Effect of enzymes and polyethylene glycol addition to Kalahari melon(*Citrullus lanatus*)-based diets on egg production performance in layer chicken

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DECLARATION

I, Mpho Darky Mauwane, declare that:

1. This thesis represents my own unique contribution.
2. With the approval of my supervisors, I submit it in pursuit of a Master of Science in Animal Science from North-West University.
3. This work has not been presented at any institution other than North-West University.
4. I acknowledge and appropriately credit all sources used in this dissertation.

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GENERAL ABSTRACT

The research is aimed to improve the nutritional quality of Kalahari melon meal (KM) in layer chicken diets by incorporating external digestive enzymes and polyethylene glycol. Chapter one outlines the study's context, issue at hand, rationale, and its intended goals and purposes. Chapter two presents a comprehensive view of layer chicken farming alongside the nutritional composition and significance of incorporating Kalahari melon in diets for chickens. It also provides a summary of the voids and contradictions discovered in the research and provides a comprehensive overview of poultry diets that contain exogenous feed enzymes. Chapters three, four and five studied the effect of polyethylene glycol and protease enzyme on FI, body weight changes, haematology and serum biochemistry, egg production parameters (internal and external), fatty acid profile, internal organs, and bone development. Five dietary treatments were formulated by including 10% KM, polyethylene glycol and protease enzyme in poultry diets. The following diet plans were developed: Control (commercial layer feed), 10%KM (10 %KM diet), PRO (10% KM diet + protease enzyme), PEG (10% KM diet + polyethylene glycol), KMEP (10% KM diet + protease enzyme + polyethylene glycol). A total of 150-leghorn layer hens at 18 weeks old were purchased for use in the research. The hens were distributed randomly among 5 different dietary plans using a completely randomized arrangement. Each of these plans consisted of 10 separate cages, with each cage housing 3 birds, serving as the experimental unit. Diets significantly affected ($P< 0.05$) average daily gain, average weekly feed intake, average daily feed intake and feed to egg ratio. The 10% Km diet had the high values for all parameters except for feed to egg ratio. The peg diet had the highest value for feed to egg ratio (31.59±2.16). The observation made on serum biochemical and haematological had no linear or quadratic responses ($P >0.05$). In comparison to other diets, the control diet exhibited elevated levels of lymphocytes, eosinophils, basophils, and red

blood cells. The KM containing diets had high values($P>0.05$) for all parameters in serum biochemistry except for blood urea nitrogen and ALKP.

With regards to egg production indices (chapter 4), diet had no influence ($P>0.05$) on all egg production indices apart from the NFEI ($P<0.05$), with the highest value in PRO diet followed by the KMEP diet. With all other parameters, the KM containing diets, whether with feed additives or not appeared to have better performances compared to the control diet while the PEG diet seemed to supress the performance of hens. Of all the diets containing KM oil cake, while the values of hens fed the PEG diet had similar performance as the control. The birds on the PRO diet experienced fewer cracked eggs in comparison to those on the KMEP and control diets. All egg external quality parameters, with the exception of the eggshell ratio, eggshell obtuse, breaking strength, and egg breadth, were unaffected by diet. The values for egg width and shell breaking strength (Shell BS) were highest ($P < 0.05$) in the commercial diet (Con), with the chickens fed the 10% KM diet following closely behind. The yolk index, haugh unit, albumen weight, and albumen ratio were the only internal parameters that were not influenced by diet. The hens fed the 10%KM diet had the highest ($P < 0.05$) yolk height (YH) and yolk weight (YWT).

KEYWORDS
Unconventional protein sources, Egg parameters, Protein inhibitors, Oil Cake, Bone development.

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DEDICATION

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ABBREVIATIONS

ANF-Anti-Nutritional Components-

ADFI-Average Daily Feed Intake-

ADG-Average Daily Gain

BW-Body weight

Ca-Calcium

CM-Canola Meal

CO_2-Carbon dioxide

CF-Crude Fat

CP-Crude Protein

CSM-Cotton Seed Meal

FCR-Feed Conversion Ratio

FAO-Food and Agricultural Organization

FM-Fish Meal

GC-Groundnut Cake

HEO-High Essential Oil

KM-Kalahari melon

KMOC-Kalahari Melon Oil Cake

MNC-Macadamia Cut Cake

PFA-Phytogenic feed additives

RM-Rapeseed Meal

SBM-Soyabean Meal

SAPA-South African Poultry Association

SFM-Sunflower Meal

CHAPTER 1

1. INTRODUCTION

1.1. Background

The term poultry collectively refers to tamed winged animals normal reared for their meat, meat products and eggs. Commonly reared poultry species include the commercial broilers and layers, indigenous chicken ecotypes, geese, guinea fowl, ducks and turkeys (Nkukwana, 2018). The majority of all poultry species reared are the chickens (63%) followed by ducks (11%), geese (9%), and turkeys (5%). However, most of the genetic variation in poultry worldwide comes from intrinsic or heritage breeds. South Africa is the biggest commercial poultry-producing nation in the African continent followed by Egypt, Morocco, Nigeria and Algeria respectively (SAPA, 2016). Poultry products such as eggs, are the most widely available, reasonably priced, and locally produced animal protein in South Africa and have the greatest potential in contribution to the country's zero-hunger goals. Nevertheless, egg production efficiency is influenced by a variety of factors including nutrition, genetic factors animal welfare (Gerber *et al*., 2015). Eggs production efficiencies can be achieved at the lowest cost per unit of output, primarily due to poultry have an advantage over other domestic animal species in that they are efficient feed converters (Gerber *et al*., 2015). In the layer production, conventional feedstuffs are commonly used however, these are increasingly becoming scarce and unaffordable due to the increasing costs of production and food-feed competition between humans and animals (Abouelezz, 2017). The use of cereals and oilseeds to produce biofuel has also exacerbated the increasing cost of traditional feed resources, such as maize and soybean meal, for layer diets. Therefore, finding alternative feed resources is crucial. This includes among others, Kalahari Melon (*Citrallus lanatus*) oil seed meal.

The Kalahari melon (KM) belongs to the family of *Cucurbitaceae* and is the ancestor of a bitter-fruit form of *C. vulgaris Schrader* (Oyewo *et al.*, 2012). The Kalahari melon (KM) originated from Africa (Iheanacho *et al.*, 2018) and is a vital source of water within the Kalahari during the dry months of the year when no surface water is accessible (Nyam, 2009a). This resource has a bitter taste which is harmful but can be used medicinally for treating worms, renal stones, dropsy, liquor harming, diabetes and as a laxative and emetic (Nyam *et al.*, 2009a). In the West Africa, there is a sizeable industry within the generation of seeds from KM, with a production of 250– 300,000 tons of seed per anum (Nyam *et al.*, 2009b). Ordinarily, the method of oil extraction from Kalahari melon seeds in Africa is done through the cold pressing of the seeds with a temperature of not < 60° C and there are no solvents or chemicals used (Nyam *et al.*, 2009b). Kalahari melon seed oil is of a particular interest in the cosmetic industry where it added to a number of conspicuous European beauty care products for moisturizing, regenerating and rebuilding skin-care (Nyam *et al.*, 2011). Like soya bean meal (SBM), the remaining cake after oil extraction can be used as an ingredient in animal diets.

1.2. Problem statement

Conventional feedstuff is in demand by both animals and humans resulting in high price for feeds and threatening food security in developing countries. One of the prospective alternative unconventional protein sources for layer diets is Kalahari melon oil cake (KMOC). It contains up to 35% crude protein which is comparable to the other protein sources apart from SBM used in layer diets such as Canola meal (Komane *et al.*, 2017). Nevertheless, KMOC contains high fibre (17.4%) and higher amounts of some secondary plant metabolites compared to SBM. It has been reported that consuming large amounts of fibre might impair digestion, which lowers animal performance (Bhuiyan *et al.*, 2021). One way of enhancing digestibility of high fibre sources is the use of enzymes (Aya *et al.*, 2013). Depending on the chemical composition of

the protein source, different blends of exogenous enzymes can be used to break down fibre enhancing the release and efficient digestion of trapped protein components with assimilation (Aya *et al.*, 2013**).** On the other hand, polyethylene glycol (PEG) may have the effect of binding on to those secondary plant metabolites including the effects of reducing palatability, digestibility and assimilation of amino acids, and increasing the utilisation of the KMOC. Currently no attempts have been made to assess the possibility of inclusion of KMOC in layer diets as a protein source. Moreover, no scientific information is available of the potential of enzymes and polyethylene glycol in improving the utilisation of novel protein sources such as KMOC. Therefore, there is the need to investigate the effect enzymes and polyethylene glycol inclusion in layer diets on egg production performance in layers.

1.3. Justification

The availability of feedstuff for poultry nutrition is becoming more competitive recently due to the use of conventional feedstuffs as main ingredients in poultry feeds because of their high nutrient content (Alshelmani *et al.*, 2021). Onunkwo (2019) reported that the rising cost of feed resources in animal production has been regarded as a genuine impediment to meeting the demand for dietary protein requirement particularly in developing countries. Therefore, there is a need to reduce competition between humans and animals for conventional feedstuff. The high cost of feed can be decreased by composing feed in a way that all required dietary nutrients should come from cheap alternative energy and protein sources. Therefore, using alternative locally available feed ingredients in poultry diet formulation is valid due to the reduction of feed costs and the enhancement of the farm's profitability and layer performance to supply animal protein (meat and eggs) to the increasing global human population. Alternative feedstuff contains a comparable amount of protein compared to conventional feedstuff. However, there is a need to improve protein digestibility by the use of enzymes and polyethylene glycol, which

will overcome feed intake limitation (Geraert *et al.*, 2003). Exogenous enzymes are primarily incorporated into diets to enhance the availability of supplements from feed constituents (Ojha *et al.*, 2019). The animal feed industry makes use of enzymes that degrade starch, fibre, proteins and phytates. Enzymes enhance the efficiency of meat and egg production by improving nutrient absorption and reducing animal waste/excreta.

1.4. Objectives

To determine the effect dietary inclusion of enzymes and polyethylene glycol on egg production performance in layers fed with Kalahari melon-based diets.

Specific objectives are:

- To evaluate the effects of dietary inclusion of enzymes and polyethylene glycol on feed intake, feed conversion efficiency, body weight changes, health and nutritional status and serum cholesterol levels in layers fed Kalahari melon meal-based diets
- To determine the effect of dietary inclusion of enzymes and polyethylene glycol on egg production; and egg quality parameters and egg sensory quality in layers fed Kalahari melon meal-based diets.
- To evaluate the effects of dietary inclusion of enzymes and polyethylene glycol on immune and bone development and welfare in layer hens fed Kalahari melon meal-based diets.

1.5. Hypothesis

H_0: The inclusion of enzymes and polyethylene has no effect on feed intake, feed conversion efficiency, body weight changes, health and nutritional status and serum cholesterol levels in layers fed Kalahari melon meal-based diets

H_0: The inclusion of enzymes and polyethylene has no effect on egg production; and egg quality parameters, fatty acid profiles and egg sensory quality in layers fed Kalahari melon meal-based diets.

H_0: The inclusion of enzymes and polyethylene has no effect on immune and bone development and welfare in layer hens fed Kalahari melon meal-based diets.

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CHAPTER 2

2. LITERATURE REVIEW

2.1. Introduction

Given the anticipated increase in the human populace over the coming years, there is a need to increase the generation of animal products for human consumption (Zadeh *et al.*, 2020). The avian species contributes greatly to human subsistence by providing nourishment (eggs and meat) that is of high quality in terms of macro and micronutrients. Moreover, one of the least expensive and least time-consuming ways to obtain animal protein is through the production of poultry (FAO, 2011). The egg production around the world has been expanding significantly over the decades. According to FAO, in 2013 the worldwide egg production reached almost 68 million tons (FAO, 2016). Eggs are consumed around the world and are a good source of vitamins, minerals, and fatty acids (Keegan *et al.*, 2019). Eggs are a nutritious, affordable, and easily available food.

2.2. Egg production in South Africa

The poultry industry is the most significant segment of the South African agricultural animal production subsector, accounting for over 16% of the gross domestic product. Nearly 100,000 individuals are employed in its value chain and related industries, both directly and indirectly (Lombard & Bahta, 2018). The South African poultry industry produces approximately 75% of its animals for flesh production, while the remaining 25% is used for egg production (SAPA, 2019). According to SAPA (2020), an average of 493 600 trays of eggs were produced per week; with a month-to-month increment of 4 700 trays (+1.0%). Eggs are produced throughout the country with Gauteng being the largest producer contributing 26.1% to the market share followed by Western Cape (14.5%), Kwa-Zulu Natal (13.7%), Free State (13.5%) and

Northwest (11.3%) (SAPA, 2016). Furthermore, Northern and Eastern Cape have the most reduced market share of 0.2% and 2.7%.



Figure 2.1: Egg production (Source: SAPA, 2018)

From Figure 2.1, the egg production industry posted a positive growth since 2005. The eggs delivered to the market in 2017 was 456 550 tons in comparison to the 375 210 tons delivered in 2005, showing an increment of 21.7% over the period. For the past 10 years (2008-2017), the egg industry was producing 450 470 tons per annum. Over the past 10 years, there have been notable breakthroughs in the production of eggs. These improvements resulted from improvements in crop yields as well as breeding and genetics of chickens, nutrition, illness prevention and management, housing technology, environmental control, and the efficient use of feed and other natural resources (Xin & Liu, 2017). Egg laying is the most critical economically important trait in layers because it straightforwardly impacts on the poultry industry`s gross domestic product (GDP) (Liu *et al.*, 2019). Enhancement of egg production is a critical challenge in the poultry industry to meet the increasing worldwide demand (SAPA, 2019).

2.3. Nutrient requirement of layers

The feed and nutrient supply are of critical importance in egg laying hens and contribute greatly to the cost of egg production. Layer diets are generally formulated to optimise egg production output at a lower cost. Inadequate nutrition in layers may result to reduction in egg production and quality. Without a doubt, the dietary protein and energy interactions and the proportion of different amino acids and mineral is critical in optimising egg productivity (Wang *et al.*, 2017). Protein and energy are the primary nutrients in the formulation of layer diets but are rather becoming expensive (Fouad *et al.*, 2018). The energy required by layers is mainly for the maintenance of metabolic body mass, and for production of eggs (Bryden *et al.*, 2021). Proteins, particularly amino acids play an essential role in ensuring normal growth, immune functions, optimum egg production, bird welfare, as well as reducing environmental risks and pollution (Alagawany, 2016). In addition, Calcium (Ca) and phosphorus (P) are key minerals in layer diets as they play numerous fundamental roles in metabolism, particularly in bone development and eggshell formation (Bryden *et al.*, 2021).

2.3.1 Macro minerals

In the diet of laying hens, calcium, phosphorus, and vitamin D3 are the three microminerals of greatest interest. Calcium (Ca) is best known for its function in the mineralization of bones and shells. In fact, calcium is one of the two minerals that are most abundantly needed by poultry, with phosphorus coming as second. There has been extensive research done on calcium levels, calcium particle sizes, and calcium sources. For most bird species, calcium carbonate is thought to make up about 98% of the eggshell even if the differences across species have not been studied (McClelland *et al*., 2021). Therefore, one of the most crucial nutritional variables in highly prolific egg-laying chickens is an adequate supply of useable calcium (Swiatkiewicz *et al.*, 2015). Kakhki *et al* (2019) stated that the daily calcium requirement recommended by the

NRC decreased from 3.85g to 3.25 of Ca/b/d. When calcium or vitamin D inclusion in diets supplied to laying hens is insufficient, calcium is mobilised from bones and deposited into the eggshell (Adhikari et al., 2020) reducing the health and welfare of the birds. Laying hens regulate their calcium balance by either absorbing food calcium in the intestine or by resorbing bone. On the other side, excessive dietary calcium can induce soft faeces, reduced feed intake, and an increase in chalky deposits in eggshells (Kakhki *et al*.,2019).

2.3.2. Micronutrients

Trace minerals are necessary for several biochemical processes and act as catalytic agents throughout metabolic pathways, making them critical for appropriate bone morphology, feather structure, enzyme structure and function (Likittrakulwong *et al*., 2021). Furthermore, they have also been shown to be important elements in the creation of bone and eggshell due to their influence on the synthesis of enzymes necessary during the mineralization process. In order to influence the quality of the eggshell, trace elements may interact with calcium crystals directly during eggshell production, act as activators or components of enzymes involved in eggshell synthesis or both (Qiu *et al*., 2022). For instance, the metalloenzymes responsible for the creation of carbonate and mucopolysaccharides require zinc and manganese as cofactors (Foley., 2021). Zinc (Zn) is a cofactor for various enzymes that include carbonic anhydrase, which plays an important role in eggshell development (Hajjarmanesh *et al*., 2023). Huang *et al* (2019) stated that Zn is a cofactor in over 200 enzymes involved in a variety of physiological processes such as immunology, antioxidant capacity, and epigenetic processes. However, to maintain the best possible level of productivity, a breeder hen diet of 50 mg of Zn per kg is recommended by the National Research Council (1994). Ultimately, lack of Zn in the breeder`s diet reduces the ability of eggs to hatch, raises embryonic mortality, and hinders sperm from penetrating eggs (Londero *et al*., 2020).

2.4. Protein requirements for layer hens

In poultry diets, soybean meal (SBM) is the most preferred protein source after energy source due to its high crude protein content (~37 to 44%) and desirable essential amino acid profiles, making it broadly utilized in animal diets (Wilkinson & Young, 2020). Worldwide soybean production has increased within the past 20 years, rising to 347 million tons in 2017/18, of which the majority (82%) was produced within USA, Brazil and Argentina (Selle *et al.*, 2020). However, the increase in SB production has generally been associated with deforestation, soil disintegration, eutrophication, broad utilization of pesticides, misfortune of biodiversity and a huge carbon dioxide (CO_2) footprint (Khan,2018). There is therefore a need to decrease the dependency on SBM by exploring alternative protein sources for layer production (Iji *et al.*, 2017; Qin *et al.*, 2017). The cost of SBM is also very high and tend to vary with changes in climatic conditions and social circumstances within the nations where it is produced (Selaledi *et al.*, 2020). Furthermore, although developing nations such as South Africa have made great strides in increasing soybean production, it is currently not self-sufficient and hence depends on imports, which increases the cost of SBM in the South African feed markets. In addition, the Food and Agriculture Organization (FAO) estimated that the demand for animal's protein is expected to increase over the coming decades (IFIF, 2021). This suggest that there is an urgent need to explore alternative and cost-effective protein sources for layer production (Teguia & Beynen, 2005).

2.5. Alternative protein sources

It has been predicted that conventional sources of protein for poultry production will become rare and more costly within the immediate future (Iji *et al*, 2017). Furthermore, besides the rising costs of feedstuffs, the conventional protein sources in poultry diets such as oil seed meals (SBM and canola meal) and fishmeal are becoming scarce and inadequate to meet the

expanding poultry industry as well as the human needs. Due to the rising costs and inadequate supply, it is critical to explore alternative protein sources that can completely or partially replace the conventional protein sources in poultry diets. These include the oilseed meals (e.g., rapeseed meal, sunflower meal, Kalahari melon meal) or pulses (peas and beans) (Winksons, 2020).

2.5.1. Sunflower meal

Sunflower meal (SFM) (*Helianthus annuus*) is a by-product obtained from the extraction of the oil from the sunflower seeds, which is used for human consumption or for the production of biodiesel. World production of sunflower seed is large and ranked fourth in oil seed production (Florou-Paneri *et al*, 2014). Generally, sunflower meal contains 35-40% of hulls, crude protein (30-34%), cellulose (20-25%) and lignin (8-10%) (Ditta & King, 2017). The high amount of fibre (26.7%) and its deficiency in lysine are the main challenges in incorporating SFM in poultry diets. According to Alagawany (2017), SFM can be used as alternative to SBM in diets of poultry due to its energy content being comparable to that of other oilseed meals and improves with higher residual oil levels and lower fibre content (Laudadio *et al*.,2014). Additionally, SFM has fewer antinutritional compounds than soybean or rapeseed meal and offers greater resistance to contaminants. Another challenge in using SFM in animal feeds when compared with SBM is the existence of certain anti-nutritional elements (lignin) and harmful compounds(gossypol) which may influence performance in layers. Therefore, SFM may be included in poultry diets at less than 10% as a replacement to SBM without any extreme impacts on growth, feed intake and egg production (Attanayaka *et al*., 2016).

2.5.2. Rapeseed meal

Rapeseed meal (RSM) (*Brassica napus*) is a by-product obtained from the extraction of oil from the rapeseed. It contains significant amounts of protein (30-40%) with a proper equilibrium of amino acid content and is currently being utilized in chicken diets (Ashayerizadeh *et al*, 2018). Nevertheless, the protein value of rapeseed meal is less than that of soybean feast, but the amino acids content, especially sulphur amino acids is comparative and indeed more superior than that in SBM (Di lena *et al*., 2021). The Ca, Phosphorous, Selenium (0.16-0.29 mg/kg) and Manganese (78.1-82.2 mg/kg) in rapeseed are exceptionally high, however only up to 65% of phosphorus is abundant in P-phytate and thus decreasing its bioavailability. Other important characteristics of RSM includes high levels of nicotinic acid (160 mg/kg), choline (6400-6700 mg/kg), low carotenoid, and low vitamin D (Feng & Zuo, 2007). However, due to the presence of anti-nutritional factors such as glucosinolates, utilization of RSM is restricted in chicken diets (Ashayerizadeh *et al*, 2018). The reduction of the effects of anti-nutritional components and the enhancement of nutrient ingestion in animals can be achieved through the use of microbial additives and enzymes.

2.5.3 Kalahari Melon oil meal

Kalahari melon oil meal is a by-product obtained from the seeds of the Kalahari melon (*Citrullus lanatus*), after the oil has been extracted. The Kalahari melon is native to the Kalahari Desert region and is related to the common watermelon, though it is more drought-resistant and grows in wild, arid environments. Kalahari melon meal represents a viable alternative protein source to reducing feed competition while maintaining egg performance in layers. The oil extracted from the seeds of the Kalahari melon is of a particular interest in most of the European cosmetic industry where it is used to make products that aid with moisturising, regenerating and restructuring skin (Nyam *et al*., 2009). Thus, resulting cake obtained from the

oil extraction has currently been identified as waste product that could constitute an environmental hazard (Iheanacho *et al*., 2018). However, the cake has some important characteristics that ultimately makes it useful as a feed ingredient for livestock. The Kalahari melon (*Citrullus lanatus*) is known as the Tsamma melon or wild watermelon and is the organic predecessor of the common watermelon presently found around the world. Unlike the common watermelon, whose tissue is sweet and ruddy, the Kalahari melon's inner flesh is pale yellow or green and tastes bitter. Kalahari melon fruits are little and circular within the wild, but bigger and oval when developed. The Kalahari melon is profoundly adjusted to surviving dry spell and the unforgiving environmental conditions. The melon seeds are rich in oil and the remaining cake has considerable amounts of protein (extending from 18-28%) and some important minerals (Iheanacho *et al.*, 2018). Citrullus *lanatus* seeds are reported to contain high levels of protein (up to 35%), essential oils (50%), and dietary fiber (5%) by Komane et al. (2017. The *C. lanatus* seed oil also contains some important unsaturated (80%) fatty acids. The major fatty acids reported were 43.3% linoleic acid, 37.36% oleic acid, 16.74% palmitic acid and 0.01% stearic acid. Despite its potential as a protein source, no studies have been carried out to assess its value as feed ingredient in layer diets.

2.6. Methods used to enhance the usage of KM cake

2.6.1. Use of enzymes

The use of enzymes in commercial layer diets has become more common in recent years as enzymes are targeted at carbohydrates (carbohydrases), proteins (proteinases) or lipids (lipases) (Roberts & Choct, 2006). Enzymes are natural catalysts that are composed of amino acids, proteins, minerals, and vitamins (Alagawany *et al*, 2018). The advantages of utilizing commercial enzymes in poultry feeds include enhanced production performance, feed usage, reduced environmental pollution due to minimized nutrient of manure. Previous studies have

shown the benefits of supplementing enzymes such as non-starch polysaccharide (NSP)-hydrolysing enzymes on egg production, feed intake, egg weight and egg mass when included in layers diets (Abd El-Hack *et al*, 2018). The inclusion of carbohydrase enzymes such as xylanase in high fibre diets such as KM based diets may reduce the negative impacts of NSPs and some antinutritional factors ultimately improving the digestion and utilisation of such diets and hence the performance of broilers and laying hens (Cimrin *et al*, 2018). In addition to improving the performance of laying, the inclusion of enzymes to high fibre diets positively impacts egg quality, which is generally influenced by minerals accessibility (primarily calcium), (Cimrin *et al.*, 2018).

2.6.2 Polyethylene glycol in layer diets

Tannins encompass a diverse array of plant secondary compounds found widely across various plant species (Nawab *et al*, 2020). According to Blackmon *et al* (2016), tannins are a group of plant polyphenols present in all vascular plants including leguminous plants, forages, flowers, fruits, grasses, and leguminous tree. Tannins are basically classified into three major groups: hydrolysable tannins (HT), condensed tannins (CT) and phlorotannins (PT) Huang (2018). According to Medugu *et al* (2012), the utilization of feeds by monogastric animals especially poultry are hindered by anti-nutritive factors knows as tannins. Tannins form complexes with protein, carbohydrates or inhibit digestive enzymes which results in depressed growth rate and feed utilization. Kalahari melon meal also contains some considerable amounts of secondary plant metabolites including tannins. Unlike ruminant animals, chickens lack microbes in the gastrointestinal tract to detoxify and reduce the effect of tannins. Therefore, inclusion of polyethylene glycol is known for its capacity to tie tannins, hence releasing proteins and other dietary components in KM based diets resulting in increased digestion and absorption of nutrients (Kumanda, 2018). Furthermore, Kumanda (2018) reported that the high tannin

affinity present in PEG breaks formed tannin-protein complexes. The inclusion of PEG in diets high in tannins allows tannins to bind more strongly to PEG than to proteins and other macromolecules, thereby preserving dietary nutrients for better processing and absorption (Mansoori et al., 2006). In this way PEG might essentially decrease the antagonistic impacts of tannins on the performance of animals and enhance their feed intake and feed conversion ratio. Including polyethylene glycol (PEG) in high-tannin diets, where tannins bind more tightly to it than to proteins and other large molecules, preserves dietary nutrients, leading to improved digestion and absorption. As a result, PEG has the capacity to significantly reduce the adverse effects of polyphenols on animal performance and enhance their feed intake (FI) and feed conversion ratios (FCRs).

2.7. Summary

Diets for laying hens are prepared to maximise egg output. Protein and energy are the major components of layer diets and hence receive a lot of consideration when formulating a diet. In poultry diets, SB seed is the most commonly used protein source because on its high value protein content. Nevertheless, SBM is becoming scarce and expensive due to a variety of factors. There is a need to reduce the dependency on SBM by exploring alternative protein sources. Research on the alternative to SBM for animals has been centred on substitution by other oilseed meals (e.g., rapeseed, sunflower) or pulses. Kalahari melon is a viable alternative protein sources to reducing feed competition while maintaining egg performance in layers. However, due to the high fibre content and some secondary plant metabolites, the study seeks to assess the strategies to improve its utilisation in layer diets through use of polyethylene glycol and enzymes.

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CHAPTER 3

THE INCLUSION OF ENZYMES AND POLYETHYLENE GLYCOL ON FEED INTAKE, FEED CONVERSION EFFECIENCY, BODY WEIGHT CHANGES, HEALTH AND NUTRITIONAL STATUS AND SERUM CHOLESTEROL LEVELS IN LAYERS FED KALAHARI MELON-BASED DIETS.

Abstract

The utilization of alternative protein source such as Kalahari melon can be used to decrease competition for the use of conventional feedstuff between humans and animals. The objective of this study was to investigate the effect of enzymes and polyethylene glycol inclusion on feed intake, feed conversion efficiency, health and nutritional status, body weight changes and serum cholesterol in layers given diets comprising cake made from Kalahari melon oil. The feeding trial used 150, 18 weeks-old leghorn hens (1.405 ± 0.105 kilograms live-weight), distributed randomly among five experimental diets excluding the control were formulated by adding 10% Kalahari melon, protease and polyethylene glycol. There was a substantial ($P<0.05$) impact of diet on the average daily feed intake (ADFI), average weekly feed intake (AWFI), average daily gain (ADG), and net feed efficient index (NFEI). There was no significant ($P>0.05$) effect on all hematology and serum parameters but Total protein (TP) and albumin (ALB) had high values for diets with KM compared to the control diet. Total protein (TP) and albumin (ALB) levels are commonly observed when animals are in a state of good health. While there was a significant effect on feed intake (FI) and body weight gain attributed to high fibre, the study showed the positive benefits of Kalahari melon in blood parameters. The overall study provides insights which contribute to understanding the potential effects of KM-based diets on poultry health and performance.

Key words; Alternative protein source, Oil cake, feed acceptance, conventional feedstuff, protein source.

3.1. Introduction

Due to the ever-increasing population across the world, there has been an expanded demand for safe, and nutritious food for human supply (Alam *et al.*, 2021). Most developing countries are suffering from malnutrition due to inadequate production of food although livestock production is currently playing an important social role in providing food, generating employment and income. The poultry industry is also playing significant role in ensuring food security worldwide especially in poor communities by providing cheap and nutritious poultry products (meat, eggs, feathers and etc.), (Kanani *et al*., 2020). However, the cost of poultry production is increasing due to the high costs of inputs especially the feed (Yadav *et al*., 2022b). One of the major nutrients in layer feeding programs is protein, normally provided by soya bean meal (SBM) (Yadav *et al*., 2022a). SBM is becoming expensive due to reduced production because of climate change and other factors (Manyeula, 2018). Therefore, there is a need to reduce competition between humans and animals for conventional feedstuff by looking for alternative protein sources that are accessible locally and comparatively more cost-effective.

Among the viable alternative sources of protein, oil cakes are desirable as they have many similarities with the SBM (Rakita *et al*., 2023). Oil cakes are the by-products of oil extraction and are commonly utilized in animal feed (Balakrishnan *et al.*, 2021). Kalahari melon oil cake (KMOC) is one of the viable promising alternative oil cakes resources for layer diets (Chharang *et al.*, 2020). The essential oil obtained from the seeds gaining is commercial significance due to their use in the cosmetic industry. After oil extraction, the remaining cake contains up to

25.4% Crude protein, but with more fibre compared to SBM. Kalahari melon has the potential to be a protein source for poultry. However, there is insufficient knowledge regarding the potential of inclusion of KMOC in layer feed as a protein source. Moreover, strategies to improve its utilisation in layer diets have not been explored. Therefore, the objective of the current study is to to evaluate the effects of enzymes and polyethylene glycol inclusion on feed intake, feed efficiency, health and nutritional status in layers fed diets containing KMOC.

3.2. Materials and Methods

3.2.1 Description of the study area

A feeding trial was conducted at the layer unit at the North-West university farm (Molelwane). The study site is located in the North West Province of South Africa. The topography of the estate was situated between 25°86'00"5 and 25°64'32"E. The annual precipitation was typically between 300 and 699 mm. In the humid damp season (November-March), temperatures range from 22°C to 38°C, while in the cool dry season (May-October), they range from 2°C to 25°C.

3.2.2 Sourcing of PEG, enzymes, Citrullus lanatus oil cake and ingredients

Kalahari melon oil cake was sourced from Highlands Essential Oils (HEO) (Ltd), who are involved in commercial production of oils from different herbal plants and seeds. The Kalahari melon oil cake is the product remaining after extraction of oils used in the cosmetics industry. Polyethylene glycol was sourced from Monitoring & Control Laboratories (MCL) (PTY) LTD, Linbro park (Johannesburg). The MCL is one of the top providers of top-notch scientific and laboratory equipment in Southern Africa. Protease enzyme was sourced from Nutroteq (PYT) LTD. Furthermore, Opti Feeds, situated in Lichtenburg, South Africa, supplied all other dietary components.

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3.2.3 Treatments

Five dietary regimens were formulated by substituting SBM with Kalahari melon oil cake at a 10% concentration, protease enzyme and polyethylene glycol as follows.

- Treatment 1- Control (Commercial layer feed)
- Treatment 2- 10% Kalahari melon diet)
- Treatment 3- 10% Kalahari melon diet + 0.3% Protease enzyme
- Treatment 4- 10% Kalahari melon diet + 0.5% polyethylene glycol,
- Treatment 5- 10% Kalahari melon diet + 0.3% Protease + 0.5% polyethylene glycol.

The inclusion was also determined as maximum permissible inclusion level from the feed formulation software done at SimpleGrow (Pvt, Ltd). The chemical composition of the C. *lanatus* oil cake is illustrated in Table 3.1, while Table 3.2 displays the ingredients and dietary treatments.

3.2.4 Animals and experimental design

For the investigation, 150 Leghorn layer chickens, each of which was 18 weeks old, were acquired. They were randomly assigned to five dietary treatments following a completely randomized design (CRD), with each treatment replicated across 10 cages. Each experimental unit comprised a cage housing three birds. These layers were housed in conventional battery cage systems, typical in commercial egg production, featuring curtains to regulate environmental factors. Each experimental cage was outfitted with a nipple drinker and an individual feeder. Feed and water were offered adlib. The hens were given a 7-day adaptation period to the diets followed by the feeding trial to evaluate their performance. The experimental was run for a period of 8 weeks. All the experimental protocols were approved by the NWU-ANIMPROD REC and the following ethics number was granted: NWU-00818-21-A5.

Table 3. 1 Chemical composition of *C. lanatus* oil cake

Parameter	Concentration
Crude fibre (%)	6.40±0.20
Crude fat (%)	49.05±0.30
Crude protein (%)	30.63±0.20
Carbohydrate (%)	7.22±0.30
Dry Matter (DM) (%)	92.90±0.40
Organic matter (OM) (%)	93.30±0.30
Energy value (kcal/100g)	592.85

Data are mean of triplicate determinations ± standard deviation.

Table 3. 2 Layer dietary ingredients and treatments

	Treatments				
Ingredient	CON	KM	PRO	PEG	KMEP
Maize	111.783	93.448	93.448	93.448	93.448
Soya Fullfat	-	20.160	20.160	20.160	20.160
Soybean Meal 46.5%	44.255	34.632	34.632	34.632	34.632
Kalahari Melon Cake	0.000	20.160	20.160	20.160	20.160
Crude Soya Oil	6.035	3.289	3.289	3.289	3.289
Calcium carbonate	21.343	-	-	-	-
L-Lysine HCL	0.040	0.040	0.040	0.040	0.040
DL-Methionine	0.342	0.449	0.449	0.449	0.449
L-Threonine	0.40	0.042	0.042	0.042	0.042
L-Tryptophan		0.015	0.015	0.015	0.015
L-Valine		0.000	0.000	0.000	0.000
Feed Lime Coarse		18.346	18.346	18.346	18.346
Feed Lime Fine		7.858	7.858	7.858	7.858
MDCP	3.158	1.489	1.489	1.489	1.489
Salt Fine	0.543	0.681	0.681	0.681	0.681
Sodium Bicarbonate	0.402	0.202	0.202	0.202	0.202
Choline Chloride 60% Powder		0.151	0.151	0.151	0.151
Phytase 10,000 Lay/Breeders (600 FTU)		0.060	0.060	0.060	0.060
Econase XT Layers (100 g/t)		0.020	0.020	0.020	0.020
Layer Premix	0.106	0.605	0.605	0.605	0.605
Polyethylene glycol	0.000	0.000	-	1.008	1.008
Protease enzyme	0.000	0.000	0.605	-	0.605

*Layer premix (mineral premix and vitamin premix)

3.2.5 Data collection and measurements

i. Feed intake and body weight changes

The variance between the offered feed and the remains was used to determine the daily feed intake. The hens' weights were initially documented at the beginning of the experiment and subsequently recorded on a weekly basis using either TSW equipment weighing scales or Adam equipment, SA. From these measurements, we computed the Average Daily Feed Intake (ADFI), Average Daily Gain (ADG), and Feed Conversion Ratio (FCR) using the following formulas:

$$\text{ADFI} = \frac{Feed\ offered - feed\ refused}{60\ days} \quad (1)$$

$$\text{ADG} = \frac{Finish\ weight - Start\ weight}{Age\ (\ days)} \quad (2)$$

$$\text{FCR (per kg egg mass)} = \frac{kg\ of\ feed\ consumed}{kg\ eggs\ produced} \quad (3)$$

3.2.6 Haematological and serum biochemical examination

15 birds from each treatment were randomly selected for blood sample collection at the conclusion of the trial. Each bird's brachial vein was punctured with a needle and syringe to obtain a 1ml blood sample. These samples were then promptly transferred into a purple tube containing an anti-coagulant for heamatological analysis. The full blood count (FBC) was analyzed using the Index lasercyte, a Heamatology analyzer. The Index lasercyte (Heamatology analyser) was used to analyse for the full blood count (FBC). Pure serum samples were pipette-aspirated and stored in 1.5 ml Eppendorf containers at -800C. They were thawed at 40C. After thawing, serum concentrations of albumin, total protein, glucose, chlorine, sodium, potassium, cortisol levels, urea, and serum enzymes; alanine amino

transferase and aspartate amino transferase were determined using commercial kits or a chemical analyser. The enzymatic determination of total cholesterol and triglycerides in serum was conducted using commercial reagent kits (ASAN Total Cholesterol, AM 202-K and ASAN TG-S, AM 157S-K, respectively, ASAN Pharm. Co. Ltd., Gyeonggi-do, Korea).

3.2.7 Statistical analyses

The general linear model (GLM) procedures of SAS (2010) were used to analyse data on feed intake, body weight changes, FCR, haematology and serum biochemistry. The model used was as follows:

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

Where,

Yijk = This represents the outcome or response variable that is being measured

μ = This is the overall average or mean value of the dependent variable across all treatments

Di = Specific effect of the i^{th} diet on the dependent variable.

Eijk = random error associated with observations

3.3 Results

3.3.1. Feed intake and body weight changes

The results on the effects of including protease and polyethylene glycol (PEG) in Kalahari melon (KM) diets on FI and BW changes are presented in Table 3.3. From the results, the experimental diets affected all the measured parameters. The hens fed the diet containing only 10%KM had the highest FW, BWG and ADG succeeded by those who were supplied the Control diet. The hens fed the 10% KM diet also had the diet exhibited the highest value for ADFI while Con had the lowest ADFI ($P<0.05$). This indicates that the inclusion of 10% KM in the diets led to increased feed consumption by the layer hens. KMEP and 10KM%+PEG

diets had the highest ($P < 0.05$) FEP while the 10%KM+PRO had the lowest. With regards to body weight changes. The initial weights were similar among all the hens used in the study. However, diets had a significant effect on final weight and total BWG. The hens fed the 10% KM diet had the highest ($P < 0.05$) FW and the total BWG succeeded by those supplied the Control diet. The hens fed the 10%KM+PRO diet had the lowest values for both the FW and total BWG respectively.

Table 3. 3 Effects of protease and polyethylene glycol on feed intake and body weight changes of layer hens fed KM diets

	Treatments				
Parameter	Con	10%KM	10KM+PRO	10%KM+PEG	KMEP
Initial Weight (kg)	4.33±0.14	4.25±0.14	4.05±0.14	4.20±0.14	4.24±0.14
Final Weight (kg)	5.07±0.19^{ab}	5.70±0.19^{a}	4.52±0.19^{ab}	5.06±0.19^{ab}	4.81±0.19^{ab}
Body Weight Gain (kg)	0.74±0.19^{ab}	1.44±0.19^{a}	0.47±0. 0.19 ^{ab}	0.86±0. 0.19 ^{ab}	0.57±0.19 ^{ab}
Average Daily Gain (g)	0.016 ± 0.02^{ab}	0.023 ± 0.02^{a}	0.011 ± 0.002^{b}	0.012 ± 0.002^{b}	0.013 ± 0.002^{b}
Average Daily Feed Intake (g)	47.43±0.53^{d}	51.51±0.54^{a}	48.61±0.52^{abc}	49.87±0.54^{c}	50.29±0.53^{ab}
Feed Egg Production	27.27±2.15^{ab}	28.45±2.16^{ab}	25.09± 2.15^{b}	31.59±2.16^{a}	31.36±2.15^{a}

$^{a, b, c, d}$ means in the same row with different superscripts are significantly different (P <0.05). Treatments; CON=Control (Commercial layer diet), 10%KM=10% Kalahari melon inclusion, 10%KM+PRO= 10% Kalahari melon + Protease, 10%KM+PEG= 10% Kalahari melon+ Polyethylene glycol, KMEP= 10% Kalahari melon + Protease+ Polyethylene glycol

3.3.2. Hematology and serum biochemistry

The presented study focused on investigating the effects of including protease and polyethylene glycol (PEG) in Kalahari melon (KM) diets on hematology and serum biochemistry of layer hens. The effect of protease and polyethylene glycol on hematology are shown on Table 3.4. While that of serum analyses are on Tables 3.5 and 3.6. Diets had no effect ($P>0.05$) on all the measured hematological parameters except the lymphocytes ($P<0.05$). The control diet led to significantly higher concentration of lymphocytes than all the KM containing diets regardless of the added feed additive. The KM containing diets had similar values for lymphocytes, eosinophils, basophils, and haemoglobin as the other treatments. With regards to serum biochemistry all the parameters were not affected apart from the Albumin/Globulin ratio. Hens fed the 10%KM+PRO diet had the highest albumin/Globulin ratio while those fed the Control diet had the lowest. The liver and digestive enzymes were not affected by diet.

3.4. Discussion

3.4.1. Feed intake and body weight changes

The amount of feed that layer chickens consume has a significant impact on their general health and productivity. The present study focused on investigating the effects of including protease and polyethylene glycol (PEG) in Kalahari melon (KM) based diets on FI and BW changes. Evidently from the findings, there was a noteworthy observation the findings. High final body weight, total BWG and ADG were observed in birds that were fed 10%KM diet. The high values were associated with a high ADFI in birds fed the same diet. The high ADFI therefore could have increased the available nutrient for hens and hence were able to cope with production resulting in some significant body weight gain when compared to other treatments. The KM cake also contains some bioactive compounds that could have increased the utilization of the diets as observed in other investigations (Marume et al., 2020; Guillon-Paez et l., 2020).

Table 3. 4 Effects of protease and polyethylene glycol on haematology of layer hens fed KM diets.

	Treatments				
Parameter	Con	10%KM	10%KM+PRO	10%KM+PEG	KMEP
Haemoglobin (g/dl)	8.19±0.990	9.49 ± 9.494	9.37 ± 1.044	9.23+1.044	9.40±1.100
White blood cells($\times 10^9$/l)	17.54 ± 4.448	18.50 ±4.688	24.89 ±4.448	21.42±4.547	22.32±4.455
Neutrophils ($\times 10^9$/l)	26.25 ±3.822	26.73 ±4.029	26.40 ± 3.822	26.30±3.822	26.55±3.822
Lymphocytes ($\times 10^9$/l)	140.17 ±45.377^{c}	61.57 ± 47.831^{a}	71.41± 47.377^{a}	76.55±46.322^{b}	67.23±46.123^{a}
Monocytes($\times 10^9$/l)	5.47± 0.967	6.00±1.019	5.85±0.967	5.53±1.019	5.91±0.967
Eosinophils($\times 10^9$/l)	3.55±0.625	2.02±0.659	2.44±0.625	2.55±0.625	2.44±0.625
Basophils($\times 10^9$/l)	0.23±0.346	0.86±0.365	0.12±0.346	0.25±0.255	0.15±0.211

$^{a, b, c, d}$ means in the same row with different superscripts are significantly different (P <0.05). Parameters; C=Count. Treatments; Con=Control (Commercial diet), 10%KM=10% Kalahari melon inclusion, 10%KM+PRO= 10% Kalahari melon + Protease, 10%KM+PEG= 10% Kalahari melon+ Polyethylene glycol, KMEP= 10% Kalahari melon + Protease+ Polyethylene glycol.

Table 3. 5 Effects of protease and polyethylene glycol on blood serum of layer hens fed KM diets.

	Treatments				
Parameter	Con	10%Km	10%KM+PRO	10%KM+PEG	KMEP
Creatine(μ/L)	0.84±0.027	0.84±0.027	0.90±0.027	0.85±0.027	0.87±0.027
Blood urea nitrogen(mmol/L)	1.73±0.135	1.47±0.135	1.81±0.135	1.64±0.135	1.79±0.135
Phosphorus(mmol/L)	3.15±0.154	3.16±0.154	3.30±0.154	3.21±0.154	3.29±0.154
Calcium(mmol/L)	57.40±2.433	59.40±2.433	60.20±2.433	57.60±2.433	59.89±2.433
Total Protein(g/L)	18.0±0.632	18.8±0.632	18.60±0.632	18.4±0.632	18.60±0.632
Albumin(g/L)	39.6±1.980	40.8±1.980	41.60±1.980	42.6±1.980	40.32±1.980
Globulin(g/dL)	0.44±0.024	0.46±0.024	0.46±0.024	0.51±2.986	0.49±2.986
Albumin/Globulin	11.0±2.986[a]	14.8±2.986[b]	16.0±2.986[c]	13.4±2.986[b]	14.9±2.986[b]

[a, b ,c , d] means in the same row with different superscripts are significantly different (P <0.05). Treatments; Con=Control (Commercial diet), 10%KM=10% Kalahari melon inclusion, 10%KM+PRO= 10% Kalahari melon + Protease, 10%KM+PEG= 10% Kalahari melon+ Polyethylene glycol, KMEP= 10% Kalahari melon + Protease+ Polyethylene glycol.

Table 3. 6. Effects of protease and polyethylene glycol on blood chemistry of layer hens fed KM diets.

	Treatments				
Parameter	Con	10%Km	10%KM+PRO	10%KM+PEG	KMEP
Alanine transaminase(U/L)	418.8±184.032	895.8±184.032	540.2±184.032	633.2±184.032	532.3±184.032
Alkaline phosphate (IU/L)	20.6±4.567	13.0±4.567	16.0±4.567	15.3±4.567	17.5±4.567
Gamma-Glutamyl Transferase	6.8±2.269	11.4±2.269	12.2±2.269	9.5±2.269	10.5±2.269
Total bilirubin (IU/L)	1.23±0.179	1.28±0.179	1.18±0.179	1.32±0.179	1.26±0.179
Cholesterol (mg/dL)	298.8±35.86	315±35.86	288.2±35.86	287.3±35.86	307.6±35.86
Amylase(U/L)	99.6±9.88	106.2±9.88	111.4±9.88	108.3±9.88	105.6±9.88

[a, b ,c , d] means in the same row with different superscripts are significantly different (P <0.05). Treatments; Con=Control (Commercial diet), 10%KM=10% Kalahari melon inclusion, 10%KM+PRO= 10% Kalahari melon + Protease, 10%KM+PEG= 10% Kalahari melon+ Polyethylene glycol, KMEP= 10% Kalahari melon + Protease+ Polyethylene glycol.

On the other hand, a reduction in performance of the birds with the inclusion of protease enzyme and PEG could be attributed to the influence of those feed additives on the bioactive compound which could have inhibited their function (Zelalem et al., 2023). Diets with elevated fibre content may lead to reduced accessibility of active ingredients for birds. Consequently, the birds consume more to meet their energy requirements for both production and maintenance. In the current study, the inclusion of KM cake at 10% did not affect the weight gain in the hens despite the KM cake having high fibre content than SBM. The increase in fibre content was therefore insignificant to cause changes in consumption and utilisation of the diets containing KM cake. The feed to egg ratio (FEP) describes the hens' overall efficiency in converting ingested feed mass into egg mass over a specific period of time (Bozkurt et al., 2014). While the enzymes inclusion improved the FEP, the inclusion of PEG and its combination with the enzymes appeared to reduce the feed efficiency. Nevertheless, the observed values for FEP are in line with previous observations (Bozkurt et al., 2014; Guillon-Paez et l., 2020).

3.4.2. Hematology

Blood parameters are a good indicator of the welfare of laying hen as they can indicate health status and immune system. Haemoglobin's role involves transporting oxygen within red blood cells throughout the body. Elevated haemoglobin levels are affected by oxygen levels and red blood cells, meaning that an increase in the quantity of red blood cells will result in high haemoglobin levels (Pantaya & Utam., 2018). The variance analysis results indicated that including protease and polyethylene glycol in KM diets did not have a significant impact on the haemoglobin and red blood cells (RBC) levels in laying hens' blood. In fact, the relationship between our results showed that a decrease in RBC resulted in increased haemoglobin. The haemoglobin range for the laying hens was between 8.19 and 9.49 grams per deciliter (g/dL)

while that of RBC ranges between 0.21 and 0.45. The range of haemoglobin level in our study falls within the normal range.According to Odunitan et al (2018) the normal range for RBC level in chicken is around 2.5 to 3.5 million cells per microliter (µL) while of haemoglobin is 7 to 13 grams per deciliter (g/dL). The haemoglobin levels within the normal range suggested that the laying hens had adequate oxygen for their body's metabolic functions (Tiya et al., 2021). According to Syahrial et al. (2013), haemoglobin serves as an indicator of the oxygen supply within the bloodstream in living organisms. The results of our study showed that diets had no significant effect on leukocytes (lymphocytes, neutrophils, eosinophils, and basophils) in laying hens' blood. The normal range of leukocyte values was observed in this study. Observations from other studies that employed comparable products are in agreement with this (Bozkurt et al., 2014; Guillon-Paez *et al*., 2020)

3.4.3. Serum biochemistry

Blood biochemical analysis is extensively employed to aid in identifying and describing diseases and nutritional adequacy among the various animal species. Additionally, it serves as a crucial tool, particularly for detecting metabolic disorders that may not be easily discernible through clinical signs alone (Andreasen et al., 1996). Furthermore, it plays a key role in monitoring the well-being of poultry animals, diagnosing, and treating diseases, and evaluating their overall health status (Hajara et al., 2020). Several serum indicators frequently mirror the health condition of animals. Elevated protein synthesis, TP (total protein), and ALB (albumin) levels are commonly observed when animals are in a state of good health (Ma et al., 2021). In our study, TP and ALB had high values for diets with KM compared to the control diet. This observation aligns with Erdoğan, et al (2010) findings, which similarly indicated that the inclusion of enzymes and polyethylene glycol in poultry diets did not have any impact on the serum total protein level. Furthermore, the Kalahari melon diets did not affect our results for

serum urea. Interestingly, our results for serum urea are in contradiction with the results of Hajara et al (2020) whereby diet containing *C.lanatus* affected serum urea in poultry diets due to the increase in protein content brought by *C.lanatus*. Numerous seeds belonging to the Cucurbitaceae family contain substantial amounts of both oil and protein (Nyam et al., 2009). Iyayi and Tewe (2010) demonstrated that the levels of serum urea and total protein are primarily influenced by the quality and quantity of protein present in the diet. Alanine Amino Transferase (ALT) and Alkaline phosphate (ALP) are liver enzymes. Increased levels of hepatic enzymes in the serum, such as AST and ALP, may suggest liver damage due to some extraneous substances (Khosravinia, et al., 2013). The findings of this research indicated that the concentrations of ALT and ALP in the serum of birds receiving varying diets containing *C. lanatus* supplementation were not significantly distinguishable when compared to the control group. Our results are similar to that of Hajara et al (2020) and Tollba et al (2010).

3.5. Conclusion

Findings from the results showed that the hens fed the 10% KM diet had higher final weight, total BWG, ADFI and ADG followed by those fed the Control diet. This was attributed to the presence of some bioactive compounds in the KM cake that could have increased the utilization of the diet. The inclusion of enzyme and PEG did not improve the utilization of the KM based diets. The results also showed no effect of diets on hematology apart from the haemoglobin. Nevertheless, the haematology values were within the expected normal range for laying hens. With regards to serum biochemistry analysis, total protein (TP), albumin (ALB), and urea levels were higher in KM-containing diets compared to the control, although not significantly different. Overall, the study's findings suggest that including KM in layer hen diets impacts feed intake and body weight, likely due to its fibre content. However, inclusion of enzymes and PEG did not improve the utilization of the KM based diets by the layer hen.

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CHAPTER 4

THE INCLUSION OF ENZYMES AND POLYETHYLENE GLYCOL INCLUSION ON EGG PRODUCTION AND EGG QUALITY LEVELS IN LAYERS FED KALAHARI MELON MEAL BASED DIETS.

Abstract

This study examined the impact of enzyme and polyethylene glycol supplementation on egg production, quality parameters, fatty acid profiles, and sensory attributes in layers fed diets containing Kalahari melon oil cake. Five dietary treatments excluding the control diet were formulated by including 10% KM, polyethylene glycol and protease enzyme in layer feed. The following diets were created as experiments: Control (commercial layer feed), 10%KM (10 %KM diet), PRO (10% KM diet + protease enzyme), PEG (10% KM diet + polyethylene glycol), KMEP (10% KM diet + protease enzyme + polyethylene glycol). A total of 150 leghorn layer hens at 18 weeks old were distributed across five dietary treatments. Data was collected on egg production indices, egg quality, and fatty acid profiles. Diet had no significant effect on production indices ($P>0.05$) apart from NFEI. Diet had a significant effect on cracked eggs, pullets, extra-large eggs and jumbo eggs ($P>0.05$). Additionally, the experimental feed did not have a significant impact on any of the egg external quality parameters ($P>0.05$), with the exception of egg width, breaking strength, shell blunt and eggshell ratio together with a noteworthy impact on every internal metric ($P<0.05$) apart from the yolk index, haugh unit, albumen weight and albumen ratio. The study revealed that the incorporation of Kalahari melon along with protease and polyethylene glycol, can have diverse effects on various aspects of egg production and quality in layer hens.

Key words: laying hens, Kalahari melon oil cake, essential oil, polyunsaturated fatty acids, laying performance, egg quality.

4.1. Introduction

Layer hens' capacity to generate a substantial quantity of eggs during an extended laying cycle is crucial to the economics of layer production. Moreover, the external and internal quality are of critical importance. Eggs with weak or cracked shells lose their potential for long-term storage or incubation, and their market price is altogether reduced (Kavtarashvili & Buyarov, 2021). Broken eggs constitute 13–20% of the total quantity of eggs produced in the layer production units, resulting in major financial losses (Wu *et al*., 2020). The most common determinant of the eggshell quality is its strength, which depends on the weight, thickness, chemical composition, and consistency of dissemination of the structural material. In addition, the eggshell should have the capacity to prevent entry and contamination of the egg internal contents, and thus eggs shell quality plays an important role in food safety (Vlčková *et al*, 2018).

The nutritional value of chicken feed is essential in determining both the egg production performance and the internal and external qualities of eggs (Berihun *et al*., 2022). An imbalance of nutrients, whether in excess or deficiency, can disrupt the absorption of other essential nutrients, resulting in deficiency diseases. Specifically, insufficient calcium can result in weaker eggshells, leading to a reduction in both eggshell weight and strength. Medicinal plants contain a variety of phytochemicals and bioactive substances (trace metal ions, alkaloids, vitamins, carotenoids, fats, polyphenols, carbohydrates, and proteins) which contribute to promoting long-term health benefits (Ashour et al., 2020). One of the medicinal plants is *C. lanatus*. There is a lot of research on using KMOC as chicken feed, yielding widely varying outcomes in terms of its impact on animal performance. For example, Marume *et al*., (2020) did not find any significant effect on most of the internal and external egg quality parameter (total NOEP, ADEP, and HDEP). However, diets had a significant on egg mass, shell weight

and shell ratio. Previous studies (Marume et al.,2020) supported the use of oil cakes and the residuals from oil pressing can be utilized to produce eggs with increased n-3 fatty acids, yet the amount included depends on the presence of antinutritional compounds, which vary across different camelina varieties and are also influenced by soil and climate conditions (Orczewska-Dudek et al., 2020). The present study evaluated the effect of enzymes and polyethylene glycol inclusion on egg production; and egg quality parameters, fatty acid profiles and egg sensory quality in layers fed diets containing KMOC. The study hypothesised that the inclusion of enzymes and polyethylene will have no effect on egg production; and egg quality parameters, fatty acid profiles and egg sensory quality in layers fed diets containing KMOC.

4.2. Methods and material

4.2.1. Description of the study area

Description of the study area was outlined in Section 3.2.1

4.2.2. Sourcing of Citrullus lanatus oil cake

Sourcing of PEG, enzymes, *Citrullus lanatus* oil cake and other ingredients was outlined in Section 3.3.2.

4.2.3. Treatments, animal and experimental design.

This was outlined in section 3.2.3.

4.2.4. Data collection and measurements

ii.Egg production indices

Every day, the total number of eggs laid—broken eggs included—was recorded. The average egg mass (AEM), feed conversion ratio (FCR), net feed efficient index (NFEI), and hen-day egg production (HDEP) were determined using the following formulas:

Hen day egg production= *Total number of eggs produced in a day/ Total number of hens present x 100%* (1)

Average egg mass = *% HDEP x average egg weight* (2)

Feed conversion ratio (per kg egg mass) = *kg of feed consumed/kg eggs produced* (3)

Net feed efficient index = *(AEM + ADG)/ADFI x 100%* (4)

iii.Egg quality measurements

Egg dimensions, such as length and width, were gauged using a vernier caliper, while the egg shape index was computed by comparing the width to the length as a percentage. The weight of the eggshell was measured post the removal of the membrane, employing a precise scale. Eggshell thickness was measured at three distinct points—blunt, middle, and sharp—utilizing a micrometer screw gauge. The eggshell index was arrived at by calculating the percentage ratio of shell weight to egg weight. Internally, eighty eggs from each treatment were collected, marked individually, weighed, and cracked onto a petri dish to determine measurements such as yolk height, yolk diameter, yolk color, yolk weight, Haugh Units, albumen height, albumen length, albumen width, albumen weight, and thickness. In the end, these measurements were used to derive the albumin ratio and index, as well as the yolk ratio and index.

4.2.5 Statistical analysis

The Proc univariate procedure of SAS (2010) was used to test for normality. Data on internal and exterior egg quality, and egg production indexes collected weekly were analysed using Proc Mixed procedures for repeated measures considering week and treatment effects.. Data on internal and exterior egg quality, and egg production indices were analysed using the GLM procedure of SAS (2010) and diet as the only fixed effect. The least square means were compared pairwise using the probability differences (PDIFF) option of SAS (2010). The quadratic response surface regression was employed to determine the optimal inclusion level of the essential oil in cases where it was significant. The statistical models used were as follows:

$$Y_{ijk} = \mu + T_i + W_j + (T \times W)_{ij} + \varepsilon_{ijk} \quad (5)$$

$$Y_{ij} = \mu + T_i + \varepsilon_{ij} \quad (6)$$

Where:

Y_{ij} = This represents the outcome or response variable that is being measured (egg production indices, internal and external egg quality).

μ = This is the overall average or mean value of the dependent variable across all treatments.

T_i = Specific effect of the i^{th} diet on the dependent variable.

W_j = specific effect of week on the dependent variable.

$(T \times W)_{ij}$ = interaction of week and diet effect on the dependent variable

ε_{ij} = random error term.

4.3. Results

4.3.1. Egg production

The findings on the effects of protease and polyethylene glycol inclusions in KM based diets on egg production indices are depicted in Table 4.1. From the table, diet had no significant effect on all egg production indices excluding NFEI. Hens fed the KM diets containing the protease enzyme appeared to have the highest NFEI followed by those having a mix of the

protease and PEG in their diets. With all other parameters, the KM containing diets, whether with feed additives or not appeared to have better performances compared to the control diet. Of all the diets containing KM oil cake, the PEG appeared to suppress the performance of the hens although the values of hens fed the 10%KM+PEG diet had similar performance as the control.

Table 4. 1 Effects of protease and polyethylene glycol on egg production indices performance of layer hens fed KM diets.

	Treatment				
Parameter	Con	10%KM	10%KM+PRO	10%KM+PEG	KMEP
AEW	58.21 ± 0.62	59.61 ± 0.62	59.03 ± 0.62	59.10 ± 0.62	58.89 ± 0.62
AEP	2.172 ± 0.072	2.287 ± 0.072	2.229 ± 0.072	2.140 ± 0.072	2.289 ± 0.072
HDEP	72.42 ± 2.42	76.24 ± 2.42	74.30 ± 2.42	71.33 ± 2.42	76.30 ± 2.42
AEM	42.15 ± 1.44	45.42 ± 1.44	43.86 ± 1.44	42.10 ± 1.44	44.92 ± 1.44
ADFI	48.33 ± 1.30^{b}	52.54 ± 1.30^{a}	49.56 ± 1.30^{ab}	50.89 ± 1.30^{ab}	51.29 ± 1.30^{ab}
NFEI	87.24 ± 1.88^{ab}	86.54 ± 1.88^{ab}	88.52 ± 1.88^{a}	82.51 ± 1.88^{b}	87.83 ± 1.88^{ab}

[a, b] means in the same row with different superscripts are significantly different ($P < 0.05$). AEW=Average egg weight, AEP = Average egg production, HDEP= Hen Day egg production, AEM= Average egg mass, ADFI= Average daily feed intake, ADG= Average daily feed intake, NFEI= Net feed efficient index. Con=Control (Commercial layer diet), 10%KM=10% KM inclusion, 10%KM+PRO=10% KM + Protease, 10%KM+PEG= 10% KM+ Polyethylene glycol, KMEP= 10% Kalahari melon + Protease+ Polyethylene glycol

4.3.2. Grading

Table 4.2 illustrates the effects of different treatments, on the number of eggs of each grade produced per treatment. From the results, the number of cracked eggs was considerably decreased when PRO and PEG were included in meals separately. The birds on 10%KM+PRO experimental diet had the lowest number of cracked eggs while those on the KMEP and the control diets had the highest number of cracked eggs. Pullet egg production was highest ($P<0.05$) in hens fed the 10%KM+PRO diet and lowest ($P<0.05$) in hens diet treated with 10%KM+PEG. The inclusion of enzymes and PEG in KM diets had no effect on the small, medium, and large egg grades although it appeared that all diets containing KM appeared to have marginally higher values for small, medium, and large egg grades compared with the control. The 10%KM+PEG diet had the highest count of extra-large eggs (8.13 ± 1.360) ($P<0.05$), followed by the PRO and KMEP diets (6.25 ± 1.360 and 6.00 ± 1.360, respectively). The Con diet had the highest number of jumbo eggs (1.63 ± 0.317), followed by the 10%KM and PRO diets (1.50 ± 0.317 and 1.13 ± 0.317) respectively. The 10%KM+PEG and KMEP diets had the lowest count of jumbo eggs (0.75 ± 0.317 and 0.38 ± 0.317, respectively). The KMEP treatment was followed closely by the chickens that were fed the 10%KM experimental feed, which had the highest total egg production ($P>0.05$). The Con, 10%KM+PRO, and 10%KM+PEG treatments had slightly lower ($P>0.05$) total egg production, with the Con treatment having the lowest.

Table 4. 2. Effects of protease and polyethylene glycol on egg grading of layer hens fed KM diets.

	Treatment				
Parameter	Con	10%KM	10%KM+PRO	10%KM+PEG	KMEP
Cracked	4.00 ± 0.91^{ab}	3.89 ± 0.91^{ab}	1.87 ± 0.91^{b}	2.87 ± 0.91^{b}	4.62 ± 0.91^{a}
Pullet	0.00 ± 0.41^{b}	0.86±0.41^{ab}	1.36 ± 0.41^{a}	0.25 ± 0.41^{ab}	0.13 ± 0.41^{b}
Small	7.00 ± 1.67	7.25±1.67	6.25±1.67	3.38 ± 1.67	8.00 ± 1.67
Medium	31.00 ± 3.22	27.00 ± 3.22	27.75 ± 3.22	29.88 ± 3.22	33.75 ± 3.22
Large	102.50 ± 16.25	110.88± 16.25	106.25±16.25	104.88 ± 16.25	104.88± 16.25
Extra large	3.63 ± 1.36^{b}	6.25 ± 1.36^{ab}	8.13 ± 1.36^{a}	5.25 ± 1.36^{ab}	6.00 ± 1.36^{ab}
Jumbo	1.63 ± 0.31^{a}	1.50 ± 0.31^{a}	1.13 ± 0.31^{ab}	0.75 ± 0.31^{ab}	0.38 ± 0.31^{b}
Total Production	149.75 ± 17.56	157.63 ± 17.56	152.75± 17.56	147.25± 17.56	157.75 ± 17.56

$^{a, b}$ means in the same row with different superscripts are significantly different (P <0.05). Con=Control (layer diet), 10%KM=10% KM inclusion, 10%KM+PRO= 10% KM + Protease, 10%KM+PEG= 10% KM+ Polyethylene glycol, KMEP= 10% Kalahari melon + Protease+ Polyethylene glycol.

4.3.3. External egg quality

The effect of feeding KM with PEG and protease enzyme supplementation on external egg quality parameters are depicted in Table 4.3. From the findings, all egg exterior quality measures were unaffected by diet, with the exception of egg diameter (length & width), breaking strength, shell blunt, and eggshell ratio. The control feed (Con) had the highest statistical significance ($P < 0.05$) values for egg width and shell breaking strength (Shell BS) followed by the layer hens fed the 10% KM experimental diet. KMEP diet had the lowest values for both egg width and shell BS. For shell strength blunt (Shell SB), the 10% KM diet emerged as the highest performing treatment, closely followed by the control diet (Con). Conversely, the 10%KM+PEG diet exhibited the lowest Shell SB values, suggesting that polyethylene glycol might have a negative impact on this aspect of shell quality. Egg shape ratio (ESR), a parameter that reflects the proportion of egg width to egg length, displayed an interesting trend. The diet made up of 10% KM had the highest ($P < 0.05$) ESR value, notably, the ESR values were lower in the control diet.

4.3.4. Internal egg qualities

The impact of feeding KM with PEG and enzyme supplementation on internal egg quality parameters is depicted in Table 4.4. From the findings, diets had a significant influence on all internal parameters apart from the yolk index, haugh unit, albumen weight and albumen ratio. The hens given the 10%KM diet had the greatest ($P < 0.05$) yolk height (YH) and yolk weight (YWT). On the contrary, the 10%KM+PEG-fed hens had the largest yolk diameter, whereas the 10%KM+PRO-fed hens displayed the greatest yolk colour. The hens fed the 10%KM+PEG diet also exhibited the highest values in albumen ratio, albumen weight and yolk index. Egg quality parameters like yolk colour and albumen length were notably higher in hens fed the 10%KM+PRO diet compared to other diets, followed by those fed the 10%KM+PEG diet.

Results showed that the hen fed the KMEP diet had on the highest YR and HU, followed by those fed the 10%KM+PRO and 10%KM+PEG diets. The hens fed the Con diet had a higher value than all the diets containing 10% KM with regards to AWID.

Table 4. 3. Effects of protease and polyethylene glycol in on external egg quality parameters of layer hens fed KM diets.

	Treatments				
Parameter	Con	10%KM	10%KM+PRO	10%KM+PEG	KMEP
Egg Length	55.06± 0.22	54.76±0.22	54.53±0.22	54.60±0.22	54.60±0.22
Egg width	42.94±0.12^{a}	42.80±0.12^{ab}	42.69±0.12^{ab}	42.70±0.12^{ab}	42.45±0.12^{b}
Egg weight	57.79±0.49	57.24±0.51	56.73±0.49	56.80±0.49	56.03±0.49
Shell Breaking Strength	5.25± 0.12^{a}	5.02± 0.12^{ab}	4.97± 0.12^{ab}	4.73± 0.12^{b}	5.20± 0.12^{ab}
Shell Weight	7.31±0.09	7.31±0.09	7.32±0.09	7.33±0.09	7.45 ± 0.09
Shell Strength Blunt	0.51±0.01^{ab}	0.52±0.01^{a}	0.50±0.01^{ab}	0.48±0.01^{b}	0.51±0.01^{ab}
Shell Strength Middle	0.50±0.31	0.90±0.32	0.47±0.31	1.13±0.31	0.50±0.31

Shell Strength Sharp	1.19±0.583	0.94±0.592	1.19±0.583	1.05±0.583	0.50±0.58
Egg Shell Index	0.78±0.03	0.78±0.03	0.78±0.03	0.78±0.03	0.78±0.03
Egg Shell Ratio	12.70 ± 0.24^{b}	13.60 ± 0.24^{a}	12.96 ± 0.24^{ab}	12.94 ± 0.24^{ab}	13.33 ± 0.24^{ab}

[a, b] means in the same row with different superscripts are significantly different (P <0.05). Con=Control (Commercial diet), 10%KM=10% KM inclusion, 10%KM+PRO= 10% KM + Protease, 10%KM+PEG= 10% KM+ Polyethylene glycol, KMEP= 10% Kalahari melon + Protease+ Polyethylene glycol

Table 4. 4 Effects of protease and polyethylene glycol in on internal egg quality parameters of layer hens fed KM diets.

	Dietary treatments				
Parameter	Con	10%KM	10%KM+PRO	10%KM+PEG	KMEP
YH	13.21± 0.176^{b}	13.89±0.179^{a}	12.95±0.176^{b}	13.28±0.176^{b}	13.39±0.176^{b}
YD	42.09±0.313^{ab}	41.61±0.318^{b}	42.36±0.313^{ab}	42.76±0.313^{a}	42.45±0.312^{ab}
YC	4.06±0.144^{b}	4.17±0.146^{ab}	4.54±0.144^{a}	3.58±0.144^{c}	4.09±0.144^{b}
YWT	15.12± 0.243^{ab}	15.33± 0.246^{a}	14.98± 0.243^{ab}	14.54± 0.243^{b}	15.20± 0.243^{ab}
YI	0.32±2.226	2.53±2.260	0.31±2.226	5.15±2.226	0.32 ± 2.226
YR	26.12±0.390^{ab}	25.68±0.397^{b}	26.39±0.390^{ab}	25.65±0.390^{b}	27.19±0.390^{a}
HU	64.98±3.872	66.98±3.932	65.79±3.782	67.32±3.932	73.86±3.872
AH	4.63±0.426^{b}	6.70±0.433^{a}	4.68±0.426^{b}	4.82±0.426^{b}	4.62±0.426^{b}
AL	85.73±1.328^{c}	85.08±1.349^{c}	91.18±1.328^{a}	90.00±1.328^{ab}	87.03±1.328^{cb}
AWID	70.28±1.147^{a}	66.73±1.164^{b}	69.94±1.147^{ab}	69.29±1.147^{ab}	68.39±1.147^{ab}
AWT	31.58±176.300	33.49±179.033	31.36±176.300	425.650±176.300	32.21±176.300
AI	2.99±0.390^{b}	4.83±0.396^{a}	2.98±0.390^{b}	3.12±0.390^{b}	3.33±0.390^{b}
AR	54.69±332.960	57.06±346.556	55.39±332.960	797.57±332.960	57.47±332.960

$^{a, b}$ means in the same row with different superscripts are significantly different (P <0.05). YH, yolk height; YD, yolk diameter; YC, yolk colour; YWT, yolk weight; YI, yolk index; YR, yolk ratio; HU, Haugh unit; AH, albumen height; AL, albumen height; AWID, albumen width; AWT, Albumen weight; AI, albumen index; AR, albumen ratio. Con=Control (Commercial layer diet), 10%KM=10% KM inclusion, 10%KM+PRO= 10% KM + Protease, 10%KM+PEG= 10% KM+ Polyethylene glycol, KMEP= 10% KM + Protease+ Polyethylene glycol.

4.4 Discussion

4.4.1. Production Indices

The lack of feed ingredients in the chicken sector is a problem for egg production (Wang *et al*., 2017). The egg farmers need to find cost-effective, environmentally friendly ways to produce eggs in order to meet the enormous challenge. To produce healthy animals and poultry (layers and broilers), several medicinal herbs have been employed, such as garlic, onion, thyme, spearmint, pepper, yucca, black cumin (black seed), and ginger (Torki et al.,2021). For example, spearmint leaves are used as a carminative, antispasmodic, and choleretic in humans. Phytogenic feed additives have been demonstrated to significantly enhance the performance, ovarian structure, levels of serum lipid, eggs sensory quality in laying hens and egg production (Marume et al., 2019). In our study, the supplementation of PEG and PRO in KM diets did not affect average egg weight, average egg production, hey-day egg production and average egg mass. The results in average egg mass could be attributed to low supply of linoleic acid. Ensuring sufficient protein (amino acids) is crucial for the size of eggs in laying hens, and it is important to consider the energy content as well to prevent the need for using protein to fulfil energy needs (Mwaniki et al., 2020). Although there were no effects of Kalahari melon oil cake on average egg weight, average egg production, hen-day egg production and average egg mass, the diets significantly($P<0.05$) affected NFEI, NOEP and ADEP. This could be attributed to a correlation between efficiency of feed conversion and number of eggs produced, whereby supplementation of KM in the diet appears to have the potential to enhance the egg-laying performance of hens. Elevated feed consumption in laying chickens can result in a higher intake of nutrients, particularly protein (Zacaria & Ampode., 2021). So, a decrease in feed intake will also result in a decreased supply of essential nutrients for the production of eggs (Liu et al., 2020).

4.4.2 Grading

Customers consider egg size as one of its most essential characteristics. As a result, while sorting and grading eggs, the size of the eggs must be considered (Soltani *et al*., 2015). Grading eggs based on their weight and size is an essential process in the preparation of eggs Grading eggs by weight and size eggs is a necessary step in preparing eggs for commercialization (Thipakorn *et al.,* 2017). The current study's findings demonstrated that, in comparison to the control diet. The utilization of protease has gained significant attention in the poultry sector due to its beneficial effects on nutritional efficiency and overcoming antinutritional factors in poor protein sources. The enzyme plays essential role in enhancing nutrient utilization and mitigating the negative impacts of secondary plant metabolites present in unconventional protein sources. This could have the effects of increasing the assimilation of egg nutrients and minerals particularly protein and calcium that is important in enhancing egg volume (Jonchère *et al.,* 2010). The PEG inclusion in diets can also be instrumental in tying down some undesirable compounds in the KMOC that could influence its utilisation in diets, ultimately enhancing egg developments as reflected by the good egg grades obtained in hens fed the diets containing PEG.

4.4.2. External parameters

The egg external quality is critical in determining egg integrity during handling procedures and storage. The observations made in the current research showed that including protease enzyme and polyethylene glycol in diets containing KMOC may produce the same egg production performance as the control diet. The inclusion of the KM in diets appeared to slightly decrease the egg quality parameters although the combined inclusion of PEG and enzymes in diet resulted in same performance as the control diet. This shows that the two feed additives don’t have complementary effects in improving egg quality when hens are fed poor quality protein

sources such as the KMOC as observed in other studies (Abdel-Wareth and Lohakare, 2020). Therefore, it is possible that the inclusion of PEG and Protease on KMOC might enhance elevation of serum calcium levels, which in turn raises shell calcification and, as a result, improves shell quality characteristics (Barbosa et al., 2022). Eggshell thickness is an essential quality criterion for table eggs and is controlled by dietary calcium and phosphorus levels while egg width and length are affected by egg weight. Eggs with poor shell quality lead to economic losses in the production of eggs for human consumption (Zelalem *et al*., 2023). Eggs that have thin shells lose more moisture than eggs with thick shells, which makes them to have shorter shelf life (Berihun *et al.,* 2022). When moving an egg from the moment it is laid until the time it is consumed, the eggshell's strength and thickness are vitally crucial. In the current research the inclusion of protease and PEG positively influenced both the shell breaking strength and the shell strength blunt implying that the eggs will be easily handled and have reduced incidences of cracking.

4.4.3. Internal parameters

The findings from the current study indicate that the inclusion of enzymes and polyethylene glycol in layer diets had varying effects on internal egg quality parameters. The outcomes of this study provide valuable insights into the intricate relationship between nutrition and internal egg quality parameters. The significant effects of diet on yolk and albumen characteristics suggest that the dietary alteration may desirably influence nutrients metabolism and assimilation ultimately influencing internal quality of eggs for the benefits of humans. The hue of the yolk is a crucial sign of an egg's quality (Wen *et al.,* 2019) and an essential egg quality attribute for both the egg industry and consumer preference (Ortiz et al., 2021). The key elements that define egg yolk color, are xanthophyll pigments and oxy-carotenoids (Cufadar *et al.,* 2021). These compounds are typically derived from the diet and are responsible for the

yellow to orange hues observed in egg yolks. In our study, adding Kalahari melon cake to the diets significantly improved yolk color. This can be attributed to the effects of some flavonoids and other desirable secondary plant metabolites contained in the KMOC that could have positively influenced egg color. These findings align with previous research indicating the influence of plant oil enhancing the yolk colour (Wen *et al.,* 2019; Cetıngul & Inal, 2009; Zhang & Kim, 2014). In another study, it was possible the inclusion of cumin seed meal (CSM) boosted bile acid production and lipase activity, resulting in improved absorption of fat, fat-soluble substances and carotenoids which lead to the improved yolk color score (Saleh *et al.,* 2020). Therefore, it is possible in our study that the inclusion of KMOC concurred the work of Saleh *et al* (2020) on the effect of CSM on yolk colour. Yolk weight linearly increased from 14.98 to 15.33 g with increased nutrient density. Likewise, Wu et al., (20007) and Wu et al., (2005b) observed that enhancing dietary energy through the inclusion of poultry oil resulted in a consistent, incremental rise in yolk weight.

4.5. Conclusion

The inclusion of enzymes (protease) and polyethylene glycol (PEG) in Kalahari melon oil cake (KMOC)-based diets demonstrated a positive impact on various aspects of egg production and quality in laying hens. While key production metrics such as average egg weight, average egg production, and hen-day egg production were not significantly affected, the improvement in net feed efficiency, number of eggs produced, and average daily egg production suggests enhanced feed conversion efficiency and nutrient utilization. Additionally, the supplementation of protease and PEG positively influenced egg grading, with eggs being more favourably sized and graded. The external quality of eggs, including shell strength and thickness, was maintained or slightly improved, which is crucial for handling and storage. Internally,

significant improvements in yolk colour and nutrient assimilation were observed, almost certainly as a result of the advantageous effects of secondary plant metabolites in the KMOC and the enhanced nutrient metabolism facilitated by the enzyme and PEG. These findings indicate that the strategic inclusion of protease and PEG in KMOC-based diets can effectively enhance both the external and internal quality of eggs, supporting their use as valuable additives in poultry nutrition, particularly when utilizing unconventional protein sources like KMOC.

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CHAPTER 5

THE EFFECTS OF ENZYMES AND POLYETHYLENE GLYCOL INCLUSION ON BONE DEVELOPMENT AND WELFARE IN LAYER HENS FED DIETS CONTAINING KALAHARI MELON OIL CAKE

Abstract

Recognizing the global welfare of layer hens welfare, this study investigates the effects of enzymes and polyethylene glycol (PEG) inclusion in diets containing KMOC on immune, bone development, and general wellbeing in layer hens. Results showed significant effect of diet on bone parameters like tibia weight, diameter, and width with the inclusion of KMOC and feed additives. Interestingly, the mineral content in tibia bones remained consistent despite slight variations in ash content among diets. Additionally, internal organ parameters, such as intestinal length and weight, were influenced by the dietary composition, possibly due to the fibre content in the Kalahari melon diet. These findings underscore the vital role of dietary components in shaping bone and internal organ parameters in layer hens, offering valuable insights for poultry nutrition and health management. The resultshighlight the potential of specific dietary inclusions, like KMOC and feed additives, in positively impacting bird welfare, bone health, and intestinal morphology competence without adversely affecting mineral content.

Keywords: breaking strength, calcium metabolism, keel bone, welfare, bone mineral density; fractures; laying hens; laying performance; osteoporosis.

5.1. Introduction

The poultry business has been optimized for extremely efficient and cost-sensitive egg and meat production in recent decades, resulting in significant changes in management methods,

including specialized and intensified production procedures. However, public awareness about the detrimental effects of intensive farming on animal welfare and food safety has risen significantly (Rovers *et al.,* 2019). The poultry industry faces modern challenges and changes to adjust to developing market interests, unfamiliar regulations and to continue improving the efficiency as well as the welfare of birds (Mohammed & Rehan, 2018). However, bird health is an important aspect of welfare and a requirement for good productivity and healthy food for humans (Hofmann *et al.,* 2021). A fully working immune system is essential for optimum health, but it can be harmed by stresses and other negative environmental factors. During production, broilers are faced up with a wide range of potential upsetting conditions that imbalance their welfare and immune framework and may disable their health. Nutrition, housing, temperature, air quality, and light regime are all examples of stressors that can have a deleterious impact on the immune system of layer hens particularly in a commercial battery system.

Layer hens' welfare is globally increasingly becoming important in poultry production (Bessei, 2018). The five freedoms are a standard paradigm for assessing welfare (Bryden *et al., 2021).* Layers in a commercial system are kept in battery cages for the rest of their production life. Their bone development, health and general welfare are critical aspects in optimising performance. In this aspect, different dietary sources may differentially provide the necessary nutrients that enhances immune competence, health, and welfare in layer hens. Calcium (Ca) and phosphorus (P) are important minerals that play a role in a variety of biological processes, including bone formation and mineralization particularly in layer hens which spend most of their productive life standing in battery cages (Proszkowiec-Weglarz *et al.,*2019). To achieve maximum egg output, bird health and welfare must be optimised (Bryden *et al.,* 2021). The inclusion of KMOC in diets and feed additives such as the enzymes and PEG may therefore

facilitate the efficient release of minerals and other nutrients that are critical to immune and bone development in broilers. Currently The effects of inclusion of KMOC and some feed additives have not been the subject of any investigations in layer diet on bone immune, bone development and welfare in layer hens. The present study conducted aims to evaluate the effects of enzymes and polyethylene glycol inclusion on immune, bone development and welfare in layer hens fed diets containing KMOC.

5.2. Methods and material

5.2.1. Description of the study area

This was outlined in section 3.2.1

5.2.2. Sourcing of Citrullus lanatus Oil cake

Sourcing of PEG, enzymes, *Citrullus lanatus* oil cake and other ingredients was explained in section 3.3.2.

5.2.3. Treatments, animal and experimental design.

This was explained in section 3.2.3.

5.2.4. Animals and experimental design

The animal and experimental design was outlined in section 3.2.4

5.2.5. Data collection and measurements

i. Tibia linear parameters

At slaughter on day 60, 6 right tibiae from each treatment were surgically removed. On day 60, during the slaughtering process, six right tibia bones were taken from each treatment group, de-fleshed and cleansed by hand on all tissue, including cartilage crowns (Periosteum), was examined and weighed to calculate the Tibia weight. The tissue was then de-fleshed and

cleaned, including the cartilage caps (Periosteum), by hand. The Tibia weight was obtained by weighing the cleansed tissue. The Toshiba-Rotanode X-ray (Toshiba Electron Tubes and Devices, Tochigi, Japan) was connected to the point-of-care (CR140) system for digital imaging to ascertain the tibia diameter proximal end, tibia width diameter , tibia length diameter , and tibia diameter distal end. Following linear measurement, each tibia was positioned on a flexible 3-point bend/snap fixture, which was attached to a robust TA XT platform of a texture analyser (model TA-XT plus, Stable Microsystmems, Surrey, UK) and broken with a 6 cm flat head probe that is connected to a 50 kg load cell, which reports the breaking force in Newtons. The bone supports were separated by 50 mm. The maximal strain prior to bone breakage was documented as the breaking bone strength (BBS).

ii. Mineral content

The tibia pieces were collected, defatted, and ashed after the breaking strength measurements were taken. The ash percentage was determined by weighing the samples after they were chilled down in a desiccator and deposited into a muffle furnace at 600°C for 24 hours. The following formula was employed to express tibia ash bone as a percentage:

$$\text{Tibia bone ash \%} = (\text{Tibia ash weight}) / (\text{Tibia dry weight}) \times 100 \quad (7)$$

The mineral content was examined at the animal health laboratory at North-West University using the dry ashing method for both macro and trace minerals, in accordance with the specifications of the Agri-laboratory Association of Southern Africa (AgriLASA 1998). Overnight, 1 ml of 55% nitric acid was used to decompose the ash, which was weighed. The samples were carefully transferred to McCartney vials without disturbing the sediment after a 24-hour period. The levels of Ca, P, Mg, Na, and K were determined using an ICP Spectrometer (Perkin-Elmer, 1982, Nex Ion 300Q). The concentration of macro minerals was measured in milligrams per litter.

iii. Internal organs

At the conclusion of the feeding experiment, 16 birds from each treatment group were randomly selected and sacrificed to assess the internal organs. The livers, spleens, and intestines were extracted and weighed during the necropsy period. Small intestine length (jejunum, duodenum, and ileum) was measured and documented. The liver weight was divided by the final avian weight to determine the hepatosomatic index (HSI), and the spleen index was computed as the ratio of spleen weight to the final bird weight.

5.2.6 Statistical analysis

The SAS (2010) Proc univariate technique was utilized to assess normality. The bone parameters data were analyzed using the Generalized Linear Model (GLM) technique of SAS (2010), with food being treated as the fixed effect. The SAS (2010) software was utilized to conduct pairwise comparisons using the Probability Differences (PDIFF) option. The statistical models used was as follows:

$$Y_{ij} = \mu + T_i + \varepsilon_{ij}$$

Where:

Y_{ij} = This represents the outcome or response variable that is being measured (bone parameters and internal organs).

μ = This is the overall average or mean value of the dependent variable across all treatments.

T_i = Specific effect of the i^{th} diet on the dependent variable.

ε_{ij} = random error term.

5.3. Results

5.3.1. Bone linear parameters

The results of the study focused on the effects of protease and polyethylene glycol (PEG) supplementation on bone parameters of layer hens fed Kalahari melon (KM) diets. The effects of including Kalahari melon in the diets along with PEG and enzyme supplementation on bone parameters are displayed within Table 5.1. The results indicates that the diet had a significant

Table 5. 1. Effects of protease and polyethylene glycol on bone parameters of layer hens fed KM diets.

	Treatments				
Parameter	Con	10%KM	10%KM+PRO	10%KM+PEG	KMEP
Tibia Weight (TW)(g)	8.55 ± 0.35^{ab}	9.41 ± 0.35^{a}	8.96 ± 0.35^{ab}	8.14 ± 0.35^{b}	8.17 ± 0.35^{b}
Tibia Diameter Proximal End (TDPE)	18.26 ± 0.61^{b}	20.78 ± 0.61^{a}	19.48 ± 0.61^{ab}	19.86± 0.61^{ab}	19.19 ± 0.61^{ab}
Tibia Diameteer Distal End (TDDE)	13.92 ± 0.52	14.74 ± 0.52	12.95 ± 0.52	14.43 ± 0.52	12.01 ± 0.529
Tibia Length (TL	118.09 ± 8.12	100.87 ± 8.12	114.92 ± 8.12	115.92 ± 8.12	113.51 ± 8.12
Tibia Width (TWID	7.46± 0.26^{ab}	7.64 ± 0.26^{a}	7.02 ± 0.26^{ab}	6.99 ± 0.26^{ab}	6.83 ± 0.26^{b}
Bone Breaking Strength (BBS)(N)	136.54 ± 15.05^{a}	141.89± 15.05^{b}	158.70± 15.05^{b}	172.05 ± 15.05^{c}	173.33± 15.052^{c}

$^{a, b}$ means in the same row with different superscripts are significantly different (P <0.05). Con=Control (Commercial diet), 10%KM=10% Kalahari melon inclusion, 10%KM+PRO= 10% Kalahari melon + Protease, 10%KM+PEG= 10% Kalahari melon+ Polyethylene glycol, KMEP= 10% Kalahari melon + Protease+ Polyethylene glycol.

effect ($P < 0.05$) on tibia weight (TW), tibia diameter at proximal end (TDPE), tibia width at intermediate distance (TWID) and BBS. However, the other parameters, tibia diameter at distal end (TDDE) and tibia length (TL) were not influenced by diets. The inclusion of KMOC in diets appeared to increase the TW, TDPE and the TWID, with the hens fed the 10%KM having the highest values. Among the treatments, the 10% KM diet had the highest TWID value, followed by the 10%KM+PEG diet, while the KMEP diet had the lowest TWID value. When feed additives and KMOC were added to the diets, bone breaking strength seemed to increase. The hens fed the KMEP diet exhibited the highest value ($P > 0.05$) for BBS, followed by the 10%KM+PEG diet. On the other hand, the control diet displayed the lowest BBS value among the treatments.

5.3.2. Mineral content of layer hen's tibia bones.

The influence of feeding KM with PEG and enzyme supplementation on mineral content parameters is shown in table 5.2. The results on table 5.2 shows that the diets had no effect($P> 0.05$) on all the parameters. Nevertheless, the hen fed KMEP diet had marginally higher values in potassium, moisture, and ash. In addition, the layer hens fed 10%KM+PRO diet had the marginally higher values for Magnesium, sodium, and phosphorus while 10% KM diet only had a high value in calcium. The Ash content appeared to vary slightly among the different diets, with values ranging from 32.48 to 35.04. The KMEP diet marginally had higher ash content, while Con diet has the lowest ash content. Calcium content in the diets was relatively consistent, with minor variations ranging between 13.10 to 13.48. The 10%KM+PEG treatment had marginally the lower calcium content at 13.10 ± 0.625.

Table 5. 2 Effects of protease and polyethylene glycol on mineral content of tibia bones layer hens fed Kalahari melon oil cakediets

	Treatment				
Parameter	Con	10%KM	10%KM+PRO	10%KM+PEG	KMEP
Ash	32.48 ± 1.387	33.22 ± 1.387	32.67 ± 1.387	32.60 ± 1.387	35.04 ± 1.387
Calcium	13.46 ± 0.625	13.48 ± 0.625	13.46 ± 0.625	13.10 ± 0.625	13.23 ± 0.625
Potassium	0.42 ± 0.028	0.41 ± 0.028	0.41 ± 0.028	0.39 ± 0.028	0.44 ± 0.028
Magnesium	0.23 ± 0.011	0.23 ± 0.011	0.24 ± 0.011	0.22 ± 0.011	0.24 ± 0.011
Sodium	0.37 ± 0.023	0.39 ± 0.023	0.42 ± 0.023	0.38 ± 0.023	0.41 ± 0.023
Phosphorus	7.11 ± 0.324	7.09 ± 0.324	7.13 ± 0.324	6.96 ± 0.324	6.71 ± 0.324
Moisture	7.17 ± 0.350	7.21 ± 0.350	6.74 ± 0.350	6.93 ± 0.350	7.47 ± 0.350

[a, b] means in the same row with different superscripts are significantly different (P <0.05). Diets: Con=Control (Commercial layer diet), 10%KM=10% KM inclusion, 10%KM+PRO= 10% KM + Protease, 10%KM+ PEG= 10% KM+ Polyethylene glycol, KMEP= 10% Kalahari melon + Protease+ Polyethylene glycol.

5.3.3. Internal organs of layer hens

The effects of diets on internal organ parameters are summarized in Table 5.3. The table indicates that the jejunum length and weight, and ileum length increased with the inclusion of KMOC in the diets. The 10% Kalahari melon oil cake diet recorded the highest values for jejunum length, followed by KMEP, PEG, and PRO, while the control diet earned the lowest rating. A comparable trend was noted for ileum length and ceca weight, with the 10% Kalahari melon diet having the highest values, followed by PEG, KMEP, PRO, and CON, respectively.

5.4 Discussion

5.4.1. Bone linear Parameters

The well-being of laying hens' bone health and metabolism is a significant focus for egg producers, veterinarians, nutritionists, and geneticists (Kolakshyapati *et al.,* 2019). Biomechanical properties of the tibia, including factors like bone mineral content, bone ash, bone density and bone breaking strength are typically employed as markers to assess the sufficiency of mineral provisions in poultry diets (Disetlhe *et al.,* 2017). In the current research, it was observed that dietary inclusion of polyethylene glycol and protease enzyme significantly affected TW, TDPE, and TWID. This may be attributed to the effects of the KM cake in increasing the availability of minerals for the development of matrix of bones. The KMOC is known to contain high levels of some important minerals such as Ca when compared to SBM. Moreover, KMOC contains some secondary plant metabolite such as flavonoids that have the effects of increasing mineral metabolism and assimilation in the gut (Abdel-Wareth and Lohakare., 2020).

Table 5. 3 . Effects of protease and polyethylene glycol in internal organ parameters of layer hens fed KM diets.

	Treatments				
Parameter	Con	10%KM	PRO	PEG	KMEP
Gizzard(g)	29.12± 1.41	32.38± 1.40	30.73± 1.40	30.86± 1.40	31.57± 1.40
Liver (g)	29.11± 0.94	28.95± 0.94	30.06± 0.94	29.82± 0.94	28.73± 0.94
Spleen (g)	2.12± 0.11	1.98± 0.11	1.83± 0.11	1.95± 0.11	1.90± 0.11
Proventiculus (g)	7.77±1.77	8.60± 1.77	8.28±1.77	8.67±1.77	3.99±1.77
Duodenum Length(cm)	29.61±1.09	30.61±1.09	30.43±1.09	31.70±1.09	29.22±1.09
Duodenum (g)	16.21±0.78	15.94±0.78	15.78±0.78	17.33±0.78	16.44±0.78
Jejunum Length(cm)	67.77 ± 2.38^{b}	74.48 ± 2.38^{a}	68.03 ± 2.38^{ab}	69.75 ± 2.38^{ab}	72.67 ± 2.38^{ab}
Jejunum Weight(g)	27.64 ± 1.31^{a}	28.30 ± 1.31^{b}	27.40±1.31	27.55±1.31	26.87±1.31
Ileum Length(cm)	60.21 ± 2.19^{b}	67.18 ± 2.19^{a}	62.41 ± 2.19^{ab}	63.97 ± 2.19^{ab}	63.70 ± 2.19^{ab}
Ileum Weight(g)	20.92±1.04	21.84±1.04	19.78±1.04	22.59±1.04	20.92±1.04
Ceca Length(cm)	21.95±1.3	23.16±1.39	21.08±1.39	20.29±1.39	24.10±1.39
Ceca Weigh(g)t	12.90 ± 0.635^{b}	14.79 ± 0.63^{a}	13.08 ± 0.63^{ab}	13.83 ± 0.63^{ab}	13.72 ± 0.63^{ab}
Intestinal pH	5.92± 0.47	5.93± 0.47	5.96± 0.47	5.85± 0.47	5.92± 0.47

[a, b] means in the same row with different superscripts are significantly different (P <0.05). Con=Control (Commercial diet), 10%KM=10% KM inclusion, PRO= 10% KM + Protease, PEG= 10% KM+ Polyethylene glycol, KMEP= 10% Kalahari melon + Protease+ Polyethylene glycol.

On the other hand, no differences were observed regarding tibia diameter distal end (TDDE), tibia length (TL) and bone breaking strength showing that the inclusion of protease enzyme and polyethylene glycol in the diets had no negative effects on bone development. Generally, poor bone development can result due to inadequate provision of particular minerals in the diet, including P and Ca. The primary reason for osteoporosis in laying hens is the significant depletion of bone minerals, primarily from the medullary bone, which occurs because of deficiencies in calcium during eggshell formation (Bain & Dunn, 2016). This condition tends to develop as hens are aging or due to inadequate nutrition, particularly when their diets lack sufficient calcium. Alfonso-Carrillo *et al* (2021) argued that there may be a negative association between egg production and skeletal integrity or bone due to a lack of calcium. On the other hand, Dunn *et al* (2021) argued that the start of puberty (the age at which the first egg is laid) should be constantly monitored, and care should be given to ensure that appropriate bone growth occurs before the chickens commence lay. The occurrence and seriousness of bone disorders are influenced by both genetic and nutritional elements (Sanchez-Rodriguez *et al.,* 2021). In fact, the adoption of novel breeding selection methods since the early 1990s, along with the use of nutritional additives, have alleviated the issue by enhancing skeletal quality alongside promoting better growth performance. These observations can be explained by considering the individual's overall body size, as bone structure adapts in response to alterations body weight, and any extra body mass increases the stress placed on the skeletal system, which in turn impacts the skeletal system's strength and composition (Kolakshyapati *et al.,* 2019).

5.4.2. Mineral content in tibia bones of layer chickens

The tibia is the bone that supports weight of laying hens. It has frequently been used as a gauge for assessing skeletal development and bone mineralization in chickens, thanks to its rapid

growth and status as one of the most heavily mineralized bones in the skeletal structure of the hens (Benavides-Reyes *et al.,* 2021). Results from the current research depicted that Kalahari-melon based diets did not negatively influence bone mineralisation. In fact, the inclusion of the KMOC and feed additives resulted in the elevation of bone mineral content which is reflective of high mineral adequacy. Inadequate dietary levels of calcium (Ca), phosphorus (P), and essential trace minerals lead to reduced bone mineral content and diminished bone strength (Güz et al., 2019). An elevated bone ash value could suggest a higher presence of macro minerals like calcium (Ca) and phosphorus (P), leading to the conclusion that bones possess increased strength and greater resistance to fractures (Evaris et al., 2021). In our research, it was noted that the ash content was elevated in diets that included KM when compared to the control diet. Although there was high ash content, the content of Ca and P were lower than the control diet and the bone breaking strength was still high in all the diets containing Kalahari melon diets highlight the desirable effects it had on bone development as observed in other studies (Liu et al., 2020).

5.4.3 Internal organs of layers

Predicting the impact of diet and its constituents on the characteristics of internal organs in laying hens is critical in assessing feed digestion and assimilation that ultimately affect performance of laying hens. In general, when organ size expands, the energy necessary for organ upkeep escalates, subsequently diminishing the energy available for productive functions (Moon *et al*., 2021). Our results revealed that dietary Kalahari melon supplementation did not affect relative weights of internal organs (gizzard, liver, spleen, proventiculus, duodenum, colon and Ileum), This suggests that incorporating Kalahari melon (KM) into the diet at levels of up to 10% did not result in any noticeable negative impacts on organ growth and development. Our results for intestinal weights of gizzard, liver, spleen, proventiculus,

duodenum, colon and Ileum are similar to the previous findings from Marume *et al*. (2020). One of the notable findings from the study is that the diets had a significant effect on several internal organ parameters, including the length and weight of the jejunum, ileum length, ceca weight, and the temperature of the intestines. The increase in the length of the jejunum and ileum in response to the 10% Kalahari melon diet is particularly interesting. An increase in the length of the intestines may suggest the necessity to extend the duration of digestion processes (Marume et al., 2020) and a high dietary fibre of Kalahari melon diet. Our results are similar to that of Mansori *et al* (2006), where it was observed that birds receiving diets rich in fibre displayed larger gizzards and extended small intestines compared to the control group fed commercial diet.

5.5. Conclusion

This study discovered that these dietary interventions considerably influenced TW, TDPE and TWID, with the 10% KM diet showing the most significant effects on these parameters. On the other hand, tibia diameter at the distal end, tibia length, and bone breaking strength did not exhibit significant differences among the treatments. However, it's worth noting that overall mineral content in the tibia bones were not affected by diet, despite some variations in ash content. This could indicate that the mineralization of bones, particularly calcium and phosphorus levels, remained consistent among the dietary treatments. Interestingly, the study also revealed significant effects on certain internal organ parameters, including the length and weight of the jejunum, ileum length, ceca weight, and intestinal temperature. The increase in intestinal length may be associated with the dietary fibre content in the Kalahari melon diet. These findings emphasize the importance of dietary composition in influencing bone and internal organ parameters in layer hens and provide valuable insights for poultry nutrition and health management.

5.6. References

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CHAPTER 6

GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

6.1. General discussion

In the South African egg production sector, around 64 to 70% of the overall costs come from feed. The presence of high-quality protein elements and grains, which make up roughly half of the ingredients, varies based on seasons and years. This fluctuation leads to frequent shifts in ingredient prices, occasionally resulting in significant changes. Another posing threat to food security is the tremendous population increase (Manyeula, 2018). Presently, there's a push globally to replace soybean meal and maize with alternative protein sources and energy in monogastric animals (Alshelmaniv et al., 2021). This study contributes to the use of alternative protein sources by studying the effect of enzymes and polyethylene glycol on egg production performance in layers fed Kalahari melon containing diets. Chapter 3 of the study hypothesized that the inclusion of enzymes and polyethylene has no effect on feed intake (FI), feed conversion efficiency, health and nutritional status, body weight changes and serum cholesterol in layers given experimental diets containing KMOC. The research is not in agreement with the theory and demonstrates that there is a significant effect on feed intake and body weight changes. The findings of a high feed intake of layers on KM diets cannot be looked away. Nevertheless, our results suggested that KM, protease enzyme, and polyethylene glycol could be incorporated into layer diets as a replacement protein source, despite the fact that they resulted in some unfavourable production outcomes. However, the study accepts the hypothesis on hematology and serum biochemistry as there is no effect observed on the parameters. The observed result depicted that the health of layer chickens did not suffer any adverse effects by the inclusion of protease and polyethylene glycol in diets containing KM.

The hypothesis tested was that the inclusion of enzymes and polyethylene has no influence on egg production; egg quality parameters and fatty acid profiles in layers fed diets made up of KMOC. It was observed in chapter 4 that diets had no effect on all egg production indices apart from the NFEI. However, the number of cracked eggs was considerably decreased when PRO and PEG were included in meals separately. However, this does not prove anything related to the shell strength as there are many factors that contribute to cracked eggs, such as handling, animal aggression and nutrition. With regards to external quality parameters, diets did not have an effect on most parameters, diet only had an effect on Eggshell ratio, SHE-SB and Shell BS. All internal parameters, with the exception of the YI, HU, AWT, and AR, were significantly influenced by diets.

The research results in bone mineralisation (Chapter 5) depicted that the layers given 10% KMdiet exhibited the lowest ($P<0.05$) value for bone calcium, whereas the KMEP diet demonstrated the lowest value for bone phosphorus in comparison to the control diet. Increased ($P>0.05$) bone Mg and K were observed in layers fed the KMEP diet as to the control diet. This implies the inclusion of polyethylene glycol and protease in KM diet lower the content of Ca and P in bones and promote Mg and K in layer bones. The results agree with the proposed hypothesis that the inclusion of enzymes and polyethylene had no impact on the welfare and bone development of layer hens that were fed diets that contained Kalahari melon oil cake. The findings on bone development showed that diets had no effect on BBS, TL and TDDE while diets had an effect on TDPE, TWID and TW. The findings imply that dietary components didn't directly affect bone strength, length and TDDE, they might have influenced the composition or weight of certain bone parts or related structures. Further analysis or research might be needed to understand the specific mechanisms behind these effects and their potential implications for bone health or development.

6.2. General Conclusion

The rise in poultry production expenses necessitates exploring alternative ingredients like KMOC, known for its advantageous bioactive compounds, to potentially lower costs and enhance overall profitability. The study's focus on KMOC as a potential alternative protein source in layer feeds revealed mixed outcomes. While the inclusion of enzymes and polyethylene glycol influenced feed intake and body weight changes, it had minimal impact on health parameters. However, the findings pointed to the potential use of Kalahari melon as an alternative protein source, despite some negative production results in egg quality and bone mineralization. The research shed light on the complexities of dietary components in influencing egg quality, shell strength, and internal parameters. Notably, the study suggested that the inclusion of polyethylene glycol and protease altered bone mineral content in layers, indicating the need for further exploration into the mechanisms affecting bone health and development. In essence, while the study's findings showcased both positive and negative implications of incorporating Kalahari melon oil cake and additives in layer diets, it emphasizes the need for continued research to comprehend the intricate effects on egg production, quality, and bone health. Such insights are critical in optimizing alternative protein sources while ensuring the overall well-being and productivity of layer chickens within the context of the evolving challenges in the egg production industry.

6.3. Research recommendations.

1. Investigate the impact of incorporating KM in the diet of newly hatched chicks until they reach maturity to gain a thorough comprehension of KM utilization.
2. Assess the utilization of nutrients, growth performance, bone strengthening, and meat quality in broilers when fed diets containing phytase-treated KM.

3. Research on the cost analysis of producing Kalahari melon oil cake compared to soybean.
4. Research on the effects of polyethylene glycol and protease on indigenous chickens fed Kalahari melon containing diets.

6.4. References

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7. List of appendices

7.1. Appendix 1: Ethics approval

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ETHICS APPROVAL LETTER OF STUDY

Based on approval by the **North-West University Animal Production Sciences Research Ethics Committee (NWU-AnimProdREC)** on 02/12/2021, 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: Effect of enzymes and polyethylene glycol on egg production performance in layers fed diets containing Kalahari Melon

Study Leader/Supervisor (Principal Investigator)/Researcher: Prof U Marume

Student: Mauwane MD

Ethics number: N W U - 0 0 8 1 8 - 2 1 - A 5
Institution Study Number Year Status

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

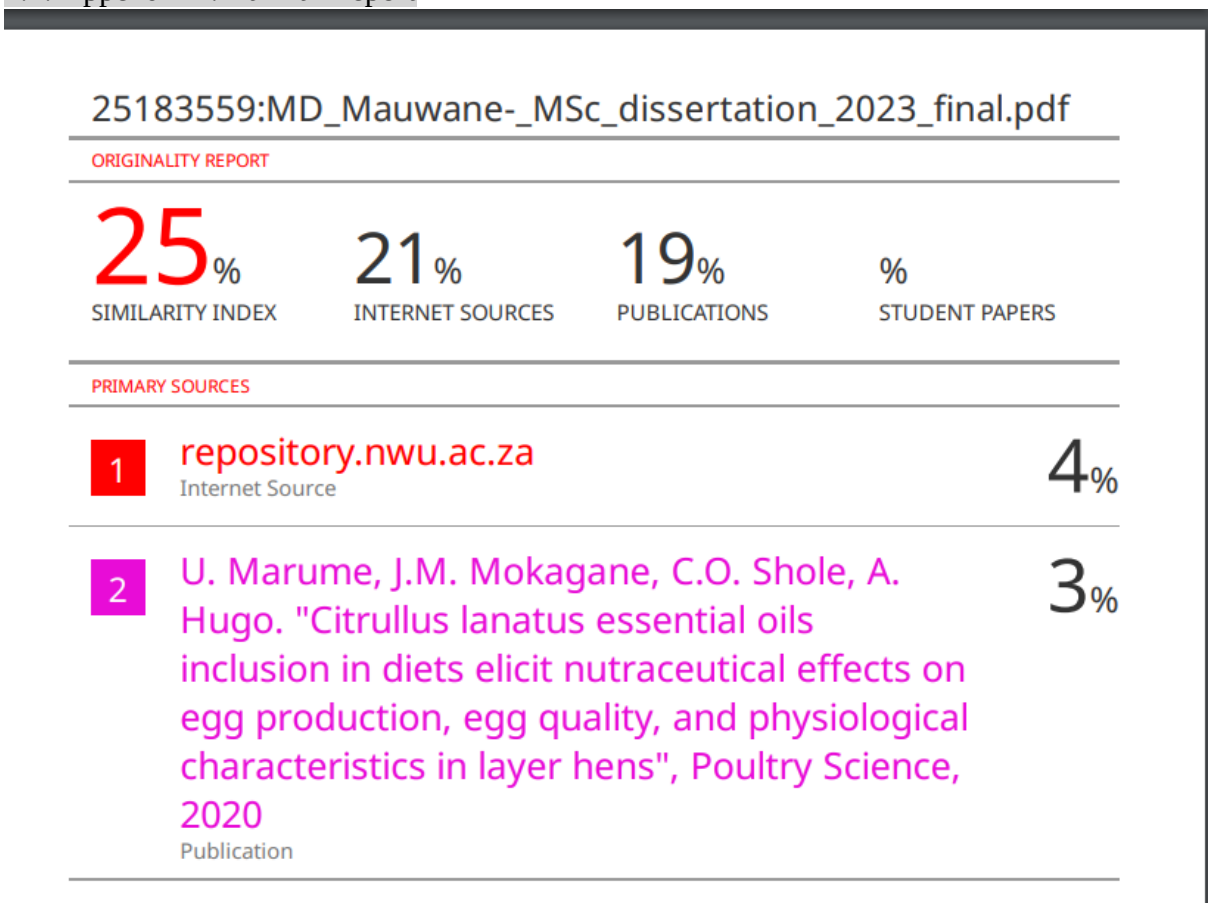
Application Type: Single Study
Commencement date: 2022/01/01 **Risk Category:** 2
Expiry date: 2022/12/31
Approval of the study is initially provided for a year, after which continuation of the study is dependent on receipt and review of the annual (or as otherwise stipulated) monitoring report and the concomitant issuing of a letter of continuation.

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

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- 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

7.2. Appendix 2: Turnitin report



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7.3. Appendix 3: Language editing certificate

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25th November 2023

Dear Sir/Madam,

RE: LANGUAGE EDITING CERTIFICATE: MD Mauwane (29632455)

This is to certify that the following Master's dissertation entitled: **Effect of enzymes and polyethylene glycol on egg production performance in layers fed Kalahari melon-based diets** written by MD Mauwane, has been edited for English language, grammar, punctuation, and spelling on November 24, 2023.

Yours faithfully,

Bukohwo Michael Esiefarienrhe, PhD
Language editor
Faculty of Natural and Agricultural Sciences.

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