Effect of dietary *Mucuna pruriens* seed meal on the serum lipid profile and bone quality traits of broiler chickens

By

Chumani Hempe: 201309192

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Department of Livestock and Pasture Science

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University of Fort Hare

Together in Excellence

Alice, Eastern Cape, South Africa

Supervisor: Prof V. Muchenje **Co-supervisor: C. S. Gajana**

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DECLARATION

I hereby declare that this dissertation is the outcome of my own study. I have accordingly acknowledged in the text where the work of others has been used.

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Chumani Hempe

I hereby certify that this statement is correct

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Prof V. MUCHENJE

……………………………………….

Mr. S. Gajana

ABSTRACT

This study aimed at evaluating the effects of heated *Mucuna pruriens* seed meal on the blood serum lipid profile and bone quality parameters of broiler chickens. A total of 120 day-old unsexed Cobb 500 broiler chicks were randomly allocated for the 4 treatment diets (T1, T2, T3 and T4) containing 0, 10, 15, and 20% heated *Mucuna pruriens* seed meal. The birds were reared on wood shaving floor pens for 35 days. Birds were humanely slaughtered by severing the jugular vein using a sharp knife. Blood was collected into a tube, centrifuge and analyzed for a lipid profile analysis (total cholesterol (TC), high density lipoproteins (HDL), low density lipoproteins (LDL), triglycerides (TCD) as well as aspartate transaminase (AST) and alanine transaminase (ALT)) by enzymatic diagnostic kits (Diasys diagnostic kits). Both drumsticks severed using a sharp knife. After boiling in the water at 100°C for 10 mins the tibia bones were taken, air-dried for 96 hrs at room temperature and measured for weights. Bone length, strength, moisture and ash content as well as morphology were measured. The blood lipid profile of birds fed T1 was high ($P < 0.05$) for all the parameters measured, while T3 had the lowest ($P < 0.05$) profile. T2 and T4 had the same profile. ALT was found to be highest ($P < 0.05$) for T4 (5.27 ± 0.207/L) and lowest ($P < 0.05$) for T2 (4.67 ± 0.333/L) while AST was highest ($P < 0.05$) for T2 (320.13 ± 28.851/L) and lowest ($P < 0.05$) for T3 (208.47 ± 10.059/L). Treatment diet 3 (T3) was observed decreased bone quality among all diets; with the lowest ($P < 0.05$) bone strength (188.55 ± 12.039N), bone length (8.59 ± 0.131cm) and morphology (1.69 ± 0.058). *Mucuna pruriens* seed meal inclusion in broiler diets reduces blood serum lipids and 20% inclusion levels have no adverse effects on the bone quality attributes.

Keywords: Velvet bean meal; Serum cholesterol; Liver function; Bone strength; Morphology

DEDICATION

To my family and dear friends I would like to dedicate my dissertation work. A special feeling of gratitude to my loving parents, Kholiswa Hempe, Zuziwe Lalendle and Milisi Mjokozeli whose words of inspiration and momentum for a drive in my ears have kept me alive. You thoroughly laid the foundation for my education giving it all it takes. You have given up very important things and lot of sacrifices to make it a point that I defeat this feat, in that regard I am and will forever be grateful. I can’t find the words that express my appreciation. My siblings Nande and Sange Hempe who always believed in me all the time and have never left my side, you two are very special. I also dedicate this dissertation to my two most precious friends Andisiwe Mpumlwana and Siyakudumisa Kwinana who have supported me throughout the process. To Nqobile Gqezuand Philisani Khangelani Ncoko I will at all times raise the value of all you have done, especially for helping me develop this dissertation and for always being there for me in the corridors of UFH. Lastly, I dedicate this work and give special thanks to my Lord and Saviour for always reminding me of the purpose behind every struggle, if it was for God I really wouldn’t have seen the end.

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Acronyms

ADF: Acid detergent fibre

ALT: Alanine transaminase

AST: Aspartic transaminase

Ca: Calcium

CP: Crude Protein

CTs: Condensed tannins

DM: Dry Matter

EE: Ether Extract

HDL: High density lipoprotein

HTs: Hydrolysable tannins

LDL: Low density lipoprotein

L-Dopa: L-3,4- Dihydroxyphenylalanine

M. pruriens: *Mucuna Pruriens*

ME: Metabolisable energy

MP: *Mucuna pruriens*

MPS: *Mucuna pruriens* seed

MPSM: *Mucuna pruriens* seed meal

N: Nitrogen

NDF: Neutral detergent fibre

NFE: Nitrogen free extract

OGM: Organic matter

P: Phosphorous

SAA: Sulphur amino acids

T1: Treatment one

T2: Treatment two

T3: Treatment three

T4: Treatment four

TC: Total cholesterol

TGs: Triglycerides

Chapter one

General Introduction

1.1 Introduction

Meat and other animal products play a remarkable role in improving the nutritional status of the consumers. According to Jayaweera *et al.* (2007) consumers nutritional status can be improved through supply of extraordinary-class and freely digestible protein that is readily available in meat. Layrisse *et al.* (1990) and reported that meat and additional meat products are noble suppliers of micronutrients. Yet an ordinary South African does not take sufficient protein of animal source (Bender, 1992). Attributed to FAO (1994) animal protein is extra competent over plant protein in making available the amino acids compulsory for development, function and repair of tissues**.** Ani and Adiegwu (2005), reported intensive poultry production as one of the ways that have been expected to supply adequate quantities of animal-derived protein for consumer consumption.

However, manifestation of cholesterol in meat and other animal manufactured products has been identified to be a factor that is limiting consumption of such goods (Jayaweera *et al.*, 2007). The major concern of people nowadays, is on associated potential health threats and nutritional quality of dietary components. Meat being nutritious and easily accessible, people have developed anxiety towards it's consumption due to high cholesterol content it contain (Abeywardena, 2003). The above circumstance has resulted to omitting of an outstanding supply of nutrients in the diet therefore leading to protein starvation particularly in developing countries. Therefore consumption of meat can be improved by gathering together ways to generate meat with minimum cholesterol level. Consequently protein undernourishment problem will be reduced as well as wellbeing susceptibility. Cholesterol dropping factors added to the diet of meat manufacturing animals, with chicken being used in this study, could be one of the ways to adopt in reducing cholesterol in meat.

Little research completed in *Mucuna pruriens* seeds demonstrated cholesterol dropping result of the beans in broilers (Iauk, 1989; Carew 1998 and Carew, 2003). According to Carew *et al*. (1998) creatinine and triiodothyronine are the notably physiological variations that showed a decrease in blood plasma levels when broilers were fed with *Mucuna* beans.

M. pruriens is a tropical legume, with little use as human food (Emenalom and Nwachukwu, 2006). The bean has tremendous potential as dietary protein source in animal feed (Emenalom and Nwachukwu, 2006). The mineral and amino acid profile of the *M. Pruriens* bean is as good as that of soybean (Iyayi and Taiwo, 2003). However, there is very little exploitation of the bean as feed ingredient in poultry industry (Eilitta and Carskey, 2003). The exploitation of *M. pruriens* bean either as food for human or as feed for animals is restricted since the untreated seed contains anti-nutritional factors such as tannins and trypsin inhibitors (Ravindran and Ravindran, 1988; Hussain and Manyam, 1997) and L-dopa anticoagulants (Houghton and Skari, 1994). Iauk *et al*. (1993) also suggested that the bean contain anti-inflammatory, analgesic and antipyretic factors and many more. Various processing techniques such as heating, autoclaving, boiling, and fermentation and roasting as well as other processing techniques can be used to improve nutritional potential of *M. pruriens* by decreasing the anti-nutritional factors of the bean to safer levels (Olaboro, *et al*., 1991). Hence, this study investigated effect of heated *M. pruriens* seed meal on lowering cholesterol levels within the blood and whether it can supply enough mineral to maintain normal bone development of broiler chickens.

1.2 Problem statement

Cost of meat and eggs has been on the rise as a consequence of ever-increasing price of poultry feeds. The increase in the price of poultry feeds has instated the need to search and develop alternative dietary protein and energy sources for use as substitute feed constituent that are cheaper, of low human first choice and locally available. Jayaweera *et al*. (2007) reported that high cholesterol found in meat and its products have been the discouraging factor for its consumption by consumers. This has resulted in the lack of protein availability in developing countries, mainly in areas where diets are based in poultry meat as the sole supply of protein.

Due to the high costs of conventional protein sources such as fish meal and soybean meal commonly used in chicken diets there is a rise in the price of poultry feed (Fagbenro *et al.*, 2003; Tacon *et al.*, 2009). The use of fish meal and soybean meal as dietary proteins has been observed to fail to drop cholesterol levels in meat. Hence there is dire need to seek alternative dietary protein sources that are cheaper and accessible in order to reduce the cholesterol. Although non-conventional plant proteins have their limitations on usage as broiler feed and other animal species for instance fish feeds, they have high potential as dietary protein and energy sources (Naylor *et al.*, 2009).

On the other side concerns from processing plants suggest that food quality is compromised as a result of leg disorders in fast growing chickens (Sullivan, 1994). Cook (2000) and Thorp (1994) noted increased economic loss through high mortality as a result of skeletal diseases. According to Pattison (1992) and Yogaratnam (1995) inability of broilers to walk near drinkers and feeders resulted to carcass condemnations which led to rise in meat losses. Hence there is a greater need to find feed alternatives that have high mineral supply of which MPS has been demonstrated to have the greater potential. Publications by Ukachukwu *et al.* (2002), Emenalon and Udedibe (2002) illustrated that there is insufficient literature on the inclusion of *Mucuna* seed meal in poultry diets and other animal species with the only available information on the use of *Mucuna* seed meals reported for fish.

1.3 Justification

One potential alternative feed ingredient is *Mucuna pruriens* seed. *M. pruriens* is an extensively available leguminous crop which blossom well in humid, subtropical and calm regions.

Ukachukwu and Obioha (1997) reported that *Mucuna* is adaptable to a large diversity of soils and resistant to pests and diseases. Raw *M. pruriens* bean contain 24-35.4% crude protein (Ukachukwu, 2006), 2570 Kcal/kg energy (Del Carmen *et al.*, 1999) and has an excellent amino acid profile (Siddhuraju *et al.*, 1996). *M.pruriens* seed meal also contains a high concentration of minerals (Vadivel *et al.*, 2007) and has significant amounts of dietary fibre (Carew *et al.*, 2002). Even though *M. pruriens* seed contains anti-nutritional factors (Udedibe and Carlini, 1998) that affect its acceptability and utilisation as poultry dietary protein source processing of the raw beans reduces the anti-nutritional factors concentration making the seed meal from the processed bean a potential dietary protein source.

High crude protein and energy content, amino acid profile, high concentration of minerals and dietary fibre make *Mucuna pruriens* seed meal a prospective replacement to soya bean meal as it benefits developed and developing broiler farmers. The expansion of the poultry industry has been described as the greatest way of improving the animal protein shortage in third world countries as a result of prominent turn-over speed linked with poultry development and subsequent economic competence (Dipeolu *et al.*, 2004).

1.4 Objective

This study ought to determine the effect of dietary *M. pruriens* seed meal on serum lipid profile and bone quality traits of broiler chicken.

1.4.1 Specific objectives

The specific objectives of the study were to:

- determine the effect of feeding broiler chickens graded inclusion levels of heated *M. pruriens* seed meal on serum lipid profile.

- ❖ determine the effect of feeding broiler chickens graded inclusion levels of heated *M. pruriens* seed meal on bone quality traits.

1.5 Null hypothesis

- ❖ Graded dietary inclusion of heated *M. pruriens* seed meal has no effect on serum lipid profile of broilers.
- ❖ Graded dietary inclusion of heated *M. pruriens* seed meal has no effect on bone quality traits.

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Chapter two

Literature review

1.1 Introduction

There is a competition between human food and animal feed industry for the utilization of obtainable conservative protein sources which has brought poor availability of dietary protein (Pugalenthi *et al*., 2005; Economist, 2011). As indicated by Baine (2013), the number of consumers getting wealth daily is increasing which put in food safety anxiety; adding to escalating requirement for protein source even though it far exceeds the ability of agriculturalists to meet. Globally, confirmation has been proposed that the lack of access to nourishing food remains a considerable challenge. According to FAO (2011), about 850 million individuals were malnourished wide-reaching for the duration of 2006-2008. The utilization of unconventional crude protein source like *Mucuna pruriens* seed meal can help to get rid of the extra use of conventional crude protein sources such as soybean (Pugalenthi *et al*., 2007).

One potential nonconventional crude protein that has been distinguished to partially replace soybean in broiler diets is *Mucuna pruriens* seeds (Sese *et al.,* 2013). *Mucuna pruriens* is a temperate legume utilized as developing crop. It belongs to kingdom Plantae, class *Dicotyledoneaen* in the family of *Fabaceae*, additionally it is also used for its diversity of medicinal uses (Probst, 2011). *Mucuna pruriens* is an annual and short-lived perennial plant which is a climber or trailer (Wulijarni-Soetjipto *et al*., 1997; US Forest Service, 2011). It has a taproot with 8-11 m long horizontal roots, the stems are thin and marginally pubertal (Wulijarni-Soetjipto *et al*., 1997). *Mucuna pruriens* seeds are different in colour; ranging from shiny black to white or brownish with black mottling

(Wulijarni-Soetjipto *et al*., 1997; FAO, 2011; US Forest Service, 2011). Despite the fact that *Mucuna pruriens* is indigenous to India, Malaysia, and China, it is now widely distributed in many tropical regions (FAO, 2011).

According to Drewnowski (2010), dry beans that are not poisonous but safe for human consumption are nutrient-rich foods. They contain variety of vitamins, minerals and other nutrients at the same time provide reasonable quantity of calories (Siddhuraju *et al*., 2000). The beans contain slight to no total fat, trans-fat, cholesterol and sodium whereas they also provide protein, fibre, folate, iron, potassium and magnesium (USDA, 2012). *Mucuna pruriens* seeds are rich in crude protein (15-30%) (Pugalenthi *et al*., 2005), starch (28%) and gross energy (10-11 MJ/kg) (Sidibé-Anago *et al*., 2009). MPS also have desirable amounts of amino acids, smaller amount of fatty acids (about 3-5%) and it also contains good mineral composition (Vadivel *et al*., 2007). Additionally, the bean has been noted to be a rich source of soluble and insoluble fibre (USDA, 2012). Association has been made between fibre consumption and decline in total and low-density lipoprotein (LDL) cholesterol and also decreasing the risk for developing metabolic syndrome, diabetes, coronary heart disease, obesity, stroke, hypertension, and some gastrointestinal diseases (Anderson *et al.*, 2009; Bourdon et al., 2001; Brown *et al*., 1999; Anderson and Bridges, 1988)

Despite being nutrient dense *M. pruriens* has a high content ratio of anti-nutritional factors, for example L-dopa, tannins, anti-tryptic and haemagglutination factors limiting its use in monogastric animals (Vadivel *et al*., 2011). According to Vijayakumari *et al*., (2007); Heuze *et al*., (2015), soaking, boiling, dry roasting, autoclaving and combinations of these techniques are important treatments that have been tested to help neutralize the effects of the bean in monogastrics. However heat treatment of *M. pruriens* beans has been reported to be the best in eliminating some harmful effects of the bean (Carew *et al*., 2006).

1.1.1 Agronomic characteristics of *Mucuna pruriens*

Mucuna pruriens is an annual or perennial, herbaceous, vigorous climbing or trailing vine which can grow up to 18m in length (Wulijarni-Soetjipto *et al.*, 1997). It's known to reach a height of 5.5m when grown on the ground although the most common height is found between 2-3m (Duke, 1981). *Mucuna* grow readily in frost-free tropical and subtropical regions. Warm temperature of 20 – 30^{0}C all through the developing period is favoured. *Mucuna* adjust to an extensive variety of soils, from sands to dirt. It endures low soil readiness, acidic soils, and dry season conditions (Hairiah *et al*., 1991; Weber, 1996). *Mucuna* inclines toward a precipitation scope of 650 to 2500 mm with normal precipitation of between 1200 - 1500 mm/yr.

1.1.2 General uses of *Mucuna pruriens* in Agriculture

Mucuna pruriens is a high yielding legume that easily grow in dry lands which on average yield 2 tons of clean bean per hector (Aguado et al., 2015; Canello et al., 2016; Yan, 2016). It is however a cheap non-conservative protein enhancement for sheep and goats (Matenga *et al*., 2003; Tuleun *et al*. 2008). It is one of the most popular medicinal plants in Africa and Asia (Mapiye *et al*., 2007). As illustrated by Del Carmen et al. (1999), in the early part in this century soybeans replaced MP which was once the common widely harvested legume in the United States. The genus *Mucuna* take account of nearly 150 species of annual and perennial legumes (Eze et al., 2017). *M. pruriens* has an immense taxonomic uncertainty around its mixture of varieties (Aguado *et al.*, 2015). However, at the moment it is acknowledged two types of *Mucuna* are in existence: *M. pruriens* var. *utilis* and *M. pruriens* var. *pruriens* (Food, 2017).

With findings from Vadivel and Biesalski (2012) legumes are an outstanding foundation of protein, micronutrients, starch, dietary fibre and bioactive compounds with a tiny fat level. Farmers adopting the MP as a skill can profit from improved drought resistance, advanced land production and well-built cash returns (de Villiers, 2014). The biological active compounds content of MPS gained the beans a historic value in Agriculture due to a number of uses (Hammerton, 2002).

Figure 2.1: ***Mucuna pruriens*** **seeds showing, with variability among varieties**

Source: www.feedipedia.org, 1997

1.2 Benefits of using *Mucuna* pruriens in agriculture

Mucuna pruriens is one of the most popular multi-purpose legumes among small farmers in the tropics because of its outstanding source of green manure, in part because of its capacity to fix atmospheric nitrogen thereby restoring soil fertility. It also adds significant qualities of organic matter (≤30 tons) to the soil (Gilbert, 2002). *M. pruriens* has the ability to resist pest and disease (Wolf et al., 2003). As a result it has gained advantage as companion crop in intercropping schemes. The crop suffocates weeds meritoriously when established (FAO, 2011). *M. pruriens* has been recommended as one of the greatest appropriate crops for recovering land taken over by weed

(Hellin, 2006; Wulijarni-Soetjipto *et al*., 1997). Furthermore, the crop can also be used to efficiently control nematodes invasion (Wulijarni-Soetjipto *et al*., 1997). Quick growth as well as extended growing period in environments that are frost free are the key qualities of *Mucuna pruriens*. *M. pruriens* is mainly grown as a cover crop and green manure because it can establish very quickly without requiring complete soil preparation (Cook *et al*., 2005). *M. pruriens* is a fast growing legume which gathers nutrients in a way of N fixation and during rainy season it shields the soil from erosion.

According to Mulyvaney, Khan and Ellsworth (2009) to restock the wasted soil, growing legumes as green manure or rotation crop was a strategy implemented and also as an organic matter required to preserve physical and chemical conditions of soil, good for continuous production of crop. To sustainable use agricultural soils as published by Ceballos *et al*. (2012) farmers in Latin America promoted use of MP state that, regardless of the dominant trend of technology, permitting them to cultivate countless crops inexpensively in rotation and inter- cropping. While stabilizing fertility of soil, it has also been promoted as bio-control of diseases, weeds with pests inclusive (Buckles *et al*., 1998). As a leguminous type MP appeared to advance soil fertility, also it make available greater than 9 t DM biomass/ha above the ground and fixes below the ground330 kg N/ha (Wilijarni-Soetjipto *et al*., 1997) and as well fixes up to 1615 kg ammonium sulfate/ha (Cook *et al*., 2005). With regards to Buckles *et al*. (1998), *M. Pruriens* similarly yields 20kg P/ha and 100 kg K/ha.

1.3 *Mucuna pruriens* for human use and animal feed

The seeds of MP are an important source of dietary protein for people in many parts of the tropics where hunger and protein malnutrition prevail (Siddhuraju *et al.*, 1996). In fact, MP has contributed to improving the food shortage problem in developing countries such as Honduras, Central America (Eilitta et al., 2002), Benin, Nigeria (Versteeg et al., 1998), Malawi (Gilbert, 2002) and

Guinea (Diallo et al., 2002). MP is used as a cover crop and as an animal feed in various parts of the world (Jayaweera et al., 2007). However, certain anti-nutritive factors such as L-dopa and trypsin inhibitors in MS need to be eliminated or neutralized before it is safe for consumption (Houghton and Skari, 1994). These valuable impacts are because of the pharmacologically active compounds in Mucuna. L-dopa (3, 4 dihydroxy-Lphenylalanine) is the main compound of interest which is utilized in the treatment of Parkinson's disease and is thought to be metabolized into dopamine or melanin in the peripheral blood system. However, ingestion of excessive quantities of the L-dopa in MP has caused vomiting, nausea, headaches, hallucination and paranoid delusions in humans (Szabo and Tebbett, 2002). Other compounds in *Mucuna*, including melanin and serotonin, are responsible for the irritating, allergenic or rubefacient effects that are associated with the hairs and sap of certain varieties. *Mucuna* is also an ornamental crop that forms a thick shade and the vine, pods and blossom are quite attractive. In addition, for ovulation MB powder is utilized as a uterine stimulant and as a sexual enhancer (Lorenzetti et al., 1998).

M. pruriens has additionally been utilized for expanding sexual potency partly because it increases sperm count and testosterone levels, which may increase deposition of protein in muscles (Siddhuraju *et al.,* 1996). Accumulation of chemical, biochemical, clinical, and epidemiological evidences indicates a positive correlation between the consumption of legume seeds and decreasing incidence of several chronic diseases, such as cancer, cardiovascular diseases, obesity, and diabetes (Vadivel and Biesalski, 2012). Such obvious health benefits of legume seeds are attributed to the presence of certain bioactive compounds, for instance phenolic acids, tannins and flavonoids (Vadivel and Biesalski, 2012).

1.4 Nutritional composition of *Mucuna pruriens*

Chemical analysis remains the best way to determine potential value of any feed type nutrient supply. Sidibé-Anago *et al*. (2009), noted that MP forage encompasses almost 15-20% protein DM basis. Seeds are rich in protein that is 24-30%, 28% starch and gross energy of 10-11 MJ/kg (Siddhuraju et al., 2000; Pugalenthi *et al*., 2005). Adebowale *et al*. (2005), MPS are rich in lipids nearby 9.65 g/100 g. According to Vadivel *et al*. (2007), the MP seeds contain excellent mineral composition and they carry desirable amino acids. The chemical composition for the MP has been shown on table 2.1.

1.4.1 Crude protein

The crude protein (CP) concentration of raw *Mucuna pruriens* has been reported to range from 21% (Flores *et al*., 2002) and 38% (Adebowale *et al*., 2005b). These differences are due to factors like variety, growth environment, and maturity. Protein concentration may decline as the bean matures, due to nitrogen availability during bean filling and the final bean size. Immature beans have been reported to have an amount of 37% CP while mature beans had 24% (Wanjekeche *et al*., 2003). Scholars such as Carew *et al*. (2002), Del Carmen *et al*. (2002), Ukachukwu *et al*. (2002),Ayala-Burgos(2003), Ezeagu *et al*. (2003), Siddhuraju and Becker (2003) argues that crude protein content in MPS grown in different places of the tropics and subtropics varies between 21.0 and 30.3%.

Mucuna pruriens contain large quantities of amino acids (Ukachukwu *et al*., 2002), with the segregation of sulphur amino acids (SAA). According to Bressani (2002) the methionine levels in MP are 1.3 g/16 g N on average. Ukachukwu *et al*. (2002) reported that value is parallel to some beans like in pigeon pea (1.2), Jack bean (1.2) and lima bean (1.5). Table 2.2 shows the types of amino-acids available both in raw and heated *Mucuna pruriens* seeds.

1.4.2 Ash content

Mineral constituent of a feed is represented by ash. Seeds of MB are found to contain about 2.9-

4.4% ash content. Vadivel and Janardhanan (2000) noted ash content levels to be high 3.3-5.5% in the bean. For maintenance of well-being, vitamins and other minerals are needed in little amounts by the body. Among the important number of reasons for ash content in feed it is a key for the worth of feeding ingredients used in poultry and other animals.

1.4.3 Fibre content

Vegetables, fruits and whole grains make available complex carbohydrates which provide fibre in the diet. Crude fibre, recognized as roughage, have cellulose and hemicellulose, an assorted group in which pentosons usually predominant over lignin, pectic, and cutin substances (Bressani, 2002). Carew *et al.* (2002) reported that the beans of *Mucuna* contain dense constituent of food namely dietary fibre primarily developed from skeletal remnants of lant cell walls ironic in cellulosic polysaccharides and lignin that counterattack hydrolysis by human intestine enzymes. The neutral detergent fibre (NDF) and acid detergent fibre (ADF) of *M. pruriens* seeds are have 9.6% and 21.3%, respectively (Siddhuraju *et al.,* 1996) and are known to contain low levels of crude fibre (1.6–7.9%) (Vadivel and Janardhanan, 2000). The neutral detergent fibre and acid detergent fibre content of MP seeds falls between 10.3–25.9% and 9.3–20.4%, respectively (Del-Carmen *et al.*,2002;; Ayala-Burgos *et al.,* 2003;). However, Castro *et al.,* (2003) found only the NDF (40.7%) and ADF was not detected. e.

1.4.4 Ether extract

The lipid concentration of *Mucuna* beans varies widely. Some researchers reported low ranges of 2.8-4.9% (Siddhuraju *et al*., 2000) but others reported higher ranges of 8.5-14.0% (Vijayakumari, *et al*., 2002). Adobowale *et al*., (2005b) confirmed that ether extract of entire cotyledon, seed and seed coat have 9.8, 9.6 and 3.0% lipid, correspondingly.

1.4.5 Minerals in Mucuna pruriens

Mucuna pruriens are rich in minerals, especially calcium, potassium, magnesium, and iron (Duke, 1981). Kay (1979) reported that *M. Pruriens* hold thiamine and riboflavin in small amounts of 13.9 and 1.8 ppm. Reports by Iyayi and Egharevba (1998); Iyayi and Taiwo (2003) suggested magnesium value as the highest mineral in *M. pruriens*. Iron concentration is of special interest because the diets of many in developing countries where *M. pruriens* is grown are deficient in this mineral (Bressani, 2002).

Table 2.1: Chemical composition of Mucuna pruriens meal

Parameter	Concentration
Proximate composition (g/kg DM)	
Crude Protein	354
Crude fibre	**77**
Ether extract	32
Ash	36
Nitrogen free-extract	479
Major Minerals (mg/kg DM)	
Potassium	14
Calcium	10
Magnesium	19
Phosphorus	8
Trace minerals (mg/kg DM)	
Zinc	13
Manganese	27
Iron	129
Copper	25
Anti-nutritional factors (mg/kg DM)	
Hydrocyanic acid	82
Tannins	21
Phytic acid	21

Source: (Iyayi and Taiwo, 2003)

Table 2.2: The values of different types of amino acids of raw and heat processed Mucuna pruriens

Components	Raw Bean (%)	Heated Bean (%)
Histidine	0.46	0.48
Isoleucine	1.03	1.09
Leucine	1.54	1.66
Lysine	1.5	1.68
Methionine	0.22	0.27
Phenylalanine	0.99	1.06
Threonine	1.04	0.84
Tryptophan	0.07	0.14
Valine	1.12	1.17
Alanine	0.88	0.94
Arginine	1.71	1.80
Aspartic acid	2.63	2.56
Cyteine	0.27	0.31
Glutamic acid	2.84	2.75
Glycine	1.00	1.02
Proline	1.36	1.45
Serine	0.93	1.04
Tyrosine	1.02	1.07
Nitrates	0.002	<0.002

Source: (Iyayi and Taiwo, 2003)

2.7 Bioactive compounds in *Mucuna pruriens* seed

According to Srindhar and Bhat, 2007), *M. pruriens* beans contain several anti-nutritional compounds namely L-Dopa, phytic acid and trypsin, tannins, amylase inhibitors and lectins. Manyam *et al*., (2004) clarified that the beans of *Mucuna* rich in alkaloids, sterols, and saponins.

2.7.1 L-DopaL-3,4- dihydroxyphenylalanine (L-DOPA)

L-dopa is an important anti-nutrient in *Mucuna pruriens* because it is a neurotoxic agent complex in fairly large volumes (Bell and Janzen, 1971; Daxenbitchler *et al*., 1971). Ensiling has been used to reduce the content of L-dopa by 11-46% in the seeds (Matenga *et al*., 2003). L-dopa well known powerful anti-nutritional element may cause serious sickness and diarrhoea in pigs fed large amount of MP seeds (Siddhuraju *et al*., 2002). In ruminants it causes less harm even though it does not give the impression to alter rumen fermentation but rumen microbial adaptation to L-Dopa have been noted (Chikagwa-Malunga *et al*., 20009b). Several studies by others such as (Castillo-Camaal *et al*., 2003a; Castillo-Camaal *et al*., 2003b;Matenga *et al*., 2003; Mendoza-Castillo *et al*., 2003; Pérez-Hernandez *et al*., 2003) performed on goats and sheep with MP seeds and pods being the major ingredients in the diets didn't point any opposing effects resulting from L-dopa.

2.7.2 Tannins

In plants the most common occurring anti-nutritional factors are tannins. These compounds exist in several shrub foliages, seeds and by-products in agro-industrial (Aregheore, Makkar and Becker, 1998, 1999). With reference to Waghorn, Red and Ndlovu (1999) livestock assembly and reproductive performance has been restricted due to reduced value of nutrition by phenolic compounds mainly tannins and lignin found in MP seeds. Hydrolysable tannins (HTs) and condensed tannins (CTs) are two classification groups of tannins. Two of them are hydrosoluble polymers that make soluble and insoluble complexes, chiefly with proteins. They furthermore have similarity towards carbohydrates, amino acids and minerals (Makkar and Becker, 1998). HTs, in contrast to CTs, are fairly degraded in the gastro-intestinal tract and numerous precursors might originate from their glucose and phenolic constituents (Makkar, 2000).

Nevertheless, most investigators come to an understanding that CTs are not tarnished in the animal gut and therefore are at all not lethalin standard nurturing circumstances once browse and tree foliage is served as a complement (Makkar, Blümmel and Becker, 1995). Yet, CTs directly disturb the nutritional assessment of feedstuffs. Ages ago, CTs were considered as unusable compounds specializing with negative effects on intake, digestion, production and reproduction in animals. However, current findings have set that CTs might as well have constructiveproperties in ruminants (Barry, and McNabb, 1999; Barry, McNeill and McNabb, 2001).

2.7.3 Alkaloids

In *Mucuna pruriens* seeds mucunain, prurienine and serotonin are notable alkaloids. Pod hairs yield mucunain which causes bad itching when in contact to the skin and severe pain on the eye should it come in contact with the hairs. Pugalenthi *et al*. (2005) suggested that L-dopa cantoo be implicated in eruptions of skin. Serotonin potentializes negative effects of mucunain (Cook *et al*., 2005). Miller, 2000 reported the consumption of hairy pods by cattle can lead to haemorrhage and death. Duke (1981) reported the occurrence of bioactive compounds like nicotine, physostinginine, and serotonin in *Mucuna* seeds. Complex of compounds is found in the roots, pods, seeds and leaves of *Mucuna,* theseinclude *N*, *N*-dimethyltryptomine, 5-oxyindole-3-alkylamines, indole-3-alkylamine, B-carboline, choline and bufotenine (Szabo, 2002).

2.7.4 Other antinutritional factors

Mucuna pruriens seeds have a number of other anti-nutritional factors: trypsin and chymotrypsininhibitors which lessen digestibility of protein, encourage pancreatic hypertrophy and hyperplasia, boost trypsin secretion and, as a result reduce N retention, development and feed conversion (van Eys *et al.*, 2004). One other factor that may perhaps decrease protein and mineral accessibility since it is found to be high in the seeds is phytate content (Pugalenthi *et al*., 2005).These anti-nutritional factors can be well reduced by a varied choice of processing methods.

2.8 Processing of *Mucuna pruriens* seed meal

According to Nyirenda *et al*. (2002) heat treatment has been identified to be the most common process of preparing *Mucuna pruriens* seeds meant for use by human and animal atmosphere. Most of the detrimental compounds found within the seeds are heat labile (Hayase *et al*., 1975). Different ways have been tried to achieve heat treatment for example, roasting at 120º C for 30 min and autoclaving for 30 min (Dossa *et al.*, 1998; Del Carmen *et al.*, 1999). Siddhuraju *et al*. (1996) reported dry heat treatment to be efficient in dropping L-Dopa in *M. pruriens*.

Heat treatment either by boiling or roasting is reported to be largely effectual in decreasing other anti-nutrients in seeds of *M. pruriens*. Nutritive quality of the beans was constant through grilling more than cooking (Dossa *et al*. 1998). However, these findings contradicts with earlier reports by Laurena *et al*. (1991) which proved boiling to be better than roasting. Levels of HCN can successfully be reduced by up to 68% by heat treatment thorough roasting and cooking. Through dry heat action phytic acid was lowered near to 36% and up to 47% phytate content was reduced through autoclaving *M. pruriens* seed (Siddhuraju *et al*., 1996).

2.9 Importance of the availability of calcium, phosphorous and vitamin D for bone development

In growing chickens the proper functioning of the skeletal system collaborate a vital role (Suchý *et al*., 2009). The genetic collection of broilers to yield bigger weight and the maturity of skeletal muscles doesn't match the expansion of bones, consequently, this can lead to the incidence of bone fractures and deformities (Suchý et al., 2009). This is led by a range of aspects of which inability of skeleton to adjust towards escalating weight of the body (Korver *et al*., 2004; Whitehead, 2004). At some stage of quick development and heritable factors, low intake of Ca and P lowers mineral consumption (Williams et al., 2000). Hence, it is important to ensure that there is enough Ca and P available in the diets. In animal species including chickens morphological properties and the internal structure of bones ought to make certain best possible balance against forces linked with the supportive and locomotor function of the skeleton (Kanis, 1994; Tatara *et al.,* 2006).

Diet is the essential factor in the development of birds, according Eastell and Lambert(2002) about 69 to 79% of bone mass is resolute genetically whereas the ensuing 21 to 31% is accredited to outer reasons such as nutrition; as it has a considerable consequence on mineralization of bone (Huyghebaert, 1997). Separately from the metabolic side merchandise of digestion and composites produced in the intestine; minerals, vitamins or some nutrient insufficiency or surplus are as well in the midst of the vital nutritional factors that may facilitate changes in the pelvic limbs. Nutrients that are in this respect found to play a key part are Ca, P and Vitamin D. Other studies by Huyghebaert (1996) and Onyango *et al.* (2003) reported that bigger volume in mineral content in the tibiotarsus was found as a result of improved levels of both Ca and P.

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Chapter 3

Effect of feeding broiler chickens with graded inclusion levels of *Mucuna pruriens* seed meal on blood serum lipid profile

Abstract

This study assessed the effects of graded dietary inclusion of heated *M. pruriens* seed meal on blood serum lipids of broiler chicken. A total of 120 day-old unsexed Cobb 500 broiler chicks were randomly assigned to four treatment diets (T1, T2, T3 and T4) containing 0, 10, 15, and 20% heated MPSM. The birds were reared on wood shaving floor pens for 35 days. Blood was collected by severing the jugular vein into blood collection tube, centrifuged and analysed for total cholesterol (TC), high density lipoproteins (HDL), low density lipoproteins (LDL), triglycerides (TCD), aspartate transaminase (AST) and alanine transaminase (ALT) by enzymatic diagnostic kits (Diasys diagnostic kits). The blood lipid profile of birds fed T1 was high ($P < 0.05$) for all the parameters measured, while T3 had the lowest ($P < 0.05$) profile. T2 and T4 had the same profile. ALT was highest ($P < 0.05$) for T4 (5.27 ± 0.207) and lowest ($P < 0.05$) for T2 (4.67 ± 0.333) while AST was highest ($P < 0.05$) for T2 (320.13 ± 28.851) and lowest ($P < 0.05$) for T3 (208.47 ± 10.059). The inclusion of heated MP in chicken broiler diets reduced both good and bad blood serum cholesterols while increasing the ALT which indicates liver dysfunction

Keywords: *Mucuna pruriens* seed meal; Serum cholesterol; Liver function

3.1 Introduction

Efficient feed conversion and fast growth rate are outstanding factors that determine success and the economic growth of broiler production. These factors can be efficiently realized not only through proper management of birds but also feeding. Feed quality and cost remain the main determinants of broiler production efficiency (Amakiri *et al.*, 2011). The cost of feed is minimized when feed conversion ratio is improved. Poultry researchers have gathered a lot of knowledge on biochemical and physiological mechanisms aimed to increase the productivity of feed consumption and wanted carcass traits in reply to altering dietary ingredients (Nkukwana, 2012).

According to the reports by Opara and Okorie (2015), the utilization of legume seeds for instance soybean into poultry diets has turned out to be very well-known since its high in protein content, about 44-47% crude protein. Up to 20% of broiler finisher diets protein concentrate is formed by soybean. The price per kg of soybean meal has now increased rapidly as a result of its high demand both for human consumption and as animal feed. The increasing price has resulted in higher value of livestock feed and increased price of poultry products. Hence, there is a necessity for the use of additional good available substitutes in order to reduce the increased feed prices.

Mucuna pruriens is one of the potential legume seed that ought to be used to substitute soybean meal in poultry diets (Anele, 2002; Ani, 2008). Among developed countries, *M. pruriens* is alternatively being used as a cover crop generally intercropped with corn (Buckles, 1995). *M. pruriens* is excellent in survival and sustainable agriculture as it is resilient, resistant to drought and defiant to insects. It is as well managed with negligible care and perk up soil fertility in the course of nitrogen fixation. This plant is habitually given as feed to animals that graze and its prolific production of beans is from time to time consumed by humans and monogastric animals like pigs and chickens (Del Carmen *et al*., 1999). Scientific findings shows that *Mucuna pruriens* seeds are poisonous when fed raw to poultry. They reduce growth, feed intake, egg production and cause mortality (Harms *et al*., 1961). Research has been focused on processing methods to reduce the toxicity of *M. pruriens* seeds in order to maximize the efficient use of *M. pruriens* in poultry.

Although scientific research has shown that feeding broiler chickens with *M. pruriens* has little or no adverse effect on growth performance with significant reduction of feed costs (Dei *et al.*, 2006; Jorge *et al.*, 2008; Vadivel *et al.*, 2011); to our knowledge little is known about the effects *M. pruriens* inclusion in broiler diets has on the blood serum lipids. Monogastrics including broiler chickens have the potential of incorporating dietary lipids into their blood (López-Ferrer *et al.*, 2001; NRC, 2012). Del Carmen (1999) and Carew (2003) highlighted that feeding *M. pruriens* to broilers has a blood serum cholesterol lowering effect. The occurrence of high cholesterol in meat and meat processed foods is a chief aspect depressing the consumption of such foods. This study evaluated the effects on feeding graded inclusion levels of heated *M. pruriens* on blood serum lipids of broiler chickens.

3.2 Methods and materials

3.2.1 Ethical clearance

Consent to carry out the study was applied for and obtained from the University of Fort Hare Ethical Clearance Committee. Ethical certificate clearance number: MUC091SHEM01

3.2.2 Study site

The study was conducted at the University Of Fort Hare, which is 123 km from East London along the eastern coastline, situated in Raymond Mhlaba Local Municipality under Amatole District, in Eastern Cape, South Africa. The geographical location of UFH is 32°47′13.4″S 26°50′56.7″E. The annual rainfall in Alice is about 386mm, of which the most rainfall occurs in summer. The midday temperature ranges between 19◦C in June to 27.6◦C in February each and every year. Altitude is 572 meters above sea level. According to Acocks, (1975), the veld of Alice is classified as False Thornveld with a savannah vegetation type. Soils are particularly heterogeneous but are dominated by sedimentary rocks with some variation when intrusions of igneous rock thus they result in red soils occurring in some areas.

3.2.3 *Mucuna* seed meal preparation

Raw *Mucuna pruriens* seeds were purchased from McDonald Seed Company in Pietermaritzburg, Kwa-Zulu Natal. The seeds were then dry heat treated at 130°C for 30 minutes using memmert laboratory oven. The treated seeds were then grinded using a laboratory mill to pass through a 1mm screen and stored in bags at room temperature. Milled samples of the seed meal were subjected to proximate composition at University of Fort Hare Nutrition Lab using (crude protein, crude fibre, ether extract) in macro and micro minerals according to AOAC (2000). Nitrogen Free Extract (NFE) was calculated as = DM - (Crude protein + Crude fibre + Ether extract + Ash) in terms of percentage. The metabolisable energy (ME) in kcal/kg of *M. pruriens* seed meal was calculated using the method by Pauzenga (1985), were ME =35 x CP % + 81.8 x EE% + 35.5 x NFE%. The total phenolic and condensed tannins content of the seed was evaluated as described by Idris *et al.* (2017) using water extracts. The oxalate and phytic acid content of the seed were determined as described by Unuofin *et al.* (2017) using water extracts.

Table 3.3: Chemical composition of heat treated *Mucuna pruriens* seed meal

Chemical nutrients (%)	Composition
Moisture	7.5
Crude protein (CP)	33.4
Crude pibre (CF)	20.0
Ether extract (EE)	8.4
Ash	3.3
Nitrogen free extract (NFE)	47.4
Total digestible nutrients (TDN)	77.4
Metabolisable Energy (kcal/kg)	3538.82
Major minerals (%)	
Calcium (Ca)	0.07
Phosphorus (P)	0.70
Potassium (K)	0.19
Magnesium (Mg)	0.09
Trace minerals (mg/kg)	
Sodium (Na)	0.0
Iron (Fe)	126
Cupper (Cu)	13
Zinc (Zn)	17
Manganese (Mn)	40

Table 3.4: Anti-nutrients of heat treated *Mucuna pruriens* seed meal

Anti-nutrients	Levels
Condensed tannins	61.95(mg CE/g)
Total free phenolic	1912.54 (mg GAE/g)
Phytic acid	19.96 (%)
Oxalate	3.81 (mg)

mg CE/g- milligram of catechin equivalent, mg GAE/g- milligram in gallic acid equivalent per grams, %- percentage, mg- milligram.

3.2.4 Dietary treatments

Four dietary treatments were formulated using Win-Feed 3.0 Formulation Software in order for diets to be iso-caloric and iso-nitrogenous and to meet the bird's nutrient requirements (NRC, 1994). The diets were designated as T1, T2, T3 and T4 with 0, 10, 15, and 20% inclusion levels of *M. pruriens* seal meal, respectively replacing soybean meal.

3.2.5 Experimental design and bird management

The experiment was carried out with 120 day-old unsexed Cobb 500 broiler chicks. These 120 chicks were randomly allocated to 20 fresh wood shavings floor pens. The 20 pens were used as experimental units and with each of the 4 dietary treatments consisting of 30 birds sub-divided into 5 replicates. Each replicate consist of 6 birds organized in a completely randomized design. The temperature was set at 34^{o}C prior to the arrival of the chick and maintained during the first week. It was decreased by 3^{o}C per week until 22^{o}C was reached and maintained at that level until the end of the experiment. Marek's disease and infectious bursal disease vaccination was carried at the hatchery. Birds were monitored daily and dead birds removed and record were kept. Birds were provided with fresh feed and water *ad libitum* throughout the experiment. The birds were fed in 2 phrases: starter 1 – 21 days and finisher 22- 35 days. The ethics of animal care in experimentation described by NRC (1994) was adhered to during this experiment.

3.2.7 Collection of blood samples

At 35 day, 15 bird per treatment were randomly selected, electrically stunned with 50 – 70 V for 3 – 5 s and the jugular vein was severed to collect blood sample. Blood samples were collected into blood test tubes, centrifuged and stored at -20^{o}C for 24 hrs before analysis of serum lipid profile.

3.2.7 Analysis of serum biochemistry

To obtain plasma the tubes containing blood were centrifuged at 3000rpm for 10 minute. The samples and "Bleed Record Sheets" were sent to Victoria Hospital in Alice, Eastern Cape province for analysis. Blood samples were subjected to lipid profile analysis [total cholesterol (TC), high density lipoproteins (HDL), low density lipoproteins (LDL), triglycerides (TGs), aspartate transaminase (AST) and alanine transaminase (ALT)) by enzymatic diagnostic kits (Diasys diagnostic kits). The cholesterol determination for each sample was made using the extraction procedure of Fisher and Leveille (1957). The cholesterol estimation was carried out according to the Liberman-Burchard method as described by Sabir *et al.* (2003).

3.2.8 Statistical analysis

The data collected was subjected to the General Linear Model (GLM) procedure of SAS (SAS, 2003) and ANOVA test was made using the following model:

$Y_{ij} = \mu + \alpha_i + e_{ij}$

Where Y_{ij} = response variable, μ = the common mean, α_i = the effect of dietary treatment (i=4; T1, T2, T3 and T4 are treatment 1, 2, 3 and 4 respectively) and e_{ij} = the random error.

Differences between means was tested according to Fisher's least significant difference (LSD) test of SAS. Least square means with the standard error were used to present data. A significant level of $P < 0.05$ was used to conclude differences.

Table 3.5: Treatment dietary formulations

Ingredients	Starter				Finisher			
	T1 (0%)	**T2 (10%)**	**T3 (15%)**	**T4 (20%)**	**T1 (0%)**	**T2 (10%)**	**T3 (15%)**	**T4 (20%)**
Yellow maize	61.00	60	53.66	53.68	70	65	58.39	52.53

Soya oil cake 47	29.09	21.83	23.89	13.86	20.63	15.80	11.73	12.53
Full fat soya	4.07	4.63	3.00	6.14	2.99	4.1	9.82	9.90
Velvet bean seed meal	0	10	15	20	0	10	15	20
Canola Oil	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Limestone fine	1.91	0.13	0.00	1.50	1.38	1.43	1.34	1.32
Mono calcium phosphate	0.59	0.00	0.84	0.85	0.65	0.47	0.50	0.51
Sodium bicarbonate	0.09	0.14	0.31	0.25	0.61	0.06	0.09	0.08
Salt fine	0.38	0.34	0.22	0.27	0.02	0.35	0.33	0.34
Lysine	0.20	0.30	0.41	0.52	1.20	0.17	0.17	0.16
Methionine	0.18	0.15	0.18	0.20	0.08	0.14	0.15	0.15
Threonine	0.00	0.00	0.00	0.22	0.00	0.03	0.03	0.03
Tryptophan	0.00	0.00	0.01	0.03	0.00	0.00	0.00	0.00
Axtra Phy broiler enzyme blend 600 px	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Aviax plus	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Surmax	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
Min-Vit Premix	0.34	0.33	0.33	0.33	0.29	0.30	0.30	0.30
Total (%)	100	100	100	100	100	100	100	100

Table 3.6: Calculated nutrient composition of the dietary treatments

Nutrients	Starter				Finisher			
	T1 (0%)	**T2 (10%)**	**T3 (15%)**	**T4 (20%)**	**T1 (0%)**	**T2 (10%)**	**T3 (15%)**	**T4 (20%)**
Protein (%)	20.0	20.0	20.0	20.0	16.3	16.3	17.00	17.00
Fat (%)	2.5	2.7	2.5	2.5	2.5	2.5	3.2	3.0
Calcium (%)	1.3	1.2	1.1	1.0	1.3	1.23	1.03	1.09
Phosphorus	0.9	0.4	0.4	1.0	0.9	0.9	0.9	0.9
Sodium (%)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
ME (MJ/kg)	9.1	9.3	9.4	9.6	11.0	11.2	11.6	11.8

3.3 Results

The feeding of broiler chickens with graded inclusion levels of *Mucuna pruriens* meal affected their blood lipid profile (Table 3.5). The blood lipid profile of birds fed T1 was high ($P < 0.05$) for all the parameters measured, while T3had the lowest ($P < 0.05$) profile. T2 and T4 had the same profile. The HDL/LDL ratio was not significant for all the dietary treatments. For the liver function tests, ALT was found to be highest ($P < 0.05$) for T4 (5.27 ± 0.207) and lowest ($P < 0.05$) for T2 (4.67 ± 0.333) while AST was highest ($P < 0.05$) for T2 (320.13 ± 28.851) and lowest ($P < 0.05$) for T3 (208.47 ± 10.059).

Table 3.7: Effect of graded inclusion of heated Mucuna pruriens meal on blood lipid of broiler chickens

Lipid fraction (mg/dl)	Dietary treatment			
	T1 (0%)	**T2 (15%)**	**T3 (15%)**	**T4 (20%)**
Lipids				
Total cholesterol	$2.81^{a} \pm 0.080$	$2.73^{a} \pm 0.065$	$2.33^{b} \pm 0.106$	$2.76^{a} \pm 0.114$
HDL	$2.27^{a} \pm 0.082$	$2.27^{a} \pm 0.054$	$1.92^{b} \pm 0.093$	$2.23^{a} \pm 0.116$
LDL	$0.38^{a} \pm 0.098$	$0.35^{ab} \pm 090$	$0.30^{b} \pm 0.077$	$0.35^{ab} \pm 0.093$
HDL/LDL ratio	6.12 ± 0.431	6.84 ± 0.576	6.48 ± 0.250	6.97 ± 0.578
Triglycerides	$0.35^{a} \pm 0.026$	$0.25^{b} \pm 0.018$	$0.17^{c} \pm 0.010$	$0.29^{b} \pm 0.018$
Liver function tests				
ALT	$5.00^{ab} \pm 0.000$	$4.67^{b} \pm 0.333$	$5.13^{ab} \pm 0.090$	$5.27^{a} \pm 0.207$
AST	$314.53^{a} \pm 19.936$	$320.13^{a} \pm 28.851$	$208.47^{b} \pm 10.059$	$242.07^{b} \pm 10.212$

Means with different [abc] within a row are significantly different at ($P < 0.05$).

3.4 Discussion

The blood serum lipid profile is very important as it has a positive relationship with the lipid profile of meat (Tohala, 2010). Replacing soybean meal with 15% *Mucuna pruriens* in the current study reduced all the blood serum lipid profile parameters measured i.e. Total cholesterol, HDL, LDL and triglycerides (Table 3.5). Previous studies have also observed a reduction in cholesterol levels when broiler chickens were fed *M. pruriens* (Carew *et al.*, 2002; 2003; Jayaweera *et al.*, 2007). Our results are also similar with earlier results of Iauk *et al.* (1989). The authors observed a reduction in cholesterols when rats were fed *M. pruriens*. The reduction of blood serum cholesterols of birds fed *M. pruriens* might be attributed to the hormonal changes. Iauk *et al.* (1989) reported changes in insulin and glucagons when rats were fed *M. pruriens*. *M. pruriens* contains lower levels of lysine: arginine ratio (Jayaweera *et al.*, 2007). Sachez and Hubbard (1991) reported that lower dietary levels of lysine: arginine ratio reduce the secretion of insulin and increases the secretion glucagons in humans. Since humans and broiler chicken are both monogastrics, the same principle might apply. The lower levels of insulin and higher glucagons have reducing factor to the synthesis of cholesterols in birds (Jayaweera *et al.*, 2007).

The concentration of AST and ALT, with more attention being given to ALT provides a good estimate of liver injury. Hepatocyte necrosis is usually observed when there are high percentage levels of these enzymes (Manterys *et al.*, 2016). Of importance, in the current study, the concentration of AST was reduced from 314.53 ± 19.936 (T1) to 208.47 ± 10.059 (T3) while the ALT was increased 5.00 ± 0.000 (T1) to 5.27 ± 0.207 (T4) where T1 is the control diet. It is also important to recap that ALT is more pronounced than AST when assessing liver damage by the two enzymes as mentioned earlier. Although the fatty acid profile of the treatment diets was not analysed, soybean and *M. pruriens* seed meal percentages were the main differentiating ingredients among the diets. Soybean contains less (6.48-11.6%) linoleic acid than *M. pruriens* seed meal (8.71 – 14.4%) (Bhat *et al.*, 2008; Banaszkiewicz, 2011; Balogun and Olatidoye, 2012). The high linoleic acid in *M.pruriens* might be the cause of increased ALT which is considered unfavorable. Studies have shown that administering 2% conjugated linoleic acid (CLA) increases AST and ALT in blood serum resulting in liver dysfunction (Kim *et al.*, 2010; Liu *et al.*, 2012).

3.5 Conclusion

The inclusion of heat treated *Mucuna pruriens* in chicken broiler diets reduced both good and bad blood serum cholesterols while increasing the ALT which indicates liver dysfunction. Treatment diet 3 (T3) would be regarded to have the best diet when considering liver function test since it had the lowest AST and moderate ALT. More so, T3 had the lowest blood serum cholesterol profile. Hence, *Mucuna pruriens* seed meal can successfully substitute soybean meal in broiler chicken diets without major adverse effects on the blood serum cholesterol and liver function. We do recommendation; evaluation of the relationship between blood serum cholesterol profile and the cholesterol in the meat of chicken broilers.

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Chapter 4

Effect of feeding chicken broilers on graded inclusion levels of heated *Mucuna pruriens* seed meal on bone quality traits

Abstract

This study evaluated the effects of graded dietary inclusion of heated *Mucuna pruriens* seed meal on bone quality characteristics of broiler chicken. A total of 120 day-olds unsexed Cobb 500 broiler chicks were randomly allocated to 4 treatment diets (T1, T2, T3 and T4) containing 0, 10, 15, and 20% heated MBSM. The birds were reared on wood shaving floor pens for 35 days. Birds were humanely slaughtered and both drumsticks severed using a sharp knife. The tibia bones were collected after boiling in water at 100°C for 10 mins, air-dried for 96 hrs and were measured weights. The bones were analysed for bone strength, length, moisture content, ash and morphology. Treatment diet 3 (T3) was observed to have decreased bone quality among all diets; with the lowest ($P < 0.05$) bone strength (188.55 ± 12.039), bone length (8.59 ± 0.131) and morphology (1.69 ± 0.058). The low bone quality of T3 fed broiler chickens might be attributed to calcium and phosphorous content to the diet. *M. pruriens* seed meal can be included in broiler chicken diets at 20% without causing an adverse effect on the bone quality

Keywords: *Mucuna pruriens* seed meal; Bone strength; bone morphology

4.1 Introduction

Considering the increase in the productivity of chicken broilers in meat production, a good functioning skeleton system is vital (Suchý *et al*., 2009). It is not solitary in poultry but in other animal groups as well whereby morphological possessions and the inner makeup of bones ought to guarantee extreme prospective balance against forces connected by way of the supportive and locomotor meaning of the skeleton (Webster, 2004; Tatara *et al*., 2006). The genetic arrangement of broilers to yield large quantity and maturity of skeletal muscles does not correspond to the development of bones. Consequently, this may lead to the incidence of bone malformation and fractures (Suchý *et a*l., 2009). Lack in adaptation of the skeleton to escalating body weight might be the major reason (Korver *et al*., 2004; Whitehead, 2004). Calcium (Ca) and phosphorous (P) are the most limiting minerals in terms of bone growth and strength (Williams *et al*., 2000). Hence, it is therefore important to ensure that there is enough Ca and P available in the diets.

According to Eastell and Lambert (2002), nutrition is the most important aspect of bone growth. Huyghebaert (1996) and Onyango *et al.* (2003) highlighted that increasing the Ca content and accessible P in the diets greatly improves the mineral content of the tibiotarsus bone. More so, Huyghebaert (1997) emphasized that nutritional contributes 20 – 30% of the bone mass with genetics taking up the other portion. Even though aspects of nutritional have been clearly noted by previous studies, there is still a lag in understanding the impact of the inclusion of heated MPSM in broiler chicken diets on bone quality parameters. *Mucunna pruriens* seed meal has been identified as the potential substitute to soybean meal in broiler diets (Sese *et al.,* 2013). MPS are rich in crude protein (15-30%), starch (28%) and gross energy (10-11 MJ/kg) (Sidibé- Anago *et al*., 2009; Pugalenthi *et al*., 2005; Siddhuraju *et al*., 2000). According to Vadivel *et al*. (2007), the composition of minerals found in *M. pruriens* is extremely great. This study aimed at evaluating the effect of feeding graded inclusion levels of heated *M. pruriens* on bone quality characteristics of broiler chickens.

4.2 Methods and materials

4.2.1 Ethical clearance

The particulars of this sector are as defined in ethical clearance section of the methodology in Chapter 3

4.2.2 Study site

The particulars of this sector are as defined in study site section of the methodology in Chapter 3

4.2.3 *Mucuna* preparation

The particulars of this sector are as defined in *Mucuna* preparation section of the methodology in Chapter 3

4.2.4 Dietary treatments

The particulars of this sector are as defined in dietary treatment section of the methodology in Chapter 3

4.2.5 Experimental design and bird management

The particulars of this sector are as defined in experimental design and bird management section of the methodology in Chapter 3

4.2.6 Slaughter

At 35 day, the birds were restricted from feed and provided water ad libitum for 12 hr. The birds were weighed (Scale Model LBK 12), electrically stunned with 50 – 70 V for 3 – 5 s and the jugular vein was severed using a sharp knife. The birds were allowed to bleed for 5 mins, submerged in a water bath at 60°C for 2 min and defeathered in a chicken mower.

4.2.7 Bone collection and measurements

After slaughter, 10 birds were randomly selected; both right and left drumsticks were dissected out. The drumsticks were immersed in boiling water (100°C) for 10 minutes, the flesh was manually removed then air dried for 96 hrs at room temperature. The right tibia was weighed using a scale (Model LBK 12) to obtain the fresh and dry weights.

4.2.8 Determination of bone strength

An Instron machine (Model 3344, crosshead speed = 400mm/min) was used to determine the breaking strength of the tibia. The right tibia of each bird was used to determine the breaking strength (EZ Test, Shimadzu, Japan) with 3-point method, 40 mm as gauge length and 5 mm velocity of the load cell per minute.

4.2.9 Bone ash content

The left tibia was grinded and ashed in a muffle furnace at 600°C for 18 hrs then cooled at room temperature for 8 hrs. The percentage ash was determined relative to dry weight of ground tibia using the formula below:

$$\textbf{Ash (\%)} = \frac{\mathbf{w3 - w1}}{\mathbf{w2 - w1}} \times \mathbf{100}$$

whereby; the crucible and lid were weighed and the combined mass was recorded as W1. Weight (W2) was obtained by weighing approximately 1g of the ground dry bone sample into the crucible (i.e. combined mass of crucible, lid and sample). Lastly, weight (W3) was recorded after the samples were ashed in the muffle furnace.

4.2.10 Statistical analysis

The data collected was subjected to the General Linear Model (GLM) procedure of SAS (SAS,

2003) and ANOVA test was made using the following model:

$Y_{ij} = \mu + \alpha_i + e_{ij}$

Where Y_{ij} = response variable, μ = the common mean, α_i = the effect of dietary treatment (i=4; T1, T2, T3 and T4 are treatment 1, 2, 3 and 4 respectively) on bone quality traits and e_{ij} = the random error.

Differences between means was tested according to Fisher's least significant difference (LSD) test of SAS. Least square means with the standard error were used to present data. A significant level of $P < 0.05$ was used to conclude differences.

4.3 Results

Feeding of broiler chickens with *Mucuna pruriens* seed meal significantly affected the bone strength by and length as well as morphology. The bone ash and moisture content were slightly affected by the inclusion of MPSM in broiler chicken diets (Table 4.1). Treatment diet 1, 2, and 4 were observed to have the highest ($P < 0.05$) bone strength and T3 had the lowest ($P < 0.05$) (188.55 ± 12.039). Diet T3 had the lowest ($P < 0.05$) bone length (8.59 ± 0.131) while T1, T2 and T4 had a similar bone length. Feeding MPSM reduced ($P < 0.05$) the bone morphology from 2.02 ± 0.109 (T1) to

1.69 ± 0.058(T3) even though T4 was insignificantly affected.

Table 3.8: Effect of dietary *Mucuna pruriens* seed meal on broiler chicken bone length, strength, ash; moisture content and morphology

	Dietary treatment			
	T1 (0%)	**T2 (10%)**	**T3 (15%)**	**T4 (20%)**
Bone length (cm)	$9.15^{a} \pm 0.173$	$9.06^{a} \pm 0.189$	$8.59^{b} \pm 0.131$	$9.39^{a} \pm 0.131$
Strength (N)	$215.86^{ab} \pm 10.511$	$205.74^{ab} \pm 20.500$	$188.55^{b} \pm 12.039$	$232.47^{a} \pm 14.073$
Ash (%)	44.94 ± 5.690	42.64 ± 0.811	45.18 ± 0.424	46.43 ± 0.491

Moisture content (%)	52.08 ± 0.835	52.96 ± 3.401	52.02 ± 2.062	54.42 ± 1.160
Morphology (g/cm)	2.02^{a} ± 0.109	1.73^{b} ± 0.051	1.69^{b} ± 0.058	1.89^{ab} ± 0.058

Means with different ^{abc} within a row are significantly different at ($P < 0.05$).

4.4 Discussion

Understanding bone quality traits like bone length, strength, ash as well as bone weight/length ratio are paramount in the broiler industry. As broiler chicken reach market weights, they become more susceptible to bone breakage and associated infections which results in mortalities and economic loss (Rath *et al.*, 2000; Świątkiewicz and Arczewska-Wlosek 2012). Although inclusion of 5, 10 and 20% MPSM in broiler diets did not show an adverse effect on the measured bone quality characteristics, 15% inclusion lowered all the measured parameters. The deterioration of the bone quality of broiler chickens fed T3 might be attributed to the content of organic and inorganic nutrients in the diet. Calcium (Ca) and phosphorous (P) are key inorganic nutrients in maintaining bone integrity as they form 95% of the mineral matrices (Rath *et al.*, 2000; Webster, 2004). In this study, even though the calculated nutrient content show balanced Ca content among treatments. It can be assumed that the analysed Ca content might have differed among diets with T3 having less content hence the deteriorated bone quality. The low bone quality noted among broiler chicken fed on diets containing 15% MPSM is an indication of poor calcification and high porosity of the bones (Webster, 2004; Williams *et al.*, 2006). This can be translated to mean that broiler chicken fed MPSM are prone to difficulties in walking, feeding and poor welfare.

4.5 Conclusion

The inclusion of heated *Mucuna pruriens* seed meal in broiler chicken diet significantly affected the quality of bones. The inclusion level of 15% MPSM deteriorated the quality of the bones. This means broiler chickens fed 15% inclusion level of MPSM are prone to bone disorders, diseases and fractures. All this can translate into poor welfare, mortality and growth performance. We can conclude these observations by saying MPSM can be included in broiler chicken diets at 20% without causing an adverse effect on the bone quality. We would recommend further research on;

- The bone minerals of broiler chickens feed graded inclusion levels of heated *Mucuna pruriens* seed meal.
- The effect of feeding >20% inclusion levels of heated *Mucuna pruriens* on bone quality of broiler chickens.

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Chapter 5

General discussion and conclusions

Growth in the volume of poultry production has been more quickly compared to other animal food manufacturing industries. This has been backed by the increasing poultry consumer base (Windhorst, 2006). However, manifestation of cholesterol in meat and meat product has been the central factor that is depressing the intake of such foods (Jayaweera *et al*., 2007). According to Windhorst (2006), existing data point out that the poultry meat business has been additional vibrant judged against the egg industry throughout the past years. Today's consumers are health conscious hence the choice of food they buy depend on the supplied nutritional content of the food, especially in developed countries. As a result, there is a need to provide adequate scientific information on the poultry meat and meat products especially those produced under novel environments like the use of *Mucuna pruriens* seed meal in broiler chicken diets.

The most important constituent of the overall cost of broiler chicken production is feed. Internationally the chief ingredients of choice for poultry feed stay beyond as corn and soybean meal. It remains without saying that at present, globally there is no applicable alternatives to these above two mentioned major ingredients (Leeson, 2004). The production of broiler chickens can significantly be enhanced by the admission of fruitful and nutritious novel feedstuffs like *Mucuna pruriens* to replace soybean meal in their diet especially for the poor resource farmers. *Mucuna pruriens* has a good nutritional profile in terms of protein content 25-36%, a good amino acid and mineral content profile as well as crude fibre (7-9% vs. 5-6%)(Iyayi and Taiwo, 2003; Vadivel and Janardhana 2005; Vadivel and Pugalenthi, 2007). Minerals perform significant and explicit roles within the body. According to Pedrosa and Castro (2005), the central purpose of calcium remains as advancement and sustenance of bone mass and density, nerve transmission, transmembrane transport, and muscle construction. Hence, the purpose of this study was to find out the effect of *M. pruriens* on serum lipid profile and bone quality traits of broilers.

This aimed at determining an effect of feeding broiler chickens with graded inclusion levels of *Mucuna pruriens* seed meal on serum lipid profile and bone quality traits (Chapter 3 and 4). In chapter 3, the effects on feeding graded inclusion levels of heated *Mucuna pruriens* on blood serum lipids of broiler chickens were evaluated. The replacing soybean meal with 15% *Mucuna pruriens* in the current study reduced all the blood serum lipid profile parameters measured i.e. Total cholesterol, HDL, LDL and triglycerides (Table 3.5). The reduction of blood serum cholesterols of birds fed *Mucuna pruriens* might be attributed to the hormonal changes. Iauk *et al.* (1989) reported changes in insulin and glucagons when rats were fed *Mucuna pruriens*. *Mucuna pruriens* contains lower levels of lysine: arginine ratio (Jayaweera *et al.*, 2007). Sachez and Hubbard (1991) reported that lower dietary levels of lysine: arginine ratio reduce the secretion of insulin and increases the secretion glucagons in humans. Since humans and chicken broilers are both monogastrics, the same principle might apply. The lower levels of insulin and higher glucagons have reducing factor to the synthesis of cholesterols in birds (Jayaweera *et al.*, 2007). These results were similar to previous studies in broiler chicken (Carew *et al.*, 2002; 2003; Jayaweera *et al.*, 2007) and rats (Iauk *et al.*, 1989). The inclusion of *Mucuna pruriens* seed meal in broiler diets also decreased the concentration of AST while increasing the concentration of ALT in the blood (Table 3.5). These two enzymes are a good predictor of hepatocytes necrosis; with the high concentration increasing susceptibility (Manterys *et al.*, 2016). It should also be noted that ALT provides more accurate prediction of hepatocyte necrosis than AST. The increase in ALT could be attributed to the dietary concentration of linoleic acid. Although the dietary fatty acids were not analysed in this study, Soybean contains less (6.48-11.6%) linoleic acid than *Mucuna pruriens* seed meal (8.71 – 14.4%) (Bhat *et al.*, 2008; Banaszkiewicz, 2011; Balogun and Olatidoye, 2012). More so, soybean and *Mucuna pruriens* seed meal percentages were the main differentiating ingredients among the diets. The high linoleic acid in *Mucuna pruriens* might be the cause of increased ALT which is considered unfavorable. Studies have shown that administering 2% conjugated linoleic acid (CLA) increases AST and ALT in blood serum resulting in liver dysfunction (Kim *et al.*, 2010; Liu *et al.*, 2012).

In chapter 4, the effect of feeding graded inclusion levels of heated *Mucuna pruriens* on bone quality traits of broiler chickens were evaluated. The inclusion of 15% heated *Mucuna pruriens* seed meal lowered all the bone quality parameters measured; 0, 10 and 20% *Mucuna pruriens* seed meal did not show any adverse effect. The deterioration of the bone quality of broiler chickens fed T3 might be attributed to the content of organic and inorganic nutrients in the diet. Calcium (Ca) and phosphorous (P) are key inorganic nutrients in maintaining bone integrity as they form 95% of the mineral matrices (Rath *et al.*, 2000; Webster, 2004). Although the calculated Ca of the diets was balanced, there might be other factors that might have caused the imbalance in the Ca absorption.

In conclusion, the inclusion of heated *Mucuna pruriens* in broiler chicken diets significantly affected the blood serum lipids and bone quality. As for blood serum lipids, T3 would be regarded observed to have the best diet when considering liver function test since it had the lowest AST and cholesterol profile as well as moderate ALT. And then for bone quality, heated *Mucuna pruriens* inclusion of level of up 20% in broiler chicken diets showed no adverse effects on bone quality and except at 15% level of inclusion. We recommend further research on:

- evaluation of the relationship between blood serum cholesterol profile and the cholesterol in the meat of chicken broilers.
- the bone minerals of broiler chickens feed graded inclusion levels of heated *Mucuna pruriens* seed meal.
- the effect of feeding >20% inclusion levels of heated *Mucuna pruriens* on bone quality of broiler chickens.

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ETHICAL CLEARANCE CERTIFICATE
AREC-150311-008

Certificate Reference Number:	MUC091SHEM01
Project title:	**Evaluating the effect of supplementing broiler chickens with different inclusion levels of *Mucuna pruriens* seed meal on serum lipid profile and bone mineralization**
Nature of Project	MSc in Animal Science
Principal Researcher:	Chumani Hempe
Supervisor:	Prof. V. Muchenje
Co-Supervisor:	Mr. C.S. Gajana

On behalf of the University of Fort Hare's Animal Research Ethics Committee (AREC) I hereby give ethical approval in respect of the undertakings contained in the above-mentioned project and research instrument(s). Should any other instruments be used, these require separate authorization. The Researcher may therefore commence with the research as from the date of this certificate, using the reference number indicated above.

Special conditions: None

Please note that the AREC must be informed immediately of

- Any material change in the conditions or undertakings mentioned in the document;

- Any material breaches of ethical undertakings or events that impact upon the ethical conduct of the research.

The Principal Researcher must report to the AREC in the prescribed format, where applicable, annually, and at the end of the project, in respect of ethical compliance. The AREC retains the right to

- Withdraw or amend this Ethical Clearance Certificate if
  - Any unethical principal or practices are revealed or suspected;
  - Relevant information has been withheld or misrepresented;
  - Regulatory changes of whatsoever nature so require;
  - The conditions contained in the Certificate have not been adhered to.

- Request access to any information or data at any time during the course or after completion of the project.

- In addition to the need to comply with the highest level of ethical conduct principle investigators must report back annually as an evaluation and monitoring mechanism on the progress being made by the research. Such a report must be sent to the Dean of Research's office.

The Animal Research Ethics Committee wishes you well in your research.

Yours sincerely

Dr. Craig Tambling
AREC Chairperson

29 November 2018

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