Optimizing voluntary intake and utilization of *Moringa oleifera* leaf meal in Jumbo quail

A Mulaudzi

orcid.org/0000-0001-7884-3217

Thesis accepted for the degree Doctor of Philosophy in Animal Science at the North-West University

Promoter: Prof CM Mnisi

Co-promoter: Prof V Mlambo

Graduation: July 2023

Student number: 24285242

DECLARATION

I, Mulaudzi Anzai, hereby declare that this thesis entitled; **Optimizing voluntary intake and utilization of *Moringa oleifera* leaf meal in Jumbo quail** is my own work, and all cited material has been fully acknowledged in the references. This work has not been submitted for a degree at any other institution of higher education other than the North-West University.

Student: Mulaudzi Anzai

Signed..... Date.....

Supervisor: Professor C.M. Mnisi

Signed..... Date.....

Co-Supervisor: Professor V. Mlambo

Signed..... Date.....

GENERAL ABSTRACT

Moringa oleifera is nutraceutical plant that possesses antimicrobial, antioxidant, and immune-boosting bioactive compounds with potential to control the growth of pathogenic microorganisms, bolster animal growth performance and enhance meat quality and shelf life. However, antinutritive components including condensed tannins (CT), glycosylates, phytic acid, and fibre restrict the amount of *M. oleifera* leaf meal (MOLM) that can be included in quail (*Coturnix coturnix*) diets. Therefore, this thesis aimed to optimize voluntary feed intake and utilization of MOLM in Jumbo quail (*Coturnix* sp.) using polyethylene glycol (PEG) and exogeneous fibrolytic enzymes. Chapter One provides the background, problem statement, justification, aim and objectives of the thesis. Chapter Two offers an overview of Jumbo quail's production as well as the nutritional profile and importance of MOLM in quail feeds. It also examines the gaps and contradictions in the available literature on the utility of MOLM in a variety of poultry species. Furthermore, it assesses the effectiveness of several exogenous feed enzymes and tannin-binding agents (including PEG) in ameliorating fibre and CT in feed resources for simple-stomached animals. In Chapter Three, a preliminary feeding trial was conducted using four hundred and thirty-two (432), two-week-old mixed-gender quail chicks (103.4 ± 12.62 g live-weight). The objective was to determine the amount of PEG required to ameliorate CT in MOLM for Jumbo quail based on growth performance, physiology, and meat quality data. Six dietary treatments were designed as follows: a standard grower diet without MOLM (CON); a standard grower diet containing 100 g/kg untreated MOLM (MPG0); and a standard grower diet containing 100 g/kg MOLM pre-treated with PEG at a rate of 2.5 (MPG25), 5 (MPG50), 7.5 (MPG75), and 10 g/kg (MPG100). The chicks were randomly allocated to 36 pens (experimental units), which were replicated six times per dietary treatment. At week 4, significant quadratic trends were recorded for weight gain and feed conversion efficiency (FCE). However, at week 5, FCE linearly declined (P <0.05) as PEG

levels increased. Based on the quadratic response (P <0.05) for weight gain, the optimal PEG pre-treatment level was calculated to be 54 g/kg. The purpose of Chapter Four was to investigate the effect of dietary treatments pre-treated with viscozyme® L multi-enzyme (VME) on growth performance, blood parameters, internal organ sizes, carcass, and meat quality characteristics in Jumbo quail. The aim was to use the VME to break down the fibre components of MOLM and subsequently improve its utilisation by the quail. Three hundred and ninety-six (396), two-week-old mixed-gender quail chicks (87.8 ± 4.40 g live-weight) were used. Six dietary treatments were formulated as follows: a standard grower diet without MOLM (CON), a standard grower diet containing 100 g/kg untreated MOLM (VME0); and a standard grower diet containing 100 g/kg MOLM pre-treated with VME at a rate of 2.5 (VME25), 5.0 (VME50), 7.5 (VME75), and 10 g/kg (VME100). In response to incremental amounts of VME, there were no linear or quadratic impacts (P >0.05) on growth performance indicators and carcass features. This suggests that the highest VME treatment rate of 10 g/kg may not have been sufficient to improve feed utilization and positively affect weight gain in Jumbo quail. It was, therefore, determined that higher levels may be needed to improve the utility of MOLM for the Jumbo quail. To simultaneously reduce the harmful effect of fibre and CT, Chapter Five examined the effect of treating MOLM with the optimum PEG concentration (54 g/kg) that was determined in Chapter Three and VME levels beyond the level (10 g/kg) used in Chapter Four. Three hundred and eighty-one (381), one-week-old mix-gender quail chicks (57.5 ± 3.95 g live-weight) were used. Six dietary treatments were formulated as follows: a standard grower diet containing untreated 100 g/kg MOLM (CON); a standard grower diet containing 100 g/kg MOLM pre-treated with 54 g/kg PEG (MPV0); and a standard grower diet containing 100 g/kg MOLM pre-treated with 54 g/kg PEG and 12.5 g/kg (MPV125), 15 g/kg (MPV150), 17.5 g/kg (MPV175) and 20 g/kg (MPV200) of VME. In response to increasing VME levels, there were no linear or quadratic effects (P >0.05) observed for overall feed intake, FCE, haematological,

carcass, or meat quality parameters. In week 3, increasing dietary VME levels resulted in a quadratic response (P <0.05) for weight gain. It can be concluded that pre-treatment of MOLM with a combination of PEG and exogenous VME is an ineffective strategy to improve its feed value in diets of Jumbo quail.

Keywords: Fibre**,** Fibrolytic enzymes, Jumbo quail, Meat quality, *Moringa oleifera*, Polyethylene glycol, Tannins

ACKNOWLEDGEMENTS

First and foremost, I praise and thank the Almighty God for his showers of blessings throughout my research and for the strength he gave me to complete this work.

I express my deep and sincere gratitude to my supervisors, Professor C.M. Mnisi and Professor V. Mlambo for giving me the opportunity to do research and providing invaluable guidance throughout. Their dynamism, vision, sincerity, and motivation have deeply inspired me. It was a privilege and honour to work and study under their guidance.

The financial support from NWU PhD bursary and National Research Foundation (NRF; Grant Number: 121399) is gratefully acknowledged.

I am thankful for the assistance received during data collection from both undergraduate (Mtshali, B., Yemama, S., Makaula, S.A., Baloyi, N.A. and Twala., Z.I.), and postgraduate students (Matshogo, T.B., Marareni, M., Mhlongo, G., Matabane, A.N. and Kwena, T.) in the Department of Animal Science, North-West University.

DEDICATION

This work is dedicated to my parents Mr and Mrs Mulaudzi for their love, prayers, caring, sacrifice and preparing me for the future. I am thankful to my siblings Thendo, Zwiswa, Lisani, Ankonisaho and my late brother Ngaahule, for their love, understanding, prayers and continuous support in ensuring that I complete this research. My special thanks go to my friends, Tshidaho, D., Rambasa, E., Nemukula, G. and Kumanda, C. for being supportive. I love you all.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

AA : Amino acids

ADF : Acid detergent fibre

AWFI : Average weekly feed intake

AGP : Antibiotic growth promoters

ALP : Alkaline phosphatase

ALT : Alanine aminotransferase

ABWG : Average body weight gain

CCW : Cold carcass weight

CT : Condensed tannins

CP : Crude protein

DM : Dry matter

FCE : Feed conversion efficiency

FI : Feed intake

GIT : Gastrointestinal tract

GLM : General linear model

HCW : Hot carcass weight

LSMEANS : Least square means

ME : Metabolizable energy

MOLM : *Moringa oleifera* leaf meal

NRC : National research council

NSP : Non-starch polysaccharides

OM : Organic matter

PEG : Polyethylene glycol

SBM : Soybean meal

SDGs : Sustainable development goals

SEM : Standard error of the mean

VME : Viscozyme® L multi-enzyme

WHC : Water holding capacity

LIST OF PUPLICATIONS

1. Mulaudzi, A., Mnisi, C.M. & Mlambo, V. 2022. Enhancing the utility of dietary *Moringa oleifera* leaf meal for sustainable Jumbo quail (Coturnix sp.) production. *Sustainability*, 14(9), 5067. **(Published)** https://doi.org/10.3390/su14095067

2. 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. (**Published**) https://doi.org/10.3390/poultry1020006

3. Mulaudzi, A., Mnisi, C.M. & Mlambo, V. 2022. Simultaneous pre-treatment of dietary *Moringa oleifera* leafmeal with polyethylene glycol and fibrolytic enzymes: Effect on growth performance, physiological indices, and meat quality parameters in Jumbo quail. *Frontiers in Animal Science*, 3, 960233. **(Published)** https://doi:10.3389/fanim.2022.960233

1 CHAPTER ONE – GENERAL INTRODUCTION

1.1 Background

The increasing human population as well as greater health concerns around red meat consumption have led to an increased demand for poultry meat worldwide. According to the Department of Agriculture, Forestry and Fisheries (DAFF, 2021) broiler meat is the most affordable dietary protein source for human consumption, amounting to over 2,924 million tons per year. According to the South African Poultry Association (SAPA, 2020) the rapidly growing human population has increased the need for poultry meat, with a per capita consumption of 38.93 kg of all poultry products. To cater for this high demand of poultry products, the poultry industry should be diversified by including a variety of bird species like Jumbo quail (*Cortunix* sp.). The Jumbo quail has the potential to radically transform the poultry market in low- and middle-income countries (Deka & Borah, 2008). Quail farming is fast gaining popularity in many regions of the world as a source of meat and eggs (Jeke *et al*., 2018). Quail birds demonstrate rapid growth, enabling them to be marketed for human consumption at 5 – 6 weeks old. It also exhibits early sexual maturity resulting in a short generation interval, high rate of lay, and much lower feed intake (30 – 35 g/day) and space requirements than other poultry species (Hemid *et al*., 2010). Under favourable environmental conditions, a quail can produce about 100 eggs per year (Arthur & Bejaei, 2017). Good management and feeding techniques are crucial for sustainable and profitable poultry production. However, the rising demand for conventional feed ingredients such as maize and soybean, which are mostly used in the formulation of commercial animal diets, has led to high market prices on a global scale

(Shi *et al*., 2012). This is because poultry production, especially in the tropics, faces the challenge of high prices of the conventional protein and energy sources. The problem has been worsened due to the increasing competition between humans and livestock for these protein ingredients as food (Nwekirk, 2010). Non-conventional feedstuffs, with no value as direct food resources for humans, need to be identified for use in poultry diets so as to reduce the cost of production. Indeed, leaf meal protein sources are amongst feed sources being investigated in recent times and do not only serve as a source of protein, but provides some necessary vitamins and minerals (Onu & Otuma, 2008) as well as bioactive compounds that have the potential to boost growth performance as well as meat quality. Thus, the use of non-conventional nutraceuticals like *Moringa oleifera* leaf meal (MOLM) has a potential to contribute towards sustainable quail production.

In many tropical and subtropical areas, *M. oleifera* has nutritional and medicinal uses due to the antimicrobial, anti-inflammatory and antioxidant properties of its bioactive components (Bennett *et al*., 2003; Ferreira *et al*., 2008). The antioxidants (phenols, vitamin C, vitamin E, alpha-carotene, zinc, selenium, and flavonoids) are known to extend the shelf life and enhance the quality of meat products (Valeria & Williams, 2011). Additionally, MOLM has antimicrobial properties that limit the activity of harmful bacteria and moulds, and thereby protect the birds from subclinical diseases and subsequently improve their performance (Khan *et al*., 2022).

1.2 Problem statement

The use of MOLM in Jumbo quail diets could control the growth of pathogenic microorganisms, boost growth performance, and enhance meat quality and shelf life (Mbikay, 2012). However, the leaf meal contains antinutritive components including glycosylates, phytic acid, condensed tannins (CT), and fibre that restrict the quantities that can be included in quail

diets. The high fibre and CT content of MOLM could hinder nutritional digestion and absorption (Chamorro *et al*., 2012). Other studies have demonstrated that tannins reduce experimental animals' feed intake, growth rate, feed efficiency, net metabolizable energy, and protein digestibility (Bele *et al*., 2010; Addisu, 2016). Tannins form complexes with proteins through covalent, hydrogen, and ionic bonding, and these complexes may only be broken under high acidity or high alkalinity conditions (Ben Salem *et al*., 2005). The pH levels in the gut affect microbial proliferation, which influences nutrients absorption and digestion (Mabelebele *et al*., 2014). The molecular weight, tertiary structure, isoelectric point, and compatibility of binding sites all affect how strongly tannins and proteins bond to one another (Silanikove *et al*., 2001).

According to Jha *et al*. (2019), dietary fibre does not undergo a lot of digestion in simple non-ruminants due to the lack of endogenous digestive enzymes that hydrolyse these insoluble non-starch polysaccharides (NSP). According to Nikam (2016), NSP can be classified as either soluble (β-glucose, arabinoxylan, arabinogalactose, xyloglucon, etc.) or insoluble (cellulose) carbohydrates. Nikam *et al*. (2017) and Khobondo *et al*. (2019) reported that soluble NSPs have the capacity to hold water in their matrix by producing a loose gel network. This increased viscosity lowers the digestion of lipids, proteins, and carbohydrates. Insoluble NSP is also harmful to quail digestive system. Several studies have shown that the amount of MOLM that can be included in chicken diets should not be more than 25 g/kg due to the negative impact of antinutritional elements (Onu & Aniebo, 2011; Hassan *et al*., 2015; Ufele & Ebenebe, 2017). Given the antinutritional effects of CT and fibre in MOLM, it is imperative that the effectiveness of tannin-binding substances like PEG and exogenous fibrolytic enzymes to enhance the utilisation of MOLM-containing diets in quail be investigated.

1.3 Justification

Moringa oleifera leaf meal has high concentrations of CT and fibre that could impair nutrient utilisation, growth performance, and meat quality, especially when added to quail diets in levels more than 25 g/kg (Hassan *et al*., 2015; Ufele & Ebenebe, 2017; Mulaudzi *et al*., 2019). It has been shown that poultry fed with diets that are high in fibre and CT experience metabolic disorders because of inadequate nutrient uptake (Khajali & Slominski, 2012). Thus, the use of PEG and exogenous fibrolytic enzymes could increase the inclusion levels of MOLM in quail diets. Exogenous fibrolytic enzymes have been shown to hydrolyse the NSP, which leads to digestion viscosity reduction, nutrient absorption improvement and better growth performance (Khattak *et al*., 2006). These fibrolytic enzymes are of fungal or bacterial origin and they can increase nutrient availability in poultry production as they can remove the antinutritional factors in diet and speed up the degradation of substrates (Salem *et al*., 2013; Mendoza *et al*., 2014; Bedford *et al*., 2022).

Furthermore, MOLM has a high concentration of polyphenolic compounds, particularly CT that inhibit their efficient use by poultry (Khoddami *et al*. 2015). This warrants the investigation on the use of tannin-binding agents to improve the nutrient utilisation of MOLM- containing diets fed to poultry. The treatment of tannin-rich leaf meals with PEG could assist feed compounders to formulate diets that would ensure optimum feed intake, without compromising quail performance (Van Niekerk *et al*., 2020). Determining the optimal level of PEG could enhance the efficient utilisation of MOLM in quail feeds. This is because PEG has the potential to neutralise the negative effects of undesirable polyphenolic compounds like CT (Hlatini *et al*., 2018).

The application of exogenous fibrolytic enzymes like VME and tannin-binding agents like PEG in diets containing MOLM could enable the birds to benefit from higher concentrations of the

beneficial bioactive components (Castillo & Gatlin, 2015). In this respect, little is known about the treatment of MOLM with PEG and exogenous enzymes because the response to the addition of these additives varies with the type of foliage as well as the animal model used. Therefore, this study sought to improve the feed value of dietary MOLM for Jumbo quail with the aid of VME and PEG using growth performance, physiological responses, and meat quality traits as response indicators.

1.4 Objectives

The study was designed to optimise voluntary feed intake and the utilisation of *Moringa oleifera* leaf meal in Jumbo quail diets using polyethylene glycol and viscozyme multi-enzyme.

The specific objectives were designed to:

i. Determine the effect of PEG pre-treatment of dietary MOLM (100 g/kg) on growth performance, haemo-biochemical parameters, carcass characteristics and meat quality in Jumbo quail.

ii. Establish the effect of VME pre-treatment of dietary MOLM on growth performance, blood parameters, carcass characteristics and meat quality traits of Jumbo quail.

iii. Determine the effect of simultaneous PEG and VME pre-treatment of dietary MOLM on growth performance, haemo-biochemical parameters, carcass characteristics, and meat quality traits of Jumbo quail.

1.5 Hypotheses

i. Pre-treatment of dietary MOLM with PEG improves growth performance, haemo-biochemical parameters, carcass characteristics, and meat quality traits in Jumbo quail.

ii. Pre-treatment of dietary MOLM with VME improves growth performance, haemo-biochemical parameters, carcass characteristics, and meat quality traits in Jumbo quail.

iii. Simultaneous pre-treatment of MOLM with PEG and VME could improve growth performance, haemo-biochemical parameters, carcass characteristics, and meat quality traits in Jumbo quail.

1.6 References

Abd El-Hack, M.E., Alagawany, M., Elrys, A.S., Desoky, E.S.M., Tolba, H.M., Elnahal, A.S., Elnesr, S.S. & Swelum, A.A., 2018. Effect of forage *Moringa oleifera* on animal health and nutrition and its beneficial applications in soil, plants and water purification. Agriculture. 8(9), 145.

Addisu, S., 2016. Effect of dietary tannin source feeds on ruminal fermentation and production of cattle; a review. Online J. Anim. Feed. Res. 6(2), 45–56.

Ahmed, W.F. & El-Rayes, T.K., 2019. Effect of using *Moringa oleifera* leaves on productive performance and some physiological parameters of Japanese quail. Egypt. Poult. Sci. J. 39(1), 193–205.

Alam, M.J., Howlider, M.A.R., Pramanik M.A.H. & Haque, M.A., 2003. Effect of exogenous enzyme in diet on broiler performance. Int. J. Poult. Sci. 2(2), 168–173.

Arthur, J. & Bejaei, M., 2017. Quail eggs. In *Egg innovations and strategies for improvements*. Academic Press, New York, United states of America. Pp. 13–21

Attia, Y.A., Zeweil, H.S., Alsaffar, A.A. & El-Shafy, A.S., 2011. Effect of non-antibiotic feed additives as an alternative flavomycin on productivity, meat quality and blood parameters in broilers. Arch. Fur Geflugelkunde. 75, 40–48.

Bele, A.A., Jadhav, V.M. & Kadam, V.J., 2010. Potential of tannins: a review. Asian J. Plant. Sci. 9(4), 209–214.

Ben Salem, H., Makkar, H.P.S., Nefzaoui, A., Hassayoun, L. & Abidi, S. 2005b. Benefit from the association of small amounts of tannin-rich shrub foliage (*Acacia cyanophylla Lindl*.) with soya bean meal given as supplements to Barbarine sheep fed on oaten hay. Anim. Feed. Sci. Technol. 122, 173–186.

Bennett, R.N., Mellon, F.A., Foid, N., Pratt, J.H., Dupont, M.S., Perkins, L. & Kroon, P.A., 2003. Profiling glucosinolates and phenolics in vegetative and reproductive tissues of the multi-purpose trees *Moringa oleifera* L. (horseradish tree) and *Moringa stenopetala*. J. Agric. Food Chem. 51, 3546–3553.

Castillo, S. & Gatlin III, D.M., 2015. Dietary supplementation of exogenous carbohydrase enzymes in fish nutrition: A review. Aquaculture. 435, 286–292.

Chamorro, S., Viveros, A., Alvárez, I., Vega, I. & Brenes, A., 2012. Changes in polyphenol and polysaccharide content of grape seed extract and grape pomace after enzymatic treatment. Food Chem. 133, 308–314.

DAFF (Department of Agriculture, Forestry and Fisheries). 2021. *Abstract of Agricultural Statistics 2012.* Pretoria, South Africa.

Deka, K. & Borah, J., 2008. Haematological and biochemical changes in Japanese quails *Coturnix coturnix japonica* and chickens due to *Ascaridia galli* infection. Int. J. Poult. Sci. 7(7), 704–710.

Ferreira, P.M.P., Davi, F.F., Jose, T.A.O. & Ana, F.U.C., 2008. *Moringa oleifera*: bioactive compounds and nutritional potential. J. Rev. Nutr. 21, 431–437.

Gakuya, D.W., Mbugua, P.N., Mwaniki, S.M., Kiama, S.G., Muchemi, G.M. & Njuguna, A., 2014. Effect of supplementation of *Moringa oleifera* (LAM) leaf meal in layer chicken feed. Int. J. Poult. Sci. 13(7), 379.

Hashemia, S.R., Zulkiflib, I., Davoodic, H., Zunitad, Z. & Ebrahimie M., 2012. Growth performance, intestinal microflora, plasma fatty acid profile in broiler chickens fed herbal plant (*Euphorbia hirta*) and mix of acidifiers. Anim. Feed Sci. Technol. 178, 167–174.

Hassan, H.M.A., El-Moniary, M.M., Hamouda, Y., El-Daly, E.F., Youssef, A.W. & Abd ElAzeem, N.A., 2016. Effect of different levels of *Moringa oleifera* leaves meal on

productive performance, carcass characteristics and some blood parameters of broiler chicks reared under heat stress conditions. Asian J. Anim. Vet. Adv. 11 (1), 60–66.

Hemid, A.E.A., El-Gawad, A.H.A., El-Wardany, I., El-Daly, E.F. & El-Azeem, N.A.A., 2010. Alleviating effect of some environmental stress factors on productive performance of laying quail. 2. Laying performance. World J. Agric. Sci. 6(5), 517–524.

Ismail, F.S.A., Hayam, M.A. & EI-Sayed, A.A.M., 2011. Performance of broiler chicks affected by dietary supplementation with commercial enzymes or probiotics. J. Anim. Poult. Prod. 2 (2), 23–32.

Jeke, A., Phiri, C., Chitiindingu, K. & Taru, P., 2018. Nutritional compositions of Japanese quail (*Coturnix coturnix japonica*) breed lines raised on a basal poultry ration under farm conditions in Ruwa, Zimbabwe. Cogent. Food. Agric. 4(1), 1473009.

Jha, R., Fouhse, J.M., Tiwari, U.P., Li, L. & Willing, B.P., 2019. Dietary fibre and intestinal health of monogastric animals. Front. Vet. Sci. 6, 48–54.

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

Khan, R.U., Naz, S., Raziq, F., Qudratullah, Q., Khan, N. A., Laudadio, V. & Ragni, M., 2022. Prospects of organic acids as safe alternative to antibiotics in broiler chicken's diet. Environ. Sci. Pollut. Res. 29, 32594–32604.

Khan, S., Ibrar, D., Bashir, S., Rashid, N., Hasnain, Z., Nawaz, M., Al-Ghamdi, A.A., Elshikh, M.S., Dvořáčková, H. & Dvořáček, J., 2022. Application of moringa leaf extract as a seed priming agent enhances growth and physiological attributes of rice seedlings cultivated under water deficit regime. Plants. 11(3), 261–272.

Khobondo, J.O., Kingori, A.M. & Manhique, A. 2019. Effect of incorporation of ground *Prosopis juliflora* pods in layer diet on weight gain, egg production, and natural antibody

titer in KALRO genetically improved indigenous chicken. Trop. Anim. Health Prod. 51(8), 2213–2218.

Khoddami, A., Truong, H.H., Liu, S.Y., Roberts, T.H. & Selle, P.H., 2015. Concentrations of specific phenolic compounds in six red sorghums influence nutrient utilisation in broiler chickens. Anim. Feed Sci. Technol. 210, 190–199.

Mabelebele, M., Alabi, O.J., Ngambi, J.W., Norris, D. & Ginindza, M.M., 2014. Comparison of gastrointestinal tracts and pH values of digestive organs of Ross 308 broiler and indigenous Venda chickens fed the same diet. Asian. J. Anim. Vet. Adv. 9 (1), 71–76.

Mbikay, M., 2012. Therapeutic potential of *Moringa oleifera* leaves in chronic hyperglycaemia and dyslipidaemia: A review. Front. Pharmacol. 3(24), 1–12.

Mulaudzi, A., Mnisi, C.M. & Mlambo, V., 2019. Dietary *Moringa oleifera* leaf meal improves growth performance but not haemo- biochemical and meat quality parameters in female Japanese quails. Pak. J. Nutr. 18, 953–960.

Newkirk, R. W., 2010. Soybean Feed Industry Guide, 1st edn.(Winnipeg, Canada: Canadian International Grains Institute)

Nikam, M., Reddy, V., Raju, M.V.L.N., Reddy, K. & Narasimha, J., 2017. Effect of non-starch polysaccharide hydrolyzing enzymes on nutrient utilization and economics of broilers reared on corn soybean meal based standard and sub-optimal diets. Int. J. Livest. Res. 7(2), 172–179.

Nikam, M.G., Ravinder Reddy., Raju, M.V.L.N., Kondal Reddy, K. & Narasimha, J., 2017. Effect of dietary supplementation of non-starch polysaccharide hydrolysing enzymes on performance of broiler fed diet based on guar meal. Int. J. Livest. Res. 7 (2), 180–190.

Nikam, M.G., Reddy, V., Reddy, K. & Narasimha, J., 2016. Effect of dietary supplementation of non-starch polysaccharide hydrolyzing enzymes on Broilers. Int. J. Agric. Sci. Res. 6

(3), 389–396.

Nkukwana, T.T., Muchenje, N., Pieterse, N.E., Masika, P.J., Mabusela, T.P., Hoffman, L.C. & Dzama, K., 2014. Effect of *Moringa oleifera* leaf meal on growth performance, apparent digestibility, digestive organ size and carcass yield in broiler chickens. Livest. Sci. 161, 139–146.

Novak, C.K., Yakout, H.M. & Remus, J., 2007. Response to varying dietary energy and protein with or without enzyme supplementation on growth and performance of Leghorns: Growing period. J. Appl. Poult. Res. 16 (4), 481–493.

Onu, P.N. & Aniebo, A.O., 2011. Influence of *Moringa oleifera* leaf meal on the performance and blood chemistry of starter broilers. Nig. Int. J. Food Agri. Vet. Sci. 1(1), 38–44

Onu, P.N. & Otuma, M.O., 2008. Utilization of heat treated sheep dropping in the diets of broiler finisher chicks. Int. J. Poult. Sci. 7(2), 169 - 173.

Onunkwo, D.N. & George, O.S., 2015. Effects of *Moringa oleifera* leaf meal on the growth performance and carcass characteristics of broiler birds. J. Agric. Vet. Sci. 8, 63–66.

Salem, A.Z.M., Odongo, N. & Pattanaik, A.K., 2013. Exogenous enzymes in animal nutrition benefits and limitations. Anim. Nutr. Feed Technol. 13(3), 335–336.

SAPA (2017). South African Poultry Association 2016. Industry Profile. Roodepoort, South Africa.

Shi, S.R., Lu, J., Tong, H.B., Zou, J.M. & Wang, K.H., 2012. Effects of graded replacement of soybean meal by sunflower seed meal in laying hen diets on hen performance, egg quality, egg fatty acid composition, and cholesterol content. J. Appl. Poult. Res. 21, 367–374.

Silanikove, N., Perevolotsky, A. & Provenza, D. F., 2001. Use of tannin-binding chemicals to assay for tannins and their negative post-ingestive effects in ruminants. Anim. Feed Sci. Technol. 91, 69–81.

Singh, A.K. & Kim, W.K., 2021. Effects of dietary fibre on nutrients utilization and gut health of poultry: a review of challenges and opportunities. Animals. 11(1), 181.

Ufele, A.N. & Ebenebe, C.I., 2017. The Effect of *Moringa oleifera* on the growth performance, packed cell volume (PCV) and laying capacity of young growing quails. Am. J. Zool. Res. 5(2), 33–37.

Valeria, V. & Williams, P., 2011. Improving meat quality through natural antioxidants. Chil. J. Agric. Res. 71(2), 18–22

Van Niekerk, R.F., Mnisi, C.M. & Mlambo, V., 2020. Polyethylene glycol inactivates red grape pomace condensed tannins for broiler chickens. Br. Poult. Sci. 61(5), 566–573.

Vooren, N.V., 2012. Flexible feed formulation with multi enzyme concept. Pak. Poult. 33, 7–11.

Wang, J.J., Garlich, J.D. & Shih, J.C.H., 2006. Beneficial effects of versazyme, a keratinase feed additive, on body weight, feed conversion, and breast yield of broiler chickens. J. Appl. Poult. Res. 15, 544–550.

Zou, J., Zheng, P., Zhang, K., Ding, X. & Bai, S. 2013. Effect of exogenous enzymes and dietary energy on performance and digestive physiology of broilers. J. Anim. Sci. Biotech. 4(1), 1–9.

2 CHAPTER TWO – LITERATURE REVIEW

2.1 Introduction

The leading agricultural sector in South Africa is poultry, which significantly improves the country's gross domestic product (GDP), creates jobs, and lowers food insecurity risks (Mohammed *et al*., 2018). Additionally, poultry farming is the quickest approach to expand the supply of high-quality protein for human consumption (Rafiullah & Sajid, 2013). The price of poultry feeds, however, has risen (22%)over time for a variety of reasons, including low production, insects, weeds, environmental concerns, cost-efficiency regimes, sustainability, shrinking area under cultivation (Kshash & Oda, 2019). According to Falodi *et al*. (2016), excessive feed cost has caused farmers to mix poultry feeds with less nutritional ingredients. Chuka (2014) and Bokau *et al*. (2018) demonstrated that due to the high cost of feed ingredients, farmers tend to feed poultry with low quality agro-industrial by-products such as palm kernel cake, rice husk and papaya leaves, which lower feed utilisation efficiency due to high tannins and fibre contents. Poultry can achieve the highest growth rates and lowest costs per unit of output, mainly because of efficient feed conversion, an advantage they have over other livestock species that cannot do this to the same extent.

To attain optimal poultry production to meet the high demands for animal protein, it is important to diversify both the bird species and the feed ingredients used. The use of alternative ingredients should seek to minimize the poultry industry's reliance on expensive ingredients such as maize and soybean grains, which have direct human food value and environmental repercussions (Mnisi & Mlambo, 2019). Quail diets are made up of grains that include anti-nutritive component such as non-starch polysaccharides (NSP), tannins, glycosylates, and phytic acid that interfere with digestive process (NRC, 2004; Khajali & Slominski, 2012). Nduka (2006) indicated that the bulk of the starch present in cereals are not easily digested by

monogastric animals, and a large proportion of the contents are found as (NSP). The use of MOLM has been limited because of low protein and energy content when compared to SBM. It has been discovered that plant derived additives contains bioactive compounds with effects similar to those of antibiotic growth promoters (AGP) in three major areas, namely gut micro-flora, antioxidant properties, and liver function that do not compromise intestinal health or the bird's genetic potential (Hernandez *et al*., 2004). Additionally, AGP may alter how cholesterol is metabolized, resulting in a food product that is significantly healthier for consumption by humans (Wallace *et al*., 2010). One such plant is *M. oleifera*, nonetheless, the inclusion of MOLM beyond 25 g/kg has detrimental effects on quail growth performance and health (Hassan *et al*., 2015; Ufele & Ebenebe, 2017. This detrimental effect might be due to the high tannins, fibre content, trypsin inhibitors and NSP, which reduce digestibility and nutrient bioavailability (Hussain, 2015). The use of PEG and exogenous enzyme supplementation to fibrous and NSP-rich diets is said to improve nutrient digestion and absorption by partially hydrolysing NSP and reducing the viscosity of gut contents (Mnisi, 2018; Kumanda *et al*., 2019).

2.2. Quail

Jumbo quail is a bigger version of the Japanese quail (Coturnix coturnix japonica) (Mbhele *et al*., 2019). The Japanese quail is a species of the Old-World quail found in East Asia that belongs to the order Galliformes and the family Phasianidae (Minvielle, 2004). The plumage color of the wild type is predominately dark cinnamon brown. However, the adult female has pale breast feathers that are speckled with dark colored spots as shown in figure 2.1. Adult males have uniform dark rusted feathers on the breast and cheek (Mizutani, 2003). Jumbo quail is fast gaining popularity as a source of meat and eggs in many parts of the world (Priti & Satish, 2014). It is also being used as a laboratory animal (Baer *et al*., 2015) with distinct characteristics such as rapid growth, enabling the bird to be marketed for human consumption

as early as 6 weeks of age. The bird exhibits early sexual maturity resulting in a short generation interval, high prolificacy and much lower feed intake (30 – 35 g/day) and space requirements than other poultry species (Hemid *et al*., 2010). Under optimal environmental conditions, the hens can produce about 100 eggs per year (Santos *et al*., 2019).

Commercial quail farming is developed for various reasons around the world; for instance, quail are reared for egg production in Asia while they are raised for meat in Europe (Brah & Mukhopadhyay, 2016). According to Nasr *et al*. (2017), when compared to other quail breeds, the Jumbo quail have a higher body weight and the best carcass features and meat quality attributes. Comparing the rearing of chicken, duck, fowl, goose, ostrich, guinea fowl, and turkey, the quail is a more profitable business endeavor (Randall & Bolla, 2008). This is explained by the quail's rapid development rate, early maturity, and small body size, which enable the upbringing of six to seven quail birds in the same area required by one adult chicken (Ali *et al*., 2012).

Plate 2.1. Jumbo quail hen

2.2.1 Importance of quail as new entrants in the poultry industry

To overcome the protein deficit or malnutrition, the rapid increase in the world's human

population has necessitated the use of additional sources of animal protein (Mohammed & Ejiofor, 2015). Quail birds are becoming more and more popular in most emerging nations as

a new diversification endeavour to meet the demand for human protein. Quail production has also become a means of generating a quick return on investment from commercial farming (Hossain *et al*., 2015). The South African poultry industry is dominated by conventional broiler and layer chickens as well as indigenous chickens, ostriches, ducks, and turkey (Thorp, 2021). To maintain a steady supply of meat and eggs for human consumption, the poultry business must be expanded (Ayasan, 2013). Raising Jumbo quail for food can be seen as an additional aspect of chicken husbandry, accompanied with advantages and difficulties. Quail farming has the advantage of supporting and allowing for flexibility in the production of chickens by supplying meat and eggs (Nguyen *et al*., 2022).

Quail farming requires little cash and labour (Sanka & Mbaga, 2015); therefore, one does not need to raise a sizeable sum of money to start a quail business. Due to the increased number of competitors in the sector and the resulting strong competition, this gives even resource-limited farmers a chance to launch their own enterprises and expand the poultry industry. Quail meat has less fat and is leaner, according to Dingle (1990), making it acceptable for consumers, who are concerned about their health (Huda *et al*., 2011). This makes the poultry sector accessible to practically all socioeconomic groups that eat meat (Erian & Phillips, 2017).

Quail products are abundant in micronutrients and several vitamins, such as the B complex, folate, vitamin E, and vitamin K. Moreover, they are safe for consumption by those that want to maintain a low cholesterol level as well as those with high cholesterol levels (Ihejirikamba, 2012). Imchen (2013) reported that quail meat has health benefits that include supporting children's body and brain development. It is also softer, juicier, and lower in lipids than chicken meat (Biswas *et al*., 2015). In addition to being vital for achieving health, good nutrition is also crucial for achieving the sustainable development goals (SDGs). Good nutrition helps achieve

other development goals that in turn have an influence on the SDGs by indirectly boosting local economies and communities (Herrerro *et al*., 2021).

2.2.2 Nutritional requirements of quail

To synthesise non-essential amino acids, poultry birds need a diet that is well-balanced, containing a precise amount of nitrogen and readily digestible essential amino acids. Sahin *et al*. (2002) and Chinrasri (2004) define nutrient requirements as the amount of nutrients needed to maintain animal performance, maximize growth and feed utilisation efficiency and optimize their body fat accumulation. Water is important despite the significance of other nutrients National Research Council (NRC, 1991). However, several factors, including temperature, humidity, salt, dietary proteins, stage of development, and the kidney's capacity to reabsorb water, may influence how much water is consumed (NRC, 1994). As a result, quail should always have access to clean, fresh drinking water. Indeed, Moundry (2016) confirmed that quail birds of all ages may die if they go without water for longer than 36 hours.

According to the (NRC, 1994), quail should be raised on diets that are high in protein, 24% crude protein (CP) during the growing phase and 20% CP during the production phase. It is essential to mention that quail raised in temperate climates can consume these recommended protein levels (NRC, 1994). In South Africa, there is currently no standard diet for broiler or layer quail (Mnisi & Mlambo, 2019), suggesting that the birds' performance may be impaired by inadequate nutrient intake. A commercial game-bird diet comprising 250 g/kg CP, 12.6 MJ/kg metabolizable energy (ME), and 10 g/kg calcium should be fed to growing quail for the best results (Randall & Bolla, 2008). Varkoohi *et al*. (2010) observed that quail consume 30 to 35 g per day and feed should always be available. For excellent performance, a game-bird diet can be fed, although it is very expensive and hard to find (Randall & Bolla, 2008).

2.2.3 Gastrointestinal physiology of quail

Jumbo quail consumes and stores the feed in the crop before passing it to the stomach through to the lower gastrointestinal tract (GIT). The crop is the diverticulum of the oesophagus, which also helps in the moistening of feed. Quail birds have no teeth; therefore, they rely on their muscular gizzard to grind and mix their feed since they are unable to do it with their mouths (Wings, 2000). The proventriculus, which is the proximal part of the quail's stomach, absorbs the feed from the crop (Zaher *et al*., 2021). The non-glandular muscular gizzard also aids in digestion by breaking down feed particles and enhancing access of digestive enzymes to substrates, while the proventriculus, which is the glandular portion, secretes hydrochloric acid and pepsin (Singh, 2018). Quail digestion is essentially similar to that of other monogastric animals. While the pancreas secretes amylase, trypsinogen, pancreatic lipase, chymotrypsinogen, procarboxypeptidase, etc., which are emptied in the duodenum and act on the chyme, the liver secretes bile that aids in the emulsification of lipids (Fieker *et al*., 2011).

The intestine secretes several enzymes, which are involved in the final stage of digestion, including maltase, isomaltase, sucrase, enterokinase, lipase, and peptidases (Harmon & Swanson, 2020). A quail has a pair of caeca responsible for fermentation of feed residues through the action of the resident microbiome (Chen *et al*., 2021). The capacity of quail caeca may not be as great as those of the other hindgut fermenters, but they can certainly provide some short-chain fatty acids that can either be used as an energy source or support mucosal epithelial health (González-Ortiz *et al*., 2020). Optimal functionality of the GIT system is essential for sustainable and profitable quail production. Diet, mucosa, and commensal microbiota are the three major factors that Conway (1994) determined as also being essential to gut health (Zheng *et al*., 2020). All these components play a critical role in the balance of gut physiology, quail health, growth performance, nutrient utilization, and welfare.

The classical role of the digestive tract is the fermentation of feed utilising enzymes and microorganisms for later nutrient absorption (Celi *et al*., 2017). The impact of diet on GIT can be directed towards various functions ranging from digestive to immune system (Mowat & Agace, 2014). Dietary fibre may have a prebiotic effect and stimulate beneficial bacteria (Jha & Berrocoso, 2015), but the constructive effect of this fibre could be offset since high fibre content could reduce the digestibility of nutrients (Freire *et al*., 2000). Therefore, feed enzymes may improve animal performance by hydrolysing feed substrates that are not or only partially broken down by endogenous enzymes, especially in young quail with relatively immature GIT (Ravindran, 2013). Additionally, a healthy gut mucosa and a balanced diet are important in the secretion of enzymes, nutrient absorption, and the harbouring of immune cells (Spencer & Belkai, 202). Moreover, the gut microbiota interacts with diet and the GIT causing rapid uptake and conversion of metabolic intermediates that are eliminated from the GIT through digestion and absorption by the host and, as a result, create a highly dynamic system (Celi *et al*., 2017).

2.2.4 Factors affecting protein digestibility and utilisation

The utilisation of protein is affected by a variety of factors, including the site of digestion in the GIT, age, dietary level of protein, carbohydrates, fibre, and lipid intake. Some of these factors are briefly described in the sections that follow.

2.2.4.1 Site of digestion

Dietary protein digestion in birds begins in the proventriculus (stomach), but it occurs primarily in the small intestine. The large intestine contains the unabsorbed peptides and undigested proteins from either dietary or endogenous origins (Bedford & Apajalahti, 2022). These components are subjected to fermentation by the intestinal microflora in the large intestine. Proteins that are not digested in the small intestine enter the caecum, where they undergo fermentation (Apajalahti & Vienola, 2016). Protein digestibility is affected by large intestine

microflora. Unabsorbed amino acids and undigested proteins enter the colon and are excreted through faeces, leading to environmental pollution (Juquiera *et al*., 2006).

2.2.4.2 Age and physiological state of the quail

Age and physiological state are known to influence the ability of birds to digest and absorb protein (Bryden & Li, 2010). The chick's digestive system and enzyme concentration improve with age. Nitsan *et al*. (1991) reported an increase in digestive enzyme concentration in the first 14 days of age. Between 1 and 14 days of development, dietary nutrients may be poorly utilised. In a study involving the effects of age on nutrient digestibility in chicks fed with different diets, Bata & Pearson (2002) concluded that the digestibility of amino acid increased and improved with age.

2.2.4.3 Dietary level of protein and amino acids

The efficiency of absorption is limited by a high dietary protein content. Protein levels less than 16% in the diets lead to higher percentages of protein being extracted by the gut (Junqueira *et al*., 2006). Joseph *et al*. (2000) found similar results on body weight when feeding a different level of protein (18% versus 16% crude protein) on breeder chickens. A previous study by Aletor *et al*. (2000) reported a significant increase in protein efficiency ratio with a reduction in the dietary crude protein content in broiler chicken diets. Patras *et al*. (2009) investigated the effects of dietary fibre and protein level on the nitrogen excretion pattern of pigs and found that total nitrogen excretion was higher in high protein diets (18.8 g/day) compared to low protein diets (12 g/day). The authors concluded that a reduction of protein content in the diet was an effective way of reducing nitrogen excretion, thereby lowering ammonia emission. Thus, the metabolic utilisation of amino acids in the gut depends on the composition of the feed with respect to the presence or absence of indispensable and dispensable essential amino

acids (Ten have *et al*., 2007). A high concentration of threonine in the ileal digesta has been

attributed to a high production of mucin, leading to a low ileal apparent digestibility coefficient for threonine in the diets (Ravindran *et al*., 2008).

2.3 *Moringa oleifera*

Moringa oleifera Lam (*Moringaceae*), as shown in plate 2.2, is a valuable plant commonly found in many tropical and subtropical countries, has a wide range of medical benefits including antibacterial and antioxidant activities (Bennett *et al*., 2003; Ferreira *et al*., 2008). Data derived from nutrient characterisation of *M. oleifera* leaves clearly shows a rich nutritional profile of essential minerals, a good source of protein and amino acids, vitamins, β-carotene and various phenols with different properties as nutritional supplements (Moyo *et al*., 2011). Numerous phytogenic feed additives have been shown to affect the palatability of the feed due to its aromatic qualities, depending on the quantity of the individual constituents (Applegate *et al*., 2010).

Tropical insular weather is optimal for the plant to flourish. It thrives in hot, arid climates and less rich soils, and it is not greatly affected by drought. It may even grow well in wet tropics (Anwar *et al*., 2007). Although nearly every part of the *M. oleifera* tree can be utilised for food, medicine, or industrial processes, it is regarded as one of the most useful trees in the world (Khalafalla *et al*., 2010). People consume its fresh pods, flowers, and leaves as vegetables, and some people feed it to their animals (Anjorin *et al*., 2010). The possible benefits of this tree include better nutrition, increased food security, and enhanced support for rural development (Hsu, 2006). Recently, in most countries where it was not a native plant, there has been a significant renewed interest in the nutritional benefits of moringa (Reyes *et al*., 2006; Oduro *et al*., 2008). Its reported nutritional, medicinal, and prophylactic benefits were shown to improve animal productivity, conferring logic to the growing interest in the tree (Fahey, 2005). The leaves are rich in vitamins, minerals, and amino acids (Isitua *et al*., 2015). Due to this, the

leaves have been used to treat malnutrition, particularly in young children and nursing women. As a short-term substitute for chemoprophylaxis, nutrition is also vital for both humans and livestock (Mushtaq *et al*., 2021). An animal's ability to fight the negative effects of parasitism and diseases is strongly influenced by nutrition (Anwar *et al*., 2007). A well-fed animal, when exposed to an infection, will resist diseases better than an animal already weakened by malnutrition. When an animal is exposed to a pathogen, its immune system responds to fight off infection (Malafaia & Talvani, 2011). This includes the formation of antibodies to fight infection and the use of white blood cells to attack pathogens (Busani *et al*., 2011). For an animal to gain immunity, it needs energy, proteins for the manufacture of antibodies and cells, minerals (zinc, copper, iron), and vitamins (A and E) in sending signals to various parts of its body to fight infections (Moyo *et al*., 2011).

Plate 2.2. *Moringa oleifera* leaves and powder.

2.3.1 Nutritional composition of Moringa oleifera

The moringa tree is particularly promising as a feed source in the tropics since it is fully leafed out at the end of the dry season, when other feeds are typically scarce (Kushwaha *et al*., 2015).

It is a tree characterised by rapid growth, drought-resistance, and its morphological components are a great source of nutrients. They contain cytokinins in the form of zeatin as well as other beneficial phytochemicals such as vanillin, beta-sitosterol, caffeoylquinic acids, kaempferol, quercetin and carotenes (Kushwaha *et al*., 2012). The leaves of the *M. oleifera* tree are a rich source of micronutrients and they are a natural source of many essential minerals (Kushwaha & Chawla, 2015). Important trace elements such as calcium, iron, magnesium, zinc, and copper are present in the leaves of the *M. oleifera* plant (Kasolo *et al*., 2010). *Moringa oleifera* also contains folic acid, pyridoxine, and nicotinic acid, and vitamins A, B, C, D, and E (Mbikay, 2012).

The presence of phytochemicals, including tannins and alkaloids, as well as anti-cancerous substances such glycosylates, isothiocyanates, glycoside compounds, and 24 glycerol 1-9-ocdecanoate, makes the leaves a viable alternative to animal feed (Berkovich *et al*., 2013). According to Bennett *et al*. (2003), Ferreira *et al*. (2008), and Moyo *et al*. (2011), MOLM contains 275 g/kg of CP, 193 g/kg of crude fibre, and 223 g/kg of crude fat. Mbikay (2012) reports that the leaves have demonstrated an ability to regulate glucose metabolism in both humans and rats, controlling complications like lipoedema and hyperglycaemia that make cells vulnerable to oxidation. According to Singh (2018), MOLM promotes increased breast milk production in young mothers of premature infants.

2.3.2 Growth promoting efficacy

In general, feed additives promote growth by lowering the host animal's immune defence stress during critical situations and increasing the intestinal capacity of essential nutrients for absorption, thus assisting animals in growing better within the framework of their genetic potential (Wendish *et al*., 2008). Previously, the use of plants in monogastric diets like *Ananas comosus* and MOLM wereprohibited due to various deleterious effects attributable to their

phytochemical composition, which varies widely due to the environment and climate (Steiner & Wegleitner, 2007; Xiao *et al*., 2012). However, according to Hernández *et al*., 2006), it is important for researchers to establish the maximum tolerance level of each phytogenic. The addition of phytochemicals into animal feeds increases the secretion of digestive juices (Jamroz *et al*., 2005; Steiner & Wegleitner, 2007) and lowers intestinal pH (Huyghebaert *et al*., 2011), thereby improving the efficiency of nutrient utilisation, reducing the incidence of digestive disorders, and increasing the performance of broiler chickens while enhancing their health (Botlhoko, 2009). It has been demonstrated that a particular class of steroids found in MOLM boosts protein synthesis, promoting the growth of muscles and bones (Bamishaiye, 2011).

Processing of broiler feeds has been confirmed to strongly influence physiological functions of the digestive tract and improve feed conversion (Engberg *et al*., 2000). In contrast, the same bioactive phytochemicals can have a strong odour or pungent taste, thereby limiting feed intake and utilisation (Windisch *et al*., 2008). Chicks that are fed with diets containing more than 5% MOLM were found to have poor feed conversion efficiency (FCE) and growth performance (Olugbemi *et al*., 2010). Ogbe & Affiku (2012) found no significant differences in carcass and organ weight in birds fed with MOLM-containing diets. Polyphenol concentrations as low as 0.05 mg/ml can exert an inhibitory effect on α-amylase, pepsin, trypsin, and lipase, reducing enzyme- mediated hydrolytic reactions and the digestibility of carbohydrates, proteins, and lipids (He *et al*., 2006). From a nutritional point of view, birds cannot reach their genetic potential if dietary nitrogen is supplied exclusively in the form of essential amino acids. In this respect, dietary composition of non-essential amino acids in the diet is also critical.

Study by Joshi & Mehta (2010) found that the protein content of MOLM is higher than that of many commonly consumed leafy green vegetables such as spinach (2%) and mint (4.8%) and is equivalent to the protein content of many legumes such as moth beans and cowpeas, which

contain 22 to 24% CP. The leaves of the *M. oleifera* plant are rich in sulphur

containing amino acids (Bennett *et al*., 2003; Ferreira *et al*., 2008; Moyo *et al*., 2011). A study by Makkar & Becker (1997) suggested that high milk producing cows could benefit from consuming MOLM as a protein supplement. They reported high levels of true protein (230 g/kg), 33% of which remains available in the intestine, adequate levels of all essential amino acids, and low levels of antinutritional factors. However, the fat content in MOLM is considerably low, while the carbohydrate content after dehydration is comparable to that of many carbohydrate-rich cereals and vegetables (Joshi & Mehta, 2010). The results of Bamishaiye (2011) show that MOLM has a very high carbohydrate content, with no significant difference between the early and middle stages, but the values decrease in the late stage, indicating a decrease and an increase, respectively, in carbohydrate and protein content as the plant ages.

2.3.3 Anti-nutrients in Moringa oleifera leaf meal

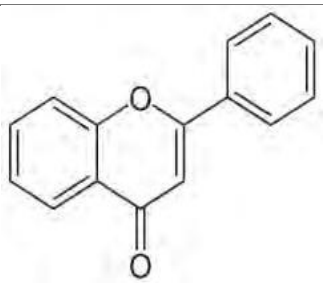
According to Gemede & Ratta (2014), anti-nutritional factors are compounds that reduce the availability of one or more nutrients when present in animal feed. The result is a reduction of feed utilisation and/or feed intake. *Moringa oleifera* contains anti-nutritional components such as Condensed tannins (CT) and fibre that may cause a decrease to its feed value (Stevens *et al*., 2015). Anti- nutritional factors may occur as natural constituents of plant and animal feeds, as artificial factors added during processing or as contaminants from the ecosystem (Jithender *et al*., 2019). Anti-nutrients are substances which interfere with the metabolism and utilisation of nutrients by the body; examples are phytates, oxalates, tannins, fibre and saponins (Thakur *et al*., 2019). Globally, *Moringa oleifera* is a popular plant species due to its usefulness as a food, medicinal, and aesthetic plant (Berushka & Himansu, 2012). However, like any other plant species, this species also contains anti-nutritional factors,

which can reduce the bioavailability of nutrients in the various parts of the plant. *Moringa oleifera* leaves have been found to contain anti-nutrients such as tannins, lectins, cyanogenic, oxalates, phytates saponins, glucoside and glucosinolate (Moyo *et al*., 2011; Lo *et al*., 2018). *Moringa oleifera* has been reported as a good source of vitamins and amino acids (Olugbemi *et al*., 2010), however the presence of these antinutrients could restrict their utilisation (Sallau *et al*., 2012). Although anti-nutritional factors play harmful functions by chelating nutrients and forming a binding factor with food values, making the nutrients non-bioavailable in the system, it is significant to mention that they can also have positive effects at certain levels (Soetan & Oyewole, 2009).

2.3.3.1 Tannins

Tannins are natural compounds found in plants and they are made up of an aromatic benzene ring substituted with hydroxyl groups (Kim *et al*., 2003; Rubanza *et al*., 2005). *Moringa oleifera* leaves have 12 g/kg tannins in a DM basis and phytates at 21 g/kg (Makkar & Becker, 1997). Tannins are naturally occurring plant polyphenols with a molecular weight greater than 500 and can bind and precipitate protein (Makkar, 2003). The amount and type of tannins synthesized by plants vary with stage of growth and environmental conditions. Tannins form complexes with proteins through covalent, hydrogen and ionic bonding (Figure 2.1), and these complexes are broken under high acidity or high alkalinity conditions (Salem *et al*., 2005). The isoelectric point, molecular weight, tertiary structure, and compatibility of the binding sites all affect how strongly tannins bind to proteins in complexes (Silanikove *et al*., 2001). Tannins can be found in almost all parts of a plant as well as in various plant tissues (Scogings *et al*., 2004). They are found in the seed, roots, stem, leaf, and bud, and they play a defensive role against predation, microbial infestation, and disease (Dutta *et al*., 2014). Tannins are divided into hydrolysable and condensed forms (Figure 2.1). Hydrolysable tannins are characterised by

a central carbohydrate core containing various phenolic carboxylic acids such as gallic acid, ellagic acid and hexahydroxydiohenic acid (Ashok & Upadhayaya, 2012; Figure 2.1).



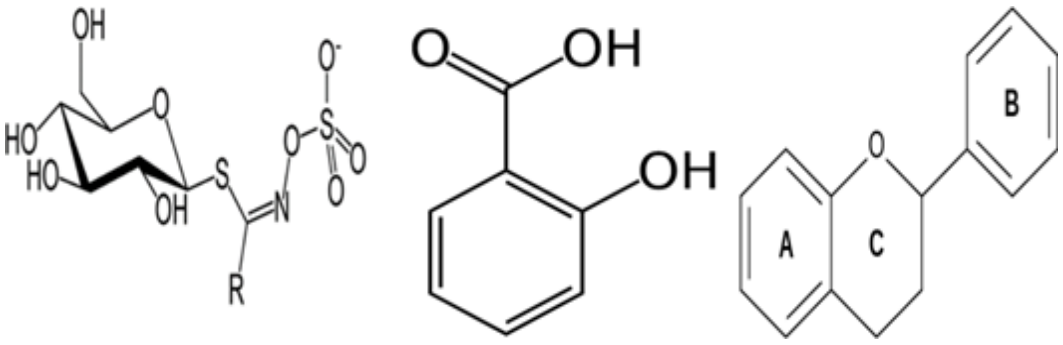
Gallic acid	Flavone
hydrolysable tannins	condensed tannins

Figure 2.1. Structure of condensed and hydrolysable tannins (Source: Ashok and Upadhayaya, 2012).

These tannins are easily hydrolysed by acids, alkali, or some enzymes. Hydrolysable tannins are not present in cereals and/or legumes. As a result, they are associated with a positive impact on animal nutrition (Mueller-Harvey, 2006). When these tannins are hydrolysed, gallic or epigallic and sugar are formed on the other hand, condensed tannins are dimers or oligomers of catechin, epicatechin or similar units (Behrens *et al*., 2003). The structure of these units is joined by carbon-carbon bonds, which can be broken by hydrolysis (Halimani *et al*., 2007). Condensed tannins have been identified as a major factor affecting productivity of birds fed with leaf meals from legume trees and shrubs. The reactivity of proanthocyanidins with molecules of biological significance has important nutritional and physiological consequences (Kawabata *et al*., 2019). Their multiple phenolic hydroxyl groups lead to the formation of complexes with proteins (Ozdal *et al*., 2013). They have a negative effect on protein metabolism and decrease palatability of feeds at high levels (Pratoomyot *et al*., 2010), but can be beneficial at very low levels.

2.3.3.2 Glucosinolates

The *M. oleifera* plant is high in compounds that contain the simple sugar rhamnose and a specific class of compounds known as glucosinolates and isothiocyanates (Fahey *et al*., 2001; Bennett *et al*., 2003). Glucosinolates are classified as a volatile anti-nutritive chemical with antimicrobial, antifungal, and antibacterial effects (Leeson & Summers, 2005). Four glucosinolates, two of which are found in huge concentrations (65.5 µmol) in *M. oleifera* leaf tissues have been identified as 4-(α-l-rhamnopyranosyloxy)-benzylglucosinolate and three of its monoacetyl isomers (Bennett *et al*., 2003). The Structural motifs and backbones of major phytochemicals found in *M. oleifera* is shown in figure 2.2. In lipopolysaccharide stimulated RAW264.7 mouse macrophage cells, numerous novel isothiocyanates have been found to inhibit inducible nitric oxide synthase expression and nitric oxide formation (Cheenpracha *et al*., 2011). Additionally, it is reported to have anti-inflammatory and chemo-defensive properties against most of cancers (Park *et al*., 2011). The impact of glucosinolate absorption is harmful in non-ruminants compared to ruminants, and higher in laying hens and turkeys compared to broilers, likely due to their short life expectancy (Tripathi & Mishra, 2007).



A B C

Figure 2.2. Structural motifs and backbones of major phytochemicals found in *M. oleifera* leaves. A = Glucosinolates; B = Phenols; C = Flavonols (Source: Mbikay, 2012).

2.3.3.3 Phenolics and flavonoids

Phenolic compounds are commonly found in both palatable and non-palatable plants, and they are known to have many organic effects, consisting of antioxidant properties (Kahkonen *et al*., 1999). There are two types of phenolic acids that can be distinguished: hydroxybenzoic acids (gallic and ellagic acids) and hydroxycinnamic acids (p-coumaric, caffeic, ferulic, chlorogenic, and sinapic acids). Hydroxycinnamic acids are more prevalent in plants than the hydroxybenzoic acids (Kasetti *et al*., 2012) and many of these have been eliminated in the leaves of *M. oleifera* (Mbikay, 2012). The phenolic content is lowest in young *M. oleifera* leaves and it slowly increases as the leaves mature (Iqbal & Bhanger, 2006), hence they should be fed in a limited amount when leaves are matured. In contrast to flavonoids, which are hydroxylated phenolic compounds that occur as a C6-C3 unit linked to an aromatic ring, flavones are phenolic molecules with one carboxyl group that produce flavonol on the addition of a three-hydroxyl organization (Cowan, 1999).

The leaf extracts from *M. oleifera* contain a variety of bioactive substances, including flavonols and phenolic compounds (Kahkonen *et al*., 1999; Lemmen, 2007). Flavonols are a category of flavonoids that have the three-hydroxyflavone spine (IUPAC name: three-hydroxy-2-phenylchromen-4-1). Among the classes of phytochemicals determined inside the *M. oleifera* plant, flavonoids hold the maximum of antioxidant capacity (Mbikay, 2012). The predominant flavonols in *M. oleifera* leaves are quercetin and kaemferol, of their 3'-O-glycoside forms (Kahkonen *et al*., 1999; Joshi & Methta, 2010; Mbikay, 2012). Together with kaempferol rhanoside, kaempferol and quercetin-glucoside; other flavonol glycosides, namely, benzoic acid glycosides and benzaldehy de-glucoside, syringric acid, gallic acid and rutin have been isolated from the defatted aqueous methanolic extract of *M. oleifera* leaves (Manguro & Lemmen, 2007). According to Xiao *et al*. (2011), phenolics and flavonoids play a pivotal role

with regards to their health benefits to animals as they have anti-inflammatory, antioxidant, and anticancer properties.

2.3.3.4 Non-starch polysaccharides

In most countries, the primary cereal grains used in diet formations are corn and soybeans (Owen *et al*., 2015). However, the least costly diet formulation commonly requires utilising other grains or agricultural by-products, which could contain less variable levels of anti-nutritive components (Ravindran, 2012). The cell wall of cereals is composed of complex carbohydrates, which are loosely termed non-starch polysaccharides (NSP) (Chotinsky, 2015). Corn derived distillers' dried grains with soluble is a by-product of ethanol and contains higher amounts of NSP relative to corn (Świątkiewicz & Koreleski, 2008). Other cereal grains such as wheat also contain higher levels of NSPs. Lee *et al*. (2017) found that arabinoxylans found in wheat, severely affected broilers' growth and FCE. In addition, carbohydrate, lipid, and protein digestion were generally inhibited by wheat pentosans (Wang *et al*., 2015). Non-starch polysaccharides increase the viscosity of the digesta, decreasing the rate at which digestive enzymes pass through, and inhibit the mixing of gut contents (Yaghobfar & Kalantar, 2017). Several other researchers have noted that the presence of NSPs can affect a bird's performance by increasing intestinal viscosity, reducing nutrient digestibility, and increasing feed conversion ratio (Khattak *et al*., 2006; Shakouri, 2009; Arczewska-Wlosek, 2019).

2.4 Tannin de-activation strategies

Several methods have been used to deactivate detrimental and toxic effects of tannins to improve the nutritive value of feeds. These methods include the use of binding agent (polyethylene glycol, polyvinylpyrrolidone, polyvinyl polypyrrolidone, acetic acid and sodium hydroxide), heating, soaking in water and drying, wood ash, chopping and storage,

urea, solid state fermentation and exogenous enzymes (Silanikove *et al*., 2001; Makkar, 2003; Ben Salem *et al*., 2005a). The methods are commonly grouped into physical and chemical methods.

2.4.1 Physical methods

Ben Salem *et al*. (2000) and Makkar (2003) observed that drying reduces levels of condensed tannins in *Acacia* foliage. For example, drying cassava and Leucaena leaves at 90°C for 24 hours decreased tannin content by 56.7% . The tannin content can be reduced also by storing fresh (40% moisture leaves) chopped leaves at 37°C prior feeding (Bhat *et al*., 2013). Grinding and chopping also decrease the negative effects of tannins in leaf meals (Abd El Tawab *et al*., 2018). Soaking is amongst the recommended methods in reducing the detrimental effects of tannins (Medungu *et al*., 2012). Some methods of physical treatment do not seem to be economically viable and are less effective due to intensive labour requirements and time consuming. However, chopping of fresh leaves, water soaking and then storage can be of practical use to resource-limited farmers as these require only minor changes in normal farm practices (Bhat *et al*., 2013). Moreover, these methods can reduce the negative effects of tannins in leaves (Ben Salem *et al*., 2005b).

2.4.2 Chemical methods

Several chemical methods from urea to tannin-binding agents for deactivating tannins have been tested (Kyarisiima *et al*., 2004; Alipour & Rouzbehan, 2007). Kyraissima *et al.* (2004) reported that pre-treatment of sorghum with wood ash reduces the level of tannins in tannin-rich feed resources. The use of urea to neutralise tannin-rich leaf meals is recommended and gives extra nitrogen to the animals (Hlatini *et al*., 2018). Moreover, the addition of urea is effective due to the higher pH caused by urea-ammoniation. Tannin-binding agents and enzymes are also used to neutralise anti-nutritive factors (Schons *et al*., 2012; Tshabalala *et al*.,

2013). Polyethylene glycol, polyvinylpyrrolidone, polyvinyl polypyrrolidone, charcoal and sodium hydroxide are common examples of tannin-binding agents (Kumanda, 2019).

Challenges of chemical methods include the loss of soluble nutrients, analyses in laboratory work and to the cost of these for resource-poor farmers. There is little information on the use of tannin-binding agents such PEG to overcome problems associated with reduced feed value, toxic effect and utilization in quail fed with MOLM-containing diets. Therefore, there is a need to explore the influence of PEG in growing quail fed with diets containing *M. oleifera* leaf meal. Animal performance is expected to increase linearly as PEG levels increase, until a point when the performance starts to decrease. The linear increase at low to moderate inclusion levels need to be investigated such that farmers could determine cost-effective and sustainable levels to optimize growth in quail.

2.4.3 Polyethylene glycol

Polyethylene glycol is a non-nutritive synthetic polymer, an oligomer or polymer of ethylene oxide with a molecular mass below 20 000 g/mol (Makkar, 2003). Polyethylene glycols of different molecular weights are available commercially, and their molecular weight ranges from as little as 200 upwards. The chemical formula for PEG is (C2H4O) n+1 H2O (Henning, 2002) where "n" represents the average number of oxethylene groups, and below 55°C it is a free-flowing white powder freely soluble in water. Molecular weight of PEG from 200 to 600 occurs as a slight vapour, colourless, slightly hygroscopic liquid with a slight characteristic odour (Pandey *et al*., 2012). The PEG with higher molecular weight is available as free-flowing white powder (1500 and 4000 molecular weights) and creamy with flakes for the range 4000 to 8000 (Gao, 1993). Bhat *et al*. (2013) reported that PEG 4000 and 6000 molecular weights have more capacity to bind with tannins than PEG of various other molecular weights. Therefore, it might be preferred to others because of its ability to bind tannins at near neutral

pH and make plant proteins to be more available for digestion and absorption by binding to tannins. Ferraz de Oliveira *et al*. (2008) used PEG 6000 to determine its effect on protein output of free-range pigs. Crude protein concentration from faeces was low, indicating availability of dietary protein (Ferraz de Oliveira *et al*., 2008). The most important property of PEG is its solubility in water, making it ideal for inclusion in leaf meals (Henning, 2002; Ben Salem *et al*., 2005b). The PEG has numerous properties such as a viscosity, melting range, hygroscopicity, solubility in organic solvents, PEG solvency, additional property and blended PEG compounds (Turner *et al*., 2011). He *et al*. (2007) reported that PEG has been used for fractional precipitation of properties. It is usually used as a substance to make proteins from ingested tannin containing feed available for utilization by livestock (Akande *et al*., 2010).

Polyethylene glycol blocks the formation of tannin-protein complex. According to Besharati & Taghizadeh (2011), PEG breaks already formed tannin-protein complexes due to its high affinity (together with larger number of atoms). It deactivates tannins over a wide pH range of 2 to 8.5. Adequate oxygen molecules from water-soluble PEG form hydrogen bonds together with phenolic and hydroxyl group in tannins (Silanikove *et al*., 2001). This phenomenon makes protein and other nutrients available for utilisation and increases the voluntary intake of leaf meals (Mantz, 2008). A minimum of 1.8 g PEG can completely reverse the binding effect of tannins (Min & Hart, 2003). Therefore, the addition of PEG in diets containing high levels of tannins improves the feed value of such diets.

2.4.4 Nutritive value of polyethylene glycol-treated leaf meals

In poultry, the effect of PEG treated MOLM on the performance of broiler chickens has been studied (Kumanda *et al*., 2019; Van Niekerk *et al*., 2020), leading to famers be able to utilize PEG in enhancing the production efficiency of poultry and this might result in the improvement of economic status of famers and also accelerating reduction in food scarcity.

. Oduguwa *et al*. (2007) reported that PEG (1 g and 10 g/kg DM + 30 g of malted sorghum with 140 g/kg tannin) had no improvement on the apparent amino acid digestibility `and true amino acid digestibility. This could be associated with tannin bound by other components such as fibre. Mansoori & Acamovic (2009) determined that PEG improved protein digestibility, utilisation and reduced negative effect of tannins in the GIT of birds fed with high tannins feeds. The PEG also increases the palatability of high tannin forages, thereby increasing their intake (Nsahlai *et al*., 2011). Detrimental effects in nutrition caused by tannins may not be completely reversed by application of PEG. However, the presence of PEG may increase astringency and appetite when low-tannin alternatives offer fewer nutrients than the high-tannin forage (Egea *et al*., 2016). Unfortunately, the information on the effect of PEG supplementation on the performance of quail fed with MOLM diet has not been verified and studies in this area are generally limited. The adaptation of birds to different types of diet and levels of nutrition is important in quail production. Hence, blood metabolites concentrations are commonly used in monitoring health (functioning of liver, heart, and kidney) and nutritional problems of the birds.

Blood metabolites are undoubtedly the best practical predictor, and it could be useful in preventing metabolic complications together with damages in animal (Khanyile *et al*., 2014). Changes in the constituent compounds of blood when compared to normal values could be used to determine metabolic state, together with feed quality. Moreover, blood indicators alongside body weight conditions of quail could detect the status of energy, protein, glucose, and early signs of metabolic disorders (Lim *et al*., 2013). Serum protein, iron, and cholesterol are vital in identifying the nutrient profile of animals (Ndlovu *et al*., 2009). Activities of aspartate aminotransferase, alkaline phosphatase and alanine aminotransferase, which are slightly

dependent on cholesterol indicate liver damage and could be used to describe the quality of feed given to the quail (Rashidi *et al*., 2020). The effect of PEG pre-treatment of MOLM diets on blood parameters of growing quail birds has not been studied. Therefore, an evaluation of the nutritional status of the quail is vital because such data is fundamental in the formulation of their quality diets.

2.4.5 Constraints to utilization of polyethylene glycol

The PEG has many oxygen atoms and contains sufficient oxygen molecules in a chain to form strong bonds with phenolics and hydroxyl groups of tannins (Ren *et al*., 2013). It has the disadvantage, however, of requiring tissue samples. Turner *et al*. (2011) discovered that the potentially unfavorable effects that might be caused by PEG can be divided into several groups. Adverse side effects in the body can be triggered by the polymer itself or by side products formed during the synthesis that led to hypersensitivity. When an excess amount is given, unexpected changes in the body of the animal can occur with PEG-based carriers. The PEG requires some skills and knowledge to use without compromising the health of quail. The PEG might be administered to animals in different ways such as spraying leaves, oral dosage and mixed with diet (Ben Salem *et al*., 2000).

Application of PEG by spraying the standing plants prior utilisation requires a lot of labour and it is time consuming, while mixing with diets is preferred. Oral dosage may include a slower onset of action, irritating and stressing to animals (Turner *et al*., 2011). Otherwise, spraying and oral dosage both require a moderate technical skill accompanied with confidence. The use of PEG needs to be performed accurately, rapidly, and humanely in animals (Hlatini *et al*., 2018). Application of PEG has been recommended even though the methods are either uneconomical and/or impractical under farmer management. There is little in the literature regarding the supplementation of PEG on leaf meal diets for the Jumbo quail.

2.5 Fibre utilisation in quail

According to McDonald & Whitesides (2002), the term fibre refers to cell walls of plant tissue, which are mostly composed of lignin, cellulose as well as hemicelluloses. Varasthegani & Dahlan (2014) have identified two terms that are used interchangeably in animal nutrition, namely crude fiber and roughage, where crude fiber refers to the structural carbohydrates of cellulose, hemicellulose and lignin in the plant cell wall, however, the composition of crude fiber in each individual plant differs from other plant species. Dietary fiber intake ranges from 3 to 4% but should not exceed 7% for optimal poultry growth performance (Varastegani & Dahlan, 2014). A lower than recommended fiber content in quail feed increases the risk of cannibalism and gastrointestinal infections.

A balanced diet is necessary for the maintenance of the health and productivity of the poultry (Ali *et al*., 2021). Some reports suggest that several factors such as the amount of fiber, species, age of the birds, the type and quantity of feed consumed, the nutritional composition of the diet, the level of anti-nutritive ingredients, management, and environmental conditions all factors affect how well quail can digest fiber (Khattak *et al*., 2006; Mateos *et al*., 2013; Bokau *et al*., 2018). Although simple non-ruminants do not digest dietary fiber as much because they lack the digestive enzymes needed to hydrolyze the insoluble NSP, it plays a significant function in the animal's digestive system (Jha *et al*., 2019).

Exogenous enzymes are added to the diet or feed by-products to enhance fiber digestion and, ultimately, improve animal performance, hence minimizing their detrimental impact on animal performance (Tufarelli *et al*., 2007; Alefzadeh *et al*., 2016). According to Nikam (2016), NSP can be classified as either soluble (β-glucose, arabinoxylan, arabinogalactose, xyloglucon, etc.) or insoluble (cellulose). Nikam *et al*. (2017) and Khobondo *et al*. (2019) indicated that soluble NSPs have the capacity to hold water in their matrix by producing a loose gel network. This

increased viscosity lowers the digestion of lipids, proteins, and carbohydrates. Additionally, dietary fiber increases the size duodenum (Oliaei *et al*., 2016), which significantly enhances nutrient digestion and levels of healthy gut microbes (Scheideler *et al*, 2005).

2.6 Poultry gut enzymes

Digestion in poultry relies on endogenous digestive enzymes. Bokau (2018) have attributed possible changes on endogenous enzymes production to the chicken birds' age, health status, consumed feed type, environmental factors including the dose of exogenous enzyme. According to Khattak *et al*. (2006), the enzymes that hydrolyse NSP in cell wall of grains are not produced by poultry. Buchanan *et al*. (2007) argues that poultry depend on acid digestion within proventriculus and microbial digestion in the caeca and large intestine. In Jumbo quail, the basal diet is composed of various feedstuffs that includes maize, soya, wheat, barley, and rice. Additionally, these feedstuffs are not readily digested by poultry hence they are not desirable for usage in metabolic process because of high NSP content (Buchanan *et al*., 2007). Kamran *et al.* (2002) attributed the lack of suitable enzymes in the GIT and anti-nutritional factors availability to impair digestion.

Buchanan *et al*. (2007) reported that the digestion of NSP can range from 13% in lupin diets to 21.9% in wheat diets excluding supplementation of enzyme, whereas maize and rice contains 9% and 25% of NSP, respectively. According to Ravindran (2013) and Nikam *et al.* (2017), the addition of enzyme in poultry diets is likely to ameliorate the negative effects of NSP by improving the efficiency of feed. Additionally, Alagawany *et al.* (2018) states that exogenous enzymes are generated from various sources such as microorganisms and plants. Enzymes over the past years have been utilised in feed industry, this includes enzymes such as cellulase (ß-glucanases), xylanases and associated enzymes, phytases, proteases, lipases, and galactosidases (Khattak *et al*., 2006).

2.6.1 Exogenous enzyme supplementation in poultry diets

The use of exogenous enzymes in poultry production has been propelled by the existence of anti-nutritional factors in poultry feeds (Lee *et al*., 2014). Several studies have shown the use of exogenous enzymes in poultry diets as the effective approach to maximize the digestibility of complex molecules, particularly in young animals without a well-developed intestinal enzyme profile (Oliaei *et al*., 2016). According to Khattak *et al*. (2006), NSP is hydrolysed by exogenous enzymes, leading to a digesta viscosity, improvement of nutrient absorption, and improved growth performance. This is further supported by Lee *et al*. (2014), who attributed the benefits of using exogenous enzymes to lower anti-nutritional factors and improve the use of dietary energy and proteins, therefore resulting in an improved poultry performance.

Additionally, Bedford & Partridge (2001) and Ismail *et al*. (2011) indicates that utilisation of exogenous enzymes is beneficial due to the ability to eliminate anti-nutritional factors, which impair normal digestive processes. This is made possible through the digestion of fibre components of the diet or creating an environment that promotes limited bacterial in the caeca (Ismail *et al*., 2011). Furthermore, Alam *et al*. (2003) reports that incorporation of enzymes in chicken diets is directly linked to reduced feed intake because they reach the required nutrient requirements through improved feed conversion ratio. The emission of nitrogen (N) in birds that are fed with enzyme-based diets has reduced while CP digestibility has increased by 1.9% (Zanella *et al*., 1999). This is contrary to a study by Buchanan (2007) who did not observe any important effect on performance or digestibility variables when an exogenous enzyme was added in a variety of poultry feeds.

2.6.2 Enzymes used in the poultry industry

Panda *et al*. (2014) defines enzymes as a biological catalyst that brings about biochemical reactions without themselves undergoing any change. In their nature, enzymes are proteins and

are made of amino acids arranged in a sequence. They speed up the chemical reactions of the living cells on specific nutrients from feedstuffs to support organs and tissues. Furthermore, enzymes are categorised based on substrates upon which they react together with their specificity (Alagawany *et al*., 2018). According to Bedford (2000), there are three types of enzymes that are currently in use within the poultry industry, such as viscous grain enzymes targeting rye, wheat, oats, and barley; non-viscous grain enzymes targeting corn and sorghum; and lastly phytase targeting phytate-rich substrates. Bedford & Partridge (2001) reported that the use of enzyme technology is the principal strategy to improve the nutritive value of feedstuffs. Low-nutrient feed cannot be used in poultry diets due to high fiber content. Enzymes may be a practical solution to the problem in poultry diets (Tavernari *et al*., 2008). The use of enzymes, according to Ravindran (2013), results in a breakdown of the integrity of the plant cell wall and the subsequent release of nutrients that were encapsulated by the cell wall.

2.6.3 Viscozyme® L supplementation in quail feeds

Poultry do not naturally acquire sufficient enzymes for the hydrolysis of NSP found in cereal grain cell walls. These NSP often remain unhydrolysed and could result in low reduced feed efficiency (Ayres, 2019). Carbohydrases can be added as a supplement to reduce the harmful effects of NSPs by decreasing intestinal viscosity, and an improving nutrient digestibility (Raza *et al*., 2012; Alagawany *et al*., 2018). Further, NSP-degrading enzymes can be synthesized by genetic modification or from microbial sources (Bedford & Cowieson, 2012).

Viscozyme® L is a multi-enzyme complex that contains a variety of carbohydrases, such as arabanase, cellulase, glucanase, hemicellulase, and xylanase (Gama *et al*., 2015). The enzyme preparation is produced from a selected strain of *Aspergillus aculeatus* (Nakazawa *et al*., 2012). Viscozyme® L was shown to be an effective enzyme for the extraction of polyphenols (Zheng *et al*., 2009). It also acts on branched pectin-like substances found in plant cell walls.

Viscozyme® L is a clear brown liquid with a density of approximately 1.2 g/ml. It is a special enzyme preparation used in the breakdown of cell walls for the extraction of useful components from plant tissues (Perussello *et al*., 2017). The optimal conditions for Viscozyme® L with its several and complex activities are a pH range of 3.3 - 5.5 and a temperature of 25 - 55 °C (de Figueiredo *et al*., 2018). Viscozyme® L is non-flammable, completely miscible with water and safe when used according to the manufacturer's directions (Kumanda, 2019).

In addition, the Viscozyme® L multi-enzyme (VME) is reported to improve the digestibility of amino acids (AA) (Romero *et al*., 2013). However, there is no supporting evidence on the positive and/or negative alteration caused by exogenous VME on endogenous secretions (Romero *et al*., 2013). Hussein *et al*. (2020) reported on the growth, carcass traits, and meat quality of broilers and found no difference in weight gain or feed conversion ratio in birds fed with a low-energy diet supplemented with a multi-enzyme. Likewise, Kumanda (2019) reported no effect on weight gain when VME was added to broilers reared on red grape (*Vitis vinifera* L.) pomace-based diet. This is not surprising as Lee *et al*. (2014) also reported similar results in broilers. Nevertheless, other scholars have reached a common understanding on the ability of VME to enhance nutrient bioavailability and thus improve weight gain and gain-to-feed ratio (G:F) in simple non-ruminant diets (Cowieson & Bedford, 2009; Hajati *et al*., 2009; Hana *et al*., 2010). Differences on the observations and findings reported by different scholars can be attributed to different study designs and environments, various types of experimental animals and different dietary treatments. It is, therefore, important to determine the effect of the VME in Jumbo quail reared on MOLM-containing diets.

2.6.4 Phytase

The MOLM contain significant amounts of phytic acid, which may inhibit nutrient absorption (Dai *et al*., 2020). According to Cowieson *et al*. (2017), phytase is currently a standard

additive in poultry diets. Considerable amounts of phytic acid in the MOLM may hinder nutrient utilisation. Therefore, having a thorough understanding of the action of the phytase and how it interacts with other enzymes is necessary for accurate interpretation of results. Myoinositol is released when phytic acid is hydrolysed by the phosphatase enzyme (phytase) to create inorganic orthophosphate (Gupta *et al*., 2015). Additionally, it functions with monoesters (phosphomonoesterase) (O'Brie & Herschlag *et al*., 2001).

A series of myoinositol phosphate intermediates, according to Vohra (2003) and Singh *et al*. (2011), helps phytases speed up the hydrolytic breakdown of phytate. There are different sources of phytases, including plants, animals, and microbes (bacteria, yeasts, and fungi). However, the most reliable source is the microbes (Ebune *et al*., 1995; Rao *et al*., 2009; Singh *et al*., 2011). The form and amount of phytase, the method of pre-treatment and inclusion (application), other feed ingredients, and the concentrations of nutrients, particularly protein and minerals, all have an influence on the efficiency and utility of phytase (Selle & Ravindran, 2007).

Since beneficial effect of exogenous phytases is attributable to the hydrolysis of phytate and the subsequent improvement of nutrient (minerals, amino acids, and energy) availability, Selle & Ravindran (2007) noted that there is still a gap in the understanding of the phytate-phytase matrix in poultry nutrition. Phytases increase the nutritional value of feeds and decrease phosphorus excretion, which is essential for the reduction of eutrophication. The use of protein and energy is enhanced by phytate-degrading enzymes (Selle & Ravindran, 2007; Oduguwa *et al*., 2007), and the digestive tract's health is enhanced due to decreased secretions (Pirgozliev *et al*., 2008). Additionally, they improve nutritional quality and lower levels of phosphorus contamination in the environment (Jain & Singh, 2016). Selle & Ravindran (2007) and Xu *et al*. (2011) reported that phytases degrade phytate-bound phosphorus by reducing phytic acid

complexes in plant ingredients, thereby increasing the bioavailability of phosphorus and calcium.

2.6.5 Protease

Exogenous protease has previously been included in commercial enzyme admixtures, but it is now only available as a mono-component enzyme (Cowieson & Adeola, 2005; Cowieson & Roos, 2016). Although there is little information on how individual proteases affect Jumbo quail reared on MOLM-containing diets, their use as single enzymes has grown. According to the theory, the protease enzyme acts in the plant cell wall by eliminating the structural proteins to promote faster digestion. According to Blazek (2008), the ability of protease to coagulate protein meals is influenced by both the type of dietary protein and the enzyme's nature. Proteases improve intestinal integrity by emulsifying fats and enhancing endogenously produced mucin by liberating fat and starch and equally reducing the antinutritional effects in the basal diet (Cowieson & Roos, 2016). Feed protease is also reported to increase the digestion of AA (Romero *et al*., 2013).

2.7 Factors affecting dietary exogenous enzyme supplementation in poultry

Digestion, absorption, animal health, and overall growth performance should all exhibit positive responses to animal production output (Khattak *et al*., 2006). According to Khattak *et al*. (2006) and Cowieson *et al*. (2006), the response to exogenous enzyme supplementation varies depending on the animal's type (poultry tended to respond effectively to dietary enzyme supplementation than pigs), age (younger animals tended to effectively respond to enzymes than older animals), species of intestinal microflora present in the GIT, the physiological state of the bird, quality or type of feed used, and levels of enzymatic. The study conducted by Zamini *et al*. (2014) established that the response is affected by concentration (relative to body weight) and whether an animal responds positively or negatively to supplementation.

2.8 Mode of action of exogenous enzymes

Different modes of action for exogenous enzymes have been reported by various researchers (Slominski, 2011; Ravindran 2013; Swiatkiewicz *et al*., 2015; Dida, 2016). Jzefiak (2007) suggested that exogenous enzymes might work by lowering the viscosity of the digesta in the GIT, since broiler feed contains mainly high fiber/starch grains in the form of NSP. These cereals include antinutritional, soluble indigestible polysaccharides like arabinoxylan, which has anti-nutritional effects in poultry. These arabinoxylans are present in cereals like wheat, rye, and triticale, according to Salami (2015) and Panda *et al*. (2014). However, NSP adversely affect the function of the GIT because they bind lots of water, increase viscosity in the gut, decrease digestion rate, growth rate, feed intake and nutrient availability (Khattak *et al*., 2006; Kalantar *et al*., 2015). Exogenous enzyme supplementation in poultry feed enhances performance of the bird by boosting the efficiency of host (endogenous) enzymes, breaking down soluble fibres, which allows the birds' digestive enzymes to utilise the substrates (Singh Kim, 2021). This increases the efficiency of digestibility of starch, protein, fat, amino acids, and energy (Bokau *et al*., 2018), changes the feed passage rate, which reduces the capacity of the intestinal contents to hold water and stimulates feed intake. It also increases the digestibility of phosphorus by 20 – 50%, which results in a significant reduction in phosphorus excretion (Woyengo & Nyachoti, 2011; Tang *et al*., 2014).

2.9 Effects of dietary protein on growth, health, and meat quality in quail.

2.9.1 Effect on growth performance

El-Katcha *et al*. (2015) conducted an experiment to investigate the effects of protein intake on growing quail and concluded that a protein supplementation of 3.2 g/bird/day is mandatory for optimum growth. Similarly, Ribeiro *et al*. (2014) observed that an improved quality of dietary protein improves body weight gain or growth rates. In an experiment conducted by Salahuddin

et al. (2012), the effects of protein on growth performance of broilers were investigated and they established that including 20% crude protein in the diets of the broilers boosted body weight gain. According to Mehaisen *et al*. (2017), feeding quail diets high in CP (25 vs. 20%) while maintaining the ambient temperature high (32°C) during the growing period enhanced weight gain. However, Khan *et al*. (2016) found no significant differences on the growth rate and feed intake of quail due to high protein and energy diet. Junqueira *et al*. (2006) supported this conclusion by observing that the performance of the birds was not affected by a higher protein content.

Additionally, the researchers reported that feeding protein in excess (more than 16%) did not affect performance but rather increased faecal nitrogen losses through faeces and contributed to environmental contamination (Kirwan *et al*., 2021; Arriaga *et al*., 2009). It was shown by Sahin & Kucuk (2001) that quail increase to a high final body weight when given a 20% protein diet with high digestibility. Kermanshashi *et al*. (2011) investigated the effects of dietary crude protein fluctuation on performance and nutrient retention in broiler chicken during a starting period and concluded that reducing crude protein essentially decreased body weight gain. According to Tarasewich *et al*. (2006), the protein content of the feed had an influence on the growth of quail and broilers, mostly in the early stages of life.

2.9.2 Effect on immunological responses and blood parameters

The defense mechanisms of poultry are severely influenced by proteins. The quantitative and qualitative aspects of the immune response to pathogens can be modified by adding enough protein in the diet (Povey *et al*., 2009). It has been demonstrated in poultry that an insufficient or excessive intake of dietary protein or amino acids alters immunological responses (Zhang *et al*., 2017). A deficiency of a dietary protein or amino acids has long been known to impair the

immune function and increase the susceptibility of animals to infectious diseases (Li *et al*., 2007).

The animal's blood parameters are essential measures for identifying any disorders (Ogunbajo *et al*., 2009; Etim *et al*., 2014). They provide information about the animal's health and nutritional status (Orawan & Aengwanich, 2007; Khawaja *et al*., 2012). The efficacy of an animal's metabolic processes and dietary intake affect its nutritional status (Etim *et al*., 2014). A lower-than-normal white blood cell count is an indication that a bird's immune system is under stress. An increase in the neutrophils:lymphocytes ratio is a good indicator of nutritional stress (Etim *et al*., 2014). According to Nwambe & Elechi (2009), packed cell volume (PCV) and haemoglobin values below the normal range indicate excessive levels of blood dilution and inefficient cellular oxygen transportation. There is a direct relationship between quality of feed and the composition of quail blood (El-Tarabany, 2016). Quail fed high protein diets had significantly higher red blood cell (RBC), haemoglobin (Hb), and PCV values than those fed low protein diets (Reda *et al*., 2020). According to Onu & Aniebo (2011), higher red blood cell counts are associated with healthy birds and high-quality protein diets. Kavitha *et al*. (2010) observed that as protein levels increase, mean corpuscular haemoglobin concentration (MCHC) values decrease. The protein intake consumed by birds has a significant effect on the levels of Hb, PCV and MCHC (Egbunike *et al*., 2009). However, little is known about the serum metabolites and haematological indices of Jumbo quail fed with various protein sources. However, the utility of MOLM as a protein source is restricted by the presence of CT, hence the utilisation of PEG is important to improve protein bioavailability and digestibility.

2.9.3 Effect on carcass characteristics

In their study, Siyadati *et al*. (2011) observed that quail fed a diet with less crude protein (18 versus of 20% CP) had lower carcass yields. Malik *et al*. (2013) examined the effects of dietary

protein levels (21% v/s 23% CP for starter diets and 19% v/s 21% CP for finisher diets) on the carcass traits of heat-stressed broiler chicks and discovered that a high dietary protein level (23% CP) resulted in higher carcass traits (drumstick, thigh, chest, back, and wing weight) as compared to broilers fed with a low dietary CP. According to Murawska (2012), breast muscles composition accounted for up to 40% of the edible meat and 50% of the edible protein in the carcass. As a result, the quality and quantity of the protein affect the traits of the carcass. According to Hickling *et al*. (1990), increasing the dietary methionine or lysine by 12% over the National Research Council recommendation could make extra l5 – 20 g of breast meat per bird to be obtained.

2.9.4 Effect on meat quality parameters

Meat quality is a set of characteristics that gives meat the ability to satisfy the needs of its consumer. It refers to the overall meat characteristics including its physical, chemical, morphological, biochemical, microbial, sensory, technological, hygiene, nutritional and culinary properties (Toughan *et al*., 2013). Colour is one of the most dominant factors in a consumer's initial decision of buying the meat (Zeng & Durif, 2019). The colour of meat samples including poultry are judged by the *L**, *a**, *b** values. The *L** is the measure of the lightness or darkness of the meat colour, while *a** is the measure of redness and *b** is the measure of yellowness (Petracci & Fletcher, 2002). Green plants such as MOLM are currently the main sources of *β*-carotene (Castañeda *et al*., 2005).

Like other meat products, poultry meat colour is influenced by the myoglobin content, chemical nature of the heme and the pH (Fletcher., 2002). Carotenoids are compounds responsible for the pigmentation of skin, shanks, and other non-feather tissues. The rest of the body colour is influenced by the presence of the muscle pigments myoglobin and haemoglobin

(Fletcher *et al*., 2000). The myoglobin content depends on the type of species, and age of the animal (Listrat *et al*., 2016). When myoglobin is exposed to oxygen the meat tends to become bright red and the meat may appear brown if myoglobin is exposed to less oxygen or when the meat colour life is exhausted late in display when the iron in the pigment becomes oxidised (Listrat *et al*., 2016).

Pre-slaughter conditions and the handling practices that the bird is subjected to also influence the colour of the breast meat (Ali *et al*., 2008). Reduced carcass fat can also affect colour and lead to lighter breast meat colour (Mir *et al*., 2017). Several studies have shown that pH has a significant negative correlation to breast meat lightness values and breast meat pH (Qiao *et al*., 2001; Fletcher, 2000; Fletcher, 2002).

Meat pH ranges from pH 5.2 to 7.0 with the highest quality meat product falling between a pH range of 5.2 and 6.0 (Glamoclija *et al*., 2015). The pH of meat may be influenced by other internal factors such as muscle type, chicken strain (Santos *et al*., 2005) and external factors including feed, fasting, electrical stimulation and chilling. The changes that occur in the muscle post-mortem can be measured by the level of pH and temperature (Deiss *et al*., 2009). Meat pH is primarily related to the biochemical state of the muscle at time of slaughter, following the development of rigor mortis (Wattanachant, 2008). Fletcher (2002) observed that muscle pH and meat colour is highly correlated. Higher muscle pH is associated with darker meat, whereas lower muscle pH values are associated with lighter meat (Fletcher, 2002). High meat pH is often characterised as being dark, firm, and dry meat and the lighter meat as being pale, soft and exudative (Wattanachant, 2008). The ultimate pH of meat is exceptionally structured upon the quantity of glycogen present inside the muscle, with the implications that pre- slaughter strain is related to muscle pH (Mir *et al*., 2017). Consequently, the pre-slaughter feed

withdrawal length has a capacity to influence glycogen levels inside the muscle resulting in pH adjustments following rigor mortis (Lesiow & Kijowski, 2003).

Drip loss is caused by myofiber leakage and loss of water, iron, and proteins from the onset of rigor mortis when muscle is being converted into meat (Ponsuksili *et al*., 2008). From the point at which a bird is slaughtered during the meat production process, it is inevitable that water will be lost (drip loss) from the carcass. Water represents between 70% and 80% of the weight of raw poultry meat. The loss of water (drip loss) is a key concern for meat producers as this water content contributes to the sensorial, organoleptic (juiciness, tenderness texture, smell, and colour) and technological quality traits of the meat products (Mir *et al*., 2017). However, this exerts an impact on consumer opinion, thus affecting demand and the saleable value (Prevolnik *et al*., 2010; Maison *et al*., 2016). According to Gil *et al*. (2008), drip loss is related to sensory qualities, such as hardness and juiciness. Muscles with a high drip loss have a high Warner-Bratzler shear force. The results of a study by Mikulski *et al*. (2012) showed that substituting soybeans with a rapeseed meal at 120 g/kg increased the drip loss as compared to the control diet.

The term water holding capacity (WHC) refers to a meat's capacity to retain moisture during production, processing, and storage. The WHC isan important meat quality attribute (Woelfel *et al*., 2002) and is influenced by several factors such as pH, muscle type, rigor mortis, processing conditions, and ingredients added to the meat. Poor WHC in raw poultry meat leads in lower visual appeal and palatability characteristics for customers, as well as lower ingredient retention, protein functionality, and product output for processors (Sarke *et al*., 2021). Pectoralis major muscles from broilers are almost 100% of fast-twitch glycolytic muscle fibers, making them particularly exposed to a rapid pH decrease after slaughter and having lower WHC characteristics (Bowker & Zhuang, 2016). Main factors effecting quail breast

muscle WHC are post-mortem pH and protein denaturation. One method used to determine WHC is expressible moisture and the other is drip loss (Woelfel *et al*., 2002). Rapid denaturation of myosin increases the likelihood of reduction in WHC (Offer, 1991).

Meat tenderness is an important trait in Jumbo quail. It is rated as the most important attribute by the average consumer (Petracci *et al*., 2011). The tenderness of meat is influenced by several factors including the type of muscle, sex of the animal, the grain of the meat, the amount of connective tissue, and the amount of fat (Listrat *et al*., 2016). Historically, meat tenderness was associated with live bird quality factors such as breed, sex, orage. The two major contributing factors to poultry meat tenderness are maturity of the connective tissues involved and the contractile state of the myofibrillar proteins (Fletcher, 2002). Collagen cross linking increases with age, consequently meat from older birds tend tobe tougher (Wattanachant *et al*., 2004). The contractile state of the myofibrillar proteins is primarily a function of the rate and severity of rigor mortis development (Huang *et al*., 2012). Factors such as feed withdrawal, environment, and struggle before slaughter have been shown to affect muscle glycogen stores at the time of slaughter (Ali *et al*., 2008). Birds with higher muscle glycogen content at slaughter have lower shear values than birds with lower muscle glycogen (Abdulla *et al*., 2017). Increasing feed withdrawal times has been shown to decrease breast muscle glycogen stores, making the meat tough (Petracci *et al*., 2010). Processing conditions such as stunning and slaughtering also affect meat tenderness. Using gas stunning such as argon and carbon dioxide, have been shown to decrease carcass damage and improve meat quality (Raj *et al*., 1990). Birds slaughtered in a gas environment appeared to have accelerated rigor mortis in breast muscle without adverse toughening (Mohan *et al*., 1990; 1991). According to the research of these authors, gas slaughtering resulted in a lower initial muscle pH, and more tender meat compared to

electrically stunned birds. Rapid pH decline at high temperature reduces post-mortem tenderisation and can lead to toughening of meat (Dransfield, 1994).

2.10 Summary

In South Africa, the largest agricultural farming enterprise is the chicken sector, which creates employment throughout the entire value chain. The cheapest source of high-quality animal protein is also poultry, which is also more affordable than red meat. A new bird species called quail has recently been introduced to the poultry industry. Quail birds are the smallest avian species noted for their fast growth rates, early sexual maturity and market age, short generation intervals, prolific laying, and resistance to several avian diseases. Due to its tender taste, the meat products from quail are currently dominating luxury markets. To profitably improve quail production to meet anticipated growth in demand, it is essential to find alternative feed ingredients. Finding alternative feed ingredients that are not used directly by humans and can only be consumed by domestic animals is a major priority for researchers. *Moringa oleifera* is a fast-growing drought-resistant tree whose leaves are an outstanding source of nutrients. They contain cytokinin in the form of zeatin as well as other beneficial phytochemicals such as vanillin, beta-sitosterol, caffeoylquinic acids, kaempferol, quercetin and carotenes.

The leaves are also rich in minerals like calcium, potassium, zinc, magnesium, iron, and copper. In addition, *M. oleifera* has high levels of crude protein, crude fibre, crude fat, and beta-carotene. However, antinutritive elements including glycosylates, phytic acid, condensed tannins (CT), and fibre restrict the quantity of MOLM that can be included in quail diets. The high fibre and CT content of MOLM may hinder nutritional absorption and digestion. If the MOLM is to be included at higher levels, there is a need to find alternative strategies that could be employed to improve its feed value and reduce its antinutritive effects. The use of feed additives such as PEG and exogenous fibrolytic enzymes in animal nutrition has been adopted

to complement endogenous digestive enzymes. These could enhance the digestibility of feed substrates, improve gut morphology, and increase growth performance of farm animals. The addition of PEG can prevent the binding of tannins to proteins and thus increase their utilisation.

Polyethylene glycol forms complexes with tannins, preventing them from being absorbed or interacting with other feed constituents and thus allowing them to be excreted in the faecal matter. The addition of MOLM treated with PEG into the diet of Jumbo quail could lead to meat with a longer shelf life and is better protected against deterioration. The response and optimum inclusion level of PEG-treated MOLM on Jumbo quail is, however, unknown.

Exogenous VME are known to cleave the viscous fibre components in carbohydrate-rich feedstuffs such as xylenes and glucans and thereby increasing their digestibility. There is also limited information on supplementing feed enzymes to *M. oleifera* leaf meal diets to improve Jumbo quail performance, haematological and serum biochemical parameters, and carcass and meat quality traits. It is, therefore, important to investigate the effects of PEG and VME on the utilisation of MOLM diets in Jumbo quail.

2.11 References

Abd El Tawab, A.M. & Khattab, M.S.A., 2018. Utilization of polyethylene glycol and tannase enzyme to reduce the negative effect of tannins on digestibility, milk production and animal performance. Asian. J. Anim. Vet. Adv. 13(3), 201–209.

Abdulla, N.R., Mohd Zamri, A.N., Sabow, A.B., Kareem, K.Y., Nurhazirah, S., Ling, F.H., Sazili, A.Q. & Loh, T.C., 2017. Physico-chemical properties of breast muscle in broiler chickens fed probiotics, antibiotics or antibiotic–probiotic mix. J. Appl. Anim. Res. 45(1), 64–70.

Agina, O.A., Ezema, W.S. & Iwuoha, E.M., 2017. The haematology and serum biochemistry profile of adult Japanese quail (*Coturnix coturnix japonica*). Not. Sci. Biol. 9(1), 67–72.

Akande, K.E., Doma, U.D., Agu, H.O. & Adamu, H.M., 2010. Major antinutrients found in plant protein sources: their effect on nutrition. Pak. J. Nutr. 9(8), 827–832.

Alagawany, M., Elnesr, S.S. & Farag, M.R., 2018. The role of exogenous enzymes in promoting growth and improving nutrient digestibility in poultry. Iran J. Vet Res. 19(3), 157.

Alam, M.J., Howlider, M.A.R., Pramanik M.A.H. & Haque, M.A., 2003. Effect of exogenous enzyme in diet on broiler performance. Int. J. Poult. Sci. 2(2), 168–173.

Alefzadeh, T., Bouyeh, M., Van den Hoven, R., Seidavi, A., Laudadio, V. & Tufarelli, V., 2016. Effect of dietary dried orange (*Citrus sinensis*) peel powder and exogenous multienzymes on growth and carcass traits and ileal microflora of broiler chickens. Pak. J. Zool. 48 (6), 1891–189.

Aletor, V.A., Hamid, I.I., Nieb, E. & Pfeffer, E., 2000. Low protein amino acid-supplemented diets in broiler chickens: Effects on performance, carcass characteristics, whole body composition and efficiencies of nutrient utilization. J. Sci. Food Agric. 80, 547–554.

Ali, A., Ponnampalam, E.N., Pushpakumara, G., Cottrell, J.J., Suleria, H.A. & Dunshea, F.R., 2021. Cinnamon: A natural feed additive for poultry health and production—A review. Animals. 11(7), 2026.

Ali, M., Kang, G.H. & Joo, S.T., 2008. A review: Influences of pre-slaughter stress on poultry meat quality. Asian-Austral. J. Anim. Sci 21(6), 912–916.

Ali, M.A., Hmar, L., Devi, L.I., Prava, M., Lallianchhunga, M.C. & Tolenkhomba, T.C., 2012. Effect of age on the haematological and biochemical profile of Japanese quails (*Coturnix coturnix japonica*). Int. J. Multidiscip. Res. 2(8), 32–35.

Alipour, D. & Rouzbehan, Y., 2007. Effects of ensiling grape pomace and addition of polyethylene glycol on *in vitro* gas production and microbial biomass yield. Anim. Feed. Sci. Technol. 137, 138–149.

Anjorin, T.S., Ikokoh, P. & Okolo, S., 2010. Mineral composition of *Moringa oleifera* leaves, pods and seeds from two regions in Abuja, Nigeria. Int. J. Agri. Bio. 12(3), 431–434.

Anwar, F., Latif, S., Ashraf, M. & Gilani, A.H., 2007. *Moringa oleifera*: a food plant with multiple medicinal uses. Phytother. Res. 21, 17–25.

Apajalahti, J. & Vienola, K., 2016. Interaction between chicken intestinal microbiota and protein digestion. Anim. Feed Sci. Technol. 221, 323–330.

Applegate, T.J., Klose, V., Steiner, T., Ganner, A. & Schatzmayr, G., 2010. Probiotics and phytogenics for poultry: Myth or reality? J. Appl. Poult. Res. 19(2),194–210.

Arczewska-Wlosek, A., Swiatkiewicz, S., Bederska-Lojewska, D., Orczewska-Dudek, S., Szczurek, W., Boros, D., Fras, A., Tomaszewska, E., Dobrowolski, P., Muszynski, S. & Kwiecien, M., 2019. The efficiency of xylanase in broiler chickens fed with increasing dietary levels of rye. Animals. 9(2), 46.

Arriaga, H., Pinto, M., Calsamiglia, S. & Merino, P., 2009. Nutritional and management strategies on nitrogen and phosphorus use efficiency of lactating dairy cattle on commercial farms: An environmental perspective. J. Dairy Sci. 92(1), 204–215.

Ashok, P.K. & Upadhyaya, K., 2012. Tannins are astringent. Anim. Feed Sci. Technol. 106, 3–19.

Ayasan, T., 2013. Effects of dietary inclusion of protexin (probiotic) on hatchability of Japanese quails. Indian J. Anim. Sci. 83(1), 78–81.

Ayres, V.E., 2019. *Evaluating the Effects of Exogenous Enzyme Supplementation on Broiler Growth*. West Virginia University. Morgantown, West Virginia, USA.

Baer, J., Lansford, R. & Cheng, K., 2015. Japanese quail as a laboratory animal model. In *Laboratory animal medicine*. Academic Press. Los Angeles, California, USA. Pp. 1087–1108.

Bamishaiye, E.I., Olayemi, F.F., Awagu, E.F. & Bamshaiye, O.M., 2011. Proximate and phytochemical composition of *Moringa oleifera* leaves at three stages of maturation. Adv. J. Food. Sci. Technol. 3(4), 233–237.

Batal, A.B. & Parsons, C.M., 2002. Effects of age on nutrient digestibility in chicks fed different diets. Poult. Sci. 81, 400–407.

Bedford, M. & Cowieson, A.J., 2012. Exogenous enzymes and their effects on intestinal microbiology. Anim. Feed. Sci. Technol. 173(1-2), 76–85.

Bedford, M.R. & Apajalahti, J.H., 2022. The role of feed enzymes in maintaining poultry intestinal health. J. Sci. Food Agric. 102(5), 1759–1770.

Bedford, M.R. & Partridage, G.G., 2001. Enzymes in farm in animal nutrition. Finn feeds, Marlborough, Wiltshire SN8 IXN, Uk, International publishing.

Bedford, M.R., 2000. Exogenous enzymes in monogastric nutrition, their current value and future benefits. Finn feeds, Marlborough, Wiltshire SN8 IXN, UK. Anim. Feed. Sci. Technol. 86, 1–13.

Behrens, A., Maie, N., Knicker, H. & Kögel-Knabner, I., 2003. MALDI-TOF mass spectrometry and PSD fragmentation as means for the analysis of condensed tannins in plant leaves and needles. Phytochemistry. 62(7),1159–1170.

Ben Salem, H., Ben Salem, I. & Ben Said, M. S., 2005a. Effect of the level and frequency of PEG supply on intake, digestion, biochemical and clinical parameters by goats given kermes oak (*Quercus coccifera L.*)–based diets. Small Rumin. Res. 56, 127–137.

Ben Salem, H., Makkar, H.P.S., Nefzaoui, A., Hassayoun, L. & Abidi, S., 2005b. Benefit from the association of small amounts of tannin-rich shrub foliage (*Acacia cyanophylla Lindl*.) with soya bean meal given as supplements to Barbarine sheep fed on oaten hay. Anim. Feed. Sci. Technol. 122, 173–186.

Ben Salem, H., Nefzaoui, A. & Tisserand, J.L., 2000. Deactivation of condensed tannins in *Acacia cyanophylla Lind1*. Foliage by polyethylene glycol in feed blocks, Effect on feed intake, diet digestibility, nitrogen balance, microbial synthesis and growth by sheep. Livest. Prod. Sci. 64, 51–60.

Bennett, R.N., Mellon, F.A., Foid, N., Pratt, J.H., Dupont, M.S., Perkins, L. & Kroon, P. A., 2003. Profiling glucosinolates and phenolics in vegetative and reproductive tissues of the multi-purpose trees *Moringa oleifera* L. (horseradish tree) and *Moringa stenopetala*. J. Agric. Food Chem. 51, 3546–3553.

Berkovich, L., Earon, G., Ron, I., Rimmom, A., Vexler, A. & Lev-Ari, S., 2013. *Moringa oleifera* aqueous leaf extract down- regulates nuclear factor- and increases cytotoxic effect of chemotherapy in pancreatic cancer cells, BMC Complement. Med. 13, 212–219.

Berushka, P. & Himansu, B., 2012. An overview of the medicinal importance of *Moringaceae*. J. Med. Plant Res. 6(48), 5831–5839.

Besharati, M. & Taghizadeh, A., 2011. Effect of tannin-binding agents (polyethylene glycol and polyvinylpyrrolidone) supplementation on *in vitro* gas production kinetics of some grape yield by products. Int. Sch. Res. Notices. http://dx.doi.org/10.5402/2011/780540.

Bhat, T.K., Kannan, A., Singh, B. & Sharma, O.P., 2013. Value addition of feed and fodder by alleviating the antinutritional effects of tannins. Agri. Res. 2(3), 189–206.

Biswas, A.K., Jairath, G., Mandal, A.B. & Khanna, S., 2015. Non-traditional small poultry species: An approach to food sustainability. Agric. Rev. 36(2)147–152.

Blazek, V., 2008. Chemical and biochemical factors that influence the gelation of soybean protein and the yield of Tofu (PhD Dissertation). University of Sydney, Sydney, Australia.

Bokau, R.J.M., Febriani, D., Indariyanti, N. & Rakhmawati, D., 2018. Dosage optimization of artificial digestive enzymes in feed to improve the digestibility and growth of *Osphronemus gourami*. Aquac. Indones. 19 (2), 83–89.

Botlhoko, T.D., 2009. Performance of *Cloristridium perfringes*-challenged broilers inoculated with effective microorganisms. MSc Dissertation. University of Pretoria. Pretoria, South Africa.

Bowker, B.C. & Zhuang, H., 2016. Instrumental texture characteristics of broiler pectoralis major with the wooden breast condition. Poult. Sci. 95(10), 2449–2454.

Brah, G.S. & Mukhopadhyay, C.S., 2016. Microsatellite-polymorphism and association with production traits in Japanese quails. India. J. Poult. Sci. 51(2), 146–150.

Bryden, W.L. & Li, X., 2010. Amino acid digestibility and poultry feed formulation: expression, limitations and application. Rev. Bras. de Zootec. 39, 279–287.

Buchanan, N.P., Kimbler, L.B., Parsons, A.S., Seidel, G.E., Bryan, W.B., Felton, E.E.D. & Moritz, J.S., 2007. The effects of non-starch polysaccharide enzyme addition and dietary energy restriction on performance and carcass quality of organic broiler chickens. J. Appl. Poult. Res. 16 (1), 1–12.

Busani, M., Patrick, J.M., Arnold, H. & Voster, M., 2011. Nutritional characterization of Moringa (*Moringa oleifera* Lam.) leaves. Afr. J. Biotechnol. 10(60), 2925–12933.

Castaneda, M.P., Hirschler, E.M. & Sams, A.R., 2005. Skin pigmentation evaluation in broilers fed natural and synthetic pigments. Poult. Sci. 84(1), 143–147.

Cheenpracha, S., Park, E.J., Yoshida, W.Y., Barit, C. & Wall, M., 2010. Potential anti-inflammatory phenolic glycosides from the medicinal plant *Moringa oleifera* fruits. Bioorg. Med. Chem. 18, 6598–6602.

Chen, S., Luo, S. & Yan, C., 2021. Gut microbiota implications for health and welfare in farm animals: A Review. Animals. 12(1), 93.

Chinrasri, A., 2004. Poultry Production Technology. Apichart Printing Press, Mahasarakam Province, Thailand. Pp. 206.

Chotinsky, D., 2015. The use of enzymes to improve utilization of nutrient in poultry diets. Bulg. J. Agric. Sci. 21(2), 429–435.

Chuka, E., 2014. Comparative study of the effects of probiotic and commercial enzyme on growth rate, haematology and serum biochemistry of broiler chicken. J. Food Sci. Technol. 5(9), 25–34.

Corso, A., Loar II, R.E., Kidd, M.T. & Burgess, S.C., 2011. Dietary protein effects on growth performance, carcass traits and expression of selected *jejunal peptides* and amino acid transporters in broiler chickens. Braz. Poult. Sci. 13, 139–146.

Cowan, M.M., 1999. Plant products as antimicrobial agents. Clin. Microbiol. Rev. 12(4), 564–582.

Cowieson, A.J. & Bedford, M.R., 2009. The effects of phytase and carbohydrase on ileal amino acid digestibility in monogastric diets: Complimentary mode of action? World. Poult. Sci. J. 65(4), 609–624.

Cowieson, A.J. & Roos, F.F., 2016. Toward optimal value creation through the application of exogenous mono-component protease in the diets of non-ruminants. Anim. Feed. Sci. Technol. 221, 331–340.

Cowieson, A.J. & Adeola, O., 2005. Carbohydrase's, protease, and phytase have an additive beneficial effect in nutritionally marginal diets for broiler chicks. Poult. Sci. 84, 1860–1867.

Cowieson, A.J., Hruby, M. & Pierson, E.E.M. 2006. Evolving enzyme technology: impact on commercial poultry nutrition. Nutr. Res. Rev. 9 (1), 90–103.

Cowieson, A.J., Ruckebusch, J.P., Sorbara, J.O.B., Wilson, J.W., Guggenbuhl, P. & Roos, F.F., 2017. A systematic view on the effect of phytase on ileal amino acid digestibility in broilers. Anim. Feed. Sci. Technol. 225, 182–194.

Dai, J., Tao, L., Shi, C., Yang, S., Li, D., Sheng, J. & Tian, Y., 2020. Fermentation improves calcium bioavailability in *Moringa oleifera* leaves and prevents bone loss in calcium-deficient rats. Food. Sci. Nutr. 8(7), 3692–3703.

de Figueiredo, V.R.G., Yamashita, F., Vanzela, A.L.L., Ida, E.I. & Kurozawa, L.E., 2018. Action of multi-enzyme complex on protein extraction to obtain a protein concentrate from okara. J. Food Sci. Technol. 55(4), 1508–1517.

Deiss, V., Temple, D., Ligout, S., Racin, C., Bouix, J., Terlouw, C. & Boissy, A., 2009. Can emotional reactivity predict stress responses at slaughter in sheep? Appl. Anim. Behav. Sci. 119, 193–202.

Dida, M.F. 2016. Review paper on enzyme supplementation in poultry ration. Int. J. Bioorg. Chem. 1, 1–7.

Duangnumsawang, Y., Zentek, J. & Goodarzi Boroojeni, F., 2021. Development and functional properties of intestinal mucus layer in poultry. Front. Immunol. 12, 745849.

Dutta, D., Puzari, K.C., Gogoi, R. & Dutta, P., 2014. Endophytes: exploitation as a tool in plant protection. Braz. Arch. Biol. Technol. 57, 621–629.

Egbunike, G.N., Agiang, E.A., Owosibo A.O. & Fatufe, A.A., 2009. Effect of protein on performance and haematology of broilers fed cassava peel-based diets. Arch. de Zootec. 58, 655–662.

Egea, A.V., Allegretti, L.I., Lama, S.P., Grilli, D., Fucili, M., Guevara, J.C. & Villalba, J.J., 2016. Diet mixing and condensed tannins help explain foraging preferences by Creole goats facing the physical and chemical diversity of native woody plants in the central Monte desert (Argentina). Anim. Feed Sci. Technol. 215, 47–57.

El-Katcha, M.I., Soltan, M., Ramdan, S.S., El Naggar, M.K. & El-Shobokshy, S.A., 2015. Growth performance, blood biochemical changes, carcass traits and nutrient digestibility of growing Japanese quail fed on various dietary protein and calcium levels. Alex. J. Vet. Sci. 44(1), 38–53.

El-Tarabany, M.S., 2016. Impact of temperature-humidity index on egg-laying characteristics and related stress and immunity parameters of Japanese quails. Int. J. Biometeorol. 60(7), 957–964.

Engberg, R.M., Hedemann, M.S., Leser, T.D. & Jensen, B.B., 2000. Effect of zinc bacitracin and salinomycin on intestinal microflora and performance of broilers. Poult. Sci. 79, 1311–1319.

Erian, I. & Phillips, C.J., 2017. Public understanding and attitudes towards meat chicken production and relations to consumption. Animals. 7(3), 20–26.

Etim, N.A.N., Enyenithi, G.E., Akpabio, U. & Offiong, E.E.A., 2014. Effects of nutrition on haematology of Rabbits. Euro. Scientific. J. 10, 413–424.

Fahey, J.W., 2005. *Moringa oleifera*: Review of the medicinal evidence for its nutritional, therapeutic and prophylactic properties. Trees life. J. 5, 2–3.

Falodi, F., Adeoye, G., Ajaja, T. & Alagbe, J., 2016. Chickens forced to skip meals as cost of poultry feed rises by 100%. Available online: https://punchng.com/chickens-forced-skip-meals-cost-poultry-feed-rises-100

Ferraz de Oliveira, M.I., Cancela d'Abreu, M. & Freitas, A., 2008. The effect of polyethylene glycol (PEG) on protein output of free range Alentejano pigs. Proceedings of the 6th international symposium Mediterranean pig, Capo d'Orlando, Messina.

Ferreira, P.M.P., Farias, D.P., Oliveira, J.T. & Carvalho, F.U., 2008. *Moringa oleifera*: bioactive compounds and nutritional potential. Rev. de. Nutr. 21 (4), 431–437.

Fieker, A., Philpott, J. & Armand, M., 2011. Enzyme replacement therapy for pancreatic insufficiency: present and future. Clin. exp. gastroenterol. 4, pp.55.

Fletcher, D.L., 2002. Poultry meat quality. World's Poult. Sci. J. 58(2), 131–145.

Fletcher, D.L., Qiao, M. & Smith, D.P., 2000. The raw broiler breast meat colour and pH to cooked pH. Poult. Sci. 79, 784–788.

Freire, J.P.B., Guerreiro, A.J.G., Cunha, L.F. & Aumaitre, A., 2000. Effect of dietary fibre source on total tract digestibility, caecum volatile fatty acids and digestive transit time in the weaned piglet. Anim. Feed. Sci. Technol. 87(1-2), 71–83.

Gama, R., Van Dyk, J.S. & Pletschke, B.I., 2015. Optimisation of enzymatic hydrolysis of apple pomace for production of biofuel and biorefinery chemicals using commercial enzymes. 3 Biotech. 5(6), 1075–1087.

Gao, J.K., 1993. *Polyethylene glycol as an embedment for microscopy and histochemistry*. CRC press. Boca Raton.

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

Gil, M., Delday, M.I., Gispert, M., Font, I., Furnols, M., Maltin, C.M., Plastow, G.S., Klont, R., Sosnicki, A.A., Carrión, D., 2008. Relationships between biochemical characteristics and meat quality of *Longissimus thoracis* and *Semimembranosus* muscles in five porcine lines. Meat Sci. 80, 927–933.

Glamoclija, N., Starcevic, M., Janjic, J., Ivanovic, J., Boskovic, M., Djordjevic, J., Markovic, R. & Baltic, M.Z., 2015. The effect of breed line and age on measurements of pH-value as meat quality parameter in breast muscles (*M. pectoralis major*) of broiler chickens. Procedia Food Sci. 5, 89–92.

González-Ortiz, G., Olukosi, O.A., Jurgens, G., Apajalahti, J. & Bedford, M.R., 2020. Short-chain fatty acids and ceca microbiota profiles in broilers and turkeys in response to diets supplemented with phytase at varying concentrations, with or without xylanase. Poult. Sci. 99(4), 2068–2077.

Gupta, R.K., Gangoliya, S.S. & Singh, N.K., 2015. Reduction of phytic acid and enhancement of bioavailable micronutrients in food grains. J. Food. Sci. Technol. 52(2), 676–684.

Hajati, H., Rezaei, M. & Sayyahzadeh, H., 2009. The effects of enzyme supplementation on performance, carcass characteristics and some blood parameters of broilers fed on corn-soybean meal-wheat diets. Int. J. Poult. Sci. 8, 1199–1205.

Halimani, T.E., Ndlovu, L.R., Dzama, K., Chimonyo, M. & Miller, B.G., 2007. Metabolic response of pigs supplemented with incremental levels of leguminous *Acacia karroo*, *Acacia nilotica* and *Colophospermum mopane* leaf meals. Livest. Res. Rural. Dev. 19, 87.

Hlatini, V.A., Ncobela, C.N., Zindove, T.J. & Chimonyo, M., 2018. Use of polyethylene glycol to improve the utilisation of leguminous leaf meals in pigs: a review. S. Afr. J. Anim. Sci. 48(4), 609–620.

Hana, A.H., Jalal, M.A. & Abu Ishmais, M.A., 2010. The influence of supplemental multi-enzyme feed additive on the performance, carcass characteristics and meat quality traits of broiler chickens. Int. J. Poult. Sci. 9, 126–133.

Harmon, D.L. & Swanson, K.C., 2020. Nutritional regulation of intestinal starch and protein assimilation in ruminants. Animal. 14(S1), s17-s28.

He, G., Ma, L.L., Pan, J. & Venkatraman, S., 2007. ABA and BAB type triblock copolymers of PEG and PLA: a comparative study of drug release properties and “stealth” particle characteristics. Int. J. Pharm. 334(1-2), 48–55.

He, Q, Lv, Y. & Yao, K., 2006. Effects of tea polyphenols on the activities of a-amylase, pepsin, trypsin and lipase. Food. Chem. 101, 1178–1182.

Hemid, A.E.A., El-Gawad, A.H.A., El-Wardany, I., El-Daly, E.F. & El-Azeem, N.A.A., 2010. Alleviating effect of some environmental stress factors on productive performance of laying quail. 2. Laying performance. World J. Agric. Sci. 6(5), 517–524.

Henning, T., 2002. Polyethylene glycols (PEGs) and the pharmaceutical industry. Fine, Specialty and Performance Chemicals. Pharmachem. 1, 57–59.

Hernández, F., Garci´a, V., Madrid, J., Orengo, J., Catala´, P. & Megi´as, M.D., 2006. Effect of formic acid on performance, digestibility, intestinal histomorphology and plasma metabolite levels of broiler chickens. Bri. Poult. Sci. 47 (1), 50–56.

Hernandez, F., Madrid, J., Garcia, V., Orengo, J. & Megias, M.D., 2004. Influence of two plant extracts on broilers performance, digestibility, and digestive organ size. Poult. Sci. 83(2), 169–174.

Herrero, M., Thornton, P.K., Mason-D'Croz, D., Palmer, J., Bodirsky, B.L., Pradhan, P., Barrett, C.B., Benton, T.G., Hall, A., Pikaar, I. & Bogard, J.R., 2021. Articulating the effect of food systems innovation on the Sustainable Development Goals. Lancet Planet. Health. 5(1), 50–62.

Hickling, D., Guenter, W. & Jackson, M.E., 1990. The effects of dietary methionine and lysine on broiler chicken performance and breast meat yield. Can. J. Anim. Sci. 70, 673–678.

Hlatini, V.A., Ncobela, C.N., Zindove, T.J. & Chimonyo, M., 2018. Use of polyethylene glycol to improve the utilisation of leguminous leaf meals in pigs: a review. S. Afr. J. Anim. Sci. 48(4), 609–620.

Hossain, M.B., Sen, P.C., Noman, M.A. al, Islam, A., Ghosh, S., Islam, S., Chakma, S. & Paul, A.K., 2015. Production performances of Japanese quail parent stock under open housing system. J. Anim. Reprod. Biotechnol. 30, 115–119.

Hsu, R., Midcap, S. & Arbainsyah, D.W.L., 2006. *Moringa oleifera*: Medicinal and Socio-Economical Uses. International Course on Economic Botany, National Herbarium Leiden, the Netherlands.

Huang, H., Larsen, M.R. & Lametsch, R., 2012. Changes in phosphorylation of myofibrillar proteins during post-mortem development of porcine muscle. Food Chem. 134(4), 1999–2006.

Huda, N., Putra, A.A. & Ahmad, R., 2011. Proximate and physicochemical properties of Peking and Muscovy duck breasts and thighs for further processing. J. Food Agric. Environ. 9(1), 82–88.

Hussain, S., Siddique, T., Saleem, M., Arshad, M. & Khalid, A., 2009. Impact of pesticides on soil microbial diversity, enzymes, and biochemical reactions. Adv. Argon. 102, 160–190.

Hussein, E.O.S., Suliman, G.M., Alowaimer, A.N., Ahmed, S.H., Abd El-Hack, M.E., Taha, A.E. & Swelum, A.A., 2020. Growth, carcass characteristics, and meat quality of broilers fed a low-energy diet supplemented with a multienzyme preparation. Poult. Sci. 99, 1988–1994.

Huyghebaert, G., Ducatelle, R. & van Immerseel, F., 2011. An update on alternatives to antimicrobial growth promoters for broilers. Vet. J. 187, 182–188.

Ihejirikamba., 2012. Benefit of quail eggs. A-living healthy.blogspot.com/2012/11/benefitof-quail-eggs.htm. World Poult. Sci. J. 65, 609–624.

Imchen, M., 2013. Sunday Post: Benefits about quail meat and egg. Retrieved from http://www.nagalandpost.com/sundaypost/LifeStyleShow.aspx?ls.

Iqbal, S. & Bhanger, M.I., 2006. Effect of season and production location on antioxidant activity of *Moringa oleifera* leaves grown in Pakistan. J. Food. Composit. Anal. 19(6-7), 544–551.

Isitua, C.C., Lozano, M.J.S., Jaramillo, C. & Dutan, F., 2015. Phytochemical and nutritional properties of dried leaf powder of *Moringa oleifera* Lam. from machala el oro province of ecuador. Asian J. Plant Sci. Res. 5(2), 8–16.

Ismail, F.S.A., Hayam, M.A. & EI-Sayed, A.A.M., 2011. Performance of broiler chicks affected by dietary supplementation with commercial enzymes or probiotics. Mansaura University. J. Anim. Poult. Prod. 2(2), 23–32.

.

Jamroz, D., Wiliczkiewicz, A., Wertelecki, T., Orda, J. & Skorupińska, J., 2005. Use of active substances of plant origin in chicken diets based on maize and locally grown cereals. Brit. Poult. Sci. 46(4), 485–493.

Jha, R. & Berrocoso, J.D., 2015. Dietary fibre utilization and its effects on physiological functions and gut health of swine. Animal. 9(9), 1441–1452.

Jha, R., Fouhse, J.M., Tiwari, U.P., Li, L. & Willing, B.P., 2019. Dietary fibre and intestinal health of monogastric animals. Front. Vet. Sci. 6, 48–54.

Jithender, B., Upendar, N.K. & Rathod, P.J., 2019. Nutritional and anti-nutritional factors present in oil seeds: an overview. Int. J. Conserv. Sci. 7(6), 1159–1165.

Joseph, N.S., Robinson, F.E., Korver, D.R. & Renema, R.A., 2000. Effects of dietary protein intake during the pullets – to-breeder transition period on egg early egg weight and production in broiler breeder. Poult. Sci. 79, 1790–1796.

Joshi, P. & Mehta, D., 2010. Effect of dehydration on the nutritive value of drumstick leaves. J. Metabolomics. Syst. Biol. 1(1), 5–9.

Joshi, P. & Mehta, D., 2010. Effect of dehydration on the nutritive value of drumstick leaves.

Józefiak, D., Rutkowski, A., Jensen, B.B. & Engberg, R.M., 2007. Effects of dietary inclusion of triticale, rye and wheat and xylanase supplementation on growth performance of broiler chickens and fermentation in the gastrointestinal tract. Anim. Feed Sci. Technol. 132(1-2), 79–93.

Junqueira, O.M., De Laurentiz, A.C., Da Silva F.R., Rodrinques, A & Casrtelli, E.A., 2006. Effects of energy and protein levels on egg quality and performance of laying hens at early second production cycle. J. Appl. Poult. Res. 15, 110–115.

Kahkonen, M.P., Hopia, A.I., Vuorela, H.J., Rauha, J.P., Pihlaja, K., Kujala, T.S. & Heinomen, M., 1999. Antioxidant activity of plant extracts containing phenolic compounds, J. Agric. Food Chem. 47, 3952–3962.

Kalantar, M., Khajali, F. & Yaghobfar, A., 2015. Different dietary source of non-starch polysaccharides supplemented with enzymes affected growth and carcass traits, blood parameters and gut physicochemical properties of broilers. Glob. J. Anim. Sci. Res. 3, 1818–1823.

Kamran, M., Pasha, T.N., Mahmud, A. & Ali, Z., 2002. Effect of commercial enzyme (Natugrain) supplementation on the nutritive value and inclusion rate of guar meal in broiler rations. Int. J. Poult. Sci. 1(6), 167–173.

Kamran, Z., Sarwar, M., Nisa, M., Nadeem, M.A., Mahmood, S., Babar, M.E. & Ahmed, S., 2008. Effect of low-protein diets having constant energy-to-protein ratio on performance and carcass characteristics of broiler chickens from one to thirty-five days of age. Poult. Sci. 87(3), 468–474.

Kasetti, R.B., Nabi, S.A., Swapna, S. & Apprao, C., 2012. Cinnamic acid as one of the antidiabetice active principle(s) from the seeds of *Syzygium alternifolium*. J. Food Chem. Toxicol. 50, 1425–1431.

Kasolo, J.N., Bimenya, G.S., Ojok, L., Ohieng, J. & Ogwalokeng, J.W., 2010. phytochemicals and uses of *Moringa oleifera* leaves in Ugandan rural communities. J. Med. Plants Res. 753–757.

Kavitha, C., Malarvizhi, A., Kumaran, S.S. & Ramesh, M., 2010. Toxicological effects of arsenate exposure on hematological, biochemical and liver transaminases activity in an Indian major carp, *Catla catla*. Food. Chem. Toxicol. 48(10), 2848–2854.

Kawabata, K., Yoshioka, Y. & Terao, J., 2019. Role of intestinal microbiota in the bioavailability and physiological functions of dietary polyphenols. Molecules. 24(2), 370.

Kermanshahi, H., Ziaei, N. & Pilevar, M., 2011. Effect of dietary crude protein fluctuation on performance, blood parameters and nutrients retention in broiler chicken during starter period. Global. Vet. 6, 162–167.

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

Khalafalla, M.M., Abdellatef, E., Dafalla, H.M., Nassrallah, A.A., Aboul-Enein, K.M., Lightfoot, D.A., El-Deeb, F.E. & El-Shemy, H.A., 2010. Active principle from *Moringa oleifera* Lam leaves effective against two leukaemia's and a hepatocarcinoma. Afr. J. Biotechnol. 9(49), 8467–8471.

Khan, R.U., Chand, N. & Akbar, A., 2016. Effect of organic acids on the performance of Japanese quails. Pak. J. Zoo, 48(6).

Khanyile, M., Ndou, S.P. & Chimonyo, M., 2014. Influence of *Acacia tortilis* leaf meal-based diets on growth performance of pigs. Livest. Sci. 167, 211–218.

Khattak, F.M., Pasha, T.N., Hayat, Z. & Mahmud, A., 2006. Enzymes in poultry nutrition. J. Anim. PI. Sci. 16, 1–7.

Khawaja, T., Khan, S.H., Mukhtar, N. & Parveen, A., 2012. Comparative study of growth performance, meat quality and haematological parameters of Fayoumi, Rhode Island Red and their reciprocal crossbred chickens. Ital. J. Anim. Sci. 11, 211–216.

Khobondo, J.O., Kingori, A.M. & Manhique, A., 2019. Effect of incorporation of ground *Prosopis juliflora* pods in layer diet on weight gain, egg production, and natural antibody titer in KALRO genetically improved indigenous chicken. Trop. Anim. Health Prod. 51(8), 2213–2218.

Kim, D., Chum, O.K., Kim, J.Y., Moon, H. & Lee, Y.C., 2003. Quantification of polyphenolics and their antioxidant capacity in fresh plums. J. Agric. Food Chem. 51, 6509–6515.

Kingori, A.M., Tuitoek, J.K., Muiruri, H.K., Wachira, A.M. & Birech, E.K., 2007. Protein intake of growing indigenous chickens on free-range and their response to supplementation. Int. J. Poult. Sci. 6, 617–621.

Kirwan, S.F., Pierce, K.M., Serra, E., McDonald, M., Rajauria, G. & Boland, T.M., 2021. Effect of chitosan inclusion and dietary crude protein level on nutrient intake and digestibility, ruminal fermentation, and N excretion in beef heifers offered a grass silage-based diet. Animals. 11(3), 771.

Kshash, B. & Oda, H., 2019. Constrains facing poultry producers. J. Agric. Ext. 23(2), 90–100.

Kumanda, C., 2019. *Enhancing the feed value of red grape pomace for broiler chickens using polyethylene glycol and fibrolytic enzymes* (Doctoral dissertation, North-West University, Mafikeng, South Africa).

Kumanda, C., Mlambo, V. & Mnisi, C.M., 2019. Valorization of red grape pomace waste using polyethylene glycol and fibrolytic enzymes physiological and meat quality responses in broilers. Animals. 9, 779.

Kushwaha, R., Rai, S.N., Singh, A.K., Chandra, G., Vaidya, M.M., Sharma, V.K., Pathan, M.M. & Kumar, S., 2011. Tanniniferous feed resources in dairy animals: a review. Agric. Rev. 32(4), 267–275.

Kushwaha, S. & Chawla, P., 2015. Impact of supplementation of drumstick (*Moringa oleifera*) and amaranth (*Amaranthus tricolour*) leaves powder on menopausal symptoms of postmenopausal women. Int. J. Sci. Res. Publ. 5, 1–10.

Kushwaha, S., Chawla, P. & Kochhar, A., 2014. Effect of supplementation of drumstick (*Moringa oleifera*) and amaranth (*Amaranthus tricolour*) leaves powder on antioxidant profile and oxidative status among postmenopausal women. J. Food Sci. Technol. 51(11), 3464–3469.

Kushwaha, S., Daniel, M. & Shakti., 2015. Potential of underutilized drumstick (*Moringa oleifera*). Int. J. Res. 2(4), 478–83.

Kyarisiima, C.C., Okot, M.W. & Svihus, B., 2004. Use of wood ash in the treatment of high tannin sorghum for poultry feeding. S. Afri. J. Anim. Sci. 34, 110–115.

Lee, K.W., Choi, E.J., Moon, S.T. Lee, H., Kang, C.W. & An, B.K., 2014. Evaluation of dietary multiple enzyme preparation (Natuzyme) in laying hens. Asian-Australas. J. Anim. Sci. 27(2), 1749–1754.

Lee, S.A., Apajalahti, J., Vienola, K., González-Ortiz, G., Fontes, C.M.G.A. & Bedford, M.R., 2017. Age and dietary xylanase supplementation affect ileal sugar residues and short chain fatty acid concentration in the ileum and caecum of broiler chickens. Anim. Feed. Sci. Technol. 234, 29–42.

Leeson, S. & Summers, J.D., 2005. In Commercial Poultry Nutrition Publ Univ Books, Guelph,Canada.

Lesiów, T. & Kijowski, J., 2003. Impact of PSE and DFD meat on poultry processing a review. Polish. J. Food Nutr. Sci. 12(2), 3–8.

Li, M., Cha, D.J., Lai, Y., Villaruz, A.E., Sturdevant, D.E. & Otto, M., 2007. The antimicrobial peptide-sensing system aps of *Staphylococcus aureus*. Mol. Microbiol, 66(5), 1136–1147.

Lim, C.K., Penesyan, A., Hassan, K.A., Loper, E.J. & Paulsen, T.I., 2013. Effect of tannic acid on the transcriptome of the soil bacterium *Pseudomonas protegens* Pf-5. Appl. Environ. Microbiol. 79, 3141–3145.

Listrat, A., Lebret, B., Louveau, I., Astruc, T., Bonnet, M., Lefaucheur, L., Picard, B. & Bugeon, J., 2016. How muscle structure and composition influence meat and flesh quality. Sci. World J. 21, 61–78.

Liu, H. & Fang, H.H., 2002. Extraction of extracellular polymeric substances (EPS) of sludges. J. Biotechnol. 95(3), 249–256.

Lo, D., Wang, H.I., Wu, W.J. & Yang, R.Y., 2018. Anti-nutrient components and their concentrations in edible parts of vegetable families. CAB Rev. 13(15), 10–1079.

López-Pedrouso, M., Cantalapiedra, J., Munekata, P.E., Barba, F.J., Lorenzo, J.M. & Franco, D., 2019. Carcass characteristics, meat quality and nutritional profile of pheasant, quail and Guinea fowl. *In More than beef, pork and chicken–The production, processing, and quality traits of other sources of meat for human diet*. Springer, Cham. 269–311.

Makkar, H.P.S. & Becker, K., 1997. Nutrients and anti-quality factors in different morphological parts of the *Moringa oleifera* tree. J. Agric. Sci. 128(3), 311–322.

Makkar, H.P.S., 2003. Effects and fate of tannins in ruminant animals, adaptation to tannins and strategies to overcome detrimental effects of feeding tannin rich feeds. Small. Rumin. Res. 49, 241–256.

Malafaia, G. & Talvani, A., 2011. Nutritional status driving infection by *Trypanosoma cruzi*: lessons from experimental animals. J. Trop. Med. 2011, 981879.

Malik, H.E.E., Ali, O.H.A., Mohamed, E.A.A. & Yousuf, I.A., 2013. Effects of season and dietary protein level on immune response of three exotic broiler strains in Sudan. J. Anim. Feed. Res. 3, 31–35.

Manguro, L.O. & Lemmen, P., 2007. Phenolics of *Moringa oleifera* leaves. Nat. Pod. Res. 21, 56–68.

Mansoori, B. & Acamovic, T., 2009. Influence of tannic acid and polyethylene glycol on the excretion and digestibility of amino acids in gelatin –fed broilers. Br. Poult. Sci. 50, 199–206.

Mantz, G.K., 2008. Using polyethylene glycol to enhance the intake of Sericea lespedeza by Cattle. UMI, United States ProQuest LLC.

Mareta, R.E., Subandiyono, E. & Hastuti, S., 2017. Effect of papain and probiotics in artificial feed efficiency utilization level and growth rate of gourami (*Osphronemous gourami*). J. Sains Akuakultur Tropis. 1, 21–30.

Mateos, G.G., Serrano, M.P., Berrocoso, J., Perez-bonilla, A. & Lazaro. R., 2013. Improving the utilization of raw materials in poultry feeding: new technologies and inclusion levels. XXIV Word's Poultry Congress. Salvador de Bahía, Brazil. 1–13.

Mbhele, F.G.T, 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.

Mbikay, M., 2012. Therapeutic potential of *Moringa oleifera* leaves in chronic hyperglycaemia and dyslipidaemia: A review. Front. Pharmacol. 3(24), 1–12.

McDonald, J.C. & Whitesides, G.M., 2002. Poly (dimethylsiloxane) as a material for fabricating microfluidic devices. Acc. Chem. Res. 35 (7), 491–499.

Medugu, C.I., Saleh, B., Igwebuike, J.U. & Ndirmbita, R.L., 2012. Strategies to improve the utilization of tannin-rich feed materials by poultry. Int. J. Poult. Sci. 11, 417–423.

Mehaffey, J.M., Pradhan, S.P., Meullenet, J.F., Emmert, J.L., McKee, S.R. & Owens, C.M., 2006. Meat quality evaluation of minimally aged broiler breast fillets from five commercial genetic strains. Poult. Sci. 85(5), 902–908.

Mehaisen, G.M., Ibrahim, R.M., Desoky, A.A., Safaa, H.M., El-Sayed, O.A. & Abass, A.O., 2017. The importance of propolis in alleviating the negative physiological effects of heat stress in quail chicks. Plos One. 12(10), e0186907.

Mikulski, D., Jankowski, I.J., Zdunczyk, Z., Juskiewicz, J. & Slominski, B.A., 2012. The effect of different dietary levels of rapeseed meal on growth performance, carcass traits, and meat quality in turkeys. Poult. Sci. 91, 215–223.

Min, B.R. & Hart, S.P., 2003. Tannins for suppression of internal parasites. J. Anim. Sci. 81(14), 102–109.

Minvielle, F., 2004. The future of Japanese quail for research and production. Worlds Poult. Sci. J. 60, 500–507.

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(10), 2997–3009.

Mizutani, M., 2003. The Japanese quail. *Laboratory Animal Research Station, Nippon Institute for Biological Science,* Kobuchizawa, Yamanashi, Japan.

Mnisi, C.M. & Mlambo, V., 2019. Canola meal as an alternative dietary protein source in quail (*Coturnix coturnix*) diets: A review. Acta Agric. Scand. Sect. A Anim. Sci. 0, 1–12.

Mnisi, C.M., 2018. *Towards the optimization of canola meal as a protein source for Japanese quails using exogenous feed enzymes* (Doctoral dissertation, North-West University, Mafikeng, South Africa).

Mohammed, A.A., Habib, A.B., Eltrefi, A.M., Shulukh, E.S.A. & Abubaker, A.A. 2018., Effect of different levels of multi-enzymes (Natuzyme Plus®) on growth performance, carcass traits and meat quality of broiler chicken. Asian. J. Anim. Vet. Adv. 13, 61–66.

Mohammed, B.R. & Ejiofor, C., 2015. The prospects and limitations of Japanese quail (*Coturnix coturnix japonica*) production in Nigeria: a Review. Int. J. Multidiscip. Curr. Res. 3, 920–926.

Mohan Raj, A.B., Grey, T.C. & Gregory, N.G., 1991. Effect of early filleting on the texture of breast muscle of broilers stunned with argon-induced anoxia. Br. Poult. Sci. 32, 319–325.

Mohan Raj, A.B., Grey, T.C., Audsely, A.R. & Gregory, N.G., 1990. Effect of electrical and gaseous stunning on the carcass and meat quality of broilers. Br. Poult. Sci. 31, 725–733.

Moyo, B., Masika, P.J., Hugo, A. & Muchenje, V., 2011. Nutritional characterization of Moringa (*Moringa oleifera Lam*) leaves. Afr. J. Biotechnol. 10, 12925–12933.

Mueller-Harvey, I., 2006. Unravelling the conundrum of tannins in animal nutrition and health. J. Sci. Food. Agric. 86(13), 2010–2037.

Murawska, D., 2012. The effect of age on the growth rate of tissues and organs and the percentage content of edible and nonedible carcass components in Pekin ducks. Poult. Sci. 91(8), 2030–2038.

Mushtaq, B.S., Hussain, M.B., Omer, R., Toor, H.A., Waheed, M., Shariati, M.A., Sergey, P. & Heydari, M., 2021. *Moringa oleifera* in malnutrition: A comprehensive review. Curr. Drug Discov. Technol. 18(2), 235–243.

Nakazawa, H., Kawai, T., Ida, N., Shida, Y., Kobayashi, Y., Okada, H., Tani, S., Sumitani, J.I., Kawaguchi, T., Morikawa, Y. & Ogasawara, W., 2012. Construction of a recombinant *Trichoderma reesei* strain expressing *Aspergillus aculeatus* β-glucosidase 1 for efficient biomass conversion. Biotechnol. Bioeng. 109(1), 92–99.

Nasr, M.A.F., Ali, E.S.M.R. & Hussein, M.A., 2017. Performance, carcass traits, meat quality and amino acid profile of different Japanese quail strains. J. Food Sci. Technol. 54, 4189–4196.

Ndlovu, T., Chimonyo, M., Okoh, A.I., Muchenje, V., Dzama, K., Dube, S. & Raats, J.G., 2009. A comparison of nutritionally-related blood metabolites among Nguni, Bonsmara and Angus steers rose on sweet-veld. Vet. J. 179, 273–281.

Nduka, U.K., 2006. The effect of enzyme supplementation on the utilization of fibrous feed ingredients in broiler diets. MSc thesis. Animal Science Department, Netherlands.

Nguyen, D.D., Di Prima, S., Huijzendveld, R., Wright, E.P., Essink, D. & Broerse, J.E., 2022. Qualitative evidence for improved caring, feeding and food production practices after nutrition-sensitive agriculture interventions in rural Vietnam. Agric. Food Secur. 11(1), 1–23.

Nikam, M., Reddy, V., Raju, M.V.L.N., Reddy, K. & Narasimha, J., 2017. Effect of non-starch Polysaccharide hydrolyzing enzymes on nutrient utilization and economics of broilers reared on corn soybean meal based standard and sub-optimal diets. Int. J. Livest. Res. 7(2), 172–179.

Nikam, M.G., Reddy, V., MVLN, R., Reddy, K. & Narasimha, J., 2016. Effect of dietary supplementation of non-starch polysaccharide hydrolyzing enzymes on broilers. Int. J. Agric. Sci. Res. 6(3), 389–396.

Nitsan, Z., Ben-Avraham, G., Zoref, Z. & Nir, I., 1991. Growth and development of the digestive organs and some enzymes in broiler chicks after hatching. Br. Poult. Sci. 32, 515–523.

NRC, 2004. *Nutrient Requirements of Poultry*, 9th ed.; National Research Council; National Academy Press: Washington, DC, USA.

NRC, 1991. Quail. In: Micro-livestock's-Little Known small animals with a promising economic future. National Research Council; National Research Council, Academy Press, Washington D.C. pp. 147–155.

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

Nsahlai, I.V., Fon, F.N. & Basha, N.A.D., 2011. The effect of tannin with and without polyethylene glycol on In vitro gas production and microbial enzyme activity. S. Afri. J. Anim. Sci. 41, 337–344.

Nwambe, R.N. & ELechi, F.N., 2009. Haematological and serum biochemical indices of broiler finisher birds fed potash boiled Bambara groundnut (*Voandzein subterranea*) (L) Thour) meal as replacement for soybean meal. Proceedings of the 34th Annual conference of the Nigerian Society for Animal Production. March 15-18, University of Uyo, Nigeria. 443–44.

O'Brie, P.J. & Herschlag, D., 2001. Functional interrelationships in the alkaline phosphatase superfamily: phosphodiesterase activity of *Escherichia coli* alkaline phosphatase. Biochem. 40(19), 5691–5699.

Oduguwa, O., Pirgozliev, V. & Acamovic, T., 2007. Energy metabolizability and digestibility of amino acids by broilers fed malted sorghum sprouts supplemented with polyethylene glycol, charcoal, phytase and xylanase. Br. Poult. Sci. 48, 55–63.

Oduro, I., Ellis, WO. & Owusu, D., 2008. Nutritional potential of two leafy vegetables: *Moringa oleifera* and *Ipomoea batatas* leaves. Sci. Res. Essays. 3(2), 057–060.

Offer, G., 1991. Modelling of the formation of pale, soft, and exudative meat: Effects of chilling regime and rate and extent of glycolysis. Meat Sci. 30, 157–184.

Ogbe, A.O. & Affiku, J.P., 2012. Effect of Polyherbal Aqueous Extracts (*Moringa oleifera*, *Gum arabic* and wild *Ganoderma lucidum*) in comparison with antibiotic on growth performance and haematological parameters of broiler chickens. Res. J. Rec. Sci. 1, 10–18.

Ogunbajo, S.O., Alemede, I.C., Adama, J.Y. & Abdullah, J., 2009. Haematology parameters of savannah brown does feed varying dietary levels of Flamboyant tree seed meal, proceed. Annu. Confers. Niger. Soci. Anim. Product. Pp. 88–91.

Oliaei, A.H., Palizdar, M.H. & Tabrizi, H.M., 2016. Inclusion a multi-enzyme (Natuzyme Plus) in broiler chicken diets containing high canola meal. Glob. Vet. 16(1), 18–25.

Olugbemi, T.S., Mutayoba, S.K. & Lekule, F.P., 2010. Effect of moringa (*Moringa oleifera*) inclusion in cassava-based diets fed to broiler chickens. Int. J. Poult. Sci. 9(4), 363–367.

Olugbemi, T.S., Mutayoba, SK. & Lekule, F.P., 2010. Effect of moringa (*Moringa oleifera*) inclusion in cassava-based diets fed to broiler chickens. Int. J. Poult. Sci. 9(4), 363–367.

Onu, P.N. & Aniebo, A.O., 2011. Influence of *Moringa oleifera* leaf meal on the performance and blood chemistry of starter broilers. Int. J. Food. Agric. Vet. Sci. 1(1), 38–44.

Orawan, C. & Aengwanich, W., 2007. Blood cell characteristics, haematological values and average daily gained weight of Thai indigenous. Thai indigenous crossbred and broiler chickens. Pak. J. Biol. Sci. 10, 302–309.

Owen, M.D., Beckie, H.J., Leeson, J.Y., Norsworthy, J.K. & Steckel, L.E., 2015. Integrated pest management and weed management in the United States and Canada. Pest Manag. Scie. 71(3), 357–376.

Ozdal, T., Capanoglu, E. & Altay, F., 2013. A review on protein–phenolic interactions and associated changes. Food. Res. Int. 51(2), 954–970.

Panda, A.K. & Cherian, G., 2014. Role of vitamin E in counteracting oxidative stress in poultry. J. Poult. Sci. 51(2), 109–117.

Pandey, S., Baker, S.N., Pandey, S. & Baker, G.A., 2012. Fluorescent probe studies of polarity and solvation within room temperature ionic liquids: A review. J. Fluoresc. 22(5),1313–1343.

Park, E.J., Cheenpracha, S., Chang, L.C., Kondratyuk, T.P. & Pezzuto, J.M., 2011. Inhibition of lipopolysaccharide-induced cyclooxygenase-2 and inducible nitric oxide synthase expression by 4-[(2'-O-acetyl-α-L-rhamnosyloxy) benzyl]-isothiocyanate from *Moringa oleifera*. Nutr. Cancer. 63 (6), 971–982.

Pathare, P.B. & Roskilly, A.P., 2016. Quality and energy evaluation in meat cooking. Food. Eng. Rev. 8(4), 435–447.

Patras, P., Nitrayova, S., Brestensky, M. & Heger, J., 2009. The effects of dietary fibre and protein level on nitrogen excretion pattern of pigs. Archia Zootechnical. 12, 5–10.

Perussello, C. A., Zhang, Z., Marzocchella, A. & Tiwari, B. K., 2017. Valorization of apple pomace by extraction of valuable compounds. Compr. Rev. Food Sci. Food Saf. 16, 776–796.

Petracci, M. & Cavani, C., 2011. Muscle growth and poultry meat quality issues. Nutrients. 4(1), 1–12.

Petracci, M. & Fletcher, D.L., 2002. Broiler skin and meat colour changes during storage. Poult. Sci. 81(10), 1589–1597.

Petracci, M., Bianchi, M. & Cavani, C., 2010. Pre-slaughter handling and slaughtering factors influencing poultry product quality. World Poult. Sci. J. 66(1), 17–26.

Pirgozliev, V., Oduguwa, O., Acamovic, T. & Bedford, M.R., 2008. Effects of dietary phytase on performance and nutrient metabolism in chickens. Br. Poult. Sci. 49, 144–154.

Ponsuksili, S., Jonas, E., Murani, E., Phatsara, C., Srikanchai, T., Walz, C., Schwerin, M., Schellander, K. & Wimmers, K., 2008. Trait correlated expression combined with expression QTL analysis reveals biological pathways and candidate genes affecting water holding capacity of muscle. BMC. Genom. 9(1), 1–14.

Povey, S., Cotter, S.C., Simpson, S.J., Lee, K.P. & Wilson, K., 2009. Can the protein costs of bacterial resistance be offset by altered feeding behaviour? J. Anim. Ecol. 78(2), 437–446.

Pratoomyot, J., Bendiksen, E.Å., Bell, J.G. & Tocher, D.R., 2010. Effects of increasing replacement of dietary fishmeal with plant protein sources on growth performance and body lipid composition of Atlantic salmon (*Salmo salar L.*). Aquaculture. 305(1-4), 124–132.

Prevolnik, M., Čandek-Potokar, M. & Škorjanc, D., 2010. Predicting pork water-holding capacity with NIR spectroscopy in relation to different reference methods. J. Food. Eng. 98, 347–352.

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

Qiao, M., Fletcher, D.L., Smith, D.P. & Northcutt, J.K., 2001. The effect of broiler breast meat colour on pH, moisture, water-holding capacity, and emulsification capacity. Poult. Sci. 80(5), 676–680.

Rafiullah, P. and Sajid, A. 2013. Effect of feed additives on the performance of broilers. J. Agric. Biol. Sci. 6, 9–14.

Raj, A.M., Grey, T.C., Audsely, A.R. & Gregory, N.G., 1990. Effect of electrical and gaseous stunning on the carcase and meat quality of broilers. Brit. Poult. Sci. 31(4), 725–733.

Randall, M. & Bolla, G., 2008. Raising Japanese quail. Primefact. 602(2), 1–5.

Rashidi, N., Khatibjoo, A., Taherpour, K., Akbari-Gharaei, M. & Shirzadi, H., 2020. Effects of licorice extract, probiotic, toxin binder and poultry litter biochar on performance, immune function, blood indices and liver histopathology of broilers exposed to aflatoxin-B1. Poult. Sci. 99(11), 5896–5906.

Ravindran, V., 2012. Advances and future directions in poultry nutrition: an overview. Korean. J. Poult. Sci. 39(1), 53–62.

Ravindran, V., 2013. Feed enzymes: The science, practice, and metabolic realities. J. Appl. Poult. Res. 22(3), 628–636.

Ravindran, V., Morel, P.C., Rutherfurd, S.M. & Thomas, D.V., 2008. Endogenous flow of amino acids in the avian ileum as influenced by increasing dietary peptide concentrations. Br. J. Nutr. 101(6), 822–828.

Raza, A., Bashir, S. & Tabassum, R., 2019. An update on carbohydrases: growth performance and intestinal health of poultry. Heliyon. 5(4), 01437.

Reda, F.M., Swelum, A.A., Hussein, E.O., Elnesr, S.S., Alhimaidi, A.R. & Alagawany, M. 2020., Effects of varying dietary DL-methionine levels on productive and reproductive

performance, egg quality, and blood biochemical parameters of quail breeders. Animals. 10(10), 1839.

Ren, B., Wu, M., Wang, Q., Peng, X., Wen, H., McKinstry, W.J. & Chen, Q., 2013. Crystal structure of tannase from *Lactobacillus plantarum*. J. Mol. Biol. 425(15), 2737–2751.

Reyes-Sánchez, N., 2006. *Moringa oleifera* and *Cratylia argentea*: Potential fodder species for ruminants in Nicaragua, Doctoral thesis No. 2001:1. (SLU Acta Universitatis *Agriculturae Sueciae, Uppsala*).

Ribeiro, T., Lordelo, M.M., Costa, P., Alves, S.P., Benevides, W.S., Bessa, R.J.B., Lemos, J.P.C., Pinto, R.M.A., Ferreira, L.M.A., Fontes, C.M.G.A. & Prates, J.A.M., 2014. Effect of reduced dietary protein and supplementation with a docosahexaenoic acid product on broiler performance and meat quality. Br. Poult. Sci. 55(6), 752–765.

Romero, L.F., Parsons, C.M., Utterback, P.L., Plumstead, P.W. & Ravindran, V., 2013. Comparative effects of dietary carbohydrases without or with protease on the ileal digestibility of energy and amino acids and AMEn in young broilers. Anim. Feed Sci. Technol. 181(1-4), 35–44.

Rubanza, K.D.C., Shem, N. M., Ichinole, T. & Fujihara, T., 2005. Polyphenolics and mineral composition of selected browse tree species leaves native to north-western Tanzania traditional fodder banks. J. Food. Agric. Envir. 4, 328–332.

Sahim, N., Akdemir, F., Orhan, C., Kucuk, O., Hayirli, A. & Sahin, K., 2008. *Lycopeneenriched* quail egg as functional food for humans. Int. Food Res. 41, 295–300.

Sahin, K. & Kucuk, O., 2001. Effects of vitamin E and selenium on performance, digestibility of nutrients, and carcass characteristics of Japanese quails reared under heat stress (34 C). J. Anim. Physiol. Anim. Nutr. 85(11-12), 342–348.

Salahuddin, M., Miah, M.A. & Ahmad, N., 2012. Effects of protein and vitamin A.D.E on growth performance and haeto-biochemical profile in broiler. Bangl. J. Vet. Med. 10, 9–14.

Salami, S.A., Agbonlahor, E.M., Atteh, J.O., Sideeq, B.A. & Salako, A.O., 2015. Effect of supplemental enzymes on 80etabolizable energy value of wheat bran and performance of broilers. University of Ilorin, Department of Animal Production, Nigeria.

Sallau, A.B., Mada, S.B., Ibrahim, S. & Ibrahim, U., 2012. Effect of boiling, simmering and blanching on the ant nutritional content of *Moringa oleifera* leaves. Int. J. Food Nutri. Safety. 2, 1–6.

Sanka, Y.D. & Mbaga S.H., 2014. Evaluation of Tanzanian local chicken reared under intensive and semi-intensive systems: II. Meat quality attributes. Livest Res. Rural Dev. 26, 156.

Santos, A.L., Sakomura, N.K., Freitas, E.R., Fortes, C.M.S. & Carrilho, E., 2005. Comparison of free-range broiler chicken strains raised in confined or semi-confined systems. Rev. Bras. Cienc. Avic. 7, 85–92.

Santos, T.C., Gates, R.S., Tinôco, I.F.F., Zolnier, S., Rocha, K.S.O. & Freitas, L.C.S.R., 2019. Productive performance and surface temperatures of Japanese quail exposed to different environment conditions at start of lay. Poult. Sci. 98(7), 2830–2839.

Scheideler, S.E., Beck, M.M., Abudabos, A. & Wyatt, C.L., 2005. Multiple-enzyme (Avizyme) supplementation of corn-soy based layer diets. J. Appl. Poult. Res. 14, 77–86.

Schons, P.F., Battestin, V. & Macedo, G.A., 2012. Fermentation and enzyme treatments for sorghum. Braz. J. Microbiol. 43, 89–97.

Scogings, P.F., Dziba, L.E. & Gordon, I.J., 2004. Leaf chemistry of woody plants in relation to season, canopy retention and goat browsing in a semiarid subtropical savanna. Austral. J. Ecol. 29, 278–286.

Selle, P.H. & Ravindran, V., 2007. Microbial phytase in poultry nutrition. Anim. Feed. Sci. Technol. 135(1-2), 1–41.

Shakouri, M.D., Iji, P.A., Mikkelsen, L.L. & Cowieson, A.J., 2009. Intestinal function and gut microflora of broiler chickens as influenced by cereal grains and microbial enzyme supplementation. J. Anim. Physiol. Anim. Nutr. 93(5), 647–658.

Silanikove, N., Perevolotsky, A. & Provenza, D.F., 2001. Use of tannin-binding chemicals to assay for tannins and their negative post-ingestive effects in ruminants. Anim. Feed Sci. Technol. 91, 69–81.

Singh, A.K. & Kim, W.K., 2021. Effects of dietary fibre on nutrients utilization and gut health of poultry: a review of challenges and opportunities. Animals. 11(1), 181–197.

Singh, A.K., 2018. *Effects of multi-enzymes on growth performance, and effects of multi-enzymes and probiotics on nutrient utilization in broilers fed different level of fibres* (Doctoral dissertation, University of Hawai‘i, Mānoa).

Singh, B., Kunze, G. & Satyanarayana, T., 2011. Developments in biochemical aspects and biotechnological applications of microbial phytases. Biotechnol. Mol. Biol. Rev. 6(3), 69–87.

Singh, V.P., Arulanantham, A., Parisipogula, V., Arulanantham, S. & Biswas, A., 2018. Moringa olifera: nutrient dense food source and world’s most useful plant to ensure nutritional security, good health and eradication of malnutrition. Eur. J. Nutr. Food Saf. 8(4), 204–214.

Siyadati, S.A., Irani, M., Ghazvinian, K., Mirzaei-Aghsaghali, A., Rezaipoor, V., Fathi, H., Alipoor, K. & Zamanzad-Ghavidel, S., 2011. Effect of varying dietary energy to protein ratio on productive performance and carcass characteristics of Japanese quail. Ann. Biol. Res. 2, 149–55.

Slominski, B.A. 2011. Review- Recent advance in research on enzymes in poultry diets. Poult. Sci. 90, 2013–2023.

Soetan, K.O. & Oyewole, O.E., 2009. The need for adequate processing to reduce the antinutritional factors in animal feeds: A review. Afr. J. Food Sci. 3, 223–232.

Spencer, S.P. & Belkaid, Y., 2012. Dietary and commensal derived nutrients: shaping mucosal and systemic immunity. Curr. Opin. Immunol. 24(4), 379384.

Steiner, T. & Wegleitner, K., 2007. Natural growth promoters for gut health management. World Poult. Magaz. 23, 22–24.

Stevens, C.G., Ugese, F.D., Otitoju, G.T. & Baiyeri, K.P., 2015. Proximate and anti-nutritional composition of leaves and seeds of *Moringa oleifera* in Nigeria: a comparative study. Agro-Sci. 14(2), 9–17.

Świątkiewicz, S. & Koreleski, J., 2008. The use of distillers dried grains with solubles (DDGS) in poultry nutrition. World Poult. Sci. J. 64(2), 257–266.

Tang, D., Shengyan, H., Guohua, L., Fang, N. & Yingjun, R., 2014. Effects of maize source and complex enzyme on performance and nutrient utilization of broiler. Asian-Australas. J. Anim. Sci. 27(12), 1755–1762.

Tarasewich, Z., Szczerbinska, D., Ligocki, M., Wiercciska, M., Majewska, D. & Romaniszyn, K., 2006. The effects of differentiated dietary protein level on the performance of breeder quails. Anim. Sci. Pap. Rep. 24, 207–216.

Tavernari, F.C., Albino, L.F.T., Morata, R.L., Dutra Júnior, W.M., Rostagno, H.S. & Viana, M.T.S., 2008. Inclusion of sunflower meal, with or without enzyme supplementation, in broiler diets. Br. Poult. Sci. 10, 233–238.

Ten Have, G.A., Engelen, M.P., Luiking, Y.C. & Deutz, N.E., 2007., Absorption kinetics of amino acids, peptides, and intact proteins. Int. J. Sport. Nutr. Exerc. Metab. 17(1), 23–36.

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

Thorp, B., 2021. The poultry industry. *Poultry Health: A Guide for Professionals*. pp. 25.

Tripathi, M.K. & Mishra, A.S., 2007. Glucosinolates in animal nutrition. A review. Anim. Feed Sci. Technol. 132, 1–27.

Tshabalala, T., Sikosana, J.L.N. & Chivandi, E., 2013. Nutrient intake, digestibility and nitrogen retention in indigenous goats fed on Acacia nilotica fruits treated for condensed tannins. S. Afri. J. Anim. Sci. 43, 458–463.

Tufarelli, V., Dario, M. & Laudadio, V., 2007. Effect of xylanase supplementation and particle-size on performance of guinea fowl broilers fed wheat-based diets. Int. J. Poult. Sci. 4, 302–307.

Turner, P.V., Brabb, T., Pekow, C. & Vasbinder, M.A., 2011. Administration of substances to laboratory animals: Routes of administration and factors to consider. J. Am. Assoc. Lab. Anim. 50, 600–613.

Van Niekerk, R.F., Mnisi, C.M. & Mlambo, V., 2020. Polyethylene glycol inactivates red grape pomace condensed tannins for broiler chickens. Br. Poult. Sci. 61(5), 566–573.

Varastegani, A. & Dahlan, I., 2014. Influence of dietary fibre levels on feed utilization and growth performance in poultry. J. Anim. Prod. Adv. 4(6), 422–429.

Varkoohi, S., Babak, M.M.S., Pakdel, A., Javaremi, A.N., Zaghari, M. & Kause, A., 2010. Response to selection for feed conversion ratio in Japanese quail. Poult. Sci. 89(8),1590–1598.

Vohra, A. & Satyanarayana, T., 2003. Phytases microbial sources, production, purification and potential biotechnological applications. Crit. Rev. Biotech. 23(1), 29–60.

Wang, S., Li, C., Copeland, L., Niu, Q. & Wang, S., 2015. Starch retrogradation: A comprehensive review. Compr. Rev. Food Sci. Food Saf. 14(5), 568–585.

Wattanachant, S., Benjakul, S. & Ledward, D.A., 2004. Composition, colour, and texture of Thai indigenous and broiler chicken muscles. Poult. Sci. 83(1),123–128.

Wattanachant, S., Benjakul, S. & Ledward, D.A., 2005. Microstructure and thermal characteristics of Thai indigenous and broiler chicken muscles. Poult. Sci. 84, 328–336.

Wiatkiewicz, S., Arczewska-Wlosek, A. & Jozefiak, D. 2015., Efficacy of feed enzymes in pig and poultry diets containing distillers dried grains with soluble: a review. J. Anim. Nutr. 100, 15–26.

Widyaratne, G.P. & Drew, M.D., 2011. Effects of protein level and digestibility on the growth and carcass characteristics of broiler chickens. Poult. Sci. 90, 595–603.

Windisch, W., Schedle, K., Plitzner, C. & Kroismayr A., 2008. Use of phytogenic products as feed additives for swine and poultry. J. Anim. Sci. 86, E140–E148.

Wings, O., 2007. A review of gastrolith function with implications for fossil vertebrates and a revised classification. Acta Palaeontol. Pol. 52(1), 14–19.

Woelfel, R.L., Owens C.M., Hirschler, E., Martinez-Dawson M. R. & Sams., A. R., 2002. The characterization and incidence of pale, soft, and exudative broiler meat in a commercial processing plant. Poult. Sci. 81, 579–584.

Woyengo, T.A. & Nyachoti, C.M., 2011. Review: supplementation of phytase and carbohydrases to diets for poultry. Can. J. Anim. Sci. 91, 177–192.

Xiao, J., Mao, F., Yang, F., Zhao, Y., Zhang, C. & Yamamoto, K., 2011. Interaction of dietary polyphenols with bovine milk proteins: Molecular structure-affinity relationship and influencing bioactivity aspects. J. Nutr. Food Res. 55, 1637–1645.

Xiao, Z., Lester, G.E., Luo, Y. & Wang, Q., 2012. Assessment of vitamin and carotenoid concentrations of emerging food products: edible microgreens. J. Agric. Food. Chem. 60, 7644–7651.

Yaghobfar, A. & Kalantar, M., 2017. Effect of non-starch polysaccharide (NSP) of wheat and barley supplemented with exogenous enzyme blend on growth performance, gut microbial, pancreatic enzyme activities, expression of glucose transporter (SGLT1) and mucin producer (MUC2) genes of broiler chickens. Braz. J. Poult. Sci. 19, 629–638.

Yancey, J.W.S., Wharton, M.D. & Apple, J.K., 2011. Cookery method and end-point temperature can affect the Warner–Bratzler shear force, cooking loss, and internal cooked colour of beef longissimus steaks. Meat Sci. 88(1), 1–7.

Zaher, M., El-Ghareeb, A.W., Hamdi, H. & Abu-Amod, F., 2012. Anatomical, histological and histochemical adaptations of the avian alimentary canal to their food habits: I-*Coturnix coturnix*. Life Sci. J. 9(3), 253–275.

Zamini, A.A., Kanani, H.G., Azam Esmaeili, A., Ramezani, S. & Zoriezahra, S.J., 2014. Effects of two dietary exogenous multi-enzyme supplementation, Natuzyme® and betamannanase (Hemicell®), on growth and blood parameters of Caspian salmon (*Salmo trutta caspius).* Comp. Clin. Path. 23(1), 187–192.

Zanella, I., Sakomura, N.K., Silversides, F.G., Fiqueiredo, A. & Pack, M., 1999. Effect of enzyme supplementation of broiler diet based on corn and soybeans. Poult. Sci. 78, 561–568.

Zeng, T. & Durif, F., 2019. The influence of consumers' perceived risks towards eco-design packaging upon the purchasing decision process: An exploratory study. Sustainability. 11(21), 6131.

Zhang, S., Zeng, X., Ren, M., Mao, X. & Qiao, S., 2017. Novel metabolic and physiological functions of branched chain amino acids: a review. J. Anim. Sci. 8(1), 1–12.

Zheng, H.Z., Hwang, I.W. & Chung, S.K., 2009. Enhancing polyphenol extraction from unripe apples by carbohydrate-hydrolyzing enzymes. J. Zhejiang Univ. Sci. B. 10(12), 912–919.

3 CHAPTER THREE - EFFECT OF PRE-TREATING DIETARY *MORINGA OLEIFERA* LEAF MEAL WITH POLYETHYLENE GLYCOL ON GROWTH PERFORMANCE, CARCASS TRAITS AND MEAT QUALITY PARAMETERS IN JUMBO QUAIL

Abstract

The inclusion of dietary *M. oleifera* leaf meal (MOLM) beyond 25 g/kg was reported to compromise growth performance in Japanese quail due to the antinutritional effects of condensed tannins. Thus, in this study, a higher level (100 g/kg) of MOLM inclusion was pre-treated with graded levels of PEG to determine quail responses. The objective of this study was to determine the amount of PEG required to ameliorate MOLM condensed tannins for Jumbo quail based on growth performance and meat quality data. Four hundred and thirty-two (432), two-week-old mixed gender quail chicks (103.4 ± 12.62 g live-weight) were randomly allocated to 36 pens (experimental unit), which were replicated six times per dietary treatment. Six dietary treatments were formulated as follows: a standard grower diet without MOLM (CON); a standard grower diet containing 100 g/kg untreated MOLM (MPG0); and a standard grower diet containing 100 g/kg MOLM pre-treated with PEG at 25 (MPG25), 50 (MPG50), 75 (MPG75), and 100 g/kg (MPG100). Experimental diets and fresh water were offered *ad libitum*. Average weekly feed intake (AWFI) and average weekly body weight gain (ABWG) were used to calculate feed conversion efficiency (FCE). All quail were slaughtered at a local abattoir at the end of 4-week feeding trial. During slaughter, blood was collected from two random quail per experimental unit for determination of haematological and serum biochemical parameters. Repeated measures analysis showed significant week × diet interaction effects on

ABWG and FCE but not AWFI. Overall feed intake linearly increased (P <0.05) with PEG levels. At week 4, significant quadratic trends were recorded for weight gain and feed conversion efficiency (FCE) but, at week 5, FCE linearly declined as PEG levels increased. Haemoglobin, phosphorus, and albumin showed quadratic trends (P <0.05), while calcium and chroma (1 h post-mortem) linearly declined (P <0.05) in response to PEG levels. Diet MPG50 promoted a higher (P <0.05) shear force value (2.41) than diets MPG0 and MPG25. The MPG50 diet promoted a similar (P >0.05) shear force as diet CON. Based on the quadratic response for weight gain, the optimal PEG pre-treatment level was calculated to be 54 g/kg. It was concluded that ameliorating MOLM condensed tannins increased feed intake but reduced feed utilization efficiency in Jumbo quail.

Keywords: Avian birds, Blood indices, Feed additives, Growth traits, Meat quality, Phytogenics

3.1 Introduction

Quail production can provide an opportunity to diversify the poultry industry and thereby increase the supply of animal protein (Deka & Borah, 2008). Indeed, the use of quail birds as a source of protein has increased worldwide (Genchev *et al.*, 2005). This has been attributed to their low maintenance, early sexual maturity, high prolificacy, short generation intervals, fast growth rates, and resistance to numerous avian diseases (Huss *et al*., 2008; Mnisi & Mlambo, 2018). However, sustainable intensification of quail birds could be restricted by high feed costs, disease outbreaks, and poor performance. Indeed, the cost of poultry feeds has remained high, especially in the tropics, due to rising prices of soybeans and maize grain, which are conventional nutrient sources in poultry diets (Marareni & Mnisi, 2020). The competition between humans and livestock for these conventional nutrient sources (Newkirk, 2010)

contributes to rising demand that fuels price increases on the world market. It is, therefore, imperative that non-conventional feedstuffs that have nutraceutical properties be identified for use in quail diets to allow for sustainable intensification and reduce production cost.

One such potential feedstuff is *Moringa oleifera* leaf meal (MOLM), which contains a variety of nutrients and bioactive compounds that could be beneficial to the Jumbo quail. *Moringa oleifera* Lam is a nutrient-rich plant that is widely distributed in tropical and subtropical countries. It is widely used in the animal, food, and pharmaceutical sectors (Bennett *et al*, 2003; Ferreira *et al*., 2008). *Moringa oleifera* by-products have bioactive agents (flavonoids and other phenolics compounds such as caffeic, ferulic, and coumaric acids) with antimicrobial, antioxidant properties, and hypocholesterolaemia effects that can enhance growth performance, health status, the shelf life of the meat, and product quality (Melesse *et al*., 2013; Nduku *et al*., 2020). *Moringa oleifera* leaves have been used as a source of nutrients in poultry (Makkar *et al*., 2007; Mahajan *et al*., 2007) because they contain high concentrations of protein, vitamins (C, K, and B complex), beta-carotene, and manganese (Leon *et al*., 2015). Moreover, the leaf meal has detergent and anti-septic properties due to the presence of different phytochemicals (Torondel *et al*., 2014).

The use of MOLM as a dietary supplement in broilers and layers has been shown to improve growth performance and egg quality (Briones *et al*., 2017). However, the amount of MOLM that can be added into Jumbo quail diets is limited by the presence of condensed tannins (CT) (12 g/kg tannins in a dry matter basis) and fibre (19.3% crude fibre) (Moyo *et al.*, 2018). Indeed, other scholars have reported that the inclusion of MOLM in poultry diets should not exceed 25 g/kg due to the presence of antinutritional factors (Hassan *et al*., 2016; Ufele & Ebenebe, 2017). Further reports have shown that high levels of CT reduce feed utilisation efficiency, growth

rate, and protein digestibility in chickens (Falowo *et al*., 2018). Moreover, high levels of tannins are harmful to the lining of the small intestines because they disturb the normal absorptive function of the gut, resulting in poor performance. Therefore, there is a need to ameliorate the negative effect of CT to allow the birds to fully benefit from the bioactive components of MOLM. One potential strategy is the use of polyethylene glycol (PEG), a tannin-inactivating compound, which has been reported to have high affinity for CT (Silanikove *et al.,* 2001).

Pre-treatment of tannin-rich feeds with PEG has the potential of reducing the negative effects of CT in the GIT of birds and improving protein digestibility (Manssori & Acamovic, 2009). Several studies have investigated the effect of PEG treatment in poultry (Kumanda *et al*., 2019; Zulkifli *et al*., 2019; Van Niekerk *et al*., 2020) and ruminant feeds (Henkin *et al*., 2009; Brown & Ngambi, 2017). However, the effectiveness of PEG in improving the feed value of MOLM in Jumbo quail diets has not been investigated, possibly because this bird is relatively new to the South African poultry industry. Moreover, the application of PEG to ameliorate the negative effects of CT may enable the inclusion of MOLM at high levels (e.g., 100 g/kg) in Jumbo quail diets without compromising their productivity and meat quality. Therefore, this feeding trial evaluated the effect of pre-treating MOLM with different levels of PEG on growth performance, physiology, and meat quality responses of Jumbo quail. The study tested the hypothesis that PEG treatment of MOLM would improve the growth performance, blood parameters, and meat quality attributes in Jumbo quail.

3.2 Material and methods

3.2.1 Ingredient sources

Moringa oleifera leaf meal (MOLM) was purchased from Origin Organics Investments (PTY)

LTD (Gauteng, South Africa). Polyethylene glycol (PEG) was bought from Sigma-Aldrich (Modderfontein, Gauteng, South Africa). The other feed ingredients were procured from Nutroteq (PTY) LTD in Centurion, South Africa.

3.2.2 Chemical analysis of MOLM

Prior to the formulation of the diet, the MOLM was subjected to an initial analysis for proximate composition using the Official Analytical Chemists International Methods (AOAC, 2005). To determine the laboratory dry matter (DM) (AOAC, 2005: method no. 930.15), 1 g of MOLM sample was weighed into pre-weighed crucibles and oven-dried at 105°C for 12 h and re-weighed. The DM was determined by subtracting the moisture content weight from the initial sample weight. The organic matter (OM) content was determined (AOAC, 2005: method no.924.05) by ashing the dried samples for 12 hours at 600°C in a muffle furnace. The weight loss was calculated as OM content while the residue was calculated as ash content. The total nitrogen (N) content was determined using the conventional macro-Kjeldahl (AOAC, 2005, method no. 984.1 3). Afterwards was converted into crude protein (CP) by multiplying N content with a factor of 6.25.

According to Van Soest *et al*. (1991), neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined using an ANKOM^{2000} Fibre Analyzer (ANKOM Technology, New York, USA). Total soluble phenolics (TSPh) were determined following the Folin–Ciocalteau method (Makkar, 2003). Absorbance was recorded at 725 nm wavelength using a spectrophotometer (T60 UV-Visible spectrophotometer, PG Instruments Limited, Lutterworth, UK) and expressed as tannic acid equivalent (g TAE/kg DM). Soluble condensed tannins (SCT) were determined using the Butanol-HCl method (Porter *et al*., 1986) and absorbance was measured at 550 nm wavelength using the spectrophotometer described above. Minerals (calcium, phosphorus, sodium, potassium, magnesium, and sulphur) were analysed using Agri-Laboratory

Association of Southern Africa guidelines (AgriLASA, 1998). The equation developed by Khalil *et al*. (1986) was used to determine the amount of metabolizable energy (ME).

3.2.3 Polyethylene glycol treatment of MOLM

The PEG (Mr 4000) was purchased from Agro-Enviro Solutions (Gauteng, South Africa). Four PEG solutions were made by dissolving 150, 300, 450, and 600 g of PEG in 6 L of distilled water. Subsequently, each PEG solution was sprayed onto 6 kg of MOLM, thus producing PEG treatment rates of 25, 50, 75, and 100 g/kg (w/w) before inclusion into the experimental diets. Untreated MOLM was sprayed with 6 L of distilled water without PEG. The mixing process was conducted as described by Van Niekerk *et al*. (2020). The untreated and treated MOLM samples were kept at an average room temperature of 25°C for a duration of 12 h so that the PEG would react with MOLM tannins. The untreated and treated MOLMs were thereafter air-dried to a constant weight, 1 mm milled (Polymix PX-MFC 90 D, Kinematica AG, Malters, Switzerland), and incorporated into a standard grower diet.

3.2.4 Formulation of experimental diets and analyses

As indicated in Table 3.1, six isonitrogenous and isocaloric experimental diets were formulated by incorporating 100 g/kg of untreated MOLM (MPG0) or MOLM pre-treated with PEG at 25, 50, 75, and 100 g/kg to a standard grower diet (CON). The nutrient composition of experimental diets are shown in Table 3.2.

Table 3.1. Ingredient composition (g/kg *as-fed* basis, unless stated otherwise) of the dietary treatments.

	[1]Diets					
Ingredients	CON	MPG0	MPG 25	MPG50	MPG75	MPG100
Polyethylene glycol	0.0	0.0	25	50	75	100
Moringa oleifera leaf meal	0.0	100.0	100.0	100.0	100.0	100.0
Yellow maize-fine	698.6	626.9	626.9	626.9	626.9	626.9
Choline powder	0.8	0.8	0.8	0.8	0.8	0.8
Full fat soya meal	50.7	148.6	148.6	148.6	148.6	148.6
Grower-phytase	1.7	1.7	1.7	1.7	1.7	1.7
Limestone powder	14.5	14.5	14.5	14.5	14.5	14.5
L-Threonine	0.4	0.4	0.4	0.4	0.4	0.4
Lysine	2.8	2.8	2.8	2.8	2.8	2.8
Methionine	1.9	1.9	1.9	1.9	1.9	1.9
Monocalcium phosphate	7.2	7.2	7.2	7.2	7.2	7.2
Olaquindox	0.4	0.4	0.4	0.4	0.4	0.4
Prime gluten 60	18.0	18.0	18.0	18.0	18.0	18.0
Salt-fine	3.2	3.2	3.2	3.2	3.2	3.2
Sodium bicarbonate	1.7	1.7	1.7	1.7	1.7	1.7
Soybean meal	96.7	70.5	70.5	70.5	70.5	70.5
Vitamin and mineral premix	0.5	0.5	0.5	0.5	0.5	0.5

[1]Diets: CON = control diet (a standard grower diet); MPG0 = control diet containing 100 g/kg *M. oleifera*; MPG25 = control diet containing 100 g/kg *M. oleifera* pre-treated with 25 g/kg polyethylene glycol; MPG50 = control diet containing 100 g/kg *M. oleifera* pre-treated with 50 g/kg polyethylene glycol; MPG75 = control diet containing 100 g/kg *M. oleifera* pre-treated with 75 g/kg polyethylene glycol; MPG100 = control diet containing 100 g/kg *M. oleifera* pre-treated with 100 g/kg polyethylene glycol.

Table 3.2. Nutritional composition (g/ kg DM, unless stated otherwise) of the dietary treatments.

		[1]Diets					
	MOLM	CON	MPG0	MPG25	MPG50	MPG75	MPG100
Dry matter (g/kg)	923.7	916.1	906.5	914.6	913.7	920.8	921.2
Ash	7.6	4.93	5.21	4.81	4.85	5.15	4.49
Organic matter	847.2	911.2	901.2	909.8	908.9	915.7	916.7
Metabolizable energy (MJ/kg)	12.0	12.07	12.07	12.07	12.07	12.07	12.07
Calculated crude protein	177.5	182.5	182.2	182.2	182.3	182.4	182.3
Calcium	7.01	7.00	7.03	7.03	7.03	7.03	7.03
Phosphorus	5.05	5.34	4.77	4.77	4.77	4.77	4.77
Potassium	3.19	3.74	2.66	2.66	2.66	2.66	2.66
Magnesium	1.05	1.21	0.88	0.88	0.88	0.88	0.88
Sulphur	0.60	0.74	0.48	0.48	0.48	0.48	0.48
Sodium	1.68	1.69	1.68	1.68	1.68	1.68	1.68
Neutral detergent fibre	211.1	150.0	142.6	142.4	139.2	105.6	140.5
Acid detergent fibre	151.5	113.9	111.8	92.17	92.2	102.2	111.0
Total phenolics (g/TAE kg DM)	41.8	9.22	19.90	20.90	20.39	18.23	18.83
Condensed tannins (AU_{550nm}/200 mg)	0. 80	0. 04	0.45	0.23	0.20	0.18	0.13

MOLM = *Moringa oleifera* leaf meal; [1]Diets: CON = control diet (a standard grower diet); MPG0 = control diet containing 100 g/kg *M. oleifera*; MPG25 = control diet containing 100 g/kg *M. oleifera* pre-treated with 25 g/kg polyethylene glycol; MPG50 = control diet containing 100 g/kg *M. oleifera* pre-treated with 50 g/kg polyethylene glycol; MPG75 = control diet containing 100 g/kg *M. oleifera* pre-treated with 75 g/kg polyethylene glycol; MPG100 = control diet containing 100 g/kg *M. oleifera* pre-treated with 100 g/kg polyethylene glycol.

3.2.5 Ethics statement and feeding trial

The North-West University's Animal Production Research Ethics Committee granted the approval (NWU-01884-19-S5) for the feeding trial, handling, and slaughter of the birds. The feeding experiment took place at the North-West University's Farm in Molelwane, South Africa, between December 2019 and January 2020. During this period, ambient temperatures ranged from 27°C to 37°C. Mixed-gender Jumbo quail chicks (n = 432; one-week old) were purchased from Golden Quail Farm (Randfontein, South Africa). The chicks were randomly and evenly allotted to 36 pens deemed as the experimental units. The experimental diets were replicated six times per experimental unit. The pens, each holding 12 chicks (60 cm Width × 100 cm Length × 30 cm Height) were built using wire mesh, and the floor was covered with removable polythene plastics used as bedding. The birds were adapted to the six experimental diets until two weeks of age while a stress pack containing water-soluble vitamins and electrolytes was given in the first three days. The experiment was conducted under natural lighting (12 h of daylight) with house temperatures ranging between 25°C and 30°C and an average indoor humidity of 60%. For the 4-week feeding period, the birds had unrestricted access to clean water and experimental diets. Average weekly feed intake (AWFI) was measured by subtracting the weight of the feed refusals (which were measured daily from beginning of week 1 to the end of week 6). Initial live weights were measured at 2 weeks of age and thereafter measured weekly until 6 weeks of age to determine average weekly body weight gain (ABWG). Feed conversion efficiency (FCE) was calculated by dividing body weight gain by feed consumed.

3.2.6 Slaughter and haemo-biochemical analyses

At six-weeks of age, all the birds were starved for 12 hours and weighed to determine their final body weights (FBW) and then transported using bakkie to a local poultry abattoir, where they were stunned and then slaughtered by cutting the jugular vein. At the time of slaughter, blood samples (4 mL) were collected into two sets of sterile whole blood and serum tubes. Purple-top tubes containing ethylene diamante tetra acetic acid as an anti- coagulant were used for haematology, whereas red-top tubes without anticoagulant was used for serum biochemical analysis. Haematological (erythrocytes, haemoglobin, haematocrits, leucocytes, lymphocytes, reticulocytes, monocytes, basophils, eosinophils, neutrophils, mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), red blood cell distribution width (RDW), white blood cells (WBC) were determined using a Haematology Analyser (lDEXX Laboratories S.A. PTY, Gauteng, South Africa). Serum biochemical parameters (albumin, alkaline phosphatase (ALP), alanine transaminase (ALT), amylase, serum cholesterol, blood glucose, lipase, phosphorus, globulin, creatinine and total protein) were determined using a Vet Test Chemistry Analysers (lDEXX Laboratories S.A. PTY, Gauteng, South Africa).

3.2.7 Carcass traits and internal organs

After slaughter, the carcasses were manually eviscerated and individually measured to determine the hot carcass weights (HCW). The carcasses were then chilled in a cold room for 24 hours and reweighed to determine cold carcass weight (CCW). Carcass yield was calculated as the proportion of HCW on final body weight (FBW). Weights of internal organs (gizzard, liver, proventriculus, colon, caecum, small and large intestine including their lengths) and carcass cuts (breast, wing, thigh, and drumstick) were measured using a digital balance (Explorer EX224, 0.01 g readability (2 decimal places), supplied by OHAUS Corp, Parsippany, NJ, USA).

3.2.8 Meat pH and temperature measurements

Meat pH and temperature were recorded 1 h and 24 h post-slaughter on the breast muscle (central area of the breast) using a Corning Model 4 pH-temperature meter (Corning Glass Works, Medfield, MA) fitted with an Ingold spear-type electrode (Ingold Messtechnik AG, Udorf, Switzerland). For shelf life, meat pH and temperature were recorded for five days post-slaughter on the breast muscle. After every 10 measurements, the pH meter was calibrated with pH 4, pH 7, and pH 10 standard solutions (Ingold Messtechnik AG, Udorf, Switzerland) meant for this purpose.

3.2.9 Meat colour attributes

Meat colour coordinates: L^* (lightness), a^* (redness) and b^* (yellowness) were measured from the breast meat samples 1 h and 24 h post-slaughter using a Minolta colour-guide 45/0 BYK-Gardener GmbH machine, with a 20 mm diameter measurement area and illuminant D65-daylight, 10º observation angle after 30 minutes blooming time. For shelf life, meat colour coordinates: L^* (lightness), a^* (redness) and b^* ($\overline{\text{yellowness}}$) were measured for five days post-slaughter on the breast muscle. Hue angle was calculated as

arc $Tan\ (\theta)\ \frac{a^*}{b^*}$ and chroma was calculated as $\sqrt{a^{*2} + b^{*2}}$ in Priolo *et al*. (2002).

3.2.10 Cooking loss and meat tenderness

Raw breast meat samples were individually weighed to obtain initial weight, and followed by cooking the samples in an oven set at 75ºC for 20 min (Honikel, 1998). The samples were allowed to cool before measuring final weight. The following formula was employed to measure the cooking loss:

$$Cooking\ loss\ (\%) = \frac{initial\ weight - final\ weight}{initial\ weight} \times 100$$

After the evaluation of cooking losses, breast samples were sheared using a Meullenet-Owens

razor shear blade set on a Universal lnstron device (crosshead speed = 200 mm/minute, one shear in the centre of each core). The reported value was the average shear force expressed in Newtons for each sample.

3.2.11 Water holding capacity

Water holding capacity (WHC) was determined by pre-weighing (~ 10 g) of freshly cut breast samples which were placed in-between a pre-weighed 18 Whatman filter-paper and pressed under a pressure of 60 kg for 5 min using dumbbell weights (Grau & Hamm, 1957). The water expressed from fresh meat was absorbed by filter bags and thereby used to calculate water holding capacity expressed in proportion of the initial weight as shown in the formula below:

$$WHC\ (\%) = [100 - \frac{initial\ weight - weight\ after\ pressing}{initial\ weight}] \times 100$$

3.2.12 Drip loss

Drip loss was determined using a method adapted from Honikel (1998), whereby duplicate samples from each pen weighing approximately 2 g (w1) each were left hanging inside an airtight container. The suspended samples were stored in a cold room that was pre-set at 4°C for 72 hours. The meat samples were then reweighed to obtain weight after drip (w2). The weight of each sample before and after drip was conveyed as percentage drip loss and calculated as follows:

$$Drip\ loss\ (\%) = \frac{w1 - w2}{w1} \times 100$$

3.3 Statistical analysis

Average weekly feed intake, average weekly body weight gain and average weekly FCE data were analysed using the repeated measures analysis (SAS, 2010). The following statistical linear model was utilised:

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

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

Feed intake, growth performance, haemo-biochemistry, and meat quality data (excluding CON diet) were evaluated for linear and quadratic effects using response surface regression analysis (SAS, 2010) using the following quadratic equation:

$$y = ax^2 + bx + c$$

Where: y = dependent variable, a and b are the coefficients of the quadratic equation; and c is the intercept; x is PEG pre-treatment levels (%) and $\frac{-b}{2a}$ is the x value for optimal response

Overall feed intake, body weight gain, FCE, serum biochemical parameters and meat quality data were analysed using the general linear model (GLM) procedure of SAS (2010), with diet as the only main factor. The linear statistical model employed was as follows:

$$Yij = \mu + Di + Eij$$

Where Yij = dependent variable, μ = population mean, Di = effect of diets, and Eij = random error associated with observation ij, assumed to be normally and independently distributed. For all statistical tests, significance was set at $P < 0.05$ and least squares means were compared using the probability of difference.

3.4 Results

3.4.1 Growth performance and blood indices

Repeated measures analysis showed significant week × diet interaction effects on ABWG (P = 0.031) and FCE (P = 0.017), but not on AWFI (P = 0.341). Table 3.3 shows that PEG pre-treatment levels resulted in a linear increase in overall feed intake (y = 676.2 (±15.95) + 0.198 (±0.763) x; R^2 = 0.329, P = 0.004). Pre-treatment of dietary MOLM with PEG resulted in quadratic trends for weight gain (y = 37.22 (±3.17) + 0.471 (±0.152) x – 0.004 (±0.001) x^2; R^2 = 0.305; P = 0.007) and FCE (y = 0.223 (±0.016) + 0.002 (±0.0008) x – 0.00002 (±0.000007) x^2; R^2 = 0.374; P = 0.002) in four-week-old quail birds, from which the optimal PEG pre-treatment level was calculated to be 54 g/kg for weight gain. A linear decrease in FCE was observed in five-week-old quail birds when PEG levels increased (y = 0.132 (±0.017) 0.001 (±0.0008) x; R^2 = 0.172; P = 0.046). Birds reared on MPG75 and MPG100 diets had higher (P

<0.05) overall FI than those reared on CON, MPG0, and MPG25, for which the overall FI did not differ (P >0.05). In week 4, diet MPG50 (57.02 g/bird) promoted the highest (P >0.05) ABWG compared with diets CON, MPG0, MPG75, and MPG100, which were statistically similar (P >0.05). The MPG50 diet promoted a similar (P >0.05) ABWG as diet MPG25. There were dietary influences (P <0.05) observed for FCE in weeks 4 and 5. In week 4, birds on diet MPG50 (0.325) had the highest FCE when compared with those on diets MPG0 and MPG100. Diet MPG50 promoted the same (P >0.05) FCE as diets CON, MPG25, and MPG75. In week 5, diets MPG0 (0.135) promoted higher FCE than diets MPG50, MPG75, and MPG100, which did not differ (P >0.05). The MPG0 diet had similar (P >0.05) FCE as diets CON and MPG25.

Table 3.3. Effect of pre-treating dietary *Moringa oleifera* leaf meal with different levels of polyethylene glycol on growth performance in Jumbo quail.

	[1]Diets							*P* values	
	CON	MPG0	MPG25	MPG50	MPG75	MPG100	[2]SEM	Linear	Quadratic
Overall FI (g/bird)	644.5^{c}	679.8^{bc}	666.9^{bc}	710.6 ^{ab}	711.5 ^{a}	745.1^{a}	14.6	0.004	0.538
Average weekly weight gain (g/bird)									
Week 3	47.6	39.3	37.1	37.1	46.7	48.3	4.27	0.139	0.179
Week 4	40.8^{b}	39.5^{b}	48.8^{a}	57.0^{a}	44.1^{b}	41.5^{b}	4.38	0.449	0.007
Week 5	20.2	26.4	19.5	17.9	17.9	17.5	3.93	0.119	0.220
Week 6	25.5	28.9	32.5	25.5	24.4	21.07	4.09	0.368	0.238
Average weekly feed conversion efficiency									
Week 3	0.371	0.289	0.287	0.269	0.004	0.330	0.031	0.771	0.450
Week 4	0.253^{abc}	0.232^{bc}	0.317^{ab}	0.325^{a}	0.256^{abc}	0.231^{c}	0.029	0.872	0.002
Week 5	0.110 ^{ab}	0.135^{a}	0.096 ^{ab}	0.087^{b}	0.087^{b}	0.080^{b}	0.018	0.046	0.198
Week 6	0.147	0.143	0.179	0.134	0.124	0.107	0.021	0.182	0.277

[a,b,c] Means in the same row with different superscripts are significantly different (P <0.05).

[1]Diets: CON = control diet (a standard grower diet); MPG0 = control diet containing 100 g/kg *M. oleifera*; MPG25 = control diet containing 100 g/kg *M. oleifera* pre-treated with 25 g/kg polyethylene glycol; MPG50 = control diet containing 100 g/kg *M. oleifera* pre-treated with 50 g/kg polyethylene glycol; MPG75 = control diet containing 100 g/kg *M. oleifera* pre-treated with 75 g/kg polyethylene glycol; MPG100 = control diet containing 100 g/kg *M. oleifera* pre-treated with 100 g/kg polyethylene glycol.

[2]SEM = standard error of the mean.

3.4.2 Haematological parameters

Table 3.4 shows that there was a significant quadratic trend for haemoglobin (y = 6.99 (±1.08) + 0.126 (±0.049) x – 0.001 (±0.0004) x^2; R^2 = 0.151; P = 0.036). No significant dietary effects were observed on all haematological parameters of Jumbo quail, except for haemoglobin. Birds reared on diet MPG0 had the least (P <0.05) amount of haemoglobin (6.84 g/dL) compared with those on diets MPG50 and MPG75, which did not differ (P >0.05).

Table 3.4. Effect of pre-treating dietary *Moringa oleifera* leaf meal with different levels of polyethylene glycol on haematological parameters in Jumbo quail.

	[1]Diets							*P* values	
[2]Parameters	CON	MPG0	MPG25	MPG50	MPG75	MPG100	[3]SEM	Linear	Quadratic
Basophils (× 10^9 /L)	0.102	0.196	0.309	0.194	0.328	0.136	0.067	0.633	0.188
Eosinophils (× 10^9/L)	0.182	0.801	0.811	0.432	0.582	0.395	0.196	0.115	0.825
Erythrocytes (× 10^9/L)	4.76	3.85	3.75	4.96	4.97	4.75	0.59	0.091	0.462
Haematocrits (L/L)	34.1	26.6	25.2	34.6	34.6	31.6	4.70	0.212	0.410
Haemoglobin (g/dL)	9.39[ab]	6.84b	9.76[a]	10.9[a]	10.2[a]	9.57[a]	1.12	0.122	0.036
Lymphocytes (× 10^9/L)	37.75	57.27	82.24	79.24	66.8	83.3	16.6	0.514	0.720
MCH (pg)	18.8	14.8	22.0	23.5	21.1	20.2	3.43	0.395	0.116
MCV (fL)	65.9	55.6	58.3	68.08	70.4	66.1	7.47	0.156	0.388
Monocytes (× 10^9 /L)	0.660	1.31	1.86	1.31	1.21	1.45	0.326	0.751	0.978
Neutrophils (× 10^9/L)	3.48	10.2	9.03	5.31	9.77	5.25	2.46	0.259	0.868
RDW (× 10^9 /L)	18.4	17.7	21.5	23.1	22.3	22.9	2.31	0.172	0.351
Reticulocytes (K/µL)	3.32	5.25	1.85	2.37	3.10	3.41	1.61	0.659	0.203
WBC (× 10^9/L)	42.1	69.9	94.2	85.6	78.7	90.5	17.6	0.663	0.771

[a,b] Means in the same row with different superscripts are significantly different (P <0.05).

[1]Diets: CON = control diet (a standard grower diet); MPG0 = control diet containing 100 g/kg *M. oleifera*; MPG25 = control diet containing 100 g/kg *M. oleifera* pre-treated with 25 g/kg polyethylene glycol; MPG50 = control diet containing 100 g/kg *M. oleifera* pre-treated with 50 g/kg polyethylene glycol; MPG75 = control diet containing 100 g/kg *M. oleifera* pre-treated with 75 g/kg polyethylene glycol; MPG100 = control diet containing 100 g/kg *M. oleifera* pre-treated with 100 g/kg polyethylene glycol.

[2]Parameters: MCV = mean corpuscular volume; MBH = mean corpuscular haemoglobin; RDW = red blood cell distribution width; WBC = white blood cells.

[3]SEM = standard error of the mean.

3.4.3 Serum biochemical parameters

Serum calcium linearly declined (y = 3.94 (±0.346) − 0.018 (±0.015) x; R^2 = 0.158, P = 0.035) as PEG levels increased (Table 3.5). However, serum phosphorus (y = 3.31 (±0.613) + 0.076 (±0.028) x − 0.0006 (±0.0002) x^2; R^2 = 0.189, P = 0.018) and albumin (y = 27.6 (±4.14) − 0.379 (±0.189) x + 0.004 (±0.001) x^2; R^2 = 0.165, P = 0.030) quadratically responded to incremental levels of PEG by first decreasing and then increasing. The diets had no (P >0.05) effect on serum biochemical parameters, except on phosphorus and albumin. However, diet CON promoted statistically similar (P >0.05) albumin and phosphorus levels as the other treatment groups.

Table 3.5. Effect of pre-treating dietary *Moringa oleifera* leaf meal with different levels of polyethylene glycol on serum biochemical parameters in Jumbo quail.

	[1]Diets							*P* values	
[2]Parameters	CON	MPG0	MPG25	MPG50	MPG75	MPG100	[3]SEM	Linear	Quadratic
Albumin (g/L)	25.9[ab]	26.6[ab]	22.7[a]	17.6b	21.6[a]b	31.0[a]	4.64	0.492	0.030
ALKP (U/L)	244.3	82.8	212.1	150.5	180.5	122.6	54.85	0.864	0.143
ALT (U/L)	65.5	31.8	43.7	50.6	56.1	55.2	12.38	0.089	0.487
Amylase (U/L)	284.1	274.8	384.2	330.5	441.4	363.9	99.79	0.462	0.559
Calcium (mmol/L)	3.71	3.87	3.69	3.09	3.05	2.94	0.361	0.035	0.584
Creatinine (mmol/L)	26.6	31.4	17.4	39.5	18.0	29.5	9.77	0.954	0.854
Globulin (g/L)	42.9	49.8	56.5	44.5	45.08	39.9	8.61	0.232	0.742
Glucose (mmol/L)	0.885	1.75	1.57	1.38	1.06	1.00	0.450	0.154	0.941
Lipase (U/L)	264.4	125.0	168.7	186.8	172.9	148.9	54.89	0.767	0.328
Phosphorus (mmol/L)	4.68[ab]	3.12b	5.20[a]	5.20[a]	5.20[a]	4.33ab	0.599	0.307	0.018
Total protein (g/L)	63.2	41.6	79.3	63.5	62.9	60.9	11.41	0.661	0.167

[a,b] Means in the same row with different superscripts are significantly different (P <0.05).

[1]Diets: CON = control diet (a standard grower diet); MPG0 = control diet containing 100 g/kg *M. oleifera*; MPG25 = control diet containing 100 g/kg MOLM pre-treated with 25 g/kg polyethylene glycol; MPG50 = control diet containing 100 g/kg MOLM pre-treated with 50 g/kg polyethylene glycol; MPG75 = control diet containing 100 g/kg MOLM pre-treated with 75 g/kg polyethylene glycol; MPG100 = control diet containing 100 g/kg MOLM pre-treated with 100 g/kg polyethylene glycol.

[2]Parameters: ALP = alkaline phosphatase; ALT = alanine transaminase.

[3]SEM = standard error of the mean.

3.4.4 Internal organs

Pre-treatment of dietary MOLM with PEG had no *(P* >0.05*)* linear or quadratic trends for internal organ sizes in Jumbo quail (Table 3.6). Similarly, no significant dietary effects were observed on internal organ sizes of the birds.

Table 3.6. Effect of pre-treating dietary *Moringa oleifera* leaf meal with different levels of polyethylene glycol on internal organ sizes in Jumbo quail.

	[1]Diets							*P* values	
[2]Parameter	CON	MPG0	MPG25	MPG50	MPG75	MPG100	[3]SEM	Linear	Quadratic
Weights (%HCW)									
Caecum	10.2	10.5	11.8	9.73	13.8	9.13	1.12	0.911	0.429
Colon	2.76	3.23	3.90	3.67	3.24	3.34	0.326	0.609	0.300
Small intestine	60.7	55.0	65.4	60.8	66.4	66.4	6.21	0.619	0.753
Lengths (cm)									
Caecum	0.979	1.36	1.33	1.50	0.824	1.18	0.153	0.136	0.911
Colon	0.620	0.255	0.224	0.395	0.564	0.322	0.160	0.258	0.352
Gizzard	2.25	2.09	2.37	2.14	2.06	2.33	0.102	0.532	0.661
Liver	2.93	2.89	2.90	3.05	3.01	3.13	0.204	0.310	0.973
Proventriculus	0.541	0.571	0.582	0.647	0.585	0.719	0.063	0.175	0.645
Small intestine	3.90	3.630	3.70	3.62	3.94	3.68	0.253	0.291	0.238

[1]Diets: CON = control diet (a standard grower diet); MPG0 = control diet containing 100 g/kg *M. oleifera*; MPG25 = control diet containing 100 g/kg *M. oleifera* pre-treated with 25 g/kg polyethylene glycol; MPG50 = control diet containing 100 g/kg *M. oleifera* pre-treated with 50 g/kg polyethylene glycol; MPG75 = control diet containing 100 g/kg *M. oleifera* pre-treated with 75 g/kg polyethylene glycol; MPG100 = control diet containing 100 g/kg *M. oleifera* pre-treated with 100 g/kg polyethylene glycol.

[2]Parameters: HCW = hot carcass weight.

[3]SEM = standard error of the mean.

3.4.5 Carcass traits

Pre-treatment of dietary MOLM with PEG had no (P >0.05) linear or quadratic trends for carcass characteristics sizes in Jumbo quail (Table 3.7). Similarly, no significant dietary effects were observed on carcass traits of the birds, except on thigh weights with birds reared on diet CON having lighter (P <0.05) thigh weights (6.03 %HCW) than those reared on diet MPG0 (6.90 %HCW).

Table 3.7. Effect of pre-treating dietary *Moringa oleifera* leaf meal with different levels of polyethylene glycol on carcass characteristics sizes (% HCW, unless stated otherwise) in Jumbo quail.

	[1]Diets							*P* values	
[2]Parameters	CON	MPG0	MPG25	MPG50	MPG75	MPG100	[3]SEM	Linear	Quadratic
Carcass yield (%)	67.3	66.2	60.5	64.0	65.3	63.9	2.03	0.794	0.391
FBW (g)	234.3	232.4	233.1	239.6	235.9	231.3	6.48	0.766	0.366
HCW (g)	157.8	153.7	142.4	152.2	153.6	147.5	4.20	0.741	0.957
CCW (g)	154.5	149.6	142.5	149.1	122.6	166.9	14.9	0.866	0.951
Breast	21.2	21.2	19.9	20.8	21.6	18.94	1.57	0.537	0.569
Wing	7.46	7.27	7.72	7.51	7.55	7.41	0.259	0.839	0.721
Thigh	6.03[b]	6.90[a]	6.52[ab]	6.34[ab]	6.20[ab]	6.21[ab]	0.280	0.050	0.404
Drumstick	4.26	4.05	4.17	4.27	3.90	4.82	0.319	0.223	0.345
Lengths (cm)									
Wing (cm)	4.16	4.46	4.50	4.54	4.61	4.48	0.244	0.882	0.209
Thigh (cm)	3.38	3.51	3.45	3.45	3.36	3.24	0.175	0.108	0.641
Drumstick (cm)	4.01	3.76	4.07	3.87	3.90	3.80	0.183	0.712	0.291

[a,b] Means in the same row with different superscripts are significantly different (P <0.05).

[1]Diets: CON = control diet (a standard grower diet); MPG0 = control diet containing 100 g/kg *M. oleifera*; MPG25 = control diet containing 100 g/kg *M. oleifera* pre-treated with 25 g/kg polyethylene glycol; MPG50 = control diet containing 100 g/kg *M. oleifera* pre-treated with 50 g/kg polyethylene glycol; MPG75 = control diet containing 100 g/kg *M. oleifera* pre-treated with 75 g/kg polyethylene glycol; MPG100 = control diet containing 100 g/kg *M. oleifera* pre-treated with 100 g/kg polyethylene glycol.

[2]Parameters: FBW = final body weight; HCW = hot carcass weight; CCW = cold carcass weight.

[3]SEM = standard error of the mean.

3.4.6 Meat quality traits

Chroma values measured 1 h post-mortem showed a linear decrease (y = 22.4 (±1.64) − 0.207 (±0.077) x; R^2 = 0.436; P = 0.049) in response to PEG pre-treatment levels (Table 3.8). The dietary treatments had significant effects on chroma of the meat. Birds reared on MPG0, MPG25, MPG50, and MPG100 diets had higher (P <0.05) meat chroma$_1$ than those reared on CON and MPG75.

Table 3.8. Effect of pre-treating dietary *Moringa oleifera* leaf meal with polyethylene glycol on meat quality parameters measured 1 h post-mortem of Jumbo quail.

	[1]Diets							*P* values	
[2]Parameters	CON	MPG0	MPG25	MPG50	MPG75	MPG100	[3]SEM	Linear	Quadratic
Meat pH$_1$	5.9	6.00	6.02	5.97	5.96	6.03	0.033	0.862	0.621
Temperature$_1$ (°C)	23.1	20.9	21.2	24.0	22.4	20.8	1.45	0.643	0.482
L^*_1	53.4	52.9	51.5	52.9	53.6	51.9	1.05	0.375	0.499
a^*_1	3.73	3.93	4.05	4.18	3.80	4.15	0.226	0.974	0.843
b^*_1	9.66	11.6	11.5	11.8	10.8	11.7	0.603	0.330	0.432
Chroma$_1$	10.3^b	12.2^a	12.2^a	12.5^a	11.4^{ab}	12.4^a	0.564	0.049	0.055
Hue angle$_1$	1.19	1.23	1.23	1.22	1.22	1.23	0.027	0.514	0.743

[a,b] Means in the same row with different superscripts are significantly different (P <0.05).

[1]Diets: CON = control diet (a standard grower diet); MPG0 = control diet containing 100 g/kg *M. oleifera*; MPG25 = control diet containing 100 g/kg *M. oleifera* pre-treated with 25 g/kg polyethylene glycol; MPG50 = control diet containing 100 g/kg *M. oleifera* pre-treated with 50 g/kg polyethylene glycol; MPG75 = control diet containing 100 g/kg *M. oleifera* pre-treated with 75 g/kg polyethylene glycol; MPG100 = control diet containing 100 g/kg *M. oleifera* pre-treated with 100 g/kg polyethylene glycol.

[2]Parameters: L^* = lightness; a^* = redness; b^* = yellowness.

[3]SEM = standard error of the mean.

Table 3.9 shows that there were significant linear and quadratic trends for breast meat hue angle$_{24}$ (R^2 = 1.00; *P* = 0.0001) as PEG levels increases. The dietary treatments had significant effects on chroma$_{24}$ and hue angle$_{24}$ of the meat. Diet MPG100 promoted the highest chroma$_{24}$ value (17.87) compared with diets CON, MPG0, MPG25, MPG50, and MPG75, which did not differ (*P* >0.05). Meat from birds reared on diet MPG100 had a lower (*P* <0.05) hue angle$_{24}$ than meat from those in MPG0 and MPG75, whose hue angle$_{24}$ did not differ.

Table 3.9. Effect of pre-treating dietary *Moringa oleifera* leaf meal with polyethylene glycol on meat quality parameters measured 24 h post-mortem in Jumbo quail.

	[1]Diets							*P* values	
[2]Parameters	CON	MPG0	MPG25	MPG50	MPG75	MPG100	[3]SEM	Linear	Quadratic
Meat pH$_{24}$	5.92	5.99	5.95	5.93	5.95	5.90	0.897	0.225	0.161
Temperature$_{24}$ (°C)	18.0	19.5	20.8	16.1	16.1	16.1	2.91	0.752	0.763
*L**$_{24}$	48.77	49.37	47.45	47.22	40.21	34.91	3.91	0.816	0.099
*a**$_{24}$	5.50	5.43	6.11	6.16	5.40	9.03	0.786	0.3470	0.7755
*b**$_{24}$	11.8	14.7	14.5	13.4	13.06	15.03	0.611	0.645	0.076
Chroma$_{24}$	13.06^{c}	15.7^{b}	15.7^{b}	14.8^{b}	14.1^{bc}	17.8^{a}	0.629	0.051	0.223
Hue angle$_{24}$	1.13^{ab}	1.21^{a}	1.16^{ab}	1.13^{ab}	1.17^{a}	1.04^{b}	0.046	0.000	0.000

[a,b,c] Means in the same row with different superscripts are significantly different (*P* <0.05).

[1]Diets: CON = control diet (a standard grower diet); MPG0 = control diet containing 100 g/kg *M. oleifera*; MPG25 = control diet containing 100 g/kg *M. oleifera* pre-treated with 2.5 g/kg polyethylene glycol; MPG50 = control diet containing 100 g/kg *M. oleifera* pre-treated with 5 g/kg polyethylene glycol; MPG75 = control diet containing 100 g/kg *M. oleifera* pre-treated with 7.5 g/kg polyethylene glycol; MPG100 = control diet containing 100 g/kg *M. oleifera* pre-treated with 10 g/kg polyethylene glycol.

[2]Parameters: *L** = lightness; *a** = redness; *b** = yellowness.

[3]SEM = standard error of the mean.

Neither linear nor quadratic trends (P >0.05) in response to the PEG levels were observed for meat quality traits of the quail except for the shear force (Table 3.10). Dietary treatments had no significant effect on the cooking loss, water holding capacity, and drip loss. Shear force showed quadratic response (y = 2.19 (±0.049) + 0.004 (±0.002) x – 0.00004 (±0.00002) x^2; R^2 = 0.130; P = 0.053) in response to PEG pre-treatment levels. Diet MPG50 promoted a higher shear force value (2.41 N) than MPG0 and MPG25 diets, which did not differ (P >0.05). The CON diet promoted similar (P >0.05) shear force values as all the other treatment groups.

Table 3.10. Effect of pre-treating dietary *Moringa oleifera* leaf meal with polyethylene glycol on meat quality parameters of Jumbo quail.

Parameters	[1]Diets						[2]SEM	*P* values	
	CON	MPG0	MPG25	MPG50	MPG75	MPG100		Linear	Quadratic
Cooking loss (%)	23.9	19.6	21.4	24.6	21.7	24.6	1.88	0.103	0.599
Drip loss (%)	33.9	30.7	30.8	31.6	31.3	31.4	1.48	0.654	0.974
Shear force (N)	2.30[ab]	2.21[b]	2.21[b]	2.41[a]	2.25[b]	2.23[b]	0.049	0.627	0.053
Water holding capacity (%)	87.9	86.9	87.5	87.0	87.3	87.4	0.978	0.076	0.220

[a,b] Means in the same row with different superscripts are significantly different (P <0.05).

[1]Diets: CON = control diet (a standard grower diet); MPG0 = control diet containing 100 g/kg *M. oleifera*; MPG25 = control diet containing 100 g/kg *M. oleifera* pre-treated with 2.5 g/kg polyethylene glycol; MPG50 = control diet containing 100 g/kg *M. oleifera* pre-treated with 5 g/kg polyethylene glycol; MPG75 = control diet containing 100 g/kg *M. oleifera* pre-treated with 7.5 g/kg polyethylene glycol; MPG100 = control diet containing 100 g/kg *M. oleifera* pre-treated with 10 g/kg polyethylene glycol.

[2]SEM = standard error of the mean.

The data measured at room temperature over 5 days showed no significant (P >0.05) effect of dietary treatment on meat shelf life (Figures 3.1–3.4). The breast meat pH for Jumbo on MPG0, MPG25, MPG50, MPG75, and MPG100 diets increased over 5 days. For all treatments, the lightness (L*) of the breast meat decreased over 5 days. The redness (a*) of the breast meat decreased over 5 days among the treatments. The yellowness (b*) of the breast meat decreased for MPG0, MPG25, MPG50 and MPG 100 while MPG75 increased over 5 days.

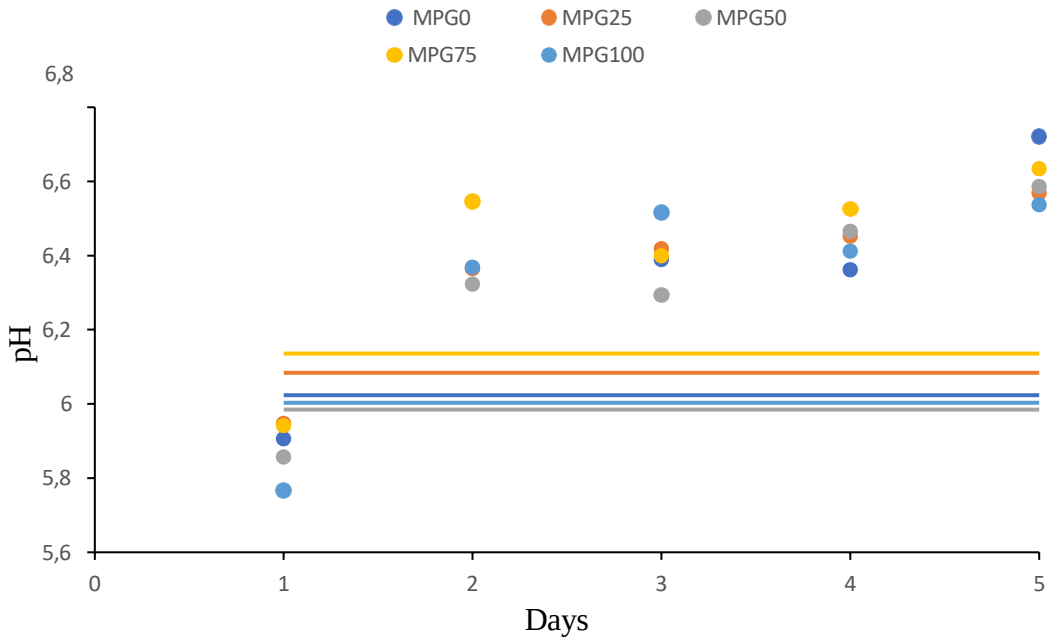


Figure 3.1. Effect of pre-treated *Moringa oleifera* leaf meal on the stability of breast meat pH upon storage at room temperature for 5 days [Diets: MPG0 = control diet containing 100 g/kg *M. oleifera*; MPG25 = control diet containing 100 g/kg *M. oleifera* pre-treated with 25 g/kg polyethylene glycol; MPG50 = control diet containing 100 g/kg *M. oleifera* pre-treated with 50 g/kg polyethylene glycol; MPG75 = control diet containing 100 g/kg *M. oleifera* pre- treated with 75 g/kg polyethylene glycol; MPG100 = control diet containing 100 g/kg *M. oleifera* pre-treated with 100 g/kg polyethylene glycol].

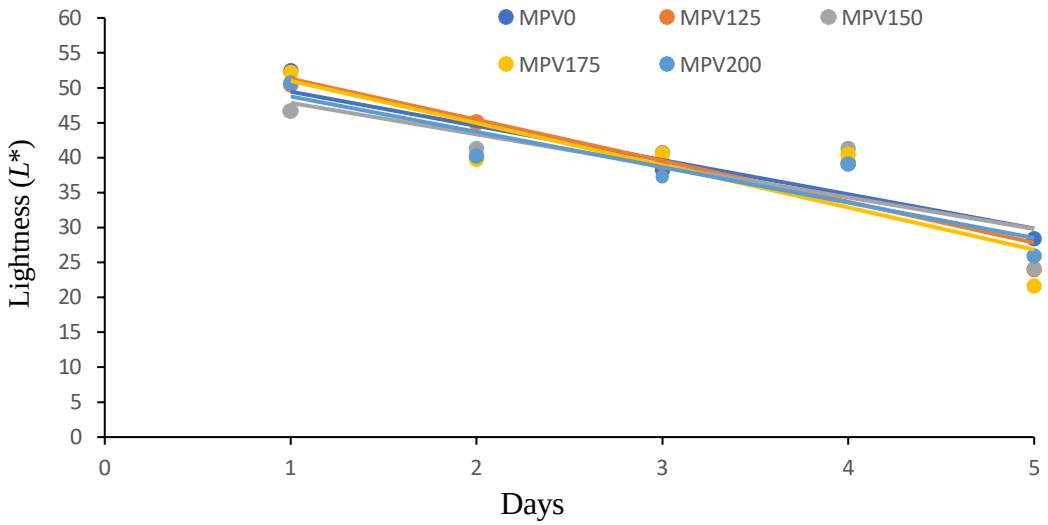


Figure 3.2. Effect of pre-treated *Moringa oleifera* leaf meal on the stability of breast meat lightness upon storage at room temperature for 5 days [*Diets*: MPG0 = control diet containing 100 g/kg *M. oleifera*; MPG25 = control diet containing 100 g/kg *M. oleifera* pre-treated with 25 g/kg polyethylene glycol; MPG50 = control diet containing 100 g/kg *M. oleifera* pre-treated with 50 g/kg polyethylene glycol; MPG75 = control diet containing 100 g/kg *M. oleifera* pre- treated with 75 g/kg polyethylene glycol; MPG100 = control diet containing 100 g/kg *M. oleifera* pre-treated with 100 g/kg polyethylene glycol].

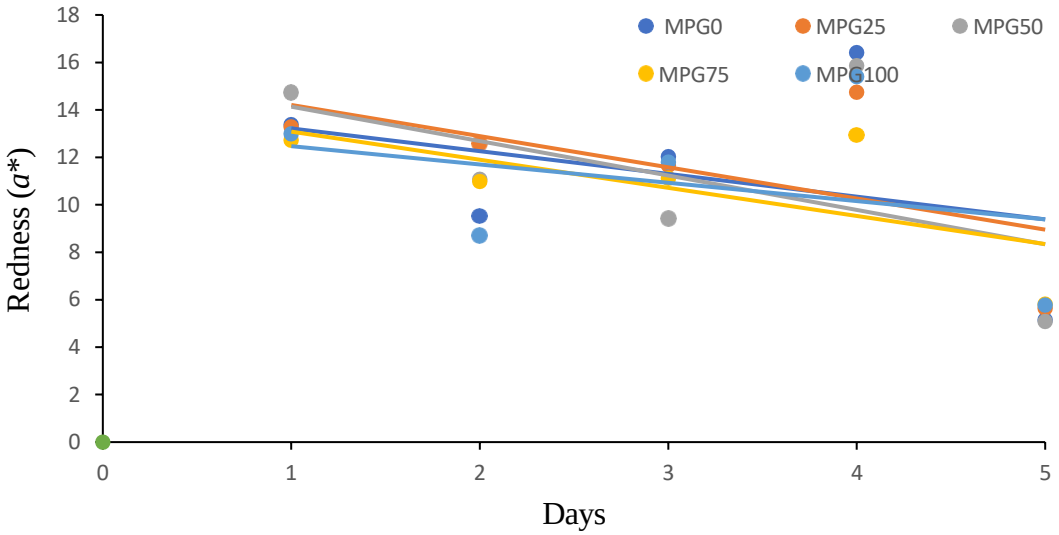


Figure 3.3. Effect of pre-treated *Moringa oleifera* leaf meal on the stability of breast meat redness upon storage at room temperature for 5 days [*Diets*: MPG0 = control diet containing 100 g/kg *M. oleifera*; MPG25 = control diet containing 100 g/kg *M. oleifera* pre-treated with 25 g/kg polyethylene glycol; MPG50 = control diet containing 100 g/kg *M. oleifera* pre-treated with 50 g/kg polyethylene glycol; MPG75 = control diet containing 100 g/kg *M. oleifera* pre- treated with 75 g/kg polyethylene glycol; MPG100 = control diet containing 100 g/kg *M. oleifera* pre-treated with 100 g/kg polyethylene glycol].

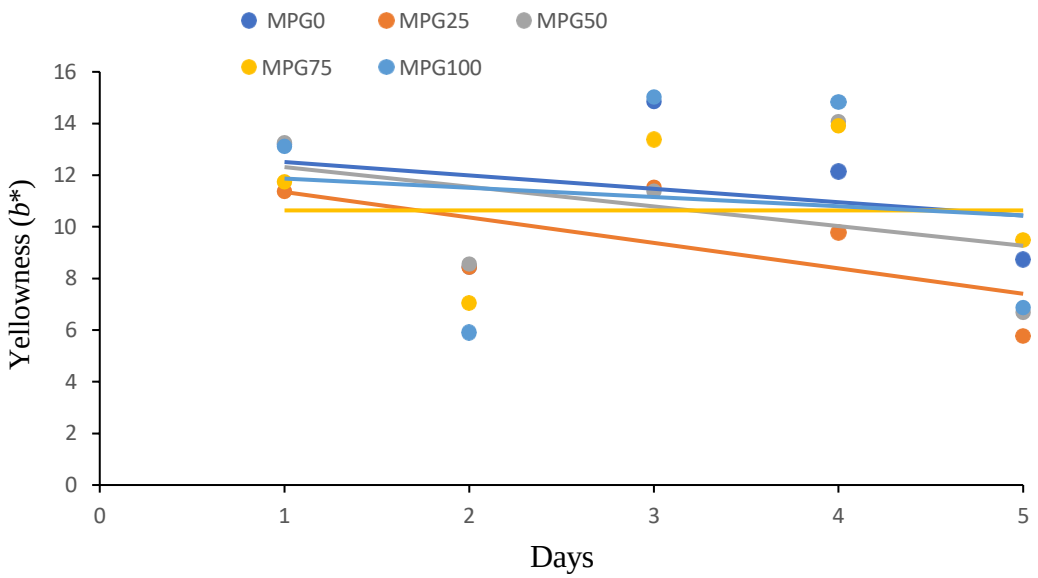


Figure 3.4. Effect of pre-treated *Moringa oleifera* leaf meal on the stability of breast meat yellowness upon storage at room temperature for 5 days [*Diets*: MPG0 = control diet containing 100 g/kg *M. oleifera*; MPG25 = control diet containing 100 g/kg *M. oleifera* pre-treated with 25 g/kg polyethylene glycol; MPG50 = control diet containing 100 g/kg *M. oleifera* pre-treated with 50 g/kg polyethylene glycol; MPG75 = control diet containing 100 g/kg *M. oleifera* pre- treated with 75 g/kg polyethylene glycol; MPG100 = control diet containing 100 g/kg *M. oleifera* pre-treated with 100 g/kg polyethylene glycol].

3.5 Discussion

3.5.1 Growth performance and haemo-biochemistry

Moringa oleifera leaf meal has nutraceutical properties that can be used in poultry diets to enhance performance, antioxidant capacity, and product quality (Ufele & Ebenebe, 2017; Falowo *et al*., 2018). However, high levels of CT in MOLM could restrict its utilisation at higher inclusion levels in Jumbo quail diets. Consequently, PEG, a tannin-binding agent, can be applied to negate the antinutritional effects of MOLM CT on quail performance (Silanikove *et al*., 2001). The PEG does not interfere with digestion processes but binds all the polyphenolic compounds including flavones, lignin, and tannins, although it has a higher affinity for CT (Hoste, 2006). However, the use of PEG to improve the feed value of MOLM at higher inclusion levels in Jumbo quail diets has not been conclusively investigated. Pre-treatment of dietary MOLM with incremental levels of PEG had no effect on the concentrations of total soluble phenolics, suggesting that most phenolics in MOLM are not CT. However, pre-treatment with PEG tended to reduce the concentrations of CT in MOLM. This is because PEG binds to CT, forming strong PEG-tannin complexes that do not react with the butanol-HCl mixture during CT assay (Makkar, 2003). Importantly, the CT in these complexes become inactive and do not reduce crude protein digestibility. In this study, repeated Repeated measures analysis showed a significant diet × week interaction effect for weight gain and FCE, indicating that the efficacy of the birds in converting the dietary treatments into body mass varied with the age of birds.

The quadratic responses observed for weight gain and FCE in week 4 only were surprising and the reasons are unknown. The significant linear increase in overall FI as PEG levels increased could indicate that the anti-nutritional effects of CT were successfully ameliorated, resulting in improved feed utilization. The untreated MOLM promoted the lowest weight gain compared

with the standard control diet and the PEG pre-treated MOLM diets, further confirming that the antinutritional effects of CT were ameliorated. These findings corroborate a report by Bhat *et al*. (2013), which indicated that PEG enhanced nitrogen digestibility in broilers reared on high-tannin sorghum diets. In contrast, pre-treating tannin-containing rapeseed meal with PEG at a rate of 1.5% had no influence on male broiler performance (Karunajeewa *et al*., 1990). The response of the quail reared on MOLM treated with less than 54 g/kg PEG may indicate that the amount of PEG administered to them was insufficient to inactivate the harmful effects of CT.

Blood parameters offer a clearer diagnosis of toxicosis and clinical surveillance of disorders as well as indicators of pathogenic and nutritional state of animals (Karesh *et al*., 1997). No diet-induced changes were observed for all haematological parameters except haemoglobin, which exhibited a positive quadratic trend as PEG levels increased. However, all the haematological parameters fell within the normal ranges reported for a healthy quail (Genchev *et al*., 2005; Huss *et al*., 2008; Mahlake *et al*., 2021). The fact that the albumin initially increased and then declined confirms that PEG treatment must be capped at 54 g/kg. Moreover, no differences were observed particularly on serum total protein as well as the liver enzymes (ALT and ALKP), further verifying that the pre-treating of MOLM with PEG did not compromise the health status of the birds.

3.5.2 Carcass and meat quality traits

Pre-treating MOLM with PEG had no influence on the size of internal organs, carcass, or meat quality attributes except chroma, hue angle$_{24}$, and shear force. These findings corroborate with those of Kumanda *et al*. (2019), who observed a lack of dietary effect on meat lightness (*L**), redness (*a**), and yellowness (*b**) in broilers fed with diets containing red grape pomace. Nonetheless, as the PEG levels increased, the redness of the meat decreased, indicating that the

highest PEG treatment of MOLM may have interfered with anthocyanin and lowered the myoglobin content of the meat.

Tenderness is an important factor that consumers evaluate when making a purchase (Siddhuraju & Becker, 2003). Because of the degradation of myofibrillar proteins, meat softness improves substantially as muscles age. The shear force value reveals how delicate the meat is, with a lower value indicating tenderness and a higher value showing toughness. As such, meat from birds reared on the diet pre-treated with 50 g/kg PEG had a higher shear force value than the meat from the birds reared on the other treatments (MPG0 and MPG25) indicating tougher meat in the former group. In comparison with the control diet, the chroma values increased considerably with the addition of PEG, which could be attributable to an increase in *a** values. As a result, PEG treatment of MOLM increased the colour intensity of Jumbo quail meat.

The ability of meat to hold water is referred to as its water holding capacity (WHC) (Pearce *et al*., 2011). It is a critical quality metric that impacts the amount of water lost during transit, storage, processing, and cooking as well as the visual attractiveness of meat (Bertram *et al*., 2003). Juices are released during cooking because of protein denaturation and muscle atrophy (Purslow *et al*., 2016). In this investigation, there was no change in WHC, cooking loss, or drip loss, indicating that utilising untreated or treated MOLM did not impact meat quality in Jumbo quail birds. For meat stability (shelf life), all meat quality parameters measured at room temperature over 5 days were not significantly affected by dietary treatments. However, the pH of the meat increased over time. In general, increasing the pH of meat is associated with improved meat quality, however, increasing pH over time also indicates microbial proliferation. These results agree with Ali *et al*. (2021), who evaluated the effect of MOLM extract on the 6-day shelf life of crustaceans.

3.6 Conclusion

Inactivating condensed tannins with polyethylene glycol in *Moringa oleifera* leaf meal boosted overall feed intake. In week 4, the average weekly weight gain and feed conversion efficiency of quail initially increased in response to polyethylene glycol pre-treatment levels before decreasing. However, blood parameters, internal organs, carcass characteristics, and meat quality attributes were not influenced by PEG treatment. We concluded that pre-treating *Moringa oleifera* leaf meal with PEG at 54 g/kg maximizes weight gain in Jumbo quail.

3.7 References

Ali, F., Oaf, G., Elmasry, A. & Hassan, A., 2021. Sensory acceptability, shelf life, and quality of crustaceans treated with *Moringa oleifera* and green tea leaf extracts versus acetic acid. J. Ad. Vet. Res. 11(2), 102–109.

AgriLASA. 1998. Feed and Plant Analysis Methods; Agri Laboratory Association of Southern Africa: Pretoria, South Africa.

AOAC. 2005. *Official Methods of Analysis of the Association of Official's Analytical Chemists.* 18th ed.; Association of Official Analytical Chemists: Arlington, VA, USA.

Bennett, R.N., Mellon, F.A., Foid, N., Pratt, J.H., Dupont, M.S., Perkins, L. & Kroon, P.A., 2003. Profiling glucosinolates and phenolics in vegetative and reproductive tissues of the multi-purpose trees *Moringa oleifera* L. (horseradish tree) and *Moringa stenopetala*. J. Agric. Food Chem. 51, 3546–3553.

Bertram, H.C., Andersen, H.J., Karlsson, A.H., Horn, R; Hedegaard, J., Nargaard, L. & Engelsen, S.B., 2003. Prediction of technological quality (cooking loss and Napole Yield) of pork based on fresh meat characteristics. Meat Sci. 65, 707–712.

Bhat, T.K., Kannan, A., Singh, B. & Sharma, O.P., 2013. Value addition of feed and fodder by alleviating the antinutritional effects of tannins. Agric. Res. 2(3), 189–206.

Briones, J., Leung, A., Bautista, N., Golin, S., Caliwag, N., Carlos, M.A., Guevarra, J., Miranda, J., Guevarra, J.K. & Pili, N.L., 2017. Utilization of *Moringa oleifera* Lam. in animal production. Acta Hortic. 1158, 467–474.

Brown, D. & Ngambi, J.W., 2017. Effect of polyethylene glycol 4000 supplementation on the performance of yearling male Pedi goats fed dietary mixture levels of *Acacia karroo* leaf meal and *Setaria verticillata* grass hay. Trop. Anim. Health Prod. 49, 1051–1057.

Chung, K.T., Wong, T.Y., Wei, C.I.; Huang, Y.W. & Lin, Y., 1998. Tannins and human

health: A Review. Crit. Rev. Food Sci. Nutr. 38, 421–464.

CIE. 1976. *Recommendations on Uniform Colour Spaces-Colour Difference Equations, Psychometric Colour Terms; Supplement No*. 2 to CIE Publication No. 15 (E-1.3.1.) 1978, 1971/(TC-1-3); Commission Internationale de I'Eclairage: Paris, France.

Deka, K. & Borah, J., 2008. Haematological and biochemical changes in Japanese quails *Coturnix coturnix japonica* and chickens due to *Ascaridia galli* infection. Int. J. Poult. Sci. 7, 704–710.

Falowo, A.B., Mukumbo, F.E., Idamokoro, E.M., Lorenzo, J.M., Afolayan, A.J. & Muchenje, V., 2018. Multi-functional application of *Moringa oleifera* Lam. in nutrition and animal food products: A review. Food. Res. Int. 106, 317–334.

Ferreira, P.M.P., Farias, D.F., Oliveir, J.T. & Carvalho, F.U., 2008. *Moringa oleifera*: Bioactive compounds and nutritional potential. Rev. de Nutr. 21, 431–437.

Ford, J.E. & Hewitt, D., 1979. Protein quality in cereals and pulses. 3. Bioassay with rats and chickens on sorghum *(Sorghum vulgare Pers.*), barley and field beans (*Vicia faba L*.). Influence of polyethylene glycol on digestibility of the protein in high-tannin grain. Br. J. Nutr. 42, 325–340.

Genchev, A.G., Ribarski, S.S., Afanasjev, G.D. & Blohin, G.I., 2005. Fattening capacities and meat quality of Japanese quails of Faraon and White English breeds. J. Cent. Eur Agric. 6, 495–500.

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

Hassan, H.M.A., El-Moniary, M.M., Hamouda, Y., El-Daly, E.F., Youssef, A.W. & Abd El Azeem, N.A., 2016. Effect of different levels of *Moringa oleifera* leaves powder on productive performance, carcass characteristics and some blood parameters of broiler

chicks reared under heat stress conditions. Asian J. Anim. Vet. Adv. 21, 60–66.

Henkin, Z., Perevolotsky, A., Rosenfeld, A. & Brosh, A., 2009. The effect of polyethylene glycol on browsing behaviour of beef cattle in a tanniferous shrubby Mediterranean range. Livest. Sci. 126, 245–251.

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

Hoste, H., Jackson, F., Athanasiadou, S., Thamsborg, S.M. & Hoskin, S.O., 2006. The effects of tannin-rich plants on parasitic nematodes in ruminants. Trends. Parasitol. 22(6), 253–261.

Huss, D., Pointer, G. & Lansford, R. 2008. Japanese quail (*Coturnix japonica*) as a laboratory animal model. Lab. Anim. 37, 513–519.

Karesh, W.B., Campo, A.D., Braselton, E., Puche, H. & Cook, R.A., 1997. Health evaluation of free ranging and hand reared Macaws (*Ara* spp.) in Peru. J. Zoo Wildl. Med. 28, 368–377.

Karunajeewa, H., Ijagbuji, E.G. & Rreece, R.L., 1990. Effect of dietary levels of rapeseed meal and polyethylene glycol on the performance of male broiler chickens. Br. Poult. Sci. 51, 545–555.

Khalil, J.K., Saway, W.N. & Hyder, S.Z., 1986. Nutrient composition of Atriplex leaves grown in Saudi Arabia. J. Range Manag. 39, 104–107.

Kumanda, C., Mlambo, V. & Mnisi, C.M., 2019. Valorization of red grape pomace waste using polyethylene glycol and fibrolytic enzymes physiological and meat quality responses in broilers. Animals. 9, 779.

Leon, A., Spada, A., Battezzati, A., Schiraldi, A., Aristil, J. & Bertoli, S., 2015. Cultivation, genetic, ethnopharmacology, phytochemistry and pharmacology of *Moringa oleifera*

leaves: An overview. Int. J. Mol. Sci. 16, 12791–12835.

Mahajan, S., Mali, R. & Mehta, A., 2007. Protective effect of ethanolic extract of seeds of *Moringa oleifera* Lam against inflammation associated with development of arthritis in rats. J. Immunotoxicol. 4, 39–47.

Makkar, H.P.S., 2003. Quantification of tannins in tree and foliage. A Laboratory Manual; Springer: Dordrecht, The Netherlands, pp. 43–54.

Makkar, H.R., Francis, G. & Becker, K., 2007. Bioactivity of phytochemicals in some lesser-known plants and their effects and potential applications in livestock and aquaculture production systems. Animal. 1, 1371–1391.

Manssori, B. & Acamovic, T., 2009. Influence of tannic acid and polyethylene glycol on the excretion and digestibility of amino acids in gelatin-fed broilers. Br. Poult. Sci. 50, 199–206.

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.

Melesse, A., Getye, Y., Berihun, K. & Banejee, S., 2013. Effect of feeding graded levels of *Moringa stenopetala* leaf meal on growth performance, carcass traits and some serum biochemical parameters of Koekoek chickens. Livest. Sci. 157, 498–505.

Mnisi, C.M. & Mlambo, V. 2018., Growth performance, haematology, serum biochemistry and meat quality parameters of Japanese quails (*Coturnix coturnix japonica*) fed canola meal-based diets. Anim. Nutr. 4, 37–44.

Moyo, B., Masika, P.J., Hugo, A. & Muchenje, V., 2011. Nutritional characterization of Moringa (*Moringa oleifera* Lam) leaves. Afr. J. Biotechnol. 10, 12925–12933.

Nduku, X.P., Mabusela, S.P. & Nkukwana, T.T., 2020. Growth and meat quality of broiler

chickens fed *Moringa oleifera* leaf meal, a probiotic and an organic acid. S. Afr. J. Anim. Sci. 50, 710–718.

Newkirk, R. 2010. Soybean: Feed Industry guide.; Canadian International Grains Institute; Winnipeg, MB, 1st ed: Canada. p.48.

Pearce, K.L., Rosenvold, K., Andersen, H.J. & Hopkins, D.L., 2011. Water distribution and mobility in meat during the conversion of muscle to meat and ageing and the impacts on fresh meat quality attributes - A review. Meat Sci. 89, 111–124.

Porter, L.J., Hrstich, L.N. & Chan, B.G., 1986. The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. Phytochem. 25, 223–230.

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(2), 179–185.

Purslow, P.R., Oiseth, S., Hughes, J. & Warner, R.D., 2016. The structural basis of cooking loss in beef: Variations with temperature and ageing. Food Res. Int. 89, 739–748.

Siddhuraju, R. & Becker, K., 2003. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. J. Agric. Food Chem. 51, 2144–2155.

Sikka, M.P. & Midha, V.K., 2019. The role of biopolymers and biodegradable polymeric dressings in managing chronic wounds. In *Advanced Textiles for Wound Care*, 2nd ed.; Woodhead Publishing: India, New Delhi.

Silanikove, N., Perevolotsky, A. & Provenza, D.F., 2001. Use of tannin-binding chemicals to assay for tannins and their negative postingestive effects in ruminants. Anim. Feed Sci. Technol. 91, 69–81. Statistical Analysis System Institute Inc. 2010. *Users Guide*, SAS: Carry, NC, USA,

Stevens, C.G., Ugese, F.D., Otitoju, G.T.& Baiyeri, K.P., 2015. Proximate and anti-nutritional composition of leaves and seeds of *Moringa oleifera* in Nigeria: A comparative study. Agro-Sci. 14(2), 9–17.

Torondel, B., Opare, D., Brandberg, B., Cobb, E. & Cairncross, S., 2014. Efficacy of *Moringa oleifera* leaf powder as a hand- washing product: A crossover-controlled study among healthy volunteers. BMC Complement. Altern. Med. 14, 57.

Ufele, A.N. & Ebenebe, C.I., 2017. The Effect of *Moringa oleifera* on the growth performance, packed cell volume (PCV) and laying capacity of young growing quails. Am. J. Zool. Res. 5, 33–37.

Van Niekerk, R.F., Mnisi, C.M. & Mlambo, V., 2020. Polyethylene glycol inactivates red grape pomace condensed tannins for broiler chickens. Br. Poult. Sci. 61, 566–573.

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

Zulkifli, I., Tan, H.S., Soleimani, A.F., Goh, Y.M., Croes, E., Partha, S.K. & Tee, A.K., 2019. The effects of glyceryl polyethylene glycol ricinoleate on growth performance, fat digestibility, apparent metabolisable energy, serum lipid profile, meat fatty acid content, and caecal microflora in male broiler chickens. Eur. Poult. Sci. 83.

4 CHAPTER FOUR - EFFECT OF PRE-TREATING DIETARY *MORINGA OLEIFERA* LEAF MEAL WITH FIBROLYTIC ENZYMES ON GROWTH PERFORMANCE, PHYSIOLOGICAL AND MEAT QUALITY PARAMETERS IN JUMBO QUAIL

Abstract

Due to the presence of non-starch polysaccharides (NSPs), *Moringa oleifera* leaf meal (MOLM) tends to be of relatively lower digestibility and promote lower feed intake. Therefore, animals fed with MOLM-containing diets could benefit from pre-treatment of the leaf meal with a viscozyme® L multi-enzyme (VME). Three hundred and ninety-six (396), two-week-old mixed gender quail chicks (87.8 ± 4.40 g live-weight) were randomly allocated to 36 pens (experimental unit), which were replicated six times per dietary treatment. Six dietary treatments were as follows: a standard grower diet without MOLM, control diet (CON), 156.5 g neutral detergent fibre/kg), a standard grower diet containing 100 g/kg untreated MOLM (VME0); and a standard grower diet containing 100 g/kg MOLM pre-treated with VME at a rate of 2.5 (VME25), 5 (VME50), 7.5 (VME75), and 10 g/kg (VME100). Experimental diets and fresh water were offered *ad libitum*. Average weekly feed intake (AWFI) and average weekly weight gain (ABWG) were used to calculate feed conversion efficiency (FCE). There were no significant linear or quadratic effects on growth performance parameters and carcass characteristics in response to incremental levels of fibrolytic enzymes. However, neutrophils linearly increased (P <0.05), while breast meat lightness and 24 h hue angle linearly declined (P <0.05) with VME levels. All the haemato-biochemical values fell within the normal ranges

for healthy quail. Quadratic effects (P <0.05) were observed on gizzard weights and 1 h hue angle in response to enzyme levels. It was concluded that the maximum fibrolytic multi-enzyme application rate of 10 g/kg may not have been adequate to enhance feed utilisation and positively affect weight gain in Jumbo quail, thus higher levels may need to be investigated further.

Keywords: Carcass characteristics, *Coturnix coturnix,* Feed additives, Growth performance, phytogenics

4.1 Introduction

Moringa foliage has been used as an organic additive in animal and human diets to supply biologically active substances such as carotenoids, ascorbic acid, and phenolic compounds (Tesfaye *et al*., 2012). In addition, *Moringa oleifera* leaf meal (MOLM) is a rich source of antioxidants (Bamishaiye *et al*., 2011), which can be used to enhance Jumbo quail meat quality and treat inflammatory conditions (Pari & Kumar, 2002). Jumbo quail is the largest and fastest-growing meat-type breed of quail, which has been recently developed from the traditional Japanese quail genealogy. The birds are noted for early attainment of sexual maturity (six weeks of age), high reproductive rates (±100 eggs per year), disease resistance and low feed requirements (35–45 g/day/bird) (Marareni & Mnisi, 2020).

The extract of MOLM contains tannins, flavonoids, and glycosides that have medicinal properties (Bamishaiye *et al*., 2011). In south Asia and some parts of Africa, MOLM is used to treat a variety of diseases (Verma *et al*., 2002), while their consumption has been reported to improve nutrient absorption, increase immunological response, strengthen immune functions, and promote health in broiler chickens. This could be attributed to the high concentrations of micronutrients and polyphenols (Nkukwana *et al*., 2014). In addition, the

MOLM have been shown to improve quality and oxidative stability of broiler meat due to their high antioxidant activity (Verma *et al*., 2002; Cui *et al*., 2018). However, the presence of antinutritional factors (ANF) such as tannins, saponins, phytate, lectins, and cyanogenic glucosides, in MOLM (Stevens *et al*., 2019) can compromise utilisation of nutrient and bioactive compounds and, consequently, quail performance. Indeed, other scholars have reported that the inclusion of MOLM in quail diets should not exceed 25 g/kg due to the presence of ANF (Hassan *et al*., 2016; Mulaudzi *et al*., 2019).

Moreover, high fibre content (300 g/kg DM) in MOLM negatively affects nutrient availability (Afuang *et al*., 2003; Richter *et al*., 2003) and limits their level of inclusion in simple non-ruminant diets (Sul & Chen, 2020). High intake of dietary fibre has been reported to reduce nutrient digestibility and cause acute toxicosis and hepatocellular damage to birds (Kim & Lillehoj, 2019), suggesting a need to ameliorate the negative effect of fibre to allow the birds to fully benefit from the bioactive components of MOLM. Indeed, poultry birds, such as the quail, have no capacity to produce the enzymes, such as beta-glucanase, hemicellulase, and cellulase, which is needed to digest β-glycosidic linkages in MOLM non-starch polysaccharides (Vooren, 2012). Fibre reduces nutrient intake and growth rate and may compromise biochemical parameters when included at high levels in animal diets (Khanyile, 2007). Indeed, dietary fibre is known to increase intestinal transit time and intestinal viscosity, which reduces diffusion and assimilation rates of nutrients and beneficial bioactive compounds in birds (Jha & Mishra, 2021). One potential solution to this problem is the use of exogenous fibrolytic enzymes to break down the cell wall components in MOLM prior to their inclusion in quail diets.

Exogenous fibrolytic enzyme supplementation reduces the intestinal viscosity and the nutrient encapsulating effect of cell walls, and it subsequently increases protein and energy utilisation

(Slominski, 2011). The use of fibrolytic enzymes could enhance the utility of MOLM as a source of nutrients and bioactive compounds in quail diets by reducing the antinutritional activities of its fibre content and thus promote similar or improved blood parameters as the control diet. Feed enzymes have become an important tool to increase the nutritional value of feed ingredients, reduce feed costs, and ensure environmental stewardship while maintaining or improving animal performance

Furthermore, using fibrolytic enzymes could allow MOLM to be included at higher amounts in Jumbo quail diets without negatively affecting their productivity and meat quality. Therefore, this study evaluated the effect of pre-treating MOLM with graded levels of viscozyme® L multi-enzyme (VME), a fibrolytic enzyme admixture, on feed utilisation and physiological and meat quality responses in Jumbo quail. The study tested the hypothesis that pre-treatment of MOLM with fibrolytic enzymes would improve growth performance, blood parameters, and meat quality attributes in Jumbo quail.

4.2 Material and methods

4.2.1 Study site and ingredient sources

The experiment was carried out from March to April 2021 during summer whereby ambient temperatures around the area ranges from 17ºC to 37ºC. The *M. oleifera* leaf meal was from the same batch as the one described in Chapter 3, Section 3.2.1. The study area was described in Chapter 3, Section 3.2.5. Viscozyme® L is a multi-enzyme complex containing a wide range of carbohydrases, including arabanase, cellulase, β-glucanase, hemicellulase, and xylanase. It has an enzyme activity of 100 fungal β-glucanase per gram and a density of 1.2 g/mL and was supplied by Sigma-Aldrich (Modderfontein, Gauteng, South Africa). The other feed ingredients were bought from Simplegrow Agric Services (Pty) Ltd and Nutroteq (PTY) LTD in Centurion, South Africa.

4.2.2 Chemical analysis of Moringa oleifera leaf meal

The MOLM was chemically analysed in Chapter 3, Section 3.2.2.

4.2.3 Enzyme treatment of moringa and analyses

The MOLM was pre-treated with Viscozyme® L at the rate of 0, 2.5, 5.0, 7.5, and 10 g/kg before being incorporated into a standard quail diet. Exactly 6 kg of milled (2 mm; Polymix PX-MFC 90D, Kinematica AG, Switzerland) MOLM per treatment was sprayed and hand mixed with 6000 mL of distilled water in which 12.5, 25, 37.5, and 50 mL of Viscozyme® L (density: 1.2 g/mL) was dissolved (Kumanda *et al*., 2019). The untreated MOLM (6 kg) was sprayed with 6000 mL of distilled water only. During this mixing process, efforts were made to avoid the leaching of MOLM chemical components by ensuring that no excess liquid ran off the samples in partially opened containers (Matshogo *et al*., 2021). Treated and untreated MOLM were then stored at room temperature (average 30˚C) for 24 h to allow time for the VME to predigest fibre (Matshogo *et al*., 2021) in MOLM. Thereafter, the untreated and treated MOLM were sun-dried until constant weight and then crushed (2 mm) before being used in diet formulation. The nutrient composition (Table 4.2) of untreated and enzyme pre-treated MOLM was determined using the methods by the Association of Official Analytical Chemists (AOAC, 2005) for dry matter (DM), ash, organic matter (OM), and crude protein (CP). The fibre detergent method (Van Soest *et al*., 1991) was used to determine the neutral detergent fibre (NDF) and acid detergent fibre (ADF). Metabolisable energy (ME) was calculated using the equation by Khalil *et al*. (1986).

Table 4.1. Nutritional composition (g/kg DM, unless stated otherwise) of untreated and Viscozyme® L enzyme pre-treated *Moringa oleifera* leaf meal

	[1]Substrates				
	MV0	MV1	MV2	MV3	MV4
Dry matter (g/kg)	918.4	921.4	925.8	913.9	910.1
Ash	9.51	9.55	9.43	8.68	8.08
Organic matter	827.3	825.8	823.3	820.5	892.2
Metabolizable energy (MJ/kg)	11.9	11.9	11.9	11.8	11.9
Crude protein	217.0	213.0	213.2	212.8	206.0
Neutral detergent fibre	165.4	161.3	160.4	155.5	159.6
Acid detergent fibre	147.2	148.2	146.6	145.7	141.9
Acid detergent lignin	134.7	132.5	131.9	130.1	133.2

[1]Diets: MV0 = MOLM without enzyme pre-treatment; MV1 = MOLM pre-treated with 2.5 g/kg fibrolytic enzyme; MV2 = MOLM pre-treated with 5 g/kg fibrolytic enzyme; MV3 = MOLM pre-treated with 7.5 g/kg fibrolytic enzyme; MV4 = MOLM pre-treated with 10 g/kg fibrolytic enzyme.

4.2.4 Diet formulation

Six experimental diets, in mash form, were formulated (Table 4.2) by hand to meet the nutritional requirements for grower quail, as guided by the National Research Council (NRC, 2004). This was accomplished by incorporating treated and untreated MOLM into a standard grower diet as follows: a standard grower diet without MOLM (CON); a standard grower diet containing 100 g/kg MOLM without VME pre-treatment (VME0); and a standard grower diet containing 100 g/kg MOLM pre-treated with 2.5 (VME25), 5.0 (VME50), 7.5 (VME75), and 10 g/kg VME (VME100). The nutritional composition (Table 4.3) of the dietary treatments was analyzed as described above for the untreated and VME-treated MOLM samples.

Table 4.2. Gross ingredient (g/kg *as fed* basis) of the dietary treatments.

Ingredients	[1]Diets					
	CON	VME0	VME25	VME50	VME75	VME100
Viscozyme® L	0.0	0.0	2.5	5	7.5	10
Moringa oleifera leaf meal	0.0	100.0	100.0	100.0	100.0	100.0
Yellow maize fine	698.6	626.9	626.9	626.9	626.9	626.9
Prime gluten 60	18.0	18.0	18.0	18.0	18.0	18.0
Full-fat soya meal	50.7	148.6	148.6	148.6	148.6	148.6
Soybean meal	196.7	70.5	70.5	70.5	70.5	70.5
Limestone powder	14.5	14.5	14.5	14.5	14.5	14.5
Monocalcium phosphate	7.2	7.2	7.2	7.2	7.2	7.2
Salt fine	3.2	3.2	3.2	3.2	3.2	3.2
Sodium bicarbonate	1.7	1.7	1.7	1.7	1.7	1.7
Choline powder	0.8	0.8	0.8	0.8	0.8	0.8
Lysine	2.8	2.8	2.8	2.8	2.8	2.8
L-Threonine	0.4	0.4	0.4	0.4	0.4	0.4
Methionine	1.9	1.9	1.9	1.9	1.9	1.9
Grower phytase	1.7	1.7	1.7	1.7	1.7	1.7
Vitamin and mineral premix[2]	0.5	0.5	0.5	0.5	0.5	0.5
Olaquindox antibiotic	0.4	0.4	0.4	0.4	0.4	0.4

[1]Diets: CON = standard quail grower diet without *M. oleifera* leaf meal; VME0 = control diet containing 100 g/kg *M. oleifera* leaf meal without viscozyme® L multi-enzyme; VME25 = control diet containing 100 g/kg *M. oleifera* leaf meal pre-treated with 2.5 g/kg viscozyme® L multi-enzyme; VME50 = control diet containing 100 g/kg *M. oleifera* leaf meal pre-treated with 5 g/kg viscozyme® L multi-enzyme; VME75 = control diet containing 100 g/kg *M. oleifera* leaf meal pre-treated with 7.5 g/kg viscozyme® L multi-enzyme; VME100 = control diet containing 100 g/kg *M. oleifera* leaf meal pre-treated with 10 g/kg viscozyme® L multi-enzyme.

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

Table 4.3. Nutrient composition (g/kg DM, unless stated otherwise) of the dietary treatments.

	Diets					
	CON	VME0	VME25	VME50	VME75	VME100
Dry matter (g/kg)	913.6	917.9	919.9	922.2	926.7	929.0
Ash	4.66	4.34	4.58	4.53	4.63	4.16
Organic matter	864.9	861.6	867.9	872.8	873.8	877.5
Metabolizable energy (MJ/kg)	11.9	11.8	11.8	11.8	11.8	11.8
Crude protein	187.2	187.7	188.6	188.4	187.7	188.8
Neutral detergent fibre	156.5	155.3	148.3	152.2	154.1	154.3
Acid detergent fibre	141.1	144.1	142.3	149.2	144.1	148.7
Acid detergent lignin	131.3	138.6	134.2	134.4	134.4	136.2

[1]Diets: CON = standard quail grower diet without *M. oleifera* leaf meal; VME0 = control diet containing 100 g/kg *M. oleifera* leaf meal without viscozyme® L multi-enzyme; VME25 = control diet containing 100 g/kg *M. oleifera* leaf meal pre-treated with 2.5 g/kg viscozyme® L multi-enzyme; VME50 = control diet containing 100 g/kg *M. oleifera* leaf meal pre-treated with 5 g/kg viscozyme® L multi-enzyme; VME75 = control diet containing 100 g/kg *M. oleifera* leaf meal pre-treated with 7.5 g/kg viscozyme® L multi-enzyme; VME100 = control diet containing 100 g/kg *M. oleifera* leaf meal pre-treated with 10 g/kg viscozyme® L multi-enzyme.

4.2.5 Feeding trial, slaughter, blood, and meat quality

The North-West University's Animal Production Research Ethics Committee granted approval (NWU-0 1884-19-S5) for the feeding trial, handling, and slaughter of the birds. The feeding trial

was conducted as described in Chapter 3, Section 3.2.5. The hematological and serum biochemical parameters were analyzed according to the description in Chapter 3, Section 3.2.6.

The carcass traits and internal organs were analyzed as described in Chapter 3, Section 3.2.7. Meat pH and temperature were recorded as described in Chapter 3, Section 3.2.8. Meat color coordinates: *L** (lightness), *a** (redness) and *b** (yellowness) were measured as described in Chapter 3, Section 3.2.9. Cooking loss and meat tenderness were conducted according to Chapter 3, Section 3.2.10. Water holding capacity was determined as described in Chapter 3, Section 3.2.11. Drip loss was determined using a method described in Chapter 3, Section 3.2.12.

4.2.6 Statistical analysis

Average weekly feed intake, BWG and FCE data were analyzed using the repeated measures analysis (SAS, 2010) described in Chapter 3, Section 3.3. Feed intake, growth performance, haemo-biochemistry, and meat quality data (excluding CON diet) were evaluated for linear and quadratic effects using response surface regression analysis (SAS, 2010) using the following quadratic equation:

$$y = ax^2 + bx + c$$

Where: y = dependent variable, a and b are the coefficients of the quadratic equation; and c is the intercept; x is VME pre-treatment levels (%) and $\frac{-b}{2a}$ is the x value for optimal response

Overall feed intake, BWG, FCE, serum biochemical parameters and meat quality data were analysed using the general linear model (GLM) procedure of SAS (2010), with diet as the only main factor. The linear statistical model was described in Chapter 3, Section 3.3.

4.3 Results

4.3.1 Growth performance

Repeated measures analysis did not show significant week × diet interaction effects on average weekly BWG (P = 0.184) and FCE (P = 0.417) but on FI (P = 0.011). Table 4.3 shows that there were neither linear nor quadratic responses (P >0.05) for average weekly FI, overall weight gain, and overall FCE to incremental levels of VME. Similarly, no enzymatic effects (P >0.05) were observed on overall weight gain, overall FCE, and FI in weeks 3, 4, 5, and 6.

Table 4.4. Effect of pre-treating dietary *Moringa oleifera* leaf meal with incremental levels of viscozyme® L multi-enzyme on growth performance (g/bird) in Jumbo quail.

[2]Parameters	[1]Diets						[3]SEM	*P* values	
	CON	VME0	VME25	VME50	VME75	VME100		Linear	Quadratic
Average weekly feed intake									
Week 3	139.5	139.2	143.1	140.5	143.8	140.0	3.564	0.082	0.443
Week 4	150.7	157.6	151.3	151.5	154.2	160.2	3.750	0.492	0.054
Week 5	232.2	229.1	223.5	231.8	230.0	223.4	3.781	0.712	0.447
Week 6	209.2	205.8	202.6	214.0	206.7	195.5	5.043	0.337	0.093
Overall BWG	148.8	137.6	146.3	145.9	138.9	144.5	4.625	0.651	0.452
Overall FCE	0.203	0.187	0.203	0.197	0.189	0.200	0.005	0.525	0.587

[1]Diets: CON = standard quail grower diet without *M. oleifera* leaf meal; VME0 = control diet containing 100 g/kg *M. oleifera* leaf meal without viscozyme® L multi-enzyme; VME25 = control diet containing 100 g/kg *M. oleifera* leaf meal pre-treated with 2.5 g/kg viscozyme® L multi-enzyme; VME50 = control diet containing 100 g/kg *M. oleifera* leaf meal pre-treated with 5 g/kg viscozyme® L multi-enzyme; VME75 = control diet containing 100 g/kg *M. oleifera* leaf meal pre-treated with 7.5 g/kg viscozyme® L multi-enzyme; VME100 = control diet containing 100 g/kg *M. oleifera* leaf meal pre-treated with 10 g/kg viscozyme® L multi-enzyme.

[2]Parameters: BWG = body weight gain; FCE = feed conversion efficiency.

[3]SEM = standard error of the mean.

4.3.2 Haematological parameters

Table 4.5 indicates that all the haematological measures parameters showed neither linear nor quadratic effects (P >0.05), except for neutrophils, which linearly increased with VME levels (y = 1.18 (±0.875) + 0.037 (±0.039) x; R^2 = 0.206; P = 0.017). Similarly, only neutrophils showed a significant dietary effect, with birds on diet VME100 having higher (P <0.05) neutrophil levels (4.47 109/L) than those on diets VME0 and VME75. However, the CON diet promoted similar (P >0.05) neutrophil levels as all the other dietary treatments.

Table 4.5. Effect of pre-treating dietary *Moringa oleifera* leaf meal with incremental levels of viscozyme® L multi-enzyme on haematological parameters in Jumbo quail.

	[1]Diets							*P* values	
Parameters	CON	VME0	VME25	VME50	VME75	VME100	[2]SEM	Linear	Quadratic
Haemoglobin (g/dL)	6.25	9.20	8.66	12.0	7.60	9.05	1.17	0.211	0.779
Neutrophils (×10^9/L)	3.36^{ab}	0.980^{b}	2.69^{ab}	3.26^{ab}	1.39^{b}	4.47^{a}	1.153	0.017	0.827
Monocytes (×10^9/L)	0.558	0.243	1.23	1.15	0.301	0.825	0.505	0.675	0.340
Eosinophils (×10^9/L)	0.826	1.002	0.773	0.282	0.407	0.669	0.340	0.237	0.862
Basophils (×10^9/L)	0.040	0.147	0.110	0.063	0.043	0.101	0.068	0.554	0.259

[a,b]Means in the same row with different superscripts are significantly different (P <0.05).

[1]Diets: CON = standard quail grower diet without *M. oleifera* leaf meal; VME0 = control diet containing 100 g/kg *M. oleifera* leaf meal without viscozyme® L multi-enzyme; VME25 = control diet containing 100 g/kg *M. oleifera* leaf meal pre-treated with 2.5 g/kg viscozyme® L multi-enzyme; VME50 = control diet containing 100 g/kg *M. oleifera* leaf meal pre-treated with 5 g/kg viscozyme® L multi-enzyme; VME75 = control diet containing 100 g/kg *M. oleifera* leaf meal pre-treated with 7.5 g/kg viscozyme® L multi-enzyme; VME100 = control diet containing 100 g/kg *M. oleifera* leaf meal pre-treated with 10 g/kg viscozyme® L multi-enzyme.

[2]SEM = standard error of the mean.

4.3.3 Serum biochemical parameters

For the serum biochemical parameters of Jumbo quail, pre-treatment of dietary MOLM with incremental levels of viscozyme® L multi-enzyme did not show any (P >0.05) linear or quadratic effects (Table 4.6). Similarly, no significant dietary effects on the birds' serum biochemistry were observed.

Table 4.6. Effect of pre-treating dietary *Moringa oleifera* leaf powder meal with incremental levels of viscozyme® L multi-enzyme on serum biochemical parameters in Jumbo quail.

	[1]Diets							*P* values	
Parameters	CON	VME0	VME25	VME50	VME75	VME100	[3]SEM	Linear	Quadratic
Glucose (mmol/L)	6.74	7.87	11.6	12.1	6.70	6.56	2.775	0.285	0.105
Phosphorus (mmol/L)	4.74	5.00	4.93	4.20	4.71	4.55	0.306	0.158	0.246
Total protein (g/L)	54.5	57.5	74.1	65.0	66.6	54.5	10.56	0.530	0.074
Albumin (g/L)	29.0	18.4	22.7	19.4	19.0	17.3	3.272	0.289	0.172
Globulin (g/L)	35.0	38.9	51.4	47.9	47.5	40.6	8.747	0.984	0.076
[2]ALKP (U/L)	123.5	232.9	197.7	141.6	149.5	201.3	44.46	0.329	0.084
Amylase (U/L)	224.7	282.0	336.7	263.8	292.6	460.3	86.20	0.167	0.220
Lipase (U/L)	332.3	238.7	244.1	220.8	201.0	198.7	31.13	0.068	0.878

[1]Diets: CON = standard quail grower diet without *M. oleifera* leaf meal; VME0 = control diet containing 100 g/kg *M. oleifera* leaf meal without viscozyme® L multi-enzyme; VME25 = control diet containing 100 g/kg *M. oleifera* leaf meal pre-treated with 2.5 g/kg viscozyme® L multi-enzyme; VME50 = control diet containing 100 g/kg *M. oleifera* leaf meal pre-treated with 5 g/kg viscozyme® L multi-enzyme; VME75 = control diet containing 100 g/kg *M. oleifera* leaf meal pre-treated with 7.5 g/kg viscozyme® L multi-enzyme; VME100 = control diet containing 100 g/kg *M. oleifera* leaf meal pre-treated with 10 g/kg viscozyme® L multi-enzyme.

[2]ALKP = Alkaline phosphatase.

[3]SEM = standard error of the mean.

4.3.4 Internal organs

As the VME pre-treatment levels increased, Table 4.7 shows that neither linear nor quadratic effects were observed for the weights of the internal organs in Jumbo quail. However, a quadratic effect was observed for gizzard weight [y = 2.02 (±0.075) + 0.009 (±0.003) x -

0.00008 (±0.00003) x^2; $R^2 = 0.181$, $P = 0.023$]. The weights of the birds' internal organs and carcass characteristics were also unaltered by the dietary treatments ($P > 0.05$).

Table 4.7. Effect of pre-treating dietary *Moringa oleifera* leaf meal with incremental levels of viscozyme® L multi-enzyme on internal organ sizes in Jumbo quail.

	[1]Diets							*P* values	
Parameters	CON	VME0	VME25	VME50	VME75	VME100	[2]SEM	Linear	Quadratic
Weights (% HCW)									
Caecum	0.820	1.62	0.852	0.909	1.00	0.722	0.222	0.016	0.172
Colon	0.174	0.375	0.195	0.197	0.166	0.192	0.089	0.111	0.183
Small intestine	4.28	3.45	4.16	3.52	3.47	4.60	0.564	0.684	0.379
Lengths (cm)									
Gizzards	2.20	1.99	2.22	2.24	2.18	2.13	0.104	0.598	0.023
Liver	2.89	2.69	2.84	2.90	2.85	2.64	0.385	0.501	0.532
Proventriculus	0.614	0.610	0.598	0.609	0.696	0.534	0.064	0.501	0.533
Caecum	16.2	8.86	17.0	12.6	14.3	13.0	0.791	0.104	0.562
Colon	2.26	3.17	2.32	3.99	2.50	2.46	0.399	0.271	0.576
Small intestine	64.0	54.7	61.3	60.7	60.0	59.0	1.701	0.081	0.10

[1]Diets: CON = standard quail grower diet without *M. oleifera* leaf meal; VME0 = control diet containing 100 g/kg *M. oleifera* leaf meal without viscozyme® L multi-enzyme; VME25 = control diet containing 100 g/kg *M. oleifera* leaf meal pre-treated with 2.5 g/kg viscozyme® L multi-enzyme; VME50 = control diet containing 100 g/kg *M. oleifera* leaf meal pre-treated with 5 g/kg viscozyme® L multi-enzyme; VME75 = control diet containing 100 g/kg *M. oleifera* leaf meal pre-treated with 7.5 g/kg viscozyme® L multi-enzyme; VME100 = control diet containing 100 g/kg *M. oleifera* leaf meal pre-treated with 10 g/kg viscozyme® L multi-enzyme.

[2]SEM = standard error of the mean.

.

4.3.5 Carcass traits

There were no (P >0.05) linear or quadratic effects for Jumbo quail carcass characteristics in response to pre-treatment of dietary MOLM with incremental levels of viscozyme® L multi-enzyme (Table 4.8). Similarly, no significant dietary effects were observed on carcass traits of the birds.

Table 4.8. Effect of pre-treating dietary *Moringa oleifera* leaf meal with incremental levels of viscozyme® L multi-enzyme on carcass sizes (%HCW, unless stated otherwise) in Jumbo quail.

	[1]Diets							*P* values	
[2]Parameters	CON	VME0	VME25	VME50	VME75	VME100	[3]SEM	Linear	Quadratic
Hot carcass weight	150.1	143.8	146.5	138.6	146.9	139.2	2.968	0.359	0.682
Cold carcass weight	146.3	139.5	141.3	137.1	142.1	134.4	2.970	0.305	0.365
Carcass yield (%)	62.8	62.6	63.0	60.1	64.9	60.5	1.493	0.648	0.751
Breast	17.0	16.4	17.0	17.6	15.0	16.4	0.701	0.400	0.577
Wing	4.37	4.70	4.91	4.79	4.73	4.80	0.137	0.988	0.676
Thigh	6.19	6.22	7.60	6.57	6.30	6.23	0.484	0.455	0.298
Drumstick	4.28	4.76	4.63	4.48	4.20	4.50	0.157	0.086	0.259

[1]Diets: CON = standard quail grower diet without *M. oleifera* leaf meal; VME0 = control diet containing 100 g/kg *M. oleifera* leaf meal without viscozyme® L multi-enzyme; VME25 = control diet containing 100 g/kg *M. oleifera* leaf meal pre-treated with 2.5 g/kg viscozyme® L multi-enzyme; VME50 = control diet containing 100 g/kg *M. oleifera* leaf meal pre-treated with 5 g/kg viscozyme® L multi-enzyme; VME75 = control diet containing 100 g/kg *M. oleifera* leaf meal pre-treated with 7.5 g/kg viscozyme® L multi-enzyme; VME100 = control diet containing 100 g/kg *M. oleifera* leaf meal pre-treated with 10 g/kg viscozyme® L multi-enzyme.

[2]SEM = standard error of the mean.

4.3.6 Meat quality traits

Table 4.9 shows that there was a significant quadratic response for breast meat hue angle measured 1 h post-mortem [$y = 1.17 (\pm 0.025) - 0.002 (\pm 0.001)\ x + 0.00002 (\pm 0.00001)\ x^2$; $R^2 = 0.166$, $P = 0.027$] in response to incremental levels of viscozyme® L multi-enzyme. Diet CON promoted a lower 1-hour hue angle value (1.02) than all the other diets, whose 1-hour hue angle value did differ ($P > 0.05$).

Table 4.9. Effect of pre-treating dietary *Moringa oleifera* leaf meal with incremental levels of viscozyme® L multi-enzyme on breast meat quality parameters measured 1 h post-mortem in Jumbo quail.

	[1]Diets							*P* values	
[2]Parameters	CON	VME0	VME25	VME50	VME75	VME100	[3]SEM	Linear	Quadratic
pH_1	5.83	5.83	5.87	5.81	5.97	5.94	0.056	0.055	0.648
L^*_1	46.4	51.3	48.4	48.1	47.3	48.9	1.270	0.164	0.084
a^*_1	5.41	5.37	5.64	5.98	5.83	5.27	0.346	0.989	0.092
b^*_1	8.89	12.5	12.0	11.8	11.0	12.4	0.507	0.484	0.127
$Chroma_1$	10.4	13.6	13.3	13.3	12.4	13.5	0.500	0.466	0.313
Hue $angle_1$	1.02^b	1.16^a	1.12^a	1.10^a	1.08^a	1.16^a	0.027	0.702	0.027

[a,b] Means in the same row with different superscripts are significantly different ($P < 0.05$).

[1]Diets: CON = standard quail grower diet without *M. oleifera* leaf meal; VME0 = control diet containing 100 g/kg *M. oleifera* leaf meal without viscozyme® L multi-enzyme; VME25 = control diet containing 100 g/kg *M. oleifera* leaf meal pre-treated with 2.5 g/kg viscozyme® L multi-enzyme; VME50 = control diet containing 100 g/kg *M. oleifera* leaf meal pre-treated with 5 g/kg viscozyme® L multi-enzyme; VME75 = control diet containing 100 g/kg *M. oleifera* leaf meal pre-treated with 7.5 g/kg viscozyme® L multi-enzyme; VME100 = control diet containing 100 g/kg *M. oleifera* leaf meal pre-treated with 10 g/kg viscozyme® L multi-enzyme.

[2]Parameters; *L** = lightness; *a** = redness; *b** = Yellowness.

[3]SEM = standard error of the mean.

Table 4.10 shows significant linear decreases for breast meat lightness (L^*) [y = 50.6 (±0.873) − 0.125 (±0.041) x; R^2 = 0.340, P = 0.0001] and for breast meat hue angle measured 24 h post-mortem [y = 1.06 (±0.033) − 0.001 (±0.001) x; R^2 = 0.187; P = 0.019] were observed as VME levels increased. Birds on diet CON had a higher 24-hour hue angle (P <0.05) than birds on diets VME0, VME50, VME75 and VME75, which had statistically similar (P >0.05) hue angle$_{24}$ values.

Table 4.10. Effect of treating dietary *Moringa oleifera* leaf meal with incremental levels of viscozyme® L multi-enzyme on meat quality parameters measured 24 h post-mortem in Jumbo quail.

	[1]Diets							*P* values	
[2]Parameters	CON	VME0	VME25	VME50	VME75	VME100	[3]SEM	Linear	Quadratic
pH_{24}	5.69	5.84	5.86	5.85	5.83	5.84	0.026	0.534	0.738
L^*_{24}	54.0	50.7	47.7	46.5	45.7	45.9	5.125	0.000	0.057
a^*_{24}	8.10	8.21	7.48	9.07	9.19	10.19	0.868	0.057	0.561
b^*_{24}	10.1	14.8	13.0	13.7	14.2	13.5	0.495	0.437	0.264
$Chroma_{24}$	13.0	16.9	15.0	16.4	17.0	17.2	0.783	0.338	0.255
Hue $angle_{24}$	0.895^{b}	1.06^{a}	1.04^{ab}	0.988^{a}	0.998^{a}	0.950^{a}	0.034	0.019	0.947

[a,b] Means in the same row with different superscripts are significantly different (P <0.05).

[1]Diets: CON = standard quail grower diet without *M. oleifera* leaf meal; VME0 = control diet containing 100 g/kg *M. oleifera* leaf meal without viscozyme® L multi-enzyme; VME25 = control diet containing 100 g/kg *M. oleifera* leaf meal pre-treated with 2.5 g/kg viscozyme® L multi-enzyme; VME50 = control diet containing 100 g/kg *M. oleifera* leaf meal pre-treated with 5 g/kg viscozyme® L multi-enzyme; VME75 = control diet containing 100 g/kg *M. oleifera* leaf meal pre-treated with 7.5 g/kg viscozyme® L multi-enzyme; VME100 = control diet containing 100 g/kg *M. oleifera* leaf meal pre-treated with 10 g/kg viscozyme® L multi-enzyme.

[2]Parameters: L^* = lightness; a^* = redness; b^* = yellowness.

[3]SEM = standard error of the mean.

Pre-treatment of dietary MOLM with VME had no (P >0.05) linear or quadratic trends for meat quality parameters of Jumbo quail (Table 4.11). Similarly, no significant dietary effects were observed on meat quality traits of the birds.

Table 4.11. Effect of pre-treating dietary *Moringa oleifera* leaf meal with incremental levels of viscozyme® L multi-enzyme on meat quality traits in Jumbo quail.

	[1]Diets							*P* values	
[2]Parameters	CON	VME0	VME25	VME50	VME75	VME100	[3]SEM	Linear	Quadratic
Cooking loss (%)	19.7	17.8	12.9	16.3	20.2	18.3	2.410	0.211	0.403
Drip loss (%)	50.9	44.8	42.7	40.0	42.6	29.5	5.645	0.102	0.441
Shear force (N)	3.26	2.85	2.51	2.69	3.10	3.53	0.468	0.197	0.319
WHC (%)	87.3	88.2	87.9	87.1	83.5	85.8	1.631	0.102	0.734

[1]Diets: CON = standard quail grower diet without *M. oleifera* leaf meal; VME0 = control diet containing 100 g/kg *M. oleifera* leaf meal without viscozyme® L multi-enzyme; VME25 = control diet containing 100 g/kg *M. oleifera* leaf meal pre-treated with 2.5 g/kg viscozyme® L multi-enzyme; VME50 = control diet containing 100 g/kg *M. oleifera* leaf meal pre-treated with 5 g/kg viscozyme® L multi-enzyme; VME75 = control diet containing 100 g/kg *M. oleifera* leaf meal pre-treated with 7.5 g/kg viscozyme® L multi-enzyme; VME100 = control diet containing 100 g/kg *M. oleifera* leaf meal pre-treated with 10 g/kg viscozyme® L multi-enzyme.[2]Parameters: WHC = water holding capacity.

[3]SEM = standard error of the mean.

The data measured at room temperature over 5 days showed no significant effect of dietary treatment (Figures 4.1 – 4.4). The breast meat pH for Jumbo on VME0, VME25, VME50, VME75, and VME100 diets increased over 5 days. For all treatments, the lightness (L*) of the breast meat decreased over 5 days. The redness (a*) of the breast meat decreased for VME0, VME25, VME75 and VME100 while VME50 increased over 5 days. The yellowness (b*) of the breast meat decreased for MVE25 and VME100 while VME0, VME50 and VME100 slightly increased over 5 days.

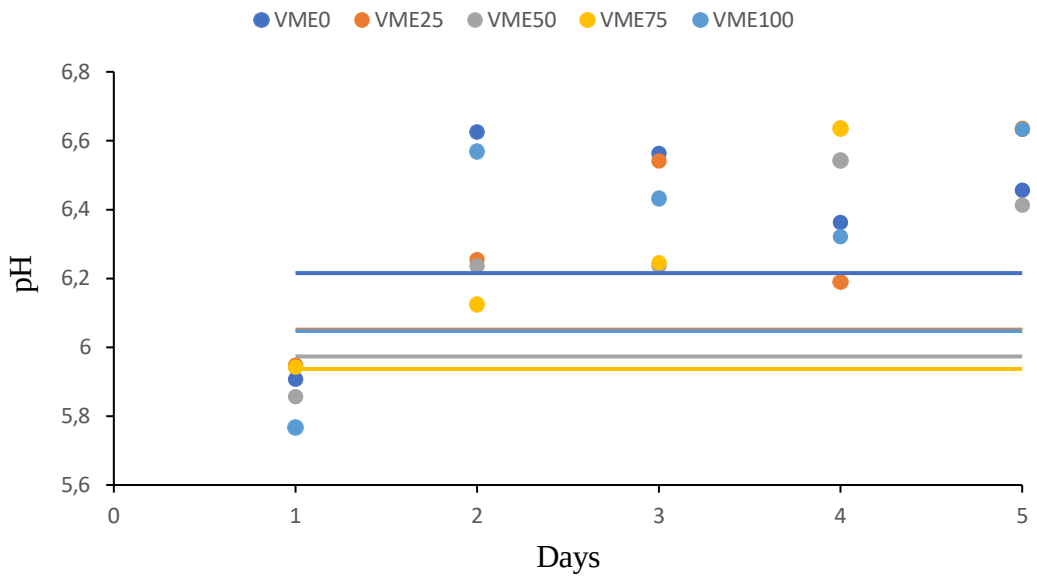


Figure 4.1. Effect of pre-treated *Moringa oleifera* leaf meal on the stability of breast meat pH upon storage at room temperature for 5 days [*Diets*: CON = standard quail grower diet without *M. oleifera* leaf meal; VME0 = control diet containing 100 g/kg *M. oleifera* leaf meal without viscozyme® L multi-enzyme; VME25 = control diet containing 100 g/kg *M. oleifera* leaf meal pre-treated with 2.5 g/kg viscozyme® L multi-enzyme; VME50 = control diet containing 100 g/kg *M. oleifera* leaf meal pre-treated with 5 g/kg viscozyme® L multi-enzyme; VME75 = control diet containing 100 g/kg *M. oleifera* leaf meal pre-treated with 7.5 g/kg viscozyme® L multi-enzyme; VME100 = control diet containing 100 g/kg *M. oleifera* leaf meal pre-treated with 10 g/kg viscozyme® L multi-enzyme].

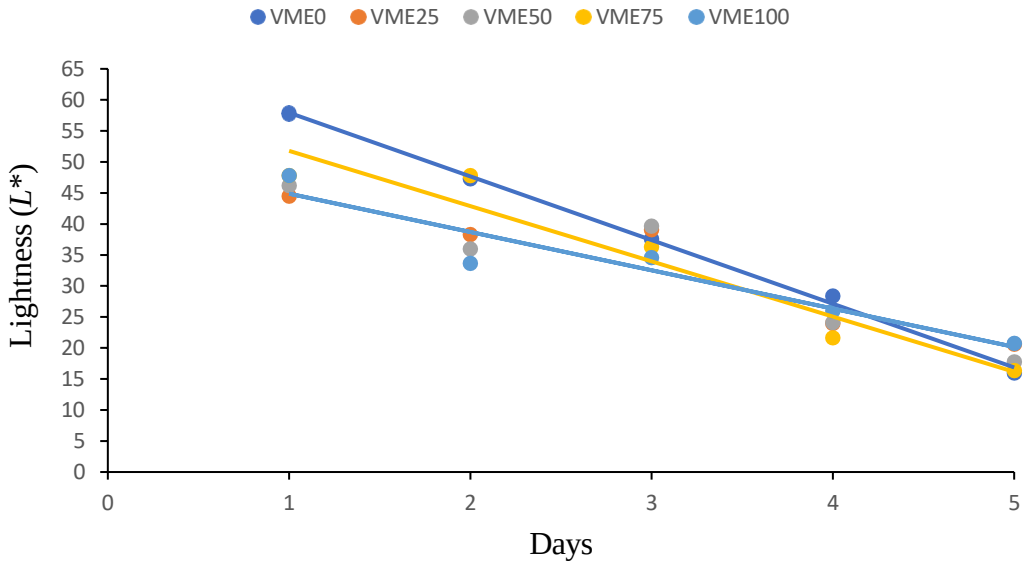


Figure 4.2 Effect of pre-treated *Moringa oleifera* leaf meal on the stability of breast meat lightness upon storage at room temperature for 5 days [*Diets*: CON = standard quail grower diet without *M. oleifera* leaf meal; VME0 = control diet containing 100 g/kg *M. oleifera* leaf meal without viscozyme® L multi-enzyme; VME25 = control diet containing 100 g/kg *M. oleifera* leaf meal pre-treated with 2.5 g/kg viscozyme® L multi-enzyme; VME50 = control diet containing 100 g/kg *M. oleifera* leaf meal pre-treated with 5 g/kg viscozyme® L multi- enzyme; VME75 = control diet containing 100 g/kg *M. oleifera* leaf meal pre-treated with 7.5 g/kg viscozyme® L multi-enzyme; VME100 = control diet containing 100 g/kg *M. oleifera* leaf meal pre-treated with 10 g/kg viscozyme® L multi-enzyme].

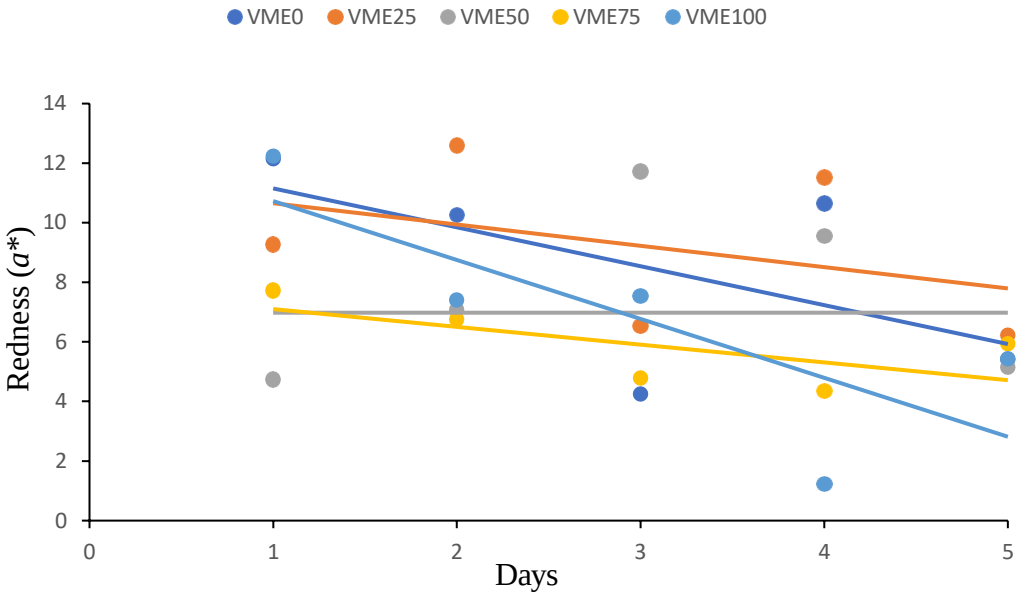


Figure 4.3. Effect of pre-treated *Moringa oleifera* leaf meal on the stability of breast meat redness upon storage at room temperature for 5 days [*Diets*: CON = standard quail grower diet without *M. oleifera* leaf meal; VME0 = control diet containing 100 g/kg *M. oleifera* leaf meal without viscozyme® L multi-enzyme; VME25 = control diet containing 100 g/kg *M. oleifera* leaf meal pre-treated with 2.5 g/kg viscozyme® L multi-enzyme; VME50 = control diet containing 100 g/kg *M. oleifera* leaf meal pre-treated with 5 g/kg viscozyme® L multi-enzyme; VME75 = control diet containing 100 g/kg *M. oleifera* leaf meal pre-treated with 7.5 g/kg viscozyme® L multi-enzyme; VME100 = control diet containing 100 g/kg *M. oleifera* leaf meal pre-treated with 10 g/kg viscozyme® L multi-enzyme].

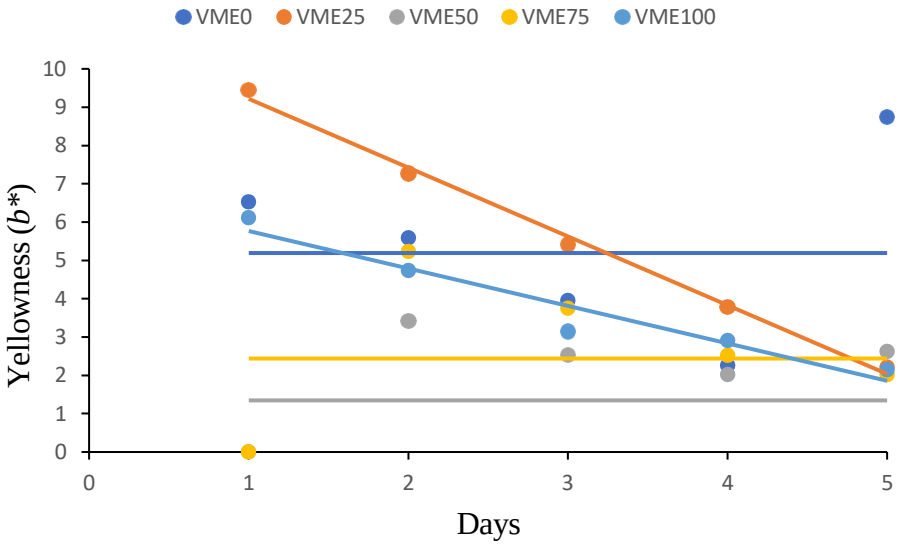


Figure 4.4. Effect of pre-treated *Moringa oleifera* leaf meal on the stability of breast meat yellowness upon storage at room temperature for 5 days [*Diets*: CON = standard quail grower diet without *M. oleifera* leaf meal; VME0 = control diet containing 100 g/kg *M. oleifera* leaf meal without viscozyme® L multi-enzyme; VME25 = control diet containing 100 g/kg *M. oleifera* leaf meal pre-treated with 2.5 g/kg viscozyme® L multi-enzyme; VME50 = control diet containing 100 g/kg *M. oleifera* leaf meal pre-treated with 5 g/kg viscozyme® L multi- enzyme; VME75 = control diet containing 100 g/kg *M. oleifera* leaf meal pre-treated with 7.5 g/kg viscozyme® L multi-enzyme; VME100 = control diet containing 100 g/kg *M. oleifera* leaf meal pre-treated with 10 g/kg viscozyme® L multi-enzyme].

4.4 Discussion

4.4.1 Growth performance and haemo-biochemistry

Moringa foliage has the potential to be used as a source of bioactive compounds and nutrients in quail diets. However, the amount of the leaf meal that could be added to quail diets is limited by its high fibre content, among other factors (Mulaudzi *et al*., 2019). High levels of dietary fibre have detrimental effects on digestion, absorption, and utilisation of nutrients and bioactive compounds in birds (Jha & Mishra, 2021; Tejeda & Kim, 2021). Thus, this study used a fibrolytic multi-enzyme to alleviate the negative effects of fibre in dietary MOLM. Repeated

measures analysis revealed a significant week × diet interaction effect only on FI, indicating that the dietary effect on feed consistent varied as the birds grew older.

It was expected that birds fed the enzyme-pretreated diets would have higher overall FI. This is due to the ability of fibrolytic enzymes to improve the digestibility of fibre-rich diets, allowing the birds to ingest more feed (Cowieson & Bedford, 2009). The results, however, did not support this hypothesis as there were no variations in FI across the experimental diets. Similarly, enzyme pre-treatment of MOLM had no effect on weight gain and FCE. These findings are in line with those of Hussein *et al*. (2020), who studied growth, carcass characteristics, and meat quality of broilers and recorded no effect on weight gain and FCR in birds fed a low-energy diet supplemented with a multi-enzyme. Contrary to these results, Hana *et al*. (2010) and Hajati *et al*. (2009) reported that adding multi-enzymes to standard poultry diets significantly improved BWG, FCR and ileal digestibility of crude protein in broilers.

Blood parameters are crucial in determining the pathophysiological status and the quality and safety of feed ingredients for farm animals (Ali *et al*., 2012; Mnisi & Mlambo, 2018). Due to the presence of antinutritional components, such as CT and fibre, it was hypothesised that MOLM would have deleterious impact on haematological and biochemical parameters. However, no diet-induced changes were observed for all haematological parameters, except neutrophils, which linearly increased in response to enzyme pre-treatment levels. Nonetheless, the CON diet promoted similar neutrophils levels as all the other dietary treatments, indicating that the increase in neutrophil levels was not induced by the MOLM. Indeed, the concentration of blood parameters found in this study were within normal ranges for healthy quail birds (Mnisi & Mlambo, 2018). Similarly, no variations in serum total protein or liver enzyme (alkaline phosphatase) were observed, with all reported values falling within the normal ranges

for adult Japanese quail (Scholtz *et al*., 2009; Ali *et al*., 2012), indicating that the diets had no negative effect on bird health.

4.4.2 Carcass and meat quality traits

In this study, the size of the gizzards showed a quadratic response by first increasing and then decreasing with enzyme levels. Theoretically, the consumption of high fibrous diets induces changes in the size of intestines and gizzards in birds as an adaptation mechanism (Kumanda *et al*., 2019). According to Musa *et al*. (2006), consuming high-fibre feed causes gizzard enlargement, which leads to improved muscular grinding of feed particles and higher nutrient digestibility. These findings also demonstrate that fibrolytic enzymes have no influence on carcass yields and any of the carcass parts, which is consistent with the results established by Saleh *et al*. (2005), who found a similar lack of fibrolytic enzyme effect on broiler carcass yields. Carcass yield is affected by genetics, feed, slaughtering conditions, body weight, and gender of birds (Brickett *et al*., 2001; Havenstein *et al*., 2003). The effects of fibrolytic enzymes on breast, wing, thigh, and drumstick weights are consistent with those reported by Hajati *et al*. (2009) in broilers given untreated and multi-enzyme-treated diets.

The growth of the liver, breast, and proventricular was not affected by the enzyme-treated MOLM diets. When injured, the liver produces enzymes, such as alanine aminotransferase (ALT) and alkaline phosphatase (ALKP), which are released into the blood (Sherwin, 2003). Elevated levels of these liver enzymes are associated with altered hepatic membrane permeability, which can arise from circulatory hypoxia, exposure to toxins and toxaemia, inflammation, metabolic abnormalities, or hepatocyte growth (Sherwin, 2003). As a result, normal levels of these enzymes in quail fed MOLM-containing diets reflect normal quail liver and intestinal processes, implying that the MOLM is a safe feed ingredient. Enzyme pre-treatment of dietary MOLM resulted in a linear decrease in breast meat lightness and hue angle

measured 24 h post-slaughter and a quadratic response for breast meat hue angle measured 1 h post-slaughter. The increased oxidative stability of breast meat seen after pre-treatment with dietary MOLM could be attributed to its antioxidant capabilities (Makkar & Becker, 1996). *Moringa oleifera* is high in metals, including selenium, manganese, copper, and zinc, all of which are important in the action of antioxidant enzymes. In addition, tocopherol, ascorbic acid, carotenoids, polysaccharide, flavonoids, saponins, phenolics, tannins, and proanthocyanins are among the natural antioxidant compounds found in MOLM (Makkar & Becker, 1996; Abuye *et al*., 2003). In contrast to the findings of this study, Kumanda *et al*. (2019) found no differences in meat lightness between broilers given red grape pomace pre-treated with a similar fibrolytic multi-enzyme and those fed an untreated control diet.

One of the most critical factors influencing meat quality indicators, such as cooking loss, tenderness, drip loss, and WHC, is the pH value of the meat (Dyubele *et al*., 2010). The pH drop after slaughter is measured as the ultimate pH. The range is influenced by the amount of glycogen in breast muscle prior to slaughter and the rate at which the remaining glycogen is converted to lactic acid after slaughter (Lonergan *et al*., 2003). Diets had no effect on meat pH, shear force, cooking loss, drip loss, or WHC. The lack of dietary effects suggests that enzyme-treated MOLM has the potential to promote normal oxidative stability for meat quality characteristics during storage. The shear force is a measure of meat toughness, with a lower shear force value suggesting tenderer meat (Pearce *et al*., 2011). However, there were no dietary effect on shear force values in this investigation.

The ability of meat to retain water is referred to as its water-holding capacity. It is a critical quality parameter that influences the amount of water lost during transit, storage, processing, and cooking (Bertram *et al*., 2003). Juices are released during cooking because of protein denaturation and muscle shrinkage (Purslow *et al*., 2016). In this investigation, there was no

difference in WHC, cooking loss, or drip loss, indicating that the untreated or pre-treated MOLM does not have any impact on meat quality. All shelf-life parameters tested over 5 days at room temperature were not significantly affected by dietary treatments. Although an increase in meat pH is linked to enhanced meat quality, an increase in pH over time may also indicate microbial growth. These results agree with Falowo *et al*. (2016), who evaluated the effect of MOLM extract on the 6-day shelf life of ground beef quality.

4.5 Conclusion

Pre-treatment of *Moringa oleifera* leaf meal with fibrolytic multi-enzymes influenced neutrophils, gizzard weights, and meat colour indicators but not growth performance and carcass parameters in Jumbo quail. The fibrolytic multi-enzyme application rate of 10 g/kg may not have been adequate to enhance feed utilisation and positively affect weight gain in Jumbo quail; thus, higher levels may need to be investigated further.

4.6 References

Abuye, C., Urga, K., Knapp, H., Selmar, D., Omwega, A.M., Imungi, J.K. & Winterhalter, P., 2003. A compositional study of *Moringa stenopetala* leaves. East Afr. Med. J. 80, 247–252.

Afuang, W., Siddhuraju, P. & Becker, K., 2003. Comparative nutritional evaluation of raw, methanol extracted residues and methanol extracts of moringa (*Moringa oleifera* Lam) leaves on growth performance and feed utilization in Nile tilapia (Oreochromis niloticus L.). Aquac. Res. 34, 1147–1159.

Ali, M.A., Hmar, L., Devi, L.I., Prava, M., Lallianchhunga, M.C. & Tolenkhomba, T.C., 2012. Effect of age on the haematological and biochemical profile of Japanese quails (*Coturnix coturnix japonica*). Int. Multidiscip. Res. J. 2, 32–35.

AOAC. 2005. *Official Methods of Analysis of the Association of Official's Analytical Chemists*, 18th ed.; Association of Official Analytical Chemists: Arlington, VA, USA.

Bamishaiye, E.I., Olayemi, F.F., Awagu, E.F. & Bamshaiye, O.M., 2011. Proximate and phytochemical composition of *Moringa oleifera* leaves at three stages of maturation. Adv. J. Food Sci. Technol. 3, 233–237.

Bertram, H.C., Andersen, H.J., Karlsson, A.H., Horn, P., Hedegaard, J., Nørgaard, L. & Engelsen, S.B., 2003. Prediction of technological quality (cooking loss and Napole yield) of pork based on fresh meat characteristics. Meat Sci. 65, 707–712.

Brickett, K.E., Dahiya, J.P., Classen, H.L. & Gomis, S., 2001. Influence of dietary nutrient density, feed form, and lighting on growth and meat yield of broiler chickens. Poult. Sci. 86, 2172–2181.

Buetow, B.S., Treuting, P.M. & van Hoosier, G.L., 1999. *The Hamster*; Loeb, W.F., Quimby, F.W., Eds.; Taylor and Francis: Philadelphia, PA, USA, 9–63.

CIE. 1976. *Recommendations on Uniform Colour Spaces, Colour-Difference Equations, Psychometric Colour Terms*; Supplement No. 2 to CIE Publication No. 15 (E-1.3.1.) 1978, 1971/(TC-1-3); Commission Internationale de l'Eclairage: Paris, France.

Cowieson, A.J. & Bedford, M.R., 2009. The effects of phytase and carbohydrase on ileal amino acid digestibility in monogastric diets: Complimentary mode of action? World Poult. Sci. J. 65, 609–624.

Cui, Y.M., Wang, J., Lu, W., Zhang, H.J., Wu, S.G. & Qi, G.H., 2018. Effect of dietary supplementation with *Moringa oleifera* leaf on performance, meat quality, and oxidative stability of meat in broilers. Poult. Sci. 97, 2836–2844.

Dyubele, N.L., Muchenje, V., Nkukwana, T.T. & Chimonyo, M., 2010. Consumer sensory characteristics of broiler and indigenous chicken meat: A South African example. Food Qual. Pref. 21, 815–819.

Falowo, A.B., Muchenje, V., Hugo, C.J. & Charimba, G., 2016. In-vitro antimicrobial activities of Bidens pilosa and *Moringa oleifera* leaf extracts and their effects on ground beef quality during cold storage. Cyta-J. Food. 14(4), 541–546.

Grau, R. & Hamm, R., 1957. About the water-binding capacity of the mammalian muscle. II. Commun. Z. Lebensm. Unters. Brisk. 105, 446.

Hajati, H., Rezaei, M. & Sayyahzadeh, H., 2009. The effects of enzyme supplementation on performance, carcass characteristics and some blood parameters of broilers fed on corn-soybean meal-wheat diets. Int. J. Poult. Sci. 8, 1199–1205.

Hana, A.H., Jalal, M.A. & Abu Ishmais, M.A., 2010. The influence of supplemental multi-enzyme feed additive on the performance, carcass characteristics and meat quality traits of broiler chickens. Int. J. Poult. Sci. 9, 126–133.

Hassan, H.M.A., El-Moniary, M.M., Hamouda, Y., El-Daly, E.F., Youssef, A.W. & Abd

ElAzeem, N.A., 2016. Effect of different levels of *Moringa oleifera* leaves powder on productive performance, carcass characteristics and some blood parameters of broiler chicks reared under heat stress conditions. Asian J. Anim. Vet. Adv. 11, 60–66.

Havenstein, G.B., Ferket, P.R. & Qureshi, M.A., 2003. Carcass composition and yield of 1957 versus 2001 broilers when fed representative 1957 and 2001 broiler diets. Poult. Sci. 82, 1509–1518.

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

Hussein, E.O.S., Suliman, G.M., Alowaimer, A.N., Ahmed, S.H., Abd El-Hack, M.E., Taha, A.E. & Swelum, A.A., 2020. Growth, carcass characteristics, and meat quality of broilers fed a low-energy diet supplemented with a multienzyme preparation. Poult. Sci. 99, 1988–1994.

Jha, R. & Mishra, P., 2021. Dietary fibre in poultry nutrition and their effects on nutrient utilization, performance, gut health, and on the environment. J. Anim. Sci. Biotechnol. 12, 51.

Khalil, J.K., Saway, W.N. & Hyder, S.Z., 1986. Nutrient composition of Atriplex leaves grown in Saudi Arabia. J. Range Manag. 39, 104–107.

Khanyile, M., Ndou, S.P. & Chimonyo, M., 2007. Influence of *Acacia tortilis* leaf meal-based diets on growth performance of pigs. Livest. Sci. 167, 211–218.

Kim, W.K. & Lillehoj, H.S., 2019. Immunity, immunomodulation, and antibiotic alternatives to maximize the genetic potential of poultry for growth and disease response. Anim. Feed Sci. Technol. 250, 41–50.

Kumanda, C., Mlambo, V. & Mnisi, C.M., 2019. Valorization of red grape pomace waste using

polyethylene glycol and fibrolytic enzymes physiological and meat quality responses in broilers. Animals. 9, 779.

Lonergan, S.M., Deeb, N., Fedler, C.A. & Lamont, S.J., 2003. Breast meat quality and composition in unique chicken populations. Poult. Sci. 82, 1990–1994.

Makkar, H. & Becker, K., 1996. Nutritional value and antinutritional components of whole and ethanol extracted *Moringa oleifera* leaves. Anim. Feed Sci. Technol. 63, 211–228.

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.

Matshogo, T.B., Mlambo, V., Mnisi, C.M. & Manyeula, F., 2021. Effect of pre-treating dietary green seaweed with fibrolytic enzymes on growth performance, blood indices, and meat quality parameters of Cobb 500 broiler chickens. Livest. Sci. 251, 104652.

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

Mulaudzi, A., Mnisi, C.M. & Mlambo, V., 2019. Dietary *Moringa oleifera* leaf meal improves growth performance but not haemo-biochemical and meat quality parameters in female Japanese quails. Pak. J. Nutr. 18, 953–960.

Musa, H.H., Chen, G.H., Cheng, J.H., Shuiep, E.S. & Bao, W.B., 2006. Breed and sex effect on meat quality of chicken. Int. J. Poult. Sci. 5, 566–568.

National Research Council. 2004. *Nutrient Requirements of Poultry*, 9th ed.; National Academy Press: Washington, DC, USA.

Nkukwana, T.T., Muchenje, N., Pieterse, N.E., Masika, P.J., Mabusela, T.P., Hoffman, L.C. & Dzama, K., 2014. Effect of *Moringa oleifera* leaf meal on growth performance,

apparent digestibility, digestive organ size and carcass yield in broiler chickens. Livest. Sci. 161, 139–146.

Pari, L. & Kumar, N.A., 2008. Hepatoprotective activity of *Moringa oleifera* on ant tubercular drug-induced liver damage in rats. J. Med. Food. 5, 171–177.

Pearce, K.L., Rosenvold, K., Andersen, H.J. & Hopkins, D.L., 2011. Water distribution and mobility in meat during the conversion of muscle to meat and ageing and the impacts on fresh meat quality attributes—A review. Meat Sci. 89, 111–124.

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.P., Oiseth, S., Hughes, J. & Warner, R.D., 2016. The structural basis of cooking loss in beef: Variations with temperature and ageing. Food Res. Int. 89, 739–748.

Richter, N., Siddhuraju, P. & Becker, K., 2003. Evaluation of nutritional quality of moringa (*Moringa oleifera* Lam.) leaves as an alternative protein source for Nile tilapia (*Oreochromis niloticus L.*). Aquaculture. 217, 599–611.

Saleh, F., Tahir, M., Ohtsuka, A. & Hayashi, K., 2005 . A mixture of pure cellulose, hemicellulase and pectinase improves broiler performance. Br. Poult. Sci. 46, 602–606.

Scholtz, N., Halle, I., Flachowsky, G. & Sauerwein, H., 2009. Serum chemistry reference values in adult Japanese quail (*Coturnix coturnix japonica*) including sex-related differences. Poult. Sci. 88, 1186–1190.

Sherwin, J.E., 2003. Liver Function. In *Clinical Chemistry: Theory, Analysis, Correlation*, 4th ed. Pages 492–506. Kaplan, L.A., Pesce, A.J., Kazmierczak, S.C., Eds.; Elsevier Science: St. Louis, MO, USA.

Slominski, B.A., 2011. Recent advances in research on enzymes for poultry diets. Poult. Sci. 90, 2013–2023.

Statistical Analysis System Institute Inc. 2010. *Users Guide*; SAS: Carry, NC, USA.

Sul, B. & Chen, X., 2020. Current status and potential of *Moringa oleifera* leaf as an alternative protein source for animal feeds. Front. Vet. Sci. 7, 53.

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

Tesfaye, E., Animut, G., Urge, M. & Dessie, T., 2012. Effect of replacing *Moringa oleifera* leaf meal for soybean meal in broiler ration. Global J. Sci. Front. Res. Agric. Biol. 12, 289–297.

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

Verma, A.R., Vijayakumar, M., Mathela, C.S. & Rao, C.V., 2009. *In vitro* and *in vivo* antioxidant properties of different fractions of *Moringa oleifera* leaves. Food Chem. Toxicol. 47, 2196–2201.

Vooren, N.V., 2012. Flexible feed formulation with multi enzyme concept. Pak. Poult. 33, 7–11.

Washington, I.M. & van Hoosier, G., 2012. *Clinical Biochemistry and Haematology*; University of Washington: Seattle, WA, USA, pp. 59–91.

5 CHAPTER FIVE - EFFECT OF POLYETHYLENE GLYCOL AND FIBROLYTIC ENZYMES PRE-TREATMENT OF *MORINGA OLEIFERA* LEAF MEAL ON GROWTH PERFORMANCE, PHYSIOLOGICAL INDICES AND MEAT QUALITY PARAMETERS IN JUMBO QUAIL

Abstract

The previous chapters showed that the utility of *Moringa oleifera* leaf meal (MOLM) as a nutraceutical source for Jumbo quail is limited by high levels of fibre and condensed tannins (CT). The treatment of *Moringa oleifera* leaf meal with polyethylene glycol (PEG) and viscozyme® L multi-enzyme (VME) alone was an ineffective strategy to increase its utilisation in Jumbo quail. The current study, therefore, evaluated the effect of simultaneous pre-treatment of MOLM with PEG and VME on growth performance, haemato-biochemical, carcass characteristics and meat quality parameters in Jumbo quail. The purpose of treating MOLM with PEG and VME was to simultaneously reduce fibre content and inactivate CT in MOLM to exploit the synergistic effects of the two. Therefore, this Chapter examined the effect of pre-treating MOLM with the optimum PEG concentration (54 g/kg) that was determined in Chapter Three and VME levels beyond the level (10 g/kg) used in Chapter Four. A total of 381, one-week-old quail chicks (57.5 ± 3.95 g live-weight), were randomly allocated to 36 pens (experimental unit), which were replicated six times per dietary treatment. Six dietary

treatments were as follows: a standard grower diet containing untreated 100 g/kg MOLM (CON); a standard grower diet containing 100 g/kg MOLM pre-treated with 54 g/kg PEG only (MPV0); and a standard grower diet containing 100 g/kg MOLM pre-treated with 54 g/kg PEG and 12.5 g/kg (MPV125), 15 g/kg (MPV150), 17.5 g/kg (MPV175) or 20 g/kg (MPV200) of VME. Experimental diets and fresh water were offered *ad libitum*. Repeated measures analysis showed a significant week × diet interaction effect on average weekly body weight gain (ABWG; $P = 0.001$), but not on average weekly feed intake (AWFI; $P = 0.356$) and feed conversion efficiency (FCE; $P = 0.321$). Graded levels of VME did not ($P > 0.05$) induce linear or quadratic effects for overall FI, FCE, haematological, carcass, and meat quality parameters in Jumbo quail. However, weight gain in week 2 quadratically responded [$R^2 = 0.117$, $P = 0.043$]. Two-week old birds reared on MPV125 had lower ($P < 0.05$) weight gains (40.9 g/bird) than those reared on the other treatment groups. Birds reared on MPV200 diet had longer caecum (14.1 cm) than those reared on MPV0, MPV125, MPV150 and MPV175, whose caeca lengths did not differ ($P > 0.05$). Birds reared on diet MPV175 had shorter small intestines (59.5 cm) than those reared on CON and MPV0 diets. It can be concluded that simultaneous pre-treatment of dietary MOLM with PEG and viscozyme® L fibrolytic enzymes did not improve growth performance, blood parameters, and carcass and meat quality traits, but affected some visceral organ sizes in Jumbo quail.

Keywords: Blood parameters, Fibre, Fibrolytic enzymes, Growth, Meat quality, Polyethylene glycol, Quail

5.1 Introduction

Sustainable Jumbo quail (*Coturnix* sp.) production has the potential to economically transform the poultry industry and contribute towards food and nutrition security for the rapidly growing human population. Thus, the use of phytogenic products in their diets could enhance feed utilisation efficiency, thereby facilitating sustainable large-scale intensification for safe, healthy, and high-quality quail products (Mahlake *et al*., 2021). Phytogenic products such as *Moringa oleifera* leaf meal (MOLM) can be incorporated in Jumbo quail diets as a source of nutrients and bioactive substances (Ahmed & El-Rayes, 2019). Unfortunately, the high levels of CT (0.070 absorbance unit (AU) 550 nm/200 mg) and fibre (157.5 g/kg DM) in MOLM reduces nutrient utilisation, growth performance, and meat quality (Mahfuz & Piao, 2019), especially when included beyond 25 g/kg in quail diets (Hassan *et al*., 2016; Ufele & Ebenebe, 2017; Mulaudzi *et al*., 2019). Indeed, poultry birds reared on diets that have high concentrations of fibre and CT have been reported to suffer from metabolic disorders due to poor nutrient uptake (Khajali & Slominski, 2012).

Undigested non-starch polysaccharides (NSP) constitute a large proportion of digesta in high fibre diets, which causes digestive upsets that result in sticky droppings and poor development in young birds (Desbruslais *et al*., 2021; Jha & Mishra, 2021). High levels of cellulose, pectin, and xylene in MOLM (David *et al*., 2012), cause the formation of high molecular weight sticky clumps in the gastrointestinal tract of the birds, which delays digesta flow and ultimately affects growth performance (Wallace *et al*., 2010). High concentrations of CT have been linked to poor FCE, low growth rates, and reduced protein digestibility in poultry birds (Redondo *et al*., 2014; Choi & Kim, 2020). Thus, the combined use of PEG, a tannin-binding agent, and viscozyme® L multi-enzyme (VME), a fibrolytic enzyme admixture, could be an efficient strategy to ameliorate the antinutritional effects of CT and fibre for improved MOLM

utilisation, growth performance, blood parameters, visceral organs, and carcass and meat quality traits in Jumbo quail. Pre-treatment of MOLM with VME could facilitate the breakdown of the cell wall matrix of NSP in MOLM that cannot be hydrolysed by endogenous digestive enzymes, thus reducing their antinutritive effects (Mousa *et al*., 2022).

On the other hand, the PEG could deactivate CT in MOLM by forming PEG-tannin complexes, and as such increase nutrient bioavailability and digestibility (Hlatini *et al*., 2018). The phenolic and hydroxyl groups in CT form hydrogen bonds with oxygen molecules from water- soluble PEG, which could explain the increase in nutrient digestibility of PEG-treated tannin- rich feeds (Abd El Tawab & Khattab, 2018)

. No known studies have investigated the simultaneous application of PEG and exogenous fibrolytic multi-enzymes as a strategy to improve the utilisation of MOLM in Jumbo quail diets. Furthermore, pre-treatment with both PEG and VME could facilitate the use of higher dietary inclusion levels of MOLM (e.g., 100 g/kg), allowing the birds to benefit from higher doses of the bioactive compounds in MOLM. This study, therefore, evaluated the effect of pre-treating MOLM with PEG (54 g/kg) and different levels of fibrolytic enzymes on growth performance, blood parameters, visceral organs, and carcass and meat quality traits in Jumbo quail. The study tested the hypothesis that pre-treating MOLM with PEG and fibrolytic enzymes would improve feed intake, physiological, and meat quality parameters of the birds.

5.2 Material and methods

5.2.1 Study site and ingredient sources

The experiment was carried out from September to October 2021 during summer, whereby ambient temperatures around the area ranged from 17ºC to 37ºC. The study area was described in Chapter 3, Section 3.2.1. The MOLM was from the same batch as the one described in Chapter

3, Section 3.2.1. The PEG, VME and other ingredients were purchased as specified in Chapter 3, Section 3.2.1.

5.2.2 Chemical analysis of MOLM

The MOLM was chemically analysed following the method described in Chapter 3, Section 3.2.2.

5.2.3 Polyethylene glycol and enzyme treatments

In this study, MOLM pre-treated with 54 g/kg PEG was further treated with 0, 12.5, 15.0, 17.5, or 20 g/kg of VME before being incorporated (100 g/kg) into a standard quail diet. Briefly, PEG solution was made by dissolving 324 g of PEG in 6 L of distilled water (5.4%). For the fibrolytic enzyme pre-treatment, 62.5, 75, 87.5, and 100 ml of viscozyme® L were dissolved in the PEG solution as described above and then sprayed on MOLM (6 kg per treatment). The untreated MOLM (6 kg) was sprayed with 6 L of distilled water only, and mixing was done by hand for all the samples. The untreated and treated MOLM samples were kept at an average room temperature of 25°C for 12 h to allow PEG and the enzymes time to react with MOLM CT and fibre, respectively. Thereafter, the untreated and treated MOLM samples were air-dried to constant weight and then milled (Polymix PX-MFC 90 D, Kinematica AG, Switzerland) before diet formulation. The proximate composition (Table 5.1) of untreated and PEG and viscozyme enzyme pre-treated MOLM was determined as described in Chapter 3, Section 3.2.2.

Table 5.1. Proximate composition (g/kg DM, unless stated otherwise) of untreated and polyethylene glycol and viscozyme® L multi-enzyme pre-treated *Moringa oleifera* leaf meal.

	[1]Diets					
	CON	PEG54	MPV125	MPV150	MPV175	MPV200
Dry matter (g/kg)	922.5	933.0	915.3	912.3	908.9	915.6
Ash	9.24	9.21	8.91	7.99	7.18	7.69
Organic matter	846.4	850.2	832.3	828.0	840.5	829.9
Calculated ME[2] (MJ/kg)	11.9	11.9	11.9	11.9	11.9	11.9
Crude protein	243.5	231.5	223.8	223.4	213.1	211.7
Neutral detergent fibre	157.5	156.6	155.3	154.6	151.3	147.7
Acid detergent fibre	138.9	138.7	136.3	135.2	130.2	125.5
[3]sCT (AU)	0.070	0.058	0.057	0.055	0.022	0.021

[1]Substrates: CON= untreated *M. oleifera*; PEG54 = *M. oleifera* pre-treated with 54 g/kg polyethylene glycol; MPV125 = *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 12.5 g/kg fibrolytic enzymes; MPV150= *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 15 g/kg fibrolytic enzymes; MPV175 = *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 17.5 g/kg fibrolytic enzymes; MPV200 = *M. oleifera* pre- treated with 54 g/kg polyethylene glycol and 20 g/kg fibrolytic enzymes.

[2]Calculated ME = calculated metabolizable energy.

[3]sCT = soluble condensed tannins.

5.2.4 Diet formulation

Six isonitrogenous and isoenergetic mash dietary treatments were formulated by hand to meet the National Research Council's (NRC, 2004) requirements for grower quail as follows: CON = a standard grower diet that contains 100 g/kg untreated MOLM; MPV0 = a standard grower diet that contains 100 g/kg MOLM pre-treated with 54 g/kg PEG; and a standard grower diet that contains 100 g/kg MOLM pre-treated with 54 g/kg PEG and 12.5 (MPV125), 15.0 (MPV150), 17.5 (MPV175), and 20 g/kg (MPV200) of VME, as shown in Table 5.2. The nutritional composition of the dietary treatments was determined as described in Chapter 3, Section 3.2.2.

Table 5.2. Gross ingredient composition (g/kg *as fed* basis, unless stated otherwise) of the dietary treatments.

	[1]Diets					
Ingredients	CON	MPV0	MPV125	MPV150	MPV175	MPV200
Polyethylene glycol	0.0	54	54	54	54	54
Viscozyme® L	0.0	0.0	12.5	15.0	17.5	20
Moringa oleifera leaf meal	100.0	100.0	100.0	100.0	100.0	100.0
Fine yellow maize	698.6	626.9	626.9	626.9	626.9	626.9
Prime gluten 60	18.0	18.0	18.0	18.0	18.0	18.0
Full fat soya meal	50.7	148.6	148.6	148.6	148.6	148.6
Soybean meal	196.7	70.5	70.5	70.5	70.5	70.5
Limestone powder	14.5	14.5	14.5	14.5	14.5	14.5
Mono calcium phosphate	7.2	7.2	7.2	7.2	7.2	7.2
Salt-fine	3.2	3.2	3.2	3.2	3.2	3.2
Sodium bicarbonate	1.7	1.7	1.7	1.7	1.7	1.7
Choline meal	0.8	0.8	0.8	0.8	0.8	0.8
Lysine	2.8	2.8	2.8	2.8	2.8	2.8
L-Threonine	0.4	0.4	0.4	0.4	0.4	0.4
Methionine	1.9	1.9	1.9	1.9	1.9	1.9
Grower-phytase	1.7	1.7	1.7	1.7	1.7	1.7
Vitamin and mineral premix[2]	0.5	0.5	0.5	0.5	0.5	0.5

[1]Diets: CON = standard grower diet containing untreated *M. oleifera* leaf meal; MPV0 = standard grower diet containing *M. oleifera* leaf meal pre-treated with 54 g/kg polyethylene glycol; MPV125 = standard grower diet containing *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 12.5 g/kg fibrolytic enzymes; MPV150 = standard grower diet containing *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 15 g/kg fibrolytic enzymes; MPV175 = standard grower diet containing *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 17.5 g/kg fibrolytic enzymes; MPV200 = standard grower diet containing *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 20 g/kg fibrolytic enzymes.

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

Calculated ME[3] = calculated metabolizable energy.

[4]sCT = soluble condensed tannins.

Table 5.3. Nutritional composition (g/kg DM, unless stated otherwise) of the dietary treatments.

	[1]Diets					
	CON	MPV0	MPV125	MPV150	MPV175	MPV200

Dry matter (g/kg)	920.0	919.9	916.9	910.6	910.3	907.3
Ash	5.82	5.42	5.46	5.35	5.11	5.05
Organic matter	868.0	858.9	862.2	872.2	878.4	855.1
Calculated ME[3] (MJ/kg)	11.9	11.9	11.9	11.9	11.9	11.9
Crude protein	184.5	184.0	184.2	184.8	184.6	184.2
Neutral detergent fibre	166.8	156.4	155.7	154.6	146.4	147.7
Acid detergent fibre	158.3	155.5	140.9	145.8	143.2	137.8
[4]sCT (AU $_{550nm/200mg}$)	0.029	0.020	0.018	0.016	0.015	0.011

[1]Diets: CON = standard grower diet containing untreated *M. oleifera* leaf meal; MPV0 = standard grower diet containing *M. oleifera* leaf meal pre-treated with 54 g/kg polyethylene glycol; MPV125 = standard grower diet containing *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 12.5 g/kg fibrolytic enzymes; MPV150 = standard grower diet containing *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 15 g/kg fibrolytic enzymes; MPV175 = standard grower diet containing *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 17.5 g/kg fibrolytic enzymes; MPV200 = standard grower diet containing *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 20 g/kg fibrolytic enzymes.

5.2.5 Feeding trial, slaughter, blood analyses, and meat quality

The North-West University's Animal Production Research Ethics Committee granted approval (NWU-01884-19-S5) for the feeding trial, handling, and slaughter of the birds. The feeding trials conducted as described in Chapter 3, Section 3.2.5. The hematological and serum biochemical parameters were analyzed according to the method specified in Chapter 3, Section 3.2.6. The carcass traits and internal organs were analyzed as described in Chapter 3, Section 3.2.7. Meat pH and temperature were recorded as specified in Chapter 3, Section 3.2.8. Meat colour coordinates: *L** (lightness), *a** (redness) and *b** (yellowness) were measured as outlined in Chapter 3, Section 3.2.9. Cooking loss and meat tenderness were conducted according to Chapter 3, Section 3.2.10. Water holding capacity was determined as described in Chapter 3,

Section 3.2.11. Drip loss was determined using the method described in Chapter 3, Section 3.2.12.

5.2.6 Statistical analysis

Average weekly FI, average weekly BWG and average weekly FCE data were analyzed using repeated measures analysis in PROC GLM (SAS, 2010) as described in Chapter 3, Section 3.3.

Feed intake, growth performance, haemato-biochemistry, and meat quality data (excluding CON diet) were evaluated for linear and quadratic effects using response surface regression analysis (SAS, 2010) using the following quadratic equation:

$$y = ax^2 + bx + c$$

Where: y = dependent variable, a and b are the coefficients of the quadratic equation; and c is the intercept; x is VME pre-treatment levels (%) and $\frac{-b}{2a}$ is the x value for optimal response.

Overall feed intake, BWG, FCE, blood parameters and meat quality data were analyzed using PROC GLM in SAS (2010), with diet as the only main factor. The linear statistical model was as described in Chapter 3, Section 3.3.

5.3 Results

5.3.1 Growth performance

Repeated measures analysis showed a significant week × diet interaction effect on ABWG (P = 0.001), but not on AWFI (P = 0.356) and FCE (P = 0.321). Table 5.4 shows that neither linear or quadratic effects (P >0.05) were recorded for overall FI and FCE as VME levels increased. However, weight gain in two-week-old quail quadratically responded [y = 53.18 (±4.63) – 0.198 (±0.081) x + 0.000848 (±0.000324) x^2; R^2 = 0.248; P = 0.016] to increasing VME pre-treatment levels. Similarly, dietary influences (P <0.05) were only observed on weight gain in week 2. T-week-old birds reared on diet MPV125 had lower (P <0.05) weight gain compared to birds reared on the other treatment groups, whose weight gain did not differ (P >0.05).

Table 5.4. Effect of pre-treating dietary *Moringa oleifera* leaf meal with polyethylene glycol and incremental levels of viscozyme® L multi-enzyme on growth performance in Jumbo quail.

	[1]Diets							*P* values	
[2]Parameters	CON	MPV0	MPV125	MPV150	MPV175	MPV200	[3]SEM	Linear	Quadratic
Overall FI (g/bird)	155.1	155.3	161.7	154.6	156.8	152.0	5.551	0.350	0.661
Overall FCE	0.268	0.268	0.281	0.259	0.263	0.269	0.007	0.536	0.712
Average weekly weight gain (g/bird)									
Week 2	46.7[a]	45.6[a]	40.9[b]	45.0[a]	48.2[a]	45.4[a]	1.357	0.383	0.016
Week 3	44.8	46.5	49.6	48.6	46.5	45.6	2.334	0.994	0.269
Week 4	41.5	46.2	42.8	39.0	42.2	40.2	2.736	0.070	0.181
Week 5	22.0	16.8	28.3	21.8	19.7	20.6	4.159	0.877	0.139

[a,b] Means in the same row with different superscripts indicate statistical differences (P <0.05).

[1]Diets: CON = standard grower diet containing untreated *M. oleifera* leaf meal; MPV0 = standard grower diet containing *M. oleifera* leaf meal pre-treated with 54 g/kg polyethylene glycol; MPV125 = standard grower diet containing *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 12.5 g/kg fibrolytic enzymes; MPV150 = standard grower diet containing *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 15 g/kg fibrolytic enzymes; MPV175 = standard grower diet containing *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 17.5 g/kg fibrolytic enzymes; MPV200 = standard grower diet containing *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 20 g/kg fibrolytic enzymes.

[2]Parameters: Overall FI = feed intake for the entire duration of the study; Overall FCE = feed conversion efficiency for the entire duration of the study.

[3]SEM = standard error of the mean.

5.3.2 Haematological parameters

For all the haematological parameters, Table 5.5 shows that there were no linear or quadratic effects (P >0.05) in response to VME levels. Similarly, no significant dietary effects were observed on haematological parameters.

Table 5.5. Effect of pre-treating dietary *Moringa oleifera* leaf meal with polyethylene glycol and incremental levels of viscozyme® L multi-enzyme on haematological parameters in Jumbo quail.

	[1]Diets							*P* values	
[2]Parameters	CON	MPV0	MPV125	MPV150	MPV175	MPV200	[3]SEM	Linear	Quadratic
Erythrocytes ($\times 10^9$/L)	4.00	3.93	4.49	6.03	3.60	5.50	0.902	0.088	0.585
Haematocrits (L/L)	25.3	28.7	36.6	46.3	18.6	43.0	9.312	0.210	0.529
Haemoglobin (g/dL)	1	9.25	11.1	10.8	11.7	11.6	1.913	0.178	0.331
MCH (pg)	26.5	23.1	24.9	17.9	32.7	21.5	2.836	0.655	0.776
MCV (fL)	60.8	70.4	81.5	76.8	52.1	78.5	8.167	0.827	0.546

[1]Diets: CON = standard grower diet containing untreated *M. oleifera* leaf meal; MPV0 = standard grower diet containing *M. oleifera* leaf meal pre-treated with 54 g/kg polyethylene glycol; MPV125 = standard grower diet containing *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 12.5 g/kg fibrolytic enzymes; MPV150 = standard grower diet containing *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 15 g/kg fibrolytic enzymes; MPV175 = standard grower diet containing *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 17.5 g/kg fibrolytic enzymes; MPV200 = standard grower diet containing *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 20 g/kg fibrolytic enzymes.

[2]Parameters: MCH = mean corpuscular haemoglobin; MCV = mean corpuscular volume.

[3]SEM = standard error of the mean.

5.3.3 Serum biochemical parameters

Table 5.6 shows that for all the serum parameters, there were no linear or quadratic effects (*P* >0.05) in response to VME levels. The serum parameters of the Jumbo quail did not show any dietary effects (*P* >0.05).

Table 5.6. Effect of pre-treating dietary *Moringa oleifera* leaf meal with polyethylene glycol and incremental levels of viscozyme® L multi-enzyme on serum biochemistry parameters in Jumbo qual.

	[1]Diets							*P* values	
[2]Parameters	CON	MPV0	MPV125	MPV150	MPV175	MPV200	[3]SEM	Linear	Quadratic
Glucose (mmol/L)	0.785	2.95	3.26	2.57	1.57	4.75	2.155	0.204	0.822
Creatinine (µmol/L)	9.00	9.00	10.4	9.00	9.08	9.00	1.410	0.792	0.234
Phosphorus (mmol/L)	5.20	5.19	5.18	4.98	4.91	4.70	0.203	0.137	0.923
Calcium (mmol/L)	2.09	2.49	2.67	2.18	2.44	2.65	0.621	0.606	0.848
Total protein (g/L)	36.5	40.0	35.8	34.6	31.9	32.6	2.663	0.230	0.807
Albumin (g/L)	12.5	15.0	12.2	12.4	12.3	15.3	2.910	0.133	0.093
Globulin (g/L)	24.0	25.0	23.5	22.2	19.7	21.5	4.178	0.330	0.858
ALT (U/L)	28.0	50.8	41.6	38.1	29.8	21.2	4.290	0.096	0.887
ALKP (U/L)	230.0	153.0	179.1	177.9	216.5	280.4	49.48	0.067	0.210
Amylase (U/L)	224.5	275.6	367.7	277.8	283.5	213.6	59.21	0.515	0.162
Lipase (U/L)	214.5	330.6	235.1	220.2	224.7	243.3	37.59	0.508	0.236

[1]Diets: CON = standard grower diet containing untreated *M. oleifera* leaf meal; MPV0 = standard grower diet containing *M. oleifera* leaf meal pre-treated with 54 g/kg polyethylene glycol; MPV125 = standard grower diet containing *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 12.5 g/kg fibrolytic enzymes; MPV150 = standard grower diet containing *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 15 g/kg fibrolytic enzymes; MPV175 = standard grower diet containing *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 17.5 g/kg fibrolytic enzymes; MPV200 = standard grower diet containing *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 20 g/kg fibrolytic enzymes.

[2]Parameters: MCH = mean corpuscular haemoglobin; MCV = mean corpuscular volume; ALT = alanine transaminase; ALKP = alkaline phosphatase.

[3]SEM = standard error of the mean.

5.3.4 Internal organs

Table 5.7 shows a linear decrease for small intestine length [y = 64.75 (±4.390) – 0.004 (±0.077) x; R^2 = 0.215; P = 0.028] and a quadratic effect for caecum length [y = 15.37 (±3.281) – 0.112 (±0.058) x – 0.0005 (±0.0002) x^2; R^2 = 0.182; P = 0.039] in response to incremental levels of VME. Birds reared on MPV200 diet had longer caeca (14.1 cm) than those reared on MPV0, MPV125, MPV150 and MPV175, whose caeca lengths did not differ (P >0.05). The control diet (CON) promoted similar caeca length *(P >0.05)* as the MPV200 diet. Birds reared on diet MPV175 had shorter small intestines (59.5 cm) than those reared on CON and MPV0 diets. The MPV0 diet promoted similar small intestine lengths *(P >0.05)* as the CON, MPV125, MPV150 and MPV200 diets.

Table 5.7. Effect of pre-treating dietary *Moringa oleifera* leaf meal with polyethylene glycol and incremental levels of viscozyme® L multi-enzyme on size of internal organs (%HCW, unless stated otherwise) in Jumbo quail.

	[1]Diets							*P* values	
Parameters	CON	MPV0	MPV125	MPV150	MPV175	MPV200	[2]SEM	Linear	Quadratic
Weights (%HCW)									
Gizzards	2.55	2.61	2.47	2.52	2.49	2.53	0.093	0.825	0.615
Liver	2.92	2.88	3.85	2.59	2.68	2.82	0.304	0.721	0.307
Proventriculus	0.63	0.61	0.61	0.60	0.60	0.63	0.022	0.493	0.457
Caecum	1.09	1.39	1.17	1.25	1.24	1.19	0.112	0.319	0.510
Small intestine	4.84	4.84	4.98	4.38	4.29	4.82	0.243	0.779	0759
Lengths (cm)									
Caecum	11.8^{ab}	10.4^{b}	9.74^{b}	9.56^{b}	10.7^{b}	14.1^{a}	1.122	0195	0.039
Small intestine	64.6^{a}	65.1^{a}	64.3^{ab}	61.9^{ab}	59.5^{b}	61.3^{ab}	1.763	0.028	0.639

^{a,b} Means in the same row with different superscripts indicate statistical difference (P <0.05).

[1]Diets: CON = standard grower diet containing untreated *M. oleifera* leaf meal; MPV0 = standard grower diet containing *M. oleifera* leaf meal pre-treated with 54 g/kg polyethylene glycol; MPV125 = standard grower diet containing *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 12.5 g/kg fibrolytic enzymes; MPV150 = standard grower diet containing *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 15 g/kg fibrolytic enzymes; MPV175 = standard grower diet containing *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 17.5 g/kg fibrolytic enzymes; MPV200 = standard grower diet containing *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 20 g/kg fibrolytic enzymes.

[2]SEM = standard error of the mean.

5.3.5 Carcass traits

Table 5.8 shows that pre-treatment of dietary MOLM with incremental levels of fibrolytic enzymes showed no significant linear or quadratic effects for carcass characteristics in Jumbo quail. Similarly, no significant dietary effects were observed on all carcass traits.

Table 5.8. Effect of pre-treating dietary *Moringa oleifera* leaf meal with polyethylene glycol and incremental levels of viscozyme® L multi-enzyme on carcass characteristics (%HCW, unless stated otherwise) in Jumbo quail.

	[1]Diets							*P* values	
[2]Parameters	CON	MPV0	MPV125	MPV150	MPV175	MPV200	[3]SEM	Linear	Quadratic
FBW (g)	211.8	206.7	218.2	211.5	210.8	208.7	7.688	0.342	0.753
HCW (g)	137.9	131.6	135.7	139.8	138.5	139.6	2.548	0.407	0.424
CCW (g)	135.2	129.2	134.1	136.0	136.6	138.1	3.164	0.055	0.091
Carcass yield (%)	65.2	68.9	62.2	66.1	64.6	63.3	2.524	0.931	0.991
Breast	18.9	18.5	18.6	18.7	18.3	18.1	0.433	0.761	0.181
Wing	4.77	4.97	4.87	4.75	4.96	4.86	0.118	0.750	0.957
Thigh	6.56	6.58	6.54	6.45	6.71	6.66	0.109	0.526	0.161
Drumstick	4.02	4.39	4.41	4.35	4.49	4.35	0.122	0.335	0.256
Lengths (cm)									
Back	9.92	10.3	10.0	9.61	9.93	9.56	0.270	0.222	0.950
Wing	10.64	10.62	10.62	10.60	10.58	10.58	0.108	0.972	0.688
Thigh	4.46	4.55	4.52	4.39	4.52	4.36	0.082	0.215	0.773
Drumstick	5.24	5.40	5.33	5.24	5.24	5.19	0.088	0.163	0.760

[1]Diets: CON = standard grower diet containing untreated *M. oleifera* leaf meal; MPV0 = standard grower diet containing *M. oleifera* leaf meal pre-treated with 54 g/kg polyethylene glycol; MPV125 = standard grower diet containing *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 12.5 g/kg fibrolytic enzymes; MPV150 = standard grower diet containing *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 15 g/kg fibrolytic enzymes; MPV175 = standard grower diet containing *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 17.5 g/kg fibrolytic enzymes; MPV200 = standard grower diet containing *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 20 g/kg fibrolytic enzymes.
[2]Parameters: FBW = final body weight; HCW = hot carcass weight; CCW = cold carcass weight.
[3]SEM = standard error of the mean.

5.3.6 Meat quality traits

According to Table 5.9, Table 5.10 and 5.11, neither of the meat quality parameters showed any linear or quadratic trends (P >0.05) in response to incremental levels of viscozyme® L multi-enzyme. Similarly, no significant dietary effects were recorded on meat quality parameters.

Table 5.9. Effect of pre-treating dietary *Moringa oleifera* leaf meal with polyethylene glycol and incremental levels of viscozyme® L multi-enzyme on breast meat quality parameters measured 1 hour post-mortem in Jumbo quail.

	[1]Diets							*P* values	
[2]Parameters	CON	MPV0	MPV125	MPV150	MPV175	MPV200	[3]SEM	Linear	Quadratic
pH_1	6.09	6.20	6.23	5.95	6.16	6.21	0.066	0.707	0.405
$Temperature_1$ (°C)	18.80	19.9	19.8	16.8	18.7	18.7	1.963	0.871	0.970
L^*_1	45.9	46.1	45.8	45.0	45.3	46.4	1.063	0.392	0.280
a^*_1	8.57	8.37	8.23	9.80	8.52	8.63	0.634	0.917	0.750
b^*_1	5.00	4.61	5.32	4.89	5.02	4.78	0.380	0.396	0.381
$Chroma_1$	10.00	9.61	9.88	10.9	9.96	9.91	0.541	0.867	0.496
$Hue\ angle_1$	0.54	0.50	0.58	0.46	0.53	0.50	0.052	0.464	0.693

[1]Diets: CON = standard grower diet containing untreated *M. oleifera* leaf meal; MPV0 = standard grower diet containing *M. oleifera* leaf meal pre-treated with 54 g/kg polyethylene glycol; MPV125 = standard grower diet containing *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 12.5 g/kg fibrolytic enzymes; MPV150 = standard grower diet containing *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 15 g/kg fibrolytic enzymes; MPV175 = standard grower diet containing *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 17.5 g/kg fibrolytic enzymes; MPV200 = standard grower diet containing *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 20 g/kg fibrolytic enzymes.
[2]Parameters: L^* = lightness; a^* = redness; b^* = yellowness.
[3]SEM = standard error of the mean.

Table 5.10. Effect of treating dietary *Moringa oleifera* leaf meal with polyethylene glycol and incremental levels of viscozyme® L multi-enzyme on meat quality parameters measured 24 hour post-mortem in Jumbo quail.

	[1]Diets							*P* values	
[2]Parameters	CON	MPV0	MPV125	MPV150	MPV175	MPV200	[3]SEM	Linear	Quadratic
pH_{24}	6.48	6.34	6.19	6.33	6.23	6.28	0.085	0.516	0.663
$Temperature_{24}$ (°C)	12.5	13.2	12.2	11.7	11.9	11.4	0.947	0.064	0.666
L^*_{24}	42.6	42.7	42.6	42.7	42.1	42.9	0.789	0.677	0.669
a^*_{24}	10.3	9.43	10.1	10.6	11.4	10.8	0.554	0.053	0.780
b^*_{24}	6.98	6.28	6.30	6.57	6.42	6.26	0.311	0.484	0.994
$Chroma_{24}$	12.2	11.8	11.8	12.5	13.1	12.5	0.516	0.113	0.784
Hue $angle_{24}$	0.59	0.58	0.55	0.55	0.51	0.52	0.032	0.406	0.824

[1]Diets: CON = standard grower diet containing untreated *M. oleifera* leaf meal; MPV0 = standard grower diet containing *M. oleifera* leaf meal pre-treated with 54 g/kg polyethylene glycol; MPV125 = standard grower diet containing *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 12.5 g/kg fibrolytic enzymes; MPV150 = standard grower diet containing *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 15 g/kg fibrolytic enzymes; MPV175 = standard grower diet containing *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 17.5 g/kg fibrolytic enzymes; MPV200 = standard grower diet containing *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 20 g/kg fibrolytic enzymes.
[2]Parameters: *L** = lightness; *a** = redness; *b** = yellowness.
[3]SEM = standard error of the mean.

Table 5.11. Effect of pre-treating dietary *Moringa oleifera* leaf meal with polyethylene glycol and incremental levels of viscozyme® L multi-enzyme on meat quality traits in Jumbo quail.

	[1]Diets							*P* values	
[2]Parameters	CON	MPV0	MPV125	MPV150	MPV175	MPV200	[3]SEM	Linear	Quadratic
Cooking loss (%)	38.5	32.2	41.8	34.5	39.8	41.1	4.992	0.107	0.224
Drip loss (%)	45.5	45.1	46.9	46.2	44.7	48.6	2.245	0.353	0.621
WHC (%)	91.8	90.3	90.7	89.4	90.4	90.0	1.306	0.861	0.512

[1]Diets: CON = standard grower diet containing untreated *M. oleifera* leaf meal; MPV0 = standard grower diet containing *M. oleifera* leaf meal pre-treated with 54 g/kg polyethylene glycol; MPV125 = standard grower diet containing *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 12.5 g/kg fibrolytic enzymes; MPV150 = standard grower diet containing *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 15 g/kg fibrolytic enzymes; MPV175 = standard grower diet containing *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 17.5 g/kg fibrolytic enzymes; MPV200 = standard grower diet containing *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 20 g/kg fibrolytic enzymes.

[2]Parameters: WHC = water holding capacity.

[3]SEM = standard error of the mean.

The data measured at room temperature over 5 days established no (P >0.05) effect of dietary treatment (Figures 5.1 – 5.4). The breast meat pH for Jumbo quail on MPV0, MPV25, MPV50, MPV75, and MPV100 diets slightly increased over 5 days. For all treatments, the lightness (L*) of the breast meat decreased over the 5-day period. For all treatments, the redness (a*) of the breast meat decreased over 5 days.

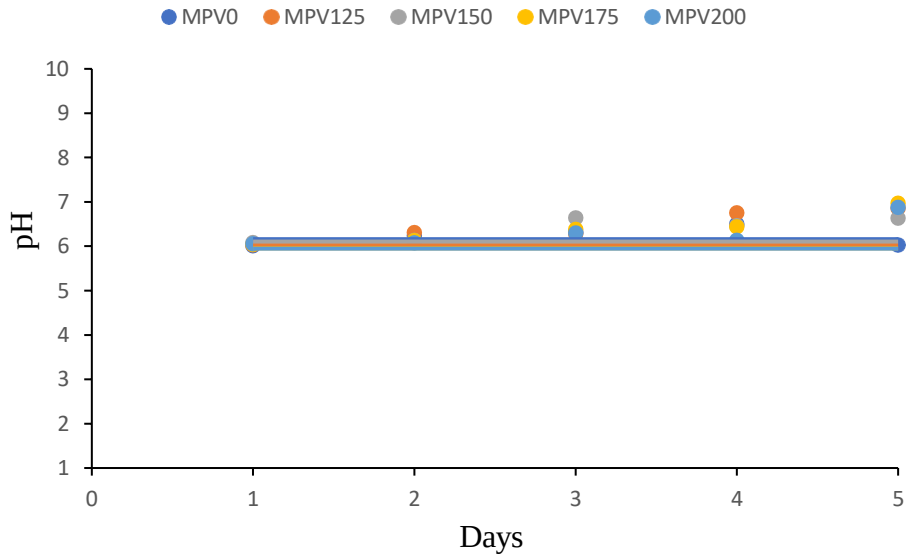


Figure 5.1. Effect of pre-treated *Moringa oleifera* leaf meal on the stability of breast meat pH upon storage at room temperature for 5 days [*Diets*: CON = standard grower diet containing untreated *M. oleifera* leaf meal; MPV0 = standard grower diet containing *M. oleifera* leaf meal pre-treated with 54 g/kg polyethylene glycol; MPV125 = standard grower diet containing *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 12.5 g/kg fibrolytic enzymes; MPV150 = standard grower diet containing *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 15 g/kg fibrolytic enzymes; MPV175 = standard grower diet containing *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 17.5 g/kg fibrolytic enzymes; MPV200 = standard grower diet containing *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 20 g/kg fibrolytic enzymes].

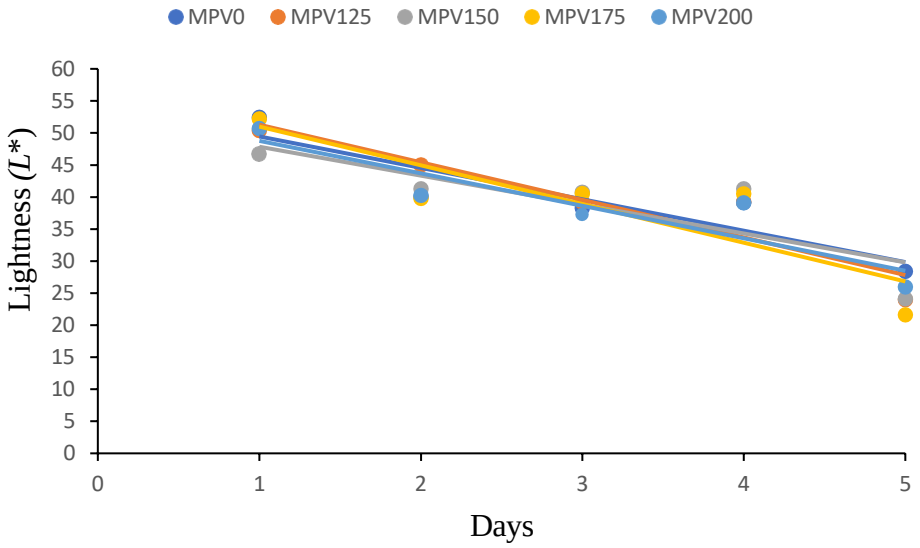


Figure 5.2. Effect of pre-treated *Moringa oleifera* leaf meal on the stability of breast meat lightness upon storage at room temperature for 5 days [*Diets*: CON = standard grower diet containing untreated *M. oleifera* leaf meal; MPV0 = standard grower diet containing *M. oleifera* leaf meal pre-treated with 54 g/kg polyethylene glycol; MPV125 = standard grower diet containing *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 12.5 g/kg fibrolytic enzymes; MPV150 = standard grower diet containing *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 15 g/kg fibrolytic enzymes; MPV175 = standard grower diet containing *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 17.5 g/kg fibrolytic enzymes; MPV200 = standard grower diet containing *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 20 g/kg fibrolytic enzymes].

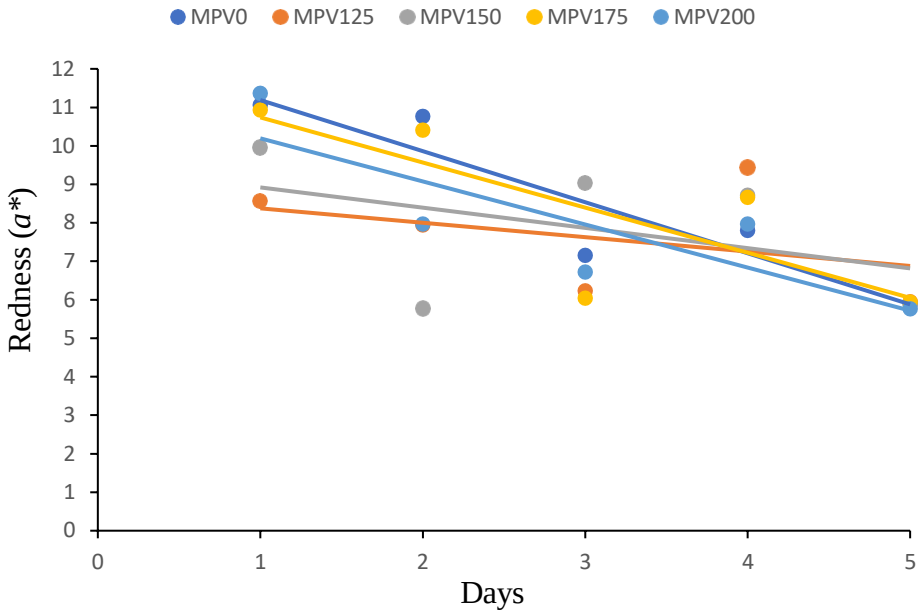


Figure 5.3. Effect of pre-treated *Moringa oleifera* leaf meal on the stability of breast meat redness upon storage at room temperature for 5 days [*Diets*: CON = standard grower diet containing untreated *M. oleifera* leaf meal; MPV0 = standard grower diet containing *M. oleifera* leaf meal pre-treated with 54 g/kg polyethylene glycol; MPV125 = standard grower diet containing *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 12.5 g/kg fibrolytic enzymes; MPV150 = standard grower diet containing *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 15 g/kg fibrolytic enzymes; MPV175 = standard grower diet containing *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 17.5 g/kg fibrolytic enzymes; MPV200 = standard grower diet containing *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 20 g/kg fibrolytic enzymes].

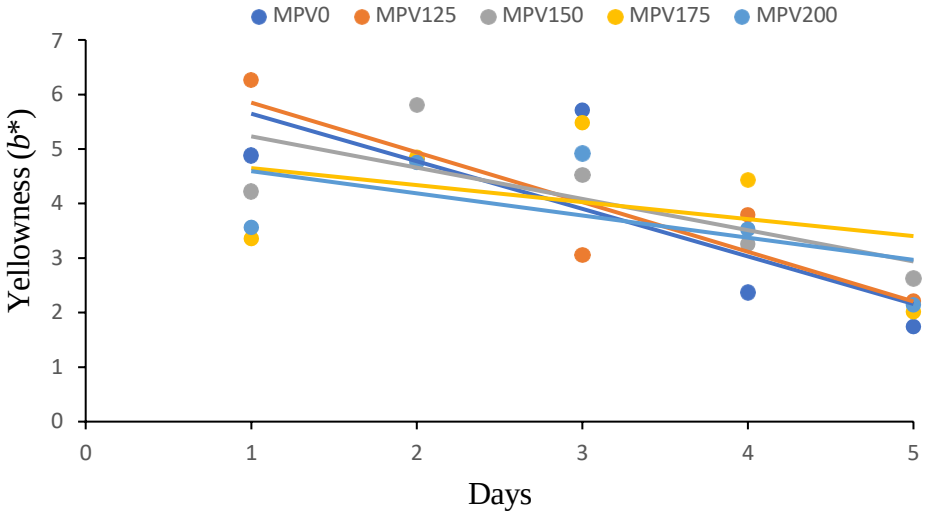


Figure 5.4. Effect of pre-treated *Moringa oleifera* leaf meal on the stability of breast meat yellowness upon storage at room temperature for 5 days [*Diets*: CON = standard grower diet containing untreated *M. oleifera* leaf meal; MPV0 = standard grower diet containing *M. oleifera* leaf meal pre-treated with 54 g/kg polyethylene glycol; MPV125 = standard grower diet containing *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 12.5 g/kg fibrolytic enzymes; MPV150 = standard grower diet containing *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 15 g/kg fibrolytic enzymes; MPV175 = standard grower diet containing *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 17.5 g/kg fibrolytic enzymes; MPV200 = standard grower diet containing *M. oleifera* pre-treated with 54 g/kg polyethylene glycol and 20 g/kg fibrolytic enzymes].

5.4 Discussion

5.4.1 Growth performance and haemo-biochemistry

Moringa oleifera leaf meal (MOLM) can supplement Jumbo quail diets with bioactive substances and nutrients required for optimum production. However, the utility of the leaf meal is limited by the high levels of fibre and CT. In my Masters' work, the inclusion of dietary MOLM beyond 25 g/kg in quail diets had adverse effects on feed utilisation efficiency and growth performance of Japanese quail (Mulaudzi *et al*., 2019). Higher dietary MOLM inclusion levels in Jumbo quail diets could promote sustainable intensification of the birds but requires pre-treatment to ameliorate the antinutritional activities of fibre and CT. In the current study,

repeated measures analysis showed a significant diet × week interaction effect on weight gain only, indicating that as the birds grew older, the ranking of dietary treatments in terms of weight gain changed. It is not clear why the birds fed with diet MPV125 had lower weight in week 2. Indeed, no dietary differences were observed in weeks 3, 4, and 5. Thus, the reasons for the reduced weight gain in the MPV125 group are unknown. In addition, pre-treatment of MOLM with PEG and VME had no effect on overall FI and FCE, signifying that both additives did not improve the feed value of MOLM.

This further suggests that there is no benefit in pre-treating high dietary MOLM (100 g/kg) levels with PEG and enzymes as it does not enhance growth performance traits in Jumbo quail. Similar findings were reported by Kumanda *et al*. (2019), who established a lack of improvement on growth performance of broiler chickens upon pre-treatment of red grape pomace waste with PEG and fibrolytic enzymes. Similarly, Lee *et al*. (2014) and Abolade (2016) did not observe any significant improvement on growth performance parameters of broiler chickens reared on enzyme-supplemented diets. Although these feed additives have been proven to individually improve nutrient utilisation and growth performance (Kaczmarek *et al*., 2015; Aziz ur Rahman *et al*., 2021), it should be noted that different methods of application could account for variation in their modes of action and ameliorative activities. This could be the reason why there are inconsistent results reported in literature regarding their effectiveness in improving poultry performance.

The evaluation of blood parameters is critical in determining the impact of untreated and PEG and VME pre-treated MOLM on the pathophysiological and health status of the birds (Saki *et al*., 2017). Because dietary MOLM contains antinutritional components such as CT and insoluble fibre, it was expected that their inclusion at a rate of 100 g/kg would have a negative impact on haematological and serum biochemical parameters. However, the selected blood

indices measured in this study confirmed that untreated and PEG- and enzyme-treated MOLM had no effect on the birds' pathophysiology and health status, with all the blood values falling within the values reported for healthy quail (Mbhele *et al*., 2019; Mahlake *et al*., 2021). The lack of negative effects suggests that higher dietary inclusion levels of MOLM do not compromise the well-being of Jumbo quail.

Cañedo-Castro *et al*. (2019) reported that serum biochemical indices provide useful information on the health and nutritional status of animals fed non-conventional feed ingredients. This further indicates that the Jumbo quail did not suffer from nutritional deficiencies. Moreover, liver enzymes such as ALKP and ALT were unaffected, signifying that the birds did not suffer from diet induced toxicosis (Bona *et al*., 2018).

5.4.2 Carcass and meat quality traits

Changes in gastrointestinal morphology associated with variation in the concentration of dietary fibre have been reported (Jha *et al*., 2019). The negative quadratic response observed for caecum length could also point to the ineffectiveness of the enzyme to improve fibre utilisation by the birds. No dietary influences were observed on the sizes of liver, gizzard, and proventriculus, which was also surprising given that the diet containing untreated MOLM supposedly contained high levels of fibre and CT. The profitability of poultry production has been strongly related to the improvements in carcass yield and composition. Thus, understanding factors that affect carcass characteristics is critically important for the success of any poultry enterprise. Unfortunately, knowledge of such factors in quail birds is limited (Dyubele *et al*., 2010). The supplementation with PEG and enzymes had no effect on carcass parameters, which is consistent with the findings of Alefzadeh *et al*. (2016) and Taheri & Shirzadegan (2017), who reported no significant differences in carcass traits of broilers reared on diets supplemented with exogenous enzymes.

The appearance, texture, and sensory characteristics of poultry and other meat products all contribute to their overall consumer acceptability (Muchenje *et al*., 2009; Yu *et al*., 2017). The colour of the meat is the most important indicator when consumers purchase meat products and is the major factor that affects consumer acceptance of the meat (Muchenje *et al*., 2009). Consumers tend to reject the products based on colour variations from the expected norm. However, no colour variations were observed in all the treatment groups, suggesting that PEG and fibrolytic enzymes do not affect the appearance of the meat. Meat pH is primarily related to the biochemical state of the muscle at the time of slaughter, resulting in the onset of rigor mortis (Ding *et al*., 2022). Chan *et al*. (2011) confirmed a correlation between the pH of the meat and its colour, with lighter meat indicating low pH, while dark meat indicating a high pH value. Kralik *et al*. (2018) reported that low pH in broiler meat stimulates the oxidation of myoglobin (pink colour) and oxyhaemoglobin (red colour) to meta myoglobin (brown meat colour). However, the dietary treatments in this study had no effect on meat pH, which further confirm that the addition of PEG and enzymes does not alter post-mortem pH.

Water holding capacity refers to the ability of the meat to retain water when external forces such as cutting, grinding, and pressing are applied (Bowker & Zhuang, 2015). The degree of water lost or retained depends mainly on the pH of the tissue for example a higher pH will result in less water loss, while a lower pH value would determine the amount of water retained by the meat (Warner, 2017). When there is low pH in meat products, it leads to protein denaturation, which further reduces the WHC of the meat (Singh & Deshpande, 2018). Thus, the lack of dietary differences on meat pH could explain why the meat had similar water-binding potential. Indeed, no differences were observed on cooking loss and drip loss, which further indicate that PEG and enzyme supplementation do not alter the organoleptic (juiciness, tenderness, texture, smell, and colour) and sensorial qualities (Maison *et al*., 2016; Choi *et al*.,

2019) of quail meat. Pre-treating MOLM with PEG and VME did not affect the meat stability (shelf life) tested over 5 days at room temperature. Although an increase in meat pH is linked to enhanced meat quality, an increase in pH over time may also indicate meat spoilage due to microbial contamination.

5.5 Conclusion

Pre-treatment of dietary *Moringa oleifera* leaf meal with 54 g/kg PEG and up to 20 g/kg exogenous fibrolytic enzymes did not improve growth performance, blood, carcass, and meat quality parameters, but affected some visceral organ sizes in Jumbo quail. In addition, an optimum could not be determined using the growth performance data, indicating that there is no benefit in pre-treating high dietary MOLM (100 g/kg) levels with PEG and enzymes as it does not enhance growth performance traits in Jumbo quail.

5.6 References

Abd El Tawab, A.M. & Khattab, M.S.A., 2018. Utilization of polyethylene glycol and tannase enzyme to reduce the negative effect of tannins on digestibility, milk production and animal performance. Asian J. Anim. Vet. Adv. 13, 201–209.

Abolade, O.J. 2016., Effect of protease supplementation on the performance of laying chickens fed marginally deficient protein diet. J. Manag. Stud. 2(11), 20–29.

Ahmed, W.F. & El-Rayes, T.K., 2019. Effect of using *Moringa oleifera* leaves on productive performance and some physiological parameters of Japanese quail. Egypt. Poult. Sci. J. 39(1), 193–205.

Alefzadeh, T., Bouyeh, M., Van den Hoven, R., Seidavi, A., Laudadio, V. & Tufarelli, V., 2016. Effect of dietary dried orange (*Citrus sinensis*) peel powder and exogenous multienzymes on growth and carcass traits and ileal microflora of broiler chickens. Pak. J. Zool. 48(6), 1891–1897.

AOAC. 2005. *Official Methods of Analysis of the Association of Official's Analytical Chemists*, 18th ed.; Association of Official Analytical Chemists: Arlington, VA, USA.

Aziz ur Rahman, M., Jamal, U., Anwar, U., Bilal, M. Q., Riaz, M., Hussain, M. & Ahmad, S., 2021. Effects of potato peels inclusion with exogenous enzymes in broiler diet on growth performance, nutrients digestibility and carcass characteristics. Sci. Prog. 104(4), 1–14.

Bona, L., Van Staaveren, N., Pokharel, B.B., Van Krimpen, M. & Harlander-Matauschek, A., 2018. The effect of low protein energy-rich diets on plasma hepatic markers, hepatic damage, and discrimination reversal learning in young female chicks. Front. Vet. Sci. 5, 107.

Bowker, B. & Zhuang, H., 2015. Relationship between water-holding capacity and protein denaturation in broiler breast meat. Poult. Sci. 94(7), 1657–1664.

Cañedo-Castro, B., Piñón-Gimate, A., Carrillo, S., Ramos, D. & Casas-Valdez, M., 2019. Prebiotic effect of *Ulva rigida* meal on the intestinal integrity and serum cholesterol and triglyceride content in broilers. J. Appl. Phycol. 31, 3265–3273.

Chan, J.T., Omana, D.A. & Betti, M., 2011. Effect of ultimate pH and freezing on the biochemical properties of proteins in turkey breast meat. Food Chem. 127(1) 109–117.

Choi, J. & Kim, W.K., 2020. Dietary application of tannins as a potential mitigation strategy for current challenges in poultry production: A review. Animals. 10(12), 2389.

Choi, Y.M., Garcia, L.G. & Lee, K., 2019. Correlations of sensory quality characteristics with intramuscular fat content and bundle characteristics in bovine longissimus thoracis muscle. Food Sci. Anim. Resour. 39(2), 197–208.

CIE. 1976. *Recommendations on Uniform Colour Spaces, Colour-Difference Equations, Psychometric Colour Terms*; Supplement No. 2 to CIE Publication No. 15 (E-1.3.1.) 1978, 1971/(TC-1-3); Commission Internationale del'Eclairage: Paris, France.

David, L.S., Vidanarachchi, J K., Samarasinghe, K., Cyril, H. W. & Dematawewa, C.M.B., 2012. Effects of moringa based feed additives on the growth performance and carcass quality of broiler chicken. Trop. Agric. Res. 24 (1), 12–20.

Desbruslais, A., Wealleans, A., Gonzalez-Sanchez, D. & Di Benedetto, M., 2021. Dietary fibre in laying hens: a review of effects on performance, gut health and feather pecking. World Poult. Sci. J. 77(4), 797–823.

Ding, Z., Wei, Q., Liu, C., Zhang, H. & Huang, F., 2022. The quality changes and proteomic analysis of cattle muscle post-mortem during rigor mortis. Foods. 11(2), 217.

Dyubele, N. L., Muchenje, V., Nkukwana, T. T. & Chimonyo, M., 2010. Consumer sensory characteristics of broiler and indigenous chicken meat: A South African example. Food Qual. Prefer. 21(7), 815–819.

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

Hlatini, V.A., Ncobela, C.N., Zindove, T.J. & Chimonyo, M., 2018. Use of polyethylene glycol to improve the utilization of leguminous leaf meals in pigs: a review. S. Afr. J. Anim. Sci. 48(4), 609–620.

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

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, 51.

Jha, R., Fouhse, J., M., Tiwari, U.P., Li, L. & Willing, B.P. 2019. Dietary fibre and intestinal health of monogastric animals. Front. Vet. Sci. 6, 48.

Kaczmarek, S.A., Bochenek, M., Samuelsson, A.C. & Rutkowski, A., 2015. Effects of glyceryl polyethylene glycol ricinoleate on nutrient utilisation and performance of broiler chickens. Arch. Anim. Nutr. 69(4), 285–296.

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

Khalil, J.K., Saway, W.N. & Hyder, S.Z., 1986. Nutrient composition of Atriplex leaves grown in Saudi Arabia. J. Range Manag. 39, 104–107.

Kralik, G., Kralik, Z., Grčević, M. & Hanžek, D., 2018. Quality of chicken meat. Anim. Husb. Nutr. 63.

Kumanda, C., Mlambo, V. & Mnisi, C.M., 2019. Valorization of red grape pomace waste using polyethylene glycol and fibrolytic enzymes: Physiological and meat quality responses in broilers. Animals. 9(10), 779.

Lee, K.W., Choi, E.J., Moon, S.T. Lee, H., Kang, C.W. & An, B.K., 2014. Evaluation of dietary multiple enzyme preparation (Natuzyme) in laying hens. Asian-Australas. J. Anim. Sci. 27(2), 1749–1754.

Mahfuz, S. & Piao, X.S., 2019. Application of Moringa (*Moringa oleifera*) as natural feed supplement in poultry diets. Animals. 9(7), 431.

Mahlake, S.K., Mnisi, C.M., Lebopa, C. & Kumanda, C., 2021. The effect of green tea (*Camellia sinensis*) leaf powder on growth performance, selected hematological indices, carcass characteristics and meat quality parameters of Jumbo quail. Sustainability. 13(13), 7080.

Maison, A., Abdullah, B., Muradov, M., Korostynska, O., Al-Shamma, A., Bjarnadottir, S.T., Lunde, K. & Alvseike, O., 2016. Theoretical basis and application for measuring pork loin drip loss using microwave spectroscopy. S*ensors*. 16, 1–13.

Makkar, H.P.S., 2003. *Quantification of Tannins in Tree and foliage*. A Laboratory Manual; Springer: Dordrecht, The Netherlands, 43–54.

Mbhele, F.G.T., 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.

Mousa, G.A., Allak, M.A., Shehata, M.G., Hashem, N.M. & Hassan, O.G., 2022. Dietary supplementation with a combination of fibrolytic enzymes and probiotics improves digestibility, growth performance, blood metabolites, and economics of fattening lambs. Animals. 12(4), 476.

Muchenje, V., Dzama, K., Chimonyo, M., Strydom, P.E., Hugo, A. & Raats, J.G., 2009. Some biochemical aspects pertaining to beef eating quality and consumer health: A review. Food Chem. 112, 279–289.

Mulaudzi, A., Mnisi, C.M. & Mlambo, V., 2019. Dietary *Moringa oleifera* leaf meal improves growth performance but not haemo-biochemical and meat quality parameters in female Japanese quails. Pak. J. Nutr. 18, 953–960.

NRC. 2004. *Nutrient Requirements of Poultry*, 9th ed.; NRC; National Academy Press: Washington, DC, USA.

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.

Redondo, L. M., Chacana, P.A., Dominguez, J.E. & Fernandez Miyakawa, M.E.D., 2014. Perspectives in the use of tannins as alternative to antimicrobial growth promoter factors in poultry. Front. Microbiol. 5, 118.

Saki, A.A., Goudarzi, S.M., Ranjbaran, M., Ahmadi, A. & Khoramabadi, V., 2017. Evaluation of biochemical parameters and productive performance of Japanese quail in response to the replacement of soybean meal with canola meal. Acta Sci. Anim. Sci. 39, 51–56.

SAS. 2010. Users Guide; Statistical Analysis System Institute Inc: Carry, NC, USA.

Singh, R. K. & Deshpande, D. 2018. Thermally induced changes in quality of chicken breast meat protein fractions. J. Nutr. Food Sci. 8, 709.

Taheri, H.R. & Shirzadegan, K., 2017. Multiple-enzyme supplementation on digestive traits, carcass characteristics, blood lipid parameters and growth performance of broilers fed a wheat-based diet. Asian-Australas. J. Anim. Sci. 30 (9), 1285–1291.

Ufele, A.N. & Ebenebe, C.I., 2017. The Effect of *Moringa oleifera* on the growth performance, packed cell volume (PCV) and laying capacity of young growing quails. American. J. Zool. Res. 5(2), 33–37.

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

Wallace, R.J., Oleszek, W., Franz, C., Hahn, I., Baser, K.H.C., Mathe, A. & Teichmann, K., 2010. Dietary plant bioactives for poultry health and productivity. Br. Poult. Sci. 51, 461–487.

Warner, R.D., 2017. The eating quality of meat-IV Water-holding capacity and juiciness. In *Lawrie's meat science* (pp. 419-459). Woodhead Publishing.

Washington, I.M. & van Hoosier, G., 2012. *Clinical Biochemistry and Haematology*; University of Washington: Seattle, WA, USA. 59–91.

Yu, Q.P., Feng, D. Y., Xiao, J., Wu, F., He, X.J., Xia, M.H. & Zuo, J.J., 2017. Studies on meat colour, myoglobin content, enzyme activities, and genes associated with oxidative potential of pigs slaughtered at different growth stages. Asian-Australas. J. Anim. Sci. 30(12), 1739–1750.

6 CHAPTER SIX - GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

6.1 General discussion

The broiler industry is rapidly developing worldwide to provide much needed animal protein to the growing human population (Nkukwana, 2018). This is because it is generally believed that white meat costs less and is healthier than red meat (Muchenje *et al*., 2009). However, given that the human population has recently breached the 8 billion marks, there is a need to ensure sustainable intensification of poultry using low-cost novel feed sources with nutraceutical properties such as *Moringa oleifera* leaf meal (MOLM). The *M. oleifera* plant has a significant amount of bioactive chemicals with antibacterial, antioxidant, and immune-stimulating activities that can be utilised to prevent the growth of harmful pathogens, improve animal performance, and prolong the shelf life and quality of meat (Mbikay, 2012).

However, antinutritional factors such as glycosylates, phytic acid, condensed tannins, and fibre limit the amount of MOLM that can be included in quail diets. Intake of nutrients and digestion may be hindered by MOLM's high fibre and CT content (Chamorro *et al*., 2012). In line with additional reports, tannins too are responsible for the reduction in feed consumption, growth rate, feed efficiency, net metabolisable energy, and protein digestibility observed in experimental animals (Bele *et al*., 2010; Addisu, 2016). According to several reports, *M. oleifera* should only make up 2.5% of a bird's diet since the plant contains antinutritional components (Onu & Aniebo, 2011; Hassan *et al*., 2015; Ufele & Ebenebe, 2017).

The inclusion of higher levels of MOLM in quail diets may compromise productivity and meat quality. In quail, there has been no known research on the effectiveness of tannin-binding compounds such as polyethylene glycol (PEG) and fibrolytic enzymes to increase voluntary feed intake and utilisation of MOLM-containing diets. Therefore, the first experiment of this

study investigated the growth performance, haematology, serum biochemistry, internal organs and carcass and meat quality parameters in Jumbo quail fed with diets containing MOLM pre-treated with incremental levels of PEG. This experiment explored the hypothesis that growth performance, and physiological and meat quality parameters in Jumbo quail would respond to incremental levels of dietary MOLM pre-treated with PEG, thereby allowing the determination of an optimum inclusion level of PEG. Two-week-old quail chicks were randomly allocated to six diets formulated by incorporating (100 g/kg) untreated MOLM (MPG0) or MOLM pre-treated with PEG at 25 g/kg (MPG25), 50 g/kg (PEG50), 75 g/kg (MPG75), and 100 g/kg (MPG100) into a standard grower diet (CON).

The first experiment demonstrated that pre-treating dietary MOLM with PEG beyond 54 g/kg decreases weight gain and thus may impair the performance of Jumbo quail. However, blood parameters, internal organs, carcass characteristics, and meat quality attributes were not influenced by PEG treatment. Pre-treatment of MOLM with PEG improved weight gain only, this indicated the efficacy of PEG to valorize the feed value of MOLM as a potential feed ingredient. However, precautions need to be taken when high amounts of *M. oleifera* and PEG are included in quail diets.

The second experiment tested the hypothesis that pre-treatment of MOLM with an exogenous fibrolytic enzyme could improve growth performance, blood parameters, internal organs, carcass characteristics, and meat quality traits in Jumbo quail. A total of 396 Jumbo quail were randomly distributed to 6 experimental diets: CON = a standard grower diet without MOLM; VME0 = CON + 100 g/kg MOLM; and CON + MOLM pre-treated with 2.5 g/kg (VME25), 5 g/kg (VME50), 7.5 g/kg (VME75) and 10 g/kg (VME100). The results showed that pre-treating MOLM with fibrolytic enzymes influenced neutrophils, gizzard weights, and meat colour

indicators but not growth performance and carcass parameters in Jumbo quail. The hypothesis in this second experiment was rejected because results from this study showed that pre-treated MOLM with viscozyme® L multi-enzyme (VME) did not affect growth performance, blood parameters, internal organs, carcass characteristics and meat quality traits in Jumbo quail.

Pre-treatment of *Moringa oleifera* leaf meal with PEG and VME alone was an ineffective strategy to increase the utilisation in Jumbo quail. It was, therefore, hypothesised that poor protein digestibility and the incalcitrant fibre fraction, could be the reason MOLM utilisation is poor in quail. To test this hypothesis, a combination of PEG and VME was evaluated as a strategy to enhance the feed value of MOLM for Jumbo quail in chapter five. A total of 381 Jumbo quail were randomly distributed to 6 experimental diets: CON = standard grower diet containing 100 g/kg MOLM untreated; MPV0 = standard grower diet containing 100 g/kg MOLM pre-treated with 54 g/kg PEG; and a standard grower diet containing 100 g/kg MOLM pre-treated with 54 g/kg PEG (MPV0) and 12.5 g/kg (MPV125), 15 g/kg (MPV150), 17.5 g/kg (MPV175), and 20 g/kg (MPV200) fibrolytic enzymes.

The results showed that pre-treatment of dietary *M. oleifera* leaf meal with 54 g/kg PEG and up to 20 g/kg fibrolytic enzymes do not improve growth performance, blood, carcass, and meat quality parameters, but affects some visceral organ sizes in Jumbo quail, which is a true reflection that MOLM can be a potential alternative although precautions prior and during feeding should be put in place to ensure excellent quail performance, health status and desirable meat quality attributes. The hypothesis in this third experiment was rejected because results from this study showed that pre-treated MOLM with PEG and VME did not improve growth performance, blood parameters, internal organs, carcass characteristics, and meat quality traits.

As an effort to improve the utilisation of MOLM at 100 g/kg inclusion level (which was shown to reduce voluntary feed intake in Chapter 3), Chapters 4 and 5 revealed that using exogenous

enzymes alone and a combination of PEG and VME does not improve the utilisation of MOLM

by quail, suggesting that other strategies are perhaps required if moringa is to be included at higher levels.

6.2 Conclusions

Pre-treating MOLM with PEG up to 54 g/kg did not have a negative impact on growth performance, blood parameters, or meat quality characteristics in the initial trial. The maximum MOLM inclusion level (100 g/kg) significantly reduced feed intake, hence inclusion levels beyond 54 g/kg could not be appropriate. Furthermore, to increase the usage of MOLM in Jumbo quail and enable its inclusion at higher levels, subsequent research was carried out using fibrolytic enzymes. The results showed that the application of fibrolytic enzyme (viscozyme® L) did not increase the utilisation of a MOLM-based quail diet and that fibrolytic enzyme-treated diets promoted similar performance in terms of growth, health, and meat quality traits as the untreated *M. oleifera*-containing diet. The results indicated that the untreated and PEG- and enzyme-treated diets promoted similar performance in terms of growth response, health status, and meat quality traits. This suggests that simultaneous pre-treatment of dietary *M. oleifera* leaf meal with polyethylene glycol and exogenous fibrolytic enzymes does not improve its utilisation in Jumbo quail.

6.3 Recommendations and prospects

The inclusion of MOLM in diets of Jumbo quail promoted similar performance, according to the study's findings, in terms of growth performance, haematology, serum biochemistry, carcass characteristics, and meat quality traits. However, PEG inclusion levels higher than 54 g/kg apparently compromised growth performance. The following further research activities are thus needed:

- To assess the oxidative stability of lipid components in quail breast meat supplemented with *Moringa oleifera* leaf meal pre-treated with PEG and VME.
- To characterize tocopherol derivatives of vitamin E in *M. oleifera* leaves and determining the extent of their involvement in oxidative stability.
- To assess the cost-effectiveness of using exogenous feed additives such as PEG and enzymes in Jumbo quail diet.

6.4 References

Addisu, S., 2016. Effect of dietary tannin source feeds on ruminal fermentation and production of cattle; a review. Online J. Anim. Feed. Res. 6(2), 45–56.

Aletor, V.A., Hamid, I.I., Niess, E. & Pfeffer, E., 2000. Low-protein amino acid-supplemented diets in broiler chickens: effects on performance, carcass characteristics, whole-body composition and efficiencies of nutrient utilisation. J. Sci. Food Agric. 80, 547–554.

Bele, A.A., Jadhav, V.M. & Kadam, V.J., 2010. Potential of tannnins: a review. Asian J. Plant. Sci. 9(4), 209–214.

Bercovici, D. & Fuller, F., 1995. Industrial amino acids in non-ruminant animal nutrition. In: Wallace, R.J. & Chesson, A., (ed.). Biotechnology in animal feeds and animal feeding, VCH, Weinheim. pp. 93–113.

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

Chamorro, S., Viveros, A., Alvárez, I., Vega, I. & Brenes, A., 2012. Changes in polyphenol and polysaccharide content of grape seed extract and grape pomace after enzymatic treatment. Food Chem. 133, 308–314.

Ding, X.M., Li, D.D., Li, Z.R., Wang, J.P., Zeng, Q.F., Bai, S.P., Su, Z.W. & Zhang, K.Y., 2016. Effects of dietary crude protein levels and exogenous protease on performance, nutrient digestibility, trypsin activity and intestinal morphology in broilers. Livest. Sci. 193, 26–31.

Hajati, H., Rezaei, M. & Sayyahzadeh, H., 2009. The effects of enzyme supplementation on performance, carcass characteristics and some blood parameters of broilers fed on corn-soybean meal-wheat diets. Int. J. Poult. Sci. 8, 1199–1205.

Hana, A.H., Jalal, M.A. & Abu Ishmais, M.A., 2010. The influence of supplemental multi-enzyme feed additive on the performance, carcass characteristics and meat quality traits of broiler chickens. Int. J. Poult. Sci. 9, 126–133.

Hassan, H.M.A., El-Moniary, M.M., Hamouda, Y., El-Daly, E.F., Youssef, A.W. & Abd ElAzeem, N.A., 2016. Effect of different levels of *Moringa oleifera* leaves meal on productive performance, carcass characteristics and some blood parameters of broiler chicks reared under heat stress conditions. Asian J. Anim. Vet. Adv. 11(1), 60–66.

Kocher, A., Choct, M., Porter, M. & Broz, J., 2002. Effects of feed enzymes on nutritive value of soyabean meal fed to broilers. Br. Poult. Sci. 43, 54–63.

Mbikay, M., 2012. Therapeutic potential of *Moringa oleifera* leaves in chronic hyperglycaemia and dyslipidaemia: A review. Front. Pharmacol. 3(24), 1–12.

Mnisi, C.M., Matshogo, T.B., van Niekerk, R.F. & Mlambo, V., 2017. Growth performance, haematological and serum biochemical parameters and meat quality characteristics of male Japanese quails fed a *Lippia javanica*-based diet. S. Afr. J. Anim. Sci. 47(5), 661–671.

Muchenje, V., Dzama, K., Chimonyo, M., Strydom, P.E., Hugo, A. & Raats, J.G., 2009. Some biochemical aspects pertaining to beef eating quality and consumer health: A review. Food Chem. 112(2), 279–289.

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), 940–947.

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.

Onu, P.N. & Aniebo, A.O., 2011. Influence of *Moringa oleifera* leaf meal on the performance and blood chemistry of starter broilers. Nig. Int. J. Food Agri. Vet. Sci. 1(1), 38–44

Puspamitra, S., Mohanty, P.K. & Mallik, B.K., 2014. Haematological analyses of Japanese quail (*Coturnix coturnix japonica*) at different stages of growth. Int. Res. J. Biol. Sci. 3(11), 51 - 53.

Randall, M. & Bolla, G., 2008. Raising Japanese quail (2nd ed.). Prime Facts 602. pp. 1 - 5. http://www.dpi.nsw.gov.au/

Ravindran, V., 2013. Poultry feed availability and nutrition in developing countries: Main ingredients used in poultry feed formulations. FAO Poultry Development Review. Available from: http://www.fao.org/docrep/019/i3531e/i3531e.pdf [cited 8 June 2017].

Ufele, A.N. & Ebenebe, C.I., 2017. The Effect of *Moringa oleifera* on the growth performance, packed cell volume (PCV) and laying capacity of young growing quails. American. J. Zool. Res. 5(2), 33–37.

7 LIST OF APPENDICES

7.1 Ethics Certificate

Private Bag X1290, Potchefstroom
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 01/11/2019, 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: Optimizing voluntary intake and utilization of *Moringa oleifera* leaf meal in Japanese quails.

Study Leader/Supervisor (Principal Investigator)/Researcher: Dr K Mnisi, Prof V Mlambo

Student: Mulaudzi A

Ethics number: N W U - 0 1 8 8 4 - 1 9 - S 5
Institution Study Number Year Status
Status: S = Submission; R = Re-Submission; P = Provisional Authorisation; A = Authorisation

Application Type: Single Study
Commencement date: 2019/11/06 **Risk Category:** 2
Expiry date: 2020/10/05
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

7.2 Language Editing Certificate 1

NORTH-WEST UNIVERSITY
NOORDWES-UNIVERSITEIT
UNIBESITI YA BOKONE-BOPHIRIMA

Office: 0183892451

FACULTY OF EDUCATION **Cell: 0729116600**

Date: 24th October, 2022

CERTIFICATE OF EDITING

I, Muchativugwa Liberty Hove, confirm and certify that I have read and edited the entire thesis, **Optimizing voluntary intake and utilization of *Moringa oleifera* leaf meal in Jumbo quail by Mulaudzi Anzai,** orcid.org/0000-0001-7884-3217, submitted in fulfilment of the requirements for the degree of ***Doctor of Philosophy*** in ***Animal Science*** at the **North-West University**.

Mulaudzi Anzai was promoted by **Professor C.M. Mnisi** and co-promoted by **Professor V. Mlambo**.

I hold a PhD in English Language and Literature in English and am qualified to edit such a thesis for language, cohesion and coherence. The views expressed herein, however, remain those of the researcher/s.

Yours sincerely

Professor M.L. Hove (PhD, MA, PGDE, PGCE, BA Honours – English)

NORTH-WEST UNIVERSITY
NOORDWES-UNIVERSITEIT
UNIBESITI YA BOKONE-BOPHIRIMA

7.3 Language Editing Certificate 2

SCHOOL OF BIOLOGICAL SCIENCES

Tel: +27 18 389 2289 **Fax:** 018 389 2134

Cell: +27 72 626 8446 **E-mail:** Oziniel.Ruzvidzo@nwu.ac.za

Date: 13rd November 2022

To Whom It May Concern,

REF: Language Editing and Proof-reading of Dissertations/Theses

Dear Sir or Madam,

This serves to confirm that I have proof-read and edited the **Doctoral thesis** of **A Mulaudzi** (Student number: **24285242**; Orcid.org/**0000-0001-7884-3217**) entitled: **Optimizing voluntary intake and utilization of *Moringa oleifera* leaf meal in Jumbo quail**. The candidate then later corrected all the identified language and technical errors to the satisfaction of the supervisor. Thus, the document presented here is of sufficient and acceptable academic standards.

Editor

Prof. O Ruzvidzo

Supervisor

Prof. KM Mnisi

7.4 Publication in Sustainability

sustainability

MDPI

Article

Enhancing the Utility of Dietary *Moringa oleifera* Leaf Meal for Sustainable Jumbo quail (*Coturnix* sp.) Production

Anzai Mulaudzi [1,2], Caven Mguvane Mnisi [1,2,*] and Victor Mlambo [3]

[1] Department of Animal Science, Faculty of Natural and Agricultural Science, North-West University, Mafikeng 2735, South Africa; anzaimatshavhange@gmail.com
[2] Food Security and Safety Focus Area, Faculty of Natural and Agricultural Science, North-West University, Mafikeng 2735, South Africa
[3] School of Agricultural Sciences, Faculty of Agriculture and Natural Sciences, University of Mpumalanga, Nelspruit 1200, South Africa; victor.mlambo@ump.ac.za
* Correspondence: 23257539@nwu.ac.za; Tel.: +27-18-389-2738

Abstract: The effect of pre-treating *Moringa oleifera* leaf powder (MOLP) with different levels of polyethylene glycol (PEG) on the growth performance, serum biochemistry, hematology, and meat quality parameters of Jumbo quail was evaluated. Two-week-old quail chicks (n = 432; 239.6 ± 6.48 g live-weight) were randomly allocated to six diets formulated by incorporating (10% w/w) untreated MOLP (PEG0) or MOLP pre-treated with PEG at 2.5% (PEG25), 5% (PEG50), 7.5% (PEG75), and 10% (PEG100) (w/w) into a standard grower diet (CON). Overall feed intake linearly increased with PEG levels. At week 4, significant quadratic trends were recorded for weight gain and feed conversion efficiency (FCE) but, at week 5, FCE linearly declined as PEG levels increased. Hemoglobin, phosphorus, and albumin showed quadratic trends, while calcium and chroma (1 h post-mortem) linearly declined in response to PEG levels. Diet PEG50 promoted a higher shear force value (2.41) than diets PEG0 and PEG25. The PEG50 diet promoted a similar ($p > 0.05$) shear force as diet CON. Based on the quadratic response for weight gain, the optimal PEG pre-treatment level was calculated to be 5.9%. It was concluded that MOLP condensed tannins negatively affect growth performance and should be ameliorated to enhance the utility of this nutraceutical source for Jumbo quail.

Keywords: avian birds; blood indices; feed additives; growth traits; meat quality; phytogenics

Citation: Mulaudzi, A.; Mnisi, C.M.; Mlambo, V. Enhancing the Utility of Dietary *Moringa oleifera* Leaf Meal for Sustainable Jumbo quail (*Coturnix* sp.) Production. *Sustainability* 2022, *14*, 5067. https://doi.org/10.3390/su14095067

Academic Editor: George K. Symeon

Received: 18 March 2022
Accepted: 12 April 2022
Published: 22 April 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

1. Introduction

The use of phytochemicals could be a long-term strategy for achieving sustainable Jumbo quail (*Coturnix* sp.) intensification for enhanced food and nutrition security. The Jumbo quail is a subspecies of the Japanese quail (*Coturnix coturnix japonica*) that was recently introduced in South Africa for meat production [1]. It is a fast-growing, large-framed (weighing up to 300 g) brownish bird [1,2]. Moreover, quail farming has the capacity to revolutionize the South African poultry industry, which relies heavily on imports to meet local consumer demand. Quail production also provides an opportunity to diversify the poultry industry to increase the supply of animal protein [3]. Currently, quail birds are steadily evolving around the world as an excellent source of protein [4]. Their evolution could be attributed to their low maintenance, early sexual maturity, high prolificacy, short generation intervals, fast growth rates, and resistance to numerous avian diseases [5,6]. However, sustainable intensification of quail birds could be restricted by high feed costs, disease outbreaks, and poor performance. Indeed, the cost of poultry feeds has remained high, especially in the tropics, due to rising prices of soybeans and maize grain, which are conventional nutrient sources in poultry diets [7]. The competition between humans and livestock for these conventional nutrient sources [8] contributes to rising demand that fuels price increases on the world market. It is, therefore, imperative that non-conventional feedstuffs that have nutraceutical properties be identified for use in quail diets to allow

7.5 Publication in Poultry

poultry

MDPI

Artide

Effect of Pre-Treating Dietary *Moringa oleifera* Leaf Powder with Fibrolytic Enzymes on Physiological and Meat Quality Parameters in Jumbo Quail

Anzai Mulaudzi [1,2], Caven Mguvane Mnisi [1,2,*] and Victor Mlambo [3]

[1] Department of Animal Science, Faculty of Natural and Agricultural Science, North-West University, Mafikeng 2735, South Africa; anzainatshavhangp@gmail.com
[2] Food Security and Safety Niche Area, Faculty of Natural and Agricultural Science, North-West University, Mafikeng 2735, South Africa
[3] School of Agricultural Sciences, Faculty of Agriculture and Natural Sciences, University of Mpumalanga, Nelspruit 1200, South Africa; victor.mlambo@ump.ac.za
* Correspondence: 23257539@nwu.ac.za; Tel.: +27-18-389-2738

Abstract: High fiber levels (165 g neutral detergent fibre (NDF)/kg DM) in *Moringa oleifera* leaf powder (MOLP) could limit its utilization as a nutraceutical source in Jumbo quail diets. Pre-treating MOLP with exogenous fibrolytic multi-enzymes could reduce the nutrient-encapsulating effect of non-starch polysaccharides and subsequently increase nutrient and bioactive compound utilization. Thus, this study investigated the effect of pre-treating dietary MOLP with an exogenous fibrolytic enzyme mixture on some physiological parameters and meat quality characteristics in Jumbo quail. A total of 396 Jumbo quail were randomly distributed to 6 experimental diets, with 6 replicate pens each and 11 birds per replicate. The experimental diets were: CON = a standard grower diet (156.5 g NDF /kg) without MOLP; ENZ0 = CON + 10% MOLP; and CON + MOLP pre-treated with 0.25% (ENZ25), 0.50% (ENZ50), 0.75% (ENZ75) and 1% (ENZ100) fibrolytic enzymes. There were no significant linear or quadratic effects on growth performance parameters and carcass characteristics in response to incremental levels of fibrolytic enzymes. However, neutrophils linearly increased, while breast meat lightness and 24 h hue angle linearly declined with enzyme levels. Quadratic effects were observed on gizzard weights and 1 h hue angle in response to enzyme levels. All the hemato-biochemical values fell within the normal ranges for healthy quail. It was concluded that the maximum fibrolytic multi-enzyme application rate of 1% may not have been adequate to enhance feed utilization and positively affect weight gain in Jumbo quail, thus higher levels may need to be investigated further.

Keywords: carcass characteristics; *Coturnix coturnix*; feed additives; growth performance; phytogenic plants

Citation: Mulaudzi, A.; Mnisi, C.M.; Mlambo, V. Effect of Pre-Treating Dietary *Moringa oleifera* Leaf Powder with Fibrolytic Enzymes on Physiological and Meat Quality Parameters in Jumbo Quail. *Poultry* 2022, *1*, 54–65. https://doi.org/10.3390/poultry1020006

Academic Editor: Shiping Bai

Received: 14 March 2022
Accepted: 30 March 2022
Published: 5 April 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

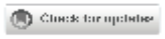
1. Introduction

In the South African poultry sector, Jumbo quail (*Coturnix coturnix*) farming is a new venture [1] that can substantially contribute to food and nutrition security, as well as economic growth, if sustainably developed. Jumbo quail is the largest and fastest-growing meat-type breed of quail, which has been recently developed from the traditional Japanese quail line. The birds are noted for early attainment of sexual maturity (six weeks of age), high reproductive rates (±300 eggs per year), disease resistance and low feed requirements (35–45 g/day/bird) [2]. However, the growth of the poultry sector has generated additional pressure on feed ingredients and increased the competition between humans and birds for conventional nutrient sources, such as maize and soybean. Therefore, emerging quail farmers struggle to profitably meet the birds' nutrient requirements for maximum production [2]. *Moringa oleifera* leaves have been found to contain substantial amounts

7.6 Publication in Frontiers in Animal Science

frontiers | Frontiers in Animal Science

TYPE Original Research
PUBLISHED 02 September 2022
DOI 10.3389/fanim.2022.960233



OPEN ACCESS

EDITED BY
Aristide Maggiolino,
University of Bari Aldo Moro, Italy

REVIEWED BY
Felicitas Mukumbo,
Rwanda Institute for Conservation Agriculture (RICA), Rwanda
Ifeanyi Ogbuewu,
Federal University of Technology Owerri, Nigeria

*CORRESPONDENCE
Caven Mguvane Mnisi
23257539@nwu.ac.za

SPECIALTY SECTION
This article was submitted to Product Quality, a section of the journal Frontiers in Animal Science

RECEIVED 02 June 2022
ACCEPTED 15 August 2022
PUBLISHED 02 September 2022

CITATION
Mulaudzi A, Mnisi CM and Mlambo V (2022) Simultaneous pre-treatment of dietary *Moringa oleifera* leaf meal with polyethylene glycol and fibrolytic enzymes: Effect on growth performance, physiological indices, and meat quality parameters in jumbo quail. *Front. Anim. Sci.* 3:960233. doi: 10.3389/fanim.2022.960233

Simultaneous pre-treatment of dietary *Moringa oleifera* leaf meal with polyethylene glycol and fibrolytic enzymes: Effect on growth performance, physiological indices, and meat quality parameters in jumbo quail

Anzai Mulaudzi[1,2], Caven Mguvane Mnisi[1,2]* and Victor Mlambo[3]

[1]Department of Animal Science, Faculty of Natural and Agricultural Sciences, North-West University, Mafikeng, South Africa, [2]Food Security and Safety Focus Area, Faculty of Natural and Agricultural Sciences, North-West University, Mafikeng, South Africa, [3]School of Agricultural Sciences, Faculty of Agriculture and Natural Sciences, University of Mpumalanga, Nelspruit, South Africa

The utility of *Moringa oleifera* leaf meal (MOLM) as a source of biologically active substances and nutrients for Jumbo quail is limited by high concentrations of condensed tannins and fiber. Simultaneous application of polyethylene glycol (PEG), a tannin-binding compound, and exogenous fibrolytic multi-enzymes could ameliorate antinutritional effects of condensed tannins and fiber thus improving MOLM utilization in quail diets. This study investigated the effect of pre-treating dietary MOLM with PEG and fibrolytic enzymes on live performance, blood parameters, visceral organs, and carcass and meat quality characteristics in Jumbo quail. A total of 381, two-week-old quail chicks (57.5 ± 3.95 g live-weight) were randomly distributed to six dietary treatments replicated six times. The treatments were: T1 = a standard grower diet containing untreated MOLM (10%); T2 = a standard grower diet containing MOLM (10%) pre-treated with 5.4% PEG; and a standard grower diet containing MOLM (10%) pre-treated with 5.4% PEG and 1.25% (T3), 1.50% (T4), 1.75% (T5) and 2.0% (T6) fibrolytic multi-enzymes. Graded levels of enzymes did not induce linear or quadratic effects for overall feed intake, feed conversion efficiency, hematological, carcass, and meat quality parameters in response to increasing fibrolytic enzyme levels. However, weight gain in week 3 quadratically responded [R^2 = 0.117, P = 0.043]. Three-week old birds reared on T3 had lower ($p < 0.05$) weight gains (40.9 g/bird) than those reared on the other treatment groups. Birds reared on T6 diet had longer caecum (14.1 cm) than those reared on T2, T3, T4 and T5 whose caeca lengths did not differ ($P > 0.05$). Birds reared on diet T5 had shorter small intestines (59.5 cm) than those reared on T1 and T2 diets. It can be concluded that simultaneous pre-treatment