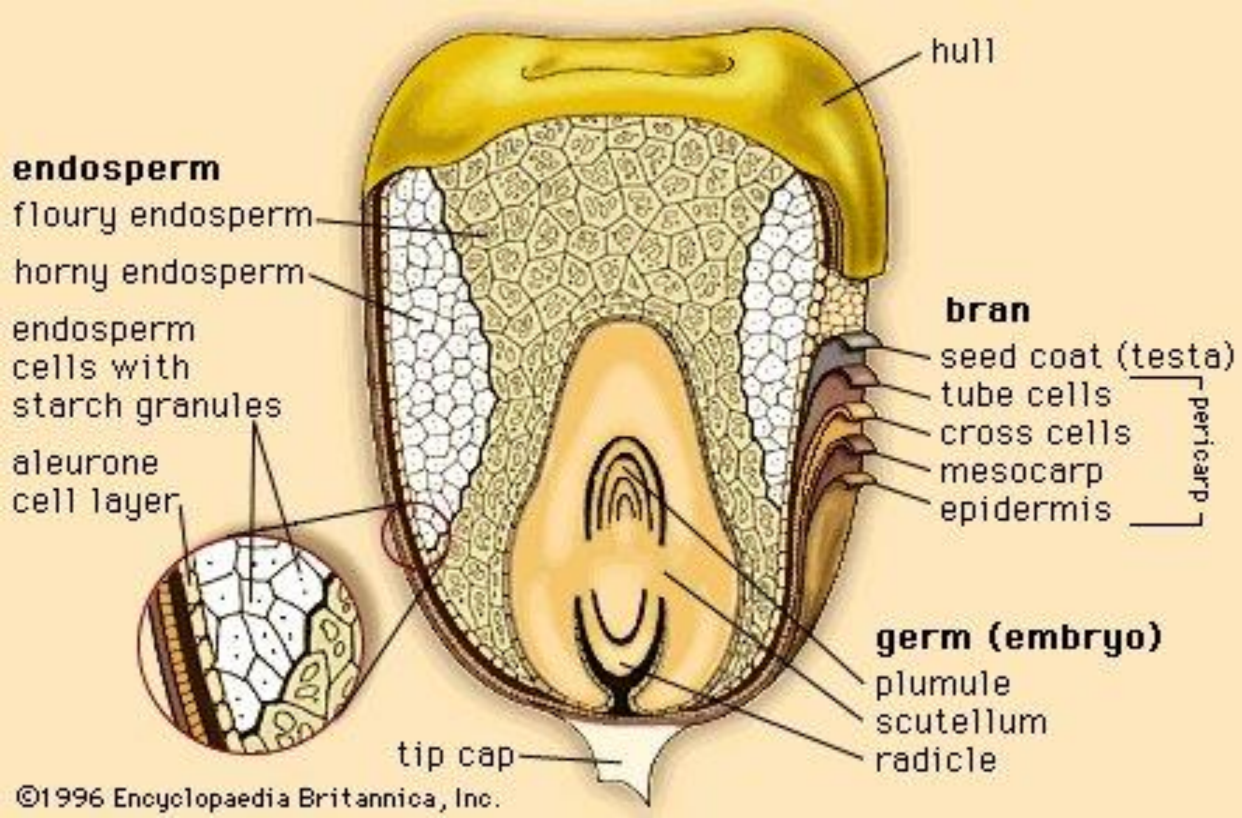

Figure 2.1.4. Structure of maize kenrnel

Source: Encyclopidia Britannica, Inc, (1996)

2.1.3. Nutritional profile of millets

Nutritionally, the macronutrients such as carbohydrates, fats and proteins, contents of millets are comparable and even superior to major cereals. They significantly contribute to human and animal diets due to their high levels of energy, calcium, iron, zinc, lipids and high-quality proteins. In addition, they are also rich source of dietary fibre and micronutrients (Ravindran, 1991; Shobana & Sreerama, 2009).

2.1.3.1 Carbohydrates

The carbohydrate components of pearl millet grains incorporate starch, dietary fibre and soluble sugars. Starch is considered a predominant component of pearl millet endosperm which comprises of glucose in the form of amylase and amylopectin. Various pearl millet grain genotypes vary in starch composition from 62.8 to 70.5% and around 71.82 to 81.02% as reported by Cheik et al. (2006), soluble sugars range from 1.2 to 2.6% and amylose from 21.9 to 28.8%. However, Suma & Urooj, (2015) recorded a low figure of 34.5 g/100 g and 39.4 g/100 g of starch for pearl millet. Starch is the major constituent of pearl millet which yields about 50-60% whole grain and contains about 20% amylose. The starch in pearl millet can be used as thickening, gelling and bulking agents of textural properties of foods (Hadimani, et al., 2001). Finger millet on the other hand has total carbohydrate content in the range of 72 to 79.5% (Bhatt et al., 2003). The detailed profile of the carbohydrates was recorded by Wankhede et al. (1979a) to be 59.5–61.2% starch, 6.2–7.2% pentosans, 1.4–1.8% cellulose, and 0.04–0.6% lignin.

Millet grain is gluten-free and consists of free sugars such as glucose, fructose, sucrose and raffinose ranging from 1.2 to 2.5%. Monosaccharides such as arabinose, xylose, glucose and uronic acids are present in the non-starch polysaccharides fraction of the millets (McDonough et al. 2000; Abdelrahman et al., 1984). The starch granules range from polygonal to round with characteristic dimensions in the range of 10- 16µm. Electron micrographs showed that some of the granules had deep indentations due to pressure exerted by protein bodies (FAO, 2007). Variation in the shape and size of starch of granules have been attributed to premature biosynthesis since the characteristics of any starch depends entirely on the time of harvesting and isolation (FAO, 2007). Based on *in vitro* digestibility observations, starch is classified as rapidly digestible (RDS), slowly digestible (SDS), and resistant starch (RS). Starch fractions such as SDS and/or RS are nutritionally important as they have significant implications for human health, mostly glucose metabolism, diabetes management, colon cancer prevention, mental performance, and satiety. However, there is limited literature on nutritional importance of millets starch fractions.

Table 2.1.2. Proximate analysis of pearl and finger millets (g/100g)

Nutrients	Pearl millet	Finger millet
Moisture	12.4	7.15 - 13.1
Protein	11.6-11.8	7.7
Fat/ lipids	4.8-5.0	1.8
Minerals	2.2-2.3	2.7
Dietary fiber	11.3	15 - 22.0
Neutral detergent fiber	9.0	12.7
Acid detergent fiber	3.3	8.7
Carbohydrates	67-67.5	75.0 - 83.3
Gross Energy (MJ/kg)	17.0	15.8
Minerals (mg/ 100 g)		
Phosphorus	296	130 - 250.0
Potassium	307	430 - 490
Magnesium	137	78 - 201
Calcium	42	398.0
Sodium	10.9	49.0
Zinc	3.1	2.3
Iron	8.0	3.3-14.89
Manganese	1.15	17.61-48.43
Copper	1.06	0.47

Source: Amadou et al. (2013).

2.1.3.2. Proteins

The second major component of millet is protein. Pear millet is believed to contain about 11.6% protein, which is higher than the protein in rice (7.2%) barley (11.5%), maize (11.1%) and that of sorghum which is (10.4%) (Jha et al., 2013). Anitha et al. (2019) recorded protein content of 9.79% in pearl millet. In comparison to maize by weight, pearl millet can be 8 to 60% higher in crude protein, 40% richer in lysine and methionine, and 30% richer in threonine (Burton et al., 1972). Finger millet in contrast, contains about 5–8% of crude protein (Chethan & Malleshi 2007a). Wafula et al. (2018) recorded the highest protein content for finger millet of about 11%. While Anitha et al. (2019) recorded a percentage of 6.32% in finger millet. The quality of protein is mainly a function of its essential amino acids (Tables 2.1.2 and 2.1.3).

McDonough et al. (1984), reported that pearl millet essential amino acids profile had more lysine, threonine, methionine, and cysteine than in sorghum and corn proteins but comparable to wheat, barley, and rice (Abdelrahman et al., 1984). Moreover, the lysine content of protein reported in pearl millet grain ranges from 1.9 to 3.9 g/100g protein. Pearl millet grains resemble maize in its distribution of proteins, particularly true prolamins, which are believed to be soluble in alcohol (Shobana, 2009). Furthermore, Shobana, (2009), reported high level of essential amino acid balance which approves of pearl millet as a nutritious and digestible source of calories and proteins for humans. Also, among the essential amino acids, arginine, threonine, valine, isoleucine, and leucine had higher digestibility in pearl millet than corn. Finger millet is relatively balanced in essential amino acids since it contains more lysine, threonine and valine compared to other millet varieties (Ravindran, 1992).

Table 2.1.3. Amino acid profiles of different millet grains variety (Pearl and Finger millet).

Amino acids (g/100g)	Pearl millet	Finger millet
Essential Amino Acid		
Isoleucine	5.1	4.3
Leucine	14.1	10.8
Lysine	0.5	2.2
Methionine	1.0	2.9
Phenylalanine	7.6	6.0
Threonine	3.3	4.3
Valine	4.2	6.3
Histidine	1.7	2.3
Tryptophan	1.2	NA
Nonessential Amino Acid		
Alanine	8.1	6.1
Arginine	0.9	3.4
Aspartic acid	6.2	5.7
Cystine	0.8	NA
Glutamic Acid	22.8	23.2
Glycine	0.7	3.3
Serine	5.4	5.3
Tyrosine	2.7	3.6
Proline	8.2	9.9

Source: Amadou et al. (2013).

2.1.3.3. Dietary Fibre

Fibre is considered important for gut health as stated by McIntosh et al. (2003) and moderate intakes of high fibre in foods could result in improvement of gut health. Likewise, fibre is important in the prevention of heart diseases, colon cancer and diabetics (Chinma et al., 2007). The high dietary fibre content in pearl millet which is 8 to 9% (Taylor, 2004), gives it the ability to improve bowl movement. In addition, because of its low digestion, it increases the transit time which reduces the rate of glucose in the blood which in turn, helps the non-insulin dependent diabetes patients. Shobana et al. (2009) reported lesser incidence of diabetes in people who eat millet. Moreover, fibre in millet may help reduce harmful cholesterol while boosting the valuable cholesterol. It also prevents the secretion of bile acids which causes gallstone in the body (Shweta, 2015). Furthermore, pearl millet with its high fibre content helps in slow movement of food from stomach to the intestine. Thus, helps in longer duration of food intervals which in turn prevents obesity. Neutral and acid detergent fibers (NDF and ADF) in pearl millets is reported by Mustafa et al. (2008) to be about 140g/kg and 62g/kg, respectively. The levels of ADF and NDF are critical because they impact animal productivity and digestion in livestock production. Thus, the NDF and ADF is higher in finger than pearl millets, validating that pearl millets can be used in poultry feeds since chickens are unable to digest fibrous feedstuffs. Moreover, higher ADF and NDF contents in the feed ingredient are simply an indication of low energy, which maybe the case with finger millets.

2.1.3.4. Lipids

The content of fat in pearl millet is up to 8% fat, which is considered more than that in wheat, rice, barley, sorghum, and maize, making it has high energy density (Shweta, 2015). The composition of fatty acid of pearl millet is high in palmitic, stearic, and linoleic acids and lower in oleic and linoleic acid in comparison to corn (McDonough et al., 1984). Pearl millet comprises of high polyunsaturated fats with Linoleic acid comprising approximately 4% of fatty acid composition. The overall lipid content in pearl millet grain ranges from 1.5 to 6.8%, which is higher than other millet varieties (Malik et al., 2002). The free and bound lipid contents of pearl millet range from 5.6 to 6.1% and 0.6 to 0.9%, respectively. With triglycerides, diglycerides and monoglycerides identified as free and bound non-polar lipid components in pearl millet (Abdelrahman et al., 1984). Sridhar & Lakshminarayana (1994) observed total lipid content in finger millet to be 5.2% (2.2% free lipids, 2.4% bound lipids, 0.6% structural lipids). The major fatty acid in finger millet was observed to be oleic acid, followed by palmitic and linoleic acids, with diminutive amount of linolenic acid. Saturated fatty acid accounts for 25.6% and unsaturated fatty (74.4%) of finger millet total fatty acids profile (Sridhar & Lakshminarayana, 1994). Total lipids are found to be at the range of 1.5-2.10% (Mahadevappa et al., 1978).

2.1.3.5. Minreals and Vitamins

Millet grain types vary in mineral composition as summarized in (Table 2.1.2). Martínez-Ballesta et al. (2010) states that environmental stresses such as high salt levels, low water accessibility and excessive temperatures are found to affect the mineral content of food. Pearl millet comprises of total mineral and trace elements, which are determined by the nature of soil. The ash content of both pearl millet and corn ranges from 1.6 to 3.6% and 0.86 to 1.35%, respectively. With high concentrations of minerals such as calcium, phosphorus, magnesium, manganese, zinc, iron, and copper in pearl millet than in corn (McDonough et al., 1984). Pearl millet is also considered a decent resource for fat- soluble vitamin E (2mg/100g) due to its high oil content. The grain is also considered a good source of the A vitamin (Malik et al., 2002). Florence et al. (2014), puts the calcium content of pearl millet at 45.6 and 48.6 mg/100g. It also has a large amount of phosphorus as well, an important mineral in the mineral matrix of bone, adenosine triphosphate or ATP, which is the energy booster in the body. It also helps in bone growth development and repair. However, some studies suggest that pearl millet has high amounts of iron along with several other factors such as phytates, oxalates and polyphones, which may decrease the bioavailability of iron (Nambiar et al., 2011).

Finger millet is rich in calcium ranging from 162 to 487 mg/100g, depending on the genotypes (Vadivoo et al., 1998). In addition, Bachar et al. (2013) reported the calcium content of finger millet to be between 189.93 to 1272.36 mg/100g. Further to this, Adéoti et al. (2017) reported that the content of calcium in pearl millet to be at the range of 31.77 mg/kg and 728.71 mg/kg. Furthermore, report by Singh & Raghuvanshi, (2012) reported the calcium content in finger millet to be 344 mg/100g. In addition, millets contain high amount of magnesium which is believed to have the ability to aid the body fight diseases such as cancer. Bachar et al. (2013) reported a magnesium content of 84.71 to 567.45 mg/100g in finger millet. Finger millet is considered a good source of natural calcium which helps in bone strengthening and reducing the risk of bone fractures. The potassium and magnesium content in millet grains is believed to lower blood pressure and reduces the risk of strokes and heart attacks.

2.1.4. Health benefits of finger and pearl millets

Millet is consumed raw with multiple health benefits; it can also be transformed into fermented spinoffs which are believed to add more benefits to the human health. Research shows that diets rich in plant food can protect against different kinds of diseases (Chandrasekara & Shahidi, 2012). It is suggested by different authors that the presence of certain nutrients in millets make them have a double benefit of nourishment and cure.

2.1.5. Polyphenols

The main polyphenols such as phenolic acids and tannins are found in abundance in millet, they are believed to act as antioxidants and plays vital role in boosting the body immune system (Chandrasekara & Shahidi, 2010). In addition, the coat of finger millet seed which contains phenols has an antibacterial effect on Bacillus cereus (Viswanath et al., 2009). Moreover, Shobana et al. (2009), indicate that millet phenolics can partially inhibit the enzymatic hydrolysis of complex carbohydrates and consequently inhibiting malt amylase, α-glucosidase, pancreatic amylase which reduces postprandial hyperglycaemia. In similar way, it is believed that ferulic and *p*-coumaric acids found in whole pearl millet have the capacity to reduce HT29 tumor cells (Chandrasekara & Shahidi, 2011a). Devi et al. (2014) reported that the phenols available in millets have antioxidant, anti-mutagenic, anti-oestrogenic, anti-inflammatory, antiviral effects, and platelet aggregation inhibitory activity. On the other hand, celiac disease is a genetically susceptible problem triggered by the consumption of gluten. As the millets are gluten free, they help in reducing the celiac disease by reducing the irritation caused by the common cereal grains which contain gluten. (Saleh et al., 2013*)*.

Millets contain phenolic acids that occur in a bound form (60%) as free molecules. The most common phenolics in millets are hydroxycinnamic acids and are present in the insoluble-bound fractions of phenolic acids (Ullah et al., 2010). Most common type of hydroxycinnamic acid is ferulic acid known as antioxidant. Antioxidants are known nutrients that help minimise free radicals damage to the body and also have anti-inflammatory activity (Sridhar et al., 1994). Also, ferulate dimers have been found in millet grains and displayed high antioxidants activity (Bhatt et al., 2003). Cereal ferulic acids displayed strong antioxidants activity when occurring in bound form thus does not require assistance of microbial activity during digestion to facilitate their release within the colon (Martínez-Ballesta et al., 2010). Millet grains contain numerous flavonoids, comprised of anthocynidins, chalcones, aminophenolics, flavanols, flavones, and flavanones (Bhatt et al., 2003). Flavonoids occurred in junction with sugars, known as glycosides of the O- or C forms, although they might be distributed as free aglycones (Vadivoo et al., 1998). Millets varieties are also reported to contain proanthocyanidis, also known as condensed tannins (Dykes & Rooney, 2006). Significant levels of tannin are mostly observed in colored millet varieties (McDonough et al., 1984). This finding was attributed to availability of condensed tannins since they contribute substantially to the grains color. However, when condensed tannins are present in sufficient quantities, may lower the nutritional value and biological availability of proteins and minerals (Chevan et al., 2001).

Table 2.1.4. Phenolic compounds present in finger millet and their functions.

Health compounds	Functions	References
Ferulic acid	Prevents tissue damage and stimulates wound healing process.	Sarita & Singh (2016).
Phytic acid	Plays important role in lowering body cholesterol.	Amadou et al. (2013), Sarita & Singh (2016), Chandra et al. (2018).
Phenols, phytates, and tannins	Critical in curing, aging and metabolic disorder. Inhibits worsening of human wellbeing, cancer, and cardiovascular illnesses. Lowering of blood pressure and diabetes. Reduces tumor.	Siwela et al. (2007), Thilagavathi et al. (2015).
Dietary fiber	Vital for hypoglycemic and hypolipidemic effect as well as cutting of serum cholesterol. Inhibits atherosclerosis, antitoxic effect and anti-cancerous effect. Energy diluents to formulate low calorie diets	Thilagavathi et al. (2015), Udeh et al. (2017). Rao et al. (2017)
Nutraceutical foods	Promotes better health by reducing the risk of chronic disease such as obesity. Lowers blood pressure, cancer, and diabetes.	Sarita & Singh (2016).
Magnesium	Reduces the risk of heart attack.	Chandra et al. (2018).
Phosphorus	Vital for the growth of body tissue and energy metabolism.	Chandra et al. (2018).

Source: Ramashia et al. (2019).

2.1.6. Antioxidants properties of millet

The generous content of phenolic compounds in millet has made it a potent source of antioxidants (Dykes & Rooney, 2006; Shahidi & Chandrasekara, 2013). Millet grains contained several natural occurring phenolic compounds which include phenolic acids, flavonoids, and tannins, in addition to xylo-oligosaccharides, insoluble fibers and peptides (Liang & Liang, 2019). Table 2.1.5 shows compounds and antioxidant properties of pearl and finger millets grains.

Table 2.1.5. Compounds and antioxidant properties of pearl and finger millets.

Millet type	Active compounds	Antioxidant property	References
Finger millet	Phenolic acids	Free radical scavenging, anti-inflammatory **activity**	Liang & Liang, (2019)
Finger millet	Phenolic compounds	High reducing power (reduction of the ferricyanide to ferrocyanide)	Kumari et al. (2017)
Pearl millet			
Finger millet	Flavonoids	inhibition of α-glucosidase and α-amylase activities Reduction of postprandial hyperglycemia	Ofosu et al. (2020)
Finger millet	Carotenoid	Quenching of single oxygen and free radicals	Viswanath et al. (2009)
Pearl millet	Phenolic acids	Metal chelating activity	Jayalaxmi et al. (2018)

2.1.7. Anti-nutritional factors present in finger and pearl millets.

Anti-nutritional factors are substances that reduce the availability of nutrients, when present in animal feed (Yacout, 2016). Their presence in pearl and finger millets is believed to limit protein and starch digestibility, hamper mineral bioavailability, and hinder proteolytic and amylolytic enzymes.

Pearl millet is gluten free and has a low glycemic index, but despite all the positive characteristics, the presence of anti-nutrients such as phytic acid, polyphenols, and tannins can limit its functions as food or feed. These factors affect the nutritional value of the grain by inhibiting protein and starch digestibility and mineral bioavailability. Studies by Anu & Kwatra, (2006) showed that pearl millet contains 354–796 mg/g^{-1} phytic acid. Phosphorus in this form is not bioavailable to non-ruminants because of lack of the digestive enzyme phytase, required to separate phosphorus from the phytate molecule. Different cultivars of pearl millet contain anti-nutritional factors as phytates (Ouattara-Cheik, et al. 2006). In addition, the presence of some goitrogenic polyphenols and C-glycosylflavones (C-GFs), such as glucosyl vitexin, glucosyl orientin and vitexin might be responsible for health problems (Suma and Urooj, 2015). Epidemiologic evidence indicated that a diet based on millet as staple food, such as in rural villages in Africa and Asia, plays a role in the genesis of endemic goiter in these areas.

Antinutritional factors present in finger millet include tannins, non-starch polysaccharides-glucans, protease inhibitors, oxalates and phytates which might inversely affect the digestibility of nutrients (Kumar et al., 2016). Tannins have been reported to be responsible for decreases in feed intake, growth rate, feed efficiency, net metabolizable energy, and protein digestibility in experimental animals (Kumar et al., 2016). Among millets, finger millet is reported to contain high amounts of tannins ranging from 0.04% to 3.74% of catechin equivalents (Ramachandra et al., 1997).

Thankfully, the proportion of these anti-nutritional factors can be reduced by applying different processing methods. Numerous processing techniques such as dehulling, milling, malting, blanching, parboiling, and acid and heat treatments, and fermentation of some forms of pearl millet seem to reduce the anti-nutrient. Sharma & Kapoor, (1996), found that germination and autoclaving, and debranning and autoclaving are effective processing treatments in reduction of phytic acid, amylase inhibitors and polyphenols. Similarly, Rathore et al. (2019) found that anti-nutritional factors can be reduced to a limited amount through the application of various processing techniques such roasting, soaking, boiling, parboiling, fermentation, milling, germination, decortications, and extrusion.

2.1.8. Millets as food and feed

Millets grains are considered unique crops because they are rich in valuable nutrients such as calcium, dietary fibre, polyphenols, and protein (Devi et al., 2011). It is a staple food to many Asian and African Countries. Most of the millet produce is mainly for human consumption, less percentage is used for livestock, beers, and bird (Obiliana, 2003). Millet is made into thin and thick porridge in some part of Africa, while in some it is made into a product called couscous (Obiliana, 2003). Different research was conducted using the whole grain or crushed and incorporated in chicken feeds. Cisse et al. (2016) confirmed that pearl millet is an effective feed ingredient for poultry production. Pearl millet grain is generally superior to sorghum as human food but at least equals maize in value as a feed grain. Whereas grain is the main purpose of cultivation in Africa and Asia, the forage at harvest is an important secondary product in subsistence agriculture for animal feed, fuel, or construction (Wafula et al., 2018). While season-specific wheat and rice might provide only food security, all-season crop millets provide food, fodder, nutrition, health, livelihood, and ecological securities. Pearl millet grains have a high potential as food for humans because they are gluten-free, higher in dietary fibre content than rice, similar in lipid content to maize and have a higher content of essential amino acids (leucine, isoleucine and lysine) than other traditional cereals such as wheat and rye (Rooney & Miller 1982).

In India where millet is highly used, it can be made to Dosa which is a flat bread made of mixture of millet and other grains, in addition to couscous, cookies, sushi, no yeast pizza and roti (ICRISAT, 2016). Madua which is a popular finger-millet-based beverage in India. Oshikundu Oshikundu is a traditional pearl millet sour–sweet beverage of Namibia, which exits in both alcoholic and non-alcoholic form (Kumar et al., 2018).

2.1.9. The use of millet grains in chicken diets

Inclusion of millet gains in animal feed has gain momentum in recent years. Research has shown that the inclusion of up to 50% whole pearl millet seeds can be used in broiler diets without adversely affecting broiler performance or the quality of feed (Hoseney, 1994). A study by Cisse et al. (2016) indicates that pearl millet varieties have produced comparable results to corn regarding metabolizable energy and digestible amino acid. Similarly, Baurhoo et al. (2011) confirmed that replacing corn with pearl millet in broiler diets resulted in significant improvements in growth and feed efficiency.

Likewise, Issa et al. (2016) found that replacement of corn with sorghum and millet up to 50% of layers diet had similar effect on the egg production rate. Rao et al. (2004), in a study on foxtail millet found that it can fully replace corn in the diets of broilers without affecting the body weight gain. In addition, feeding of pearl millet to laying hens is believed to have additional benefit in that; the eggs contain higher omega-3 fatty acids and lower omega-6 than (Ejeta et al., 1987).

Furthermore, a study by Amadou et al. (2013) concludes that broilers fed diets containing 25 or 50% millet had a final body weight equal to those fed the control diet, which contained maize. The males fed the 25% millet diet had a slightly higher percentage carcass yield than the control-fed males, clearly indicating that millet can be a replacement for maize. Similarly, in a study by McIntosh et al. (2003), it was suggested that pearl millet could replace 25–50% of maize in the broiler ration without affecting the performance of the broilers.

Table 2.1.6. Responses to replacement of maize with different inclusion levels of millet on feed intake, feed conversion ratio, and body weight of chickens.

Study/Millet	Millet inclusion level	Duration (days)	Feed intake (g/bird)	Body weight (g/bird)	Feed conversion ratio
Medugu et al. (2010)	100%	42	3949.26	2167.7	2.24
Tadele et al. (2018)	25%	90	8216.0	1177.0	6.70
	50%		8461.0	1178.0	7.22
	75%		8547.0	1167.0	5.15
	100%		7864.0	1178.0	5.82
Bulus et al. (2014)	100%	56	1177.40	2451.0	2.41
AL-Shwilly et al. (2019)	100%	32	3030.0	1660.0	2.08
Cisse et al. (2016)	25%	42	4368.0	3150.0	1.79
	50%		4452.0	3038.0	1.86
	75%		4494.0	2992.0	1.93
Issa et al. (2016)	50%	---	9540.0	1061.0	Ns
	100%		9450.0	1062.0	Ns
Bala et al. (2017)	25%	42	3634.60	2012.50	1.82
	50%		3628.30	2049.10	1.78
	75%		3609.30	2009.4 0	1.80

2.1.10. The use of millet grains in the ruminants' diets

Millet grain and in this case, the finger and pearl millets were evidently used to replace conventional grains, in the feed of small ruminants. An early study conducted by Haggblade & Holzapfel (2004) on lactating and growing goats, found that the feed intake and milk production, were not affected upon the replacement of corn with pearl millet. However, they have recorded a depression on the daily growth rate and feed – to - gain ratio when corn was completely replaced with the pearl millet. They concluded that although pearl millet could be a useful source of alternative energy feed for mature goats, it might not be useful for the growing goats. It is worth noting that the majority of the studies on millet as a replacement for maize were conducted on poultry in comparison to the ruminants. Pearl millet was the most used in these studies, probably for the reasons that it is the most popular among the millets and also its superior nutritional values. As stated by Davis et al. (2003), pearl millet has proven itself to be equivalent to corn or even superior.

Millet grain is also considered useful in replacement of maize in large ruminants. Study by Mugula et al. (2003), observed that pearl millet grain could fully replace maize in high supplement diets for confined cattle. Millet is found to be beneficial to the animals, fed whole grain or ground. Several research were conducted to establish the most effective way of feeding of millet. A study by Mugula et al. (2003), found that processing of millet grain increases the digestibility of dry matter and dietary nutrients of grazing beef cattle during dry season. Table 2.1.7 summarizes different studies on replacement of maize with millet in ruminant animals.

Table 2.1.7. Pearl and finger millet inclusion and their effect on ruminant's performance.

Millet type	Inclusion level	Effect on performance	References
Pearl millet	25% 50% 75% 100%	No adverse effect on body weight gain and feed efficiency Significant depression on growth and feed efficiency at levels higher than 25% inclusion levels.	Rao et al. (2003)
Pearl millet	30%	No adverse effects on milk yield or milk composition	Mustafa, (2009)
Pearl millet	20%	No negative effects on performance, carcass yield, or organ weight	Torres et al. (2013)
Pearl millet	10% 20% 100%	Similar body weights, overall weight gain, carcass weight, and digestibility	Safalaoh & Kavala (2020)
Pearl millet	50% 100%	Increased digestibility of starch and Ether Extracts Reduced ruminal ammonia concentration	Goncalve et al. (2010)
Pearl millet	79%	Similar performance indicators to those obtained with corn and sorghum	Hill et al. (1996)
Pearl millet	25% 50% 75% 100%	No effect on dry matter intake, milk yield and milk fat percentage	Ribeiro et al. (2004)
Pearl millet	33% 665 100%	Performances were not negatively affected by the substitution of maize with pearl millet	Alonso et al. (2017)
Finger millet	16.0% 32.5%	Reduced digestibility of the dry matter No effect on nutrients intake	Dos Santos et al. (2008)

	48.0% 67.0%		
Pearl millet	40%	Digestion coefficients for DM, GE, CP, and NDF were reduced by over 10 percentage units with partial or complete replacement of corn by pearl millet.	Haggblade & Holzapfel (2004)

2.1.11. Food applications

As the continent of origin of millets, African ancestors have different by products produced out of millets and passed down to the consecutive generations. Many beverages produced of millet, alcoholic and non-alcoholic do exist, unfortunately their composition and the potential benefits to the human body is still to be explored. Traditional African beverages making involves several processes, like soaking, drying, and fermenting. Haggblade et al. (1993) clarify that the African beer differs from the Western beer in that, it is sour, less carbonated, and often unrefined. Many beverages which include but not limited to; *Togwa*, which is a lactic acid fermented beverage (Mugula et al., 2003). This beverage is mostly found in Tanzania. *Bushera*, which is a lactic acid fermented beverage, is found in Uganda, it can be consumed by both adults and children when it is fresh. *Masvusvu* is a sweet beverage made traditionally from malted finger millet, mainly in Zimbabwe; the second product is *Mangisi* which is a sweet-sour product which results from natural fermentation of sieved *masvusvu* (Zvauya, 1997). *Marrisa* is a local Sudanese alcoholic drink which is made from sorghum or millet using the fermentation method (Dirar, 2006). *Cipumu* is produced from finger millet in Tanzania, it plays an important role as a ritual and as a source of for the locals.

In Tanzania finger millet is utilized as an ingredient of four kinds of foods (ugali, uji, togwa and pombe) in some parts of Tanzania, finger millet is considered the main ingredient in an alcoholic beverage called pombe. Three kinds of pombe is produced using finger millets, namely kimpumu, komoni and kiambule (Kubo, 2016).

This is recognized by the villagers as a desirable feature of kimpumu, attributed to finger millet. Inkomoni and kiambule production, germinated finger millet is selectively used as an amy-lase source, instead of germinated maize. To justify the selective use of germinated finger millet, the villagers mentioned that it brings a strong taste (makali)to pombe. In this context, a strong taste might mean a high content of ethanol (Kubo, 2016).

In Nigeria, pearl millet is used to make a fried cake called *masa*. The flour is used to make *tuwo* which is a thick binding paste (Izge & Song, 2013). The green fodder is usually fed to the animals.

In Zimbabwe, Phiri et al. (2019) noted that there is a unwillingness from customers when it comes to the use of millet as a basic food. They attributed this to colour, taste and flavour of the millet, in addition to general practices and lifestyles of some families, which led to the farmers not keen to produce more of the millet products and the crop is mainly produced and used for preparing traditional beer brands like Chibuku (Phiri et al., 2019).

2.1.12. Challenges of food security in the developing countries

Food security is defined as a situation whereby, people have physical and economic access to safe and nutritious food to meet their dietary requirements (World Food Summit, 1996). Food security challenges are often narrowed to supply of agricultural produce such as livestock, as pointed out by Hatab et al. (2019). However, the challenges are believed to be more complex than just increasing the supplies. Many factors such as urbanization and accessibility are some of the constrains described by (Hatab et al. 2019). Furthermore, institutional failures as well structure and processes which that are governing economies and societies are also listed as some of the factors causing food insecurity (Pangaribowo et al., 2013). In a study by Abegaz, (2017), on food security status in Ethiopia, the first rain shock which is a product of the climate change is considered one of the main impactors of food security. Fraval et al. (2020) noted that the casual intervention to prevent food insecurity is not necessarily straightforward; therefore proxies of interventions and greater understanding of different proposed pathways are important in successful interventions. These complex hindrances require multisectoral approaches and planning to resolve them. According to Pangaribowo et al. (2013), the ideal way to deal with the issues of food and nutrition security is to couple the indicators of food insecurity together with available socioeconomic and environmental indicators of a particular entity. Regardless of the challenges of food insecurity in the developing countries, the contribution of underutilized and locally produced grains such as millet cannot be overlooked. The conventional grain crops are not sufficient to overcome some of these challenges (Muthamilarasan & Prasad, 2020). Tying in situations such as the current unprecedented COVID-19 pandemic, development of minor grains such as millet has the ability to elevate poverty among poor population (Muthamilarasan & Prasad, 2020). From the climatic change context, millets are seen as the crops with the potential to survive harsh conditions and contribute to the stability of food security. Padulosi et al. (2009), reported that minor millets such as finger, kodo, foxtail, little, proso and barnyard, have the ability to grow successfully in diverse soils, varying rainfall regimes, diverse photoperiods and in marginal, due to their genetic adaptation. These characteristics qualify the millets to replace commodities like wheat and rice in harsh climatic zones, eventually leading to food security in these areas. However, millet is considered a neglected agro-biodiversity, though it has the potential to contribute to agricultural system and food security among the poor population in Sub- Saharan Africa.

2.1.13. The cost benefit of using millet

Millet is a gluten-free and low-cost cereal with an estimated cost of 40% lower than corn (Gomes et al., 2008). Silva et al. (2014), has put the trade value of pearl millet to be less than or equal to 77.78% of the cost of the corn grain. The protein content of pearl millet grain is higher than in maize, which may allow formulation of diets without supplementation of protein, consequently reducing the cost of food and feed.

In addition, the cost of producing millet is less than producing other grains such as maize and sorghum. For example, pearl millet water-use is more efficient than sorghum and maize grown in semi-arid regions of Brazil (56 ± 2.8 kg DM/ha/mm (Kilograms of dry matter per hectare per millimetre) water for the Brazilian pearl millet cultivars v. 45 ± 1.9 kg DM/ha/mm water for sorghum; Silva et al. (2014); and 21 ± 2.4 kg DM/ha/mm water for the Brazilian maize cultivars (Dos Santos et al. 2010). In a study by Gomes et al. (2008), the total replacement of maize with pearl millet, was found to be the most economical in the diet of feedlot cattle. The items which influenced the financial indicator were reported to be the price of lean and fat cattle, initial weight, final weight, and cost of concentrate, cost of roughage, consumption of concentrate and consumption of roughage (Silva et al., 2020). It is also logical to assume that positioning of millet as competitive grain to maize, will tilt the weight of the supply, which will consequently relieve the pressure on maize consumption, resulting in price reduction. In another study by Rama Rao et al. (2002), reported that the cost of feed required to produce one kg of live weight gain in maize fed group of chickens was higher than in pearl millet, finger millet and sorghum fed groups. Medugu et al. (2010) confirmed that it is more economical and cost effective to produce broiler chicken, as the cost per kg feed and cost of feed per unit weight gain are lowest in millet grains feed. Wilson et al. (2007) estimated that total net profit from the use of pearl millet as the sole feedstock was $25,175,000 per year compared to $23,758,000 for maize feedstocks, about $1.4 million advantage.

2.1.14. Conclusions

It has been demonstrated from the studied literature that pearl and finger millets have the potential to be used as an alternative source of energy in poultry diets. It has competitive nutrients equal to, or in some instances more than, conventional cereals such as maize, wheat and rice. In addition, the presence of nutraceuticals in the millets gives them extra importance in terms of health benefits, especially for humans. The inclusion of up to 100% of millets can be added to broiler diets without having negative effects on the performance of chickens. Inclusion of millet in ruminants' animals' diets also had noticeable improvements on the performance parameters. This inclusion could eventually reduce the cost of feed for livestock production and consequently reduce the cost of livestock products for people who rely on it as source of protein. Because the grain is gluten free, it is considered one of the most suitable grains for the people with celiac diseases. Further to this, millet grains contain antinutrients that can have an adverse impact on nutrient bioavailability. However, different processing methods have been proven to reduce the adverse effects of the antinutrients. Further study is necessary to establish the optimum inclusion level of millets in animal diets. In addition to that, creation of awareness to stress on the importance of these millets for human health is highly encouraged.

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Review

The Effects of Tannins in Monogastric Animals with Special Reference to Alternative Feed Ingredients

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Abstract: Over recent years, the monogastric animal industry has witnessed an increase in feed prices due to several factors, and this trend is likely to continue. The hike in feed prices is mostly due to extreme competition over commonly used conventional ingredients. For this trend to be subdued, alternative ingredients of both plant and animal origin need to be sourced. These types of ingredients are investigated with the aim of substituting all or some of the conventional compounds. However, alternative ingredients often have a double-edged sword effect, in that they can supply animals with the necessary nutrients although they contain antinutritional factors such as tannins. Tannins are complex secondary metabolites commonly present in the plant kingdom, known to bind with protein and make it unavailable; however, recently they have been proven to have the potential to replace conventional ingredients, in addition to their health benefits, particularly the control of zoonotic pathogens such as Salmonella. Thus, the purpose of this review is to (1) classify the types of tannins present in alternative feed ingredients, and (2) outline the effects and benefits of tannins in monogastric animals. Several processing methods have been reported to reduce tannins in diets for monogastric animals; furthermore, these need to be cost-effective. It can thus be concluded that the level of inclusion of tannins in diets will depend on the type of ingredient and the animal species.

Keywords: antinutrients; feedstuffs; plant extracts; monogastric animals' nutrition; tannins; health benefits

1. Introduction

Monogastric animal production, in particular the poultry production sector, is growing continuously, driven mostly by the demand for meat and eggs. However, this rapidly growing industry and the increasing demand for poultry feeds have led to a considerable increase in feedstuff prices. The gap between demand and supply of balanced feed is expected to increase, and consequently increase the cost of production. On the other hand, the conventional feed ingredients such as maize, wheat and rice can no longer meet the poultry industry's demand for feed. In addition, in-feed antibiotics have been used over a period of time as growth promoters, which positively aids in feed conversion rates and consequently reduces the cost. However, it was discovered recently that the inclusion of the antibiotics could leave residue in the meat and consequently cause resistance to some bacteria in humans [1]. These multifaceted challenges compelled the concerned researchers

2.2. THE EFFECTS OF TANNINS IN MONOGASTRIC ANIMALS WITH SPECIAL REFERENCE TO ALTERNATIVE FEED INGREDIENTS

2.2.1. Introduction

The monogastric animal production sector in particular the poultry production sector is growing continuously, driven mostly by the demand for meat and eggs. However, this rapidly growing industry and the increasing demand for poultry feeds have led to a considerable increase in feedstuff prices. The gap between demand and supply of balanced feed is expected to increase, and consequently increase the cost of production. On the other hand, the conventional feed ingredients such as maize, wheat and rice can no longer meet the poultry industry's demand for feed. In addition, in-feed antibiotics have been used over a period of time as growth promoters, which positively aids in feed conversion rates and consequently reduce the cost. However, it was discovered recently that the inclusion of the antibiotics could leave residue in the meat and consequently cause resistance to some bacteria in humans (Redondo et al., 2014). These multifaceted challenges compelled the concerned researchers to look for alternative ingredients which can fill the gap. Tannins are considered valid alternatives to the conventional feed ingredients and as an anti-pathogen, which can be used as an alternative ingredient.

The mechanisms with which tannins promote growth in the monogastric animals are not as clear as in ruminants (Huang et al., 2017). The popular suggestion is that inclusion of tannins in low concentrations, lead to increase in feed intake and consequently the performance of monogastric animals (Huang et al., 2017). There is also a suggestion that the improvement in performance comes as a result of creation of balance between the negative effects of tannins on feed palatability and nutrient digestion and the positive effects on promoting the health status of the intestinal ecology (Huang et al., 2017). A study by Wang et al. (2008), found that the condensed tannins available in the extract of grape seed reduces the faecal shedding of *E. Tenella*, and an increased growth performance of broiler chickens infected with *E. Tenella*.

To render tannins available to the monogastric animals, different processing methods to reduce the anti-nutrients are recommended. For example, the reduction of the tannin component of sorghum has improved its nutritional quality to become the closest alternative feed ingredient to maize in poultry diets (Maunder, 2002). Lately, different processing methods were introduced to reduce the tannin content in feed ingredients. The main methods used are cooking, dehulling, autoclaving, toasting, soaking, using wood ash, adding tallow, and using tannin-binding agents and enzymes. Hence, the aims of this review are 1) to elaborate on the use of tannins as alternative ingredient in monogastric animals' feed; 2) to identify different structures and types of tannins; and 3) to identify successful processing methods to reduce the harmful effects of tannins.

2.2.2. Methodology

This review was conducted according to the reporting items for systematic reviews and meta-analyses (PRISMA) statement guidelines (Moher et al., 2009). A comprehensive search was conducted to identify eligible studies. Databases, namely Web of Science, Science Direct, Google Scholar, PubMed and Wiley Online Database were searched to obtain all relevant studies that were published before September of 2020. The search strategy used involved a combination of the keywords "tannins", "alternative ingredients", "monogastric animals", "health benefits", "condensed tannins", "hydrolysable tannins", medicinal uses of tannins", "antinutrients in tannins", "antibiotic resistance" and "tannin processing methods". Furthermore, the researchers narrowed their search to time scale 1977–2020 to include old and new studies to draw a comparison between the uses of tannins in monogastric animals with the current use. The search was not restricted by language, date, or study type. Total of

315 records were screened after removal of duplicates. Later, 218 records were excluded because they were irrelevant. Total of 97 records were initially used to prepare the review.

In the second stage, extra records were searched to include ‘’antibiotic resistant strains’’ to add to the knowledge regarding the antibiotic resistant’s and the health benefits of tannin. The overall records used to prepare this review were 122 records.

2.2.3. Structural properties of tannins

The physical and chemical properties of tannins differ according to the plant species (Mangan, 1988). Tannins are classified into two main parts– the hydrolysable tannins (HTs) and condensed tannins (CTs), also known as proanthocyanidins) (Haslam, 1979; Hagerman & Butler, 1991). Hydrolysable tannins, as the name indicates, can be hydrolyzed by acids or enzymes. Their structure is characterized by a polyol core (Barbehenn & Constable, 2011). On the other hand, the condensed tannins are non-hydrolysable oligomeric and polymeric proanthocyanidins (Würdig et al., 1989). Condensed tannins are where the coupling of the single units is by positioning of *C*-4 of the first unit with *C*-8 or *C*-6 of the second unit (Porter, 1989). The two most common condensed tannins are the procyanidins and the prodelphinidins (Barbehenn & Constable, 2011). There are three types of hydrolysable tannins, which include: gallotannins, ellagitannins, and complex tannins and condensed tannins, called procyanidins (Khanbabaee & Van Ree, 2001), (Figure 2.2.1). Gallic acid is mainly found in rhubarb and clove, while ellagic acid is found in eucalyptus leaves, myrobalans and pomegranate bark. To explain further, figure 2 shows the chemical structure of common molecules found in tannins (Corral et al., 2020).

Further to this, recent research showed that tannins are produced inside an organelle named tannosome, which is believed to arise in cell plastids occurring in the green parts of plants that contain chlorophyll pigments. After creation, the tannosome is encapsulated in a membrane, and later transported to a plant vacuole for safe storage (Brillouet et al., 2013). According to (Barbehenn & Constable, 2011), the structures of the condensed tannins from different species can be differentiated based on the proportion of trihydroxylated subunits, ratio of cis:trans monomers, and the degree of polymerization. Figure 2.2.1 shows classification of tannins into different cla

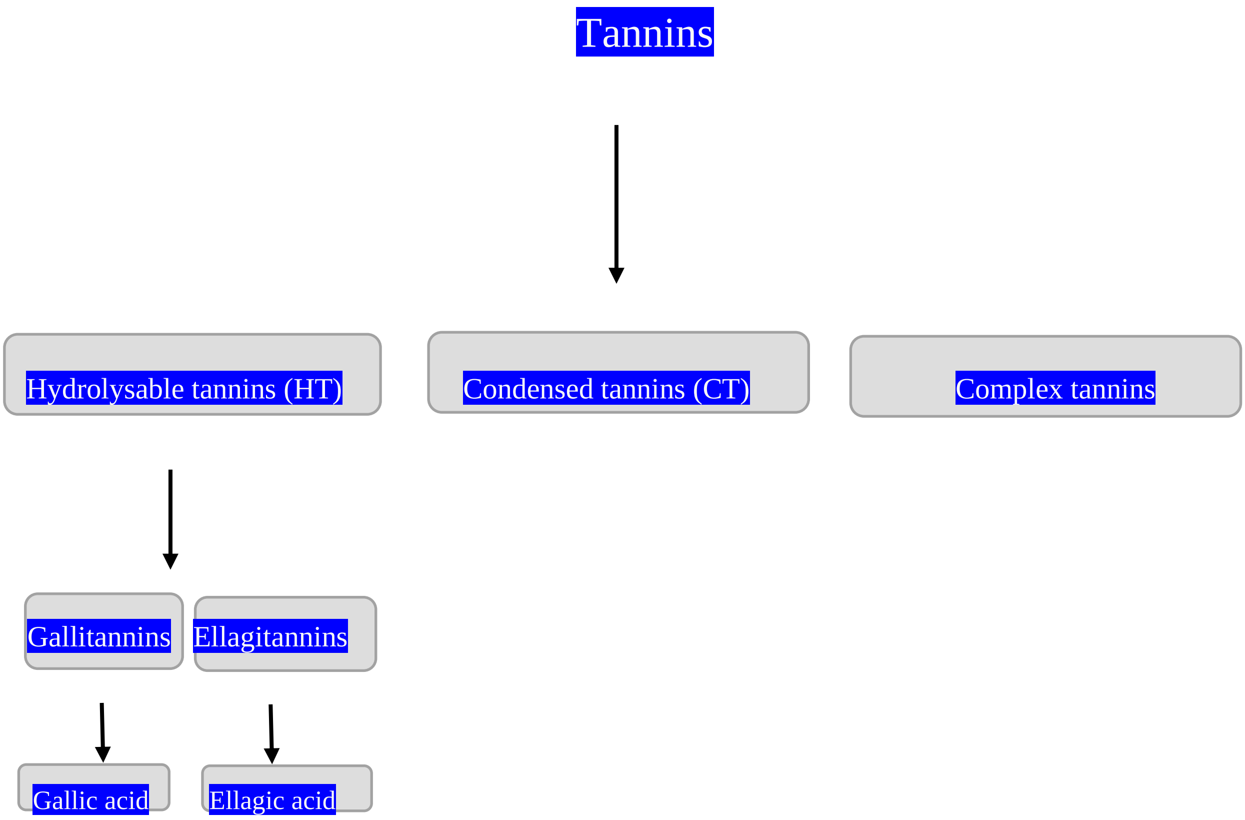

Figure 2.2.1. Classification of Tannins. Sources: Huang et al. (2017); Czochanska, et al. (1980).

2.2.4. Mode of action and functions of tannins

Tannins are a complex group of polyphenolic compounds found in a wide range of plant species. They are characterized by astringency and tanning properties, which are believed to be associated with the higher molecular weight proanthocyanidins (Hagerman, 1992). Elgailani & Ishak, (2014), reported the molecular weight of tannins to be between 500 and 5 000 Da. They are found in wood, bark, leaves and fruits; however, acacia species which belong to the family of Leguminosae in the plant kingdom are considered the most common sources of tannins (Taktak, et al., 1991). Previously, harmful nutritional consequences have been attributed to tannins because they can precipitate proteins, inhibit digestive enzymes, and decrease the utilization of vitamins and minerals (Koleckar et al., 2008). In addition, it was assumed that tannins are unabsorbable due to their high molecular weight and the ability to form insoluble structures with components of food such as proteins (Hassan et al., 2003). Hagerman & Butler, (1991), reported that tannins in poultry feed affect dry matter intake and consequently the weight gain. Tannins that can be hydrolyzed are found in smaller amounts in plants, while the condensed tannins are found in abundance. The concentration of tannins is dependent on the plant genotype, tissue developmental stage, and the environmental conditions (Barbehenn & Constable, 2011).

Biologically, tannins are significant in that they provide protection for the plant while still in the plant and have potential effect after the plant has been harvested (Galloway, 1989). In recent research tannins have been proposed as an alternative to antibiotics because of their antimicrobial properties of tannins, which is the ability to inhibit the extracellular microbial enzymes. In addition, hydro soluble tannins could be used in lieu of antibiotics, because bacteria such as *Clostridium perfringens* cannot develop a resistance to them. However, their use in animal feed is discouraged because they impact nutrition negatively. Their use has been linked with lower feed intake and digestibility and leads to poorer animal performance.

Tannins have numerous applications that benefit humans. Some of the applications of tannins include their use as nutraceuticals to prevent, for example cancer, cardiovascular disease, kidney disease, and diabetes (Sing & Kumar, 2019). They are also used for tanning leather, and manufacturing ink and wood adhesives. Medicinally, tannins are homeostatic, antidiarrheal, and a remedy for alkaloid and heavy-metals toxicity. In the lab, tannins are used as reagent for protein detection, alkaloids, and heavy metals due to their precipitating properties. In the food industry tannins are used to clarify wine, beer, and fruit juices. Other industrial uses of tannins include textile dyes, and as coagulants in rubber production.

2.2.5. Medicinal uses of tannins

Tannins in plants are believed to function as chemical guards that protect the plants against pathogens and herbivores, as stated by (Minussi et al., 2003). Furthermore, the properties of tannins as antioxidants and reducing scavenging activities were also reported by (Haslam, 1996). The ability of tannins to chelate metals, their antioxidant activity, antibacterial action, and complexation, are believed to be the mechanism of action behind the tannins' ability to treat and prevent certain conditions such as diarrhea and gastritis (Chung, et al., 1998). On the other hand, tannins' mechanisms of antimicrobial activity include inhibition of extracellular microbial enzymes, deprivation of the substrates required for microbial growth, or direct action on microbial metabolism through inhibition of oxidative phosphorylation. Sieniawska & Baj, (2017), state that the antimicrobial properties of tannins are believed to be associated with the hydrolysis of ester linkage between gallic acid and polyols hydrolyzed after the ripening of many edible fruits, which enables the tannins to function as a natural defense mechanism against microbial infections. Table 2.2.1 demonstrates some of the medicinal uses of tannins (Graziani et al., 2004).

Table 2.2.1. Uses of tannins as medicinal sources and industrial agents.

Components	Medicinal uses	References
Sweet chestnut extracts	*Escherichia coli, Bacillus subtilis, Salmonella enterica* serovar Enteritidis	Li & Song, (2004)
Extract of chestnut shell	Enteritidis, *Clostridium perfringens, Staphylococcus aureus,* and *Campylobacter jejuni.*	Özcan, (2003)
Gall nuts	Treatment of diarrhea and dermatitis	Kaur et al. (2005)
Acacia Nilotica	Antimutagenic and cytotoxic effects	Costabile et al. (2011)
Sweet chestnut extracts	*Reduction of Salmonella infection*	Attia, e al. (2016)
Quebracho Tannins	Reduction of worm eggs counts and inhibition of development of nematodes and lungworms	Tosi et al. (2013)
Chestnut extracts	Control of *Clostridium perfringens*	Hur et al. (2005)
Pine needles and dry oak leaves	Control of coccidian infection	Zhou & Du, (2019)

2.2.6. Tannins as adhesives

Tannins are used as a partial or complete substitute for phenols in wood adhesives in the form of tannin resin because of its phenolic structure (Custers et al., 1979). The use of tannin adhesives was first successfully traded in South Africa in early 1970s (Saayman, & Brown

1977). It is documented that previous research in the field of fortified starch adhesives with wattle bark tannin was carried out in South Africa (Mugedo & Waterman, 1992). Mimosa tannin adhesives were used instead of synthetic phenolic adhesives to manufacture particle-board and plywood for external and marine applications (Custers et al., 1979). In Kenya, the commercial wattle (*Acacia mearnsii*) is a well-known tannin-rich species and tannin-based adhesive (Pizzi, 2019). Current industrialized technologies are based mostly on paraformaldehyde or hexamethylene tetraamine, which are considered more environmentally friendly (Moubarik et al., 2010). The drive to create more environmentally friendly adhesives has led to different forms of research in the field; for example, the creation of corn-starch-tannin adhesives in a study by Medugu et al. (2012), in a bid to replace synthetic resins has shown that it has excellent structural stability.

2.2.7. Nutritive and anti-nutritive effects of tannins

Tannins, commonly found in most cereal grains and legume seeds, as already indicated, are considered anti-nutritional factors that hamper the use of some feeds by monogastric animals. It has been reported that tannins bind protein, and as a result weakens protein digestion (Butler et al., 1984). Tannins are blamed for the bitter taste of the feed, resulting in lowering feed consumption due to reduced palatability (Bhat et al., 2013). They are regarded as polyphenolic secondary metabolite; however, some reports have shown recently that low concentrations of some tannin sources can improve the nutrition and health status of monogastric animals (Huang et al., 2017). Anti-nutrients are commonly known as natural or synthetic compounds that interfere with the absorption of nutrients. Condensed tannins are known to inhibit several digestive enzymes, including amylases, cellulases, pectinases, lipases, and proteases (Garcia et al., 2004). They have a major anti-nutritive effect that can influence the nutrient digestibility of lipids, starch, and amino acids negatively (Brestenský et al., 2012; Liu et al., 2012). Tannins are a heterogeneous group of phenolic compounds, found in nature in many different families of plants. In Oakwood, Trillo, Myrobalaen and Divi-Divi they occur in almost every part of the plant, such as the leaves, fruits, seed, bark, wood, and roots.

Supplementation of chestnut HT at the concentration of 0.5% and 1.0% on rabbit feed had no effect on growth performance (Zoccarato et al., 2008). However, Schiavone et al. (2008) found different results when chestnut HT was included in rabbit feed at levels of 0.45% and 0.5%, as it increased feed intake and the live weight of rabbits. Similarly, Rezar & Salobir, (2014), reported that adding 0.20% of chestnut, the tannin increased average daily gain and daily feed intake of broilers. Lee et al. (2010) reported that when the sweet chestnut wood extract was used as a supplement at 0.07% and 0.02% for broiler chickens no anti-nutritive activity was observed, and the crude ash, crude protein, calcium, and phosphorus were not affected. The addition of tannic acid (HT) at a dietary level of 0.0125% and 0.1%, showed a negative impact on hematological indices and plasma iron of pigs (Iji et al., 2004). According to Kumar et al. (2007), ideal digestibility of energy, protein, arginine and leucine were lowered in broiler chickens as dietary tannin levels rose to 20g/kg diet and beyond, while phenylalanine and methionine were affected negatively only at tannin levels of 25g/kg diet. In another study with broiler chickens, Kyarisiima et al. (2004) reported that the tannin content of 16g/kg in red sorghum had no effect on phosphorus, calcium, and nitrogen retention in chickens. High-tannin sorghum treated with wood ash extract improves its nutritive value (Bilic´-Šobot et al., 2016). Tannins can act as a double-edged sword; therefore, a tannin content-specific solution could have an effect on their utilization. Although tanninferous feed and forages containing >5% tannin dry matter is not safe to be used as animal feed, low to moderate (<5% dry matter) is safe for animal consumption (Garcia et al., 2004). Table 2.2.2 shows the anti-nutritive and nutritive effects of tannins from different plant sources.

Table 2.2.2. Nutritive and anti-nutritive effects of tannins in monogastric animals.

Plant source/tannin	Animal (monogastric)	Concentration/application	Effects	Reference
Chestnut (*Castanea*) HT	Swine/pig	1%, 2% and 3%	Liver not affected. Changes in the intestine: villus height increased, mucosal thickness and villus perimeter; reduced large intestinal apoptosis and mitosis	Ebrahim et al. (2015)
Sweet chestnut wood extract	Chickens (broilers)	0.07% and 0.2%	No anti-nutritive effects	Lee et al. (2010)
Tannic acid (TA)	Chickens (broilers)	1% Tannic acid different climatic conditions	Better quality of fatty acid profile of breast muscle of broilers	Antongiovanni et al. (2015)
Chestnut (*Castanea*) HT	Chickens (layers)	0.20%	Increased monounsaturated fatty acid and reduced cholesterol content of eggs	Minieri et al. (2016)
Chestnut tannin extract (*Castanea sativa* Miller) HT	Chickens (layers)	2 g/kg	Unsaturated fatty acids increased; cholesterol significantly decreased: −17% in WLT and −9% in MUT	Lee et al. (2016)
High-tannin red sorghum (*Sorghum vulgaris*) HTS	Chickens (broilers)	16g/kg (reconstituted red sorghum)	Utilisations of phosphorus, nitrogen and calcium retention were similar	Kyarisiima et al. (2004)
Chestnut (*Castanea*)	Pigs	0, 5, 10 and 15%	Reduction in digestibility of dry matter, crude protein, ether extract, crude ash and tannin decreased linearly ($p < 0.05$) with increasing chestnut meal supplementation	Chamorro et al. (2015)

2.2.8. Influence of tannins on the productivity of monogastric animals

Tannins have been classified as an "anti-nutritional factor" for monogastric animals with negative effects on feed intake, nutrient digestibility, and production performance (Redondo et al., 2014). Currently most researchers have revealed that some tannin can improve the intestinal microbial ecosystem, enhance gut health, and hence increase productive performance when applied appropriately in monogastric diets (Zoccarato et al., 2008; Ebrahim et al., 2015; Houshmand et al., 2015). Strong protein affinity is a well-recognized property of plant tannins, which has successfully been applied to monogastric animals' nutrition. However, adverse effects of high-tannin diets on monogastric animals' performance have been reported by many researchers (Antongiovanni et al., 2015). In monogastric animals the main effects of tannins are related to their protein-binding capacity and reduction in protein, starch, and energy digestibility (Tapiwa, 2019; Ravindran et al., 2006). According to Hassan et al. (2003) and Maertens & Štruklec, (2006), dry matter intake, bodyweight, feed efficiency and nutrient digestibility were reduced when chickens were fed diets with tannins, whilst Ebrahim et al. (2015) and Antongiovanni et al. (2015), reported a decrease in body weight gain and feed intake. However, Minieri et al. (2016) and Houshmand et al. (2015) reported no effects on growth performance and on egg weight, cell thickness or yolk colour of layers. Several studies showed that low concentrations of tannins improved feed intake, health status, nutrition, and animal performance in monogastric farm animals (Huang et al., 2018; Brus et al., 2013; Prevolnik et al., 2012). According to Bee et al. (2016), supplementing pigs' diet with 0.2% chestnut wood extract rich in tannins had no effect on growth rate, carcass traits or meat quality of pigs raised up to 26 weeks of age; whereas Bee et al. (2016) reported that pigs that were fed diets rich in 3% of hydrolysable tannins from chestnuts showed no negative effects in terms of growing performance raised from day 105 until 165. Hur et al. (2005) reported an increase in small intestinal villus height, villus perimeter and mucosal thickness in pigs that were fed diets having 3% of hydrolysable tannins from chestnuts. Moreover, Brus et al. (2013), reported increased growth performance in pigs aged 23–127 days when fed chestnuts rich in tannins at 0.91% supplementation level.

In rabbits, Zoccarato et al. (2008) observed no difference in the performances of rabbits fed diets supplemented with up to 10g of tannins from chestnuts. Moreover, they reported that no improvements were observed in health status, diet nutritive value, growth performance, carcass traits and oxidative stability of rabbits fed up to 400g/100kg of hydrolysable tannins originated from chestnuts. According to Mancini et al. (2019), rabbits fed diets with 4% of tanniniferous browsers of *Acacia karroo, Acacia nilotica* and *Acacia tortilis* showed no significant differences in intake and digestibility. Mancini et al. (2019) also reported no significant difference in growth rate, feed intake or feed conversion ratio and carcass traits of rabbits fed a mixture of quebracho and chestnut tannins. Moreover, Agume et al. (2017) observed no significant difference in growth rate, feed intake or feed conversion ratio of rabbits fed low-tannin sorghum grains. Thus, tannins, when included in monogastric animal diets, can have both positive and negative effects on animal performance, depending of the amount of concentration. Therefore, it is important to minimise the inclusion or supplementation of feedstuffs containing high concentrations of tannins in monogastric animals, or to take measures to decrease their concentrations. In Table 2.2.3, the effect of tannins on productivity of monogastric animals is reported.

Table 2.2.3. Effects of tannins on productivity of monogastric animals.

Tannin concentrations	Tannin source	Monogastric animal	Influenced/affected parameter	References
0.16-0.19 %	Chestnut	Pigs	Increased growth performance;	Brus et al. (2013)
0.71 -1.5%	Chestnut	Pigs	No effect on feed intake, body weight gain and carcass traits; reduced feed efficiency	Cappai et al. (2014)
1-3%	Chestnut	Pigs	Increased small intestinal villus height, villus perimeter and mucosal thickness	Ebrahim et al. (2015)
5-10%	Grape pomace	Broilers	No effect on growth performance; increased oxidative stability and polyunsaturated fatty acids content of thigh meat	Houshmand et al. (2015)
1%	Tannic acid	Broilers	Decreased body weight gain and feed intake; improved the fatty acid profile of breast muscle	Antongiovanni et al. (2015)
	Chestnut	layers	No effect on egg weight, cell thickness or yolk colour, reduced cholesterol content	Minieri et al. (2016)
0.45% and 0.5%	Chestnut	Rabbits	Increase live weight gain and feed intake of rabbits	Prevolnik et al. (2012), Vagadia et al. (2017)
0.5% and 1.0%	Quebracho, chestnut	Rabbits	Had no effect on growth performance	Zoccarato et al. (2008), Al-Mamary et al. (2001)
4%	*Acacia karroo*, *Acacia nilotica* and *Acacia tortilis*	Rabbits	No significant differences in intake and digestibility	Mancini et al. (2019)

2.2.9. Processing techniques used to reduce effects of tannins

Several processing techniques to reduce tannin levels in different feedstuffs, more especially unconventional ingredients, have been suggested by most researchers (Avil´es-Gaxiola et al., 2018; Schons et al., 2012). Processing is an act of applying suitable techniques to reduce or eliminate tannins present in alternative feedstuffs. These techniques include enzyme supplementation, soaking, dehulling, alkali treatment, extrusion, and germination.

2.2.10. Enzyme supplementation

Supplementation of enzymes to reduce the tannins content is an effective method although it might be the most economical. It is proven to reduce tannins in a very outstanding manner than other processing methods such as soaking, dehulling etc. Several studies have shown that enzyme supplementation has been effective in reducing tannins in alternative energy and protein feedstuffs (Iji et al., 2017; Vadivel & Pugalenthi, 2008). A study by Iji et al. (2017), found that treatment of sorghum with both polyphenoloxidase and phytase enzymes showed a decrease in hydrolysable and condensed tannins of 72.3% and 81.3% respectively. Moreover, Vadivel & Pugalenthi, (2008), reported a decrease in both hydrolysable and condensed tannins by 40.6%, 38.92% and 58.00% respectively when sorghum grains were treated with the three enzymes tannase, phytase and paecilomyces variotii.

2.2.11. Soaking

Soaking is one of the cheapest traditional methods which animal nutritionists have used for many years. A study found that addition of sodium bicarbonate, prolonged time of soaking, or higher temperature has proved to be effective during soaking process (Sunil et al., 2018). Kyarisiima et al. (2004) reported that high-tannin sorghum soaked in wood ash extract showed a decreased level of tannins without lowering the nutrient content of sorghum grains. Authors stated that tannin level did not only decrease with the soaking technique, but also with roasting. The decrease in tannins during soaking may result from leaching into the soaking water (Ravindran et al., 2006). Moreover, Mittal et al. (2012) reported a decrease of about 73–82% in velvet beans.

2.2.12. Dehulling

Dehulling is a process of ramming the outer coat/hull of a seed (Alonso et al., 2000). Most seeds of alternative feedstuffs have seed coats/hulls which are normally concentrated with tannins. If tannins are removed, feedstuffs have shown to have a significant increase in protein digestibility and protein content in the legume seed meal. Navale et al. (2015), reported that dehulling reduced tannins in chickpea without lowering protein digestibility, whereas in faba beans 92% of tannins has shown to decrease with dehulling (Kaur et al., 2015).

2.2.13. Extrusion

The extrusion method is used to decrease levels of tannins in feedstuffs. According to Rahul & Uday, (2016), extrusion cooking is a high-temperature, short-time process in which starchy food materials are plasticized and cooked by a combination of moisture, pressure, temperature, and mechanical shear. Extrusion has shown the ability to inactivate anti-nutritional elements (Muhammad et al., 2014; Kumar et al., 2018). For example, Singh et al. (2017), reported that extrusion showed a significant reduction in tannins with minimum oil loss in flaxseed meal. Rusydi & Azlan, (2012), reported that lentil splits showed a reduction in tannins after treated by using extrusion techniques. Moreover, Agwunobi et al. (2002), reported reduction to the extent of 34.52% to 57.41% in sorghum.

2.2.14. Germination

During the germination process, complex sugars are converted into simple sugars (Sunil et al., 2018). Tannin content has shown to be reduced by the germination process, which is one of the cheapest methods. A maximum reduction in tannins of up to 75% has been observed when pearl millets were treated by using the germination method (Abeke & Out, 2008). Rusydi & Azlan, (2004), observed a reduction of 57.12% when peanuts were treated by using germination. The reduction of tannins may improve the nutritional quality of feedstuffs. Thus, processing techniques may help to remove or reduce tannin levels in different feedstuffs, which might be favorable for animal production (Table 2.2.4).

2.2.15. Cooking

Cooking is considered important in reducing antinutrients activities in tannins. As stated by Vitti et al. (2005) cooking reduces the antinutrients present in tuber crops like cocoyam.

2.2.16. Autoclaving

Autoclaving is found to be one of the effective methods in the elimination of antinutrients, although it might not cost effective because of its reliability on electricity (Elizondo et al., 2010).

2.2.17. Grinding

Grinding is considered an effective method in reducing the tannin content because it increases the surface area which in turn reduces the contact between tannins and the phenolic oxidase in the plant (Anderson et al., 2012; Van Parys et al., 2010).

Table 2.2.4. Different processing techniques used to reduce the effects of tannins in alternative feedstuffs.

Processing technique	Feedstuff	Effectiveness	References
Enzyme supplementation	Sorghum	The enzyme tannase reduced both hydrolysable and condensed tannins by 40.6%	Iji et al. (2017); Vadivel, & Pugalenthi, (2008)
Dehulling	Chickpeas	Reducing tannin level without lowering the nutrient content of the grain	Navale et al. (2015)
	Faba beans	Reduced about 92% of tannins	Kaur et al., (2015)
Soaking	Sorghum	Reducing tannin level without lowering the nutrient content of the grain	Kyarisiima et al. (2004) Ravindran et al. (2006)
	Velvet beans	Decreased about 73–82% of tannins	Mancini et al. (2019)
Alkali treatment	Sorghum	Reducing tannin level without lowering the nutrient content of the grain	Ravindran et al. (2006)
Extrusion	Flaxseed	Significant reduction of tannins with minimum oil loss in flaxseed meal	Singh et al. (2017)
	Lentils	Reduced the tannin content in lentil splits	Rusydi & Azlan, (2012)
	Sorghum	Reduction to the extent of 34.52 to 57.41%	Agwunobi et al. (2002)
Germination	Pearl millets	Maximum reductions in tannins up to 75%	Abeke and Out, (2008)
	Peanuts	Reduction of tannins by 57.12%	Manach et al. (2004)
Cooking	Cocoyam	Reduction of antinutrients in tuber crops	Vitti et al. (2005)
Autoclaving	Sorghum	Reduction to the extent of 34.52 to 57.41%	Agwunobi et al. (2002)
Germination	Pearl millets	Maximum reductions in tannins up to 75%	Abeke & Out, (2008)
	Peanuts	Reduction of tannins by 57.12%	Manach et al. (2004)

2.2.18. Health benefits of tannins in monogastric animal production

Tannins are plant extracts that can be used as additives in monogastric animal feed to control diseases (Redondo et al., 2014). In vitro studies have shown that most tannin has antiviral, antibacterial and antitumor properties (Khanbabaee & Van Ree, 2001). Tannins have shown a favorable outcome in the preferment of gut health when used with other antimicrobials as growth-promoting factors (AGP) such as probiotics (Redondo et al., 2014). Condensed tannins extracted from green tea or quebrachos have shown to have some antimicrobial substances Corder. However, Phytolab reported that condensed tannins may have less effect than hydrolysable tannins in controlling *Campylobacter jejuni* in the present of high concentration of amino acids. Moreover, tannins derived from chestnuts (*Castanea sativa*) can inhibit the in vitro growth of *Salmonella typhimurium* (Kovitvadhi et al., 2016). Several in vitro studies have revealed that polyphenols of the procyanidins (CT) have an antioxidant property while tannic acid has anti-enzymatic, anti-bacterial and astringent properties,as well as constringing action on mucous tissues (Bole-Hribovsek et al., 2012). The ingestion of tannic acid causes constipation, so it can be used to treat diarrhoea in the absence of inflammation (Kamijo et al., 2008). Kumar et al. (2007) reported that the tannin content of 16g/kg in red sorghum had no effect on certain animal welfare parameters of broiler chickens. Similarly, globulin, protein, plasma albumin, phosphorus, glucose, calcium, and uric acid levels were not affected, even when maize is replaced 100% with red sorghum. However, mild histopathological changes in kidney and liver tissues as well as high cell-mediated immune response were detected when raw red sorghum containing 23g tannins/kg was fed to the same group of broiler chickens. The supplementation of purple loosestrife (*Lythrum salicaria*) in rabbits has led to a significant increase in the total white blood cells and higher concentrations of volatile fatty acids and acetic acid, therefore a low level of loosestrife supplementation (< 0.4%) has been suggested to gain health benefits and prevent adverse effects on animal health and performance (Girard & Bee, 2020). Farmatan tannin concentrations of 0.05, 0.025 and 0.0125% can inhibit the growth of *Clostridium perfringens* by more than 54-fold (Jamroz et al., 2009). Another in vitro study was conducted to evaluate the effects of tannins from chestnuts and quebracho, or a combination of both, on *Clostridium perfringens.* All three products reduced the presence of *C. perfringens*. When the comparative analysis was conducted, it was discovered that the concentrations of quebracho tannin were more effective in inhibiting the growth of *C. perfringens*as compared to chestnut tannin. Commensal bacteria such as *Bifidobacterium breve* or *Lactobacillus salivarius* are very useful and their growth or presence should not be inhibited by the tannin. Ellagitannins isolated from *Rosa rugose* petals have some antibacterial activities against pathogenic bacteria such as *Salmonella* sp, *Bacillus cereus*, *S. aureus* and *E. coli* but they had no effect on beneficial bacteria. Most in vitro results are supported by in vivo experiments that the inclusion of tannin in monogastric animals can lower the occurrence and severity of diarrhoea (Viveros et al., 2011). However, the efficiency of adding tannins that shows robustness in inhibiting pathogens in in vitro studies needs to be evaluated further in the experimental set-up (in vivo) involving poultry and pigs. These disparities in terms of types of tannins that are efficient in combating certain pathogens warrant further research. Table 2.2.5 shows different health benefits of tannins in monogastric animals.

Table 2.2.5. Health benefits of tannins in monogastric animals.

Plant source/tannin	Animal/monogastric	Application rates	Health benefits	Reference
Chestnut tannin (HT)	Chickens	0, 250, 500 and 1000mg/kg	reduced number of *E. coli* and coliform bacteria in small intestine. Greatest number of *Lactobacillus* observed in supplementation of 1000mg/kg	Jamroz et al. (2009)
Purple loosestrife (*Lythrum salicatia*)	Rabbit	0.2%, 0.4% and 0.3%	Increased total white blood cells in rabbit	Kovitvadhi et al. (2015)
Chestnut (HT)	Chickens (broiler)	0.15% to 1.2%	Reduced bacteria in the gut. *Clostridium perfringens* (*Eimeria maxima*, *Eimeria tenella* and *Eimeria acervulina*)	Tosi et al. (2013)
Grape pomace (CT)	Pigs	2.80%	Reduction in the absorption of mycotoxins in the gastrointestinal surface	Gambacorta et al. (2016)
Grape pomace (CT)	Chickens (broiler)	6%	Increased commensal bacteria (*Lactobacillus*) and decreased the counts of clostridium bacteria in ileal content	Viveros et al. (2011)

2.3. Conclusions

In the quest to find alternative feed ingredients in the monogastric animals' production, the effects of tannins have proven to be of value. Tannins can be beneficial in both as feed ingredients and a valuable ingredient in animal health. Although tannins contain antinutrients, different processing methods have proofed to be effective in reduction or elimination of these antinutrients. This review has provided extensive literature on the benefits and impacts of tannins in poultry production. Furthermore, it has elaborated on different processing methods which can be employed to reduce the negative effects of tannins. The methods chosen should be cost-effective, easy to use and should not defeat the purpose of alternative feed ingredients. Even though tannins can act as feed additives, their inclusion level will depend on the source, age and species of poultry. Thus, future research should focus on the optimum tannin inclusion level in poultry and more cost-effective processing methods, especially for small-scale poultry keepers who mostly utilize these alternative feed ingredients. Development of more convenient readily available products of tannins ready to be incorporated in the monogastric animal feed is encouraged.

CHAPTER 3

GENERAL MATERIALS AND METHODS

3.1. Introduction

The experimental procedures were conducted in accordance with the University of South Africa's (UNISA) Ethics code for the use of live animals in research, ethics reference number 2019/CAES/051. The experiments are presented here as chapters. Each chapter consists of specific objective, materials and methods used to achieve these objectives and the results obtained.

3.2. Study site

The feeding trial experiment was conducted at the University of Limpopo Experimental Farm, in Limpopo, South Africa. The farm is situated 10 km North-west of the Turfloop campus, of the University of Limpopo. The ambient temperature at the study site ranges between 20 and 36°C in summer (November - January) and between 5 and 25 °C in winter (May – July). Mean annual rainfall ranges between 446.8 and 468.44 mm. The proximate analysis of the millet varieties was conducted at the University of Pretoria, South Africa, while the phenolic compounds analysis was conducted at the University of Stellenbosch, South Africa.

3.3. Experimental animals

For a 6-week feeding trial experiment, a total of 200 Ross 308 chicks with an average weight of 44.07g, were used in feeding trial experiment. The total number of chickens is then divided into different experiments as indicated below, using different protocols in partial replacement of maize with different inclusion levels of pearl millet in the diets. The chicks were purchased from a local hatchery in the Limpopo province. The birds were individually weighed and kept together in a brooding house, they were transferred on day 14 into 20 pens, with ten chicks per each pen. They were fed on the experimental diets until the end of the experimental period, which was 42 days.

3.3.1. Experimental diets

The diets used in this experiment consisted of maize and millet. For proximate analysis experiment, the finger and pearl millet samples were milled and stored for further analysis. To determine the phenolic compounds in the millet samples, the millet was ground finely and stored ready for analysis. The feeding trial experiment only used the pearl millet type, which was ground at the Limpopo University facility. The feed was allocated to different inclusion levels.

3.4. Data analysis

All the data collected were subjected to completely randomized design analysis using the General Linear Model procedure (PROC GLM) of Statistical Analysis System, (SAS, 2011). Means were separated the least significant difference, at ($p<0.05$) the means were considered significant.

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Evaluating the physical and chemical contents of millets obtained from South Africa and Zimbabwe

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ABSTRACT
Millets which are considered the third most important cereal in Africa have remained underutilised for food and feed. Therefore, the core aim of this study was to evaluate types of millets obtained from South Africa and Zimbabwe for physical and chemical characterization. Catechin and epicatechin were higher for finger millet types. Crude protein and gross energy were similar for all the millets. However, the starch content of the South African finger millet was higher compared to the Zimbabwean type and the pearl millets from both regions. The macro-minerals of the four millets varied with calcium and magnesium being higher in finger millets. All the millet types had abundant potassium which ranged from 3864.60 to 4899.30 mg/kg. Furthermore, the essential amino acids of the millet types differed greatly. Overall, a greater impact on physical and chemical characteristics was influenced by millet type, even though some locational differences were observed.

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Evaluación del contenido físico y químico del mijo obtenido de Sudáfrica y Zimbabue

RESUMEN
A pesar de ser el tercer cereal más importante de África, el mijo sigue siendo infrautilizado para la alimentación humana y animal. El objetivo central de este estudio fue evaluar diferentes tipos de mijo obtenidos en Sudáfrica y Zimbabue para analizar su caracterización física y química. En los tipos de mijo dedo se constató que la catequina y la epicatequina eran más altas, mientras que la proteína bruta y la energía bruta eran similares para todos los mijos. Además, se comprobó que el contenido de almidón del mijo dedo sudafricano es mayor en comparación con el tipo zimbabuense y los mijos perlados de ambas regiones. Por otra parte, la presencia de macrominerales en los cuatro mijos varía, encontrándose que los mijos dedo tenían mayor contenido de calcio y magnesio. Todos los tipos de mijo tienen un contenido abundante de potasio, el cual oscila entre 3864.60 y 4899.30 mg/kg. Además, los aminoácidos esenciales presentes en los diversos tipos de mijo son muy diferentes. En general, se constató que los tipos de mijo se diferencian más por aspectos atribuibles a su caracterización física y química, aunque se observaron algunas diferencias atribuibles a su lugar de origen.

1. Introduction

Cereal grains have been part of human's diet over the longest period, as source of energy, carbohydrate, protein, and fibre, as well as micronutrients (MCkevith, 2004). The most common cereals used by human include maize, rice, barley, sorghum, wheat, and millets. Although, millets *(Panicum miliaceum)* are not widely recognized, they are important crops in the semi-arid tropics of Africa and Asia, known for their ability to flourish in harsh climatic conditions (Dykes & Rooney, 2007). Due to their nutritional composition, they are considered the most competitive grains as alternatives to most popular cereals like wheat and rice as food (Parameswaran & Sadasivam, 1994; Saleh et al., 2013). In addition, millets could resist pests and diseases with a short growing season compared to other cereals resulting in low production costs (Devi et al., 2011). Further to this, pearl millet is known to be the most drought-tolerant cereal and can yield more grain and thrive under rainfall as low as 200 to 250 mm (Bidinger & Hash, 2003). In Africa, a total of 19 million ha (60% of total land use) is used to grow millet (Macauley, 2015; Orr et al., 2016). Pearl, finger, proso and foxtail millets are the main types of millets (Gomez & Gupta, 2003).

According to Anitha et al. (2019) and Food and Agriculture Organization (FAO) (2017), millets can be an important source of essential nutrients such as amino acids, mineral and trace elements. However, there are, wide variations evident in the chemical composition of pearl and finger millet (FAO, 2017). Shweta (2015) reported that pearl millet contains higher energy compared to cereal grains like rice and wheat, and are important source of thiamine, niacin, and riboflavin (Taylor, 2004). Moreover, the content of minerals such as calcium, iron and phosphorus in pearl millet is like those found in other cereals (Adeola & Orban, 1995). Furthermore, Ali et al. (2003) reported nutritional value for pearl millet of about 92.5% dry matter, 2.1% ash, 2.8% crude fibre, 7.8% crude fat, 13.6% crude protein, and 63.2% starch. In addition, the starch content of millet was recorded by

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CHAPTER 4

EVALUATING THE PHYSICAL AND CHEMICAL COMPOSITION OF MILLETS OBTAINED FROM SOUTH AFRICA AND ZIMBABWE

4.1. Abstract

Millets (*Panicum miliaceum*) which are considered the third most important cereal in Africa, are known to be abundant in nutrients and are drought tolerant, which makes them climate-smart crops in these regions. Irrespective of their importance and health benefits, they remain underutilised for food and feed. Therefore, the core aim of this study was to evaluate varieties of millets grown in two locations for physical and chemical characterization. Catechin and epicatechin was observed to be higher ($P < 0.05$) for finger than pearl millets irrespective of their growing location. Crude protein and gross energy values were similar ($P > 0.05$) for all the millets grown in both South Africa and Zimbabwe. However, the starch content of the South African finger millet was significantly higher ($P < 0.05$) compared to the Zimbabwean type and the pearl millets from both regions. The macro-minerals of the four millets varied significantly with calcium and magnesium being higher ($P < 0.05$) in finger than pearl millets. Although, pearl millets exhibited higher ($P < 0.05$) phosphorus values than their counterparts. Furthermore, the essential amino acids of the millet types differed greatly. Lysine was higher ($P < 0.05$) for the pearl millets whereas finger millets were shown to have higher ($P < 0.05$) methionine values. Pearson correlations and principal component analysis (PCA) has further confirmed significant correlations among the varieties. Overall, a greater impact on physical and chemical characteristics was influenced by millet type, even though some locational differences were observed. Moreover, millet type should be considered when selecting for use as food or feed.

4.2. Introduction

Cereal grains have been part of human's diet over the longest period, as source of energy, carbohydrate, protein, and fibre, as well as micronutrients (MCkevith, 2004). The most common cereals used by human include maize, rice, barley, sorghum, wheat and millets. Although, millets (*Panicum miliaceum*) are not widely recognized, are important crops in the semi-arid tropics of Africa and Asia, known for their ability to flourish in harsh climatic conditions (Dykes & Rooney, 2007). Due to their nutritional composition, they are considered the most competitive grains as alternatives to most popular cereals like wheat and rice as food, (Parameswaran & Sadasivam,1994; Saleh et al., 2013). In addition, millets have the ability to resist pests and diseases and have short growing season compared to other cereals resulting in low production costs (Devi et al., 2011). In Africa, a total of 19 million ha (60% of total land use) is used to grow millet (Macauley, 2015; Mwema, 2016). Pearl, finger, proso and foxtail millets are the main varieties of millets (Gumez & Gupta, 2003).

According to Anitha, (2019) and FAO, (2017), millets can be an important source of essential nutrients such as amino acid, mineral and trace elements. There are, however, wide variations evident in the chemical composition of pearl and finger millet (FAO, 2017). Shweta, (2015) reported that pearl millet contains higher energy compared to cereal grains like rice and wheat, and are important source of thiamine, niacin, and riboflavin (Taylor, 2004). Moreover, the content of minerals such as calcium, iron and phosphorus in pearl millet is like those found in other cereals (Adeola & Orban, 1995). Pearl millet is also considered a good source of fat-soluble vitamin E (2mg/100g). Furthermore, Ali et al. (2003) reported nutritional value for pearl millet of about 92.5% dry matter, 2.1% ash, 2.8% crude fibre, 7.8% crude fat, 13.6% crude protein, and 63.2% starch. Furthermore, the starch content of millet was recorded by Krishnakumari & Thayumanavan, (1995) to be at the range of 64-79%.

Finger millet, however, was recorded to have 81.5% carbohydrates, 9.8% protein content, 4.3% crude fibre, and 2.7% minerals. Its protein contains more lysine, threonine, and valine than other millets (Ravindran, 1991). In addition, Mustafa et al. (2008) reported values for neutral detergent fibre (NDF) and acid detergent fibre (ADF) to be (145, 138, 137, 134, 145, g kg-1 DM) and (67, 56, 63, 65, 59, g kg^{-}1 DM) respectively, for pearl millet varieties. Different factors contribute to these variations, for instance the type of soil that the millet is grown in, is considered one of the determining factors of its mineral content (Wafula et al., 2018).

In addition, millets are also believed to have nutraceutical health benefits, which includes but not limited to, digestive system wellbeing, reduction of cholesterol in the body, prevention against heart diseases, protects from diabetes, lowers the risk of cancer, increases energy levels and improves muscular systems (Amadou et al., 2013; Manach et al., 2005; Chandra et al., 2018).

These characteristics ought to put such grains at the right position in terms of alternative crops; however, due to lack of attention millet was termed the lost crop (Lost crop of Africa, 1996). Given the current challenges of sustainable food production, climatic changes, and water scarcity, coupled with overpopulation, interests have been developed towards millet. This has provided an opportunity for farmers, nutritionists, food and feed manufacturers to engage in research in quest of the nutritional and functional characterization of millet grains.

With advancing competition on the uses of maize in different industries, it is vital to find competitive unconventional crops for human and livestock consumption (FAO, 2017). Thus, this study is aimed to investigate the nutritional composition of millet varieties grown in different locations as food for humans and feed for livestock.

4.3. Materials and Methods

4.3.1. Sourcing of millets

South African pearl millet *(Pennisetum glaucum)* was obtained from ARC-Grain Crops Institute, Potchefstroom in the North-West Province whilst finger millet *(Eleusine coracana)* variety was obtained from a local market in Johannesburg, Gauteng province of South Africa. The Zimbabwean pearl and finger millet varieties were purchased from a local market in Zimbabwe. The grains were cleaned to remove any foreign material, then divided into two samples which were stored in 45 ml tubes in a dry and cool place until the time of further analysis.

4.3.2. Physical characteristics determination

A random selection of 2 replicates for each species of millet (20 grains each) was used to determine the thousand kernel weight. The two species were manually counted, and their weights determined several times using digital electronic balance with 0.01 g accuracy (Adam CPW plus-150p, USA), the average weight was then determined for both pearl and finger millet varieties. The colour of the grains was determined by the means of visual observation. To complement, Hunter Lab test L* (lightness), a* (redness) and b* (green) colour values were measured with a Konica Minolta CR-400 c camera (Konica Minolta, Sakai, Osaka, Japan).

A total of 20 seeds from each grain species were cut in half with a blade, and kernel texture were assessed for the proportion of corneous and floury endosperms using the method of (Waniska et al., 1992). The exterior layer (pericarp) of kernels of the four millet types was scratched using a scalpel and observed under the microscope to check for a pigmented grain. The bleach test was used to detect millet grains with a pigmented testa (Taylor, 2001). The millet varieties were also tested for the presence or absence of tannins; the grains were submerged in five grams sodium hydroxide dissolved in 100 ml of 3.5% sodium hypochlorite solution, in a beaker following the method of (Waniska et al., 1992). The mixture was left to rest for more than an hour. Brown sorghum was used as control.

4.3.3. Epicatechin and catechin determination

The extracts were prepared by using 2g dry millet material + 15 ml 50% methanol/1% formic acid in water with ultrasonication for 1 hour and standing overnight, followed by centrifugation and transfer of the supernatant to a glass vial ready for the LC-MS analysis. The samples were then analyzed by LC-MS method using a Waters Synapt G2 Quadrupole time-of-flight (QTOF) mass spectrometer (MS) connected to a Waters Acquity ultra-performance liquid chromatograph (UPLC) (Waters, Milford, MA, USA) was used for high-resolution UPLC-MS analysis. Electrospray ionization was used in negative mode with a cone voltage of 15 V, desolvation temperature of 275 °C, desolvation gas at 650 L/h, and the rest of the MS settings optimized for best resolution and sensitivity. Data were acquired by scanning from m/z 150 to 1500 m/z in resolution mode as well as in MSE mode.

4.3.4. Proximate analysis

To determine the nutritional composition, four samples of millet varieties were prepared in duplicate, then analysed using the methods of AOAC, (2000), to determine the dry matter, fat, ash, crude fibre, total nitrogen and protein (N x 6.25) content which was determined by the Kjeldahl using (AOAC, 2000). N2 was freed by pyrolysis and subsequent combustions, is swept by CO2 carrier into nitrometer. CO2 is absorbed in KOH and volume residual N2 is measured and converted to equivalent protein by numerical factor. Ether Extracts was determined using Soxtec solvent extraction systems, the samples were extracted through the three automatic steps of boiling, rinsing and recovering, which took about an hour. Calcium, Magnesium, Copper, Iron, Manganese, Zinc, Sodium and Potassium was determined by Atomic Absorption using the method of Varian SpektrAA. Test portion containing 2g dry material was dried to constant weight at 95˚-100˚C, under pressure ≤100mm Hg (ca 5hrs). Loss on drying (LOD) was reported as an estimate of moisture content.

Amino acid separation and detection was performed using a Waters Acquity Ultra Performance Liquid Chromatograph (UPLC) fitted with a photodiode array (PDA) detector.1 µl of sample/standard solution is injected into the mobile phase which conveys the derivatized amino acids onto a Waters UltraTax C_{18} column (2.1 x 50mm x 1.7µm) held at 60°C. Elution of analytes off the column is performed by running a gradient. Analytes eluting off the column are detected by the PDA detector, with each amino acid coming off the column at a unique retention time. Fat and ether extract lipid content was estimated using TecatorSoxtec.

4.3.5. Statistical analysis

The data obtained was analysed using descriptive statistics (means and standard deviation), using the General Linear Model procedure (PROC GLM) of Statistical Analysis System, version 24.0 (SAS, 2011). Mean separation was conducted using Duncan's multiple range tests at the level of ($P < 0.05$). Pearson's correlation coefficient was used to determine the level of correlation between physical characteristics.

4.4. Results

4.4.1. Physical properties of millet varieties

Results on physical properties (colour, endosperm texture, and 1000 kernel weight (TKW) and phenolic compounds) of millet varieties are presented in Table 4.1. Pearl and finger millets exhibited green and red colour, respectively during visual observation. When the hunter Lab test was conducted, all millet varied significantly. The values ranged from 39.39

to 54.32 for L* (lightness), the highest was observed for the pearl millet South Africa (PMSA), whilst no significant difference observed between FMSA and FMZM. On the other hand, the values for a* (redness) ranged from 1.99 to 12.50, lowest and highest values were observed in PMSA and FMZM, respectively. The values for b* (green) ranged between 9.19 to 14.24, highest value was recorded in PMZM. Pear millet types had higher L* values, indicating their lightness in comparison to the finger millet varieties. Contrary, the finger millet varieties had a* value indicating their redness over the pearl millet types. The values for b* were somewhat similar among the varieties. The texture of the grains differed (P < 0.05) as South African pearl millet was corneous and almost completely floury for the Zimbabwean type. The 1000 kernel grain weight for the South African (14.42g) was higher (P < 0.05) compared to the Zimbabwean (9.71g,) pearl millet. However, the South African finger millet (FMSA) resulted in lower (P < 0.05) kernel weight than that of the Zimbabwean type (FMZM). FMZM variety tested positive for tannins while other varieties tested negative when the bleach test was used. Furthermore, FMSA and FMZM resulted in higher (P < 0.05) catechin and epicatechin values compared to pearl millets varieties in different locations.

Table 4.1 Physical characteristics of millet varieties.

Parameters									
							Hunters LAB		
Millet	VKC	TKW	Tannin	Texture	Catechin	Epicatechin	L	a	b
FMSA	Red	2.59^{d}	-	4	610.43^{b}	99.10^{a}	39.39^{d}	12.47^{b}	10.74^{c}
FMZM	Red	3.20^{c}	+	4	675.06^{a}	13.50^{b}	39.39^{d}	12.50^{a}	9.19^{d}
PMSA	Green	14.42^{a}	-	2	12.64^{c}	1.80^{c}	54.32^{a}	1.99^{d}	11.93^{b}
PMZM	Green	9.71^{b}	-	4	2.530^{d}	1.20^{c}	53.46^{b}	3.83^{c}	14.24^{a}

Hunter LAB test: L: lightness, a: redness, b: green colour value. TKW: 1000 kernel weight (g). VKC: Visual kernel colour. $^{a, b, c, d}$: Means in the same column with different superscripts are significantly different at (p <0.05). Endosperm texture (2 = corneous, 4 = floury). Tannin + and – indicate presence and absence of tannins in the samples respectively.

4.4.2. Pearson's correlation of physical parameters

Results on correlation among physical parameters are shown in Table 4.2. Thousand kernel weight (TKW) had a strong negative correlation with L (lightness) and a negative correlation with b (green), however, there was a strong positive correlation between TKW and a* (redness) hunter lab values. A strong negative correlation was also observed between TKW and both the catechin and epicatechin. On the other hand, texture (TXT) had a negative correlation with TKW and a*(redness) hunter lab value. A positive correlation was observed between texture, L*(lightness), catechin and epicatechin and a positive correlation with b (green) colour values.

Table 4.2. Pearson correlation matrices between the physical characteristics and Catechin and Epicatechin.

Parameters	TXT	TKW	L	a	b	CAT
TKW	-0.26					
L	0.66^{*}	-0.89^{***}				
A	-0.57^{*}	0.93^{***}	-0.99^{***}			
B	0.73^{*}	-0.67^{*}	0.85^{***}	-0.78^{*}		
CAT	0.57^{*}	-0.94^{***}	0.98^{***}	-0.98^{***}	0.87^{*}	
EpiCAT	0.56^{*}	-0.90^{***}	0.95^{***}	-0.93^{***}	0.93^{***}	0.98^{***}

Hunter LAB test: L: lightness, a: redness, b: green colour value, TKW: 1000 kernel weight (g). CAT: Catechin, EpiCAT: Epicatechin, * indicate a significant correlation ($P < 0.05$); *** indicate a highly significant correlation ($P < 0.01$)

4.4.3. Nutrient composition

Chemical composition was significantly different (*P* < 0.05) across millet varieties except for crude protein and gross energy which was similar (*P* < 0.05) irrespective of location (Table 4.3). Meanwhile, the crude protein content was 6.960 for FMSA,6.430g/100g for FMZM whereas 8.390 was recorded for PMSA and 9.410 for PMZM. The gross energy was somewhat similar across the varieties, ranging between 15.54 to 17.00g/100g with the lowest value recorded for FMZM. Numerically, pearl millets exhibited higher crude protein and gross energy than the finger millet varieties. Crude fibre content, ADF and NDF were higher (*P* < 0.05) for the FMSA compared to the other varieties. Similarly, the contents of crude fibre, ADF and NDF for the FMZM were higher (*P* < 0.05) than that of the pearl millets irrespective of location.

FMSA showed the highest (*P* < 0.05) starch content of 58.73 g/100g compared to FMZM and all the other pearl millets. Moreover, the lowest starch content of (40.95 g/100g) was recorded for the PMSA. The ether extracts mean values ranged between 0.650 to 4.52 g/100g. The lowest value was observed in the FMZM, whilst the highest was recorded in PMSA.

Table 4.3. Nutrient composition of millet grains (g/100g).

	Millet varieties					
	FMSA	FMZM	PMSA	PMZM	SEM	Probability
DM	89.73[c]	88.93[d]	90.15[b]	90.58[a]	0.043	0.0001
CP	6.960	6.430	8.390	9.410	0.526	0.0564
CF	5.670[a]	5.200[b]	3.870[c]	2.270[d]	0.050	0.0001
NDF	12.76[a]	12.51[b]	11.64[c]	9.010[d]	0.043	0.0001
ADF	8.710[a]	7.910[b]	4.190[c]	3.310[d]	0.050	0.0001
GE	15.83	15.54	17.00	17.00	0.035	0.0001
EE	0.900[c]	0.650[d]	4.520[a]	3.800[b]	0.004	0.0001
Starch	58.73[a]	38.04[d]	40.95[c]	48.86[b]	0.035	0.0001

Values are means of duplicate analysed millet samples. [a, b, c, d]; Means followed by the same superscript in a row, are not significantly different ($P < 0.05$).

4.4.4. Mineral composition of millet varieties

The results of the mineral composition of finger and pearl millets grown in different locations are presented in Table 4.4 Calcium and magnesium were observed to be lower ($P < 0.05$) in pearl millet varieties compared to finger millets which exhibited higher ($P < 0.05$) values. The lowest sodium content was observed in the PMZM variety at 2.00 mg/kg than all the other millet types. Potassium was the amplest mineral found in the millet grains ranging from 3864.6 to 4899.3mg/kg. The highest (P <0.05) potassium value was recorded for the PMSA with the lowest ($P < 0.05$) figure observed in the PMZM. Phosphorus was higher ($P < 0.05$) for the pearl millet varieties compared to the other types. Trace-mineral manganese was higher ($P < 0.05$) in the finger millet varieties with mean values of 205.7 (FMZM) and 350.05 (FMSA) for finger millet types and 9.17 (PMSA) -16.09 (PMZM) for pearl millet. The iron content was similar ($P < 0.05$) among the varieties, ranging from 71.28 to 71.96 mg/kg. The highest zinc was observed for PMSA (52.66 mg/kg) and lowest for FMSA (17.65 mg/kg).

Table 4.4. Mineral composition of millet grains varieties (mg/kg).

	FMSA	FMZM	PMSA	PMZM	SEM	probability
Macro-minerals						
Calcium	3443.91^{a}	3291.10^{b}	82.990^{c}	32.980^{d}	0.0000	0.0001
Phosphorus	2625.44^{d}	2749.6^{c}	3831.9^{a}	3226.4^{b}	0.0000	0.0001
Magnesium	1635.36^{a}	1588.9^{b}	1343.1^{c}	1279.3d	0.0355	0.0001
Potassium	4429.79^{c}	4796.7^{b}	4899.3^{a}	3864.6^{d}	0.0000	0.0001
Sodium	11.490^{c}	18.320^{a}	18.240^{b}	2.000^{d}	0.0005	0.0001
Trace-minerals						
Copper	6.660^{c}	6.660c	7.330^{a}	7.330^{b}	0.0000	0.0002
Manganese	350.1^{a}	205.7^{b}	9.170^{d}	16.09^{c}	0.0050	0.0001
Iron	71.28^{d}	72.95^{b}	77.66^{a}	71.96^{c}	0.0000	0.0001
Zinc	17.65^{d}	19.99^{c}	52.66^{a}	39.31^{b}	0.0000	0.0001

Values are means of duplicate analysed millet samples. $^{a, b, c, d:}$ Means followed by the same superscript in a row are not significantly different (P < 0.05).

4.4.5. Amino acid composition

Table 4.5 shows the limiting and non-limiting amino acids of millet types grown in different locations. Both finger millets irrespective of their growing area had higher (P < 0.05) essential amino acids than those occurring in pearl millets. However, in pearl millets lysine was higher (P < 0.05) compared to that of the finger millets. PMZM (0.48 g/100g) had the highest lysine value followed by PMSA (0.35g/100g) whereas; finger millet varieties ranged from 0.19 to 0.22 g/100g. Amino acid proline was the most plentiful (4.214g/100g) and significantly higher (P < 0.05) in the finger millet types. While the amino acid glutamine was the second highest among the millet varieties with an average mean of 1.64g/10

Table 4.5. Amino acid composition of millet grains (g/100g CP).

	Millet varieties					
	FMSA	FMZM	PMSA	PMZM	SEM	Probability
Essential amino acids						
Histidine	0.23^{b}	0.28^{a}	0.19^{c}	0.19^{c}	0.00133	0.0001
Arginine	0.60^{b}	0.66^{a}	0.53^{c}	0.36^{d}	0.00005	0.0001
Threonine	0.44^{a}	0.41^{b}	0.28^{c}	0.26^{d}	0.00020	0.0001
Lysine	0.19^{d}	0.22^{c}	0.35^{b}	0.48^{a}	0.00004	0.0001
Tyrosine	0.55^{a}	0.43^{c}	0.51^{b}	0.39^{d}	0.00005	0.0001
Methionine	0.57^{b}	0.91^{a}	0.20^{d}	0.23^{c}	0.00005	0.0001
Valine	0.56^{b}	0.53^{d}	0.57^{a}	0.54^{c}	0.00004	0.0001
Leucine	0.58^{a}	0.46^{b}	0.34^{d}	0.37^{c}	0.00005	0.0001
Non- Essential amino acids						
Serine	0.43^{c}	0.37^{d}	0.45^{b}	0.47^{a}	0.00005	0.0001
Glycine	0.36^{c}	0.23^{d}	0.49^{a}	0.39^{b}	0.00005	0.0050
Aspartic acid	0.51^{c}	0.35^{d}	0.75^{b}	0.97^{a}	0.00005	0.0001
Glutamine	1.93^{a}	1.16^{b}	1.48^{c}	1.99^{a}	0.00005	0.0001
Alanine	0.47^{c}	0.43^{d}	0.71^{b}	0.82^{a}	0.00005	0.0001
Proline	4.21^{a}	3.45^{b}	1.91^{c}	1.75^{d}	0.00000	0.0001
Isoleucine	0.49^{a}	0.23^{b}	0.11^{d}	0.15^{c}	0.00020	0.0001
Phenylalanine	0.92^{a}	0.82^{d}	0.85^{b}	0.83^{c}	0.00003	0.0001

Values are means of duplicate analysed millet samples. $^{a, b, c, d}$: Means followed by the same superscript in a column are not significantly different ($P < 0.05$).

4.5. Discussion

This study investigated different varieties of millet grain to identify their strength in relation to competing grains like maize and to compare varieties among themselves. The present study indicated that the kernel weights of pearl millet types were higher than in the finger millet types. This substantial variation in the thousands kernel weights could be attributed to the grain size; with finger millet being very minute in size in comparison to the pearl millet. Dholakia et al. (2003), states that kernel weight is associated with kernel size traits such as kernel length, kernel width and kernel thickness. In addition, kernel weight of grains is be believed to be related to the grain yield and milling quality (Botwright et al., 2002). The endosperm texture of PMSA was corneous, while that of FMSA, FMZM and PMZM were floury. Catechin and epicatechin were higher in the finger millet types, corresponding the colour red. Previous studies confirmed high presence of catechin and epicatechin in finger millet varieties (Xiang, 2018). Hunter value L* was higher in the pearl millet varieties than it was in the finger millet, which shows that pearl millet had a lighter coloured pericarp than the finger millet. The mean values of L* are slightly lower than the range of 68.47 to 74.00 reported by Ramashia, et al. (2018) and the values of 80.50 to 80.73 reported by (Gull et al., 2015). The value a* which stands for redness was understandably higher in the finger millet types; the values were higher than the report by (Ramashia et al., 2018). The variations might be attributed to the different cultivars used.

The results in the current study also observed that there is an inverse correlation between the catechin and epicatechin contents and the total kernel weight (TKW), however, this relationship is not seen as a causative correlation but attribute it to the varietal differences. Xiang, (2018) reported that catechin and epicatechin are the predominant flavonoids in finger millet varieties. This property of finger millet variety makes them beneficial for human health as catechins are considered natural antioxidants that help prevent cell damage and provide other health benefits in the body as stated in (Fan et al., 2017).

Generally, the nutrient composition reported in this study, was within the range of report by (Ali et al., 2003). Highest protein content was observed in the Zimbabwean pearl millet

(9.41g/100g), which was within the range of the protein content of 4.6 - 9.9% (Hadimani et al., 1995; Kiprotich et al., 2015; and Anitha, 2019). The lowest protein content observed in finger millet Zimbabwe (6.43) was also in agreement with the findings of (Anita, 2019; Thippeswamy, 2016) but lower than 8.13 to 8.74% range reported by (Jayawardana et al., 2019). The crude fibre content of finger millet is higher than what was reported by David et al. (2014) in finger millet, but in agreement with the report of (Shrestha & Karki 2019; Jayawardana et al., 2019). Abdalla et al. (1998) also reported the same results for fibre in pearl millet. Fibre is considered important for gut health as stated by McIntosh et al. (2003) and moderate intakes of high fibre in foods could result in improvement of gut health. Likewise, fibre is important in the prevention of heart diseases, colon cancer and diabetics (Chinma et al., 2007). Furthermore, ether extract for pearl millet in the current study was higher than what was stated by David et al. (2014) on studies on finger millet but it was somewhat the same in the finger millet types. Moreover, the levels of ADF and NDF are critical because they impact animal productivity and digestion in livestock production. Thus, the NDF and ADF in the current study were higher in finger millet varieties validating those finger millet varieties. The higher ADF and NDF contents in the feed ingredient are simply an indication of low energy, which was the case in finger millets.

The starch content is known as an important determinant of grain yield and quality. In the present study the starch content was lower than what was reported by (Shrestha & Karki 2019; Mustafa et al., 2008). The content was the highest in the finger millet types; however, it was lower than the 63.43 and 73.29% of carbohydrates reported by (Thippeswamy, 2016). Furthermore, the starch content was also lower than report by Ali et al. (2003) for pearl millet but within the range of the report of (Balasubramanian et al., 2014).

Wafula et al. (2018) observed a significant variation in calcium content of finger millet cultivated in different locations, which was attributed to the calcium content in the soil in these different locations. Some of the differences among the millet grains could be credited to the varietal differences as well. Although the current study observed lower essential nutrients like calcium (32.98 to 82.99 mg/kg) for pearl millet when compared to finger millet varieties, the calcium content was still within the range of (31.77mg/kg to 728.71mg/kg) recorded by (Adeoti et al., 2017). Kumar et al. (2018) stated that calcium content of finger millet is about eight times higher than wheat making it the richest source of calcium (348 mg/100 g), calcium in diets has the ability to prevent osteoporosis in humans and animals equally. Adeoti et al. (2017) also reported magnesium content for pearl millet that ranged from 340.27 mg/kg to 4769.9 mg/kg. Phosphorus was found in abundance in the varieties investigated, pearl millet types had the highest share, and the result is in the same range of (295.55 mg/100gm) reported by Mariod et al., (2017), for pearl millet varieties. Phosphorus which is considered the precursor to energy in the body, Devi et al. (2011) has the benefit of boosting the energy content of the millet. The fluctuations in minerals contained in pearl millet, might have been caused by the biggest factor nature of the soil (Kiprotich et al., 2015). Potassium on the other hand, was the amplest mineral in the current study, the varieties however, contain less than what was reported by Mariod et al. (2017) but more than Abdulrahman & Omoniyi, (2016) who reported the value of 366.67mg/100g in millet varieties. Manganese content was higher in the finger millet varieties; the result is higher than the values reported by Sanusi et al. (2019). The iron content of the current study was also reported to be higher than the report of 1.25 to 1.46 by Sanusi et al. (2019).

The amino acid content of the varieties investigated recorded lower values than reports by other researchers. Amino acid histidine was the least, however lysine was also limiting.

Although the amino acid contents in the varieties investigated were quite low in comparison to what have been reported in literature (Macrae et al., 1993; Obadina et al., 2016) it contains most of the essential and non-essential range. The result is within the range of amino acid content reported in a study by Vasan et al. (2008) and higher than the findings of Mohammed Nour et al. (2015) in a study conducted on pearl millet supplemented with fenugreek seeds.

4.6. Conclusions

The present study concluded that millets contain good source of energy, starch, protein, fibre, and other essential nutrients which could be of benefit to both humans and livestock. This study, therefore, supports the suggestions that millet could be utilized as partial replacement crop for other common cereals, as source of energy and can also contribute to the improvement of communities who consume it as staple food.

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ORIGINAL RESEARCH

Assessment of the phenolic compounds of pearl and finger millets obtained from South Africa and Zimbabwe

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Abstract

Millet grains are rich in phenolic compounds which have health benefits. This study aims to elucidate the phenolic properties of pearl and finger millet grown in South Africa and Zimbabwe. The milled samples were analyzed by Waters Synapt G2 Quadrupole time-of-flight (QTOF) mass spectrometer (MS). A total of eight phenolic compounds were detected and quantified in the millet varieties, which included derivatives of benzoic acid such as protocatechuic and p-hydroxybenzoic acids. Flavonoids such as catechin, epicatechin, procyanidin B1, procyanidin B2, and kaempferol glycoside were also detected. Generally, catechin was the dominant phenolic compound, followed by epicatechin. The mean values for catechin ranged from 2.50 to 12.6 mg/kg for the pearl millet and 610.4 to 675.1 mg/kg for the finger millet. While the epicatechin mean values ranged between 1.2 to 1.8 for pearl millet and 99.1 to 139.5 for finger millet. Procyanidins B1 and B2 were only detected and quantified in the finger millet types, While Kaempferol glycoside was only recorded in the pearl millets with mean values of 196.0 mg/kg for pearl millet South Africa and 213.6 mg/kg for pearl millet Zimbabwe. There was a difference among the varieties for the content of kaempferol glycoside. Protocatechuic and p-hydroxybenzoic acids were only present in the finger millet types, their mean values were (20.9, 23.7 mg/kg) and (16.8,13.5 mg/kg) respectively. It can be substantiated from the outcome of this study that millet can be used as a source of valuable phenolic compounds and that the variety of millet is the determining factor of the phenolic compound content.

KEYWORDS

flavonoids, high-resolution UPLC-MS, millet, phenolic acids, phenolic compounds

1 | INTRODUCTION

Millet is regarded as one of the economically important cereals after maize, rice, wheat, barley, and sorghum (Prasad & Staggenborg, 2009). It is mostly grown in semi-arid zones and is used for human food and livestock feed (Amadou, Le, Amza, Sun, & Shi, 2013). Nutritionally, millet is on par with other popular cereals and its grains are abundant in phytochemicals making it very vital as feed and food (Chandrasekara, Naczk, & Shahidi, 2012; Shahidi & Chandrasekara, 2013). Furthermore, they are natural sources of antioxidants in food and biological systems which have health benefits that may work against several pathophysiological conditions (Shahidi

CHAPTER 5

ASSESSMENT OF THE PHENOLIC COMPOUNDS OF PEARL AND FINGER MILLETS OBTAINED FROM SOUTH AFRICA AND ZIMBABWE

5.1. Abstract

Millet grains, in addition to containing valuable nutrients, are also rich in phenolic compounds which have health benefits for human and livestock. This study aims to investigate the phenolic compounds properties of pearl and finger millet grown in South Africa and Zimbabwe. The samples were analyzed by Waters Synapt G2 Quadrupole time-of-flight (QTOF) mass spectrometer (MS) connected to a Waters Acquity ultra-performance liquid chromatograph (UPLC) (Waters, Milford, MA, USA) for high-resolution UPLC-MS analysis. Total of 8 phenolic compounds were detected and quantified in the millet varieties, which included derivatives of benzoic acid. such as, protocatechuic and p-hydroxybenzoic acids, flavonoids such as catechin, epicatechin, procyanidin B1, procyanidin B2 and kaempferol glycoside, in addition to amino acid tryptophan. Generally, catechin was the dominant phenolic compound, followed by epicatechin. Their mean values ranged from (2.50, 12.6, 610.4 and 675.1mg/kg) and (1.20, 1.80, 99.1 and 139.5mg/kg) respectively, the highest values were recorded in the finger millet types; there was highly significant difference for the content of Catechin and Epicatechin across the varieties ($P < 0.05$). Procyanidin B1 and procyanidin B2 were only detected and quantified in the finger millet types. While Kaempferol glycoside was only recorded in the pearl millet type with mean values of 196.0 and 213.6 mg/kg, the highest value was detected in pearl millet Zimbabwe. There was a significant difference among the varieties for the content of Kaempferol glycoside ($P < 0.05$). Protocatechuic and p-hydroxybenzoic acids which were the only phenolic acids detected in the millet varieties, were only present in the finger millet types,

their mean vales were (20.9, 23.7 mg/kg) and (16.8,13.5 mg/kg) there was a highly significant difference among the treatments ($P < 0.05$). It can be substantiated from the outcome of this study that millet can be used as a source of valuable phenolic compounds and that the variety of millet is the determining factor of the phenolic compounds content.

5.2. Introduction

Millet is one of the economically important cereals after maize, rice, wheat, barley and sorghum, it is mostly grown in semi-arid zones, Amadou et al. (2013) as human food and for livestock feed. It is a smallseeded cereal, which belongs to the grass family *Poaceae (Gramineae*). Nutritionally, millet is on par with other popular cereal grains. In addition to that, the grains also have an abundance of phytochemicals, mainly phenolic compounds, making them very vital as feed and food (Chandrasekara et al., 2012; Shahidi & Chandrasekara, 2013). Their ability to flourish in unfavorable climatic conditions has encouraged scientists to further study other properties of these small but valuable seeds. Furthermore, they are natural sources of antioxidants in food and biological systems which has health benefits that may work against several pathophysiological conditions (Shahidi & Chandrasekara, 2013). Among the physiognomies of millet which need to be explored are the phenolic compounds.

Phenolic compounds are a class of secondary metabolites found in plants and further divided into phenolic acids and polyphenols. Furthermore, phenolic acids are divided into two classes: hydroxybenzoic and hydroxycinnamic acids (Dykes & Rooney, 2006). The main dietary phenolic compounds include the phenolic acids, flavonoids, and tannins (King & Young, 1991). The phenolic acids and flavonoids are also considered vital in promoting health by reducing the risk of metabolic syndrome and the related complications of type 2 diabetes (Lin et al., 2010). In addition, these compounds can act as antioxidants and UV screens like the flavonoids (Lin et al., 2016). To date, there is no uniform set of identified and quantified phenolic compounds in different millet varieties.

Chethan et al. (2008) identified nine phenolic acids which include gallic acid, protocatechuic acid, p-hydroxybenzoic acid, vanillic acid, ferulic acid, syringic acid, trans-cinnamic acid and p-coumaric acid in millets. On the other hand, Chandrasekara & Shahidi, (2011) found that hydroxycinnamic acids and their derivatives were the main contributors to the total phenolic compounds of insoluble bound phenolic fraction of millet varieties. However, in another study by Xiang et al. (2019), flavonoids were found to be the predominant phenolic compound in different millet varieties. Whereas Sharma et al. (2017) reported higher amounts of phenolic content and antioxidant activity in methanolic extracts of kodo millet grains. In like manner, Pradeep & Sreerama, (2015) found that Kaempferol was the most abundant flavonoid in raw millet varieties. In comparison, the most dominant phenolic compounds in maize varieties are Phenolic acids followed by flavonoids (García-Salinas et al., 2017). It is, however, important to realize that different analysis methods also affect the total phenolic compound contents of plants. Chettan & Malishi, (2007), argue that although different solvents are being used to extract phenolic compounds from plant foods, acidified methanol is the best organic solvent for extraction of phenolic compounds from millets. Other factors such as environmental conditions, cultivar of a plant, processing conditions, and storage are also found to affect the quantity of phenolic compounds in plants. Environmental factors such as sun exposure, soil type, and rainfall influence the phenolic content of plants (Manach et al., 2004). Similarly, Shahidi & Naczk (2004) also reported that the type and the content of the phenolic compounds of grains depends on the type of millet, variety, part of the grain, climatic conditions and cultivation practices.

More studies to expansively profile the phenolic compounds of millet types, in different locations are therefore warranted. With all these interesting incites and the importance of millet grains mentioned above, studies on phenolic compounds in millet are limited, particularly in the Southern Africa region. This study aims to investigate the phenolic compounds of pearl and finger millet grains obtained from South Africa and Zimbabwe by LC-MS method using the Synapt G2 qTOF from Waters (Milford, USA).

5.3. Materials and Methods

5.3.1. Plant materials

Pearl millet (*Pennisetum glaucum*) used in this study was obtained from ARC (Agricultural Research Council) Grain crop institute in Potchefstroom, in the North West province. The variety, ARC-PM 01, was imported from ICRISAT-Bulawayo in 2005, among other pearl millet germplasm, for performance test under South African condition. After testing over three locations in South Africa (Potchefstroom, Taung, Polokwane) over two seasons, the variety was selected as top performer among 36 varieties based on agronomic desirability and grain yield. Variety ARC-PM 01 was further undergone regress selection over the years through conventional breeding and submitted for variety listing for production in SA. The variety was grown during 2017/18 growing season at the ARC-Grain Crops Experimental Farm, Potchefstroom, North West Province. The finger millet (*Elusine coracana*) was sourced from a local market in Johannesburg. The Zimbabwean millet samples were obtained from local market in Harare. The samples were milled using a grinder and sifted to produce a fine texture. The samples were prepared in duplicate and ready for further analysis.

5.3.2. Extraction of phenolic compounds

The extracts were prepared by using 2g dry millet material + 15 ml 50% methanol/1% formic acid in water with ultrasonication for 1 hour and standing overnight, followed by centrifugation and transfer of the supernatant to a glass vial ready for the LC-MS analysis.

5.3.3. Liquid Chromatography Mass Spectrometry (LC-MS) analysis

The samples were analyzed by LC/MS quadrupole time-of-flight (QTOF)) mass spectrometer (MS) connected to a Waters Acquity ultra-performance liquid chromatograph (UPLC) (Waters, Milford, MA, USA) was used for high-resolution UPLC-MS analysis. Electrospray ionization was used in negative mode with a cone voltage of 15 V, desolvation temperature of 275 °C, desolvation gas at 650 L/h, and the rest of the MS settings optimized for best resolution and sensitivity. Data were acquired by scanning from m/z 150 to 1500 m/z in resolution mode as well as in MSE mode. In MSE mode two channels of MS data were acquired, one at a low collision energy (4 V) and the second using a collision energy ramp (20−60 V) to obtain fragmentation data as well. Leucine enkaphalin was used as lock mass (reference mass) for accurate mass determination and the instrument was calibrated with sodium formate. Separation was achieved on a Waters HSS T3, 2.1 × 100 mm, 1.7 μm column. An injection volume of 2 μL was used and the mobile phase consisted of 0.1% formic acid (solvent A) and acetonitrile containing 0.1% formic acid as solvent B. The gradient started at 100% solvent A for 1 min and changed to 28% B over 22 min in a linear way. It then went to 40% B over 50 s and a wash step of 1.5 min at 100% B, followed by re-equilibration to initial conditions for 4 min. The flow rate was 0.3 mL/min, and the column temperature was maintained at 55 °C.

5.3.4. Statistical analysis

Data obtained were subjected to a one-way Analysis of Variance (ANOVA) of Statistical Analysis Software (SAS), version 24.0.Duncan's multiple comparison tests was used to compare the means. The mean values were statistically significant at (P < 0.05). Principal component analysis (PCA) is a multivariate analysis used to emphasize variation and bring out strong patterns in a dataset. The PCA analysis was conducted using PAST version 4.02, (2020), software for scientific data analysis, with functions for data manipulation, plotting, univariate and multivariate statistics analysis.

5.4. Results

Table 5.1 shows the retention time, fragments, elemental composition, and tentative identification of phenolic compounds detected in the four millet varieties. Total of eight (8) phenolic compounds were detected and quantified in the millet types, which included derivatives of benzoic acid such as, protocatechuic acid and p-hydroxybenzoic acid, flavonoids such as catechin, epicatechin, procyanidin B1, procyanidin B2 and kaempferol glycoside, in addition to amino acid tryptophan. Citric acid was also detected but not quantified in any of the varieties. Numerous unknown compounds were also detected during the analysis which was later assigned to suggested compounds using the elemental composition, through SCI- FINDER application. Generally, finger millet varieties had more phenolic compounds than the pearl millet.

5.4.1. Total flavonoids

The contents of flavonoids in the millet varieties determined by the LC-MS method are shown in (Table 5.2). The flavonoids in finger millet were more than what is recorded in the pearl millet types, indicating differences. Catechin was the dominant flavonoid (Figure 5.6), followed by epicatechin, finger millet varieties had the highest average mean values of 642.75mg/kg in finger millet South Africa (FMZA) and 119.3 mg/kg in finger millet Zimbabwe (FMZM). There was highly significant difference ($P < 0.05$), for the content of the catechin and epicatechin across the varieties. In addition, kaempferol glycoside was only detected in the pearl millet types, with mean values of 196.0 and 213,6 mg/kg. The highest value was detected in pearl millet Zimbabwe (PMZM) and lowest in pearl millet South Africa (PMZA).

Moreover, it can be seen in (Figure 5.6) that catechin was recorded at 10.45 minutes retention time and [M-H] of 289, 0673 while epicatechin peaked at retention time of 12, 46 minutes and [M-H] of 289, 0704 for the finger millet type. Consecutives chromatograms of pearl millet types showed no presence of the two compounds but high peaks for the flavonoids kaempferol glucoside at the retention rate of 14,78 and [M-H] of 609,1454 (Figures 5.8 and 5.9). The predominance of the catechin in finger millet varieties is clearly indicated by the high peaks as shown in ((Figure 5.6 and 5.7, and a high percentile in Figure 5.1). While p-hydroxybenzoic acid and procyanidin B2 registered their presence at the bottom of the percentile. In addition, it can also be noted that many peaks with almost similar retention time and M-H was captured but not identified, indicating the possibility of other dimers of catechin and epicatechin might exist in the millet types investigated. Compounds 6 and 7 (catechin and epicatechin) were detected at the same M-H formula at 289, suggesting that the two compounds are isomers. Likewise, compounds 5 and 8 (Procyanidin B1 and Procyanidin B2) were detected at the same M-H formula of 577.

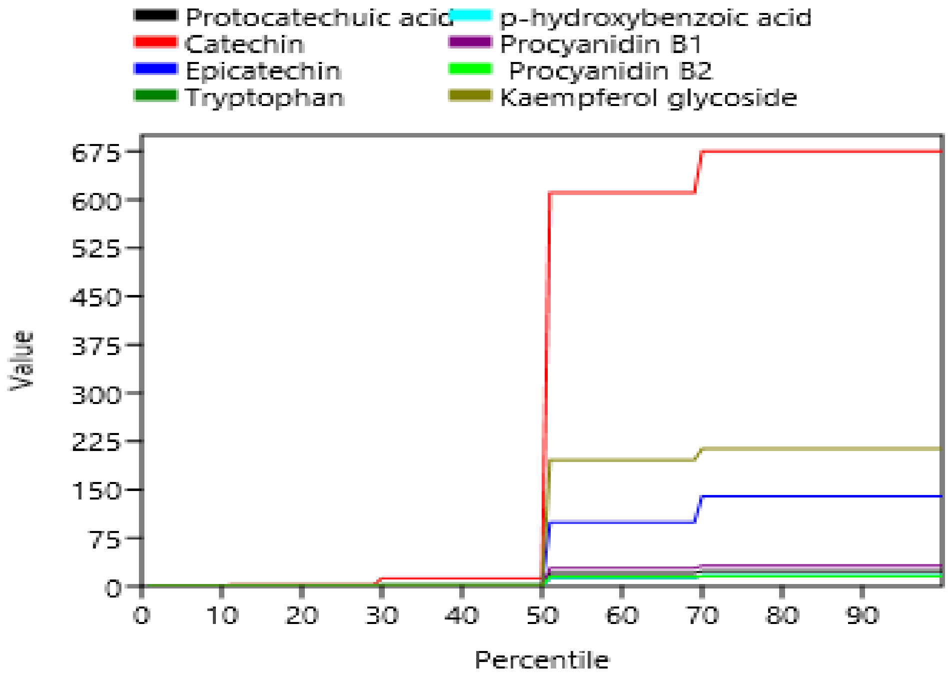

Figure 5.1 Percentile plot of the phenolic compounds

Table 5.1. Phenolic compounds identified in the extracts of millet grains using LC-MS methods.

No	Retention time	[M-H]- formula	Fragments	[M-H] elemental composition	UV$_{ma}$	Tentative identification
1	1,690	191,018	Nd	C6H7O7	265	Citric acid
2	7,350	153,019	153,109	C7H5O4	258,294	Protocatechuic acid
3	8,540	203,0811	190	C11H11N2O2	278	Tryptophan
4	9.178	137,0235	128,117	C7H5O3	277,320	P-hydroxybenzoic acid
5	9,820	577,137	289,161,125	C30H25O12	278	Procyanidin B1
6	10,45	289,0673	Nd	C15H13O6	279	Catechin
7	12,46	289,0704	Nd	C15H13O6	279	Epicatechin
8	13,93	577,1281	Nd	C30H25O12	278	Procyanidin B2
9	14,78	609,1454	489,429,327,309,297	C27H29O16	268,347	Kaempferol glycoside

The table shows the retention time, the M-H formula, fragments, and elemental composition. Nd; not detected.

5.4.2. Phenolic acids

The first compounds to be identified was citric acid at a retention time of 1.69 minutes, however, its fragment was not detected, and it was not quantified in any of the millet types, as shown in Table 5.1. However, p-hydroxybenzoic acid, which is a monohydroxy benzoic acid, a phenolic derivative of benzoic acid was quantified but only detected in the finger millet varieties with the mean values of 13.5 and 16.8 mg/kg. The highest mean value was in the finger millet grown in Zimbabwe. Protocatechuic acid, on the other hand, was the predominant phenolic acid found in finger millets ranging from 20, 9 to 23,7mg/kg. There was no presence of this acid detected in the pearl millet types. Protocatechuic and p-hydroxybenzoic acids were detected at the retention times of 7, 35 and 9,178 and [M-H] formula of 153,019 and 137, 0235.

Table 5. 2. Individual phenolic compounds in millet varieties (mg/kg).

Samples	FMZA	FMZM	PMA	PMZM	SEM	Probability
Protocat	20.90^{b}	23.70^{a}	Nd	Nd	0.0500	0.001
Cat	610.4^{b}	675.1^{a}	12.6^{c}	2.50^{d}	0.0025	0.001
Epicat	99.10^{b}	139.5^{a}	1.80^{c}	1.20^{d}	0.0050	0.001
Trypt	3.000^{a}	2.100^{b}	16.5^{c}	12.5^{d}	0.0500	0.001
p-hydroxbenz	16.80^{a}	13.50^{b}	Nd	Nd	0.0354	0.001
ProcB1	28.40^{b}	31.90^{a}	Nd	Nd	0.0354	0.001
ProcB2	15.50^{b}	15.80^{a}	Nd	Nd	0.0354	0.001
Kaemp-glyco	Nd	Nd	196.0^{b}	213.6^{a}	0.0289	0.001

Mean values of individual polyphenols. a, b, c, d: Means in the same row with different superscripts are significantly different at (P <0.05). Abbreviations: Protocat: Protocatechuic acid, Cat; Catechin, Epicat; Epicatechin, Trypt.; Tryptophan, p-hydroxbenz; p-hydroxybenzoic acid, ProcB1; Procyanidin B1, ProcB2; Procyanidin B2, and Kaemp-glyco; Kaempferol glycoside.

5.4.3. Principal component analysis (PCA)

Figure 5.2 showed a loading of procyanidin B1 and B2, and p-hydroxybenzoic acid in the same area on the right middle side of the score plot, indicating possible link between these phenolic compounds. In the same way, catechin, tryptophan and kaempferol glycoside clustered at the right topside of the plot. In the model plotted, the principal components (PCs) explained variance of 99.96% of the data. The two principal components pc1 and pc2 had variabilities of 99.8990 and 0.05817, respectively as listed in table 4. The loading plots shown in Figure 5.2, indicates that a positive correlation exist between (CP1) and catechin, epicatechin, p-hydroxybenzoic acid, kaempferol, procyanidin B1 and B2; moreover, negative correlation was observed between CP1 and the protocatechuic. High component loadings for (CP1), was contributed by finger millet Zimbabwe (FMZM) variety. Epicatechin was the last phenolic compound appeared at the bottom of the plot. However, (PC2) inversely correlated with most of the phenolic compounds with the exception of the protocatechuic acid.

Pearson correlation plot in (Figure 5.3, Table 5.3) showed a significant negative correlation between p-hydroxybenzoic acid and both epicatechin and tryptophan as indicated by the blank space in the figure. A general positive correlation among the phenolic compounds is evident as indicated by the blue circles in the figure.

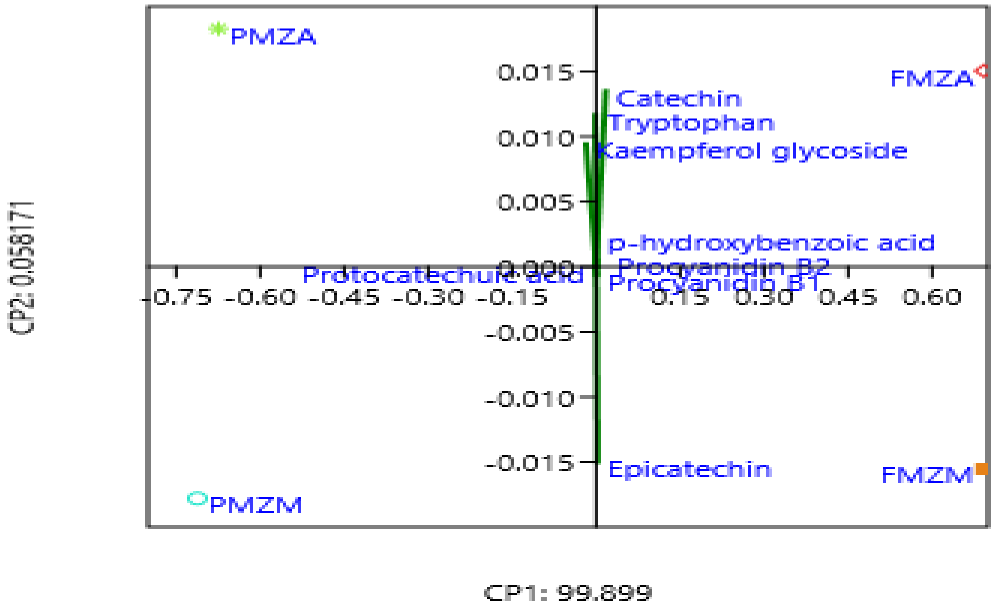

Figure 5.2 Principal component analysis (PCA) scatter plot of the phenolic compounds.

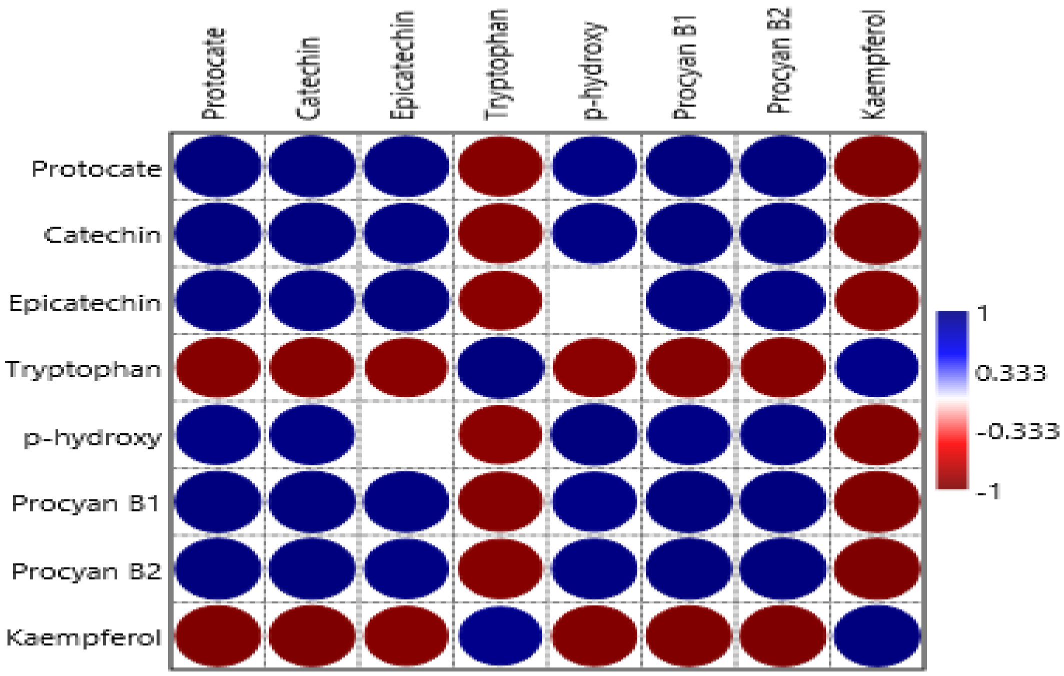

Figure 5.3. Pearson correlation plot for the phenolic compounds.

Protocate= Protocatechuic acid, Procyan B1= Procyanidin B1, Procyan B2= Procyanidin B2, p-hydroxy= p-hydroxybenzoic acid.

5.4.5. Pearson correlation between the phenolic compounds

Pearson correlation was performed to investigate the correlations among the individual phenolic compounds. The results indicate that strong positive correlation exists between protocatechuic acid and catechin, epicatechin, p-hydroxybenzoic acid, procyanidin B1 and B2. Exception was observed between protocatechuic acid and both tryptophan and kaempferol where a strong negative correlation existed. The same results were observed for catechin and epicatechin. There was also strong correlation between p-hydroxybenzoic acid strongly correlated positively with procyanidin B1 and B2 and strong negative correlation with kaempferol.

Table 5. 3. Pearson correlation between the phenolic compounds

	Protocate	**Catechin**	**Epicatechin**	**Tryptophan**	**phydroxybenzoic**	**Procyanidin B1**	**Procyanidin B2**	**Kaempferol**
Protocatechuic		0.0009	0.0125	0.0337	0.0281	0.0007	0.0028	0.0144
Catechin	0.9990		0.0156	0.0448	0.0248	0.0008	0.0024	0.0007
Epicatechin	0.9875	0.9843		0.0484	0.0771	0.0135	0.0270	0.0144
Tryptophan	-0.9662	-0.9551	-0.9516		0.0624	0.033	0.0369	0.0346
phydroxybenzoic	0.9719	0.9752	0.9229	-0.9375		0.0265	0.0131	0.0253
Procyanidin B1	0.9999	0.9991	0.9865	-0.9662	0.9735		0.0023	0.0007
Procyanidin B2	0.9972	0.9975	0.9729	-0.9631	0.9869	0.9977		0.0019
Kaempferol	-0.9999	-0.9993	-0.9856	0.9654	-0.9747	-0.9999	-0.9980	

As shown in Table 4, the principal component1 (PC1), had the highest eigenvalue of 1.64066, which explains the highest variance of 99.899% in the principal component1 (PC1). In this study, the variations are caused by only a few phenolic compounds. In the case of the (PC1), the variation was influenced by catechin and kaempferol glycoside, which had a negative loading as shown in Figure 5.4. The major contributors to variation in (PC2) are catechin, epicatechin, tryptophan and kaempferol glycoside, epicatechin had a negative loading. (CP3) was influenced by epicatechin, tryptophan and kaempferol glycoside. It is noticeable that kaempferol glycoside contributed significantly to the variations of all the three components.

Table 5. 4. Principal component analysis of the phenolic compounds showing their percentage contribution to the total variations.

	PC 1	PC 2	PC 3
Protocatechuic acid	0.02453	-0.02285	-0.00366
Catechin	0.68074	0.53444	0.28301
Epicatechin	0.12519	-0.59342	0.77301
Tryptophan	-0.04816	0.46168	0.39881
p-hydroxybenzoic acid	0.01682	0.09318	-0.15252
Procyanidin B1	0.03317	-0.02493	-0.01318
Procyanidin B2	0.01726	0.01875	-0.05059
Kaempferol glycoside	-0.71855	0.37269	0.37054
Eigenvalue	1.64066	1.00370	0.90270
% variance	99.8990	0.05817	0.04265
Cumulative %	99.8990	99.9140	100

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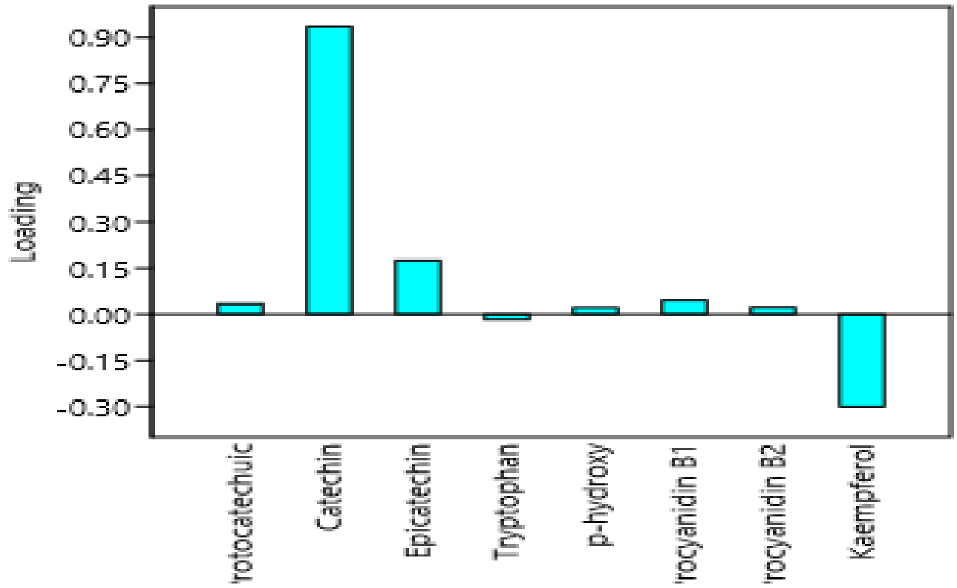


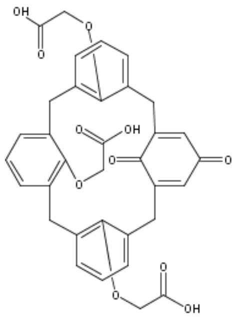
Figure 5.4. Loadings plot of the phenolic compounds and their percentage contribution to the total variations.

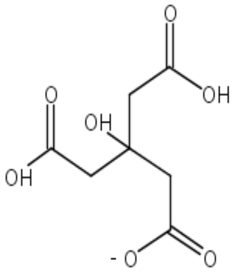
5.4.6. Unknown compounds

A total of 4 unknown compounds with identified elemental formulas were also detected during the analysis. These compounds as presented in (Figure 5.5) were assigned to possible compounds using SCI-FINDER®. They include Acetic acid, 2,2',2"-(5,28-dioxopentacyclo)**,** glutaric acid, Benzopyran and b-D-Glucopyranoside.

1. C34 H28 O11

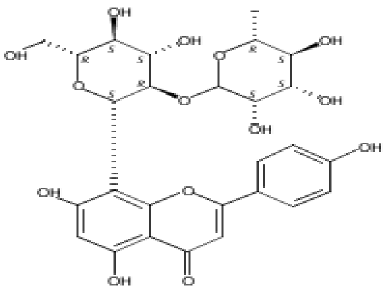
Acetic acid, 2,2',2''-(5,28-dioxopentacyclo)



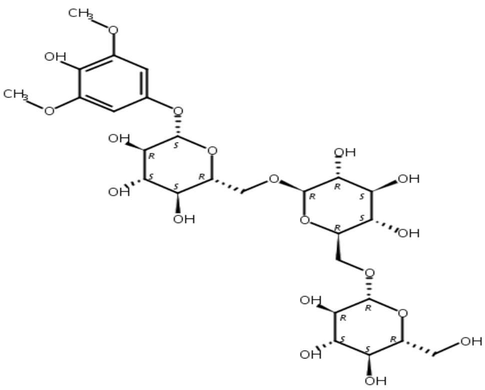


2. **C7 H10O7**

3-carboxymethyl-3-hydroxy-glutaric acid



3. C27 H30 O14 4H-1- Benzopyran-4- one, 8-[2-O-(6-deoxy-Dmannopyranosyl)-b-D-glucopyranosyl]-5,7-dihydroxy-2-(4-hydroxyphenyl)-



4. C26 H40 O19. b-D-Glucopyranoside, 4-hydroxy-3,5-dimethoxyphenyl O-b-D-glucopyranosyl-(1®6)-O-b-D-glucopyranosyl-(1®6).

Figure 5.5. Possible fragmentation structures for compounds unknown

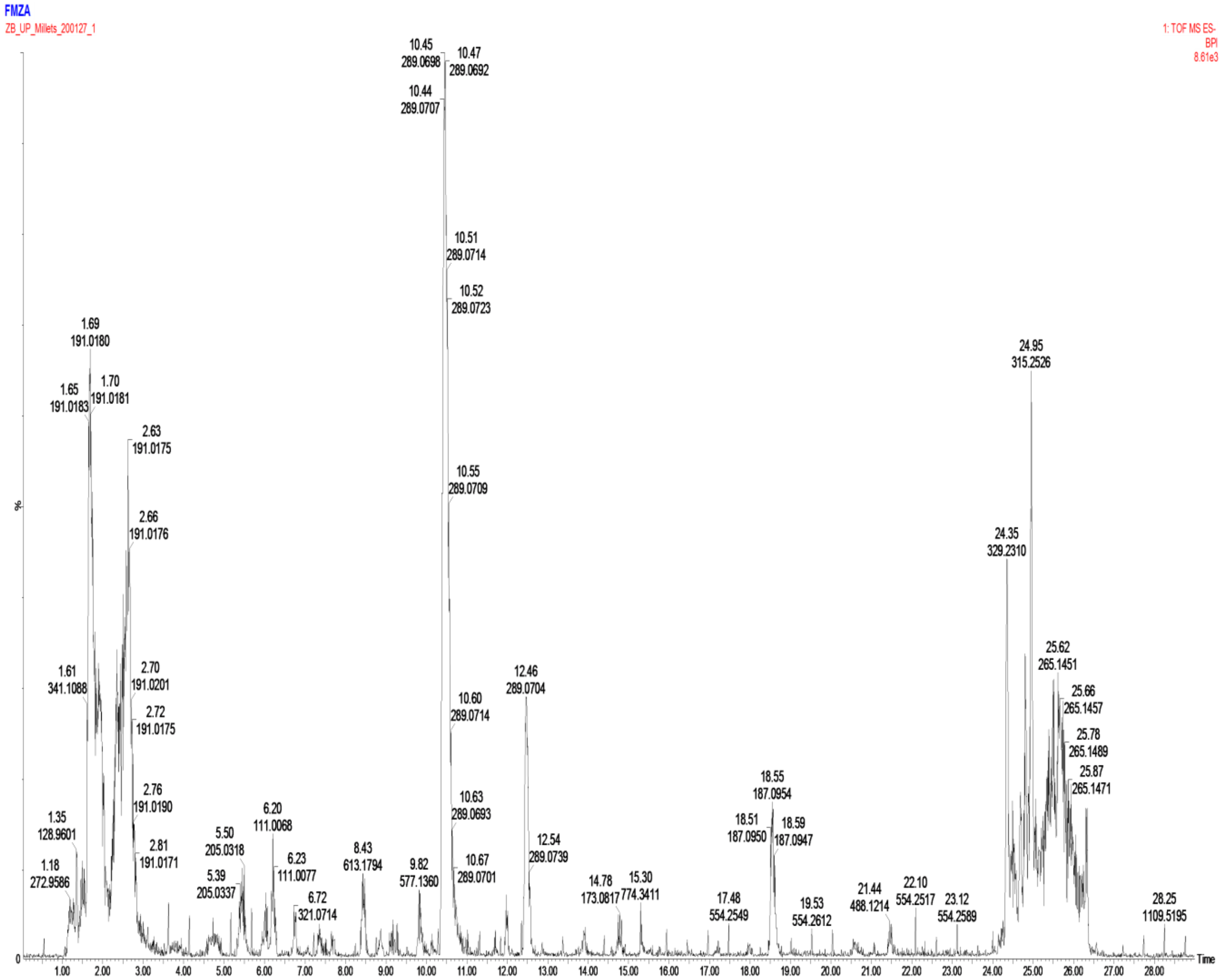


Figure 5.6. Finger Millet South Africa LC-MS Chromatogram.

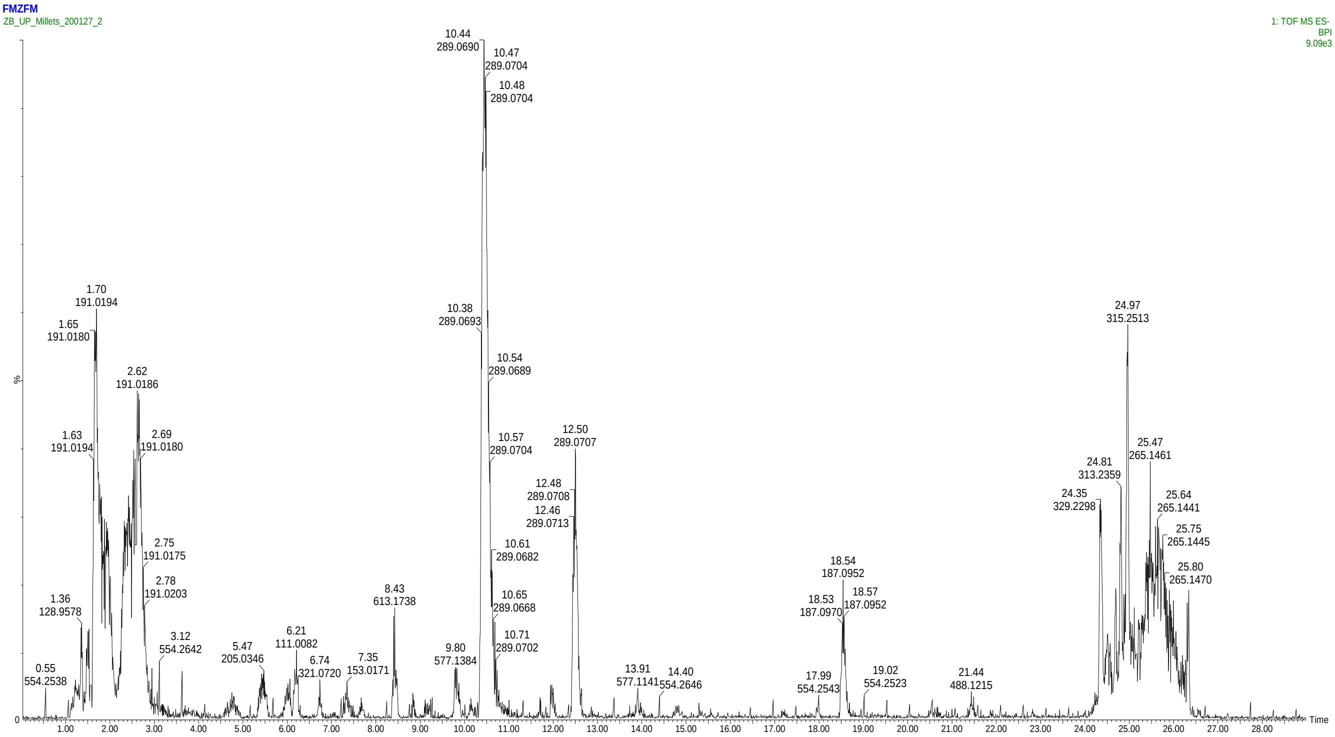


Figure 5.7. Finger Millet Zimbabwe LC-MS Chromatogram

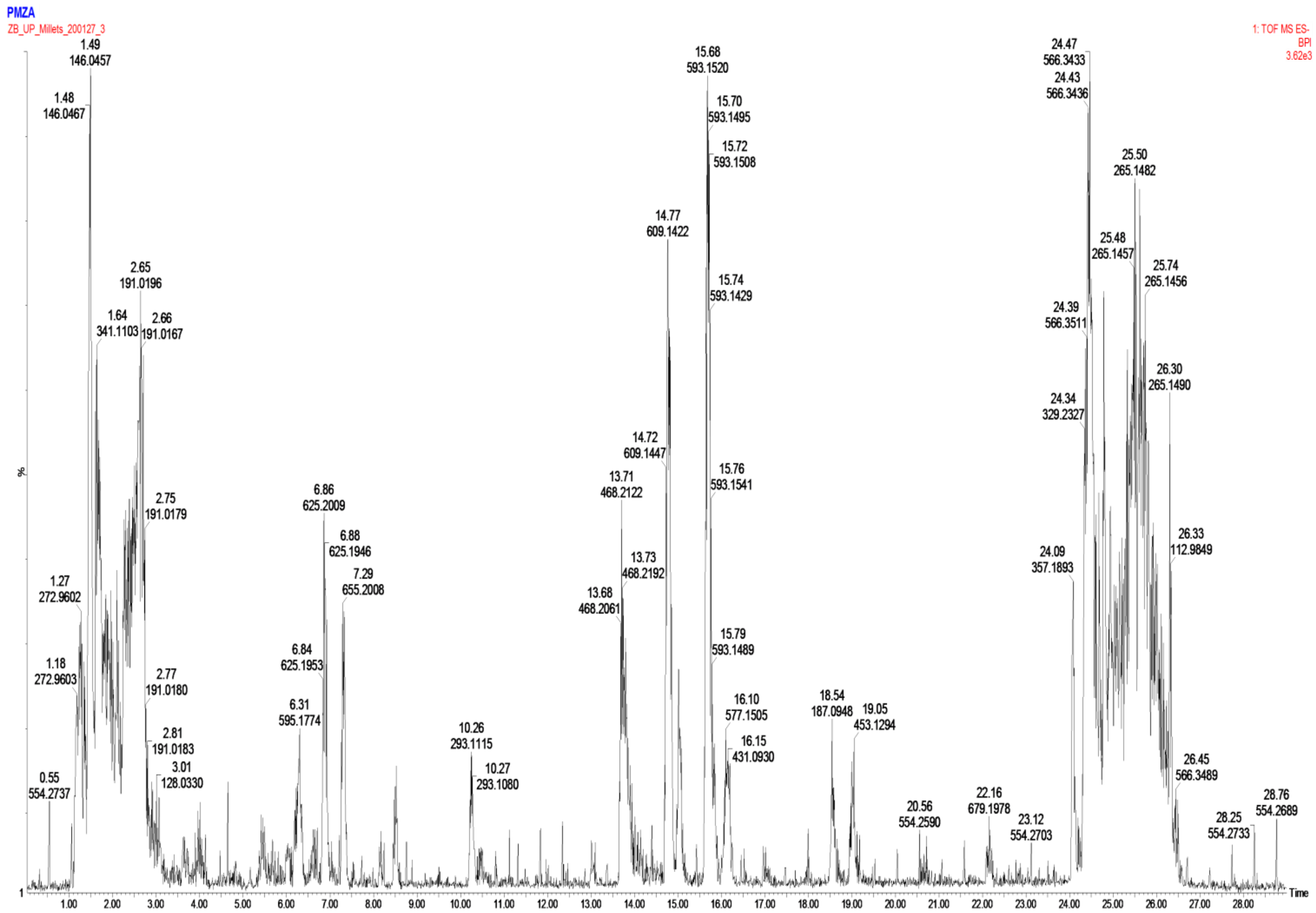


Figure 5.8. Pearl Millet South Africa LC-MS Chromatogram.

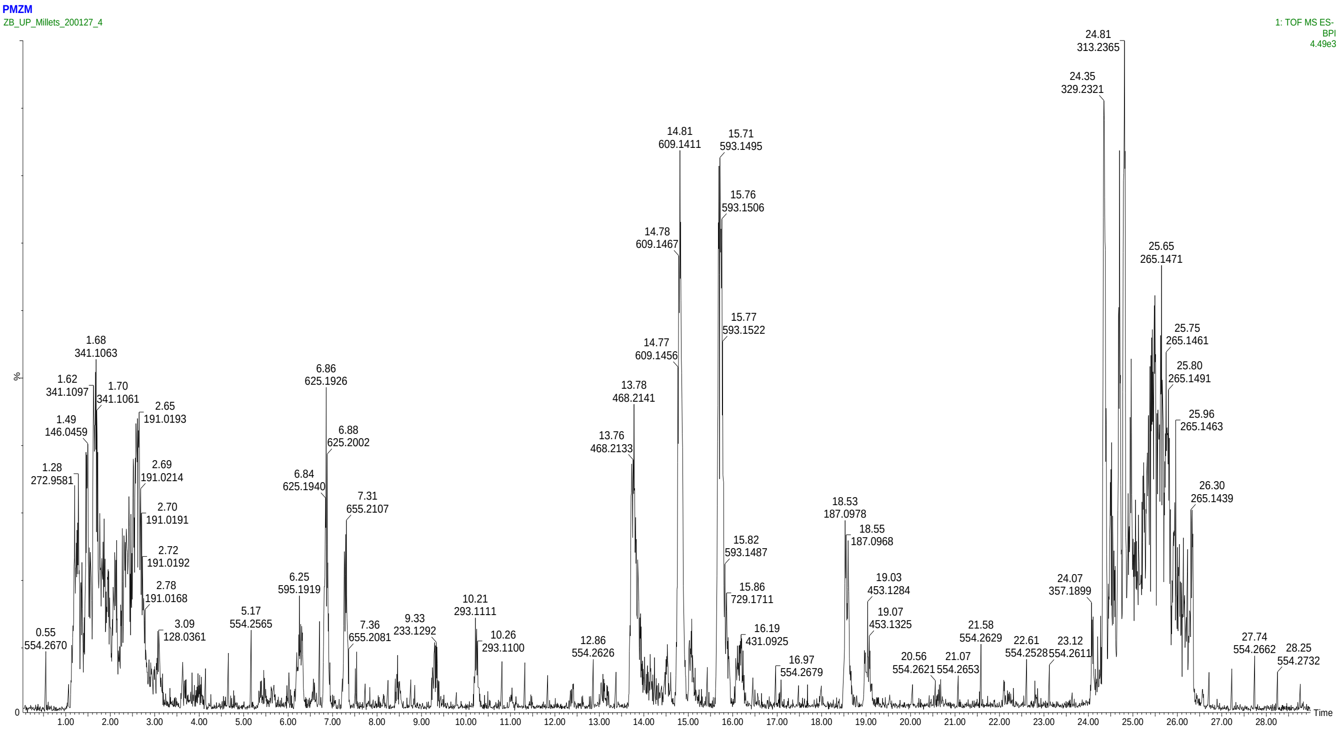


Figure 5.9. Pearl Millet Zimbabwe LC-MS Chromatogram.

5.5. Discussion

In this study, most of the detected and quantified phenolic compounds were from the finger millet types. The results of this investigation showed that the variety of the millet has influence on the content and type of phenolic compounds more than the place of cultivation. A finger millet type was found to have the highest percentage of the flavonoids such as catechin and epicatechin. This finding is consistent with Xiang et al. (2019), where catechin and epicatechin were the abundant flavonoids in finger millet varieties. Surprisingly, these findings are in disagreement with the findings of Rao & Muralikrishna, (2002) which states that the phenolic acids and tannins are the main polyphenols in cereals and that flavonoids are present in small quantities. This could be attributed to different locations and the place of origin of the millets used in the study. Protocatechuic acid was detected and quantified in this study, knowing that it is a phenolic compound with pharmaceutical properties, such as antiatherosclerosis, antiviral, antifibrotic and anticancer (Kakkar & Bais, 2014), (Chandrasekara & Shahidi, 2011a), the presence of these compounds indicates possible health benefits when these grains are used as food. The data presented, further substantiate the health benefit associated claims of finger millet consumption with regards to its flavonoid components.

Contrary to the findings by Ralph et al. (2004) who state that Hydroxycinnamic acids were the most common phenolics present in the insoluble-bound fractions of the phenolic acids from the millet grains and also to the findings of Chandrasekara & Shahidi, (2011), this study did not record any presence of Hydroxycinnamic acids in the varieties investigated. The most logical explanation would be the methods of analysis used in these studies. Interestingly, during the course of this study, the two most common identified phenolic acids are

Protocatechuic and p-hydroxybenzoic acids, this might indicate the ease of detection of these two acids using different analytical methods.

Chandrasekara & Shahidi, (2011) had reported the presence of procyanidin B1 and procyanidin B2 in finger millet; likewise, this study recorded the presence of procyanidin in the finger millet variety. In this experiment, along with cyanidins, a few pelargonidins and one delphinidin were also identified; the possibilities of occurrence of all these compounds in the millet genotype are therefore justifiable.

Principal component analysis showed that most of the phenolic compounds in the millet varieties have clustered on the (PC1) axis, which may indicate homogeneity among the investigated varieties. Finger millet varieties were seen as the main contributor to the clustering on the right side of the cluster.

The results of the current study demonstrated difference among the varieties of millet rather than the environmental effects, as we see that finger millet varieties had more phenolic compounds than the pearl millet varieties regardless of the area of cultivation.

5.6. Conclusions

This study reports the profile of the phenolic compounds in the millet grains which confirms that the LC-MS-based profiling is a powerful technique for the phenolic characterization. It also substantiates the fact the millet grains are good source of nutrition and also beneficial for the health status of those who rely on it as staple food, due to the presence of nutraceutical components such as antioxidants and polyphenols. Because millet flavonoids play important roles in the prevention and management of type 2 diabetes, the finger millet varieties fit perfectly in terms of health benefit, as it contains the flavonoids more than the pearl millet types. The results of this study confirm that the type of millet was the determining factor of the phenolic contents. These results have provided useful information for effective utilisation of millets as functional food ingredients for promoting health. Broader profiling of different millet varieties to include most of the varieties present in the Southern African region, is encouraged.

CHAPTER 6

THE USE OF THE SOUTH AFRICAN PEARL MILLET IN DIETS FOR BROILER CHICKENS

6.1. Abstract

The aim of this study was to determine the performance of Ross broiler chickens d=fed different inclusion levels of pearl millet. The chickens were divided into four treatments each having 50 chickens (n=200). Maize was replaced with pearl millet on an equal weight bases at inclusion levels of 0%, 25%, 50% and 75%. The chickens were raised for six weeks where feed intake and weight gain were measured on a weekly basis and used to calculate feed conversion ratio. The statistical analysis was performed using the GLM procedure of SAS (2011). All the data collected were analysed using completely randomized design. The mean was separated using Duncan test. The result showed significant differences in feed intake, weight gain, feed conversion ratio and the internal organs. However, mortality rate and bones characteristics of the chickens were not significantly different. Bones characteristics were also determined; the results on the weight, length, and width of the tibia, femur and fibula bones did not show a significant difference among the treatments at (P > 0.05). The results have shown that, although maize is the more desired cereal by birds in early days, there was an observed adoptability with age. In this experiment, the cost of replacing maize with pearl millet in the diets of Ross 308 broiler chickens was determined, to establish the cost benefit of using pearl millet instead of maize. The net gain or loss due to replacement of maize with pearl millet was investigated. The cost of feed was used as a variable cost. Pearl millet-based diets were the cheapest according to the findings of this study. The improved pearl millet

variety which is developed in South Africa has the potential to replace maize in the diet of broiler chickens with almost similar performance.

In this experiment, the cost of replacing maize with pearl millet in the diets of Ross broiler chickens were determined, in order to establish the cost benefit of using pearl millet instead of maize. Partial budget was used to determine the profitability of pearl millet. The net gain or loss due to replacement of maize with pearl millet was investigated. The cost of feed was used as a variable cost. The return which would have been accrued as a result of selling of the broiler chickens was estimated and recorded. The cost of production was subtracted from the gain of the chickens to calculate the net gain. The net gain or lose because of replacing pearl millet for maize, was analysed by using feed cost as the variable cost, taking into consideration the feed expense as a variable cost and sale of the broiler chickens as a return. Pearl millet-based diets were the cheapest according to the findings of this study. The quadratic analysis of the effect of substitution of maize with pearl millet had positive relationships with the performance parameters.

6.2. Introduction

Poultry sector plays a very important role in supplying human population with a reliable source of protein. However, the sustainability of the sector is threatened by the ever-increasing human population which has led to high demand. Coupled with that, the supply of maize as a major source of energy has been disturbed due to manifold uses of the grain. Recently, a concern developed around the overuse of maize in different industries, besides the livestock and human nutrition. The wide use of maize as the chief source of energy in livestock feed, is believed to be due to its high energy content, palatability, low fiber, and essential fatty acids (Panda et al., 2020). The high demand for maize consequently led to high prices of the commodity leading to high cost of production. Feeding is considered the major expenditure of production as it accounts for 70-85% of the cost (Sanni & Ogundipe, 2005).

Alternative feed is an area exploited by researchers in a bid to find solution for the overuse of maize. Many cereals emerged as candidates in replacement of maize. Pearl millet is among the cereals which have proven to have potential in terms of nutrient content and least cost. For example, its protein and lysine content are considered higher than the content in maize (Adeola & Rogler, 1994). Pearl millet, in addition to its high nutritional value, it is believed to have low tannin content, in comparison to maize and sorghum. It ranked as the fourth most important tropical cereal, as stated by Andrews & Bramel, (1994). Recent trends in animal nutrition and alternative energy sources for chicken has shown interest in developing pearl millet as an alternative source in replacement of maize most due to its favourable performance in comparison to maize (Davis & Dale, 2003). The move is part of research looking into the use of non-conventional animal feeds in a bit to reduce the cost of nutrition. The inclusion level of pearl millet in the chicken feeds was studied by different researchers and different recommendations for inclusion level have been issued. A study by Tornekar et al. (2009) has put the optimum level of inclusion of pearl millet at 25 – 50% in the broiler ration. Rao et al. (2003) reported that body weight gain and feed efficiency of broiler chickens fed different levels of peral millet were not affected at inclusion of up to 25%, although higher inclusion levels affected the body weight gain.

There is lack of uniformity regarding the optimum inclusion of the pearl millet in the feed of the chicken. Adeola & Rogler, (1994); Amato & Forrester, (1995), reported that the protein content of millet in general is higher than in maize. This favourable content of protein makes millet to be a perfect candidate in replacement of maize, not only as source of energy but potentially as a better choice for protein content.

However, in the Southern Africa millet is not commonly used as in other African and Asian countries, which rely on millet as staple food. This research is a long journey to unearth the whodunnit around the production of pearl millet in South Africa. Its effect on performance of the chicken is also studied to determine its efficiency in poultry industry.

In addition, the cost of producing millet is less than producing other grains such as maize and sorghum. For example, in a study by Silva et al. (2020), the total replacement of maize with pearl millet, was found to be the most economical in the diet of feedlot cattle. Medegu et al. (2010) confirmed that it is more economical and cost effective to produce broiler chickens, using pearl millet, as the cost per kg feed and cost of feed per unit weight gain are lowest in millet grains feed.

The cost of poultry production has become a major challenge for the industry, mainly because of the cost of feed. The cost of conventional grains such as maize steadily increases year after year. It has therefore become very difficult for the producers to attain meaningful profit margin. The strategy to develop some of the locally produced cereals becomes imperative in order to reduce the cost of production. If the farmers have to consider looking for different and improved alternative nutrition, at least it has to be profitable. The cost per kilogram of feed and feed cost per kilogram of weight gain were calculated to indicate the economics of production using mixed methods.

In addition, different models are used to determine the optimum substitution levels. Quadratic programming is among the models used to investigate optimization. Sterling et al. (2005), states that using a maximum profit model such as the quadratic programming could generate more profit for broiler production.

6.2.1. The cost benefit of using millet

Millet is a gluten-free and low-cost cereal with an estimated cost of 40% lower than corn (Gomes et al., 2008). Silva et al. (2014) has put the trade value of pearl millet to be less than or equal to 77.78% of the cost of the corn grain. The protein content of pearl millet grain is higher than in maize, which may allow formulation of diets without supplementation of protein, consequently reducing the cost of food and feed. As stated by Farooq et al. (2001) the cost of production and net profit of poultry determine the fate of the production.

In addition, the cost of producing millet is less than producing other grains such as maize and sorghum. For example, pearl millet water-use is more efficient than sorghum and maize grown in semi-arid regions of Brazil (56 ± 2·8 kg DM/ha/mm water for the Brazilian pearl millet cultivars v. 45 ± 1·9 kg DM/ha/mm water for sorghum; Silva et al. (2011); and 21 ± 2·4 kg DM/ha/mm water for the Brazilian maize cultivars; (dos Santos et al., 2010). In a study by Silva et al. (2020), the total replacement of maize with pearl millet, was found to be the most economical in the diet of feedlot cattle. The items which influenced the financial indicator were reported to be the price of lean and fat cattle, initial weight, final weight, and cost of concentrate, cost of roughage, consumption of concentrate and consumption of roughage (Silva et al., 2020). It is also logical to assume that positioning of millet as competitive grain to maize, will tilt the weight of the supply, which will consequently relieve the pressure on maize consumption, resulting in price reduction. In another study by Rama Rao et al. (2002) reported that the cost of feed required to produce one kg of live weight gain in maize fed group of chickens was higher than in pearl millet, finger millet and sorghum fed groups. Medegu et al. (2010) confirmed that it is more economical and cost effective to produce broiler chicken, using pearl millet, as the cost per kg feed and cost of feed per unit weight gain are lowest in millet grains feed. Wilson et al. (2007), estimated that total net profit from the use of pearl millet as the sole feedstock was $25,175,000 per year compared to $23,758,000 for maize feedstocks, a $1.4 million advantage.

6.3. Materials and Methods

Study Site

The study was conducted at the University of Limpopo Animal unit (latitude 27.55°S and 24.77°E). The ambient temperature at the study site ranges between 20 and 36°C in summer

(November - January) and between 5 and 25 °C in winter (May – July). Mean annual rainfall ranges between 446.8 and 468.44 mm. This study was conducted during the winter period.

6.3.1. Physiochemical characteristics of the pearl millet

In our previous study Hassan et al. (2020), the chemical and physical characteristics of the pearl millet used in this study, were investigated. The results of the investigation showed that pearl millet had ample energy content and reasonable protein content. Dry matter content was 90.15, the crude protein, 8.39, and gross energy content of 17.00 g/100g (Hassan et al., 2020).

6.3.2. Preparation of the house

The chickens were housed in an open sided structure; the long axis was situated along east-west direction for proper ventilation. Twenty 1m^2 pens were constructed inside the poultry house using wire mesh. The poultry house was cleaned and disinfected before the arrival of the birds. Wood shaved saw dust was used as bedding for the chickens. Equipment like feeders and drinkers were washed with disinfectants drinkers were washed and cleaned daily in the morning and feeders were cleaned weekly before being used. Strict sanitary measures were followed during the experimental period. Paraformaldehyde was used to disinfect the poultry house two weeks prior to the start of the experiment. All the trials were carried out in accordance with the University of South Africa ethical practices of animal care in research programs.

Data on body weight gain, feed conversion ratio were calculated following the below mentioned formulae.

$$\text{Feed Conversion Ratio (FCR)} = \frac{\text{Feed intake (g)}}{\text{Live weight (g)}}$$

6.3.3. Experimental Diets

The pearl millet used in this study was planted in Agricultural Research Council (ARC) Grain Crops Division, Potchefstroom, in the North-West Province of South Africa. The period of planting was from June to July 2019. Feed and water were given to the chicken's *ad-libitum*, liveweight; feed intake and mortality were recorded on a weekly basis.

The feeding protocol consisted of the commercial maize feed and a gradual replacement of maize with pearl millet on an equal weight basis (Table 2). The feeding group consisted of T1 which was the control that contains maize as the source of energy. Graded levels of pearl millet were added to three treatment groups T_2 (25%), T_3 (50%), and T_4 (75%) as shown in Table 1. The pearl millet used in this experiment was analysed to determine their, Dry matter, crude protein, energy, crude fiber, calcium, and phosphorus using the method of (AOAC, 2011). The pearl millet used in this study was milled using a grinder at the University of Limpopo, South Africa. The treatments were mixed according to the proposed inclusion levels of maize and pearl millet.

6.3.4. Birds management and Experimental design

The chickens were arranged in a completely randomized design into four different treatments with each replicated 5 times. Total of 20 pens were used, each pen had 10 chicks with the total of 200 chicks. One day old, unsexed Ross 308 chicks were purchased from a local hatchery. The initial mean weight of the chicks was approximately equal at day one and at 14 days. The experimental period lasted for 42 days, the first 14 days the chicks were fed on the control diet and on day 15 the experimental diets were introduced. Two feeding periods were adopted, the first one started at Day 14-21, grower feeds were administered, the second phase started at Day 22-42, the experimental diets were administered. Feed and water were provided *ad libitum* throughout the experimental period.

6.3.5. Experimental layout

The experiment was designed to accommodate 200 birds in total, with 50 chicks per treatment as shown in table 6.1.

Table 6.1. Experimental layout

Treatments	Replicates	Number of chickens
T1= Rations containing 100% maize + 0% pearl millet	5	10 = (50 chicks)
T2= Rations containing 75% maize + 25% pearl millet	5	10 = (50 chicks)
T3= Rations containing 50% maize + 50% pearl millet	5	10 = (50 chicks)
T4= Rations containing 25% maize + 75% pearl millet	5	10 = (50 chicks)

Table 6.2. Composition of the experimental diet

	Treatment (Diets)			
Ingredients	**T1**	**T2**	**T3**	**T4**
Yellow maize	52.50	40.42	26.25	13.47
Pearl millet	0.00	13.47	26.25	40.42
Soya beans meal	42.00	42.00	42.00	42.00
Canola oil	3.18L	3.18L	4.03L	5.73L
Potassium carbonate	72.95	72.95	69.62	67.66
Monocalcium phosphate	1.04	1.04	0.11	0.11
Salt	0.32	0.32	0.32	0.32
Premix	0.42	0.42	0.42	0.42
Lysine	0.19	0.19	0.19	0.19
Methionine	0.30	0.30	0.30	0.30
Limestone powder	12.92	12.92	10.19	2.70
Analyzed chemical composition (%)				
DM	90.58	88.93	90.15	90.58
CP	9.41	6.43	8.39	9.02
CF	2.27	5.20	3.87	4.09
NDF	9.01	12.51	11.64	9.01
ADF	3.31	7.91	5.19	4.31
GE	17.00	15.54	17.23	17.09
EE	3.80	3.65	4.52	3.80
Starch	48.86	38.04	40.95	47.77

6.3.6. Data collection

Feed intake and body weight were recorded weekly per each pen for a period of 6 weeks. The feed conversion ratio was then calculated, and ratios recorded for each treatment. 40 chickens were then slaughtered by cervical dislocation method, following the recommendations of the University of Limpopo ethical guidelines. The birds were immersed in hot water to remove the feather, then cleaned up and dissected to harvest the internal organs such as liver, gizzard, heart, lungs, and intestine. The weight of the carcass was also recorded.

Furthermore, the tibia bone samples were cleaned from remnants and stored in isotonic saline at the temperature of -250 C. The geometric characteristics of the tibia bones were assessed by using the method of (Muszyński et al., 2017). The mechanical characteristics were assessed using force-displacement curves method of OriginLab, Northampton, MA, USA. Toughness modulus and ultimate strength was determined using the method of (Tomaszerwska et al., 2016). The content of minerals such as Calcium and Potassium were determined using the method of atomic absorption spectrometry (AOAC, 2011).

6.3.7. Cost benefit analysis

The principles used here are adopted from (Upton, 1979). The calculation was done by using the formulae:

Marginal Rate of Return=Δ Variable cost/Δ Net Return;

Net Return=Total Return-Total variable cost.

Feed cost per live weight gain will also be calculated as follows as an indicator of cost and biological efficiency.

Feed cost per live weight gain

$$= \frac{\text{Cost of feed consumed}}{\text{Live weight gain (kg)}} \times 100$$

The cost of maize and pearl millet was obtained, and calculations conducted were, (The price of maize, the price of maize mix with pearl millet. To explain further, one kg of maize costs 3.23 rands, to calculate the quarter quantity mixed with pearl millet, we divided by 4 (3.23/4) = 0.81 rands. The same for pearl millet (the price per kg is 1.76 rands, divided by 4 =0.44. It was observed that the greater the substitution level in the feed mixture, the lower the cost feed is. The net return is the return from selling the chickens at the price of 60 rands each (50 chicken's times 60 rands).

6.3.8. Statistical analysis

The statistical analysis was performed using the GLM procedure of SAS, (2011). All the data collected were analysed using completely randomized design. The mean was separated using Duncan test. ANOVA was used to test the significant difference among the treatment means.

The quadratic models were fitted to the experimental data by the non-linear model (NLIN) procedure of SPSS, (2007). The response in optimum feed intake, body weight, internal organs weight of the broiler chickens, due to the replacement of maize with pearl millet in different inclusion levels, were modeled using the following quadratic equation:

$$Y= a+ b_1x+ b_2x^2$$

Where y = optimum, a = intercept; b = coefficients of the quadratic equation; x = pearl millet replacement level and $-b_1/2b_1$ = x value for optimum response. The quadratic equation was preferred model as it gives the optimum fit.

6.4. Results

Performance results of broiler chickens

The results of the effect of different inclusion levels of pearl millet in replacement of maize are recorded. Body weight, weight gain, feed conversion ratio, mortality, internal organs, bones characteristics, and cost analysis were determined.

6.4.1. Body weight

The results of the final body weight and body weight gain of the Ross 308 broiler chickens fed on maize and different inclusion levels of pearl millet are shown in Table 6.3 and Figure 6.1. The weight had increased steadily from week 3 up to week 6 of the experiment. Significant difference was observed among all treatment groups. Treatment 3, which was a 50% mixture of maize and pearl millet has shown better body weight. The mean final body weight ranged between 2773, 2871, 2877 and 2897 g for treatments T2, T1, T4 and T3, respectively. The highest value was recorded in T3, whilst the lowest value was recorded in T2 (25% pearl millet). The substitution of 50% maize with 50% pearl millet in diets of broiler chickens appeared to be superior in comparison to the maize diets and the other inclusion levels of pearl millet. Dietary treatments had significant effect on feed intake, body weight, and feed conversion ratio.

6.4.2. Weight gain

It was statistically evident that the weight gain was affected by the gradual replacement of maize with pearl millet grain at ($P < 0.05$). The weight gain for the Ross 308 broiler chickens progressively decreased at week three for all the treatment groups. Later, the weight gain increased in week 4, week 5 and week 6 (Figure 6.1). The total body weight gain ranged between 2352, 2471, 2441 and 2460 grams (Table 6.3). The results showed that the broilers fed the diet with 75% inclusion level attained higher total weight gain compared to the birds fed the control diet and other pearl millet inclusion levels. The results clearly indicate that the birds fed the diets containing graded levels of pearl millet tend to have higher weekly live weight gain.

6.4.3. Feed conversion ratio

Feed conversion ratio of the Ross 308 broiler chickens which were fed on the diet containing maize and different levels of pearl millet were significantly different ($P < 0.05$). The feed conversion ratios were 1.16, 1.30, 1.19 and 1.15 for T1, T2 T3 and T4, respectively. The highest ratio was recorded in T2 which contains 25% pearl millet whilst the lowest was reported in T4 which contains 75% pearl millet inclusion.

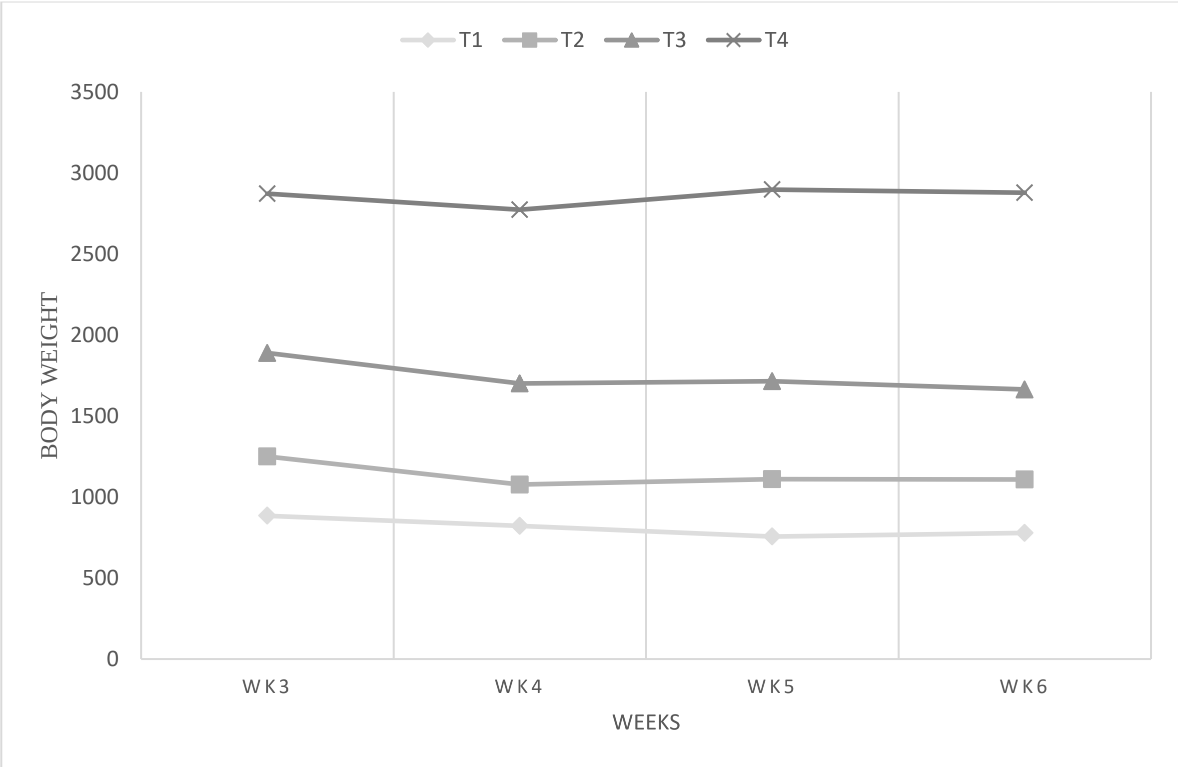


Figure 6.1. Weekly body weight of T1; control diet, T2; = 25% of maize replaced with pearl millet, T3; = 50% of maize replaced with pearl millet, and T4; 75% of maize replaced with pearl millet. WK3= Week3; WK4= Week4; WK5= Week5; and WK6= Week6.

Table 6.3. Performance of broiler chickens fed different maize and pearl millet diets for 42 days.

	Treatment (Diets)					
Parameters	T1	T2	T3	T4	Probability	SEM
Mean initial live weight (g)	430.0^{a}	421.0^{b}	396.0^{d}	417.0^{c}	<.0001	17.042
Final body weight (g)	2871^{c}	2773^{d}	2897^{a}	2877^{b}	<.0001	20.452
Total body weight gain (g)	2441^{c}	2352^{d}	2471^{a}	2460^{b}	<.0001	23.452
Total feed intake (g)	2824.08^{d}	3063^{a}	2924.04^{b}	2841.72^{c}	<.0001	27.544
Daily feed intake (g)	67.24^{d}	72.95^{a}	69.62^{b}	67.66^{c}	<.0001	2.345
Daily weight gain (g)	58.12^{c}	56.0^{d}	58.83^{a}	58.57^{b}	<.0001	2.452
Feed conversion ratio	1.16^{c}	1.30^{a}	1.19^{b}	1.15^{d}	<.0001	0.9564

T1= control diet; T2 = 25% of maize replaced with pearl millet; T3 = 50% of maize replaced with pearl millet, and T4 = 75% of maize replaced with pearl millet. $^{a, b, c, d}$. Means followed by the same superscript in a row, are not significantly different ($P > 0.05$). SEM − standard error of the mean.

6.4.4. Carcass parts characteristics

The inclusion of pearl millet in the diets of broiler chickens had no effect on breast, thighs, drums, wings, and neck ($P > 0.05$. However, treatment 1, the control diet had higher quantity in comparison to the other treatments (Table 4). The average breast weight was 479.56g. The highest was recorded in treatment 3 and the lowest recorded in treatment 4. Moreover, Thighs, drums, wings, and neck weights were observed to be low in treatment 4.

Table 6.4. Effect of pearl millet levels on the performance of carcass parts weights of Ross 308 at 42 days.

	Treatment (Diets)					
Parameters	T1	T2	T3	T4	Probability	SEM
Breast weight (g)	488.09	469.55	492.71	467.91	0.3221	12.233
Thighs weight (g)	204.41	211.09	199.70	198.04	0.4014	10.303
Drums weight (g)	199.44	197.78	200.00	198.80	0.4014	10.421
Wings weight (g)	161.77	159.06	158.02	158.00	0.5510	9.003
Neck weight (g)	108.03	99.67	100.54	98.34	0.3512	7.144

T1; control diet, T2; = 25% of maize replaced with pearl millet, T3; = 50% of maize replaced with pearl millet, and T4; 75% of maize replaced with pearl millet. SEM − standard error of the mean.

6.4.5. Mortality

The highest mortality recorded in this experiment was in treatment 1 (T1), which was the treatment with 100% maize inclusion, however, it was not statistically different from the other treatment groups. It is worth noting that most of the mortalities occurred in the first three week of the experimental period, which could be attributed to the cold weather condition.

6.4.6. Internal organs

The internal organs showed significant difference ($P < 0.05$) among the treatments except for heart and intestine weight which were not affected by the dietary treatments. The average gizzard weight was 70.25g; the highest was recorded in T3 which contains 50% of pearl millet whilst the lowest was recorded in T4, treatment which contains 75% of pearl millet. The weight of the lungs was almost similar across the treatment with no significant difference at ($P > 0.05$). The intestine length registered the longest of 243 cm in the diet containing maize, whilst the shortest of 211 cm was in the diet containing 25% (T2) of pearl millet.

Table 6.5. Performance of visceral parts of broiler chickens fed on maize and pearl millet diets for 42 days.

	Treatment (Diets)					
Parameters	T1	T2	T3	T4	Probability	SEM
Mean Gizzard weight (g)	63^{c}	57^{d}	89^{a}	77^{b}	<.0001	9.033
Mean Lungs weight (g)	8	8	7	6	0.4014	1.101
Mean Liver weight (g)	54^{c}	48^{d}	61^{a}	57^{b}	<.0001	10.004
Mean Heart weight (g)	15^{a}	9^{d}	14^{b}	14^{c}	0.0010	1.006
Mean Intestine weight (g)	17	15	16	16	0.351	7.144
Mean Intestine length (cm)	243^{a}	211^{d}	215^{c}	217^{b}	<.0001	16.344

T1= control diet; T2 = 25% of maize replaced with pearl millet; T3 = 50% of maize replaced with pearl millet, and T4 = 75% of maize replaced with pearl millet. $^{a, b, c, d}$. Means followed by the same superscript in a row, are not significantly different ($P > 0.05$). SEM − standard error of the mean.

6.4.7. Bones structure characteristics

The results on the weight, length, and width of the tibia, femur and fibula bones did not show a significant difference among the treatments at ($P > 0.05$), as shown in Table 6. The tibia bones were heavier and longer in treatment 1 (T1), with values of 17.23 g and 116.10 mm, respectively. While treatment 2 had the highest values of weight, length, and width for the femur bones. Furthermore, the fibula weight, length and width parameters were higher in treatment 1 (T1).

Table 6.6. Effect of dietary replacement of maize with pearl millet on the weight (g), length (mm) and width (mm) of tibia, femur and fibula bones of Ross 308 broiler chickens.

Treatment (Diets)

Parameters	T1	T2	T3	T4	Probability	SEM
Tibia weight (g)	26.23	24.02	22.8	22.0	<.0.072	11.031
Tibia length (mm)	116.10	111.21	113.00	115.11	<.0.408	10.452
Tibia width (mm)	10.11	10.01	9.60	9.46	<.0.065	08.332
Femur weight (g)	19.24	19.95	18..64	18.76	<.0.0802	07.544
Femur length (mm)	94.44	96.43	95.99	96.01	<.0.408	12.441
Femur width (mm)	11.86	11.89	11.92	11.95	<.0.406	12.452
Fibula weight (g)	3.88	3.32	3.39	3.39	<.0.408	0.0654
Fibula length (mm)	90.76	90.31	88.61	89.55	<.0.065	14.221
Fibula width (mm)	4.66	4.20	4.01	4.54	<.0.065	0.0554

T1= control diet; T2 = 25% of maize replaced with pearl millet; T3 = 50% of maize replaced with pearl millet; and T4 = 75% of maize replaced with pearl millet.

6.4.8. Flock uniformity

The flock uniformity which is the spread of live weight above and below the flock average, was not statistically different ($P > 0.05$), among the treatments. It was observed that the reduction in flock uniformity was recorded among the treatments which included different dietary levels of pearl millet as shown in figure 6.1.

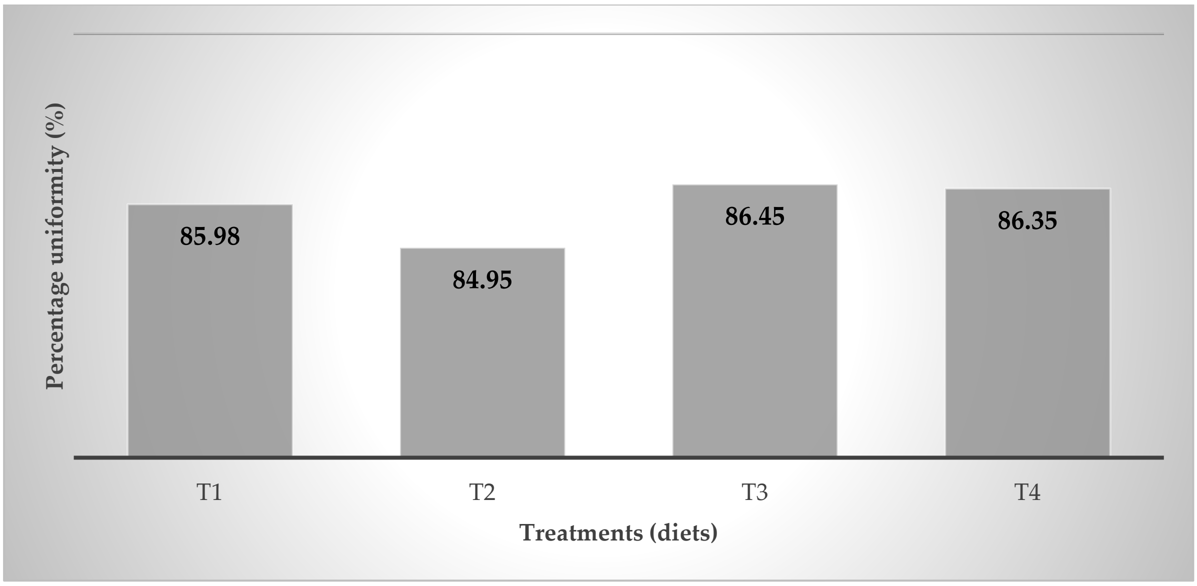


Figure 6.2. Flock uniformity of final body weight of different experimental diets. T1= control diet; T2 = 25% of maize replaced with pearl millet; T3 = 50% of maize replaced with pearl millet, and T4 = 75% of maize replaced with pearl millet.

6.4.9. Relative weights and lengths of Duodenum, Ilium, Jejunum and Caeca

The results of the current study showed no significant difference (p > 0.05) in the relative weights and length of the small intestine segments.

Table 6.7. Relative weight (g/kg Body weight) and relative length (cm/kg Body weight) of small intestine parts of broiler chickens fed different levels of pearl millet.

	Treatment (Diets)					
Parameters (Segments)	T1	T2	T3	T4	Probability	SEM
Duodenum weight	4.24	3.99	3.90	4.00	<.0.070	1.332
Duodenum length	11.28	11.66	10.34	10.99	<.0.502	1.332
Ilium weight	3.98^{c}	3.92^{d}	4.13^{a}	4.00^{b}	<.0.054	1.544
Ilium length	10.08	11.00	10.45	10.87	<.0.080	2.441
Jejunum weight	3.09	4.02	4.00	4.00	<.0.408	2.452
Jejunum length	5.94	6.11	5.76	5.40	<.0.404	2.044
Caeca weight	2.22	2.19	3.00	2.89	<.0.400	1.022
Caeca length	3.20	3.15	3.71	3.88	<.0.400	1.054

T1; control diet, T2; = 25% of maize replaced with pearl millet, T3; = 50% of maize replaced with pearl millet, and T4; 75% of maize replaced with pearl millet. SEM − standard error of the mean.

6.4.10. Minerals content of bones

The minerals content of the broiler chicken bones is shown in Table 9, the results showed no significant difference ($p > 0.05$) in the ash, calcium, and phosphorus contents of the bones. The calcium content ranged from 444.81, 455.33, 456.45 and 458.20 g/kg for the tibia bones. The lowest was in treatment 1 (T1) which is the control diet. Phosphorus content for tibia bones ranged between 186.09, 187.11, 188.65 and 198.28 g/kg. The lowest content of ash was 35.21% was recorded in treatment 4 (T4). For the femur bones, the calcium content ranged from 447.09, 457.34, 488.60 and 495.10 g/kg. The highest quantity was recorded in treatment 4. Whiles the phosphorus content of femur bones ranged from 179.09, 187.02, 198.72, and 199.08 g/kg. The calcium content for fibula bones ranged from 477.00, 477.01, 485.11 and 498.62 g/kg. Phosphorus content ranged between 189.08, 188.02, 188.72 and 189.09 g/kg for the fibula bones.

Table 6.8. Ash, Calcium and Phosphorus content of bones

Parameters	T1	T2	T3	T4	Probability	SEM
	Treatment (Diets)					
Tibia bones						
Ash (%)	38.70	36.00	36.77	39.21	<.0.077	2.332
Ca (g/kg)	444.81	456.45	455.33	458.20	<.0.408	12.044
P (g/kg)	198.28	187.11	188.65	186.09	<.0.065	11.001
CA/P ratio	2.24	2.44	2.41	2.46	<.0.0802	1.095
Femur bones						
Ash (%)	39.22	41.00	39.09	42.12	<.0.408	3.332
Ca (g/kg)	457.34	488.60	447.09	495.10	<.0.406	13.544
P (g/kg)	199.08	198.72	187.02	179.09	<.0.065	11.441
Ca/P ratio	2.30	2.46	2.40	2.76	<.0.0802	1.184
Fibula bones						
Ash (%)	38.22	41.11	39.04	43.12	<.0.408	2.222
Ca (g/kg)	477.00	498.62	477.01	485.11	<.0.406	11.544
P (g/kg)	189.08	188.72	188.02	189.09	<.0.408	08.441
Ca/P ratio	2.52	2.64	2.54	2.57	<.0.406	1.368

T1 = control diet; T2 = 25% of maize replaced with pearl millet; T3 = 50% of maize replaced with pearl millet, and T4 = 75% of maize replaced with pearl millet. SEM − standard error of the mean.

Table 6.9. Bone geometric analysis of the tibia, femur and fibula of Ross 308 broiler chickens fed varying levels pearl millet.

	Treatment (Diets)					
Parameters	T1	T2	T3	T4	Probability	SEM
Tibia seedor index	0.2259	0.2159	0.2017	0.1911	<.0.077	2.332
Femur seedor index	0.2037	0.2069	0.1941	0.1954	<.0.408	12.044
Fibula seedor index	0.0427	0.0367	0.0383	0.0379	<.0.065	11.001
Tibia robusticity index	39.075	38.544	39.850	41.081	<.0.0802	1.095
Femur robusticity index	35.244	35.554	36.203	36.133	<.0.408	3.332
Fibula robusticity index	57.472	60.537	58.86	5.612	<.0.406	13.544

T1 = control diet; T2 = 25% of maize replaced with pearl millet; T3 = 50% of maize replaced with pearl millet, and T4 = 75% of maize replaced with pearl millet. SEM − standard error of the mean.

6.4.11. Cost benefit of replacing maize with pearl millet in chicken diets

Higher profit which is the net return of production was highest in treatment 4 which was the treatment with maize and 75% of pearl millet (ZAR 26.61/chick) as shown in Table 6.11, however it was only slightly higher than the other treatment groups. It was observed that higher net return increased with the increase of pearl millet diets. In addition, the variable cost was lower in the pearl millet mixtures in comparison to maize only. The variable cost ranged between 32.15, 33.39, 34.45, and 36.82 Rands. The highest was registered in treatment1 whilst the lowest was recorded in treatment 4. Therefore, pearl millet can be used to replace maize in broiler chicken diets resulting in higher body weight gain and profit.

Table 6.10. Economic value of replacing maize with different levels of pearl millet on broiler chickens.

	Treatment (Diets)			
Parameters	T1	T2	T3	T4
Cost of feed/ kg (ZAR)	3.23	2.87	2.50	2.13
Cost of day-old chick (ZAR)	9	9	9	9
Total cost of feed/chick (ZAR)	24.17	22.18	21.52	20.67
Cost of feed/daily gain	0.42	0.40	0.37	0.35
Total variable cost	36.82	34.45	33.39	32.15
Gross return (chicken sale)	60	60	60	60
Net return (ZAR)	23.18	25.55	26.61	27.85
Change in total variable cost	-	-2.37	-3.43	-4.67
Change in gross return	-	0	0	0
Change in net return	-	2.37	3.44.	4.67
Marginal rate of return	-	1	.99	1

T1= control diet; T2 = 25% of maize replaced with pearl millet; T3 = 50% of maize replaced with pearl millet, and T4 = 75% of maize replaced with pearl millet.

6.4.12. Optimization analysis

The quadratic analysis of the effect of substitution of maize with pearl millet had positive relationships with the performance parameters of the Ross 308 broiler chickens, as shown in Table 6.11. The coefficient of determination for body weight and feed intake of the Ross 308 broiler chickens were (r^2 = 0.244 and 0.350, respectively) at the age of 42 days. Positive relationships were observed between the level of replacement of maize with pearl millet and drumstick and thigh of Ross 308 broiler chickens (Figures 6.7 and 7.8, respectively). The optimization occurred at 13.567 and 21.500, respectively.

Body weight of Ross 308 broiler chickens was optimized (r^2 = 0.244) at pearl millet replacement level of 28.225, while feed intake was optimized at the replacement of 36.027 as shown in Figures 6.4 and 6.5, respectively. Positive relationships (r^2 = 0.651) were also observed between level of replacement of maize with pearl millet and the liver weight (Figure 6.6).

A Positive relationship (r^2 = 0.467) was also observed between level of replacement of maize with pearl millet and the gizzard weight of Ross 308 broiler chickens as shown in (Figures 6.9 and Table 6.11).

Furthermore, the heart weight of the broiler chickens and replacement level of pearl millet had a positive relationship (r^2 = 0.417), the optimization was at 42.750 (Figure 6.7). The intestine had a positive relationship (r^2 = 0.549), with an optimization at 22.457 (Figure 6.8). It was noted that most of the optimum levels of performance occurred at the lower inclusion levels of pearl millet in the diets of Ross 308 broiler chickens.

Feed conversion ratio related positively (r^2 = 0.643), optimization occurred at 11.957 upon replacement of maize with pearl millet in the diet of Ross 308 broiler chickens (Figure 6.7).

Table 6.11. Quadratic relationship between pearl millet inclusion levels and performance parameters of broiler chickens at 42 days of age.

Parameter	Formula	r^2	Probability
Body weight	$Y=2915.42-8248x+19.365x^2$	0.244	0.806
Feed intake	$Y= 2484.495-79.897x+19.499x^2$	0.350	0.806
FCR	$Y=1.0115. +0.209x+0.015x^2$	0.643	0.598
Gizzard (g)	$Y=45.504-14.893x+1.499x^2$	0.467	0.737
Drum (g)	$Y=199.488.-0.535x+113x^2$	0.220	0.990
Thigh (g)	$Y=200.488+7.389x-2.087x^2$	0.624	0.614
Heart (g)	$Y=19.981-7.277x+1.496x^2$	0.417	0.764
Liver (g)	$Y=38.522+8.979x-0.997x^2$	0.651	0.591
Intestine (g)	$Y=16.784-0.725x-245x^2$	0.549	0.840

r^2 = coefficient of determination

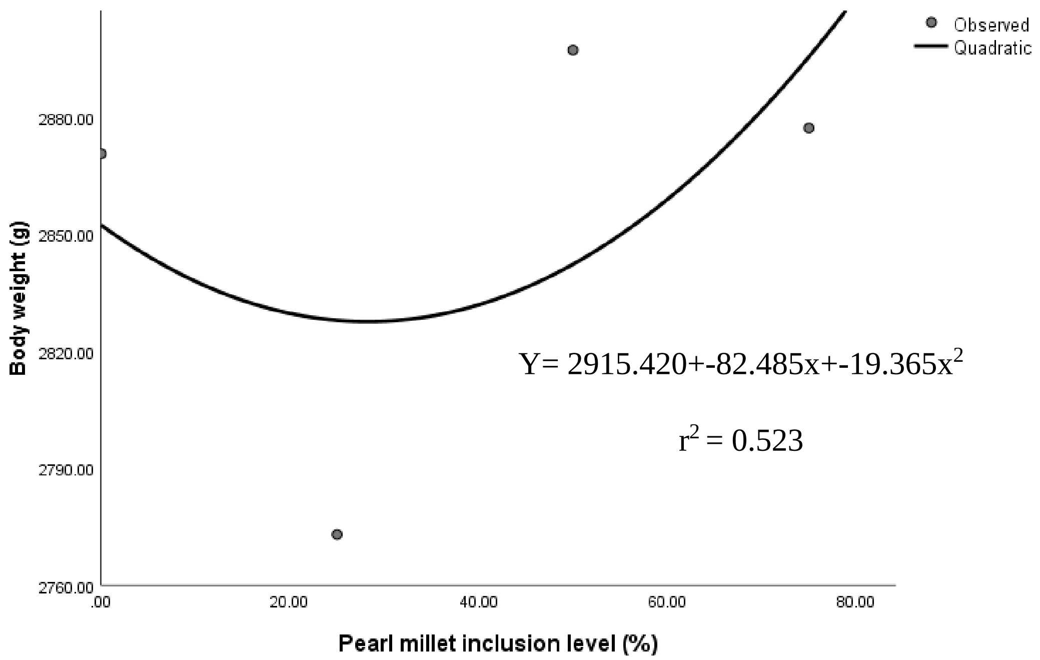


Figure 6.3. Quadratic function best fitting optimum pearl millet level and body weight of Ross 308 broiler chickens**.**

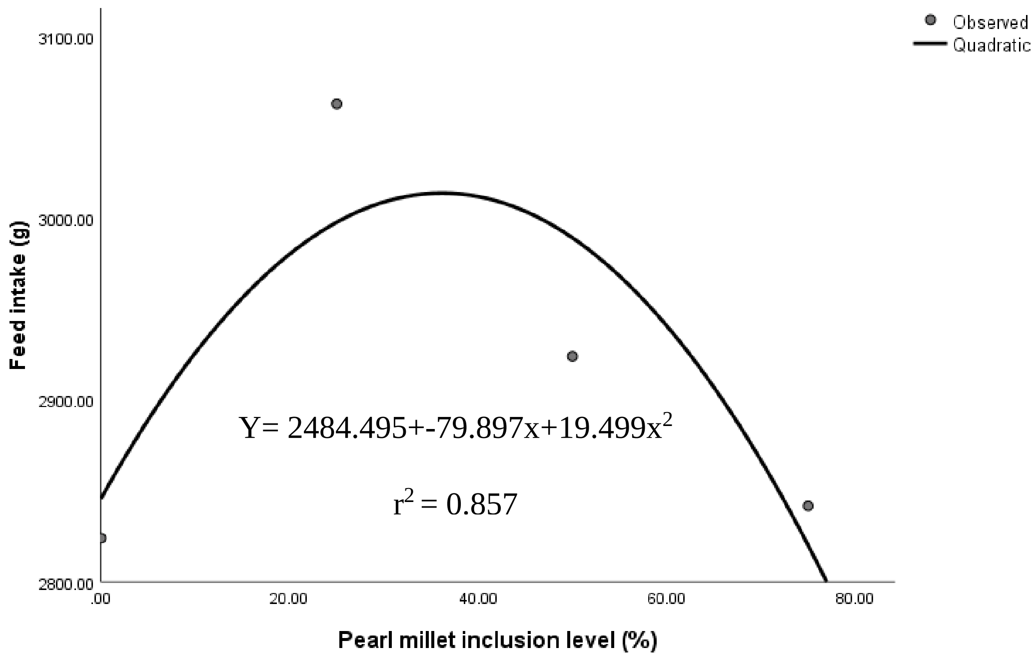


Figure 6.4. Quadratic function best fitting optimum pearl millet level and feed intake of Ross 308 broiler chickens**.**

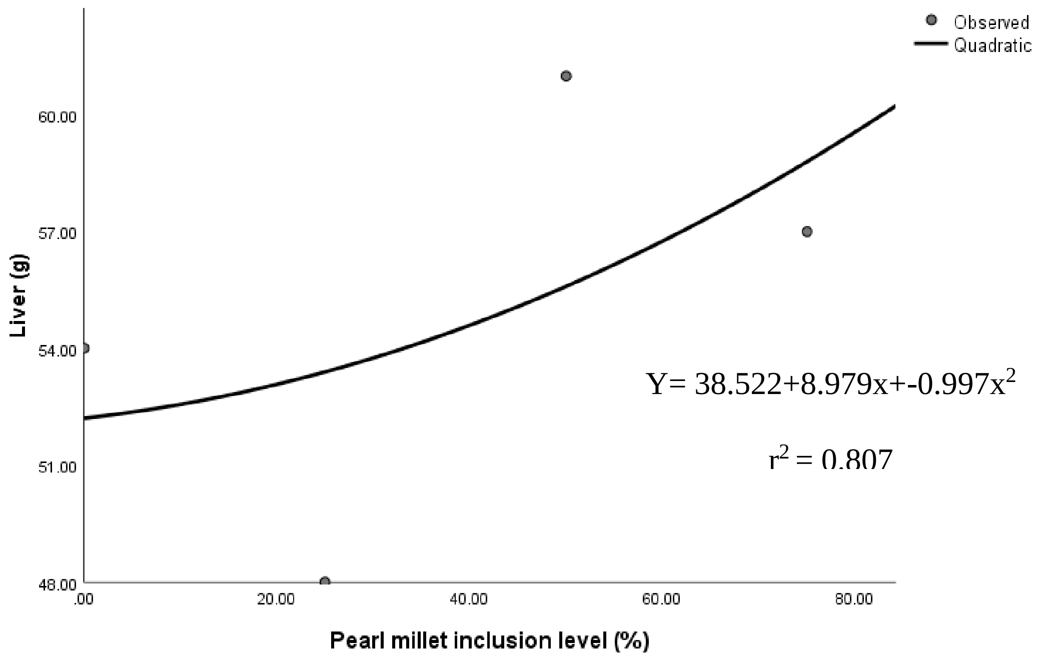


Figure 6.5. Quadratic function best fitting optimum pearl millet level and liver of Ross 308 broiler chickens**.**

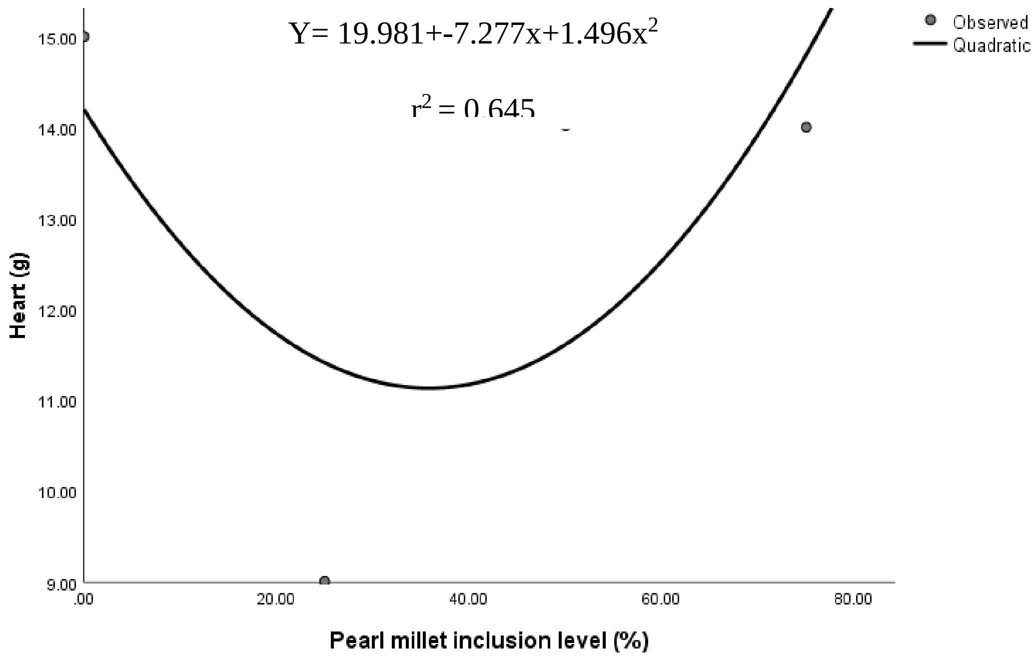


Figure 6.6. Quadratic function best fitting optimum pearl millet level and heart of Ross 308 broiler chickens**.**

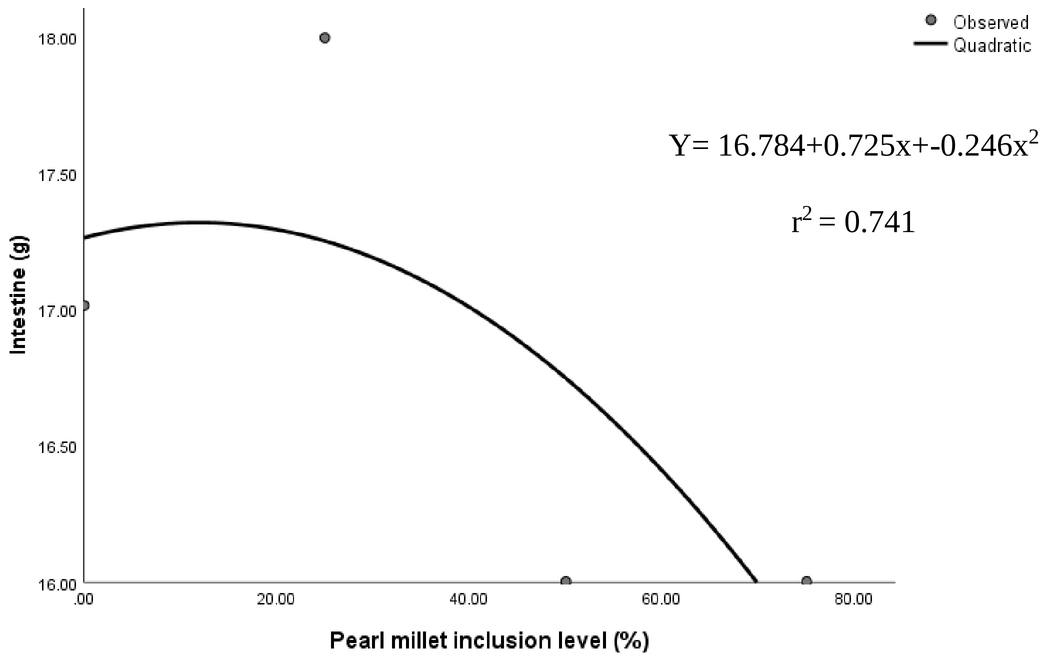


Figure 6.7. Quadratic function best fitting optimum pearl millet level and Intestine of Ross 308 broiler chickens**.**

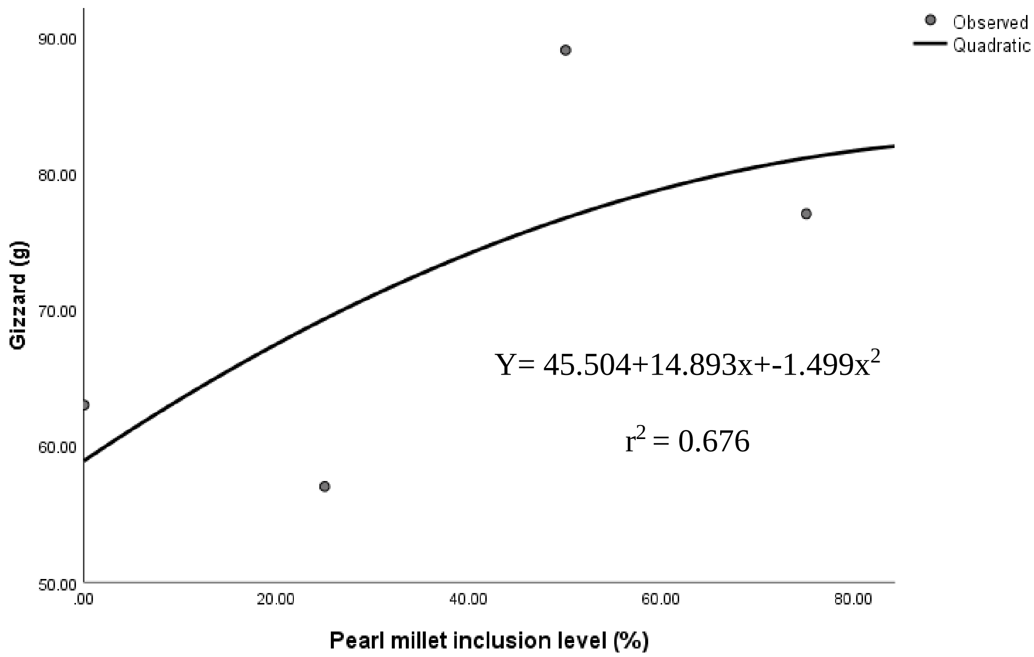


Figure 6.8. Quadratic function best fitting optimum pearl millet level and gizzard of Ross 308 broiler chickens**.**

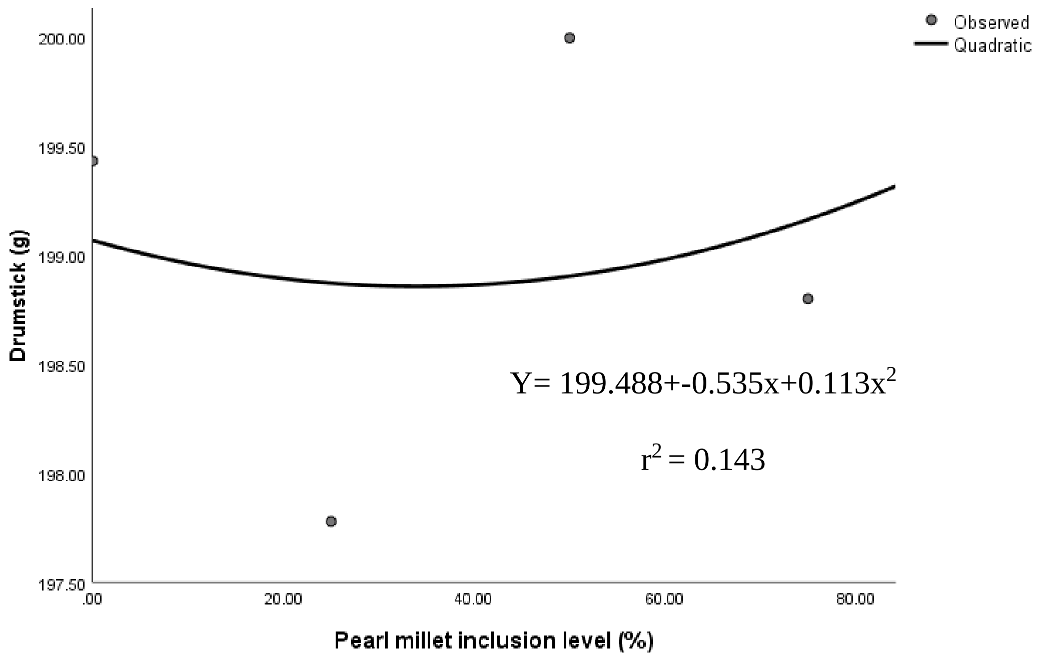


Figure 6.9. Quadratic function best fitting optimum pearl millet level and drumstick of Ross 308 broiler chickens**.**

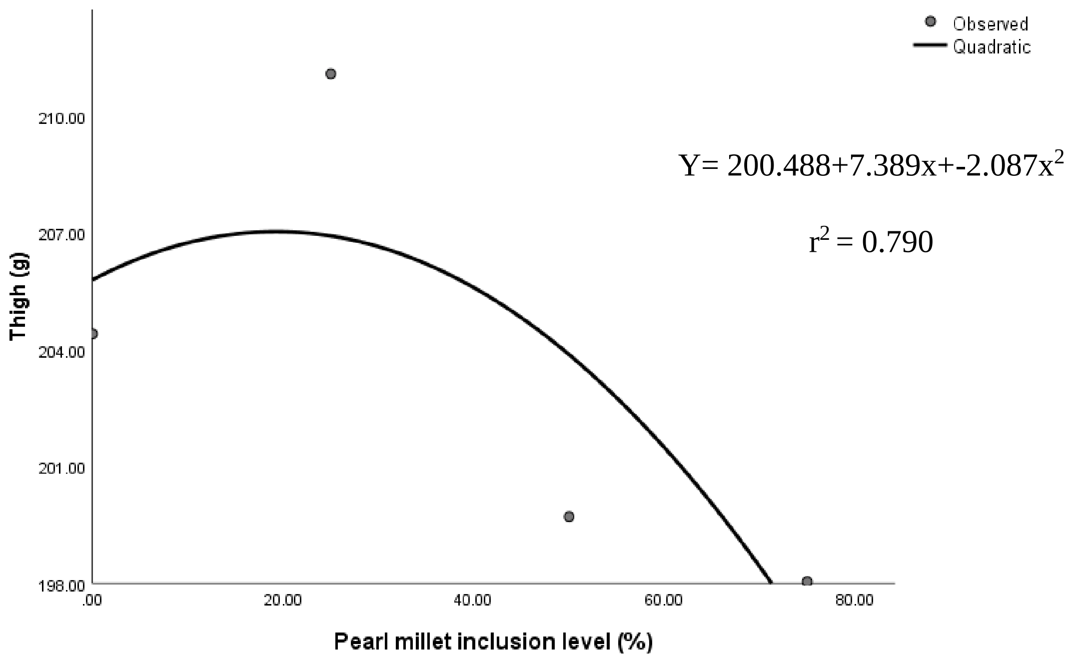


Figure 6.10. Quadratic function best fitting optimum pearl millet level and thigh of Ross 308 broiler chickens**.**

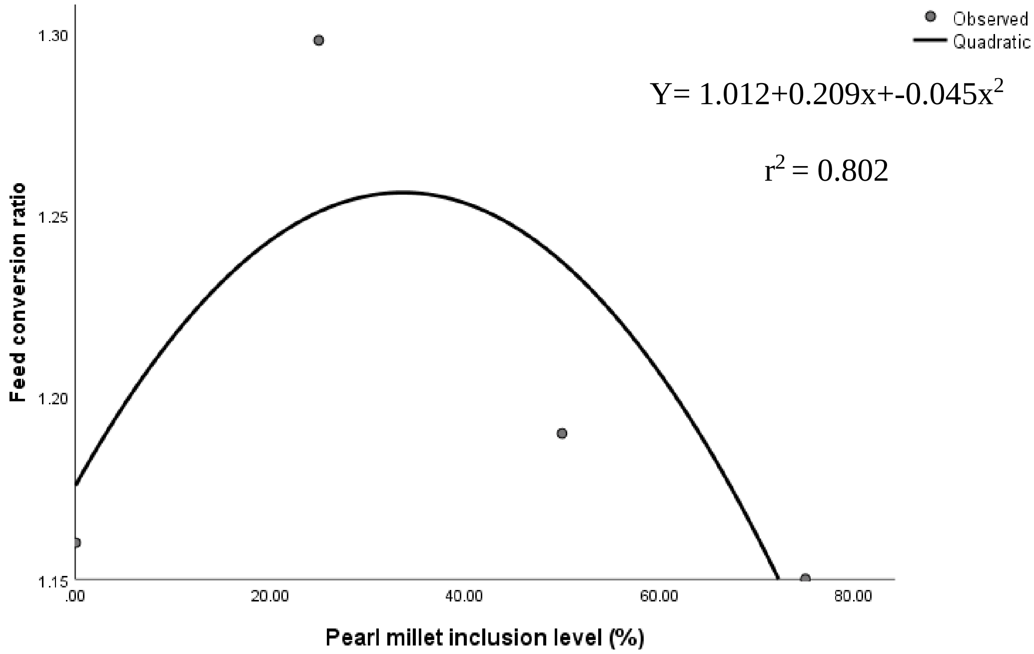


Figure 6.11. Quadratic function best fitting optimum pearl millet level and feed conversion ratio of Ross 308 broiler chickens**.**

6.5. Discussions

Pearl millet, an essential cereal grain, which has the potential to provide a low-cost solution for replacement of maize in the diet of chickens, was investigated in this study to determine its suitability as alternative energy source. The aim of this study was to assess the potential of pearl millet grains grown in South Africa, in replacing maize grains as source of energy in the diet of broiler chickens. In addition to that to assess the economic viability of the substitution of maize with pearl millet. The findings revealed that pearl millet can indeed replace maize in the diets of broiler chickens with no negative effects on performances. Previous studies conducted on pearl millet as poultry feed, has proved that pearl millet can replace maize in the diet of chickens at different inclusion levels without having negative effect on performance (Hidalgo et al., 2001; Tornekar et al., 2009; Abubakar et al., 2011; Baurhoo et al., 2011; & Ibitoye et al., 2012). In addition, Medugu et al. (2010) found no significant difference in live and slaughter weights of chickens fed on pearl millet, on total replacement of maize. The current study demonstrated that pearl millet inclusion of up to 75% in the broiler diet had no adverse significant effect on the performance of broiler chicken. The performance indices were either similar or superior to the maize diets. Furthermore, the weights of organs such as gizzard, liver and heart were not significantly affected by the energy source of the diet. In agreement, Raju et al. (2004), recorded no significant difference on the gizzard weight, length of small and large intestine of chicken fed pearl millet as a replacement of maize. Davis et al. (2003), reported significant improvement in body weight gain and feed conversion ratio for replacement of maize with pearl millet up to inclusion level of 50%.

Total feed intake in this study was significantly affected by the dietary treatments, the results is in disagreements with the findings of (Abdelrazig & Elzubeir, 1998), who reported that feeding pearl millet did not statistically affect the mean total feed intake of the birds. It is worth mentioning that the difference was in favor of pearl millet inclusion. As stated by Bastos et al. (2005), pearl millet cultivars differ in chemical compositions resulting in different nutritional values. It is therefore imperative to study the cultivars available in South Africa to establish their suitability as animal feed. The gizzard weights were higher in the diet with higher inclusion levels of pearl millet. Previous studies have also recorded higher weights for gizzard because of using whole grain cereals (Kiiskinen, 1996). Raju et al. (2004) reported no difference on the weight of gizzard and giblets, length of small intestine, caeca, and large intestine among the treatments in weight-by-weight replacement of maize with pearl millet. In the results presented, the performance of broiler chickens fed maize, was almost equal to the mixtures of maize and millet dietary replacements. Medugu et al. (2010), reported no significant difference on neck, wings, thigh, drumstick of broilers fed different inclusion levels of maize and pearl millet. This shows that although maize is the grain of choice when it comes to the best energy source, pearl millet can also play the role of the nearest alternative. Pearl millet showed a better feed conversion ratio (FCR) and lower cost per weight gain, in comparison to maize.

Willaiams et al. (2000), reported on poor calcification and porosity of bones in the modern poultry production, which leads to poor walking ability, which was attributed to either inadequate dietary supply of minerals such as Calcium and Phosphorus or impaired utilization of the minerals due to a rapid growth rate or genetic factors. However, the finding of the current study on the tabia bones characteristics show no difference among the treatments, which agreed with the findings of Chiripasi et al. (2013), on a study on guinea fowl fed millet, sorghum, and maize diets. Likewise, Manyelo et al. (2019), found that the difference in ash and mineral content of tibia bone were not significant when maize was replaced with sorghum in the diet of Ross 308 broiler chickens.

Generally, the characteristics of the bones of the broiler chickens were not affected by the inclusion of pearl millet in the diets of Ross broiler chickens. It was also noted that although the quantity of calcium in pearl millet was low Hassan et al. (2020), it had no significant effect on the availability of calcium in the broiler bones. This finding agrees with Swiatkiewicz & Arczewska-Wlosek, (2012), who reported that using a diet with low levels of Ca and P had no negative effect on the performance indices.

Flock uniformity as an indicator of profitably was clearly affected by the nutritional levels in this current study. Gous, (2017), reported on the nutritional and the environmental effect on the flock uniformity. Study by Adeleye & Oladotun, (2019) found that inclusion of 50%

whole millet in the diet of broiler chicks resulted in low flock uniformity. Mabelebele et al. (2018) reported no change in flock uniformity when whole sorghum was incorporated at 25-75% in the diet of broiler chickens.

Furthermore, it was also observed in the current study that, the colour of the meat was somehow pale in the diets containing millet in comparison to the diet with maize as the source of energy. Amini & Ruiz-Feria, (2008), reported lack of pigmentation of the egg yolk which was attributed to the absence of xanthophylls in pearl millet.

During the current experiment it was realized that pearl millet is not conveniently available to the consumers in comparison to the availability of maize. This is likely one of the reasons the grain is not so popular. Davis et al. (2003), reported on low production volume as an obstacle to demand for the use of pearl millet as alternative animal feed. Another observation is that those who received the slaughtered chicken preferred the yellow looking chicken meat of the pale looking meat, giving maize a space of an only winner. The researchers also found that maize is easily available to the farmers in comparison to pearl millet. The recommendation is to boost the production of pearl millet, to further reduce its prices. Coupled to this, the farmers also need to be sensitized on the benefits of pearl millet as an alternative energy source, in animal feed.

Based on the results obtained in this experiment and the cost analysis of the benefit of using pearl millet instead of maize. It is safe to say that all being equal, it is more profitable to use pearl millet as source of energy for feeding chickens. The net gain was highest at the inclusion level of 75% of pearl millet. This is a direct result of the lower prices of pearl millet, compared to the price of maize. Similar results were obtained by Medugu et al. (2010), Ibitoye et al. (2012) and Etuk et al. (2012) confirming the profitability of replacing maize with pearl millet in the diets of chickens. In addition, high protein content in pearl millet in comparison to maize is advantageous, because it may lead to reduction in the inclusion levels of other protein sources, which will eventually reduce the cost of feed.

Furthermore, from the results obtained in this experiment and the cost analysis of the benefit of using pearl millet instead of maize. It is safe to say that all being equal, it is more profitable to use pearl millet as source of energy for feeding chicken. The net gain was highest at the inclusion level of 75% of pearl millet. Similar results were obtained by (Medegu et al., 2010; Ibitoye et al., 2012 and Etuk et al., 2012), confirming the profitability of replacing maize with pearl millet in the diets of chickens.

6.6. Conclusions

In conclusions, it is evident the results presented in this study, that pearl millet grain can fully replace maize in chicken rations. It neither reduced the feed-conversion efficiency nor the rate of weight gain. The improvement in the overall weight gain of the chickens upon the nutritional intervention, is an indication that the grain has the potential to be a valuable alternative feed.

In addition, the use of pearl millet as a replacement of maize can result in low cost of production in broiler chicken diets resulting in more profits. Profits from chicken sales over feed cost were directly proportional to the inclusion of pearl millet in the diet of the chicken. More research is recommended in order to investigate the peak weight of the chicken at which the profit is at its highest point. In Countries where production of maize does not flourish, pearl millet can come in as the cost-effective alternative.

CHAPTER 7

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

The present study was intended to investigate the nutritional composition of millet grown in South Africa and Zimbabwe, to determine their phenolic characteristics and to investigate the effects of feeding graded levels of pearl millet on growth performance, of broiler chickens. Pearl millet was incorporated in broiler diets using different feeding protocols, at levels of 0, 25, 50 and 75% of the diet in replacement of maize in a basal control diet which were administered to the experimental chickens, during the period of the experiment which lasted 42 days. The results on the physical and chemical properties of the millet revealed that millet grains contain valuable nutrients which qualify it to be a complementary grain for maize, as an energy source. Phenolic compounds characterisation revealed that millet contains valuable phenolic compounds which are beneficial for livestock as well as human. In addition, the experimental results revealed that, feeding pearl millet produced the same results if not superior in terms of the performance of the chickens, as the maize diets. Although feed intake was affected at the third week of the experiment, the chicken quickly adapted to the new feed and picked up on the intake and the weight gain. It was noticed that growth performance was generally, improved by feeding the experimental pearl millet cultivars diets. This improvement can be attributed to improvement in the efficiency of feed and nutrients utilization from the pearl millet diets. This explanation has been suggested in many reports, showing that the efficiency of feed and nutrients utilization of pearl millet is greater than that for maize (Adeola, et al., 1994).

Based on these findings and results of the present study it can be implied that replacement of maize by graded levels (25, 50, and 75 %) of pearl millet in the diet of the broiler chickens would improve growing performance and feed conversion ratio. Further to this, Pear millet is a hardy crop which can grow in harsh environment in comparison to other cereals such as maize. At the end of the day, producing pearl millet to partially replace maize in the diets of poultry, not only is beneficial in terms of cost, but also in terms of being sensitive to the environment by producing cereals which is not as demanding in terms of water consumption and other scarce resources.

Millet as an alternative is a chance to take off pressure from the use of maize, this will in turn reduce the demand for maize and consequently reduce the price of maize. Environmentally, it is good to have a diversified option, in this case pearl millet, even though not at the same level as the maize, it stands a chance to replace the maize.

7.1. Conclusions and Recommendations

The implication of this study is that pearl millet, as a crop which can thrive in adverse climatic condition, than maize, has the capacity to reduce the total dependency on maize as sole energy source in poultry feeds. Findings from this study showed that pearl millet has the potential to improve the performance of the broiler chickens and can be incorporated in the feeds to replace the needed maize which is an expensive alternative. The study revealed that the inclusion level of 75% had high performance as evidenced by higher feed intake (FI) and body weight (BW) and net return in comparison to the other levels of inclusion. Sensitization of the farmers about the importance of pearl millet as a valuable alternative to maize is highly recommended. Link between the producers of pearl millet and the producers of chickens needs to be established in order to create a communication channel which will increase the awareness on the importance of pearl millet for the industry

CHAPTER 8

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