Enhancing the feed value of Marama bean for Jumbo quail using thermal treatments

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DECLARATION

I, Mary Funmilayo Fatoki, hereby declare that this dissertation, in its entirety has been written by me under the guidance of my supervisors and that the work contained herein is my own, except, where acknowledged by form of using a reference. This work has not been submitted for any other degree or academic qualification in any university other than the North-West University.

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GENERAL ABSTRACT

The study evaluated the efficacy of thermal processing methods to improve the feeding value of Marama bean (*Tylosema esculentum*) in diets of Jumbo quail (*Coturnix* sp.). Five isonitrogenous and isoenergetic experimental diets were formulated as follows: a standard grower diet without MBM (CON); = a standard grower diet with 99.98 g/kg of raw (MBMR), cooked (MBMC), autoclaved (MBMA), and toasted MBM (MBMT). A total of 306, one-week-old mixed-gender Jumbo quail with initial live-weights of 30.97 ± 1.79 g/b were distributed to 5 dietary treatments in a completely randomised design with 8 or 9 birds per cage and each replicated 7 times. Effect of diets on growth performance, apparent retention (AR) of amino acids (AA), serum biochemical and haematological parameters, carcass characteristics and quality, and internal organ sizes in Jumbo quail was tested. Daily feed intake and weekly live-weights were measured until the quail reached 5 weeks of age. Blood samples were collected during slaughter, and other parameters were measured postmortem. The AR of AA was assessed from weeks 5 to 6 using 70 quail hens (two birds per experimental cage). The diets significantly affected (P <0.05) the AR of lysine, methionine, threonine, and otheressential amino acids. The CON diet resulted in the highest (P <0.05) overall feed intake (FI) followed by MBMC and MBMA, while the MBMR and MBMT groups had the lowest overall FI. Quail birds fed MBMR and MBMT diets showed significantly lower overall body weight gain (BWG) (P <0.05) compared to those fed CON, MBMC and MBMA diets. Moreover, quail on the MBMR diet had the lowest overall gain-to-feed ratio (G:F) (P <0.05) compared to those reared on the other diets, which had statistically similar G:F values (P >0.05). The MBMR diet also resulted in higher (P <0.05) lipase level than the other treatments. The CON diet produced higher (P <0.05) haematocrit level than the other treatments. Quail birds fed MBMR and MBMT diets had significantly lower (P <0.05) final body weights compared to those fed the CON, MBMC and MBMA diets.

Additionally the MBMR diet resulted in the heaviest (P <0.05) relative gizzard weights than all other dietary treatments. The MBMT and MBMR groups had significantly heavier (P <0.05) relative spleen weights compared to the other treatment groups. However, diet MBMT led to significantly heavier (P <0.05) relative proventriculus weights compared to the other dietary groups. Diet CON improved 1-hour breast meat redness more than the other dietary treatments. Furthermore, the MBMC, MBMA and CON diets significantly enhanced (P <0.05) 24-hour breast meat redness compared to the MBMR and MBMT diets. It was concluded that relative to the CON diet, cooked and autoclaved MBM treatments improved apparent AA retention, overall growth performance, carcass weights, and meat redness than the raw and toasted MBM treatments.

Keywords: Blood parameters, Growth performance, Heat treatment, Jumbo quail, Marama bean, Meat quality

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF PLATES

LIST OF FIGURES

PEER-REVIEW ARTICLE FROM THIS DISSERTATION

Fatoki, M.F., Kiarie, E.G. & Mnisi, C.M., 2023. Jumbo quail responses to diets containing raw or heat-treated Marama bean (*Tylosema esculentum*) meal. Translational Animal Science, 10, 10014. https://doi.org/10.1093/tas/txad136 [**Published**]

LIST OF ABBREVIATIONS

AA	:	Amino acids
ADF	:	Acid detergent fibre
ADL	:	Acid detergent lignin
ANF	:	Anti-nutritional factors
AR	:	Apparent retention
AOAC	:	Association of Official Analytical Chemists
CCW	:	Cold carcass weight
CF	:	Crude fibre
CL	:	Cooking loss
CP	:	Crude protein
CT	:	Condensed tannins
DL	:	Drip loss
EE	:	Ether extract
EU	:	European Union
FBW	:	Final body weight
G:F	:	Gain to feed ratio
HCW	:	Hot carcass weight
MB	:	Marama bean
MBM	:	Marama bean meal
NDF	:	Neutral detergent fibre
NRC	:	National Research Council
SAS	:	Statistical Analysis System
SBM	:	Soybean meal
WHC	:	Water holding capacity
TI	:	Trypsin inhibitors

1 CHAPTER ONE - GENERAL INTRODUCTION

1.1 Background

The use of indigenous legume plants as sources of nutrients in animal feeds has become crucial in the quest to establish sustainable animal agricultural production systems and reduce malnutrition and food security risks in developing countries (Jeke *et al*., 2018). Due to the rapidly growing human population, which is mostly in developing countries, the demand for animal protein has increased in recent years (Erdaw & Beyene, 2022). Poultry meat and eggs are among the most common animal products consumed globally, by a wide diversity of cultures, traditions and religions, making them key to food and nutrition security (Mottet & Tempio, 2017). Thus, the use of non-conventional alternative feed sources in poultry production is imperative to meet the increasing demand of poultry products, especially in the African continent (Dalle Zotta *et al*., 2019). To complement chicken production, quail farming could be a feasible strategy to address issues of animal protein deficiency, especially in sub-Saharan Africa, where a large segment of the population experiences persistent undernourishment and malnourishment (Jeke *et al*., 2018).

Quail (*Coturnix spp*.) are diversified small-sized, birds with short legs and varied plumage reared for meat and egg production (Mondry, 2016; Lukanov, 2019). They are rapidly gaining popularity among poultry consumers because their meat and eggs are delicious and have high nutritional value with beneficial bioactive substances that can prevent and cure various ailments (Wamuyu *et al*., 2017). Quail are tough and resilient to many diseases that affect poultry birds, which minimises on vaccination and veterinary costs (Guri *et al*., 2017). The Jumbo quail is the biggest *Coturnix* quail breed, which was bred from the Japanese quail (*Coturnix coturnix japonica*) line for meat production. It is currently the fastest growing quail breed that attains sexual maturity at six weeks of age with body weights ranging from 150 to

200 g (Marareni & Mnisi, 2020; Lamba *et al*., 2022). According to Altine *et al*. (2016), optimal quail production requires feeds of high quality that contains high levels of protein and energy. However, the over-reliance on conventional protein and energy sources such as soybeans and maize pose socio-economic and environmental challenges for large-scale quail intensification due their incessant market prices and other factors (Abd El-Moneim & Sabic, 2019).

1.2 Problem statement

Globally, soybean meal (SBM) is dominant in the feed market as a source of vegetable protein because of its high protein content (average 47.4%) compared to other plant sources. This makes it an outstanding constituent of feed during formulations (Ravn *et al*., 2015; Shafigullin *et al*., 2020). However, the over-dependence on SBM is unsustainable for large-scale quail production given its negative impact on the environment, which is associated with its cultivation (Gadzirayi *et al*., 2012; Parolini *et al*., 2020). Briefly, several inputs such as pesticides, fertilizers, machinery, and fuel, among others, are required for the cultivation of soybeans, which pose environmental sustainability challenges (Dlamini *et al*., 2014; Raucci *et al*., 2015). Unlike other traditional leguminous plants, like soybeans that require large acres of arable agricultural land for its cultivation, with land preparation resulting in deforestation, soil erosion, and compaction due to heavy machinery (Kuss *et al*., 2015; Almeida *et al*., 2018). Marama plant grows even in poor quality soils. Therefore, exploring local legumes that have high nutritional value and little-to-no food value to the South African general citizenry has the potential to deliver sustainable quail production (Sekali *et al*., 2020).

Presently, Marama bean (MB) is one of the underutilized protein sources in South Africa (Trytsman *et al*., 2019). Hence, its evaluation as an alternative nutrient source is imperative to ensure sustainable Jumbo quail production. However, the use of raw Marama beans in quail diets could be limited by the presence of trypsin inhibitors (TIs), which makes up to 20% of total protein in MB (Alabi *et al*., 2022). Further, the presence of other anti-nutritional factors (ANFs) like saponins of 55-63 mg/L (Nyembwe *et al*., 2015), phenols up to 24.6 mg/g catechin equivalent (Gulzar & Minaar, 2017) and condensed tannins CT, 200 mg/g catechin equivalent (Shelembe *et al*., 2012) can reduce MB protein digestibility by suppressing digestive enzymes and, subsequently, increase faecal nitrogen (Tadele, 2015).

According to Gemede & Ratta (2014) and Samtiya *et al.* (2020), TIs reduce protein digestibility and mineral absorption causing toxicity, micronutrient malnutrition, mineral deficiency, and decreased growth rates of livestock, especially when present in high concentrations. On the other hand, saponins reduce nutrient absorption, depress growth rates, and inhibit enzyme functions and smooth muscle activity (Abu Hafsa *et al*., 2022). All these effects could place limitation on its use as a dietary protein source in simple non-ruminants like quail. However, the adverse effects of these ANFs can be reduced or eliminated by applying effective processing techniques like boiling or combination of techniques and thereby improve the nutritive value of feed sources (Tadele, 2015; Batista *et al*., 2020). Likewise, the inactivation and/or removal of anti-nutritional components are vital to improve the nutritional potential of MB in quail nutrition.

1.3 Justification

Marama bean is a high source of protein, amino acids and lipids that compares relatively well with soybean (Ikhajiagbe *et al*., 2021). The MB contains 30 - 39% crude protein and 30 - 43% crude oil comparable to 33 - 48% crude protein and 15 - 24% crude oil in soybean (Petrusan *et al*., 2016; Chimwamurombe, 2016; Miao *et al*., 2020; Omotayo & Aremu, 2021). It also contains dietary crude fibre of up to 8% (Amonsou *et al*., 2011) and approximately 75% of unsaturated fatty acids (Holse *et al*., 2012), with 87% of those being a combination of oleic, linoleic, and palmitic acids (Holse *et al*., 2011). Marama bean is a rich source of potent phytochemicals including phenolic acids, flavonoids and phytosterols, as well amino acids (arginine, alanine, isoleucine, cysteine, and lysine), magnesium (274.5 mg /100 g) and iron (3.95 mg /100 g) (Omotayo & Aremu, 2021). Some of the MB bioactive compounds have increasing proof of possible health benefits with regards to diet related non-communicable diseases (Duodu & Apeah-Bah, 2017).

However, the presence of TIs and CTs in MB (Tibe *et al*., 2016) calls for pre-processing measures to improve and safeguard its utilization in quail diets. The most effective way of ameliorating thermos-labile ANFs is through heat processing (Aviles-Gaxiola *et al*., 2017). Heat treatments can inactivate ANFs (Nyembwe *et al*., 2015) and enhance antioxidant activities (Kim *et al*., 2019) by denaturing the heat-labile proteins like oxalates, tannins and trypsin inhibitors through reactions that involve destruction of disulphide bonds and splitting of covalent bonds (Irakli *et al*., 2020). Thermal treatments like roasting, dry or wet heating, micronization or physical treatment involving pressure processing are necessary to deactivate a variety of ANFs (Gulzar & Minnaar, 2017). In addition, processing techniques such as soaking, fermentation, debraning, autoclaving, to mention but a few, are used to reduce the anti-nutritional activities in feedstuff (Samtiya *et al*., 2020). Common treatment processes involving soaking, boiling, cooking, extrusion and autoclaving, have been shown to inhibit or reduce the anti-nutritive activities of tannins, phytic acids, and TIs (Abbas & Ahmad, 2018). For example, TI activities reduced when thermal treatments were employed on soybeans (boiled for 9 min at 100°C and autoclaved for 15 min at 121°C) and peanuts roasted for 20 min at 180°C (Aviles-Gaxiola *et al*., 2017). Nyembwe *et al*. (2015) found that roasting MB for 20, 25, or 30 min at 150°C resulted in a change of sensory properties and its saponin content. However, the feeding value of heat-treated (cooked, autoclaved and toasted) MB is unknown for the Jumbo quail. Thus, it is important to establish the feed value of raw or heat-treated Marama bean meal (MBM) for Jumbo quail.

1.4 Aim and objectives

This study intended to enhance the feed value of Marama bean (*Tylosema esculentum*) meal (MBM) as a potential nutrient source in Jumbo quail diets using three thermal treatments.

The specific objectives stated below guided the feeding experiment:

i. To determine the proximate constituents and amino acid composition of raw or heat-treated MB substrates.
ii. To determine the effect of raw or heat-treated MBM on apparent retention of amino acids, growth performance, and blood parameters in Jumbo quail.
iii. To determine the effect of raw or heat-treated MBM on carcass characteristics, internal organ sizes, and meat quality and stability traits in Jumbo quail.

1.5 Hypothesis

This study tested the following alternative hypotheses (H_A):

i. H_A: Heat treatment of raw MB substrates would improve proximate constituents and the availability of its amino acids.
ii. H_A: Heat treatment of MBM would improve the apparent retention of amino acid, growth performance and blood parameters in Jumbo quail compared to the soybean-based and raw MBM-based control diets.
iii. H_A: Heat treatment of MBM would enhance internal organs, carcass features, and meat quality and stability parameters in Jumbo quail compared to the soybean-based and raw MBM-based control diets.

1.6 References

Abbas, Y. & Ahmad, A., 2018. Impact of processing on nutritional and anti-nutritional factors of legumes: A review. Ann. Food Sci. Technol. 19(2), 199-215.

Abdul El-Moneim, A.E. & Sabic, E.M., 2019. Beneficial effect of feeding olive pulp and *Aspergillus awamori* on productive performance, egg quality, serum/yolk cholesterol and oxidative status in laying Japanese quails. Anim. Feed Sci. 28(1), 52-61.

Abu Hafsa, S.H., Hassan, A.A., Elghandour, M.M., Barbabosa-Pliego, A., Mellado, M. & Salem, A.Z., 2022. Dietary anti-nutritional factors and their roles in livestock nutrition. In Sustainable Agriculture Reviews Anim. Biotechnol. 57(2), 131-174.

Alabi, F., Kiarie, E.G., Mnisi, C.M. & Mlambo, V., 2022. Physical treatment reduces trypsin inhibitor activity and modifies chemical composition of Marama bean (*Tylosema esculentum*). Molecules. 27(1), 4451.

Almeida, W.S., Panachuki, E., de Oliveira, P.T.S., da Silva Menezes, R., Sobrinho, T. A. & Carvalho, D. F., 2018. Effect of soil tillage and vegetal cover on soil water infiltration. Soil Tillage Res. 175(1), 130-138.

Altine, S., Sabo M.N., Muhammad, N., Abubakar, A. & Saulawa, L.A., 2016. Basic nutrient requirements of the domestic quails under tropical conditions: A review. World Sci. News. 2(49), 223-235.

Amonsou, E., Taylor, J. & Minnaar, A., 2011. Microstructure of protein bodies in Marama bean species. LWT-Food Sci.Technol. 44(1), 42-47.

Aviles-Gaxiola, S., Chuck-Hernandez, C. & Saldivar, S.O.S., 2017. Inactivation methods of trypsin inhibitor in legumes: A Review. J. Food Sci. 83(1), 17-29.

Batista, K.A., Pereira, W.J., Moreira, B.R., Silva, C.N.S. & Fernandes, K.F., 2020. Effect of autoclaving on hard to cook common beans (*Phaseolus vulgaris*). Int. J. Agric. Environ. Biotechnol. 5(1), 22-30.

Chimwamurombe, P.M., 2016. Domestication of *Tylosema esculentum* (Burchell Schreiber) Marama bean: A work in progress in Namibia. Biosci. Biotechnol. Res. Asia. 8(2), 549 - 556.

Dalle Zotte, A., Singh, Y., and Michaels, J. & Cullere, M., 2019. Black Soldier fly (*Hermetia illucens*) as dietary sources for laying quails: Live performance and egg physico-chemical quality, sensory profile and storage stability. Animals. 9(3), 115.

Dlamini, T.S., Tshabalala, P. & Mutengwa, T., 2014. Soybeans production in South Africa. OCL. 21(2), 1-11.

Duodu, K.G. & Apea-Bah, F.B., 2017. African legumes: Nutritional and health promoting attributes. Woodhead Publishing Series in Food Sci. Technol. Nutri. Cambridge, United Kingdom. Pp. 223-269.

Erdaw, M. M. & Beyene, W. T., 2022. Trends, prospects and the socio-economic contribution of poultry production in sub-Saharan Africa: a review. World Poult. Sci. J. 78(3), 835-852.

Gadzirayi, C.T., Masamha, B., Mupngwa, J.F. & Washaya, S., 2012. Performance of broiler chickens fed on mature Moringa oleifera leaf meal as a protein supplement to soybeanmeal. Int. J. Poult. Sci. 11(1), 5-10.

Gemede, H.F. & Ratta, N., 2014. Antinutritional factors in plant foods: Potential health benefits and adverse effects. Int. J. Food Sci. Nutr. 3(4), 284-289.

Gulzar, M. & Minaar, A., 2017. Underutilized protein resources from African legumes. Sustainable Protein Sources Academic Press, Cambridge, USA. Pp. 197-208.

Guri, B., Hassan, S., El-Yuguda, A., Sidouin, M., Ibrahim, U. & Mbaya, A., 2017. Effects of ascorbic acid and levamisole on the course of Newcastle disease in adult Japanese quails. Microbiol. Res. J. Int. 21(2), 1-7.

Holse, M., Larsen, F.H., Hansen, A. & Engelsen, S.B., 2011. Characterization of Marama bean (*Tylosema esculentum*) by comparative spectroscopy: NMR, FT- Raman, IR and NIR. Food Res. Int. 44(1), 373-384.

Holse, M., Petersen, M.A., Maruatona, G.N. & Hansen, A., 2012. Headspace volatile composition and oxidative storage stability of pressed Marama bean (*Tylosema esculentum*) oil. Food Chem. 132(4), 1749-1758.

Ikhajiagbe, B., Ogwu, M.C., Ogochukwu, O.F., Odozi, E.F., Adekunle, I.J. & Omage, Z.E., 2021. The place of neglected and underutilized legumes in human nutrition and protein security in Nigeria. Crit. Rev. Food Sci. Nutr. 62(14), 3930-3938.

Irakli, M., Lazaridou, A. & Biliaderis, C.G., 2020. Comparative evaluation of the nutritional, antinutritional, functional, and bioactivity attributes of rice bran stabilized by different heat treatments. Foods. 10(1), 57.

Jeke, A., Phiri, C., Chitindingu, K. & Taru, P., 2018. Ethnomedicinal use and pharmacological potential of Japanese quail (*Cournix cournix japonica*) bird meat and eggs and its potential implications on wild quail conservation in Zimbabwe. Cogent Food Agric. 4(1), 1507305.

Kuss, V.V., Kuss, A.V., Da Rosa, R.G., Aranda, D.A.G. & Cruz, Y.R., 2015. Potential of biodiesel production from palm oil at Brazilian Amazon. Renew. Sustain. Energy Rev. 50, 1013-1020.

Lamba, P., Goswami, S. C., Jhirwal, A. K., Saharan, V., Choudhary, S., Meena, P. & Jhajhria, K., 2022. Effect of photoperiod on weight and age at sexual maturity of Japanese quail. J. Pharm. Innov. 11(6), 2830-2832.

Lukanov, H., 2019. Domestic quail (*Coturnix japonica).* Is there such farm animal? World Poult. Sci. J. 75(4), 547-558.

Marareni, M. & Mnisi, C.M., 2020. Growth performance, serum biochemistry and meat quality traits of Jumbo quails fed with mopane worm (*Imbrasia belina*) meal-containing diets. Vet. Anim. Sci.10, 100141.

Miao, L., Yang, S., Zhang, K., He, J., Wu, C., Ren, Y., Gai, J. & Li, Y., 2020. Natural variation and selection in GmSWEET39 affect soybean seed oil content. New Phytol. 225(4), 1651-1666.

Mondry, R., 2016. Quail Farming in Tropical Regions: A repository of Agricultural Outputs. CTA and ISF Cameroon Pro-Agro series Collection, Cameroon.

Mottet, A. & Tempio, G., 2017. Global poultry production: Current state and future outlook and challenges. Worlds Poult. Sci. J. 73(2), 245-256.

Nyembwe**,** P., Minnaar**,** A., Duodu**,** K.G. & De Kock**,** H.L., 2015. Sensory and physicochemical analyses of roasted Marama beans [*Tylosema esculentum* (Burchell) A. Schreiber] with specific focus on compounds that may contribute to bitterness. Food Chem. 178, 45-51.

Omotayo, A.O. & Aremu, A.O., 2021. Marama bean (*Tylosema esculentum*) Burch. A.Schreib: an indigenous plant with potential for food, nutrition, and economic sustainability. Food Funct. 12(6), 2389-2403.

Petrusan, J. I., Rawel, H. & Huschek, G., 2016. Protein-rich vegetal sources and trends in human nutrition: A review. Curr. Top. Pept. Protein Res. 17, 1-19.

Raucci, G. S., Moreira, C. S., Alves, P. A., Mello, F.F.C., De Almeida Frazao, L., Cerri, C.E.P. & Cerri, C.C., 2015. Greenhouse gas assessment of Brazilian soybean production: a case study of Mato Grosso State. J. Clean. Prod. 96(1), 418-425.

Ravn, J.L., Martens, H.J., Pettersson, D. & Pedersen, N.R., 2015. Enzymatic solubilisation and degradation of soybean fibre demonstrated by viscosity, fibre analysis and microscopy. Int. J. Biol. 7(9), 1-13.

Samtiya, M., Aluko, R.E. & Dhewa, T., 2020. Plant food anti-nutritional factors and their reduction strategies: An overview. Food Prod. Process Nutri. 2(1), 1-14.

Shafigullin, D.R., Gins, M.S., Pronina, E.P., Romanova, E.V. & Soldatenko, A.V., 2020. Crude protein accumulation in vegetable soybean samples grown in the central region of the non-chernozem soil zone. Russ. Agric. Sci. 46, 213-217.

Shelembe, J.S., Cromarty, D., Bester, M.J., Minnaar A. & Duodu, K.G., 2012. Characterisation of phenolic acids, flavonoids, proanthocyanidins and antioxidant activity of water extracts from seedcoats of Marama bean (*Tylosema esculentum*)-an underutilised food legume. Int. J. Food Sci. Technol. 47(3), 648-655.

Tadele, Y., 2015. Important anti-nutritional substances and inherent toxicants of feeds. Food Sci. Qual. Manag. 36, 40-47.

Tibe, O., Legwatagwata, B. & Motlogewa, M., 2016. The effect of processing on the anti-nutrients in selected legumes in Botswana. Asian J. Agric. Food Sci. 4, 265-269.

Trytsman, M., Masemola, E.L., Muller, F.L., Calitz, F.J. & Van Wyk, A.E., 2019. Assessing legumes indigenous to South Africa, Lesotho and Swaziland for their pasture potential. Afri J. Range Forage Sci. 36(1), 27-40.

Wamuyu, L., Mberu, M., Imboma, T., Obanda, V., Agwanda, B., Lichoti, J. & Ommeh, S.C., 2017. Phenotypic variations between wild and farm reared quails of Kenya. Livest. Res. Rural Dev. 29(6), 111.

2 CHAPTER TWO - LITERATURE REVIEW

2.1 Introduction

The growing human population drives the need to increase food production, especially protein-rich food products (Wang *et al*., 2021). Broiler quail production offers an opportunity to meet the demand for animal protein. However, large-scale production of Jumbo quail is limited by high feed costs due to the use of expensive dietary energy (maize) and protein (soybean) sources during feed formulations. The food industry and researchers are placing emphasis on the potential use of indigenous plant-based protein sources like Mucuna, chickpeas, faba beans among others which could be cheaper, sustainable and ecologically friendly (Akhtar *et al*., 2022). These protein sources have little-to-no direct food value for humans and their production does not require arable land compared to soybean cultivation. These plants are rich in crude protein and oil required for healthy quail production (Nalluri & Karri, 2021). Several studies have evaluated the use of unconventional protein sources like Mucuna, pea, and canola meals as alternatives to soybean in poultry diets however; there is scarcity of information on the use of Marama. Marama (*Tylosema esculentum*) is a local drought-tolerant and naturally growing legume that is well-adapted to dry or low moisture conditions (Omotayo & Aremu, 2021). It has pods which contain seeds that could be used as an alternative source of nutrients in Jumbo quail diets because of its outstanding nutritional profile similar to legume crops like peanut, soybean and navy bean (Gulzar & Minaar, 2017; Omotayo & Aremu, 2021). The Marama bean (MB) is rich in crude protein (30 - 39%) with crude oil content of 30 - 43% (Chimwamurombe, 2016) as well as essential minerals including calcium content (up to 241 mg/100 g) needed for bone development, zinc (6.2 mg/ 100g) which aids protein metabolism, iron (of about 3.95 mg/100 g) and magnesium (274.5 mg per 100 g), required for several metabolic processes (Omotayo & Aremu, 2021). The aforementioned minerals are important and significant for optimum quail performance. The

Marama plant is currently growing without human intervention, thus its large-scale propagation is highly feasible given that it thrives in sandy soils with less water and no fertilizers (Mataranyika, 2019). Accordingly, the use of MB can reduce current feed-food competitions and ensure the development of profitable and sustainable quail agro-businesses (Parisi *et al*., 2020). However, the presence of anti-nutritional factors (ANFs) like tannins, saponins and phenolic compounds can limit its utilization (Nyembwe *et al*., 2015). It has been established that MB contains high activity of trypsin inhibitors (TI) (20% of total protein), which can be destroyed by heat processing (Alabi *et al*., 2022), hence, thermal treatments would be a strategic and ideal technique to ameliorate the activity of TIs in MB to improve its utilization. Heat treatment is an easy and low-cost method that shows positive effects on improving the quality and digestibility of legume seeds (Ohanenye *et al*., 2022). Moreover, heat treatment is inexpensive when compared to the use of other technologies such as mineral supplementation, plant bio fortification that requires regular and frequent applications, use of heavy and bulky products which could potentially pose some negative effects on the environment and increase cost (Vas Patto *et al*., 2014). Therefore, this research aims to evaluate the use of three heat treatment methods (cooking, autoclaving and toasting) for improving Marama bean in Jumbo quail diets.

2.2 Overview of the South African poultry industry

The poultry industry contributes significantly to food security through the provision of cheap energy, protein as well as essential micro-nutrients from poultry meat and eggs (Mottet & Tempio, 2017). Poultry is currently one of the fastest growing agricultural industries, especially in developing countries like South Africa, Nigeria and Algeria (Nkukwana, 2018). Furthermore, it can help to alleviate poverty and livelihood of the people in marginalized communities by providing income and employment (Mottet & Tempio, 2017; Etuah *et al*., 2020).

Recently, it has been documented that that South African poultry industry ranks as the fifth most efficient in the world. It is also the largest commercial poultry producer in Africa, followed by Egypt, Morocco, Nigeria, and Algeria. In South Africa, the poultry industry contributes more than 20% of its share of gross domestic product (GDP) and over 40% (as shown in Figure 2.1) of all animal products making it the largest agricultural sector (SAPA, 2022). In 2022, farm income for poultry meat and eggs increased by 17.0% yielding about R71.62 billion compared to R61.21 billion in 2021 (SAPA, 2022). Poultry meat and eggs are generally the most affordable and consumed animal proteins than red meat, which is important to meet the zero-hunger goals set by government (Nkukwana, 2018). However, the recent outbreak of avian influenza has led to an increase in the price of these products. The South African poultry industry is dominated by a few fully integrated, large-scale commercial producers, alongside numerous small-scale producers. These smaller producers, who may be either contract growers or independent operators, primarily supply the informal market (Nkukwana, 2018).

Demand for conventional feed ingredients for animal feeds exceeds the supply, hence, South Africa import its major feed ingredients from countries such as Brazil because it does not produce enough ingredients for conventional feed manufacturers. In contrast, Brazil and the United States of America produce sufficient quantities of subsidized cereal grains and oilseeds for poultry farming. Thus, it has become difficult for the South African poultry industry to remain competitive in the global market (Nkukwana, 2018). Hence, the demand for poultry products exceeds the supply which necessitates importation of poultry meat and eggs from other countries. Brazil is the main country exporting poultry products to South Africa, accounting for over 54% of poultry imports (SAPA, 2020). Between the years 2017 to

2021, the South African poultry industry experienced a downward trend in imports (chicken and egg imports decreased by 11.4% and 39.2%, respectively) due to adjusted import tariffs but tariff increase is still insufficient to stem flow of imports as feed cost is still unbearable (SAPA, 2022). High feed cost threatens the economic viability and future certainty of the industry and should be an eye opener for South African policymakers to consider issues that makes it difficult for local producers to thrive such as high feed costs (Karodia, 2017). Conventional poultry feeds are largely formulated using maize and soybean meal (SBM), which is the reason for the escalation in feed costs because these ingredients are currently available at exorbitant market prices. Feed costs, which currently occupy more than 70% of total production costs, have a major impact on the profitability of the poultry industry (Lombard & Bahta, 2018).

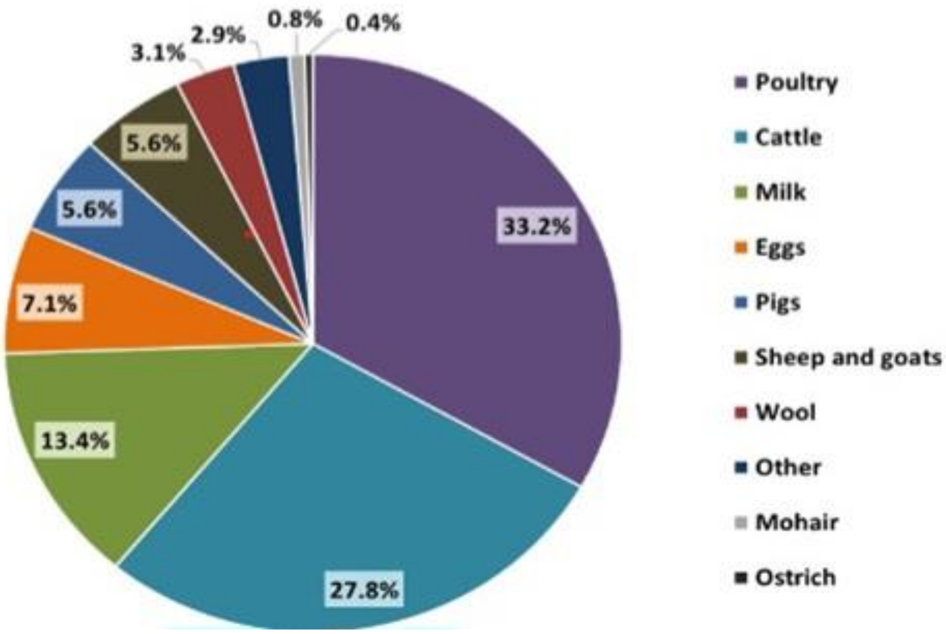

Figure 2.1. Gross value of animal products (Source: SAPA, 2017).

2.3 Challenges faced by poultry producers

The poultry industry is currently faced with lots of problems relating to high feed costs, among other issues, which remains a major challenge (Nkukwana, 2018). Bounds and Zinyemba (2018) report that the South African poultry industry is challenged by high input costs, fluctuations in import prices and exchange rates, limited capital or investment, and inadequate marketing and technical skills. Moreover, South Africa experiences exclusion from exporting its poultry products to other developed countries due to trade bans (SAPA, 2021). Nevertheless, sustainable agricultural practices like no tillage machinery, use of crop residue can be adopted to increase production of feed ingredients required for poultry farming (Sodhi *et al*., 2023).

Drought occurrences in some parts of the country have also exacerbated these challenges, which increased the cost of feed and other important inputs (Karodia, 2017). The numerous challenges ranging from rising feed costs, exchange rate fluctuations, import penetration, rising electricity tariffs, load shedding, and access to finance and markets has hindered its growth potential and ability to compete with global market (Lombard & Bahta, 2018). On the other hand, there are unprecedented riots and lootings leading to poultry farm infrastructure damage, large-scale theft, and massive interruptions to supply chains and transport systems (SAPA, 2022). Insufficient or limited state veterinary resources while there is prevalence of infectious and zoonotic diseases, outbreak of diseases like avian flu results in high mortalities and culling of birds, which have negative effects on chick production and slaughter volumes (SAPA, 2020). It has been proposed that adoption of smart farming could be a viable solution to this challenge using remote sensors for monitoring and early disease detection in poultry (Sharma *et al*., 2022).

South Africa is not an exception among the countries that depends on Soybean meal (SBM) as a dietary source of protein in poultry farming because of its high-quality that is required for optimum livestock production (Pereira *et al*., 2020). The demand for soybean in Africa does not meet with its supply and as a result, the shortage is mostly covered by importation (Murithi *et al.*, 2016). Unfortunately, high global tariffs on soybean markets result in higher prices for consumers, leading to net losses and economic inefficiency (Sabala & Devadoss, 2019). The European Union (EU) animal feed market is also not self-sufficient in SBM production (3%) and depends highly on imports which makes the sector very vulnerable to trade distortions and unstable price of soybeans (Pereira *et al*., 2020). This could lead to intense inadequacy of protein supply in the near future posing a risk of malnutrition and food insecurity, especially in developing countries where the population is on the increase (Hashemian *et al*., 2019). The use of soybean as feedstuff for animals has become unsustainable because it serves other purposes like feeding humans, which will pose threats to the food supply in the nearest future. It is expedient for researchers to identify and evaluate alternative protein sources for animal production that have no or little food value for humans (Parisi *et al*., 2020).

Climate change and increasing water scarcity and shortage remains a constant challenge in South Africa (Bonetti *et al*., 2022). The impacts of climate change have been felt across the country in a form of drought conditions, severe floods, and wildfires, which increases the production cost of feed ingredients. This results in negative economic consequences thus driving a need for efficient water systems (Maulu *et al*., 2021; Pengelly *et al*., 2021). However, unlocking opportunities for the use of drought tolerant crops like Marama, which is a climate-smart plant that has the ability to retain water in the root zone, can mitigate some of these challenges. Government interventions in making policies that could drive the uptake of drought tolerant crops and improved extension services through awareness programmes would also bring improvement to this sector (Pengelly *et al*., 2021). While implementing these policies, it is necessary to look into ways to diversify the poultry industry through production of other avian species like the quail, which can serve as a complementary source of meat and eggs.

2.4 Quail farming

Quail are small-framed birds, belonging to the *Phasianidae* family, which were domesticated for the first time in Japan many decades ago for meat and egg production (Priti & Satish, 2014; Mondry, 2016). Quail meat is a tasty and delicious white-game with extremely low skin fat and low cholesterol value (Priti & Satish, 2014; Redoy *et al*., 2017). Likewise, the eggs, which can be boiled or made into egg pickles, have low cholesterol content compared to chicken eggs. Quail meat and eggs have been reported to facilitate brain and body development hence, they are recommended for pregnant and breast-feeding women (Priti & Satish, 2014). Quail farming, which is also part of aviculture, requires less rearing space, water, and feed, and is environmentally friendly due to the lower carbon footprint (Mnisi & Mlambo, 2019). However, they require high quality diets containing 18-24% crude protein, 2 600-3 000 Kcal/kg ME, 0.6-1% calcium, and 0.5% phosphorus for maintenance and rapid growth (Tarasewicz *et al*., 2006; Smith & Tabler, 2016). However, there has not been a standard ration established for quail birds in South Africa (Marareni, 2021).. Therefore, a well-balanced protein-to-energy ration is important for optimum performance quail. Quail birds are reared in cages and/ or on floor pens with or without bedding material, however with a lower density of occupancy to avoid stress and reduce cannibalism and mortality (Mondry, 2016).


Plate 2.1. Jumbo quail raised in a cage (Source: North-West University research farm, 2023)

Quail farming requires less capital and investment and provides quick returns due to the birds’ early attainment of sexual maturity and high prolificacy. It also provides higher cost-benefit ratio compared to chicken production due to lower production cost (Redoy *et al*., 2017). Quail rearing is cost-effective compared to other poultry birds because they have strong disease resistance and requires little or no medication and vaccines (Samanta *et al*., 2018). Moreover, quail litter has high fertilizer value and can be used on farmlands to increase crop yields.

There are many different species of quail including the *Coturnix coturnix japonica* commonly called Japanese quail, *Coturnix chinensis* called king quail, and *Coturnix coturnix* called common quail (Mondry, 2016). The Jumbo quail (*Coturnix* sp.) is a meat-type breed that is known for its fast growth rate. It is a larger and meatier version bred from the Japanese quail line (Mbhele *et al*., 2019) and has now become a very important poultry species intensively reared for meat in South Africa (Masenya *et al*., 2021).

In South Africa, commercial quail farming is gradually being recognized as a potential source of animal protein, which can be a tool to alleviate protein-energy malnutrition and food insecurity, especially in rural areas (Mnisi *et al*., 2021). According to Santhi & Kalaikannan (2017), commercial quail farming is profitable and gaining popularity across nations, thus the production of high-quality quail meat is very important to improve sales. However, the problems associated with low demand for quail products, high feed costs, poor health care, and disease outbreaks, among other things (Mnisi *et al*., 2021), require urgent intervention.

2.5 Soybean production

Soybean is naturally a rich source of protein and oil and it is one of the largest sources of vegetable oil and protein globally (Sugiyama *et al*., 2015; Pagano & Miransari, 2016). Soybean is largely used as a source of protein in animal feeds, human diet, and in the production of biofuel (Pereira *et al*., 2020). This increases its demand and, consequently, results in high market prices (Sekali *et al*., 2020). Countries such as Brazil, China, USA, Argentina, and India have the highest rate of soybean production (Pagano & Miransari, 2016) and SBM remains the main protein source for animal feeds globally (Raucci *et al*., 2015).

The increased dependence on soybean has added more pressure on its cultivation, requiring the use of fertilizer or bio-fertilizers and large amounts of nitrogen (N) to acquire huge yields (Pagano & Miransari, 2016; Pereira *et al*., 2020). The production of soybean depends on inputs like pesticides, fertilizers, machinery, and fuel, among others, which contributes to increased carbon footprint and atmospheric greenhouse gas emissions (Raucci *et al*., 2015). Soybean cultivation requires extensive farming areas leading to deforestation, loss of biodiversity, and environmental and social issues in many countries (Pereira *et al*., 2020),. Even though, there are several efforts being promoted to lessen the impact of soybean cultivation on the environment including the reduction of Soy Moratorium development and adoption of no-tillage systems (Raucci *et al*., 2015). Reports from the European Unioin (EU) state that soybean production accounts for 60% of deforestation experienced around the world (Karlsson *et al*., 2021).

2.6 Alternative protein sources

The major sources of protein for livestock feeds are grains from leguminous plants, by-products from the oil industries, and several oilseed cakes. Even though these grains play integral part in feed formulation, it is expedient to consider other sources that can be produced sustainably since the production of soybean is not sustainable in the poultry feed industry especially in sub-Saharan Africa (Mazizi *et al*., 2019; Parisi *et al*., 2020). It is expected that the demand for high nutritive animal food products will increase in the future, necessitating the need to explore new alternative high-quality protein sources for use as feedstuffs. In the bid to reduce the on-going competition between humans and animals for plant-based diets, economic and environmental issues must be considered (Parisi *et al*., 2020). There is a need to evaluate alternative protein sources, without any direct food value for humans to build sustainable intensive quail production systems. There is an active consideration on the use of indigenous underutilised plant seeds (Plate 2.2) as alternative protein sources, even though, they present some challenges (majorly presence of anti-nutrients) that need to be addressed before they can become economically viable and safe for use as feed ingredients (Salter & Lopez-Viso, 2021). One of such underutilised crops that could replace soybean as a protein source in quail feed is Bambara groundnut.

Bambara groundnut (*Vigna subterranea*) is a legume which is commonly consumed by subsistence farmers in Western Africa, but it is now popular across the world (Salter & Lopez-Viso, 2021). It is usually cultivated for its seeds which could be processed into feed for animals because of its high (17-25%) crude protein content (Manyeula *et al*., 2019). Several processing methods, including soaking, fermentation or treatment with exogenous enzymes have been shown to reduce the anti-nutrient content of this legume (Salter & Lopez-Viso, 2021). This is a clear indication that Bambara groundnut has the potential increase the supply of protein available for poultry production when appropriate processing techniques are put in place. Winged bean (*Psophocarpus tetragonolobus*) symbolizes another underutilised legume that may be used as animal feed. It is a rich source of plant protein (about 30 g/100 g) and essential amino acids that have the potential to replace SBM commonly used as animal feed (Salter & Lopez-Viso, 2021). There is a wide range of other ingredients among plant sources that can supply protein for poultry with substantial production outputs instead of relying entirely on SBM (Iji *et al*., 2017). A good example of such ingredients is *Mucuna pruriens* (velvet beans) which is a tropical legume with low preference for food but a high potential as a source of energy and protein in animal nutrition (Balogun *et al*., 2016). According to, Mucuna is an alternative feedstuff locally available in South Africa with a high range of protein (25-30 g/100g) content as well as essential amino acids like lysine, methionine, and other bioactive compounds that has great potential to enhance desirable meat quality in birds (Zungu *et al*. (2023). Its high protein concentration and medicinal value having anticholesterolemic, antioxidant and anti-inflammatory effect will be of great help in reducing the protein deficiency gaps and limit and/ prevent diseases (Muoni *et al*., 2019; Pathania *et al*., 2020). The use of velvet beans in diets for simple stomached animals like quail could be limited by the presence of antinutritional factors like trypsin inhibitors, phytic acids, hydrocyanic acid and tannin (Balogun *et al*., 2016). However, research studies show that when pre-treatments like soaking seeds in water for 48 hours, autoclaving (at 121°C for 90 min) and fermentation (with *Rhizopus spp* for 72 hours) were applied, and toxic antinutrients were effectively reduced and its nutritional status improved (Balogun *et al*., 2016; Muoni *et al*., 2019; Das *et al*., 2022). Chickpea (*Cicer arietinum*) is also a good source of protein and energy for livestock with protein content ranging from 13.25% to 26.77% (Jadhav *et al*., 2015). Reports by previous scholars indicate that chickpea is rich in lysine (6 - 7% of total protein) and could be used as an alternative source of energy and protein to SBM in feeding animals because its nutritional value can be helpful in increasing sustainability in livestock production. Similar to other legumes, chickpea seeds contain ANFs like tannins, trypsin inhibitors and oxalic acid, which can be inactivated by dehulling and thermal treatments (Iji *et al*., 2017; Parisi *et al*., 2020). Lupine (*Lupinus spp*.) is another crop of interest as a valuable source of protein in animal nutrition. Its seeds are rich in protein (35 - 40% of the dry weight) which is comparable to soybean in terms of vitamins such as thiamine, niacin, riboflavin and tocopherols, as well as carotenoids, and minerals like iron, zinc, and manganese (Pihlanto *et al*., 2017). Lupine is limited by the high content of alkaloids (1-3%) especially in the wild bitter species but hardly contain any trypsin inhibitor activity and only low levels of saponins (Iji *et al*., 2017; Boukid & Pasqualone, 2022). Breeders have successfully improved on selection of lupine varieties with low-alkaloid content called the “cultivated sweet” lupine with an average range of 0.3 - 0.5% of alkaloids in their seeds; however, treatment methods like dehulling, soaking, cooking and fermentation are still required to remove the bitterness in these seeds to ensure their safety in animal nutrition (Boukid & Pasqualone, 2022). Canola (*Brassica napus*) meal is another by-product obtained after extracting oil from canola seed and can be potentially used to replace SBM because the amino acid profiles in the two products after oil extraction are comparable (Chakraborty *et al*., 2019; Mnisi & Mlambo, 2019). Another by-product of oil extraction is sunflower (*Helianthus annuus L*.) meal which is a good alternative protein source in poultry feeding and it is available in significantly high quantities throughout the year in South Africa (Mbukwane *et al*., 2022). Sunflower meals can be made from whole or decorticated seeds and can be extracted mechanically or by using solvents (Iji *et al*., 2017). Protein content in the meal ranges from 32 - 37% for non-dehulled meals and 35 - 39% for dehulled and partially dehulled meals. Moreover, an inclusion level of 20 - 30% in chicks’ starter and up to 13.5% in broiler finisher diets can be utilized without any negative effect on birds’ performance (Iji *et al*., 2017; Mbukwane *et al*., 2022). Alternative crops have an ability to adapt to pedoclimatic conditions. Often, these so called neglected and minor crops have some good characteristics including being flexible to changes in the environment, thus, becoming more attractive for use in new systems of production.

Plate 2.2. Examples of alternative protein sources (a = sunflower seeds, b = chickpeas, c = lupines, d = velvet beans, e = Bambara groundnuts).

2.6.1 Cultivation of alternative protein sources

Majority of farmers in southern Africa are faced various production constraints ranging from poor rainfall distribution, high drought frequencies, poor soil fertility status, climate change, limited access to adequate inputs and lack of technical know-how among others that have resulted in high food insecurity problems (Muoni *et al*., 2019). Cultivation of underutilized alternative crops is recommended to improve food security, enhance soil conservation and boost livestock feed since they are able to grow naturally and tolerate arduous conditions without human intervention; therefore, do not require use of inputs and heavy machineries as opposed to the cultivation of soybean (Muoni *et al*., 2019; Carrascosa *et al*., 2023). Moreover, their cultivation promotes sustainable and resilient agricultural system which necessitates consideration of the cultivation practices of potential alternative crop species. These crops are widely distributed across tropical Africa and can be propagated vegetatively or through seeds. Jideani & Diedericks (2014) and Ajilogba *et al*. (2022) reported that

Bambara groundnut (*Vigna subterranea*), a legume originally from West Africa, is now widely grown across tropical Africa, South America, parts of Asia, and northern Australia. It can be propagated through seeds on level seedbeds or ridges and is known for its early maturity, taking only 3-6 months to harvest.According to Hanum (2021), velvet bean (*Mucuna utilis*) is cultivated from seeds and stem cuttings which is preferable due to greater leaf growth than seedling and it is a highly productive legume with an average yield of 200 - 600 kg seed/ha This crop is native to South Asia and Malaysia, and at present is widely grown throughout Africa. It is a vigorously growing and twining annual plant with trailing veins and leaves grown mostly for green manuring or temporary pasture. It is a hardy crop which requires minimum care with a high resistance to drought, and great nitrogen fixing (34 - 108 kg atmospheric nitrogen) ability for soil improvement (Sese *et al*., 2013; Muoni *et al*., 2019). Irrigation is one of the factors important for crop production. Alternative crops like Bambara groundnut, winged bean, and sunflower grow under hot and humid conditions and drought-stricken places with low requirements for water and other external inputs which minimises the impact of its production on the environment and makes it an attractive source of sustainable protein (Salter & Lopez-Viso, 2021; Mbukwane *et al*., 2022). Fertilization is essential for crop cultivation, but alternative crops like mucuna, sunflower, Bambara groundnut and lupines have demonstrated high suitability for low input agriculture owing to their high adaptability to low-fertile soils, and high nitrogen fixation ability (Sese *et al*., 2013; Muoni *et al*., 2019; Boukid & Pasqualone, 2022). In addition, alternative crops can be grown as a sole crop or integrated into other farming systems. For instance, Jideani & Diedericks (2014) describe Bambara groundnut as an ideal crop for intercropping with maize and sorghum highlighting its usefulness in improving soil fertility during crop rotation.

In addition, velvet beans can be grown solely or included in a crop rotation, intercropping or relay cropping plan. Also, its high growth rate and biomass production helps to reduce weed and promotes plant canopy growth to cover the soil thereby reducing surface run off and erosion (Muoni *et al*., 2019). It is important to note that all the aforementioned alternative crops have ability to survive in extreme weather conditions without any need for irrigation which is advantageous considering the current climatic changes and cost of irrigation. More so, no heavy tillage practices or use of fertilizers is required as they can thrive on shallow poor soils with less vulnerability to fungal infestation (Mbukwane *et al*., 2022), therefore, reducing greenhouse gas emission that maybe a resultant effect of using heavy machines during tillage practices, cost of fumigation and residual effect of chemicals on the soil. These alternative crops are gaining traction as a more sustainable and non-genetically modified alternative to soybean (Boukid & Pasqualone, 2022); thus, cultivation of alternative protein sources will ensure food security at a reduced cost.

2.6.2 Cost-effectiveness of alternative protein sources in poultry

There are several alternative protein sources with good potential that can be used in poultry nutrition. To utilize these resources properly, it is imperative to have a good understanding of their properties and biological values. A shift in using other ingredients apart from SBM will significantly reduce the pressure on this gold standard protein source (Iji *et al*., 2017). As a commodity, the price of SBM is set internationally in dollars, invariably leading to its over-valuation especially in countries with weaker currencies like South Africa, making it difficult for small scale farmers to gain access to its use. This necessitates the need to evaluate the use of locally available unconventional protein sources as a substitute for SBM (Ferreira *et al*., 2020). These unconventional feed ingredients like Mucuna, Bambara groundnut, cowpea, pigeon pea, and lupine among others are inexpensive and readily available and may be included at recommended levels without any harmful effect on birds to cut down on the quantity of conventional protein sources and increase the profit of production (Iji *et al*., 2017).

The inherent anti-nutritional factors in them can be subjected to various processing methods which will improve their quality and safety as feed ingredients, thus, creating a business chain for livestock feed manufacturers who will concentrate on the production of these ingredients (Olarotimi & Adu, 2017). Locally available feedstuffs especially those with great potentials for improving poultry performance and less value as food for humans reduce biodiversity and greenhouse gas emissions that could result from deforestation (Leinonen *et al*., 2013).

Feed supplementation with native legumes is viable and provides additional proteins, minerals, energy and improves the overall nutritional status of livestock in developing countries (Sese *et al*., 2013). Due to shortage and high cost of conventional feed ingredients especially energy and protein ingredients, the utilization of alternative ingredients could make a substantial contribution towards a profitable poultry production system (Thirumalaisamy *et al*., 2016). Latino *et al*. (2020) reported that livestock production will double by 2050, thus alternative protein sources of comparable nutritive value to SBM are urgently needed to ascertain the sustainability of the South African poultry industry. Therefore, to meet the future requirements for animal protein, locally available and naturally growing protein sources should be exploited to develop profitable poultry production systems (Khan, 2018; Buccioni *et al*., 2020).

2.6.3 Effects of alternative protein on quail production

The use of alternatives is gaining popularity due to their high crude protein content comparable to soybean that could have positive impact on quail production. Several studies have evaluated the effect of incorporating alternative protein sources like pigeon pea, canola marula nut to mention a few in quail feed and they have shown variable effects on quail performance, meat quality and health status in response to the inclusion of these alternatives. These effects being primarily determined by the form and quantity incorporated into the diet of these animals (Matabane, 2022). The inclusion level of 125 g/kg canola meal was found to be adequate for optimum growth performance, health and quality of meat of quail while inclusion levels beyond 125 g/kg resulted in poor feed intake (Mnisi & Mlambo, 2018). Toro *et al*. (2020) reported that the inclusion of roasted pigeon pea (*Cajanus cajan*) meal at 100 g/kg in layer quail feed had no effect on production and quality indicators of the egg. Mazizi *et al*. (2019) also reported that 100% replacement of SBM with marula nut meal in quail diets did not compromise their growth performance and internal organs at grower and finisher phases. Nnadi *et al*. (2021) stated that inclusion level of 250 g/kg rubber seed cake meal did not affect body weight, feed conversion ratio and carcass yield in quail.

Furthermore, some of these alternatives have been shown to possess nutraceutical properties that can be used as growth enhancers and antimicrobial agent to improve the overall health status of animals, promote organic production as well as enrich their products with health-promoting bioactive compounds for consumers (Yadav & Jha, 2019). For example, Mulaudzi *et al*. (2022) found that including pre-treated moringa leaves in the diet of quail enhanced the oxidative stability of their meat, attributing this improvement to the antioxidant properties of the moringa leaves. Likewise, using 1 g/kg moringa seeds increased egg production, improved hatchability and significantly reduced blood urea and lipid profile (cholesterol and triglycerides) concentrations of layer quail (Ashour *et al*., 2020). However, the utilization of alternatives maybe limited based on the presence of ANFs and dietary fibre in the feed because the presence of ANFs like tannins forms complexes that reduces protein digestibility. In a study conducted by Abdulrazaq *et al*. (2020), it was reported that ANFs (glucosinolate and sinapine) found in canola were responsible for the reduction in quail's feed intake at a 30% inclusion level. Eratak *et al*. (2021) stated that the anti-nutritive factors such as trypsin inhibitors and tannins present in lentil by-products at 20% inclusion level resulted in increased pancreas weight of quail birds. Mulaudzi *et al*. (2022) also stated that high fibre levels in moringa leaf could limit its utilization as a nutraceutical source in Jumbo quail diets. Likewise, incorporating sunflower meal at levels exceeding 150 g/kg in growing quail diets negatively impacted performance and chymotrypsin activity, this was attributed to the high fiber content (Tuzun *et al*., 2020). Many alternatives have been used in quail diets but the feed value of Marama in Jumbo quail diet remains unknown.

2.7 Marama bean

Marama (*Tylosema esculentum*) is a perennial legume crop (Plate 2.3) that is native to Southern Africa. It naturally grows in the arid and semi-arid environments of Kalahari Desert, South Africa and surrounding countries like Namibia and Botswana, sparingly cultivated in Mozambique and Zambia. Currently, the Marama is being cultivated for experimental purposes in countries like Kenya, Israel, Australia, and the United States of America as shown in Figure 2.3 (Omotayo & Aremu, 2021). Marama have some special morphological and physiological characteristics, such as the use of giant tubers that store water to help them survive in harsh environments and thrive under prolonged, blazing drought and heat conditions, where traditional crops cannot grow (Cullis *et al*., 2019; Adeboye & Emmambux., 2021). Its seeds are entrenched in reddish-brown hardy pods (Plate 2.4) which can be roasted and eaten as snack, or ground to flour to make porridge (Omotayo & Aremu, 2021), they are also used in producing oil and butter (Maruatona *et al.*, 2010). These seeds (Marama beans, MB) can be used as a protein source in animal nutrition; however, they need to be processed further (decorticated and crushed) as shown in Plate 2.5. Marama grows on sandy soils having little organic matter during summer with a temperature range of 28 - 37°C and produces seeds continuously throughout the growing period which can be harvested at any convenient time (Cullis *et al*., 2019). MB have the potential to be a valuable feed ingredient in quail diets due to their high protein content, energy density, and other beneficial nutrients. Overall incorporating MB into quail diets can be a strategy for improving quail performance, but it is important to take steps to minimize anti-nutritional factors ans balance the diet appropriately.

Plate 2.3. Marama plant (Source: Tropical plants, 2022).

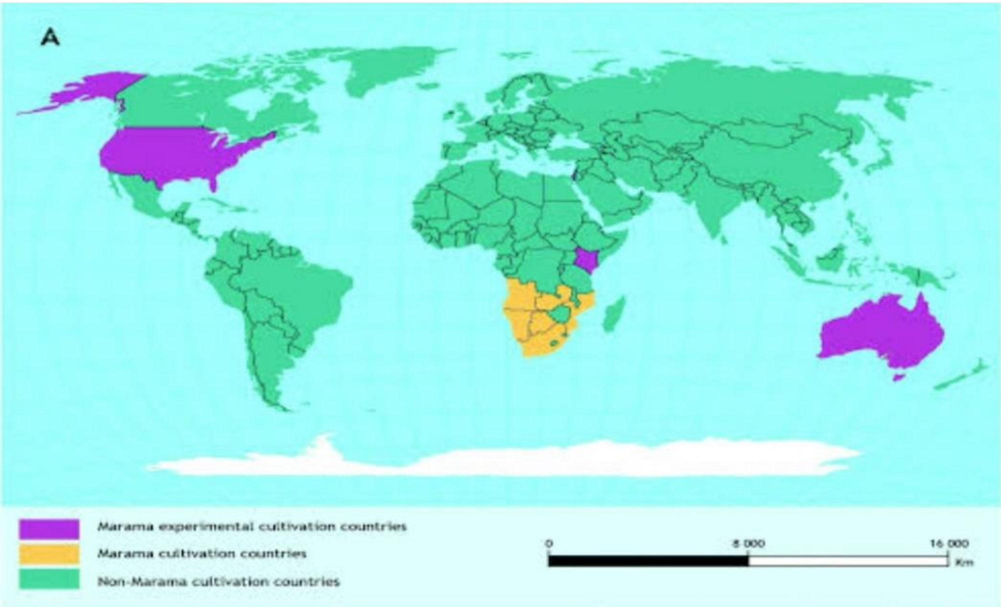

Figure 2.2. Global distribution of Marama (Source: Omotayo & Aremu, 2021).

Plate 2.4. Whole Marama bean (Source: DAFF, 2022).

Plate 2.5. Decorticated and crushed Marama seeds (Source: North-West University research farm, 2022)

2.7.1. Nutritional profile of Marama bean

MB contain a crude protein content that ranges between 29 - 34%, with the lipids content of 35-43%, dietary fibre of 19-27% and dry matter and ash contents of 90.22% and 4.57%, respectively (Holse et al., 2010; Cullis et al., 2023; Alabi *et al*., 2022). The beans also contain minerals like iron (~3.95 mg/100 g), which is important for preventing anaemia, zinc (~6.2 mg / 100 g), as well as rich calcium content (~241 mg/100 g), (Omotayo & Aremu, 2021). The MB is a good source of vitamin B, C necessary for optimal poultry health and E which acts as an antioxidant. MB also, contains α-, β- and γ-tocopherols in measurable quantities being dominated by γ-tocopherol with 59-234 mg/g followed by α- and β-tocopherols with 14 - 48 mg/g and 1.1 - 3.3 mg/g, respectively (Holse *et al*., 2010). Furthermore, the bean possesses glutamic, aspartic, tyrosine, alanine, arginine, aspartic, cysteine, isoleucine, and leucine, which are somewhat important in quail nutrition (Amonsou *et al*., 2012). However, MB is low in methionine content (1.2%) which is the first limiting amino acid with a significant effect on growth (Table 2.1) (Amonsou *et al*., 2012; Ullrich *et al*., 2019); this value is still within the minimum (0.45 - 0.5%) dietary requirements for quail (Smith & Tabler, 2016).

Table 2.1. Chemical composition (%, DM, unless mentioned otherwise) of Marama bean and soybean.

Components	Marama bean	Soybean
[1]*Proximate*		
Dry matter	94.03	90.22
Gross Energy (Kcal/kg)	2 798	2 280
Crude protein	32.72	34.79
Crude fat	38.67	17.22
NDF	7.51	14.73
ADF	3.43	12.0
Ash	2.88	4.57
[1]Amino acids		
Arginine	2.71	2.48
Histidine	1.07	0.94
Isoleucine	1.54	1.54
Leucine	2.41	2.71
Lysine	2.04	2.18
Phenylalanine	2.02	1.77
Threonine	1.22	1.32
Histidine	1.07	0.94
Valine	1.77	1.75
[2]Methionine	1.2	1.5
[2,3]Minerals (g/kg)		
Phosphorus	10.65	10.32
Calcium	1.57	0.74
Magnesium	3.58	2.02

Sources: [2]Amonsou (2010); [3]Biel *et al*. (2018); [1]Alabi *et al*. (2022).

2.7.2 Anti-nutritional factors in Marama bean

One of the main challenges with using MB in quail diets could be their high levels of anti-nutritional factors (ANFs), such as protease inhibitors, condensed tannins, lectins, phytic acid and saponins, which can reduce nutrient absorption and growth (Aviles-Gaxiola et al., 2017).

ANFs are metabolites that are produced naturally in plants to offer protection against pest attacks or herbivory (Aviles-Gaxiola *et al*., 2017). The ANFs can be divided into heat-stable group (phytic acid, tannins, alkaloids, saponins, non-protein amino acids) which are sensitive to normal processing temperatures substances and heat labile group (lectins, cyanogenic glycosides, protease inhibitors and toxic amino acids) which are resistant to normal processing temperatures (Thakur & Kumar, 2017). Generally, ANFs reduce feed intake, feed efficiency, and growth rate as well as inhibiting the digestibility and bioavailability of nutrients especially protein in experimental animals (Gemede & Ratta, 2014; Abbas & Ahmad, 2017).

The MB contain high amounts of condensed tannins (CT, 200 mg/g CE) (Shelembe *et al*., 2012), which are known to be inhibitors of lipase, amylase, trypsin and chemotrypsin interfering with absorption of iron and decreasing quality of protein in food (Gemede & Ratta, 2014). Thakur *et al.* (2019) indicated that high tannin concentration in diet may cause depression in the activities of microbial enzyme, and intestinal digestion by reducing nutrient absorption and increasing the removal of nutrients through defaecation. Thus, increasing faecal nitrogen which can lead to increased environmental pollution and reduced nutrient utilization efficiency. Phytic acids in MB reduce the nutrient bioavailability by binding to minerals like zinc, calcium and iron (Kapewangolo, 2010). This causes a reduction in gut absorption, leading to micro nutrient deficiencies and impaired growth (Kumar *et al*., 2021). Likewise, MB contain saponins concentration of 55 - 63 mg/L (Nyembwe *et al*., 2015), which inhibit enzymes and cholesterol absorption (Del Hierro et al., 2018). However, saponin increases permeability in the intestines, thereby allowing interaction between toxins and gut bacteria within the immune system of livestock (Del Hierro *et al*., 2018). The presence of amylase inhibitors in feeds have been reported to reduce feed intake and/or nutrient utilization of products in animals (Tadele, 2015). Protease inhibitors, which constitute about 10.5% of the total protein in MB (Avrelija & Walter, 2010), can bind with proteins and reduce the activity of digestive enzymes, impairing protein digestibility and utilization in animals (Khajali & Slominski, 2012). Similarly, lectins can decrease feed efficiency, cause goitrogenic responses, induce pancreatic hypertrophy, and damage the liver in poultry (Parisi et al., 2020). Lectins also have the ability to attach to the lining of the digestive tract, altering gut morphology and function, which impairs nutrient absorption and growth (Yin et al., 2019; Banti & Bajo, 2020).Hence, the presence in high concentrations of most common ANFs (saponins, tannins, phytate, polyphenolic compounds, trypsin and protease inhibitors) in plant materials like MB interfere with its nutritional value of by reducing mineral absorption, protein digestibility and causing toxicity and health disorders (Samtiya *et al*., 2020). The combined effect of endogenous loss of essential amino acids (particularly sulphur containing amino acids) and reduced intestinal proteolysis may result in reduced growth performance of birds (Clarke & Wiseman, 2007).

However, tannins and saponin at lower concentrations possess anti-microbial, anti-viral, antioxidant, and neuroactive actions that provide beneficial contribution towards health in animals (Alorkpa *et al*., 2016). This means Marama has a potential as a nutraceutical and could prevent infections in birds. The indication of tannins and saponin present in MB requires to be investigated further for quail nutrition. Adverse effects that ANFs have on animal’s growth and health performance are summarized in Table 2.2.

Table 2.2. A summary of the reports of effects of anti-nutritional factors

Anti-nutrient	Activity	Reference
Phytates	Reduces mineral bioavailablity, inhibits calcium and iron absorption.	Gemede & Ratta, 2014.
Tannins	Reduce protein digestibility, decrease bioavailablity of essential amino acids, causes loss of appetite and body weight, breathing problems and cardiac complications.	Thakur *et al*., 2019; Samtiya *et al*., 2020.
Saponins	Negatively affect absorption of lipids, vitamins A and E, alters the integrity of intestinal epithelial cells.	Samtiya *et al*., 2020
Lectins and hemaglutinins	Causes impaired growth, hypertrophy and hyperplasia of pancreas and reduction in nutrient absorption.	Grant *et al*., 1995; Muramoto, 2017.
Cyanide	Respiratory inhibitors, causes oxidative stress in poultry, rapid ingestion leads to death.	Gemede & Ratta, 2014; Kadiri & Asagba, 2019.
Enzyme inhibitors e.g.plant serpins also known as "suicide inhibitors"	Inhibit trypsin and chymotrypsin activity, causes pancreatic hypertrophy and hyperplasia, growth retardation and reduction in protein digestibility.	Samtiya *et al*., 2020

According to Parisi *et al*. (2020), post-harvest treatments can help reduce the amount of ANFs in leguminous grains. Different processing techniques commonly applied to legumes before consumption includes soaking, boiling, microwaving, cooking, extrusion, autoclaving, germination, to mention a few. These techniques are proven to reduce ANFs, enhance the sensory value, nutritive value and physical attributes of legume seeds (Abbas & Ahmad 2018; Ohanenye *et al*., 2022).

2.7.3 Evidence and effects of Marama bean meal in poultry diets

Presently, there are a few literatures regarding the use of dietary MB in poultry production. Likewise, the available studies to this end reported the use of raw MB in other poultry species with none on quail birds which indicates that the use of MB in poultry is still gaining popularity. The inclusion of raw MB improved microbial communities and their functional and metabolic pathways in male Ross 308 broilers (Montso *et al.*, 2022). Furthermore, Manyeula *et al.* (2019), reported that dietary inclusion of MBM in Tswana hens' diet did not affect their bone chemical composition, dry matter composition or organoleptic qualities of the meat . However, the minerals like phosphorus, calcium and potassium decreased over time due to utilization in egg production. In addition, Alabi (2023) concluded that the inclusion of raw MB at a higher level (190 g/kg) in to broiler diets compromised their growth performance, health status and meat quality properties. The possible explanation for this could be linked to the presence of antinutrients like trypsin inhibitors, tannins and possibly phytates. This results in growth inhibition, hypertrophy, and hyperplasia of visceral organs such as the pancreas. Although the use of MBM is limited by the presence of ANFs, no studies have investigated methods to improve the nutritive and feed values of MBM for Jumbo quail.

2.8 Thermal treatments

The most important aspects of food safety and nutrition security is the use of pre-processing techniques to improve consumer acceptability and the nutritional value of the product (Nkhata *et al.*, 2018). Generally, processing of food is aimed at increasing overall digestibility by affecting the internal (amino acid sequence of the proteins, protein folding and cross linking) and external factors (intact cell structures and presence of ANFs) (Joye, 2019). Although legumes contain high concentrations of macro- and micro-nutrients, they also constitute considerable amounts of ANFs that reduce the digestibility of proteins, vitamins and minerals (Thakur & Kumar, 2019; Mahanta *et al.*, 2020). This may also cause gastrointestinal upset that may lead to high faecal excretion of nutrients (Nkhata *et al.*, 2018).

Furthermore, it has been reported that anti-nutrients reduce availability and absorption of minerals (Samtiya *et al*., 2020).

There are various traditional methods and technologies, to process and reduce the levels of ANFs in feed ingredients. These methods include fermentation, germination, debranning, autoclaving, and boiling, soaking and genetic manipulation. However, most of the ANFs can be removed by using single traditional methods while others require combining several processes and thermal treatments (Thakur & Kumar, 2019; Samtiya *et al*., 2020).

Legumes crops are processed through traditional techniques such as soaking, fermentation, malting, and roasting. Processing techniques help to increase the availability of nutrients by lowering the levels of ANFs such as phytates, tannins, TI and lectins that interfere with mineral availability and protein digestibility (Mahanta *et al*., 2020). Grains are also subjected to these processing treatments to impart special characteristics, improve organoleptic propertiesby reducing phytochemicals and ANFs (Arbab-Sakandar *et al*., 2023). Pre-processing methods such as roasting and cooking have been proven to decrease ANFs through degradation of phytic acids (Oghbaei & Prakash, 2016; Dhuli *et al*., 2022). Heat processing is widely accepted as effective means of improving the quality of alternative protein sources, removing pathogens and inactivating the thermo-labile ANF of legume grains (Iji *et al*., 2017). Thermal treatments have greater effects on dissipation of ANFs and pesticide residues and a combination of processing techniques renders grains safe for consumption by humans and animals (Kaushik *et al*., 2018). Therefore, the nutritive value of legumes can be improved by subjecting them to various processing treatments such as milling, soaking, germination, and autoclave to overcome the effects of these food anti-nutrients (Samtiya *et al*., 2020).

Moreover, the application of suitable thermal processing treatments would increase opportunities for the versatile utilization of under-utilized legume seeds (Vadivel & Biesalski, 2012). Marama beans (*Tylosema esculentum*) contain numerous ANFs that can be lowered through thermal treatment, such as boiling, roasting, or autoclaving. Thermal treatment helps in reduction of tannins since MB contains high levels of tannins, which can bind to proteins and reduce their digestibility (Alabi *et al*., 2012). It has been reported that boiling significantly improved the protein digestibility of beans by inactivating protease inhibitors that reduces activity of digestive enzymes and decrease protein digestion (Duodu & Apea-Bah, 2017; Kaewtapee *et al*., 2017).

Furthermore, MB contains phytic acid, which can bind to minerals such as calcium, zinc, and iron, making them unavailable for absorption (Mokhele, 2018). Boiling and roasting the beans reduced the levels of phytic acid and improved mineral availability (Dhuli *et al*., 2022). MB also contains lectins that bind to the lining of the digestive tract and interfere with nutrient absorption (Muramoto, 2017). Thermal procedures such as boiling and pressure cooking have been found to inactivate these lectins and reduce their anti-nutritional effects (Patterson *et al*., 2017). Jackson *et al*. (2010) and Maphosa & Jideani (2017) observed that boiling MB increased feed intake in animals through reduced bitterness and soften the texture of the beans, making them more palatable. Therefore, thermal treatments could help detoxify ANFs in MB, and thus reduce the risk of illnesses that could be caused by food poison or toxicity, making it safer and more nutritious for animal consumption (Muramoto, 2017). Overall, thermal treatment is an important step in processing MB for use in animal feed, as it can help reduce the levels of ANFs, improve the nutritional value of the beans, and improve their safety and palatability.

2.8.1 Cooking

This is the simplest method of thermal processing used for legumes, which is done by placing the seeds in excess warm water and slowly bringing it to boil or putting it directly into a pot of boiling water at a temperature of 100°C (Drulyte & Orlien, 2019). Cooking is an effective way of removing or reducing anti-nutrients such as tannins, phytic acid and trypsin inhibitors in beans (Fabbri & Crosby, 2016). Thermal processes involved in deactivating these compounds are expensive (Zawoska-Zakrzewska *et al.*, 2020) hence, cooking provides the best and cheaper option because it does not only reduce ANFs, but also preserves the highest minerals in the seeds (Rondanelli *et al.*, 2017; Shen, 2020) (). Patterson *et al.* (2017) also reported that cooking improved the nutritional value of pulses (peas, beans, lentils, chickpeas, and faba beans) and reduced anti-nutritional contents such as tannins and trypsin inhibitors. The time for the cooking process varies between 20 and 40 min and it is an important factor which depends on the type of legume and the type of pre-processing techniques (e.g., soaking, drying etc.) (Drulyte & Orlien, 2019). Tibe et al. (2016) noted that MB must be well-cooked to maximize its high protein content. However, Snyman (2015) found that cooking at temperatures as high as 150°C for 30 minutes can lead to protein denaturation and reduce cysteine and arginine levels, thus, decreasing the overall protein content. Therefore, it is necessary to cook MB at a lower temperature for Jumbo quail.

2.8.2 Toasting

This is a traditional method that improves food flavour and helps to release phenolic acids (Shahidi *et al.*, 2021). Drying and toasting improves the nutritional quality of the meal and degrades ANFs (Snyman, 2016). Toasted seeds can be used to produce flour with modified functionality (Obasi *et al*., 2014). Toasting lowered moisture content of seeds and increased protein and mineral content in pigeon pea (Akubor, 2017). Increased inclusion levels (37.5%) of pigeon pea (*Cajanus cajan*) meal toasted at 105°C for 7 minutes reduced ANFs, increased most of the essential amino acids consequently improved the growth performance and yield in broiler chicks (Ojediran & Emiola, 2021).

Toasting MB is more effective, reducing trypsin inhibitor activity by 5% and tannin content by 41% (Tibe *et al*., 2016). Roasting MB at 150°C for 20 minutes has been shown to improve their sensory properties and increase their acceptance by consumers (Kayitesi *et al*., 2010). However, roasting of MB should not exceed 20 min to prevent it from getting a dark colour and bitter taste (Nyembwe *et al*., 2015). Additionally, improper heating may lead to low quality meal for the animals, because a significant amount of the ANF will still be active (Snyman, 2015; Tadele, 2015) which necessitates the need to toast the MB for the Jumbo quail.

2.8.3 Autoclaving

Autoclaving is a process that is generally used on cereals and other plant-based foods, to activate the phytase enzyme as well as increase acidity (Ertop & Bektaş 2018). It is a high intensity thermal method that is done by boiling in a sealed pressure chamber called autoclave, which is aimed at going beyond normal ambient boiling point of water. The procedure involves exposing the required item to a pressurized saturated steam of about 121°C with time duration varying from 7 mins to 1 h depending on quantity and type of legume (Drulyte & Orlien, 2019). Autoclaving common beans reduced trypsin inhibitors by 59.8%, amylase inhibitors by 42.2%, and resistant starch content by 45.9% (Batista *et al*.,2020).. Most ingredients have been found to show health benefits after being processed through autoclaving (Samtiya *et al*., 2020). This process improves and preserves the nutritional and functional properties of hard to cook seedsas well as improves nutrient bioavailability, texture and palatability compared to uncooked seeds. Introduction of this procedure in feed preparation could boost the nutritional and functional quality of beans (Batista *et al*., 2020). Therefore, it is necessary to evaluate the role of autoclaving in improving MB in Jumbo quail diet.

2.9 Summary

Globally, poultry products are the most consumed because they are considered a cheap source of high-quality animal protein. Quail birds are the latest addition into the South African poultry industry. To ascertain optimum performance, they require highly nutritious diets that are composed of maize, soybean and other nutritious ingredients. However, the demand for these maize and soybean is very high due to its several applications in the food, feed, and biofuel sectors. Moreover, their production has a negative impact on socio-economic and environmental sustainability. Thus, underutilized legumes such as MB can be used as alternative nutrient source. MB is a nutraceutical plant that is rich in protein, amino acid (especially lysine), oil, and bioactive compounds. Incorporating MBM into Jumbo quail diets can ensure sustainable intensification of the birds. However, the presence of ANFs can limit its utility in their diets. Thus, this necessitates MB pre-processing to reduce the anti-nutritional activities of secondary metabolites and improve their overall utilisation. This can be achieved through thermal treatments (cooking, autoclaving and toasting), which underpins the aim of this study.

2.10 References

Abbas, Y. & Ahmad, A., 2018. Impact of processing on nutritional and anti-nutritional factors of legumes: A review. Ann. Food Sci. Technol. 19(2), 199-215.

Abdulrazaq, H.S., Mahmud, S.D., Abdulrahman, J.N. & Sardare, S.Y., 2020. Productive performance, some hematological traits and genetic relationship in different local quail affected by dieting the rapeseed (canola) seeds powder. Mesop. J. Agric. 48(2), 33-49.

Adeboye, A.S. & Emmambux, N.M., 2021. Composition, functional and nutritional quality of Marama (*Tylosema esculentum*) storage root. J. Food Sci. Technol. 58, 4391-4402.

Ajilogba, C.F., Babalola, O.O., Adebola, P. & Adeleke, R., 2022. Bambara groundnut rhizobacteria antimicrobial and biofertilization potential. Front. Plant Sci. 13, 854937.

Akhtar, S., Ismail, T., Layla, A., Hussain, M. & Qamar, M., 2022. An overview of plant-based protein rich products. Plant protein foods. Springer Nature, Basel, Switzerland. Pp. 27-60.

Akubor, P.I., 2017. Effect of processing methods on the chemical composition and functional properties of pigeon pea seed. Asian J. Adv. Agric. 2(2), 1-8.

Alabi, F., Kiarie, E. G., Mnisi, C. M. & Mlambo, V., 2022. Physical treatment reduces trypsin inhibitor activity and modifies chemical composition of Marama bean (*Tylosema esculentum*). Molecules. 27(14), 4451.

Alabi, F.A., 2023. Evaluation of Marama bean as an alternative to soybeans in broiler nutrition. Doctoral dissertation, North-West University, Mafikeng, South Africa.

Alorkpa, E.J., Boadi N.O., Badu, M. & Saah, S.A., 2016. Phytochemical screening, anti-microbial and antioxidant properties of assorted *Carica papaya* leaves in Ghana. J. Med. Plants Stud. 4(6), 193-198.

Amonsou, E.O., 2010. Characterisation of Marama bean protein. Doctoral dissertation, University of Pretoria, Pretoria, South Africa.

Amonsou, E.O., Taylor J.R.N., Beukes, M. & Minnaar, A., 2012. Composition of Marama bean protein. Food Chem. 130(3), 638-643.

Arbab Sakandar, H., Chen, Y., Peng, C., Chen, X., Imran, M. & Zhang, H., 2023. Impact of fermentation on anti-nutritional factors and protein degradation of legume seeds: A review. Food Rev. Int. 39(3), 1227-1249.

Ashour, E.A., El-Kholy, M.S., Alagawany, M., Abd El-Hack, M.E., Mohamed, L.A., Taha, A.E., El Sheikh, A.I., Laudadio, V. & Tufarelli, V., 2020. Effect of dietary supplementation with *Moringa oleifera* leaves and/or seeds powder on production, egg characteristics, hatchability and blood chemistry of laying Japanese quails. Sustainability. 12(6), 2463.

Aviles-Gaxiola, S., Chuck-Hernandez, C. & Saldivar, S.O.S., 2017. Inactivation methods of trypsin inhibitor in legumes: A Review. J. Food Sci. 83(1), 17-29.

Avrelija, C. & Walter, C., 2010. Antimicrobial agents deriving from indigenous plants. Recent Pat. Food Nutr. Agric. 2(1), 83-92.

Balogun, O.I., Olatidoye, O.P. & Otunola, E.T., 2016. Effects of fermentation with *R. oligosporus* and *R. stolonifer* on proximate and pasting properties of dehulled and undehulled velvet beans (*Mucuna utilis*) flour. J. Sci. Res. Phar. Chem. Bio. Sci. 1(2), 29-39.

Banti, M., & Bajo, W., 2020. Review on nutritional importance and anti-nutritional factors of legumes. Int. J. Food Sci. Nutr. 9, 138-149.

Batista, K.A., Pereira, W.J., Moreira, B.R., Silva, C.N.S. & Fernandes, K.F., 2020. Effect of autoclaving on hard to cook common beans (*Phaseolus vulgaris*). Int. J. Agric. Environ. Biotechnol. 5(1), 22-30.

Beski, S.S., Swick, R.A. & Iji, P.A., 2015. Specialized protein products in broiler chicken nutrition: A review. Anim. Nutr. 1(2), 47-53.

Biel W., Gawęda D., Jaroszewska A. & Hury G., 2018. Content of minerals in soybean seeds as influenced by farming system, variety and row spacing. J. Elem. 23(3), 863-873.

Biesek, J., Kuzniacka, J., Banaszak, M., Kaczmarek, S., Adamski, M., Rutkowski, A., Zmudzińska, A., Perz, K. & Hejdysz, M., 2020. Growth performance and carcass quality in broiler chickens fed on legume seeds and rapeseed meal. Animals. 10(5), 846.

Bonetti, S., Sutanudjaja, E. H., Mabhaudhi, T., Slotow, R. & Dalin, C., 2022. Climate change impacts on water sustainability of South African crop production. Environ. Res. Lett. 17(8), 084017.

Boukid, F. & Pasqualone, A., 2022. Lupine (*lupinus* spp.) proteins: Characteristics, safety and food applications. Eur. Food Res. Technol. 248(2), 345-356

Bounds, M. & Zinyemba, O., 2018. Poultry Farming: Lessening poverty in rural areas. S. Afr. J. Agric. Ext. 46(1), 59-70.

Buccioni, A., Brajon, G., Nannucci, L., Ferrulli, V., Mannelli, F., Barone, A., Daghio M., Secci, G., Rapaccini S., Gatta, D., Falce, M. & Minieri, S., 2020. Cardoon meal (*Cynara cardunculus var. altilis*) as alternative protein source during finishing period in poultry feeding. Sustainability. 2, 5336.

Carrascosa, A., Pascual, J.A., Ros, M., Petropoulos, S.A. & Alguacil, M.D.M., 2023. Agronomical practices and management for commercial cultivation of *Portulaca oleracea* as a crop: A Review. Plants. 12(6), 1246.

Chakraborty, P., Mallik, A., Sarang, N. & Lingam, S.S., 2019. A review on alternative plant protein sources available for future sustainable aqua feed production. Int. J. Chem. Stud. 7(3), 1399-1404.

Chimwamurombe, P.M., 2016. Domestication of *Tylosema esculentum* (Burchell Schreiber) Marama bean: A work in progress in Namibia. Biosci. Biotechnol. Res. Asia. 8(2), 549-556.

Clarke, E. & Wiseman, J., 2007. Effects of extrusion conditions on trypsin inhibitor activity of full fat soybeans and subsequent effects on their nutritional value for young broilers. Br. Poult. Sci. 48(6), 703-712.

Cullis, C., Lawlor, D.W., Chimwamurombe, P., Bbebe, N., Kunert, K. & Vorster, J., 2019. Development of Marama bean, an orphan legume, as a crop. Food Energy Secur. 8(3), e00164.

Cullis, C., Chimwamurombe, P., Kunert, K. & Vorster, J., 2023. Perspective on the present state and future usefulness of Marama bean (*Tylosema esculentum*). Food Energy Secur. 12(2), e422.

DAFF, 2022. Department of Agriculture Forestry and Fisheries, South Africa. https//woodcropd.org/crops/Maramabean [Accessed: on 28 October 2023]

Das, G., Sharma, A. & Sarkar, P.K., 2022. Conventional and emerging processing techniques for the post-harvest reduction of antinutrients in edible legumes. Appl. Food Res. 2(1), 100112.

Del Hierro, J.N., Herrera, T., Fornari, T., Reglero, G. & Martin, D., 2018. The gastrointestinal behavior of saponins and its significance for their bioavailability and bioactivities. J. Funct. Foods. 40, 484-497.

Dhuli, S.B., Kidwai, M. K., Noor, R., Chawla, P. & Rose, P. K., 2022. A review of nutritional profile and processing of faba beans (*Vicia faba L*). Legume Sci. (4)3, e129.

Drulyte, D. & Orlien, V., 2019. The effect of processing on digestion of legume proteins. J. Food Process Preserv. 8(6), 224.

Duodu, K. G., & Apea-Bah, F. B., 2017. African legumes: nutritional and health-promoting attributes. In Gluten-free ancient grains. Woodhead Publishing, Cambridge, United Kingdom. Pp. 223-269.

Ertop, M. H. & Bektas, M., 2018. Enhancement of bioavailable micronutrients and reduction of anti-nutrients in foods with some processes. Food Qual. Saf. 4(3), 159-165.

Etuah, S., Ohene-Yankyera, K., Liu, Y., Mensah, J.O. & Lah J., 2020. Determinants of cost inefficiency in poultry production: Evidence from small-scale broiler farms in Ashanti region of Ghana. Trop. Anim. Health Prod. 53(3), 1149-1159.

Fabbri, A.D.T. & Crosby, G.A., 2016. A review of the impact of preparation and cooking on the nutritional quality of vegetables and legumes. Int. J. Gastron. Food Sci. 3, 2-11.

Ferreira M.L., da Silva, F.M., Dos Santos M. C., Lucena, J.E.C., Ricardo Yuji Sado, R.Y. & De Almeida Bicudo, A.J., 2020. Heat-treated bean (*Phaseolus vulgaris*) residue meal as an alternative protein source in pelleted diets for Nile tilapia fingerlings: growth, body composition, and physical characteristics of diets. Trop. Anim. Health Prod. 52, 2443-2450.

Gemede, H.F. & Ratta, N., 2014. Antinutritional factors in plant foods: Potential health benefits and adverse effects. Int. J. Food Sci. Nutr. 3(4), 284-289.

Grant, G., Dorward, P.M., Buchan, W.C., Armour, J.C. & Pusztai A., 1995. Consumption of diets containing raw soya beans (*Glycine max*), kidney beans (*Phaseolus vulgaris*), cowpeas (*Vigna unguiculata*) or lupin seeds (*Lupinus angustifolius*) by rats for up to 700 days: effect on body composition and organ weights. Br. J. Nutr. 73, 17-29.

Hanum, C., 2021. Growth pattern of shoot and root *Mucuna bracteata* from seeds and cuttings. IOP Conf. Ser.: Earth Environ. Sci. 782 (4), 042068.

Hashemian, M., Ahmadzadeh, H., Hosseini, M., Lyon, S. & Pourianfar, H.R., 2019. Production of microalgae-derived high-protein biomass to enhance food for animal feedstock and human consumption. In advanced bioprocessing for alternative fuels, biobased chemicals, and bioproducts. Woodhead Publishing, Sawston, England. Pp. 393-405.

Iji, P. A., Toghyani, M., Ahiwe, E.U., Omede, A.A. & Applegate T., 2017. Alternative sources of protein for poultry nutrition. Achieving sustainable production of poultry meat. University of New England, Burleigh Dodds Science Publishing Limited, Armidale, Australia. Pp. 1-13.

Jackson J.C., Duodu, K.G., Mette Holse, M., Lima de Faria, M. D., Jordaan, D., Chingwaru, W., Hansen, A., Avrelija C., Kandawa-Schultz, M., Selalelo, M., Mpotokwane, S.M., Chimwamurombe, P., De Kock, H.L. & Minnaar, A. 2010. The morama bean (*Tylosema esculentum*): A potential crop for Southern Africa. Adv. Food Nutri. 61, 187-246.

Jadhav, A.A., Rayate, S.J., Mhase, L.B., Thudi, M., Chitikineni, A., Harer, P.N., Jadhav, A.S., Varshney, R.K. & Kulwal, P.L., 2015. Marker-trait association study for protein content in chickpea (*Cicer arietinum L*.). J. Genet. 94, 279-286.

Jideani, V.A. & Diedericks, C.F., 2014. Nutritional, therapeutic, and prophylactic properties of *Vigna subterranea* and *Moringa oleifera*. In Antioxidant-antidiabetic agents and human health, Intech, Rijeka, Croatia. Pp.187-201.

Joye, I., 2019. Protein digestibility of cereal products. Foods, 8(6), 199.

Kadiri, H.E. & Asagba, S.O., 2019. The chronic effects of cyanide on oxidative stress indices in the domestic chicken (*Gallus domesticus L*.) J. Basic Appl. Zool. 80, 1-11.

Kaewtapee, C., Eklund, M., Wiltafsky, M., Piepho, H.P., Mosenthin, R. & Rosenfelder, P., 2017. Influence of wet heating and autoclaving on chemical composition and standardized ileal crude protein and amino acid digestibility in full-fat soybeans for pigs. J. Anim. Sci. 95, 779-788.

Kapewangolo, P.T., 2010. Biochemical characteristics of Marama bean (*Tylosema esculentum*): Quality evaluation and health potentials in functional cell models. Doctoral dissertation, University of Namibia, Windhoek, Namibia.

Karlsson, J.O., Parodi, A., Van Zanten, H.H., Hansson, P.A. & Roos, E., 2021. Halting European Union soybean feed imports favours ruminants over pigs and poultry. Nat. Food. 2(1), 38-46.

Karodia, A.M., 2017. The South African Poultry Industry is in Dire Straits and is facing Collapse: Is it a management and political issue. Int. J. Agric. Sci. Res. 3(5), 12-24.

Kaushik, G., Singhal, P. & Chaturvedi, S., 2018. Food processing for increasing consumption: the case of legumes. Hand book of food bioengineering, Academic Press, Cambridge, Massachusetts, USA. Pp. 1-28.

Kayitesi, E., Duodu, K.G., Minnaar, A., & de Kock, H. L., 2010. Sensory quality of Marama/sorghum composite porridges. J. Sci. Food Agri. 90(12), 2124-2132.

Khajali, F. & Slominski, B.A., 2012. Factors that affect the nutritive value of canola meal for poultry. Poult. Sci. 91(10), 2564-2575.

Khan, S.H., 2018. Recent advances in role of insects as alternative protein source in poultry nutrition. J. of Appl. Anim. Res. 46(1), 1144-1157.

Kumar, A., Singh, B., Raigond, P., Sahu, C., Mishra, U.N., Sharma, S. & Lal, M.K., 2021. Phytic acid: Blessing in disguise, a prime compound required for both plant and human nutrition. Food Res. Int. 142, 110193.

Latino, L.R., Pica-Ciamarra, U. & Wisser, D., 2020. Africa: The livestock revolution urbanizes. Glob. Food. Sec. 26, 100399.

Leinonen, I., Williams, A.G., Waller, A.H. & Kyriazakis, I., 2013. Comparing the environmental impacts of alternative protein crops in poultry diets: The consequences of uncertainty. Agric. Syst. 121, 33-42.

Lombard, W.A. & Bhata, Y.T., 2018. Overview of the South African poultry industry. Afr. J. Agric. Res. 4(2), 6-9.

Mahanta, B., Manhot, N., Deva, M. & Sing V., 2020. Weaning foods: Various methods of preparations focusing on traditional steps. Indian J Agric. Biochem. 33 (1), 11-19.

Manyeula, F., Tsopito, C.M., Mogotsi, K., Nsoso, S.J., Kamau, J.M. & Moreki, J.C., 2019. Chemical composition and sensory evaluation of meat of Tswana hens fed different protein sources: mopane worm, Bambara groundnut and morama bean. J. Anim. Feed Res. 9(2), 92-102.

Maphosa, Y. & Jideani, V.A., 2017. The role of legumes in human nutrition. Functional food: Improve health through adequate food. InTech, Rijeka, Croatia. Pp. 103-122.

Marareni, M., 2021. Inclusion of mopane worm meal in Jumbo quail diets: Nutrient utilization, physiological and meat quality responses. MSc dissertation, North-West University, Mafikeng, South Africa.

Maruatona, G.N., Duodu, K.G. & Minnaar, A., 2010. Physicochemical, nutritional and functional properties of Marama bean flour. Food Chem. 121(2), 400-405.

Masenya, T.I., Mlambo, V. & Mnisi, C.M., 2021. Complete replacement of maize grain with sorghum and pearl millet grains in Jumbo quail diets: Feed intake, physiological parameters, and meat quality traits. PLoS ONE 16(3), 0249371.

Matabane, A.N., 2022. Feed utilization, physiological and meat quality responses in Jumbo quail reared on apple pomace-containing diets. MSc dissertation, North-West University, Mafikeng, South Africa.

Mataranyika, P.N., 2019. Comparative nutritional analysis of *Tylosema esculentum* (Marama bean) germplasm collection in Namibia. MSc dissertation, Namibia University of Science and Technology, Windhoek, Namibia.

Maulu, S., Hasimuna, O.J., Haambiya, L.H., Monde, C., Musuka, C.G., Makorwa, T.H., Munganga, B.P., Phiri, K.J. & Nsekanabo, J.D., 2021. Climate change effects on aquaculture production: sustainability implications, mitigation, and adaptations. Front. Sustain. Food Syst. 5, 609097.

Mazizi, B.E., Moyo, D., Erlwanger, K.H. & Chivandi, E., 2019. Effects of dietary *Sclerocarya birrea caffra* (Marula) nut meal on the growth performance and viscera macromorphometry of broiler Japanese quail. J. Appl. Poult. Res. 28(4), 1028-1038.

Mbhele, F.G.T., 2019. Insect meal (*Hermetia illucens*) as an alternative protein source for Jumbo quails: physiological and meat quality traits. MSc dissertation, North-West University, Mafikeng, South Africa.

Mbukwane, M.J., Nkukwana, T.T., Plumstead, P.W. & Snyman, N., 2022. Sunflower meal inclusion rate and the effect of exogenous enzymes on growth performance of broiler chickens. Animals. 12(3), 253.

Mfulwane, P.H., 2023. An exploration of awareness and causes of financial distress of poultry farmers in the North West Province. MCom dissertation, North-West University, Potchefstroom, South Africa.

Mnisi, C.M. & Mlambo, V., 2018. Growth performance, haematology, serum biochemistry and meat quality characteristics of Japanese quail (*Coturnix coturnix japonica*) fed canola meal-based diets. Anim. Nutr. 4(1), 37-43.

Mnisi, C.M. & Mlambo, V., 2019. Canola meal as an alternative dietary proteins quail (*Coturnix coturnix*) diets – A review. Acta Agric. Scand. Sect. A. Anim. Sci. 68(4), 207 -218.

Mnisi, C.M., Marareni, M., Manyeula, F. & Madibana, M.J., 2021. A way forward for the South African quail sector as a potential contributor to food and nutrition security following the aftermath of COVID-19: A review. Agric. Food Secur. 10(1), 1-12.

Mokhele, T.M., 2018. The development of amadumbe (*Colocasia esculenta* (L.) *schott*)-soya composite biscuits with improved nutritional and sensory properties. MSc dissertation, University of South Africa, Johannesburg, South Africa.

Mondry, R., 2016. Quail Farming in Tropical Regions: A repository of agricultural outputs. CTA and ISF Cameroon pro-agro series collection, Yaounde, Cameroon. Pp. 5-29.

Montso, P.K., Mnisi, C.M. & Ayangbenro, A.S., 2022. Caecal microbial communities, functional diversity, and metabolic pathways in Ross 308 broiler chickens fed with diets containing different levels of Marama (*Tylosema esculentum*) bean meal. Front. Microbiol. 13, 1009945.

Mottet, A. & Tempio, G., 2017. Global poultry production: Current state and future outlook and challenges. Worlds Poult. Sci. J. 73(2), 245-256.

Mulaudzi, A., Mnisi, C.M. & Mlambo, V., 2022. Effect of pre-treating dietary *Moringa oleifera* leaf powder with fibrolytic enzymes on physiological and meat quality parameters in Jumbo quail. Poultry. 1(2), 54-65.

Muoni, T., Barnes, A.P., Oborn, I., Watson, C.A., Bergkvist, G., Shiluli, M. & Duncan, A.J., 2019. Farmer perceptions of legumes and their functions in smallholder farming systems in East Africa. Int. J. Agric. Sustain. 17(3), 205-218.

Muramoto, K., 2017. Lectins as bioactive proteins in foods and feeds. Food Sci. Tech. Res. 23(4), 487-494.

Murithi, H.M., Beed, F., Tukamuhabwa, P., Thomma, B.P.H.J. & Joosten, M.H.A.J., 2016. Soybean production in eastern and southern Africa and threat of yield loss due to soybean rust caused by *Phakospora pachryhizi*. Plant Pathol. J. 65(2), 176-188.

Nalluri, N, & Karri, V.R., 2021. Grain legumes and their by-products: As a nutrient rich feed supplement in the sustainable intensification of commercial poultry industry. In: Sustainable Agriculture Reviews. Vol 51. Springer, Cham. Switzerland. Pp. 51-96.

Nkhata, S.G., Ayua, E., Kamau, E.H. & Shingiro, J., 2018. Fermentation and germination improve nutritional value of cereals and legumes through activation of endogenous enzymes. Food Sci. Nutr. 6(8), 2440-2458.

Nkukwana, T.T., 2018. Global poultry production: Current impact and future outlook on the South African poultry industry. S. Afr. J. Anim. Sci. 48(5), 869-884.

Nyembwe**,** P., Minnaar**,** A., Duodu**,** K.G. & De Kock**,** H.L., 2015. Sensory and physicochemical analyses of roasted Marama beans [*Tylosema esculentum* (Burchell) A. Schreiber] with specific focus on compounds that may contribute to bitterness. Food Chem. 178, 45-51.

Obasi, N.E., Unammal, N.C. & Nwofia, G.E., 2014. Effect of dry heat treatment (toasting) on the cooking time of cowpeas (*Vigna unguiculata* L. Walp). Niger. Food J. 32, 16-24.

Oghbaei, M. & Prakash J., 2016. Effect of primary processing of cereals and legumes on its nutritional quality: A comprehensive review. Cogent Food Agric. 2(1), 1136015.

Ohanenye, I.C., Ekezie, F.G.C., Sarteshnizi, R.A., Boachie, R.T., Emenike, C.U., Sun, X., Nwachukwu, I.D. & Udenigwe, C.C., 2022. Legume seed protein digestibility as influenced by traditional and emerging physical processing technologies. Foods. 11(15), 2299.

Ojediran, T.K. & Emiola, I.A., 2021. Growth response, flock uniformity, organ weight, abdominal fat, carcass yield and hepatic histology of finisher broiler chicken fed toasted pigeon pea seed meal. J. Agric. Sci. Belgr. 66(4), 371-382.

Olarotimi, O.J. & Adu, O.A., 2017. Potentials of non-conventional protein sources in poultry nutrition. Archivos de Zootecnia, Universidad de Córdoba, Córdoba, España, 66(255), 453-459.

Omotayo, A.O. & Aremu, A.O., 2021. Marama bean (*Tylosema esculentum*) Burch. A. Schreib: an indigenous plant with potential for food, nutrition, and economic sustainability. Food Funct. 12(6), 2389-2403.

Pagano, M.C. & Miransari, M., 2016. The importance of soybean production worldwide. Abiotic and biotic stresses in soybean production. Academic Press. 1, 1-26.

Parisi, G., Tulli, F., Fortina, R., Marino, R., Bani, P., Dalle Zotte, A., De Angelis, A., Piccolo, G., Pinotti, L., Schiavone, A., Terova, G., Prandini, A., Gasco L., Roncarati, A. & Danieli, P.P., 2020. Protein hunger of the feed sector: the alternatives offered by the plant world. Ital. J. Anim. Sci. 19(1), 1204-1225.

Pathania, R., Chawla, P., Khan, H., Kaushik, R. & Khan MA., 2020. An assessment of potential nutritive and medicinal properties of Mucuna pruriens: a natural food legume. 3 Biotech. 10(6), 261. doi: 10.1007/s13205-020-02253-x.

Patterson, C. A., Curran, J. & Der, T., 2017. Effect of processing on anti-nutrient compounds in pulses. Cereal Chem. 94(1), 2-10.

Pengelly, C., Shai, T. & Kuschke, I., 2021. Market Analysis: Determining the extent and potential of a water-smart agriculture market in South Africa. Report to the water research commission, Pretoria, South Africa. www.wrc.org.za. [Accessed: on 23 October 2023]

Pereira, H., Sardinha, M., Santos, T., Gouveia, L., Barreira, L., Dias, J. & Varela, J., 2020. Incorporation of defatted micro algal biomass (Tetraselmis sp. CTP4) at the expense of soybean meal as a feed ingredient for juvenile gilt head seabream. Algal Res. 47, 101869.

Pihlanto, A., Mattila, P., Makinen, S. & Pajari, A.M., 2017. Bioactivities of alternative protein sources and their potential health benefits. Food Funct. 8(10), 3443-3458.

Priti, M. & Satish, S., 2014. Quail farming: An introduction. Int. J. Life Sci. 2(2), 190-193.

Raucci, G.S., Moreira, C.S., Alves, P.A., Mello, F.F.C., De Almeida Frazao, L., Cerri, C.E.P. & Cerri, C.C., 2015. Greenhouse gas assessment of Brazilian soybean production: a case study of Mato Grosso State. J. Clean. Prod. 96(1), 418-425.

Redoy, M.R.A., Shuvo, A.A.S. & Al-Mamum, M., 2017. A review on present status, problems and prospects of quail farming in Bangladesh. Bangl. J. Anim. Sci. 46(2), 109-120.

Rondanelli, M., Daglia, M., Meneghini, S., Di Lorenzo, A., Peroni, G., Faliva, M.A. & Perna, S., 2017. Nutritional advantage of sous-vide cooking compared to boiling on cereals and legumes: Determination of ashes & metals content in ready- to- eat products. Food Sci. Nutr. 5(3), 827-833.

Sabala, E. & Devadoss, S., 2019. Impacts of Chinese tariff on world soybean markets. J. Agric. Resour. Econ. 44(2), 291-310.

Salter, A.M. & Lopez-Viso, C., 2021. Role of novel protein sources in sustainably meeting future global requirements. Proceedings of the Nutrition Society, Cambridge University Press, Cambridge, UK. Pp. 186-194.

Samanta, I., Joardar, S.N. & Das, P.K., 2018. Biosecurity strategies for backyard poultry: a controlled way for safe food production. In Food control and biosecurity. Academic Press. Pp. 481-517

Samtiya, M., Aluko, R.E. & Dhewa, T., 2020. Plant food anti-nutritional factors and their reduction strategies: An overview. Food Prod. Process Nutri. 2(1), 1-14.

Santhi, D. & Kalaikannan, A., 2017. Japanese quail (*Coturnix coturnix japonica)* meat: characteristics and value addition. J. World Poult. Sci. 73 (2), 337-344.

SAPA, 2017, South African Poultry Association: Broiler and egg industry stats summary, Pretoria, South Africa.

SAPA, 2020. South African Poultry Association: Summary of poultry imports: Industry profile, Pretoria, South Africa.

SAPA, 2022. South African Poultry Association: Industry stats summary, Pretoria, South Africa.

Sekali, M., Mlambo, V., Marume, U. & Mathuthu, M., 2020. Replacement of Soybean meal with heat-treated canola meal in finishing diets of meat master lambs: physiological and meat quality responses. Animals. 10(10), 1735.

Sese, B.T., Okpeku, M. & Patani, I., 2013. Tropical velvet bean (*Mucuna utilis*) leaf meal as unconventional protein supplement in the diet of Broiler. J. Anim. Sci. Adv. 3(11), 575-583.

Sharma, V., Tripathi, A.K. & Mittal, H., 2022. Technological revolutions in smart farming: Current trends, challenges & future directions. Comput. Electron. Agric. 107217.

Shashidi, F., Danielski, R. & Ikeda, C., 2021. Phenolic compounds in cereal grains and effects of processing on their composition and bioactivities: A review. J. Food Bioact. 15.

Shelembe, J.S., Cromarty, D., Bester, M.J., Minnaar A. & Duodu, K.G., 2012. Characterisation of phenolic acids, flavonoids, proanthocyanidins and antioxidant activity of water extracts from seedcoats of Marama bean (*Tylosema esculentum*)-an underutilised food legume. Int. J. Food Sci. Tech. 47(3), 648-655.

Shen, B., 2020. The saponin composition of common Canadian pulses. MSc dissertation, University of Alberta, Edmonton, Canada.

Smith, T.W. & Tabler, T., 2016. Feeding quail. Mississippi State University Extension Service, MS, USA. 2383, Pp. 1-4.

Snyman N., 2015. The use of enzymes to improve amino acid digestibility of soya oilcake. AFMA Matrix. 24(3), 25-27.

Sodhi, G.P.S., Singh, R.K., Dhillon, G.S., Ahuja, S., Kaur, A., Kaur, T., Murai, A.S., Singh, R. & Kaur, S., 2023. Adoption behaviour of climate-resilient agricultural practices in Punjab under NICRA project. Indian J. Ext. Educ. 59(2), 46-50.

Sugiyama, A., Ueda, Y., Takase, H. & Yazaki, K., 2015. Do soybeans select specific specie of *Bradyrhizobium* during growth? Communi. Integr. Biol. 8(1), e992734.

Tadele, Y., 2015. Important anti-nutritional substances and inherent toxicants of Feeds. Food Sci. Qual. Manag. 36, 40-47.

Tarasewicz, Z., Szczerbinska, D., Ligocki, M., Wiercinska, M., Majewska, D. & Romaniszyn, K., 2006. The effect of differentiated dietary protein level on the performance of breeder quails. Anim. Sci. Pap. Rep. 24, 207-216.

Thakur, A., Sharma, V. & Thakur, A., 2019. An overview of anti-nutritional factors in food. Int. J. Chem. Stud. 7(1), 2472-2479.

Thakur, N.S. & Kumar, P., 2017. Anti-nutritional factors, their adverse effects and need for adequate processing to reduce them in food. Agric. Int. 4(1), 56-60.

Thirumalaisamy, G., Muralidharan, J., Senthilkumar, S., Hema Sayee, R. & Priyadharsini, M., 2016. Cost-effective feeding of poultry. Int. J. Sci. Environ. 5(6), 3997-4005.

Tibe, O., Legwatagwata, B. & Motlogewa, M., 2016. The effect of processing on the anti-nutrients in selected legumes in Botswana. Asian J. Agric. Food Sci. 4, 265-269.

Toro, B., Cepeda, M., Chacon, E., Sambache, J.E., Martinez, M., Bastidas, H.P., Brito, G.G., Calderon, C. & Silva, L.M., 2020. Effect of the use of *Cajanus cajan* (pigeon pea) meal on productive indicators of quails. Cuba. J. Agric. Sci. 54(2).

Tropical plants database, 2022. Ken.fern.tropical.theferns.info/viewtropical.plants.phid [Accessed: on 27 November 2023]

Tuzun, A.E., Olgun, O, Yıldız A.O. & Senturk, E.T., 2020. Effect of different dietary inclusion levels of sunflower meal and multi-enzyme supplementation on performance, meat yield, ileum histomorphology, and pancreatic enzyme activities in growing quails. Animals. 10(4), 1-14.

Ullrich, C., Langeheine, M., Brehm, R., Taube, V., Rosillo Galera, M., Rohn, K., Popp, J. & Visscher, C., 2019. Influence of different methionine sources on performance and slaughter characteristics of broilers. Animals. 9(11), 984.

Vadivel, V. & Biesalski, H. K., 2012. Effect of certain indigenous processing methods on the bioactive compounds of ten different wild type legume grain. J. Food Sci. Technol. 49(6), 673-684.

Vaz Patto, M. C., Amarowicz, R., Aryee, A.N.A., Boye, J.I., Chung, H.J., Martin-Cabrejas, M.A. & Domoney, C., 2014. Achievements and challenges in improving the nutritional quality of food legumes. Crit. Rev. Plant. Sci. 34(1-3), 105-143.

Wang, Y., Tibbetts, S.M. & McGinn, P.J., 2021. Microalgae as sources of high-quality protein for human food and protein supplements. Foods. 10(12), 3002.

Yadav, S. & Jha, R., 2019. Strategies to modulate the intestinal microbiota and their effects on nutrient utilization, performance, and health of poultry. J. Anim. Sci. Biotechnol. 10(1), 1-11.

Yin, J., Li, F., Kong, X., Wen, C., Guo, Q., Zhang, L., Wang, W., Duan, Y., Li, T., Tan, Z. & Yin, Y., 2019. Dietary xylo-oligosaccharide improves intestinal functions in weaned piglets. Food Funct. 10(5), 2701-2709.

Zaworska-Zakrzewska, A., Kasprowicz-Potocka, M., Wisniewska, Z., Rutkowski, A., Hejdysz, M., Kaczmarek, S., Nowak, P., Zmudzinska, A. & Banaszak, M., 2020**.** The chemical composition of domestic soybean seeds and the effects of partial substitution of soybean meal by raw soybean seeds in the diet on pigs' growth performance and pork quality (*M. longissimus lumborum*). Ann. Anim. Sci. 20(2), 521-533.

Zungu, P.N., Mthiyane, D.M., Mazibuko-Mbeje, S.E., & Ogwuegbu M.C., 2023. Dietary supplementation of low levels of unprocessed *Mucuna pruriens utilis* seed meal induces mild antinutritional entero-physio-metabolic perturbations without compromising performance and meat quality in finisher broilers. Trop. Anim. Health Prod. 55, 336.

3 CHAPTER THREE – APPARENT RETENTION OF AMINO ACIDS, GROWTH PERFORMANCE, AND BLOOD INDICES IN JUMBO QUAIL FED DIETS CONTAINING RAW OR HEAT-TREATED MARAMA BEAN MEAL

Abstract

This study was carried out to assess the effect of raw or heat-treated Marama bean meal (MBM) on apparent retention of amino acids, growth performance, and blood parameters in Jumbo quail. A total of 306, one-week-old mixed-gender Jumbo quail chicks (30.97 ± 1.79 g live weight) were randomly and evenly distributed into five isonitrogenous and isoenergetic experimental diets, which were replicated seven (7) times per experimental diet. The diets were formulated as follows: a standard grower diet without MBM (CON); a standard grower diet with 99.98 g/kg of raw (MBMR); cooked (MBMC); autoclaved (MBMA); and toasted MBM (MBMT). The diets caused varied responses (P <0.05) on apparent retention (AR) of lysine, methionine, threonine, among others. In week 2, diet MBMC promoted higher weight gain (34.81 g/bird) than MBMR (19.63 g/bird). Two-week-old quail birds reared on diet CON had the highest G:F (P <0.05) followed by those fed with MBMC and MBMA while diets MBMR and MBMT had the lowest G:F (P <0.05). However, in week 3, quail birds fed with diet MBMT showed the highest (P <0.05) G:F. CON resulted in higher (P <0.05) haematocrits than MBMR and MBMT. Diet MBMR promoted the highest (P <0.05) lipase levels and the lowest were on diets CON and MBMC. It was concluded that relative to the CON diet, cooking and autoclaving treatments of MBM enhanced apparent retention of amino acids, overall growth performance, carcass weights and meat redness than the raw and toasting treatments of MBM.

Keywords: Amino acids, Blood biochemistry, Growth performance, Heat treatment, Jumbo quail, Marama beans

3.1 Introduction

The Jumbo quail is an excellent meat-type breed recently introduced into the poultry industry due to its fast growth rate, early maturity, and high environmental resistance (Mbhele *et al*., 2019). It is, therefore, expedient to intensify the production of the Jumbo quail to meet the increasing global demand for protein (Mnisi & Mlambo, 2018; Ismail *et al*., 2020). However, the cost of commercial quail production is on the increase primarily due to the need for feed and dietary protein, which generally reflect the high cost of major protein sources such as soybean meal (SBM) (Nasar *et al*., 2016; Babatunde *et al*., 2021). There is a shortage of SBM as a feed ingredient for livestock because it has direct food value for humans and many other industrial biofuel applications, which make its market price to be relatively high (Mnisi & Mlambo, 2018). Consequently, feeding costs can be reduced by using non-conventional feedstuff of local origin, which has comparable nutritional profile and quality as SBM. Even though, there are several non-conventional plant-based protein sources (faba beans, field peas and chickpeas) that gave positive results when used in poultry feeds (Babatunde *et al*., 2021), the feed value of Marama bean (*Tylosema esculentum*) in Jumbo quail diet has not been investigated.

Marama bean (MB) is a hardy leguminous perennial plant that grows widely across Southern Africa, producing tubers and seeds that are of economic importance. It has been recognized as a valuable crop for subsistence diets because of its high protein and oil content, comparable to that of soybeans. It also possesses notable nutraceutical properties. (Cullis *et al*., 2019). Previous reports show that MB contain bioactive compounds that can stimulate digestion, improve gut health and increase immune response when included in poultry diets (Stastnik *et al*., 2021; Popoola *et al*., 2023). Despite their nutraceutical properties, the presence of ANFs in MB could limit their use in quail production.

Thus, this chapter determined the effects of three heat treatment methods to enhance the feed value of MBM in Jumbo quail on apparent retention (AR) of amino acid (AA), growth performance, and haematology and serum biochemistry. The hypothesis was that thermal treatment of MB would improve growth performance, AA utilisation, and blood parameters of the birds.

3.2 Materials and methods

3.2.1 Animal rights statement

The study protocol approved by the Research Ethics Committee for Animal Production studies of the North-West University (approval no. NWU-00811-22-A6) according to the guidelines established for use and care of animals in research.

3.2.2 Study area

The experiment was carried out in summer (between January and February 2023) at Molelwane Research Farm of the North-West University with geographical coordinates of 25°40.459′S, 26°10.563′E, situated in the semi-arid region of the North West province of South Africa. This area experiences ambient temperature ranging between 17 and 37°C in summer and between -3 and 17°C in winter. Annual rainfall ranges between 300 and 600 mm.

3.2.3 Ingredients, preparation, and pre-processing of Marama

Whole MB were collected from Malwelwe Village, Kweneng district of Botswana (23.94282° S; 1999° E), growing in Kalahari sands. Annual rainfall in the village ranges between 350 and 450 mm. This area experiences ambient temperature ranging between 18 and 36°C in summer and between 0 and 25°C in winter.

Soybean products as well as other ingredients were procured from Nutroteq and Simplegrow Agic Services (PTY) LTD both located in Centurion, in the Gauteng province of South Africa. The beans were dehulled using a J500 grain cleaner (JAPS Engineering, Benoni, SA), crushed and divided into four groups where three groups were subjected to different heat treatments (cooking, toasting, and autoclaving), and one group was not processed (raw). Dehulled MB seeds were divided and subjected to the different thermal procedures:

1) Cooking: Weighed MB seeds were poured into an aluminium pot containing 600 mL (1:10) of water on an electric stove and heated to boiling point (100°C), then cooked for 20 min, which was the optimum temperature and duration that yielded greatest reduction of trypsin inhibitor activity (TIA) (Alabi *et al*., 2022). After which the cooked seeds were rinsed, drained and dried at room temperature until constant weight.

2) Toasting: Weighed MB seeds were roasted in a forced convection continuous tumble roaster (Roastech, Bloemfontein, South Africa) at 150°C for 20 min, which is the recommended time and temperature required to deactivate trypsin inhibitors in MB (Nyembwe *et al*., 2015).

3) Autoclaving: Weighed MB seeds were poured into conical flasks at a seed to water ratio of 1:7.5 (w/v) and placed in an autoclave (3870E Heidolph, Tuttnaeur, New York, USA) for 15 min at 121°C (7psi), which is effective in reducing TIA and enhancing the nutritional value of MB (Alabi *et al*., 2022). Autoclaved MB seeds were spread in aluminium bowl and air dried. All pre-processed MB were milled with a Polymix PX-MFC 90 D (2 mm) to produce MBM before blending with other ingredients.

3.2.4 Chemical analysis

Prior to dietary formulation, the milled raw and heat-treated MBM samples were analyzed for chemical composition following the Association of Official Analytical Chemists methods (AOAC, 2005). The dry matter (DM) was determined (AOAC, 2005: method no. 930.15) by measuring approximately 1 g of the samples (4 subsamples) in pre-weighed crucibles, placed in an oven and dried at 105°C for 12 h. Afterwards, the samples were removed and cooled in desiccators then weighed. The DM was calculated by finding the difference between the initial weight and moisture content. Organic matter (OM) content determination (AOAC, 2005: method no. 942.05) was done by placing the dried samples in a muffle furnace incinerated at 600°C for 12 h. The loss in weight was measured as OM while the remaining residues measured as ash. Crude fibre (CF), neutral detergent fibre (NDF), and acid detergent fibre (ADF) were determined using an ANKOM^{2000} Fibre Analyser (ANKOM Tech. NY, USA) by adapting the detergent methods of Van Soest *et al*. (1991). The NDF and ADF were determined by refluxing about 0.45 – 0.5 g samples with a neutral detergent solution for 1 h and acid detergent solution for 1 h 15 min. A heat stable α-amylase was used in determining NDF and was expressed inclusive of residual ash. The CF was determined using crude fibre acid solution (0.255 N) afterwards the crude fibre base solution (0.313 N). Acid detergent lignin (ADL) content was determined by treating the dried ADF residue bags with sulphuric acid (72%) for 3 hours and thereafter cleaned using tap water and oven-dried for overnight. The standard macro-Kjeldahl method (AOAC, 2005: method no. 984.13) was used to determine the total N content, which was then converted to CP by multiplying percentage N by the factor 6.25. Ether extract (EE) was determined using an ANKOM XT 20 extractor (ANKOM Technology, NY, USA). Metabolizable energy (ME) was estimated using the following formula: ME (kcal/kg) = 2778 – 66 (CF × 88% DM). Samples were digested in 2.5 mL of concentrated HCl for 18 h at 110 °C (AOAC method 982.30) for amino acid analysis, followed by neutralization with 6 N NaOH, and cooled to room temperature. Thereafter, AAs were subjected to a chromatographic analysis using a Waters Acquity Ultra Performance Liquid Chromatograph (UPLC) fitted with a photodiode array (PDA) detector (AccQ-Tag Ultra; Waters Corporation, Milford, MA, USA).

3.2.5 Diet formulation

The study was composed of five dietary treatments which were formulated (Table 3.1) to meet the nutritional requirements for grower quail (Smith and Tabler, 2016). The experimental diets were formulated to be isocaloric and isonitrogenous in a marsh form by including MBM in a standard grower diet as follows: CON = a standard grower diet without MBM; MBMR = a standard grower diet with 99.98 g/kg of raw MBM; MBMC = a standard grower diet with 99.98 g/kg of cooked MBM; MBMA = a standard grower diet with 99.98 g/kg of autoclaved MBM; MBMT = a standard grower diet with 99.98 g/kg of toasted MBM. The chemical composition of the diets was carried out as described for the MBM samples in Section 3.2.4.

Table 3.1. Gross ingredient composition (g/kg *as is*) of the experimental diets.

Ingredients	CON	MBM
Marama bean meal	0	99.98
Yellow maize (8.0%)	552.2	584.5
Full-fat soya	120.0	28.78
Soya oilcake (45.74%)	266.9	244.7
Crude soya oil mixer	20.95	0
Limestone	12.44	12.0
Monocalcium phosphate	8.98	9.96
Salt (fine)	2.71	2.48
Sodium bicarbonate	1.27	1.63
DL-Methionine	4.14	4.42
L-Threonine	1.12	1.30
L-Tryptophan	0.09	0.05
Lysine HCl	2.85	3.82
Lignobond	2.0	2.0
Grower premix[2]	3.0	3.0
Axtraphytase (1000 FTU/g)	0.10	0.10
Antioxidant	0.25	0.25
Salinomycin (12%)	0.50	0.50
Zinc bacitracin	0.50	0.50
Calculated values (g/kg *as fed*)		
Dry matter	888.8	892.5
ME (Kcal/kg)	3,102	3,102
Crude protein	215.0	215.0
Lysine	12.2	12.2
Methionine	7.15	7.15
Threonine	8.10	8.10
Tryptophan	2.30	2.30
Crude fat	67.18	57.75
Crude fibre	36.63	35.72

CON = a standard grower diet without Marama bean meal; MBM = a standard grower diet containing 99.98 g/kg of Marama bean meal.

[2]Premix: vitamin A (11000 IU), vitamin D3 (2500 IU), vitamin E (25 IU), vitamin K3 (2.0 mg), vitamin B1 (2.5 mg), vitamin B2 (4.5 mg), vitamin B6 (5.1 mg), niacin (30 mg), pantothenic acid (10 mg), folic acid (0.7 mg), biotin (0.12 g), copper sulphate (8.0 mg), potassium iodide (0.34 mg), ferrous sulphate (80 mg), magnesium sulphate (100 mg), sodium selenite (0.25 mg), and zinc sulphate (79 mg).

3.2.6 Feeding trial and bird management

Three hundred and six (306), day-old mixed-gender Jumbo quail chicks were obtained from Golden Quail Farm in Randfontein, Gauteng province of South Africa. The chicks were offered stress pack: soluble vitamins and electrolytes for poultry (Virbac) for the first three days and raised on a standard commercial starter meal for four days, after which experimental diets were introduced until day 7, giving the birds 3 days to adapt to the treatments. For the experiment, chicks were evenly and randomly assigned to 35 cages (8/9 birds/cage) that were distributed to the 5 diets in such a way that each dietary treatment was replicated 7 times. The cages were constructed with wire mesh in which polythene sheets were laid as bedding. Each cage, measuring 100 cm in length × 60 cm width × 30 cm height, was the experimental unit; the cages were all placed in one room. The experiment lasted for 35 days during which feed and water were offered on an *ad libitum* basis. The birds were reared under natural lighting (12 hours daily) throughout the feeding trial.

3.2.7 Growth performance

Live weight of chicks was recorded at the beginning of the experiment (one-week-old) to determine initial body weights (30.97 ± 1.79 g live weight) and subsequent weekly measurements were done to estimate average weekly body weight gain. Collection and measurement of feed refusals was done daily before giving fresh feeds during the experiment to estimate feed intake per cage. Feed intake was estimated by finding the difference between weights of feed offered and refusal which was divided by total number of birds per cage. The body weight gain (BWG) and feed intake (FI) data was used to calculate average weekly gain-to-feed ratio (G:F). Mortality was recorded on occurrence and the data was used to calculate the corrected G:F.

3.2.8 Apparent amino acid retention

At the end of the experiment (35 days), two quail birds were randomly selected from each cage pooled to a total of 14 birds per treatment for the measurement of apparent retention (AR) of amino acids (AA). The birds were given a three-day adjustment period before excreta collection was done for five days. Feed offered and feed refusals was weighed to determine feed consumed. Excreta was collected in the morning every day, wrapped and stored in a refrigerator at -15°C per cage. The samples were then analysed for their amino acid composition with a focus on the essential amino acids since there is limited data on AR of essential AA in quail. Afterwards, AR of AA values were calculated as follows:

$$Apparent\ AA\ retention\ (\%) = \frac{AA\ intake - faecal\ AA}{AA\ intake} \times 100$$

3.2.9 Slaughter procedure and blood collection

The remaining birds (42–45 quail per treatment) were weighed to determine the final body weight (FBW) (at 35days) and then transported to a local abattoir where they were electrically stunned and slaughtered by slicing the jugular vein and allowed to bleed. While bleeding, blood was allowed to drip in sterilised containers and samples (up to 4 ml) were collected using 5 ml syringe and 23 *g* needle into two different test tubes from the freshly cut vein (two randomly selected birds in each replicate) for blood analysis. The tubes had two distinguished lid colours; purple ones contained ethylene-diamanie-tetra-acetic acid (EDTA), which is an anti-coagulant used for haematological analysis. The tubes having red lids did not have any anticoagulant and were used for serum biochemical analysis. The tubes containing the blood were stored in a cooler bag and the haematological samples were analysed within 24 h after collection (Washington & Hoosier, 2012).

3.2.10 Blood analysis

The collected samples were immediately taken to the laboratory for blood analysis. Whole blood counts such as platelets, heterophilis, lymphocytes, monocytes, and haematocrits, were determined by using an automated IDEXX LaserCyte Haematology Analyser (IDEXX Vet Diagnostics, S.A. PTY, Midrand, South Africa). Serum biochemistry analysis for total protein, total bilirubin, lipase, glucose, albumin, cholesterol, amylase, alkaline phosphatase

(ALP), serum symmetric dimethylarginine (SDMA) and alanine transaminase (ALT) content was determined by placing the clotted blood samples in a centrifuge (Hermle Labortechnik GmbH, Wehingen, Germany) for 15 min at 1000 g following the Washington & Hoosier (2012) guidelines, afterwards analysed in an automated IDEXX Vet Test Chemistry Analyser (IDEXX Laboratories S.A. PTY, Midrand, South Africa).

3.2.11 Statistical analysis

Data for repeatedly measured parameters (average weekly feed intake, average weekly body weight gain and average weekly G:F) were subjected to repeated measures analysis option in the General Linear Model procedure (PROC GLM) of the Statistical Analysis System (SAS), version 9.4 of 2013. The following statistical linear model was used:

$$Y_{ijk} = \mu + D_i + W_j + (D \times W)_{ij} + E_{ijk},$$

Where, Y_{ijk} = dependant variable, μ = overall population mean, D_i = effect of diet, W_j = effect of week (quail age), $(D \times W)_{ij}$ = effect of interaction between diets and week, E_{ijk} = random error associated with observation *ijk* which is assumed to be normally and independently distributed.

Data for overall feed intake, AR of AAs, growth performance (overall body weight gain and average weekly G:F), serum biochemistry and haematology were analysed using one-way ANOVA (PROC GLM) in SAS. The following model was employed for the analysis.

$$Y_{ij} = \mu + D_i + E_{ij}$$

Where, Y_{ij} = the dependant variable, μ = overall population mean, D_i = effect of diet, and E_{ij} = random error term associated with observation *ij* which is assumed to be normally and independently distributed. Separation of means was compared using the probability of difference option in SAS. Significance for all statistical tests was declared at P <0.05.

3.3 Results

The cooked Marama bean substrate tended to have the lowest DM, OM, ash and CP contents, while the toasted substrate had the highest values (Table 3.2). Autoclaving resulted in the highest EE, while cooking had the lowest EE value. The results show that raw marama substrates tended to have higher ADL contents than all the heat-treated substrates. However, cooking resulted in the lowest fibre contents (CF, NDF, ADF, and ADL), hence the cooked Marama bean substrate had a higher calculated ME value. Compared to the raw substrate, all the heat treatments tended to have a negative effect on AA values of the substrates with cooking resulting in the lowest values.

Table 3.2. Chemical composition and metabolizable energy values (g/kg DM, unless mentioned otherwise) of raw and heat-treated Marama substrates.

	[1]Substrates			
[2]Components	RMBS	CMBS	AMBS	TMBS
Dry matter (g/kg)	948.5	895.8	951.60	969.1
Organic matter	921.1	872.6	923.10	940.1
Ash	27.43	23.17	28.45	29.04
Crude protein	309.9	306.0	309.2	326.4
Ether extract	380.1	373.0	386.7	380.5
Crude fibre	252.5	110.8	298.2	314.7
NDF	329.7	154.3	362.0	327.4
ADF	181.8	144.5	172.4	174.9
ADL	165.3	69.70	134.7	162.9
ME (Kcal/kg)	2 763	2 772	2 761	2 760
Amino acids				
Arginine	31.41	27.13	31.19	30.61
Histidine	12.15	10.11	12.10	11.20
Isoleucine	15.56	15.57	15.85	15.81
Leucine	23.72	23.15	25.20	24.86
Lysine	11.04	12.61	13.00	14.33
Methionine	4.57	2.71	4.36	3.42
Threonine	10.84	10.97	11.86	12.03
Valine	16.76	17.25	17.41	17.68
Phenylalanine	23.57	20.04	22.44	21.68

[1]Substrates: RMBS = raw Marama bean substrate; CMBS = cooked Marama bean substrate; AMBS = autoclaved Marama bean substrate; TMBS = toasted Marama bean substrate.
[2]Components: NDF = neutral detergent fibre; ADF = acid detergent fibre; ADL = acid detergent lignin; ME = metabolizable energy.

The average chemical composition of diets containing raw and treated MB as well as the diet containing SB is presented in Table 3.3. There were notable differences as compared with the diet MBMR which indicates the effect of the heating techniques differed on all of the chemical components across the treatments. Cooking showed higher DM values than other heat treatments, while the highest EE was recorded in the diets that contained toasted MB. In addition, CP values showed a slight variation for the analyzed values as a result of the heat treatment on amino acids. Cooking treatment tended to slightly reduce the entire essential AA compared to the raw diet, except for histidine and phenylalanine content of the diets which were similar and greater than the control diet respectively. Autoclaving showed higher AA values for all the AAs except threonine which is lower than the diets that contained raw MB. Likewise, the highest values for methionine and threonine were recorded in diets containing toasted MB.

Table 3.3. Averaged chemical composition (g/kg *as fed*) and metabolizable energy of diets containing heat-treated Marama bean meal.

	[1]Diets				
[2]Components	CON	MBMR	MBMC	MBMA	MBMT
Dry matter	867.8	907.2	897.0	872.5	872.7
Organic matter	818.1	862.0	859.2	817.8	824.4
Ash	49.68	45.15	37.72	54.69	48.33
ME (Kcal/kg)	2,741	2,739	2,743	2,741	2,743
Crude protein	184.0	207.3	185.0	214.2	215.9
Ether extract	26.35	73.35	60.53	60.13	64.90
Crude fibre	62.54	65.85	60.85	62.59	60.70
NDF	139.0	149.8	143.3	157.5	207.7
ADF	80.72	115.2	102.2	91.99	103.2
ADL	31.35	33.13	33.48	30.50	33.80
		Amino acids			
Arginine	16.74	18.64	17.20	21.30	18.39
Histidine	6.62	7.23	6.62	7.71	7.68
Isoleucine	8.70	9.72	8.46	10.35	9.55
Leucine	18.89	19.87	18.35	22.03	19.71
Lysine	11.68	10.87	9.28	12.70	11.03
Methionine	10.26	8.82	8.73	9.39	9.56
Threonine	10.27	9.93	9.45	9.77	10.16
Valine	9.79	10.86	8.73	12.03	10.72
Phenylalanine	12.94	14.44	13.53	16.26	14.42

[1]Diets: CON = a standard grower diet without Marama bean meal; MBMR = a standard grower diet containing 99.98 g/kg of raw Marama bean meal; MBMC = a standard grower diet containing 99.98 g/kg of cooked Marama bean meal; MBMA = a standard grower diet containing 99.98 g/kg of autoclaved Marama bean meal; MBMT = a standard grower diet containing 99.98 g/kg of toasted Marama bean meal.
[2]Components: ME = metabolizable energy, NDF = neutral detergent fibre, ADF = acid detergent fibre, ADL = acid detergent lignin.

3.3.1 Apparent amino acid retention

Table 3.4 shows dietary treatment had varied effects on apparent amino acid retention of Jumbo quail. Birds fed CON, MBMC and MBMA had higher (*P* <0.05) AR of arginine than birds fed MBMR and MBMT. Diets CON and MBMT had the highest (*P* <0.05) AR of histidine followed by diets MBMC and MBMA and the lowest AR of histidine was from MBMR. . Quail birds offered diet CON had the highest (*P* <0.05) AR of isoleucine followed by MBMA diet, and the lowest was from diet MBMR. Diet CON promoted the highest (*P* <0.05) AR of leucine and phenylalanine followed by diets MBMC, MBMA and MBMT while diet MBMR promoted the lowest retention. The highest (*P* <0.05) AR of lysine was observed for birds fed CON and MBMC diets followed by MBMT and MBMR while the lowest AR for lysine was from MBMA. Diets CON and MBMC promoted higher (*P* <0.05) AR of methionine compared with the other diets, which did not differ (*P* >0.05).Birds fed CON had the highest (*P* <0.05) AR of threonine while those fed diet MBMR had the least AR of threonine. The lowest AR of valine was observed in birds fed MBMR and MBMC (*P* <0.05) followed by MBMA and MBMT birds, and the highest (*P* <0.05) AR of valine was from the CON group.

Table 3.4. Effect of diets containing raw or heat-treated marama bean meal on apparent retention of amino acids (g/kg DM) in Jumbo quail (n = 70).

	[1]Diets						
Parameters	CON	MBMR	MBMC	MBMA	MBMT	[2]SEM	*P* value
Arginine	774.1[a]	693.7[b]	742.9[a]	762.5[a]	722.6[b]	9.074	0.001
Histidine	811.3[a]	666.1[c]	767.9[b]	747.8[b]	820.7[a]	9.284	0.001
Isoleucine	695.1[a]	505.7[d]	615.6[b]	598.4[c]	568.7[c]	10.15	0.001
Phenylalanine	734.6[a]	557.2[c]	683.8[b]	704.3[b]	671.0[b]	10.20	0.001
Lysine	769.3[a]	691.2[b]	735.4[a]	641.5[c]	722.3[b]	9.961	0.001
Methionine	826.9[a]	777.1[b]	813.5[a]	802.9[b]	791.1[b]	10.56	0.022
Threonine	642.2[a]	434.7[d]	553.9[c]	522.5[c]	555.6[b]	8.146	0.001
Valine	673.0[a]	466.5[c]	506.3[c]	576.7[b]	537.3[b]	13.52	0.001
Leucine	702.3[a]	530.8[c]	648.4[b]	621.4[b]	652.8[b]	9.496	0.001

[a,b,c,d] Means, in a row, with similar superscripts are not statistically different ($P > 0.05$)
[1]Diets: CON = a standard grower diet without Marama bean meal; MBMR = a standard grower diet containing 99.98 g/kg of raw Marama bean meal; MBMC = a standard grower diet containing 99.98 g/kg of cooked Marama bean meal; MBMA = a standard grower diet containing 99.98 g/kg of autoclaved Marama bean meal; MBMT = a standard grower diet containing 99.98 g/kg of toasted Marama bean meal.
[2]SEM: standard error of the mean.

3.3.2 Growth Performance

Repeated measures analysis showed that there was no time (quail age in weeks) × diet interaction effects on FI (P = 0.076) and mortality (P = 0.898). However, significant interaction effects were observed on weight gain (P <0.0001) and G:F (P <0.0001). Table 3.5 indicates that dietary treatments had no effect on overall mortality. CON promoted the highest (P <0.05) overall FI followed by diets MBMC and MBMA, and the lowest overall FI was from the MBMR and MBMT groups. Two-week-old quail fed with diet CON had the highest weight gain (39.72 g/bird) followed by those fed with diets MBMC (34.81 g/bird) and MBMA (31.89 g/bird) while those fed with MBMR (19.63 g/bird) had the lowest weight gain. In week 3, MBMR promoted the lowest weight gain followed by MBMT and the highest weight gain was from diets CON, MBMA and MBMC, which did not differ (P >0.05). In week 4, birds reared on MBMT and MBMR had lower (P <0.05) weight gain than those reared on CON, MBMA and MBMC. No significant dietary effects (P>0.05) were recorded on weight gain in five-week-old Jumbo quail.

Two-week-old quail birds reared on diet CON had the highest G:F (P <0.05) followed by those fed with MBMC and MBMA ,MBMR and MBMT groups had lower G:F which did not differ (P>0.05). In week 3, quail birds fed with diet MBMT showed the highest (P <0.05) G:F followed by the MBMC and CON groups, and the lowest G:F was from the MBMR and MBMA groups. There was no significant dietary treatment effects recorded on G:F for four-week-old quail. Five- week-old quail fed MBMT and MBMA diets had the highest (P <0.05) G:F followed by MBMR and the lowest G:F was from quail birds reared on diets CON and MBMC, which did not differ (P >0.05).

Table 3.5. Growth performance (g/bird) in Jumbo quail (n = 221) fed with diets containing raw or heat-treated Marama bean meal.

	1Diets						
2Parameters	CON	MBMR	MBMC	MBMA	MBMT	2SEM	P value
Overall FI (g/bird)	504.0^{a}	425.0^{c}	461.5^{b}	458.^{9b}	409.9^{c}	11.32	<0.0001
Overall mortality (%)	3.17	10.18	1.59	3.17	6.35	2.830	0.238
Overall BWG(g/bird)	181.3^{a}	138.5^{b}	170.8^{a}	168.9^{a}	148.0^{b}	3.912	<0.0001
Overall G:F	0.360^{a}	0.327^{b}	0.370^{a}	0.368^{a}	0.365^{a}	0.005	<0.0001
Initial BW (g/bird)	31.26	30.88	30.57	31.26	30.97	0.730	0.956
Final BW (g/bird)	215.1^{a}	174.8^{b}	203.2^{a}	202.3^{a}	183.7^{b}	4.271	<0.001
		Average body weight gain (g/bird)					
Week 2	39.72^{a}	19.63^{d}	34.73^{b}	31.89^{b}	26.54^{c}	1.058	0.0001
Week 3	48.20^{a}	32.63^{c}	44.12^{a}	44.41^{a}	38.94^{b}	1.156	0.0001
Week 4	52.21^{a}	40.22^{b}	48.22^{a}	47.65^{a}	42.62^{b}	1.452	0.0001
Week 5	41.17	40.26	43.68	44.91	44.09	2.134	0.443
		Average gain-to-feed ratio (G:F)					
Week 2	0.529^{a}	0.349^{c}	0.506^{b}	0.476^{c}	0.446^{c}	0.010	0.0001
Week 3	0.468^{b}	0.368^{c}	0.478^{b}	0.457^{c}	0.485^{a}	0.016	0.0001
Week 4	0.368	0.346	0.375	0.362	0.363	0.012	0.363
Week 5	0.224^{c}	0.263^{b}	0.254^{c}	0.276^{a}	0.289^{a}	0.012	0.003

^{a,b,c,} Means, in a row, with similar superscripts are not statistically different (*P* > 0.05).
[1]Diets: CON = a standard grower diet without Marama bean meal; MBMR = a standard grower diet containing 99.98 g/kg of raw Marama bean meal; MBMC = a standard grower diet containing 99.98 g/kg of cooked Maramabean meal; MBMA = a standard grower diet containing 99.98 g/kg of autoclaved Marama bean meal; MBMT = a standard grower diet containing 99.98 g/kg of toasted Marama bean meal.
[2]Parameters: FI = feed intake; BW = body weight.
[3]SEM: standard error of the mean.

3.3.3 Blood parameters

There were no significant dietary treatment effects (P>0.05) on haematological parameters of Jumbo quail apart from haematocrits (Table 3.6). However, birds fed with diet CON had higher (*P* <0.05) haematocrits compared to those fed with the other dietary treatments, whose haematocrits did not differ (*P* >0.05).

Table 3.6. Haematological parameters in Jumbo quail fed with diets containing raw or heat-treated Marama bean meal.

[3]Parameters	[1]Diets					[2]SEM	*P* value
	CON	MBMR	MBMC	MBMA	MBMT		
Haematocrits (%)	48.08[a]	42.64[b]	44.07[b]	44.86[b]	42.43[b]	1.160	0.019
WBC ($\times 10^9$/L)	14.74	15.18	13.76	16.11	12.84	1.119	0.298
Platelets ($\times 10^9$/L)	25.30	26.78	29.42	26.94	25.44	1.727	0.479
Heterophils (%)	8.45	9.83	7.42	9.42	8.22	0.772	0.211
Lymphocytes (%)	5.55	4.68	5.62	5.83	3.97	0.702	0.320
Monocytes (%)	0.90	0.70	0.69	0.86	1.07	0.227	0.754

[a,b,c,] Means, in a row, with similar superscripts are not statistically different ($P > 0.05$)
[1]Diets: CON = a standard grower diet without Marama bean meal; MBMR = a standard grower diet containing 99.98 g/kg of raw Marama bean meal; MBMC = a standard grower diet containing 99.98 g/kg of cooked Marama bean meal; MBMA = a standard grower diet containing 99.98 g/kg of autoclaved Marama bean meal; MBMT = a standard grower diet containing 99.98 g/kg of toasted Marama bean meal.
[2]SEM: standard error of the mean.
[3]Parameters: WCC = white cell count.

The effect of dietary treatments on serum biochemical parameters in Jumbo quail shows that diet had no influence (P>0.05) on all serum biochemical parameters except on lipase (P <0.001) (Table 3.7). Diet MBMR promoted the highest (P <0.05) lipase levels followed by MBMA and MBMT diets and the lowest lipase levels were on diets CON and MBMC.

Table 3.7. Serum biochemical parameters in Jumbo quail fed with diets containing raw or heat-treated Marama bean meal.

[2]Parameters	[1]Diets CON	MBMR	MBMC	MBMA	MBMT	[3]SEM	*P* value
Glucose (mmol/L)	11.09	11.84	11.47	11.66	10.59	15.43	0.766
SDMA (μg/dL)	27.71	27.64	23.93	27.71	30.10	3.871	0.869
Creatinine (μmol/L)	18.56	8.84	8.84	8.84	44.2	0.161	0.410
Phosphorus (mmol/L)	0.74	12.54	11.29	11.19	10.95	0.737	0.163
Calcium (mmol/L)	0.59	0.54	0.59	0.54	0.49	0.782	0.499
Total protein (g/L)	6.06	7.06	4.07	4.22	3.96	1.091	0.197
Albumin (g/dL)	2.05	1.81	1.53	1.57	1.91	0.229	0.460
Globulin (g/dL)	3.01	3.26	2.54	2.79	2.01	0.338	0.142
ALB/GLOB	0.64	0.58	0.66	0.59	0.62	2.619	0.331
ALT (U/L)	77.29	63.79	49.43	48.00	90.08	23.13	0.685
ALKP (U/L)	298.9	215.4	306.7	272.2	283.9	38.36	0.486
TBIL (mmol/L)	0.22	0.07	0.08	1.49	1.66	10.24	0.301
Cholesterol (mmol/L)	9.80	7.50	9.29	9.03	9.57	18.34	0.540
Amylase (U/L)	325.9	452.4	416.3	497.1	487.9	46.91	0.102
Lipase (U/L)	211.4^{c}	775.6^{a}	298.1^{c}	581.5^{b}	551.8^{b}	71.49	<0.001

[a,b,c] Means, in a row, with similar superscripts are not statistically different ($P > 0.05$).
[1]Diets: CON = a standard grower diet without Marama bean meal; MBMR = a standard grower diet containing 99.98 g/kg of raw Marama bean meal; MBMC = a standard grower diet containing 99.98 g/kg of cooked Marama bean meal; MBMA = a standard grower diet containing 99.98 g/kg of autoclaved Marama bean meal; MBMT = a standard grower diet containing 99.98 g/kg of toasted Marama bean meal
[2]Parameters: SDMA = serum symmetric dimethyl arginine, ALB/GLOB = albumin/globulin ratio, ALT = alanine transaminase, ALKP = alkaline phosphatase, TBIL = total bilirubin.
[3]SEM: standard error of the mean.

3.4 Discussion

3.4.1 Growth performance

Repeated measures analysis showed that there was no significant time (quail age in weeks) and diet interaction effect on feed intake, which indicates that the ability of Jumbo quail birds to utilise the dietary treatments were not influenced by their age. However, significant diet and week interaction effects were observed on weight gain and G:F, which suggests that the ability to efficiently convert the dietary treatments into body mass depended on the bird's age. Although no dietary influences were observed on overall mortality, the MBMR treatment tended to have the highest mortalities of 10.18%, indicating the need for pre-processing of MB prior to feeding Jumbo quail. Results from the current study showed that inclusion of MBMC and MBMA in quail diet does not compromise overall feed intake and it yielded a better performance. Indeed, the MBMC and MBMA treatments boosted overall feed intake than MBMR and MBMT, further indicating the efficacy of these treatment methods (cooking and autoclaving) to improve the utility of MB (Alabi *et al*., 2022). The increase in feed consumption could also be attributed to the enticing nutty odour, improved flavour and palatability of cooked or autoclaved Marama beans (Kayitesi *et al*., 2010). In agreement, El Boushy & Van der Poel (2012), noticed that feed consumption in poultry is greatly influenced by the sensory quality of feed ingredients and concluded that in order to improve performance, producers need to improve feed palatability and acceptability to increase the feed intake in birds. The poor FI in the MBMR group was expected due to the presence of ANF (phenolic and saponin), which contributes to the beans' bitterness (Wang *et al*., 2022). Likewise, toasted Marama bean has been reported to have more intense burnt, bitter taste due to increased level of phenolics (Nyembwe *et al*., 2015) resulting in poor feed utilization. Weight gain at two weeks of age in MBMC and MBMA fed quail birds were higher than birds fed with MBMR diet but were comparable to the soybean-based control diet. At three and four weeks of age, similar weight gain was observed in MBMC, MBMA and the soybean-based control diet, suggesting that cooking and autoclaving improved feed utilization. This showed that the negative effect of ANFs in MB has been successfully ameliorated, which promoted better performance in birds fed MBMC and MBMA diets than MBMR. This is attributable to the presence of TI in raw MB (Alabi *et al*., 2022). It can also be attributed to the fact that heat processing has been indicated to improve protein quality in MB, which improved the birds' performance. Likewise, MBM has been shown to improve caecal microbiota that in turn resulted in the improvement of poultry nutrition and growth performance (Maruatona *et al*., 2010; Montso *et al*., 2022). However, at week 5, there was no significant dietary effect on weight gain for Jumbo quail indicating that feed utilization had improved as the birds advanced in age due to the development of a better digestive system. Body weight has been associated with improved feed intake and growth rate (Metwally *et al*., 2020). Therefore, MBM could be included in quail diets without any adverse effects on weight gain at week 5. The inclusion of MBMC and MBMA showed higher G:F and heavier final body weights indicating that cooking and autoclaving treatment of MB promotes similar performance as the soybean control diet without any compromise on performance. These findings agree with previous authors who noted that replacing SBM with other legumes does not have any hazardous effect on growth performance of quail birds (El-Hack *et al*., 2016). Low final body weight of birds in the MBMR and MBMT could be attributed to low feed intake due to the presence of TI, which corroborates the findings of Erdaw *et al*. (2017) that feeding diets with high levels of TI had negative effects on growth and feed efficiency of broiler chickens. This further confirms that cooking and autoclaving were more effective in ameliorating the negative activities of ANF in MBM than the toasting method.

3.4.2 Apparent amino acid retention

Apparent retention of nutrients does not account for any nutrients that are lost in the urine or gases produced during digestion (Nhlane *et al*., 2020; Zhang *et al*., 2023). This measurement is important for understanding the nutritional value of feed and it is commonly used to evaluate the quality and nutrient utilisation of feed ingredients (Kil *et al*., 2013; Rojas & Stein, 2015). In this research, focus was on AR of AA because there is a scarcity of data in quail birds (Ghazaghi *et al*., 2023). This study showed that MB contains all the essential AA (arginine, methionine, lysine, threonine histidine, isoleucine, leucine, phenylalanine, and valine), which meets the minimum biological requirements needed for growing quail (Gulzar & Minnaar, 2017). The results of this study revealed that heat treatment of MBM tended to reduce the AA contents of MB. However, diet MBMR resulted in poor AR for the reported essential AA. Relative to CON diet, the cooking treatment enhanced the AR of threonine, lysine, methionine, isoleucine, and leucine, which explains the positive effect on growth performance, since methionine, lysine and threonine are major limiting AA in poultry diets (Mehri *et al*., 2012; Ghazaghi *et al*., 2023). The high retention of these AA could be due to the solubilisation of crude protein (CP) and fibre during cooking. Autoclaving treatment also improved the AR of arginine, valine and phenylalanine indicating that this method could be important in increasing the bioavailability of these essential AA, which are required for energy and protein synthesis, metabolism, and efficient neurotransmission (NRC, 1994) in Jumbo quail. The toasting treatment increased AR of histidine only, which is important for the stimulation of thyrotropin-releasing hormone, immune responses, renal function (NRC, 1994), among others. The poor AR of AA in the MBMR group was anticipated due to the presence of ANF such as trypsin inhibitor (TI) and condensed tannins (CT) in MB (Alabi *et al*., 2022) that have been reported to reduce AA utilization (Gilani *et al*., 2012). Overall, the cooking followed by autoclaving treatments of MB enhanced AR of AA, indicating the potential of these methods to ameliorate the adverse effects of TI and CT in MB.

3.4.3 Haematological parameters

In poultry production, blood analysis is a valuable tool used to assess nutritional and health status, diagnose diseases and identify breeding problems of birds (Obese *et al*., 2018). Since the blood serves as transport system that conveys nutrients to different parts of an animal body, factors affecting blood, either in terms of nutrition or medication would certainly have overall effect on the entire body regarding health, growth, maintenance and reproduction (Etim *et al*., 2014). The fundamental information on animal blood helps to measure the level of nutrients in feed, trace physiological changes that can indicate the presence of various infections, hence detect subclinical diseases, and measure hormonal levels which can indicate fertility of poultry birds (Matabane, 2023). All of these can help producers to make informed management decisions about their flock and ensure their health and productivity (Zaitsev, 2016). The general trend in quail production is to ensure diets are safe, effective for optimum growth performance, and do not compromise the blood profile of the birds (Mnisi & Mlambo, 2018; Elnesr *et al*., 2019). Blood analysis has been recommended as a fast, safe and readily available means of evaluating nutritional health status of animals during feeding trials because consumption of dietary feed treatments have measurable effects on their blood parameters. Haematological indices are one of the critical pointers to the physiological or pathophysiological state of the animal body (Onasanya *et al*., 2015). There are indications from several studies conducted by various researchers that diets have different effects on haematological parameters of different poultry species. Thus, proper considerations should be given to the types of diets and their effects on blood parameters, as well as the physiology of birds (Etim *et al*, 2014; Alagawany *et al*., 2020). The results of this study showed that haematological parameters did not differ significantly among birds fed with the different dietary treatments in Jumbo quail apart from haematocrits. Haematocrit count denotes the percentage of blood that is composed of red blood cells which is usually affected by the number and size of these red blood cells (Alagawany *et al*., 2020). Moreover, it is a measure of oxygen-carrying capacity of the blood; hence, it is considered a key determinant of aerobic performance related to movement in birds (Kobayashi *et al*., 2022; Soulsbury *et al*., 2022). Observed hematocrits of quail birds fed with diet CON were higher than other dietary treatments, which were statistically similar. This means that the percentage of red blood cells in the CON-based quail birds was high. Higher haematocrit percentage could be attributed to higher growth rate, increased metabolic activities and production of gonadotropins and metabolic hormones because all of these require more blood supply to the muscle tissues (Sujata *et al*., 2014; Alagawany *et al*., 2020; Gharaoghlan *et al*., 2022). However, the birds fed diets containing MBM could have experienced insufficient iron absorption due to the presence of tannins leading to reduced production of red blood cells (Gupta, 2014). According to Shubham *et al*. (2020), excessive levels of phytates and tannin in the diet inhibit iron absorption which can lead to iron deficiency. Nonetheless, it was expected that thermal treatment of MBM would greatly improve iron utilization due to amelioration of tannins, but haematocrits value across the treatments were not different.

3.4.4 Serum biochemical parameters

Diet is one of the many factors that affect serum biochemical parameters in animals. Serum biochemical parameters in this study did not differ significantly except for lipase. Quail fed the MBMR diet had higher lipase levels compared to those fed with other dietary treatments, which were beyond the reported values for healthy quail (Mulaudzi *et al*., 2022). Lipase is an enzyme that is involved in the breakdown of water-insoluble fats molecules and is important for the digestion, hydrolysis of fat and absorption of fat-soluble vitamins (Pirahanchi & Sharma, 2019; Lim *et al*., 2022). Several digestive enzymes, including lipase are produced and secreted in the pancreas (Erdaw & Beyene, 2018). The presence of TI negatively affects this function whereby extreme lipase level causes damage to the pancreas or acute pancreatitis, which was expected given the high concentration of TI in MB. Lower levels of lipase observed in quail fed MBMC implies that cooking successfully ameliorated the negative effect of TI. The diets had no effect on creatinine which is the functional biomarker of the kidney (Kashani *et al*., 2020); neither on liver enzymes like ALKP and ALT, meaning that inclusion of MBM in quail feed did not affect kidney and liver function which was quite surprising given the high concentrations of CT and fibre in the diet containing raw MBM. Total protein and albumin were also not influenced by the treatments; this is because the diets were isonitrogenous. However, the blood parameters fell within the normal range reported for healthy quail birds (Mnisi & Mlambo 2018).

3.5 Conclusion

Cooking and autoclaving treatments enhanced apparent amino acid retention, overall growth performance, and carcass weights of Jumbo quail compared to raw diet. Although Jumbo quail reared on soybean-based control diet showed best performance, cooking followed by autoclaving treatments of Marama bean was more effective than toasting.

3.6 References

Alabi, F., Kiarie, E. G., Mnisi, C. M. & Mlambo, V., 2022. Physical treatment reduces trypsin inhibitor activity and modifies chemical composition of Marama bean (*Tylosema esculentum*). Molecules. 27(14), 4451.

Alagawany, M., Nasr, M., Al-Abdullatif, A., Alhotan, R. A., Azzam, M.M. & Reda, F.M., 2020. Impact of dietary cold-pressed chia oil on growth, blood chemistry, haematology, immunity and antioxidant status of growing Japanese quail. Ital. J. Anim. Sci. 19(1), 896-904.

AOAC. 2005. Official Methods of Analysis of Association of Official Analytical Chemists International, 16th ed. Association of Official Analytical Chemists, Arlington, VA, USA.

Babatunde, O.O., Park, C.S. & Adeola, O., 2021. Nutritional potentials of atypical feed ingredients for broiler chickens and pigs. Animals. 11(5), 1196.

Cullis, C., Lawlor, D.W., Chimwamurombe, P., Bbebe, N., Kunert, K. & Vorster, J., 2019. Development of Marama bean, an orphan legume, as a crop. Food and Energy Secur. 8(3), e00164.

El Boushy, A.R.Y. & Van der Poel, A.F.B., 2012. Handbook of poultry feed from waste: processing and use. Springer Science and Business Media, New York, USA. Pp.405-423.

El-Hack, M.E.A., Alagawany, M., Saeed, M., Arif, M., Arain, M.A., Bhutto, Z.A. & Fazlani, S.A., 2016. Effect of gradual substitution of soyabean meal by *Nigella sativa* meal on growth performance, carcass traits and blood lipid profile of growing Japanese quail. J. Anim. Feed Sci. 25(3), 244-249.

Elnesr, S.S., Ropy, A. & Abdel-Razik, A.H., 2019. Effect of dietary sodium butyrate supplementation on growth, blood biochemistry, haematology and histomorphometry of intestine and immune organs of Japanese quail. Animal. 13(6), 1234-1244.

Erdaw, M.M. & Beyene, W.T., 2018. Anti-nutrients reduce poultry productivity: influence of trypsin inhibitors on pancreas. Asian J. Poult. Sci. 12(1), 14-24.

Erdaw, M.M., Wu, S. & Iji, A.P., 2017. Growth and physiological responses of broiler chickens to diets containing raw, full-fat soybean and supplemented with a high-impact microbial protease. Asian Aust. J. Anim. Sci. 30: 1303-1313.

Etim, N.A.N., Akpabio, U., Okpongete, R.O. & Offiong, E.E.A., 2014. Do diets affect haematological parameters of poultry? Br. J. Appl. Sci. Technol. 4(13), 1952-1965.

Gharaoghlan, M.F., Bagherzadeh-Kasmani, F., Mehri, M. & Ghazaghi, M., 2022. The effect of short, long, natural, and intermittent short photoperiods on meat-type Japanese quails. Int. J. Biometeorol. 66, 1737-1745.

Ghazaghi, M., Hassanabadi, A. & Mehri, M., 2023. Apparent and standardized ileal amino acid digestibilities of corn, wheat, soybean meal, and corn gluten meal in quail chicks. Poult. Sci. 102(2), 102314.

Gilani, G.S., Xiao, C.W. & Cockell, K.A., 2012. Impact of antinutritional factors in food proteins on the digestibility of protein and the bioavailability of amino acids and on protein quality. Br. J. Nutr. 108 (2), 315-332.

Gulzar, M. & Minaar, A., 2017. Underutilized protein resources from African legumes. Sustainable protein sources. Academic Press. Elsevier Inc. Cambridge, MA, United States. Pp. 197-208

Gupta, C.P., 2014. Role of Iron (Fe) in Body. J. Appl. Chem. 7(11), 38-46.

Ismail, B. P., Senaratne-Lenagala, L., Stube, A. & Brackenridge, A., 2020. Protein demand: Review of plant and animal proteins used in alternative protein product development and production. Anim. Front. 10(4), 53-63.

Kashani, K., Rosner, M.H. & Ostermann, M., 2020. Creatinine: From physiology to clinical application. Eur. J. Intern. Med. 72, 9-14.

Kayitesi, E., Duodu, K.G., Minnaar, A. & de Kock, H.L., 2010. Sensory quality of Marama/sorghum composite porridges. J. Sci. Food Agric. 90(12), 2124-2132.

Kil, D.Y., Kim, B.G. & Stein, H.H., 2013. Feed energy evaluation for growing pigs. Asian-australas. J. Anim. Sci. 26(9), 1205.

Kobayashi, S., Qasimi, M.I. & Watanabe, G., 2022. The positive relationship between androgens and hematocrit levels in the Japanese quail (*Coturnix japonica*). J. Exp. Zool. A: Ecol. Integr. Physiol. 337(3), 195-198.

Lim, S.Y., Steiner, J.M. & Cridge H., 2022. Lipases: it's not just pancreatic lipase! Am. J. Vet. Res. 83(8), doi: 10.2460/ajvr.22.03.0048.

Maruatona, G.N., Duodu, K.G. & Minnaar, A., 2010. Physicochemical, nutritional and functional properties of Marama bean flour. Food Chem. 121(2), 400-405.

Matabane, A.N., 2023. Feed utilisation, physiological and meat quality responses in Jumbo quail reared on apple pomace-containing diets (MSc. dissertation, North-West University, Mafikeng, South Africa.

Mbhele, F.G., Mnisi, C.M. & Mlambo, V., 2019. A nutritional evaluation of insect meal as a sustainable protein source for jumbo quails: Physiological and meat quality responses. Sustainability. 11(23), 6592.

Mehri, M., Davarpanah, A.A. & Mirzaei, H.R., 2012. Estimation of ideal ratios of methionine and threonine to lysine in starting broiler chicks using response surface methodology. Poult. Sci. 91(3), 771-777.

Metwally, A.E., Abdel-Wareth, A.A., Saleh, A.A. & Amer, S.A., 2020. Are the energy matrix values of the different feed additives in broiler chicken diets could be summed? BMC Vet. Res. 16(1), 1-11.

Mnisi, C.M. & Mlambo, V., 2018. Growth performance, haematology, serum biochemistry and meat quality characteristics of Japanese quail (*Coturnix coturnix japonica*) fed canola meal-based diets. Anim. Nutr. 4(1), 37-43.

Mnisi, C.M., Mlambo, V., 2018. Protease treatment of canola meal-containing Japanese quail diets: Effect on physiological parameters and meat quality traits. J. Appl. Anim. Res. 46, 1389-1394.

Montso, P.K., Mnisi, C.M. & Ayangbenro, A.S., 2022. Caecal microbial communities, functional diversity, and metabolic pathways in Ross 308 broiler chickens fed with diets containing different levels of Marama (*Tylosema esculentum*) bean meal. Front. Microbiol. 13, 1009945.

Mulaudzi, A., Mnisi, C.M. & Mlambo, V., 2022. Effect of pre-treating dietary Moringa oleifera leaf powder with fibrolytic enzymes on physiological and meat quality parameters in Jumbo quail. Poult. 1(2), 54-65.

Nasar, A., Rahman, A., Hoque, N., Talukder, A.K. & Das, Z.C., 2016. A survey of Japanese quail (*Coturnix coturnix japonica*) farming in selected areas of Bangladesh. Vet. World. 9(9), 940.

Nhlane, L.T., Mnisi, C.M., Mlambo, V. & Madibana, M.J., 2020. Nutrient digestibility, growth performance, and blood indices of Boschveld chickens fed seaweed-containing diets. Animals, 10(8), 1296.

NRC. 1994. Nutrient Requirements of Poultry (9th rev. ed.), National Research Council, National Academy Press, Washington, DC, USA.

Nyembwe, P., Minnaar, A., Duodu, K.G. & Kock, H.L., 2015. Sensory and physicochemical analyses of roasted Marama beans [*Tylosema esculentum* (Burchell) A. Schreiber] with specific focus on compounds that may contribute to bitterness. Food Chem. 178, 45-51.

Obese, F.Y., Ali, Z.S., Naazie, A. & Ayizanga, R.A., 2018. Effect of age, breed and sex on haematological and blood biochemical parameters in helmeted guinea fowl (*Numida meleagris*). Comp. Clin. Pathol. 27, 901-909.

Onasanya, G.O., Oke, F.O., Sanni, T.M. & Muhammad, A.I., 2015. Parameters influencing haematological, serum and bio-chemical references in livestock animals under different management systems. Open J. Vet. Med. 5(8), 181.

Pirahanchi, Y. & Sharma, S., 2019. Biochemistry, Lipase. Stat Pearls Publishing, Treasure Island (FL), USA. PMID: 30726031.

Popoola, J.O., Ojuederie, O.B., Aworunse, O.S., Adelekan, A., Oyelakin, A.S., Oyesola, O.L., Akinduti, P.A., Dahunsi, S.O., Adegboyega, T.T., Oranusi, S.U. & Ayilara, M.S., 2023. Nutritional, functional, and bioactive properties of African underutilized legumes. Front. Plant Sci. 14, 1105364.

Rojas, O.J. & Stein, H.H., 2015. Effects of reducing the particle size of corn grain on the concentration of digestible and metabolizable energy and on the digestibility of energy and nutrients in corn grain fed to growing pigs. Livest. Sci. 181, 187-193.

SAS. 2010. Users Guide: Statistics. Version 9.3. Cary: SAS Institute, NC, USA.

Shubham, K., Anukiruthika, T., Dutta, S., Kashyap, A.V., Moses, J.A. & Anandharamakrishnan, C., 2020. Iron deficiency anemia: A comprehensive review on iron absorption, bioavailability and emerging food fortification approaches. Trends Food Sci. Technol. 99, 58-75.

Smith, T. W. & Tabler, T., 2016. Feeding quail. Mississippi State University Extension Service, MS, USA. 2383, 1-4.

Soulsbury, C.D., Dobson, J., Deeming, D.C. & Minias, P., 2022. Energetic lifestyle drives size and shape of avian erythrocytes. Integr. Comp. Biol. 62(1), 71-80.

Stastnik, O., Novotny, J., Roztocilova, A., Kouril, P., Kumbar, V., Cernik, J., Kalhotka, L., Pavlata, L., Lacina, L. & Mrkvicova, E., 2021. Safety of mealworm meal in layer diets and their influence on gut morphology. Animals. 11(5), 1439.

Van Soest, P.J., Robertson, J.B. & Lewis, B.A., 1991. Methods for dietary fibre neutral detergent fibre and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583-3597.

Wang, Y., Tuccillo, F., Lampi, A.M., Knaapila, A., Pulkkinen, M., Kariluoto, S., Coda, R., Edelmann, M., Jouppila, K., Sandell, M. & Piironen, V., 2022. Flavor challenges in extruded plant-based meat alternatives: A review Compr. Rev. Food Sci. Food Saf. 21(3), 2898-2929.

Washington, I. M. & van Hoosier G., 2012. Clinical biochemistry and haematology. University of Washington, Seattle, WA, USA. pp. 59-91.

Zaitsev, S., 2016. Dynamic surface tension measurements as general approach to the analysis of animal blood plasma and serum. Adv. Colloid Interface Sci. 235, 201-213.

Zhang, C., Hao, E., Chen, X., Huang, C., Liu, G., Chen, H., Wang, D., Shi, L., Xuan, F., Chang, D. & Chen, Y., 2023. Dietary fibre level improves growth performance, nutrient digestibility, immune and intestinal morphology of broilers from day 22 to 42. Animals. 13(7), 1227.

4 CHAPTER FOUR – CARCASS CHARACTERISTICS, VISCERAL MORPHORMETRY AND BREAST MEAT QUALITY OF JUMBO QUAIL REARED ON DIETS CONTAINING RAW OR HEAT-TREATED MARAMA BEAN MEAL

Abstract

This study investigated the effect of including raw or heat-treated Marama bean meal (MBM) inclusion in grower Jumbo quail diets on carcass characteristics, visceral organs, and meat quality attributes. Five isonitrogenous and isoenergetic experimental diets were formulated as follows: CON (a standard grower diet without MBM; MBMR (a standard grower diet with only 99.98 g/kg of raw MBM); MBMC (a standard grower diet with only 99.98 g/kg of cooked MBM); MBMA (a standard grower diet with only 99.98 g/kg of autoclaved MBM); and MBMT (a standard grower diet with only 99.98 g/kg of toasted MBM). Jumbo quail chicks (n = 306) were randomly and evenly distributed to five dietary treatments which were replicated 7 times. At the end of the 35-day experiment, all the quail birds were removed from the cages with the exception of 2 birds per cage and the rest (221) birds were slaughtered at an abattoir to examine carcass characteristics, internal organs, and breast meat quality. Dietary treatments influenced (P <0.05) final body weight (FBW), hot carcass weight (HCW) and cold carcass weight (CCW). Quail birds fed diets MBMC and MBMA had higher (P <0.05) FBW and CCW than those fed diet MBMR and MBMT. Diet MBMC promoted higher (P <0.05) HCW in quail when compared to diets MBMR, MBMA and MBMT. Quails fed diet MBMR had the heaviest (P <0.05) relative gizzard weight (2.81 g /100 g CCW) than those fed diets CON, MBMC, MBMA and MBMT. . Birds fed diet MBMT had the heaviest (P <0.05) relative proventriculus weight followed by MBMR and MBMC groups, while the CON and MBMA groups had the lightest proventriculus weights. Jumbo quail fed diets MBMT and MBMR had higher relative spleen weights (P <0.05) than CON,

MBMC and MBMA treatments. Breast meat from quail fed the CON, MBMC, and MBMA diets had similar 24-hour redness, which was higher ($P < 0.05$) than that of the MBMR and MBMT groups. The CON and MBMC diets promoted higher ($P < 0.05$) 24-hour chroma values compared to all other treatments. Additionally, the MBMR and MBMT diets resulted in higher ($P < 0.05$) hue angle values than all other dietary treatments.It is worth noting that the inclusion of heat-treated MBM in Jumbo quail diets improved the carcass characteristics, and meat quality traits, however, slight alteration was observed on some internal organ sizes.

Keywords: Carcass, Marama bean meal, Meat colour, Quail meat, Visceral organs

4.1 Introduction

The Jumbo quail is of economic importance in the poultry industry as it has been identified for its high prolificacy and fast growth rate that could increase the supply of animal protein (Mulaudzi, 2019). Quail production is gaining ground in the South African poultry industry because of the nutritional benefits and the possibility to alleviate food insecurity risks, especially in rural areas (Mnisi *et al*., 2021). The quail meat is a desirable alternative poultry meat among consumers because it is tasty, low in fat and rich in essential nutrients such as protein, vitamins, and minerals (Santhi & Kalaikannan, 2017; Mnisi *et al*., 2021). To produce high quality meat and meat products that are acceptable by consumers, it is needful to incorporate alternative feed ingredients with antioxidants, antimicrobial and meat-enhancing properties into quail diets (Marareni, 2021). Furthermore, the use of locally available non-conventional alternative protein sources such as Marama bean meal (MBM) would reduce feed costs and competition with humans (Mnisi *et al*., 2022; Montso *et al*., 2022).

MB is a local drought-tolerant leguminous plant that grows naturally, with a superior nutritional value similar to soybean plant (Cullis *et al*., 2019; Omotayo & Aremu, 2021). It is rich in protein with good essential amino acids profile, polyunsaturated fatty acids and bioactive compounds such as antioxidants. However, these phytochemicals are in the form of ANFs, which interfere with nutrient digestibility and limit the use of MB in quail diets (Maphosa & Jideani, 2017; Soetan, 2018). Processing techniques such as cooking and steam-autoclaving have been found to be an effective method to reduce some antinutritional factors (ANFs) in MB, thus enhancing its nutritional value (Alabi *et al*., 2022).

Therefore, including heat-treated MBM in quail diet could be a viable approach to improve the quality of quail meat and acceptability. Thus, this study aimed to improve the feed value of MBM using different thermal procedures. Therefore, this research represents the first attempt to evaluate the effect of raw or heat-treated (cooked, toasted and autoclaved) MBM in diets of Jumbo quail on internal organs, carcass yield, meat quality parameters and shelf-life indicators. It was hypothesized that the inclusion of heat-treated MBM would not affect internal organs, carcass and meat quality parameters as the soybean-based control diet.

4.2 Materials and methods

4.2.1 Study area, diet formulation and bird management

The study was carried out in Molelwane farm as described in Chapter 3, Section 3.2.1. Diet formulation and experimental design were also as mentioned in Chapter 3, Sections 3.2.5 and 3.2.6.

4.2.2 Feeding trial and slaughter procedure

The feeding trial, bird management and slaughter procedure were as detailed in Chapter 3, Sections 3.2.6 and 3.2.9.

4.2.3 Carcass performance and internal organs

After slaughter, the carcasses were plucked and plucking manually eviscerated by hand. The carcasses were packed and placed inside plastic bags that were labelled according to each experimental unit. The carcasses were weighed immediately to determine hot carcass weight (HCW). After which, they were stored in the cold room for 24 hours at 4°C, then reweighed to determine cold carcass weight (CCW). A digital weighing scale (Explorer® EX224, OHAUS Corporation, Parsippany, NJ, USA) was used to weigh HCW, CCW, carcass cuts (wing, drumstick, breast, and thigh) as well as internal organs (liver, gizzard, proventriculus, spleen, pancreas, small intestine and large intestine) and expressed as a proportion of CCW (g /100 g CCW). Dressing percentage was calculated as the proportion of CCW on slaughter weight (final body weight).

4.2.4 Breast meat colour, pH and temperature

Measurements for meat colour coordinates – luminosity (L^*), redness (a^*) and yellowness (b^*) were taken with the use of spectrometer (BYK-Gardener GmbH, Geretsried, Germany) with 20 mm diameter D65-day light illuminant and 10° observation angle 1 hour and 24 hours after slaughter. The colour meter was calibrated before measurements were taken. Hue angle and chroma was calculated using the formula $\tan^{-1}(b^*/a^*)$ and $\sqrt{a*^2 + b*^2}$ as guided by Priolo *et al*. (2002). Breast meat pH and temperature were taken 1 hour and 24 hours *post-mortem* using a Corning Model 4 pH-temperature meter (Corning Glass Works, Medfield, MA) fitted with an Ingold spear-type electrode. The pH meter was calibrated with pH of 4, 7, and 10 standard solutions before data collection and recalibrated after every 10 measurements.

4.2.5 Cooking loss

Raw breast meat samples were weighed individually, and oven broiled in a foil plate at a temperature of 75℃ for 20 minutes using the methods by Honikel (1998). These samples were cooled and reweighed after cooking. Cooking loss was calculated as follows:

$$Cooking\ Loss(\%) = \frac{W_i - W_f}{W_i} \times 100$$

Where, W_i = initial weight before cooking, W_f = final weight after cooking.

4.2.6 Meat tenderness

Measurement of meat tenderness was taken by shearing raw breast samples perpendicular to the direction of muscle fibres with the aid of a Meullenet-Owens razor shear blade which was mounted on a Texture Analyser (TA-XT plus, Stable Micro Systems, Surrey, UK) and obtained values which signify average peak shear force per sample were measured in Newton (N).

4.2.7 Water holding capacity and Drip loss

Water holding capacity (WHC) was determined using the methods by Grau & Hamm (1957), where W_i of breast meat samples was taken from each cage and thereafter placed in-between two filter papers and subjected to pressure (60 kg pressure) from dumbbell weights for five minutes. The W_f of the meat samples were also measured after applying pressure. The WHC was measured as follows:

$$WHC\ (\%) = 100 - [\frac{W_i - W_f}{W_i} \times 100]$$

Where: W_i = initial weight before pressure; W_f = final weight after pressure.

Drip loss (DL) was determined using the method adapted from Zhang *et al*. (2009) in which breast meat samples were cut into pieces of 2 - 3 g of weight from each cage and used as W_i. Then, the samples were placed on a hook and suspended with wire steel inside a plastic bottle which was appropriately sealed so that samples do not touch the sides of the bottle. The bottles were chilled at 2℃ for 72 hours; afterwards, samples were weighed again. The following equation was used to determine DL:

$$DL\ (\%) = \frac{W_i - W_f}{W_i} \times 100$$

Where: W_i = initial weight before suspension, W_f = final weight after suspension.

4.2.8 Shelf life

Breast meat samples were selected randomly from each experimental unit and used to determine shelf life of the breast meat. These meat samples were placed individually in foil plates, labelled accordingly and stored in a cool dry place inside the Meat Science laboratory of the North-West University at room temperature (20 - 27°C). Records of meat temperature, pH and colour (L*, *a**and *b**) were taken daily for 3 consecutive days only because the breast meat samples had rotten by day 4. Hue angle and chroma values were calculated for 1h and 24 h postmortem as described by Priolo *et al*. (2002).

4.2.9 Statistical analysis

Data for carcass characteristics, internal organs and meat quality parameters were analysed using one-way ANOVA in the procedure of General Linear Model (PROC GLM) of the Statistical Analysis System (SAS), version 9.4 of 2010 to determine the effects of the diets. The following model was employed for the analysis.

$$Y_{ij} = \mu + D_i + E_{ij}$$

Where, Y_{ij} = the dependant variable, μ = overall population mean, D_i = effect of diets, and E_{ij} = random error term associated with observation *ij* which is assumed to be normally and independently distributed. Separation of means was done using the probability of difference option in SAS. Significance for all statistical tests was declared at P <0.05.

4.4 Results

4.4.1 Carcass characteristics, internal organs and meat quality

Diet had significant impacts (P <0.05) on final body weight (FBW), HCW and CCW (Table 4.1). The CON, MBMC and MBMA groups had significantly higher (P <0.05) FBW and CCW compared to MBMR and MBMT groups. Jumbo quail fed the CON and MBMC diets had higher (P <0.05) HCW than those fed the MBMR, MBMA and MBMT diets.

Table 4.1. Carcass characteristics (g /100 g CCW, unless stated otherwise) in Jumbo quail fed with diets containing raw or heat-treated Marama bean meal.

	[1]Diets						
[3]Parameters	CON	MBMR	MBMC	MBMA	MBMT	[2]SEM	*P* value
FBW	215.1[a]	174.8[b]	203.2[a]	202.3[a]	183.7[b]	4.271	<0.001
HCW	145.8[a]	121.2[b]	137.2[a]	135.7[b]	127.4[b]	3.771	0.001
CCW	144.3[a]	117.6[b]	136.4[a]	134.6[a]	125.5[b]	3.798	0.003
Dressing (%)	67.14	67.57	67.12	67.00	68.49	1.780	0.980
Breast	19.77	18.49	18.14	18.92	17.36	1.064	0.620
Drum (cm)	5.730	5.473	5.626	5.712	5.457	0.097	0.182
Drum	4.637	4.598	4.586	4.603	4.798	0.116	0.728
Thigh (cm)	4.531	4.395	4.520	4.701	4.863	0.156	0.310
Thigh	6.634	6.538	6.711	6.749	7.590	0.334	0.249
Wing (cm)	11.33	10.59	11.22	11.54	10.22	0..523	0.407
Wing	4.644	4.969	4.752	4.812	5.652	0.258	0.098
Back (cm)	10.26	9.139	9.676	9.628	10.39	0.365	0.144

[a,b] Means, in a row, with similar superscripts are not statistically different ($P > 0.05$)
[1]Diets: CON = a standard grower diet without Marama bean meal; MBMR = a standard grower diet containing 99.98 g/kg of raw Marama bean meal; MBMC = a standard grower diet containing 99.98 g/kg of cooked Marama bean meal; MBMA = a standard grower diet containing 99.98 g/kg of autoclaved Marama bean meal; MBMT = a standard grower diet containing 99.98 g/kg of toasted Marama bean meal.
[2]SEM: standard error of the mean.
[3]Parameters: FBW = final body weight; HCW = hot carcass weight; CCW = cold carcass weight.

The different organ weights measured were not influenced (P >0.05) by the dietary treatments except gizzard, proventriculus and spleen (Table 4.2). Diet MBMR promoted the heaviest (P <0.05) relative gizzard weight (2.808 g /100 g CCW) and diet CON promoted the lightest relative gizzard weight (2.179 g /100 g CCW). Jumbo quail fed diets MBMT and MBMR had higher relative spleen weights than those fed diet the CON, MBMA and MBMC treatments, which were statistically similar to other diets (P >0.05). Birds fed diet MBMT had the heaviest (P <0.05) relative proventriculus weight followed by MBMR and MBMC treatment groups and the lightest relative proventriculus weights were from the CON and MBMA treatment groups.

Table 4.2. Effect of diets containing raw or heat-treated Marama bean meal on internal organ sizes (g /100 g CCW, unless stated otherwise) in Jumbo quail.

	[1]Diets						
[3]Parameters	CON	MBMR	MBMC	MBMA	MBMT	[2]SEM	*P* value
Liver	2.591	2.633	2.606	2.697	2.500	0.124	0.872
Gizzard	2.179^{e}	2.808^{a}	2.506^{c}	2.453^{d}	2.654^{b}	0.074	<0.001
Spleen	0.094^{b}	0.115^{a}	0.097^{b}	0.106^{b}	0.134^{a}	0.007	0.005
Proventriculus	0.541^{c}	0.624^{b}	0.590^{b}	0.563^{c}	0.639^{a}	0.021	0.009
Pancreas	0.164	0.288	0.289	0.301	0.335	0.040	0.056
SI (cm)	50.63	57.41	55.12	57.57	45.93	4.479	0.360
SI	2.561	3.262	2.951	2.907	3.964	0.387	0.178
LI (cm)	9.620	10.36	9.709	10.19	8.736	0.713	0.582
LI	0.744	1.200	0.823	0.897	3.869	1.083	0.279

[a,b,c,d,e] Means, in a row, with similar superscripts are not statistically different (P > 0.05)
[1]Diets: CON = a standard grower diet without Marama bean meal; MBMR = a standard grower diet containing 99.98 g/kg of raw Marama bean meal; MBMC = a standard grower diet containing 99.98 g/kg of cooked Marama bean meal; MBMA = a standard grower diet containing 99.98 g/kg of autoclaved Marama bean meal; MBMT = a standard grower diet containing 99.98 g/kg of toasted Marama bean meal.
[2]SEM: standard error of the mean.
[3]Parameters: SI = small intestine, LI = large intestine.

There were significant dietary treatment effects on $a*_1$, $a*_{24}$, chroma$_{24}$, hue angle$_1$, and hue angle$_{24}$ (Table 4.3). The CON diet promoted higher 1-h redness (8.427) when compared to all the other dietary treatments, which did not differ (P >0.05). Breast meat from the CON, MBMC and MBMA groups had higher (P <0.05) 24-h redness than meat from the MBMR and MBMT groups. Breast meat from birds fed with diets CON and MBMC had higher (P <0.05) 24-h chroma values than meat from the MBMR, MBMA and MBMT groups, which did not differ (P >0.05). The MBMR and MBMT diets resulted in higher (P <0.05) 1-h and 24-h hue angle values when compared to the CON, MBMC and MBMA diets, which did not differ statistically (P >0.05).

Table 4.3. Effect of diets containing raw or heat-treated Marama bean meal on meat quality parameters of Jumbo quail.

[3]Parameters	[1]Diets CON	MBMR	MBMC	MBMA	MBMT	[2]SEM	*P* value
$Temp_1$	27.31	27.0	27.78	27.88	27.75	0.422	0.537
$Temp_{24}$	15.02	16.37	13.94	19.72	14.41	2.585	0.528
pH_1	5.991	6.089	5.983	6.037	6.027	0.086	0.911
pH_{24}	5.829	5.860	5.876	5.810	5.832	0.035	0.695
L^*_1	46.03	47.65	46.81	47.95	47.73	0.503	0.061
L^*_{24}	46.11	48.22	47.81	47.23	48.32	0.657	0.137
a^*_1	8.427^a	7.156^b	8.195^b	7.997^b	7.745^b	0.300	0.052
a^*_{24}	9.950^a	8.643^b	9.911^a	9.580^a	9.074^b	0.228	0.001
b^*_1	0.953	1.474	1.325	1.276	1.530	0.143	0.063
b^*_{24}	2.236	3.153	2.762	2.799	3.268	0.264	0.077
$Chroma_1$	8.485	7.329	8.311	8.105	7.902	0.290	0.074
$Chroma_{24}$	10.21^a	9.22^b	10.32^a	10.00^b	9.656^b	0.243	0.021
Hue $angle_1$	0.114^b	0.205^a	0.160^b	0.160^b	0.200^a	0.021	0.030
Hue $angle_{24}$	0.220^b	0.350^a	0.271^b	0.284^b	0.344^a	0.025	0.005
Drip loss (%)	16.30	15.73	18.64	16.89	19.76	1.485	0.332
CL (%)	25.10	26.67	24.31	26.07	35.66	3.756	0.279
WHC (%)	86.18	91.81	87.15	83.19	83.19	2.497	0.129
Shear force (N)	5.168	5.111	3.573	5.091	4.037	0.639	0.277

[a,b] Means, in a row, with similar superscripts are not statistically different ($P > 0.05$)
[1]Diets: CON = a standard grower diet without Marama bean meal; MBMR = a standard grower diet containing 99.98 g/kg of raw Marama bean meal; MBMC = a standard grower diet containing 99.98 g/kg of cooked Marama bean meal; MBMA = a standard grower diet containing 99.98 g/kg of autoclaved Marama bean meal; MBMT = a standard grower diet containing 99.98 g/kg of toasted Marama bean meal.
[2]SEM: standard error of the mean.
[3]Parameters: Temp = temperature; L^* = lightness; a^* = redness; b^* = yellowness; CL = cooking loss; WHC = water holding capacity.

4.4.2 Breast meat shelf-life measurement

The results on the effects of dietary treatment on breast meat shelf-life of Jumbo quail birds are presented. In Figure 4.1, the meat pH values (6.300 and 7.565) were higher (P <0.05) in birds fed diet MBMA on days 1 and 2 of storage respectively than those fed diets CON, MBMR, MBMC and MBMT which did not differ (*P* >0.05). The meat pH value (7.838) were higher (P <0.05) in birds fed diet MBMR on day 3 of storage than all other dietary treatments, which did not differ (*P* >0.05). On the other hand, Figure 4.2 shows there were no significant dietary effects (*P* <0.05) on the breast meat temperature of Jumbo quail birds. Figure 4.3 shows the effect of dietary treatment on breast meat redness (*a**) of Jumbo quail birds. Breast meat sample of Jumbo quail reared on diet CON promoted lesser lightness (45.9) on day 1 of storage compared to those reared on MBMR, MBMC, MBMA and MBMT diets, which did not differ (*P* <0.05). Likewise, dietary treatment had no significant effects (*P* <0.05) on breast meat redness (*a**) of Jumbo quail birds (Figure 4.4).

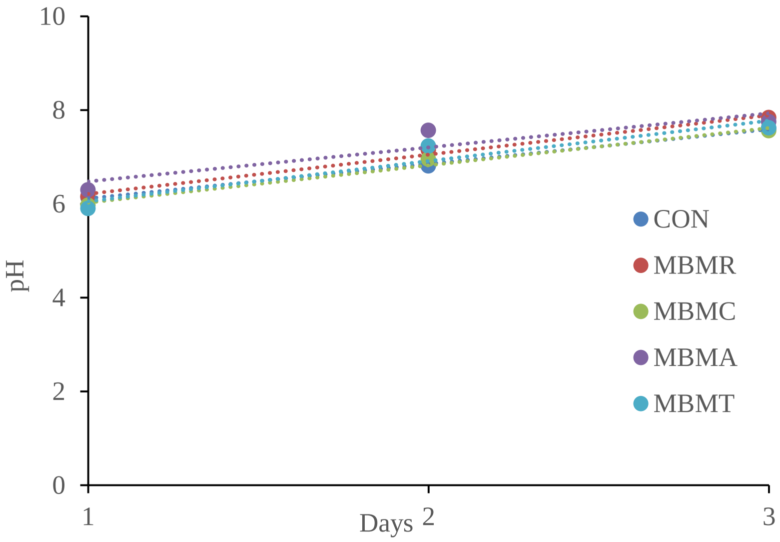

Figure 4.1. The effect of diets containing raw or heat-treated Marama bean meal on the stability of breast meat pH stored at room temperature for 3 days [CON = a standard grower diet without Marama bean meal; MBMR = a standard grower diet containing 99.98 g/kg of raw Marama bean meal; MBMC = a standard grower diet containing 99.98 g/kg of cooked Marama bean meal; MBMA = a standard grower diet containing 99.98 g/kg of autoclaved Marama bean meal; MBMT = a standard grower diet containing 99.98 g/kg of toasted Marama bean meal].

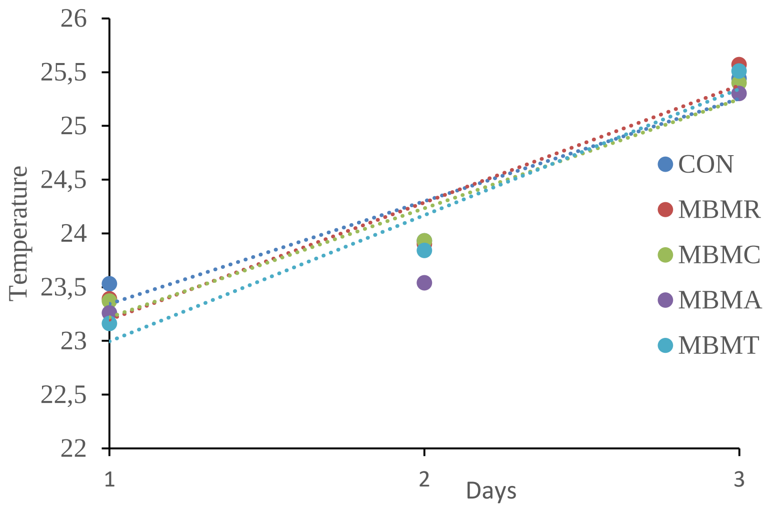

Figure 4.2. The effect of diets containing raw or heat-treated Marama bean meal on the stability of breast meat temperature stored at room temperature for 3 days [CON = a standard grower diet without Marama bean meal; MBMR = a standard grower diet containing 99.98 g/kg of raw Marama bean meal; MBMC = a standard grower diet containing 99.98 g/kg of cooked Marama bean meal; MBMA = a standard grower diet containing 99.98 g/kg of autoclaved Marama bean meal; MBMT = a standard grower diet containing 99.98 g/kg of toasted Marama bean meal].

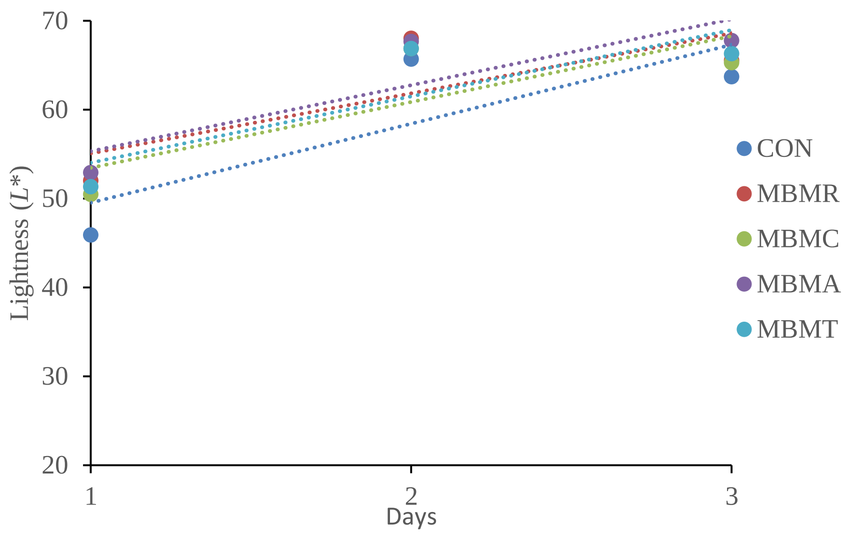

Figure 4.3. The effect of diets containing raw or heat-treated Marama bean meal on the stability of breast meat lightness stored at room temperature for 3 days [CON = a standard grower diet without Marama bean meal; MBMR = a standard grower diet containing 99.98 g/kg of raw Marama bean meal; MBMC = a standard grower diet containing 99.98 g/kg of cooked Marama bean meal; MBMA = a standard grower diet containing 99.98 g/kg of autoclaved Marama bean meal; MBMT = a standard grower diet containing 99.98 g/kg of toasted Marama bean meal].

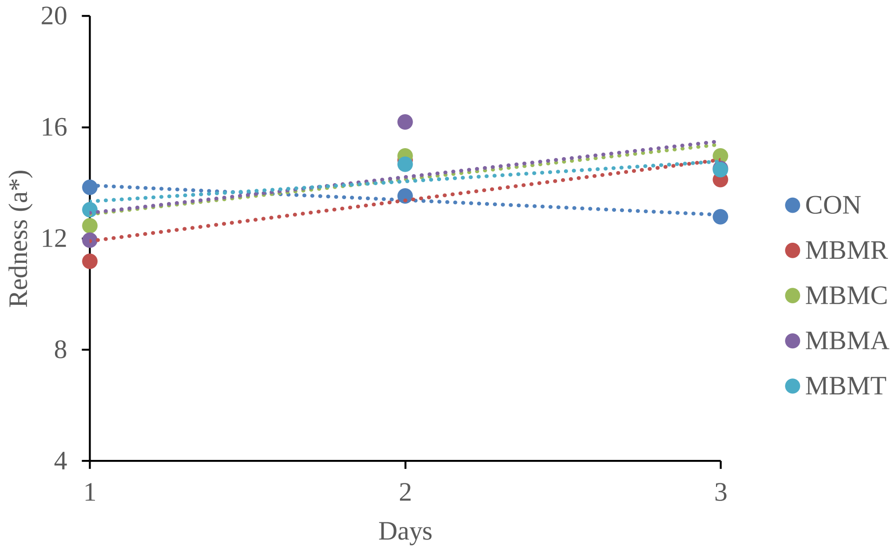

Figure 4.4. The effect of diets containing raw or heat-treated marama bean meal on the stability of breast meat redness stored at room temperature for 3 days [CON = a standard grower diet without Marama bean meal; MBMR = a standard grower diet containing 99.98 g/kg of raw Marama bean meal; MBMC = a standard grower diet containing 99.98 g/kg of cooked Marama bean meal; MBMA = a standard grower diet containing 99.98 g/kg of autoclaved Marama bean meal; MBMT = a standard grower diet containing 99.98 g/kg of toasted Marama bean meal].

Figure 4.5 shows the effect of dietary treatment on breast meat yellowness (*b**) of Jumbo quail birds. Diets MBMR, MBMA and MBMT promoted more ($P < 0.05$) yellowness on day 1 of storage than other dietary treatments, which did not differ ($P > 0.05$). However, on day 3 of storage, diet MBMA promoted ($P < 0.05$) more yellowness (8.25) than all other diets, which did not differ ($P > 0.05$).

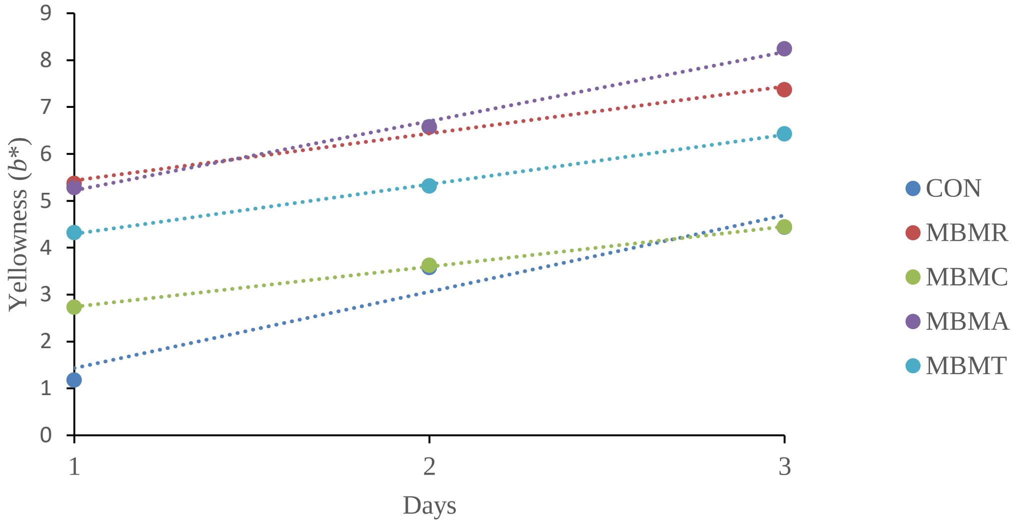

Figure 4.5. The effect of diets containing raw or heat-treated marama bean meal on the stability of breast meat yellowness stored at room temperature for 3 days [CON = a standard grower diet without Marama bean meal; MBMR = a standard grower diet containing 99.98 g/kg of raw Marama bean meal; MBMC = a standard grower diet containing 99.98 g/kg of cooked Marama bean meal; MBMA = a standard grower diet containing 99.98 g/kg of autoclaved Marama bean meal; MBMT = a standard grower diet containing 99.98 g/kg of toasted Marama bean meal].

4.5 Discussion

4.5.1 Carcass characteristics and internal organs

Carcass characteristics including live weight and dressed weight are spontaneous sensory indicators that influence the choice of consumers (Wu *et al*., 2021; Yuan *et al*., 2022). In livestock production, carcass weight and yield are important traits used in the grading system and has direct effect on consumers' acceptability (Holland & Loveday, 2013; Bown *et al*., 2016).

In the current study, inclusion of MBMC and MBMA increased HCW and CCW of jumbo quail. This could be attributed to the increased overall FI and AA utilisation observed in Chapter 3. This is in accordance with by Ballitoc & Sun (2013) and Schiavon *et al*. (2018) who reported that an increase in feed consumption increase body weight, dressed percentage yield and weight of internal organs in broilers. Another possible explanation could be due to reduced ANF which was achieved by heat processing techniques. This agrees with the findings by Lilly *et al*. (2011) and Kopmels *et al*. (2020) that feeding diets containing high amino acids and low tannin enhanced performance, carcass properties, and meat yield in broiler production. Hence, this result indicates that cooking and autoclaving are effective heat treatment methods that can be used to ameliorate the negative effect of ANFs in MBM, thus quail birds can efficiently utilize diet ultimately improving their muscle tissue.

Generally, the gut of an animal determines its feed efficiency and overall health (Tejeda & Kim, 2021). In this study, dietary treatment had an impact on the size of internal organs of quail. The birds fed with diet MBMR had the heaviest gizzards while the lightest was from those on diet CON. The increase in gizzard weight could be due to higher fibre content of the diet as determined in Chapter 3. The gizzard is the organ that orchestrates digestion process in poultry, and it has an incredible ability to grind feed particles, which improves the gizzard musculature and can lead to rapid and conspicuous enlargement of the gizzard (Svihus, 2011; Abdollahi et al., 2019). Hence, a higher fibre intake means enhanced activity of the gizzard which increases the rate at which the muscles contract to cope with the extra grinding demand from feed (Kheravii et al., 2017). Likewise, quail birds reared in the MBMT group had the heaviest relative proventriculus weights and the lightest were from the CON and MBMA groups. The proventriculus, located anterior to the gizzard, is a distinctive part of the digestive tract responsible for secreting digestive enzymes such as pepsinogen and hydrochloric acid. Most of the hydrolysis of ingested feed occurs in the gizzard due to the proventriculus's short retention time and small capacity. The roles of these two organs in digestion and nutrient absorption are crucial and are greatly influenced by diet (Svihus, 2014). Moreover, the proventriculus/gizzard area depends on structural components to develop (Svihus, 2011). Since the structures of the digestive tract in birds vary as a result of several factors which include diet, health, physiological status, body size, species, sex and age among others, the type and amount of feed ingested is one of the most important factors that mainly concerns changes in weight, length and width of the different sections of the digestive tract (Kokoszynski *et al.*, 2017). Therefore, an increase in gizzard and proventriculus weight observed in this research could be attributed to its anatomical response to higher fibre component of the dietary treatments (MBMR and MBMT) as a coping mechanism to degrade the fibre. This is in line with findings from De Verdel *et al*. (2012) who highlighted that the gizzard functions to aid digestion by causing a reduction of particle size and chemical degradation of nutrients. This means that heat-treated MBM did not compromise digestive function of the proventriculus and gizzard in Jumbo quail birds and it is supported by previous findings that the gizzard mass and musculature are mostly influenced by the nature of the structural components of diet such as coarse fibres which stimulate gizzard development. Thus, a high feed intake of diets that contains fibre with coarse particles leads to enlarged gizzard (Svihus, 2011, Singh *et al*., 2014; Abdollahi *et al*., 2019; Tejeda & Kim, 2021).

In poultry birds, the spleen is the largest secondary immune organ, and it helps to regulate the immune system (Xu *et al*., 2018). The immune status of the spleen can be impaired by restraint stress or heat stress, and this could result in changes in the immune cells of the spleen consequently reducing its relative weight (Xu *et al*., 2018; Jiang *et al*., 2020). Likewise, previous reports showed that higher activities of serum enzymes affect weight of internal organs such as the spleen, which might indicate immune system response (Barszcz *et al*., 2018; Yang *et al*., 2019). In this study, higher relative spleen weights were recorded in the birds fed diets MBMT and MBMR which could have prevented proper protein utilization and increased serum lipase levels compromising the immune status of quail. This could be attributed to the presence of TI and CT. Lighter relative spleen weights in quail fed MBMC and MBMA diets means cooking and autoclaving did not induce any negative effects nor changed the immune status of the birds. Likewise, the increased serum lipase explains the heavier pancreas weights in birds fed diet MBMR although not statistically significant; it could have been an anatomical response to secrete more lipase. This is in line with findings by Erdaw and Beyene (2018) that high levels of raw soybean meal in poultry diets, increases the volume of pancreas and other internal organs, including the duodenum, gizzard, and proventriculus; which is a coping mechanism to synthesize and secrete sufficient digestive enzymes (Erdaw *et al.,* 2017). This corroborates the report of Rocha *et al*. (2014) that pancreatic hypertrophy and hyperplasia were observed in broilers fed diets containing raw soybean due to the presence of TI. Thus, inclusion of heat-treated MBM (cooked and autoclaved) to quail diet did not have any negative effect nor compromised the overall health of the Jumbo quail birds.

4.5.2 Meat quality

Globally, consumers' attitude towards meat consumption is changing rapidly due to increased awareness on meat superiority in terms of healthiness and flavour (Wideman *et al*., 2016; Purslow, 2022). Meat quality is a complex concept and most direct indicator of poultry meat consumption that encompasses a variety of aspects including sensory attributes (colour, texture, juiciness, and flavour), functional properties (shear force, water holding capacity, cooking loss, and shelf life), and microbiological safety (Berri *et al*.,2019; Matabane, 2023). It is usually influenced by factors such as diet, breed, and age of animal as well as slaughter procedure (Mir, *et al*., 2017; Yuan *et al*., 2022). Meat freshness is paramount to its acceptance by end-consumers (Bekhit *et al*., 2021).

In spite of the high nutritive value of MBM, inclusion of either raw or processed MBM did not affect meat quality (lightness and yellowness) in Jumbo quail. However, inclusion of heat-treated MBM had a significant effect on 1-hour and 24-hour redness, 24-hour chroma, 1-hour and 24-hour hue angle. The CON group had highest 1-h redness, comparable to others and 24-h redness increased in quail birds fed with CON, MBMC and MBMA than meat from MBMR and MBMT. The effect of dietary treatment observed on colour of quail' breast meat could be due to myoglobin content of the meat which has a direct effect on meat color (Mir *et al*., 2017). According to Bhekit *et al*. (2017), myoglobin is the primary pigment associated with meat; likewise, myoglobin content of the meat has a direct effect on meat colour (Mir *et al*., 2017).Colour is the most important quality attribute of poultry meat because consumers associate it with the product's wholesomeness, and the decision to purchase the meat product is usually grounded on their opinion of its attractiveness. Lipid soluble pigments like carotenoids in the feedstuff and antioxidants determine concentration of pigment. Although MB is rich in carotenoids, and exhibits high antioxidant activity (Kuvare *et al*., 2015; Popoola *et al*., 2023), the colour of meat ultimately depends on the amount and state of pigments in meat, namely, myoglobin, haemoglobin and cytochromes. Thus, the increase in meat redness observed in this study could be attributed to increased oxygenation of the myoglobin.

This is supported by report from Ribeiro *et al*. (2021) that high pH is an implication of decreased oxygenation of myoglobin leading to dark meat while low pH stimulates the oxidation of myoglobin and oxyhaemoglobin. However, the pH (5.82 – 6.08) values observed from this study were within the normal range for fresh quail meat (Mulaudzi *et al*., 2022). The dietary treatments had no effect on meat pH, drip loss, water holding capacity, shear force, and cooking loss which is similar to previous findings by Mbhele *et al*. (2019) and Marareni & Mnisi (2020) who made similar observations when Jumbo quail were fed insect meals. This means feeding raw or heat-treated MBM does not compromise meat quality in Jumbo quail.

4.5.3 *Meat shelf-life*

Shelf life can be defined as a time period in which the food under pre-defined storage conditions follows safety, nutritional, regulatory, and sensorial standards previously established by producers, governments, public and private agencies, and consumers (Corradini, 2018). Marama beans has a high profile of bioactive compounds such as polyphenols, alkaloids, saponins, carotenoids, omega-3 fatty acids, flavonoids, oleic, stearic and palmitic acids (Popoola *et al*., 2023). This could improve meat stability by preserving its quality and extending shelf life (Awad et al., 2022)). The results of the study indicated that there was significant effect of dietary treatments on pH and colour of breast meat samples during the 3-day storage period, which are indicators of shelf life. The higher pH values from quail meat fed with MBMA on days 1 and 2 of storage could be due to putrefaction, which increases with the length of storage time (Lee & Shin, 2019). During shelf life, a set of chemicals, physical, and biological reactions can occur in food products decreasing their safety and quality until the product is considered unacceptable. This phenomenon is known as food spoilage and is caused mainly by microorganisms, enzymes, and physical and chemical degradation, which are critically associated with pH and temperature (Abdel-Aziz *et al*., 2016; Soro *et al*., 2021). Meats have physico-chemical characteristics that promote microbial activity, these includes pH, protein concentration and fats (Comi, 2017). Meat quality is generally reflected by traits like meat colour and pH which is the determinant for acid accumulation in meats; hence, variation in muscle pH can alter meat colour (Pieterse et al., 2014; Bahadori et al., 2017; Yuan et al., 2022). However, on day 3, higher pH value was observed on the MBMR group, which could indicate low glycogen levels in the quail before slaughter. The meat pH increased over the3-day storage period in all dietary treatments which could be associated with meat deterioration. Indeed, changes in pH are associated with meat spoilage, which comes as a result of microbial deterioration, lipid oxidation, and autolytic enzymatic spoilage (Alessandroni *et al*., 2022), meaning the increased pH could indicate microbial proliferation. This affected lightness an indicator of meat freshness on day 1 of storage only. Even though, the *L** value for the control diet was lower than recorded value for all other dietary treatments, there was no significant effect of dietary treatments on lightness for the remaining days of storage. Storage temperature, pH, water availability, presence of spoilage microorganisms influences the rate of meat spoilage which is characterized by changes in colour, texture, and flavour (Dirpan & Hidayat, 2023).

Yellowness another indicator of meat freshness was influenced by the dietary treatments; whereby MBMR, MBMA and MBMT increased *b** on day 1. However, on day 3 of storage, increased *b** value was recorded in diet MBMA than other dietary treatments. This can be explained by the concentration of pigments in MBM. Marama bean is rich in carotenoids and exhibits high antioxidant activity (Kuvare *et al*., 2015; Popoola *et al*., 2023). Hence, the effect of dietary treatment observed on yellowness of quail meat could be due to high level of carotenoids.

Meat is a perishable product and during storage, the actions of microorganisms and endogenous enzymes (proteases and lipases) result in chemical compositional changes (Bekhit *et al*., 2021). This causes deterioration in meat structures and promotes oxidation. Lipid and protein oxidation are the major cause of deterioration of meat quality and shelf life leading to negative changes in nutritive value, sensory and physicochemical properties of meat (Horbanczuk *et al*., 2019). Several factors affect meat shelf-life, such as the presence of oxygen, storage temperature, endogenous enzymes, exposure to light, and microorganisms, leading to deterioration and reduction of meat shelf life (Stopforth, 2017). The fact that dietary treatments had no significant effect on meat redness indicates that antioxidant compounds in MBM preserved the meat during storage, by delaying oxidation. This is supported by Horbanczuk *et al*. (2019), who stated that plant materials are rich in bioactive compounds that have the potential to inhibit or delay oxidation thereby play antioxidant, antimicrobial and preservative role in meat products during processing and storage. These findings revealed that MBM contains bioactive compounds which may act as natural antioxidants in quail feeding. However, these antioxidants may not be enough to prevent spoilage beyond three days of storage.

4.6 Conclusion

The inclusion of raw MBM compromised the immune status, increased the serum lipase levels and altered internal organs of the Jumbo quail. However, cooked and autoclaved MBM enhanced carcass characteristics, sizes of internal organs and meat quality and promoted similar meat quality as the soybean-based control diet. Therefore, quail diets can be supplemented with cooked or autoclaved MBM without any negative effect on carcass characteristics, internal organ sizes and meat quality.

4.7 References

Abdel-Aziz, S.M., Asker, M.M., Keera, A.A. & Mahmoud, M.G., 2016. Microbial food spoilage: Control strategies for shelf-life extension. In: Microbes in food and health, Springer, Cham, Switzerland. Pp. 239-264.

Abdollahi, M.R., Zaefarian, F., Hunt, H., Anwar, M.N., Thomas, D.G. & Ravindran, V., 2019. Wheat particle size, insoluble fibre sources and whole wheat feeding influence gizzard musculature and nutrient utilisation to different extents in broiler chickens. J. Anim. Physiol. Anim. Nutr. 103(1), 146-161.

Alabi, F., Kiarie, E.G., Mnisi, C.M. & Mlambo, V., 2022. Physical treatment reduces trypsin inhibitor activity and modifies chemical composition of Marama bean (*Tylosema esculentum*). Molecules. 27(14), 4451.

Alessandroni, L., Caprioli, G., Faiella, F., Fiorini, D., Galli, R., Huang, X., Marinelli, G., Nzekoue, F., Ricciutelli, M., Scortichini, S. & Silvi, S., 2022. A shelf-life study for the evaluation of a new biopackaging to preserve the quality of organic chicken meat. Food Chem. 371, 131134.

Awad, A.M., Kumar, P., Ismail-Fitry, M.R., Jusoh, S., Ab Aziz, M.F. & Sazili, A.Q., 2022. Overview of plant extracts as natural preservatives in meat. J. Food Process. Preserv. 46(8), e16796.

Bahadori, Z., Esmaielzadeh, L., Karimi-Torshizi, M.A., Seidavi, A., Olivares, J., Rojas, S., Salem, A.Z.M., Khusro, A.L. & Opez, S., 2017. The effect of earthworm (*Eisenia foetida*) meal with vermihumus on growth performance, haematology, immunity, intestinal microbiota, carcass characteristics, and meat quality of broiler chickens. Livest. Sci. 202, 74-81.

Ballitoc, D.A. & Sun, S., 2013. Ground yellow mealworms (*Tenebrio molitor L.)* feed supplementation improves growth performance and carcass yield characteristics in broilers. Open Sci. Reposit. Agric. 18, e23050425.

Barszcz, M., Taciak, M., Tusnio, A. & Skomial, J., 2018. Effects of dietary level of tannic acid and protein on internal organ weights and biochemical blood parameters of rats. PLoS ONE. 13(1), 10.1371.

Bekhit, A.E.D.A., Holman, B.W., Giteru, S.G. & Hopkins, D.L., 2021. Total volatile basic nitrogen (TVB-N) and its role in meat spoilage: A review. Trends Food Sci. Technol. 109, 280-302.

Berri, C., Picard, B., Lebret, B., Andueza, D., Lefèvre, F., Le Bihan-Duval, E., Beauclercq, S., Chartrin, P., Vautier, A., Legrand, I. & Hocquette, J.F., 2019. Predicting the quality of meat: myth or reality? Foods. 8(10), 436.

Bown, M.D., Muir, P.D. & Thomson, B.C., 2016. Dairy and beef breed effects on beef yield, beef quality and profitability: a review. New Zealand J. Agric. Res. 59(2), 174-184.

Comi, G., 2017. Spoilage of meat and fish. In The microbiological quality of food. Food Sci.Technol. Nutr. Woodhead Publishing, Sawston, England. Pp.179-210

Corradini, M.G., 2018. Shelf life of food products: from open labelling to real-time measurements. Annu. Rev. Food. Sci. Technol. 9, 251-269.

Cullis, C., Lawlor, D. W., Chimwamurombe, P., Bbebe, N., Kunert, K. & Vorster, J., 2019. Development of Marama bean, an orphan legume, as a crop. Food Energy Secur. 8(3), e00164.

De Verdel, H., Mignon-Grasteau, S., Bastianelli, D., Meme, N., Le Bihan-Duval, E. & Narcy, A., 2013. Reducing the environmental impact of poultry breeding by genetic selection. J. Anim. Sci. 91, 613-622.

Dirpan, A. & Hidayat, S.H., 2023. Quality and shelf-life evaluation of fresh beef stored in smart packaging. Foods. 12(2), 396.

Erdaw, M.M. & Beyene, W.T., 2018. Anti-nutrients reduce poultry productivity: Influence of trypsin inhibitors on pancreas. Asian J. Poult. Sci. 12(1), 14-24.

Erdaw, M.M., Wu, S. & Iji, A.P., 2017. Growth and physiological responses of broiler chickens to diets containing raw, full-fat soybean and supplemented with a high-impact microbial protease. Asian Aust. J. Anim. Sci. 30, 1303-1313.

Grau, R. & Hamm, R. 1957. About the water-binding capacity of the mammalian muscle. II. Communication. Zeitschrif Lebensm Unters Briskly. 105, 446.

Holland, R. & Loveday, D., 2013. Understanding yield grades and quality grades for value-added beef producers and marketers. Institute of Agriculture, University of Tennessee, Knoxville, USA. Pp. 1-7.

Honikel, K.O., 1998. Reference methods for the assessment of physical characteristics of meat. Meat Sci. 49, 447-570.

Horbanczuk, O.K., Kurek, M.A., Atanasov, A.G., Brncic, M. & Brncic, S.R., 2019. The effect of natural antioxidants on quality and shelf life of beef and beef products. Food Technol. Biotechnol. 57(4), 439.

Jiang, W., Li, Y., Wei, W., Li, J., Li, L., Zhang, C., Zhang, S., Kong G. & Li, Z., 2020. Spleen contributes to restraint stress induced hepatocellular carcinoma progression. Int. Immunopharmacol. 83, 106420.

Kheravii, S. K., Swick, R. A., Choct, M. & Wu, S. B., 2017. Coarse particle inclusion and lignocellulose-rich fibre addition in feed benefit performance and health of broiler chickens. Poult. Sci. 96(9), 3272-3281.

Kokoszynski, D., Bernacki, Z., Saleh, M., Steczny, K. & Binkowska, M., 2017. Body conformation and internal organs characteristics of different commercial broiler lines. Braz. J. Poult. Sci. 19, 47-52.

Kopmels, F.C.; Smit, M.N.; Cho, M.; He, L. & Beltranena, E., 2020. Effect of feeding 3 zero tannin faba bean cultivars at 3 increasing inclusion levels on growth performance, carcass traits, and yield of saleable cuts of broiler chickens. Poult. Sci. 99, 4958–4968.

Kuvare, A., Kandawa-Schulz, M. & Kapewangolo, P.T., 2015. Comparative study of antioxidant properties, polyphenols and flavonoid contents of the tuber and seed extracts of Marama bean (*Tylosema esculentum*). Int. Sci. Technol. J. Namibia, 6, 21-31.

Lee, E.J. & Shin, H.S., 2019. Development of a freshness indicator for monitoring the quality of beef during storage. Food Sci. Biotechnol. 28, 1899-1906.

Lilly, R.A., Schilling, M.W., Silva, J.L., Martin, J.M. & Corzo, A., 2011. The effects of dietary amino acid density in broiler feed on carcass characteristics and meat quality. J. Appl. Poult. Res. 20(1), 56-67.

Mancini, R., 2013. Meat colour. The science of meat quality. John Wiley & Sons, New York, USA. Pp. 177-198.

Maphosa, Y. & Jideani, V. A., 2017. The role of legumes in human nutrition. Functional food-improve health through adequate food. InTech. Rijeka, Croatia. pp. 103-122.

Marareni, M. & Mnisi, C. M., 2020. Growth performance, serum biochemistry and meat quality traits of Jumbo quails fed with mopane worm (*Imbrasia belina*) meal-containing diets. Vet. Anim. Sci. 10, 100141.

Marareni, M., 2021. Inclusion of mopane worm meal in Jumbo quail diets: Nutrient utilization, physiological and meat quality responses. MSc dissertation, North West University, Mafikeng, South Africa.

Matabane, A.N., 2023. Feed utilisation, physiological and meat quality responses in Jumbo quail reared on apple pomace-containing diets, MSc dissertation, North West University, Mafikeng, South Africa.

Mbhele, F.G., Mnisi, C.M. & Mlambo, V., 2019. A nutritional evaluation of insect meal as a sustainable protein source for Jumbo quails: Physiological and meat quality responses. Sustainability. 11(23), 6592.

Mir, N.A., Rafiq, A., Kumar, F., Singh, V. & Shukla, V., 2017. Determinants of broiler chicken meat quality and factors affecting them: a review. J. Food Sci. Technol. 54, 2997-3009.

Mnisi, C.M., Marareni, M., Manyeula, F. & Madibana, M. J., 2021. A way forward for the South African quail sector as a potential contributor to food and nutrition security following the aftermath of COVID-19: a review. Agric. Food Secur. 10(1), 1-12.

Mnisi, C.M., Oyeagu, C.E. & Ruzvidzo, O., 2022. Mopane worm (*Gonimbrasia belina* Westwood) meal as a potential protein source for sustainable quail production: A review. Sustainability. 14(9), 5511.

Montso, P.K., Mnisi, C.M. & Ayangbenro, A.S., 2022. Caecal microbial communities, functional diversity, and metabolic pathways in Ross 308 broiler chickens fed with diets containing different levels of Marama (*Tylosema esculentum*) bean meal. Front. Microbiol. 13, 1009945.

Mulaudzi, A., 2019. Assessment of growth performance, haemo-biochemistry and meat quality of Japanese quails fed on *Moringa oleifera*. MSc dissertation, North-West University, Mafikeng, South Africa.

Mulaudzi, A., Mnisi, C.M. & Mlambo, V., 2022. Effect of pre-treating dietary *Moringa oleifera* leaf powder with fibrolytic enzymes on physiological and meat quality parameters in Jumbo quail. Poultry. 1(2), 54-65.

Omotayo, A.O. & Aremu, A.O., 2021. Marama bean (*Tylosema esculentum*) Burch. A. Schreib: an indigenous plant with potential for food, nutrition, and economic sustainability. Food Funct. 12(6), 2389-2403.

Pieterse, E., Pretorius, Q., Hoffman, L.C. & Drew, D.W., 2014. The carcass quality, meat quality and sensory characteristics of broilers raised on diets containing either *Musca domestica* larvae meal, fish meal or soya bean meal as the main protein source. Anim. Prod. Sci. 54, 622-628.

Popoola, J.O., Ojuederie, O.B., Aworunse, O.S., Adelekan, A., Oyelakin, A.S., Oyesola, O.L., Akinduti, P.A., Dahunsi, S.O., Adegboyega, T.T., Oranusi, S.U. & Ayilara, M.S., 2023. Nutritional, functional, and bioactive properties of African underutilized legumes. Front. Plant Sci. 14, 1105364.

Priolo, A., Micol, D., Agabriel, J., Prache, S. & Dransfield, E., 2002. Effect of grass or concentrate feeding systems on lamb carcass and meat quality. Meat Sci. 62, 179–185.

Purslow, P., 2022. Introduction to meat quality. New aspects of meat quality. Woodhead Publishing, Sawston, United Kingdom. Food Sci. Technol. Nutr. 1-12.

Ribeiro, C.C.D.S., Contreras-Castillo, C.J., Santos-Donado, P.R.D. & Venturini, A.C., 2021. New alternatives for improving and assessing the color of dark–cutting beef–a review. Sci. Agric. 79(1), 10.1590

Rocha, C., Durau, J.F., Barrilli, L.N.E., Dahlke, F. & Maiorka, P., 2014. The effect of raw and roasted soybeans on intestinal health, diet digestibility and pancreas weight of broilers. J. Applied Poult. Res. 23, 71-79.

Santhi, D. & Kalaikannan, A., 2017. Japanese quail (*Coturnix coturnix japonica*) meat: characteristics and value addition. Worlds Poult. Sci. J. 73(2), 337-344.

Schiavon, S., Dalla Bona, M., Carco, G., Carraro, L., Bunger, L. & Gallo, L., 2018. Effects of feed allowance and indispensable amino acid reduction on feed intake, growth performance and carcass characteristics of growing pigs. PloS ONE 13(4), e0195645.

Singh, Y., Amerah, A.M. & Ravindran, V., 2014. Whole grain feeding: Methodologies and effects on performance, digestive tract development and nutrient utilisation of poultry. Anim. Feed Sci. Technol. 190, 1-18.

Soetan, K., 2018. Antinutritional factors of five selected underutilized legumes. Food Sci. Qual. Manag. 76, 92-100.

Soro, A.B., Noore, S., Hannon, S., Whyte, P., Bolton, D.J., O'Donnell, C. & Tiwari, B.K., 2021. Current sustainable solutions for extending the shelf life of meat and marine products in the packaging process. Food Packag. Shelf Life. 29, 100722.

Stopforth, J.D., 2017. Preservation methods for meat and poultry. In: Microbial control and food reservation. Theory and Practice. Springer. New York, USA. Pp. 225-254

Svihus, B., 2011. The gizzard: function, influence of diet structure and effects on nutrient availability. World Poult. Sci. J. 67(2), 207-224.

Svihus, B., 2014. Function of the digestive system. J. Appl. Poult. Res. 23, 306-314.

Tejeda, O.J. & Kim, W.K., 2021. Effects of fibre type, particle size, and inclusion level on the growth performance, digestive organ growth, intestinal morphology, intestinal viscosity, and gene expression of broilers. Poult. Sci. 100(10), 101397.

Tejeda, O.J. & Kim, W.K., 2021. Role of dietary fibre in poultry nutrition. Animals. 11(2), 461.

Wideman, N., O'bryan, C.A. & Crandall, P.G., 2016. Factors affecting poultry meat colour and consumer preferences-A review. Worlds Poult. Sci. J. 72(2), 353-366.

Wu, J., Lin, Z., Chen, G., Luo, Q., Nie, Q., Zhang, X. & Luo, W., 2021. Characterization of chicken skin yellowness and exploration of genes involved in skin yellowness deposition in chicken. Front. Physiol. 12, 585089.

Xu, Y., Lai, X., Li, Z., Zhang, X. & Luo, Q., 2018. Effect of chronic heat stress on some physiological and immunological parameters in different breed of broilers. Poult. Sci. 97, 4073-4082.

Yang, L., Liu, G., Zhu, X., Luo, Y., Shang, Y. & Gu, X.L., 2019. The anti-inflammatory and antioxidant effects of leonurine hydrochloride after lipopolysaccharide challenge in broiler chicks. Poult. Sci. 98(4), 1648-1657.

Zhang, L., Wang, Y.J., Hu, M.H., Fan, Q.X., Cheung, S.G., Shin, P.K.S., Li, H. & Cao, L., 2009. Effects of the timing of initial feeding on growth and survival of spotted mandarin fish *Siniperca scherzeri* larvae. J. Fish Biol. 75, 1158-1172.

5 CHAPTER FIVE – GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

The rapid increase in the world's human population has an associated effect on food security and demand for animal protein to meet dietary requirements. This adds a lot of pressure on producers, especially poultry farmers since poultry products has the highest consumption per capita (SAPA, 2020). Quail production could serve as a complementary source of protein that can be used to diversify the pool of animal protein sources (Matabane, 2023). This is because it requires less start-up capital compared to other poultry birds and makes quick returns due to the short generation interval and high prolificacy of the quail birds. Moreso, quail's resistance to most poultry diseases helps to reduce cost of medications and vaccines and promotes organic production (Redoy *et al*., 2017; Matabane, 2022). Thus, quail meat and eggs could serve as additional sources of poultry products to address the prevailing situation of protein malnutrition, especially in the sub-Saharan regions like South Africa. However, quail farming could be restricted by the over-reliance on conventional feed ingredients (Mnisi *et al*., 2021). Thus, the use of locally available and naturally growing feed ingredients in place of conventional feed ingredients is critical to the quail industry to reduce feed-food competition, cut down on the use of expensive conventional feed ingredients and formulate sustainable diets. This is a crucial aspect of production in a quest to meet the demand for animal protein. According to Cullis *et al*. (2023), marama bean (MB, *Tylosema esculentum*) has been identified as a sustainable and environmentally friendly crop that can be incorporated as alternative ingredients in feed formulations; though it is still in the process of domestication in South Africa, which is aimed at increasing its productivity. Reports from previous scholars revealed that MB has nutraceutical values and a high nutritional profile containing rich protein similar to soybean and a higher oil content (Cullis *et al*., 2019;

Omotayo & Aremu, 2021; Alabi *et al*., 2022). However, the use of MB in Jumbo quail diets is limited by the presence of anti-nutritional factors (ANFs) including condensed tannins and trypsin inhibitors (Alabi *et al*., 2022). Therefore, this study employed thermal processing techniques (cooking, autoclaving, and toasting) to ameliorate the negative effects of ANFs in Marama bean meal (MBM) to ensure safe utilisation in Jumbo quail diets.

In Chapter 3, the objective was set out to evaluate the effect of including raw or heat-treated MBM in a diet on apparent amino acid retention, growth performance and haemato-biochemical parameters of Jumbo quail. It was hypothesised that heat treatment of MBM would improve amino acid utilisation, growth performance, and blood parameters of the birds. The diets were formulated as follows: a standard grower diet without MBM (CON); = a standard grower diet with 99.98 g/kg of raw (MBMR), cooked (MBMC), autoclaved (MBMA), and toasted MBM (MBMT), which were fed to Jumbo quail birds. Apparent retention (AR) of amino acid (AA), feed intake, body weight gain, haematology and serum biochemistry were determined.

The results showed that diets had varied effect on AR of amino acids. Inclusion of cooking treatment improved AR of threonine, lysine, methionine, isoleucine and leucine which indicates cooking increased solubilization of crude protein and fibre resulting in an improvement in AA digestion and absorption in quail birds. Autoclaving treatment enhanced AR of valine, arginine and phenylalanine, which shows the importance of this method in increasing bioavailability of these indispensable AAs required by quail for protein synthesis, molecular signalling, neurotransmission, and regulation of metabolic activities (He *et al*., 2021). The toasting treatment improved AR of histidine only, a limiting factor for carnosine synthesis, with a strong antioxidant effect (Moro *et al*., 2020). The observed poor AR of AA in the MBMR group was expected due to the presence of ANFs (trypsin inhibitors and tannins) in MB (Alabi *et al*., 2022) that have been reported to impair AA utilization and reduce growth performance (Gilani *et al*., 2012; Jha & Mishra, 2021). This demonstrates that raw MBM compromised amino acid utilization in Jumbo quail.

Inclusion of heat-treated MBM (cooking and autoclaving) boosted gain-to-feed ratio (G:F) while raw MBM reduced weight gain and G:F, which is line with the report by Nalluri & Karri (2021) that inclusion of raw chickpeas in broiler feed had adverse effect on the body weight gain and feed conversion ratio. This could be due to poor feed intake and high fibre content of the diets since fibre has been reported to reduce growth performance in birds (Jha & Mishra, 2021). Moreover, the poor feed intake in MBMT group could be attributed to the bitter taste of toasted MB due to increased level of phenolics (Nyembwe *et al*., 2015).

Blood parameters indicate the health status of the birds; hence, they are determined to ensure the well-being of birds (Bello *et al*., 2019). Dietary treatments did not affect white blood cell count, monocytes, and platelets, but influenced haematocrits and lipase. Birds fed diets containing heat-treated MBM had lower haematocrits compared to CON which could be induced by tannins. According to Gupta (2014) and Shubham *et al*. (2020), high levels of phytates and tannins cause poor absorption of iron which results in poor red blood cell synthesis. Thus, low haematocrits can be attributed to the ANFs in MB, which could have interfered with absorption of iron.

Lipase levels in birds fed raw MBM were higher than all other dietary treatments which were anticipated given the high ANFs and fibre in MB. This suggests that inclusion of raw MBM in Jumbo quail diets could have increased secretion of lipase in the pancreas (Zhu *et al*., 2021). Thus, the pancreas devices a coping mechanism against the negative effect of ANFs in MB (Erdaw *et al*., 2017), leading to the observed heavier pancreas weights in birds fed MBMR. Similarly, Rocha *et al*. (2014) reported pancreatic hypertrophy and hyperplasia in broilers fed diets containing raw soybean due to the presence of TI. Relative to the MBMR, lower levels of lipase observed in quail fed MBMA and MBMT indicates that autoclaving and toasting inactivated the TIs, but the lowest lipase levels were from the MBMC group, which implies that cooking successfully ameliorated the negative effect of TI and presented the best response than all other diets. Since most blood parameters fell within the normal range reported for healthy quail (Mnisi & Mlambo 2018), it means that MBM can be included in quail diets without compromising the health status of the birds, but pre-processing is recommended for better performance. The hypothesis tested that heat treatment of MBM would improve the apparent retention of amino acid, growth performance and blood parameters in Jumbo quail was accepted for cooking and autoclaving treatments but not for toasting treatment.

In Chapter 4, the objective was to determine the effect of including raw or heat-treated MBM in a diet on carcass characteristics, internal organs and breast meat quality of Jumbo quail. Relative to the toasted and raw MBM, the inclusion of cooked and autoclaved dietary MBM enhanced carcass weights of the Jumbo quail which was comparable to the soybean-based control diet. This can be associated to the improved performance in these treatment groups, which was similar to the findings of Lilly *et al*. (2011) and Kopmels *et al*. (2020). These authors reported that diets containing high AA and low anti-nutrients fed to broilers resulted in enhanced meat yield and quality.

Furthermore, the proventriculus, gizzard and spleen were negatively affected by dietary treatments; which was due to high fibre content of diets containing raw and toasted MBM, which significantly resulted in poor digestibility of protein. Similarly, Alabi (2023) recorded heavier visceral organs in birds fed diets containing raw MB and attributed this to poor nutrient digestibility. However, all the other internal organs were not influenced by the dietary treatments. These findings show that raw and toasted MBM compromised the internal organ sizes of Jumbo quail.

The results on breast meat quality measurements indicated a significant dietary effect on redness (a^*_1 and a^*_{24}), chroma$_{24}$, and hue angle ($_1$ and $_{24}$) but had no effect on drip loss, cooking loss, WHC and shear force of meat. The observed poor meat colour attribute (redness) in diets containing raw and toasted MBM could be attributed to the presence of trypsin inhibitors and phytates, which have been reported to reduce protein digestibility and amino acid availability thus impairing meat quality traits in birds (Amonsou *et al*., 2011; Alabi, 2023). Additionally, the significant effect on some meat quality attributes during storage indicates that cooked and autoclaved MBM have potential to promote normal oxidative stability for quail meat. However, shelf life lasted 3 days because meat samples started to rot indicating that MB antioxidants were not effective in delaying oxidation of lipids in quail meat. Thus, the hypothesis that heat treatment of MBM would enhance internal organs, carcass features, and meat quality and stability parameters in Jumbo quail was not entirely accepted due to its ineffectiveness on meat stability.

5.2 Conclusion

Jumbo quail fed diets containing cooked and autoclaved MBM showed significantly improved performance in terms of growth, carcass yield and meat quality than those fed toasted MBM. However, the inclusion of raw MBM suppressed performance, and had a negative effect on some blood parameters, and internal organs of the Jumbo quail. The poor performance of birds fed toasted MBM could be associated to low feed intake. This study also showed that the treatment of MBM did not improve the meat shelf life and its use in feeding birds could not maintain oxidative stability in the quail meat. It can be concluded that cooking and autoclaving are the ideal heat treatments that could boost the utility of MB in Jumbo quail diets.

5.3 Recommendation for future studies

The use of alternative unconventional plant-based protein sources (like Marama beans, peas, faba bean, lupin, Mucuna, chickpea) in poultry production is crucial to meet the world's growing population and need for animal protein. Most of these plant-based sources including MB contain ANFs, which inhibit their use in poultry diets. As a result, heat treatments are considered one of the most effective methods to ameliorate the negative effect of these ANFs, thereby improve the availability of nutrients in these alternatives when fed to birds. However, the extent to which heat treatment inactivated the negative effect of ANFs in MB was not determined for this particular study. Thus, based on the findings and limitations of this study, the following future studies are recommended to find out the feed value of MB:

- To evaluate the effect of heat treatment on saponins and tannins in MBM for Jumbo quail.
- To determine the effect of heat treatments on mineral content and fatty acids profile of MBM for Jumbo quail.
- To assess the effect of heat-treated MBM containing diets on amino acids profile, fatty acids profile and organoleptic properties of the meat in Jumbo quail.
- To determine the potential effect of dietary inclusion of heat-treated MBM on production performance and egg quality indicators in layer Jumbo quail birds.
- To determine a cost-benefit analysis of including Marama bean meal in Jumbo quail diets.

5.4 References

Alabi, F., Kiarie, E.G., Mnisi, C.M. & Mlambo, V., 2022. Physical Treatment reduces trypsin inhibitor activity and modifies chemical composition of Marama bean (*Tylosema esculentum*). Molecules. 27(1), 4451.

Alabi, F.A., 2023. Evaluation of Marama bean as an alternative to soybeans in broiler nutrition. Doctoral dissertation, North-West University, Mafikeng, South Africa.

Amonsou, E., Taylor, J. & Minnaar, A., 2011. Microstructure of protein bodies in Marama bean Species. LWT - Food Sci. Technol. 44, 42-47.

Bello, K.O., Ogunsola, O.E., Yusuf, A.O., Adeleye, O.O., Oladeji, T.E. & Famakinde, S.A., 2019. Health status of indigenous and exotic chickens sold in live bird markets. Comp. Clin. Path. 28, 633-641.

Cullis, C., Lawlor, D. W., Chimwamurombe, P., Bbebe, N., Kunert, K. & Vorster, J., 2019. Development of Marama bean, an orphan legume, as a crop. Food Energy Secur. 8(3), e00164.

Cullis, C., Chimwamurombe, P., Kunert, K. & Vorster, J., 2023. Perspective on the present state and future usefulness of Marama bean (*Tylosema esculentum*). Food Energy Secur. 12(2), e422.

Erdaw, M. M., Wu, S. & Iji, A.P., 2017. Growth and physiological responses of broiler chickens to diets containing raw, full-fat soybean and supplemented with a high-impact microbial protease. Asian-Australas. J. Anim. Sci. 30, 1303-1313.

Gilani, G.S., Xiao, C.W. & Cockell, K.A., 2012. Impact of antinutritional factors in food proteins on the digestibility of protein and the bioavailability of amino acids and on protein quality. Br. J. Nutr. 108(2), 315–332

Gupta, C.P., 2014. Role of iron (Fe) in body. J. Appl. Chem. 7(11), 38-46.

He, W., Li, P. & Wu, G., 2021. Amino acid nutrition and metabolism in chickens. Amino acids in nutrition and health: Advances in experimental medicine and biology. Vol. 1285, Springer Cham, New York, USA. Pp.109-131.

Jha, R. & Mishra, P., 2021. Dietary fibre in poultry nutrition and their effects on nutrient utilization, performance, gut health, and on the environment: a review. J. Anim. Sci. Biotechnol. 12, 1-16.

Kopmels, F.C., Smit, M.N., Cho, M., He, L. & Beltranena, E., 2020. Effect of feeding 3 zero tannin faba bean cultivars at 3 increasing inclusion levels on growth performance, carcass traits, and yield of saleable cuts of broiler chickens. Poult. Sci. 99, 4958-4968.

Lilly, R.A., Schilling, M.W., Silva, J.L., Martin, J.M. & Corzo, A., 2011. The effects of dietary amino acid density in broiler feed on carcass characteristics and meat quality. J. Appl. Poult. Res. 20(1), 56-67.

Matabane, A.N., 2023. Feed utilization, physiological and meat quality responses in Jumbo quail reared on apple pomace-containing diets. Msc dissertation, North-West University, Mafikeng, South Africa.

Mnisi, C. M., Marareni, M., Manyeula, F. & Madibana, M. J., 2021. A way forward for the South African quail sector as a potential contributor to food and nutrition security following the aftermath of COVID-19: a review. Agric. Food Secur. 10(1), 1-12.

Mnisi, C.M. & Mlambo. V., 2018. Growth performance, haematology, serum biochemistry and meat quality characteristics of Japanese quail (*Coturnix coturnix japonica*) fed canola meal-based diets. Anim. Nutr. 4(1), 37-43.

Moro, J., Tome, D., Schmidely, P., Demersay, T.C. & Azzout-Marniche, D., 2020. Histidine: A systematic review on metabolism and physiological effects in human and different animal species. Nutrients, 12(5), 1414.

Nalluri, N, & Karri, V.R., 2021. Grain legumes and their by-products: As a nutrient rich feed supplement in the sustainable intensification of commercial poultry industry. In: Sustainable Agriculture Reviews. Vol 51. Springer, Cham. Switzerland. Pp. 51-96.

Nyembwe, P., Minnaar, A., Duodu, K.G. & Kock, H.L., 2015. Sensory and physicochemical analyses of roasted Marama beans [*Tylosema esculentum* (Burchell) A. Schreiber] with specific focus on compounds that may contribute to bitterness. Food Chem. 178, 45-51.

Omotayo, A.O. & Aremu, A.O., 2021. Marama bean (*Tylosema esculentum*) Burch. A. Schreib: an indigenous plant with potential for food, nutrition, and economic sustainability. Food Funct. 12(6), 2389-2403.

Redoy, M.R.A., Shuvo, A.A.S. & Al-Mamum, M., 2017. A review on present status, problems and prospects of quail farming in Bangladesh. Bangl. J. Anim. Sci. 46(2), 109-120.

Rocha, C., Durau, J. F., Barrilli, L. N. E., Dahlke, F. & Maiorka, P., 2014. The effect of raw and roasted soybeans on intestinal health, diet digestibility and pancreas weight of broilers. J. Appl. Poult. Res. 23:71-79.

SAPA, 2020. South African Poultry Association: Summary of poultry imports: Industry profile, Pretoria, South Africa.

Shubham, K., Anukiruthika, T., Dutta, S., Kashyap, A.V., Moses, J.A. &. Anandharamakrishnan, C., 2020. Iron deficiency anemia: A comprehensive review on iron absorption, bioavailability and emerging food fortification approaches. Trends Food Sci. Technol. 99, 58-75.

Zhu, G., Fang, Q., Zhu, F., Huang, D. & Yang, C., 2021. Structure and function of pancreatic lipase-related protein 2 and its relationship with pathological states. Front. Genet. 12, 693538.

6 LIST OF APPENDICES

6.1 Ethics certificate

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South Africa 2520

Tel: 018 299-1111/2222
Fax: 018 299-4910
Web: http://www.nwu.ac.za

Research Ethics Regulatory Committee
Tel: 018 299-4849
Email: nkosinathi.machine@nwu.ac.za

ETHICS APPROVAL LETTER OF STUDY

Based on approval by the **North-West University Animal Production Sciences Research Ethics Committee (NWU-AnimProdREC)** on 01/09/2022, the NWU Animal Production Sciences Research Ethics Committee hereby **approves** your study as indicated below. This implies that the North-West University Senate Committee for Research Ethics (NWU-SCRE) grants its permission that, provided the special conditions specified below are met and pending any other authorisation that may be necessary, the study may be initiated, using the ethics number below.

Study title: Enhancing the feed value of Marama bean (Tylocema esculentum) for Jumbo quail using thermal treatments

Study Leader/ Supervisor (Principal Investigator)/Researcher: Prof K Mnisi

Student: Fatoki MF

Ethics number: N W U - 0 0 3 1 1 - 2 2 - A 5

Institution | Study Number | Year | Status

Status: S = Submission; R = Re-Submission; P = Provisional Authorisation; A = Authorisation

Application Type: Single Study
Commencement date: 2022/09/28
Expiry date: 2023/09/26

Risk Category: 2

Approval of the study is initially provided for a year, after which continuation of the study is dependent on receipt and review of the annual (or as otherwise stipulated) monitoring report and the concomitant issuing of a letter of continuation.

Special in process conditions of the research for approval (if applicable):

- Any research at governmental or private institutions, permission must still be obtained from relevant authorities and provided to the NWU-AnimProdREC. Ethics approval is required BEFORE approval can be obtained from these authorities.

General conditions:

While this ethics approval is subject to all declarations, undertakings and agreements incorporated and signed in the application form, the following general terms and conditions will apply:

- *The study leader/supervisor (principle investigator)/researcher must report in the prescribed format to the NWU-AnimProdREC:*
  - *annually (or as otherwise requested) on the monitoring of the study, whereby a letter of continuation will be provided, and upon completion of the study; and*
  - *without any delay in case of any adverse event or incident (or any matter that interrupts sound ethical principles) during the course of the study.*
- *The approval applies strictly to the proposal as stipulated in the application form. Should any amendments to the proposal be deemed necessary during the course of the study, the study leader/researcher must apply for approval of these amendments at the NWU-AnimProdREC, prior to implementation. Should there be any deviations from the study proposal without the necessary approval of such amendments, the ethics approval is immediately and automatically forfeited.*
- *Annually a number of studies may be randomly selected for an external audit.*
- *The date of approval indicates the first date that the study may be started.*
- *In the interest of ethical responsibility, the NWU-SCRE and NWU-AnimProdREC reserves the right to:*

1

6.2 Language editing certificate

UNIVERSITY OF MPUMALANGA

Creating Opportunities

Faculty of Agriculture & Natural Sciences
School of Agricultural Sciences
P. Bag x11283
Riverside
Nelspruit 1200

20th November 2023

TO WHOM IT MAY CONCERN

RE: ENGLISH LANGUAGE EDITING – MF FATOKI [Student No: 39501663; ORCID: 0000-0002-1074-7939]

This serves to confirm and certify that the language and grammatical errors in the thesis by the candidate at caption entitled "**Enhancing the Feed Value of Marama Bean for Jumbo Quail Using Thermal Treatments**" have been corrected. The candidate has been advised on the correct usage of English and the necessary amendments have been made.

Sincerely,

Prof. Victor Mlambo, Pr. Sci. Nat. (Animal Science)
Tel: +27 13 002 0249
E-mail: Victor.Mlambo@ump.ac.za

6.3 Publication in Translational Animal Science

Translational Animal Science, 2023, **7**, txad136
https://doi.org/10.1093/tas/txad136
Advance access publication 1 December 2023
Non Ruminant Nutrition

Jumbo quail responses to diets containing raw or heat-treated Marama bean (*Tylosema esculentum*) meal

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Abstract

Growth performance, apparent retention (AR) of amino acids (AA), blood, and meat quality parameters were determined in grower Jumbo quail-fed diets containing raw or heat-treated marama bean (*Tylosema esculentum*) meal (MBM). The experimental diets were formulated to contain 99.98 g/kg of raw (MBMR), cooked (MBMC), autoclaved (MBMA), and toasted MBM (MBMT) in a standard grower diet (CON). A total of 306, 7-d-old Jumbo quail chicks (30.97 ± 1.79 g live weight) were placed in 35 cages (8/9 birds per cage). The five diets were allocated to the cages to give seven replicates per diet. The diets had varied responses ($P < 0.05$) on AR of lysine, methionine, and threonine, among others.The CON diet promoted the highest ($P < 0.05$) overall feed intake (FI) followed by MBMC and MBMA, and the lowest overall FI was from MBMR and MBMT. Quail-fed MBMR and MBMT diets had the least ($P < 0.05$) overall body weight gain and final body weights than those fed with the CON, MBMC, and MBMA diets. Moreover, quail on MBMR diet had the lowest ($P < 0.05$) overall gain-to-feed ratio (G:F) than those reared on the other diets, whose G:F values were statistically similar ($P > 0.05$). Similarly, quail on MBMR diet had the highest ($P < 0.05$) concentration of serum lipase and the heaviest gizzard than quail fed the other diets. However, quail-fed MBMT diet had heavier ($P < 0.05$) relative proventriculus weight compared to quail-fed other diets. Heavier ($P < 0.05$) relative spleen weights were observed for the MBMT and MBMR groups compared to the other groups. The CON diet had higher ($P < 0.05$) hematocrits and 1-h breast meat redness compared to the other diets. However, CON, MBMC, and MBMA diets increased ($P < 0.05$) 24-h breast meat redness than MBMR and MBMT diets. In conclusion, feeding diets with cooked or autoclaved marama bean meal resulted in an improvement in AA utilization, growth performance, and indices of carcass quality. However, raw and toasted marama beans had negative effects on performance parameters suggesting that optimal thermal treatment is critical for marama bean utilization.

Lay Summary

Naturally growing and locally available sources of nutrients (e.g., protein, lipids and minerals, and among others) could support sustainable quail production. marama bean (MB) (*Tylosema esculentum*) is one such source of essential nutrients that has high protein and fat content. However, the presence of antinutritional factors such as trypsin inhibitors and condensed tannins could limit its utilization in Jumbo quail diets. Heat treatments including cooking, autoclaving, and toasting could potentially reduce the antinutritional activities of these anti-nutrients and improve its utility without jeopardizing the health status of the birds. In this study, a soybean-based diet, as well as raw or heat-treated (cooked, autoclaved, and toasted) MB meal-containing diets were fed to 306 one-week-old Jumbo quail. The feeding experiment lasted for 35 d, after which the birds were slaughtered for measurements of blood, internal organs, carcass, and meat quality parameters. Overall, cooking followed by autoclaving treatments resulted in better performance metrices than the raw or toasted MB treatments. However, the soybean-based diet promoted the best performance results suggesting a need to explore other strategies to further enhance the utility of MB meal in Jumbo quail.

Key words: blood parameters, carcass quality, growth performance, plant protein, quail, thermal treatment

Introduction

Jumbo quail (*Coturnix* sp.) is one of the emerging poultry species that is currently produced in South Africa to bridge the demand gap for animal protein. Unlike chickens, the quail has less space requirement, low initial investment, early sexual maturity, shorter production cycles, and high disease resistance (Marareni and Mnisi, 2020; Mahlake et al., 2021). Despite these attributes, sustainable quail production is restricted by the ever-increasing feed cost (Mulaudzi et al., 2022). Indigenous protein sources such as marama bean (MB, *Tylosema esculentum*) can be used to deliver sustainable quail production systems. This leguminous plant is native to Southern Africa and grows naturally in adverse tropical weather conditions (Omotayo and Aremu, 2021) that are not suitable for soybean production. MB seeds have high crude protein (30% to 39%) and oil (32% to 42%) contents (Chimwamurombe, 2016; Ogbole et al., 2023), which can be essential in the formulation of high-performing quail diets (Marareni and Mnisi, 2020). However, its utility in Jumbo quail diets could be limited by the presence of antinutritional factors (ANF) such as trypsin inhibitors (TI) and condensed tannins (CT) (Alabi et al., 2022). TI constitutes about 20% of the total protein in MB and disrupts the formation of protease by binding with trypsinogen to form irreversible complexes (Alabi et al., 2022). Therefore, overconsumption of diets high in TI could cause pancreatic hyperplasia or hypertrophy due to a reduction in proteolytic activities in the gastrointestinal

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6.4 Turnitin report

23257539:Fatoki_MF_39501663_MSc_Animal_Science_Disse...

ORIGINALITY REPORT

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