PERFORMANCE OF IMPROVED INDIGENOUS GROWER CHICKEN IN KENYA FED ON TREATED MORINGA *(Moringa oleifera)* LEAF MEAL-BASED DIETS

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DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has not, wholly or in part, been presented for the award of a degree in any other University

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Recommendation

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DEDICATION

This work is dedicated to my Dad Mr. Davis Kiumbe Muremera, who sacrificed everything to ensure that I get an education and for his unwavering support during the entire period of my studies. The Almighty God bless you abundantly.

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ABSTRACT

Indigenous chicken (IC) contribute significantly as a source of animal protein to human population. However, productivity in Kenya is low due to inadequate supply of quality feed and high cost of commercial feeds. Moringa leaf meal *(*MOLM) is a locally available feed resource that can be utilized by chicken, however high fibre content in the meal leads to its low digestibility. This study tested the hypothesis that treatment of MOLM by fermentation with and without *Saccharomyces cerevisiae* and enzyme treatment would improve digestibility and thus improve performance of improved indigenous grower chicken. Data for the *in-vitro* digestibility and feeding trial was analyzed using the general linear model (GLM) procedure of the statistical analysis system version 9.1. Significant means were separated using Tukey's test at (p<0.05). In experiment one, the chemical composition, *in-vitro* dry matter and crude protein digestibilities of untreated, fermented and enzyme-treated MOLM were determined. Data was collected through an *in-vitro* digestibility procedure in a completely randomized design. In the fermented MOLM the content of crude protein significantly increased relative to the unfermented. The crude fibre, neutral detergent fibre, acid detergent fibre, acid detergent lignin, cellulose and hemi-cellulose content were significantly lower (p<0.05) in enzyme-treated MOLM relative to control. The *in-vitro* dry matter digestibility of enzyme-treated MOLM (60.3) was significantly different (p<0.05) compared with control (53.6%). Crude protein digestibility of enzyme-treated MOLM (63.1) was significantly different (p<0.05) compared to control (42.5). In experiment two, ninety (90) improved indigenous grower chicken were assigned to six treatment diets in a completely randomised design with a factorial arrangement where each treatment was replicated three times with 5 chicken per replicate. The feed intake decreased significantly (p<0.05) with the increasing level of MOLM in comparison to control. The ADG of the chicken fed 40% MOLM diets decreased significantly (p<0.05) compared to the control. There was a significant reduction (p<0.05) in the point of lay of the chicken fed enzyme-treated MOLM-based diets in comparison to control. The study concluded that enzyme pre-treatment was superior in improving digestibility and degradability of MOLM compared to all other treatments tested. Diets with 20-40% enzyme-treated MOLM were more expensive than the control diet and did not improve performance. Therefore, lower inclusion levels of 20-40 % enzyme-treated MOLM should be in-cooperated in the diets of improved indigenous grower diets for better performance and profits.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS AND ACRONYMS

ADF	Acid detergent fibre
ADG	Average daily gain
ADL	Acid detergent lignin
ANFs	Antinutritional factors
CF	Crude fibre
CP	Crude protein
DCP	Crude protein digestibility
DFI	Daily feed intake
DM	Dry matter
DMD	Dry matter digestibility
EE	Ether extract
FCR	Feed conversion ratio
FMOLM	Fermented *Moringa oleifera* leaf meal
GE	Gross energy
IC	Indigenous chicken
IVDMD	*In-vitro* dry matter digestibility
KALRO	Kenya Agricultural Livestock Research Organisation
KES	Kenyan shillings
ME	Metabolizable energy
MOLM	*Moringa oleifera* leaf meal
MT	Metric tonne
NCFR	Non-conventional feed resources
NDF	Nutrient detergent fibre
NFE	Nitrogen free extract
NSP	Non starch polysaccharides
SBM	Soybean meal
SSF	Solid state fermentation

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Agriculture contributes 25% of GDP in Kenya with poultry playing a major role, representing 30% of the agricultural contribution to Gross Domestic Product (GDP) (FAO, 2007). Kenya has an estimated poultry population of 31 million chicken. Of these, 75% consist of indigenous chicken, 22% of broilers and layers and 1% of breeding stock. Other poultry species like ducks, geese, turkeys, pigeons, ostriches, guinea fowls and quails make up 2% of the poultry production (MOLFD, 2012). The poultry sector is important for income generation especially in rural areas), food security and economic growth. It plays a very important role in sustaining the livelihood of many communities in African countries. Those reared in the rural areas account for 80% of the world's poultry stocks in many developing countries (Akinola & Essien, 2011). Local communities in many African countries consider poultry a very valuable asset as it contributes between 19-50% of the rural family income (Mottet & Tempio, 2017). Poultry play important socio-economic roles in developing countries because chicken are important for providing a cheap source of animal protein e.g. eggs and meat, generates income, and is a source of employment to the people (Olwande *et al*., 2010). The purpose of modern poultry production systems is to obtain maximum profit at minimum production cost, of which 60-70 % of this production cost consists of the feed cost (Tesfaye *et al*., 2013). The high cost of feed is attributed to competition with humans for protein sources, most of which are imported. Fishmeal and soybean meal are key protein sources for commercial poultry diets. However, their scarcity, high price, and increased demand have become a great impediment to the growth of the poultry industry (Adeniji, 2007). To lower this cost, there is a need to urgently explore alternative affordable protein sources that are not in competition with the humans for food.

Soybean meal and fishmeal have been widely and successfully used as conventional protein sources for livestock (Say, 1987). However, the prices of these protein sources have been escalating continuously in recent times, while availability is often erratic. The high interest in soybean is due to its high protein content of about 40% in the seed that is an important source of protein for human food and animal diet (Hungria *et al*., 2006). Popularity of soybean is expected to grow in the near future because of increasing need for food and fodder (Mugendi *et al*., 2010). The problem has been worsened by the increasing competition between humans and livestock for these protein ingredients as food. According to Odunsi

(2003) the rapid growth of the human and livestock population, which has created increased demand for food and feed in the less developed countries, requires that alternative feed resources must be identified and evaluated.

In most developing countries, poultry production sectors are facing some challenges such as increase in the feed cost because the major sources of protein (soybean meal, fishmeal) used to prepare feed in this sector are usually scarce and expensive (Nuhu, 2010). This situation has created the need to look for alternative affordable, locally available substitutes for some ingredients of poultry feed, in particular, sources of protein (Gadzirayi *et al*., 2012). In this context, one of the alternative cost-effective sources of protein that can be used in poultry nutrition is the leaves of tropical legumes such as Moringa (Makkar & Becker, 1997).

Moringa (*Moringa oleifera* L.) leaves are a good source of protein, vitamins A, B complex, C, and E, various phenolic compounds, and minerals such as calcium, iron, and potassium that make them a potential replacement for soybean meal in non-ruminant diets (Yang *et al*., 2006). Sarwatt *et al*. (2004) reported that Moringa leaves are a potential inexpensive protein source (26-27% CP) for livestock feeding. It is a perennial plant whose leaves can be harvested several times in one growing season. However, it has high dietary fibre that affects nutrient utilization by binding or encapsulating to or with amino acids, minerals and fats reducing the accessibility by endogenous enzymes hence reduced nutrients absorption. This study determined the *in-vitro* digestibility and effect of feeding treated and untreated Moringa leaf meal based diets to improved indigenous grower chicken on feed intake, feed conversion ratio, average daily gain, point of lay and the gross margin in Kenya.

1.2 Statement of the Problem

Soybean meal and fishmeal are the main protein sources for non-ruminant livestock feed. Their prices are high; the supplies are often erratic and are of poor quality due to adulteration. There is also increasing competition between humans and livestock for these protein ingredients as food and feed. *Moringa oleifera* leaf meal (MOLM) has been identified as a locally available alternative protein feed resource. However, high inclusion levels in diets negatively influence animal growth performance. This is because it contains high crude fibre and other associated anti-nutritive factors. However, its treatment with exogenous enzyme or fermentation has been suggested to improve digestibility. These interventions have not been conclusively evaluated in grower chicken as a possible means to improve utilization

of MOLM. Therefore, this research was carried out to determine the effect of fermentation and enzyme treatment on the utilization of the meal.

1.3 Objectives

1.3.1 Broad Objective

To contribute to sustainable chicken production for food and nutrition security and improved livelihood by incorporating MOLM as a protein source in improved grower IC diets.

1.3.2 Specific Objectives

i. To determine the effect of fermentation and enzyme treatment on the chemical composition, *in-vitro* dry matter and crude protein digestibility of Moringa (*M. oleifera*) leaf meal.
ii. To determine the effect of feeding enzyme-treated MOLM-based diets on feed intake, feed conversion ratio, average daily gain and point of lay of improved indigenous grower chicken.
iii. To determine the economic implication of feeding enzyme-treated MOLM-based diets to improved indigenous grower chicken in Kenya.

1.4 Hypotheses

i. Incorporation of fermented and enzyme-treated Moringa (*M. oleifera*) has no significant effect on the chemical composition, *in-vitro* dry matter and crude protein digestibility.
ii. Incorporation of enzyme-treated MOLM in the diet of improved indigenous grower chicken has no significant effect on feed intake, feed conversion ratio, average daily gain and point of lay.
iii. Incorporation of enzyme-treated MOLM-based diets to improved indigenous grower chicken has no significant effect on the economics of production.

1.5 Justification

The rapid growth of the human and livestock population has created increased demand for food and feed in the less developed countries. The importance of IC in wealth creation, food nutrition, security at national and household levels in Kenya is well recognized (Magothe *et al*., 2012). Consequently, IC contributes over 40% and 60% of the chicken eggs and meat produced in the country, respectively (Wanjohi, 2015). This low productivity has been attributed to poor quality feed since they depend on scavenging feed resources which may be inadequate in nutrient supply which has led to researchers looking for alternative feed to improve productivity in improved IC and shift from scavenging to semi-intensive systems due to high demand for eggs and meat. Performance of improved indigenous chicken can be achieved through improved nutrition. Conventional protein sources e.g. soybean meal and fishmeal are expensive, erratic in supply and are often of poor quality and this has led to increased importation of soybean meal, hence the need for search and evaluation of locally available alternative sources of protein (Kim *et al*., 2019).

Moringa leaf meal has been identified as an alternative protein source (26-27 % CP) but has high fibre content (11.4-19.2% CF) (Gakuya *et al*., 2014). Dietary fibre and the associated anti-nutritional factors e.g. tannins, saponin, oxalates and phytates adversely affect nutrient utilization by binding or encapsulating to or with amino acids, minerals and fats, reducing the accessibility by endogenous enzymes. This reduces nutrients absorption, hence the need to treat the meal with a commercial enzyme. Enzymes break down the NSPs, reduce intestinal viscosity, and subsequently get better nutrients digestibility by improving gut performance (Amerah, 2015). They cause the disruption of the plant cell wall integrity and consequent release of nutrients encapsulated by the cell wall (Ravindran, 2013).

Non starch polysaccharides (NSP) e.g. cellulose, hemicellulose and pectin which are in MOLM reduce effective energy and nutrient utilization by non-ruminant animals. They lack the enzymes needed for breaking down the complex cell wall structure that encapsulate other nutrients. Growing chicken tolerate crude fibre of up to 3-4% in feed, levels beyond this limit impacts negatively on voluntary feed intake, nutrient digestibility and growth rate (Mateos *et al*., 2013). This study evaluated the *in-vitro* digestibility of fermented, enzyme treated and untreated MOLM and performance of improved grower IC fed on treated MOLM based diets in Kenya. Results obtained from this study have provided a guide in the treatment

and use of treated MOLM as a protein ingredient in poultry feed. In terms of policy, it will guide on the production and utilization of MOLM as a feed resource.

1.6 Definition of Terms

Indigenous chicken: It is a pure breed of chicken unadulterated by research. They mature within a period of six months, are disease resistant and are raised for both meat and eggs

Improved indigenous chicken: This is a type of chicken that came about as a result of breeding research by the Kenya Agricultural &Livestock Research Organization (KALRO). It is the result of breeding different types of indigenous chicken in Kenya from different areas. In this research KALRO sought to look for and marry unique characteristics in the chicken e.g. high egg production, faster growth and good feed to meat conversion.

Rainbow roosters: It is a multicolored, dual purpose crossbreed indigenous chicken that is as a result of Indian research at the Indro Research Breeding farm in Hyderabad. It is the result of breeding fast growing broilers, high laying brown chicken and disease resistant indigenous chicken germ plasm. They mature within a period of four months and have low resistance to diseases.

Kuroiler: It is a hybrid breed of chicken developed in India by Kegg Farm Limited. They are derived from crossing either colored broiler males with Rhode Island Red females or White Leghorn males crossed with Rhode Island Red females. They are dual-purpose breed producing meat and eggs, medium feeders and have low resistance to diseases.

Kenbro: It is a dual-purpose chicken breed which is a product of Kenchic Limited. They are heavy feeders, have a maturity period of four to five months and have low resistance to diseases.

Sasso: It is a dual-purpose chicken breed from the France-based SASSO poultry breeding company. They mature within a period of six months, adapt to hot and humid conditions and are resistant to diseases.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of Chicken Production in Kenya

Poultry, particularly chicken are the most widely and numerous kept livestock species in the world (Moreki, 2014). In Africa, indigenous chicken makes up over 70% of total chicken population (Thornton *et al*., 2021). Kenya has an estimated poultry population of 28.5 million. Of these 22 million (76%) are free-ranging indigenous and 6.5 million (24%) exotic chicken and plays a very central role as far as household income is concerned while contributing to food security and the improvement of nutrition (Mwobobia *et al*., 2016).

2.1.1 Overview of Indigenous Chicken Production in Kenya

Indigenous chickens are widely distributed in rural and peri-urban areas where they play the important role of income generation and food production (Moreki *et al*., 2010). They are various indigenous breeds which include rainbow rooster, Kuroiler, Kenbro and improved IC. Kenya Agricultural Research Institute (KARI) - Naivasha developed "improved IC" through years of intensive research under the National Poultry Development Programme. Improved IC were developed as a result of breeding different types of indigenous chicken in Kenya from different areas. In this research Kenya Agricultural Research Institute sought to look for and marry unique characteristics in the chicken. They are easy to establish for low-income families, develops faster, is highly resistant to diseases and has high productivity compare to the pure indigenous chicken. About 90% of small-scale farmers in Kenya rear poultry, majority of which are indigenous chicken followed by exotic chicken breeds (King'ori *et al*., 2010). The chicken are hardy and thrive under harsh environment with minimal inputs. They get most of their feed from scavenging and may occasionally benefit from kitchen and other household wastes. Eggs and chicken meat contribute to the protein nutrition of the rural population thus alleviating malnutrition. Productivity is low due to factors such as genotype, poor nutrition, diseases and management. Promotion of indigenous chicken production therefore economically empowers the rural youth and women (Guèye, 2009). There is potential for increasing production and productivity of indigenous chicken by improving housing, disease control, nutrition and genetics (Okitoi & Mukisira, 2001).

2.1.2 Nutrient Requirements of Indigenous Chicken

There are many inconsistencies documented in the nutrient requirements for indigenous scavenging chicken. The feed efficiency improved as the protein level of the diet increased. This is in agreement with the findings of Morris *et al*. (1987) who reported progressive reduction in the efficiency of utilization of lysine as the CP concentration of the diet increased from 140 to 280 g/kg. The similar gain and FCR observed for chicken offered diets containing 160 requirements for growing indigenous chicken. This may have led to increased amino acid degradation, resulting in no further increase in growth and efficiency of feed utilization. As the supply of an amino acid is in excess of that required for protein synthesis, amino acid catabolism increases. This ensures that when amino acids are in short supply they are preferentially used for body protein synthesis (Kim *et al*., 1983). The activity of most amino acid catabolizing enzymes increases with increased dietary supply of protein. Ndegwa *et al*. (2001) reported that indigenous growing chicken fed diets containing 170-230 g CP/kg had similar growth rates and feed intake, suggesting that the 170 g CP/kg diet was sufficient for these chicken. Chemjor (1998) reported that a dietary protein level of 130 g/kg during the 14-21 weeks' growth phase was adequate for indigenous chicken.

Crude protein (CP) requirements for growing indigenous chicken are 20, 16 and 14%, during week 5-8, 8-14 and 14-21. Energy requirements during the same growth period are approximately 3000, 2600 and 2400 kcal/kg ME, respectively. Indigenous chicken during the 8 to 14-week growth phase require a 16% CP concentration of approximately 75g/day of 16% CP (King'ori *et al*., 2010). Table 2.1 shows the nutrient requirement of indigenous chicken.

Table 2.1 Nutrient requirements of indigenous chicken

Nutrient	**Week 5-8**	**Week 8 -14**	**Week 14-21**
Energy (ME kcal/kg)	3000	2600	2400
Crude protein (%)	20	16	14

Source: King'ori *et al*. (2010)

2.1.3 Challenges Facing Chicken Production

Chicken production in Kenya is low due to many factors such as poor nutrition, poor genotype, diseases, harsh environment, predation, high feed costs, marketing challenges and poor poultry management e.g. poor brooding and housing, poor litter management and poor biosecurity measures (King'ori *et al*., 2014). Poor nutrition and high feed costs are the focus of this research and aim to provide knowledge on the locally available alternative source of protein supplement (MOLM) for feed formulation. The performance, cost of feeding and point of lay of chicken fed on diets based on this ingredient will be determined.

2.1.4 Conventional Protein Sources

The main protein ingredient for poultry diets globally are of plant or animal origin e.g. soybean meal and fishmeal (Chisowa, 2002). Much of the fishmeal and soybean meal used in Kenya are imported. They are also a major source of protein in humans, are expensive and their use as animal feed ingredients is limited. The main source of plant protein in Kenya is soybean meal while others include sunflower cake, cottonseed cake, lupins and to a lesser extent groundnut cake (FAO, 2002). Soybean meal accounts for more than 70% of the protein source used in compounded feeds for poultry and other livestock (Ravindran, 2013). The presence of anti-nutritive factors in most of the legume protein sources such as trypsin inhibitors may limit their use in animal feeds due to the added cost of processing to get rid of the anti-nutritive factors (Kalimbira, 2000).

Soybean has a high commercial value and contains all the amino acids required by the human body except methionine, usually found in cereals such as maize (Osho, 1995). Under-processing may lead to deleterious levels of anti-nutritional factors e.g. tannins, oligosaccharides, phytates that form complexes with minerals calcium and phosphorous reducing their bioavailability. Trypsin inhibitors reduce protein digestibility and saponin decrease feed palatability which may impact negatively on the growth and performance especially in young animals. Say (1987) reported that soybean meal must be subjected to heat treatment that improves its digestibility and destroys some of the toxic factors present in the raw soybean.

2.1.5 Non-conventional Protein Sources

Non-conventional feed resources (NCFR) refer to all those feeds that have not been traditionally used for feeding livestock and are not commercially used in the production of livestock feeds (Amata, 2014).The potential of NCFR that can be used to either replace or

supplement the nutrition of livestock include agro-industrial by products e.g. Wheat bran, maize bran, copra meal, cotton seed cake, ground nut cake, rice bran and soybean hulls, some common tropical browse plants and leaf meals which include *Gliricidia sepium* leaf meal, mulberry leaf meal, Moringa leaf meal, plant algae, croton seeds, palm leaf meals, palm press fibre, cassava foliage, spent brewer's grains, sugar cane bagasse, rubber seed meal and some aquatic plants (Chadhokar, 1984).

The scarcity of feed resources for livestock and poultry feeding has diverted majority of research in the field of animal nutrition to look into possibilities to overcome this nutritional crisis. One approach is to exploit the available NCFR in livestock production systems. Most of these feed materials are low in energy, protein, minerals and contain high amounts of anti-nutritional components (Salem *et al.*, 2005). The major constraints to the use of NCFR are collection, storage, dehydration due to high moisture content, high crude fibre content and detoxification processes (Devendra, 1985). There is an urgent need for development of processing techniques that are economical and practicable. With the unfolding trend of increased prices and scarcity of the conventional protein sources, the global market is highly primed to embrace alternative non-conventional protein sources. In this context, one of the alternative cost-effective sources of protein that can be used in poultry nutrition is the leaves of tropical legumes such as Moringa (Makkar & Becker, 1997).

2.2 Origin and Distribution of Moringa *(M. oleifera)*

Moringa belongs to the moringaceae family, and is considered to have its origin in the north-west region of India, south of the Himalayan Mountains. It is now widely cultivated and has become naturalized in many locations in the tropics (Fahey *et al.*, 2001). It is widely cultivated because it is a multipurpose tree whose leaves are rich in protein, vitamins and phytochemicals that are used as a dietary supplement in animals and humans.

2.2.1 Climatic Requirements of Moringa Production in Kenya

Moringa is a highly valued plant and can easily be established in the field, has a good coppicing ability, as well as good potential for forage production. It mostly grows in Makueni, Kilifi, Busia, Tharaka Nithi and Taita Taveta Counties in Kenya. There is the possibility of obtaining large amounts of high quality forage without expensive inputs due to favourable soil and climatic conditions since it grows best in a wide range of soil conditions. Moringa tree grows best in 25 to 35^{o}C, altitudes up to 1200 m above sea level and tolerates a pH range of 5.0-9.0, meaning it can grow in acidic and alkaline soils. Only 250 to 3000 mm

of annual rainfall is required and can be grown in well-drained sandy loam and dry clay (Thurber & Fahey, 2009).

A study consisting of many trials to determine the optimum density at which Moringa should be planted to produce a maximum amount of fresh green matter was conducted by Makker and Becker (1997). Leaves can be harvested within a year of planting and flowers and pods can be harvested after two years. Within three years, one tree will produce 300-400 pods while a mature tree can yield up to 1000. It grows up to 12 meters in height. Spacing in the trials ranged from 1 m × 1 m or 10,000 plants per ha to 3.0 cm × 3.0 cm or 16,000,000 plants per ha. The optimum density in sandy, well drained and fertile soils was found to be 10 cm ×10 cm or 1,000,000 plants per ha.

Mendieta (2013) reported that whether produced for use as a green manure, for livestock or human consumption, Moringa can be grown intensively with yields up to 650 metric tons of green matter per ha. A study conducted by Akinbamijo *et al*. (2004) reported that Moringa under high density cultivation using a planting density of 15 cm × 15 cm, biomass yields in excess of 15 tons DM per ha in a 60-day growing cycle was obtained at the International Trypano-tolerance Centre in the Gambia.

2.2.2 Nutrient Composition of Moringa Leaf Meal

The chemical composition of Moringa leaf meal was shown to be: dry matter (DM) 93.63%, protein (CP) 27.1%, fibre (CF) 19.2%, fat (EE) 6.41%, energy (GE) 14.790 (MJ/kg) and fatty acids 2.31%, calcium 2.37%, potassium 0.97%, phosphorus 0.2 %, magnesium 0.4% copper 6.1%, vitamins 6.241%, phytate 2.57%, trypsin inhibitors 3.0%, saponins 1.60% and oxalates 0.45% (Ogbe *et al*., 2012). The nutrient composition is presented in Table 2.2

Table 2.2 Chemical composition of MOLM

Chemical component (%)	Moringa leaf meal
Dry Matter	93.63
Gross energy(MJ/kg)	14.790
Crude protein	27.1
Ether extract	6.41
Crude fibre	19.2
Fatty acids	2.31
Vitamin B_1	1.003
Vitamin B_2	1.21
Vitamin B_3	1.01
Vitamin C	1.02
Vitamin B_{12}	1.002
Vitamin E	0.915
Calcium	2.37
Magnesium	0.4
Phosphorous	0.2
Potassium	0.97
Copper	6.1
Phytates	2.57
Trypsin inhibitors	3.0
Saponins	1.6
Oxalates	0.45

Source: Ogbe *et al.* (2012)

The seeds and leaves contain all essential amino acids and these are at higher than adequate concentrations when compared with the recommended amino acid pattern of requirements for most farm animals (Oliveira *et al*., 1999). Essential amino acid contents of Moringa leaves and seeds are shown in Table 2.3.

Table 2.3 Amino acid Composition of Moringa Leaves and Seeds

Essential amino acids	Leaves (g/kg)	Seeds (g kg)	Requirements(g/kg)
Histidine	2.2	2.1	1.7
Isoleucine	4.2	2.3	4.0
Leucine	7.0	6.8	6.7
Lysine	4.4	4.2	4.5
Methionine	1.2	1.0	2.0
Phenylalanine	4.0	3.5	3.6
Threonine	2.2	3.0	3.7
Tryptophan	4.4	4.3	1.1

Source: Oliveira *et al*. (1999) and NRC (1984)

2.2.3 Potential Use of Moringa Leaf Meal as a Feedstuff in Poultry Diets

The increased consumption of livestock, poultry, and fish products in people's diet threatens to drive production toward the use of more and more conventional crops in animal feeds. In this context, alleviating the tightening grain crop supply and ensuring the healthy development of animal husbandry through innovations in protein feedstuff production remain a considerable challenge (FAO, 2002). *Moringa oleifera* is a tree species with abundant nutrients and high biological value protein as it promotes animal growth and production. As a potential protein feedstuff, MOLM has great potential in alleviating the feed supply and price challenges.

Chicken will not voluntarily consume Moringa leaves or Moringa leaf powder alone because of the presence of secondary plant metabolites. Glucosinolates reduce palatability whereas thiocyanates, isothiocyanates and nitriles interfere with iodine availability and affect kidney and liver function, hence decrease growth and production. However, about half the protein content can be extracted from the leaves in the form of a concentrate that can be added to the chicken feed (Price, 2007). According to Fuglie (2000) the nutrient value of Moringa leaves for chicken can be improved through the addition of phytase to break down phytate, leading to increased absorption of phosphorus. The study conducted by Tesfaye *et al*. (2013) reported that Moringa leaf meal positively affects the average final body weight, average daily feed intake and feed conversion of broilers as compared to the control diet. The study also investigated the effects of 0%, 5%, 10%, 15% and 20% MOLM on the growth

performance of broilers. They reported that MOLM can substitute soybean meal in the broilers diet up to 5% of the total ration without a negative effect on the performance in terms of feed intake and weight gain of chicken since it did not exceed the crude fibre limits.

David *et al*. (2012) reported that the addition of the two levels (0.05% and 0.1%) of Moringa leaf powder in broiler rations improved the growth performance and carcass yield of broilers. Unlike these studies, Paguia *et al*. (2014) reported that the addition of Moringa leaf powder in broiler diets did not significantly influence the broiler's feed intake, body weight gain, feed conversion ratio, final weight, feed cost per kg of broiler produced and income over feed and chick cost. Annongu *et al*. (2014) observed that the growth performance of the broiler decreased following the increasing level of Moringa leaf meal in the diet. However, a study by Gadzirayi *et al*. (2012) reported that the addition of Moringa leaf meal as a protein supplement in broiler diets at 25% promoted more growth than commercial diets. Kakengi *et al.* (2007) reported that Moringa leaf meal could be incorporated up to 10 -15 % in laying hen rations

2.2.4 Effect of Moringa Leaf Meal on Performance of Chicken

Briones *et al*. (2015) stated that Moringa leaves can be applied as a dietary supplement in layers and broilers due to high production performance and improved egg quality. However, still, there are many debates on the chicken's performance with different doses of Moringa in the previous studies. There are also many variables on doses and part of the plant used, such as leaves, extract, pods, or seeds. Many scientists agreed that Moringa leaves might have a positive role in improving the production performance and health status in chicken. Further studies are still needed to detect the actual doses of application for optimum performance in chicken.

Alabi *et al*. (2017) used aqueous *M. oleifera* leaf extracts in broiler chicken and the study demonstrated that average daily body weight gain and final body weight were higher in 120 ml/litre extract-supplemented groups than the control. The feed conversion ratio was lower in chicken on 90 ml/litre and 120 ml/litre of leaf extracts fed groups. The authors suggested that Moringa leaf extracts can be added up to 90 ml/litre in broiler chicken for optimum performance. The author stated that Moringa leaf meal can be applied as a natural source of protein in broiler diets where inclusion of *M. oleifera* leaves at levels of up to 20% in broiler diets resulted in a higher growth rate and better health status in broilers (Alnidawi *et al*., 2016). In addition, dietary supplementation of *M. oleifera* leaves up to 20% level showed higher growth performance in broilers (Moreki & Gabanakgosi, 2014). The Final live

weight, average weight gain, and FCR were higher in 10% Moringa leaf meal supplemented diets than the control through a 35-day trial period. Feeding *M. oleifera* leaf powder at 10% level improved live weight, body weight gain, dressing percentage and FCR in broilers (David *et al*., 2012). Onunkwo *et al*., (2015) stated that Moringa leaf meal can be used at the level of 10% in chicken diets to reduce the production cost.

Gadzirayi *et al.* (2012) replaced conventional soybean meal with Moringa leaf meal in broiler diets at 0%, 25%, 50%, 75%, and 100% level and reported no significant differences in feed intake and body weight gain between control and 25% level of Moringa supplementation. The study suggested using Moringa leaf meal at a 25% level to promote growth rate in broilers. In addition, Ayssiwede *et al.* (2011) reported that dietary inclusion of 24% Moringa leaf meal had no adverse effects on body weight, average daily weight gain, FCR, mortality, and the weight of organs in broilers compared to the control diet. Olugbemi *et al*. (2010) stated that the average daily growth rate was lower with inclusion of Moringa leaf meal at below 5% in diets, and suggested that using a maximum level of 5% would have no harmful effects on growth performance and FCR in broilers. These findings confirmed that feeding with Moringa leaves had no deleterious effects on normal physiology and growth in the experimental broilers. However, some authors collectively suggested that use of the *M. oleifera* leaf meal up to 10% level would not have any adverse effects in broilers (Abou-Elezz *et al*., 2011).

2.2.5 Effect of Moringa Leaf Meal on Broiler Meat Quality

Dietary manipulation is an important way to improve meat quality in poultry (Cheng *et al*., 2019). Tenderness, pH of meat, colour (lightness, redness, and yellowness), and water holding capacity are very important meat quality characteristics to the consumers. An experiment on the supplementation of Moringa leaf powder on the quality of meat in broilers indicated that supplementation of leaf powder at 12 g/kg of diet increased pH, water holding capacity, and muscle fibre diameter in the breast muscle of the broilers. Also, higher weight, ash percentage, and the density of tibia bone in broilers fed on diets with Moringa leaf meal were reported by Rehman *et al*. (2018).

In contrast, Nkukwana *et al*. (2014) reported that Moringa leaf meal had no effects on tibia bone characteristics but improved body weight gains and FCR. These differences might be related to inclusion levels of Moringa in broiler diets. Dietary antioxidants can modify the meat colour, minimize the rancidity and retard lipid peroxidation, resulting in well-maintained meat quality. Therefore, dietary supplementation of antioxidant-enriched Moringa

leaves would be a potential strategy to improve the meat quality in broilers. The inclusion of Moringa leaf meal improved the fatty acid profile and reduced lipid oxidation in breast muscle of broilers (Nkukwana *et al*., 2014). In the study reported here, higher saturated fatty acids in meat from MOLM supplemented chicken was noted. Gallic acid and linoleic acid was observed to increase arachidonic and docosahexaenoic acids in broiler meat (Jung *et al*., 2010). Qwele *et al*. (2013) concluded that the percentage lipid oxidation inhibition observed in the meat from MOLM supplemented animals indicated the defence mechanism in the animal system to prevent the formation of excessive free radicals. Antioxidant activity in *M. oleifera* leaves is further enhanced by the presence of glucosinolates, which carry a benzyl-glycoside and hydrolyses to isothiocyanates, thiocyanates, or nitriles upon enzymatic hydrolysis (Mbikay, 2012).

2.2.6 Effect of Moringa Leaf meal on Health Status of Chicken

Moringa leaves contain antioxidant properties, phytochemicals (carotenoids, flavonoids, chlorophyll, phenolics, xanthins, cytokines, alkaloids, etc.) that might have a role in improving health status (Falawo *et al*., 2014). The extract from the Moringa leaves has a potential role as an anti-bacterial and antioxidant (Sreelatha *et al*., 2016). The authors stated that Moringa leaf meal should be used within the 10% level in broiler diets. Oluduro *et al*. (2010) and Pandey *et al*. (2012) have highlighted that *M. oleifera* exhibited 4-(α-L-rhamnopyranosyloxy) benzyl isothiocyanates, methyl N-4-(α-L-rhamnopyranosyloxy) benzyl carbamate, and 4-(α-D-glucopyranosyl-1→4-α-L-rhamnopyranosyloxy) benzyl thiocarboxamide that were able to play antimicrobial properties. The antimicrobial activities of the MOLM may be due to presence of lipophilic compounds and different metabolites (carboxylic acid, 2,4-diacetyl phloroglucinol, enzymes, and chitinases) in plant cell walls (Jabeen *et al*., 2008).

Analysing blood parameters is very important in detecting the health status of chicken (Voemesse *et al*., 2019). Serum albumin level was higher in laying hens fed on diets with 3% level of Moringa leaf meal than the control group, but the number of white blood cells, red blood cells, lymphocytes, and the packed cell volume were lower in groups fed Moringa based diets than the control diet. The authors assumed that lower white blood cells and lymphocytes in chicken fed Moringa based diets may be due to the antimicrobial activity of phytochemicals e.g. alkaloids, saponins and flavonoids in the Moringa leaves. It is known that a high white blood cell count is related to an infection caused by bacteria in the host. A lower level of cholesterol content in serum with dietary supplementation of Moringa pod

meal was observed, which might be influenced by antioxidants (flavonoids and carotenoids) and high fibre content in the Moringa pod meal in the experimental diets (Ahmad *et al*., 2017). Moringa is an effective phytobiotic and is known to possess broad-spectrum antibacterial properties and immuno-modulatory functions (Lurling & Beekman, 2010).

2.2.7 Effect of Moringa Leaf Meal on Egg Production and Quality in Hens

The egg quality parameters, including egg size, shape, colour, shell thickness, and egg yolk cholesterol, directly and indirectly, influence egg consumer preference. Moringa leaf meal was used in layer chickens' diet from day-old old to 55 weeks of age to investigate the effects of the meal on growth performance, egg production performance, and blood parameters. The leaf meal was used at 0, 1, and 3% (Voemesse *et al*., 2019). In the growing period from day-old to 20 weeks of age, there were no significant differences in feed intake, but average daily body weight gain, final body weight, and FCR were improved in *M. oleifera*-supplemented groups. In the laying period, from 21 to 55 weeks, feed intake was lower in groups fed Moringa supplemented diets, but the laying percentage and FCR were higher in supplemented fed groups than the non-supplemented group. The higher body weight gain and egg production may be related to improved digestibility in supplemented groups due to different active components including vitamins, phenolic acids, flavonoids, isothiocyanates, tannins and saponins in Moringa leaves. The authors concluded that feeding Moringa leaf meal supplemented diets at 1% level had positive effects on the growth and egg production in laying hens. Moreki and Gabanakgosi (2014) reported that inclusion of *M. oleifera* at 10% level resulted in higher egg production in laying hens.

According to Abou-Elezz *et al*. (2011), *M. Oleifera* supplementation improved the egg production, egg mass, and egg yolk colour scores compared with the non-supplemented groups. The improvement of yolk color scores could be due to high carotene content in Moringa leaves. Feed intake, feed conversion ratio, and laying percentage were not influenced by adding Moringa leaf meal at a 10% level. However, the inclusion of 10% Moringa leaf meal increased the Roche egg Roche colour (Olugbemi *et al*., 2010). A similar report on decreased egg mass and egg production percent with Moringa leaf meal supplementation at higher levels (>10%) in laying was recorded. Moringa leaf meal at 5% level increased the egg weight, but decreased egg weight was found when the inclusion level was at 20%. The authors assumed that higher feed intake; FCR with lower egg production percent, egg mass, and egg weight at a higher-level supplementation was due to poor

digestibility of nutrients because of presence of different anti-nutritional phytochemicals in Moringa leaves (Kakengi *et al*., 2007).

2.3 Dietary Fibre in Poultry Nutrition

Fibre refers to cell walls of plant tissue that mostly consist of lignin, cellulose and hemicelluloses. It is a composition of plant cell that is resistant against enzymes in the small intestine. Moreover, from the chemical viewpoint, fibre is illustrated as non-starch polysaccharides (NSP) (McDonald *et al*., 2002). Researcher precisely recognize the percentage of crude fibre for poultry; could be in a range from 3 to 4% for a long period while it could be 5% for layers and must be kept below 7% in poultry feed. Fibre is viewed to be negative as it declines growth performance and production in chicken growth by reducing effectiveness of feed utilization, therefore this has led to a more targeted use of nutraceuticals e.g. NSP-degrading enzymes and gut enhancers. Research conducted with poultry has considered dietary fibre as diluents of the diet with negative impact on voluntary feed intake and nutrient digestibility (Mateos *et al*., 2013).

Feeding of fibre to poultry has generally been discouraged primarily because of the negative effects that fibre exerts on performance and nutrient utilization. As indicated earlier, cellulose and hemicellulose are not well digested. Inclusions of high fibre ingredients are usually limited because of the poor metabolizable energy contents. Non-starch polysaccharides are considered to cause adverse reactions in the digestive tract of chicken. As reviewed by Bedford (1996), viscous grains e.g. barley, sorghum have numerous effects on the digestive tract of the chicken and nutrient utilization. Grains, which increase the viscosity of contents in the intestine, are rye, barley, oats, triticale and wheat. In wheat and rye, arabinoxylans are the predominant NSP and β-glucans in barley. Increasing the viscosity decreases nutrient utilization. An increase in viscosity of digesta may limit mixing of nutrients with pancreatic enzymes and bile acids (Edwards *et al*., 1988).

Moreover, movement of nutrients towards the gastrointestinal wall is reduced by an increase in digesta viscosity, which consequently limits digestion and absorption (Fengler & Marquardt, 1988). Feeding of such grains also increases the size of the digestive tract and pancreatic mass. Often, the inclusion of these ingredients such as rye will degrade the litter through increased moisture. Research by Langhout *et al.* (2000) indicated that the anti-nutritive effects may be related to gut microflora as conventional chicks fed a diet high in pectin had reduced digestibilities of fat, starch and amino acids as well as reduced N retention and metabolizable energy compared to feeding of the NSP to germ-free broiler chicks. The

effect of the microflora may be the result of where fermentation takes place. Work by Choct *et al*. (1996) indicated that a large amount of fermentation as the result of feeding soluble NSP occurred in the small intestine whereas with enzyme supplementation fermentation was shifted from the small intestine to the caecum. Enzyme supplementation is used to improve the digestibility when such grains are used in poultry diets. In wheat-based diets, utilization of a xylanase supplement decreased intestinal viscosity and improved apparent metabolizable energy content and starch digestibility (Choct *et al*., 1999).

2.4 Strategies to Improve Utilization of Fibrous Diets by Chicken

Some feed ingredients and additives are reported to modulate gut microbiota and immune system of the host (Jha, 2015). Antibiotics have been used to modify gut microbiota and were revered by farmers as they promote growth performance of poultry. However, concern about antibiotic resistance and other negative impacts of the use of antibiotics as a growth promoter, have forced poultry farmers to stop or limit their use in feed. Feed additives and supplements like probiotics, prebiotics, organic acids, and exogenous enzymes are used as an alternative to antibiotics to modulate the gut microbiota with some success. So far, the nutritional value of Moringa leaves has mainly been improved by heating, grinding, cooking, physical and chemical means (Vongsak *et al*., 2013). Although some of the ANF can be removed, the nutrient content may also be destroyed by these processes. Therefore, a more suitable process for improving MOLM feed quality is needed.

2.4.1 Use of Exogenous Enzyme Treatment

Enzymes are specialized proteins that catalyse or accelerate the chemical reaction. The enzyme activity may be substrate dependent or through the particular site on substrates such as fat, protein, or carbohydrate. Commonly used exogenous enzymes in poultry diets are β-glucanase, xylanase, amylase, α-galactosidase, protease, lipase, and phytase (Adeola *et al*., 2011). The role of exogenous enzymes is to fulfil the absence of endogenous enzymes, to counter the anti-nutritional factors present in conventional and unconventional poultry diet. These exogenous enzymes, in combination with non-conventional ingredients, are used to reduce the cost of feeding and to utilize the non-conventional feed ingredients efficiently as non-conventional feedstuffs are typically high in fibre and are not degraded by endogenous enzymes of poultry (Costa *et al*., 2008).

A portion of starch and protein of these non-conventional feedstuffs are entrapped in the fibre matrix, making it unavailable for degradation by endogenous enzymes of the

animals, but these nutrients can be made available for utilization by use of exogenous enzymes (Jha, 2015). Enzyme supplementation is also essential for environmental issues such as pollution of soil and water with nutrients, pathogens, fouling of environment and heavy metals which occur due to poor excreta management, as it may reduce the pollutant potential of excreta (Costa *et al*., 2008). Supplementation of multienzyme (xylanase, amylase and protease) optimized the utilization of high fibre diets, leading to better growth performance of broiler chicken (Singh *et al*., 2017).

When exogenous enzymes were supplemented to degrade NSP in a barley-based diet, gut microbial communities varied significantly among gut sections except between the duodenum and jejunum (Torok *et al*., 2008). Yang *et al*. (2006) reported the growth-promoting effects of enzymes linking it to the mucosal morphology of the small intestine. They also stated that the crypt depth of the jejunum was reduced along with an increase in the membrane enzyme activity and role in the last step of digestion causing the improved growth of chicken by supplementing xylanase in diets.

Cowieson *et al*. (2016) noted the beneficial role of exogenous protease by decreasing undigested protein from diet or endogenously produced to reach the caudal gut, reducing inflammation and maintaining tight junction integrity. Exogenous enzymes are multifactorial inaction due to its role in the partitioning of nutrients and help in the growth of specific microbiota by producing nutrients for them (Bedford & Cowieson, 2012). These enzymes are being used as an integrated solution to reduce the economic burden not just by limiting GIT pathogens but also by reducing medication costs, variability in animal performance, and reducing mortality by improving the gut health (Kiarie *et al*., 2013). Although the exogenous enzyme has many benefits to the poultry, there are still some limitations imposed to health condition, disease challenge, and quality of feed, pH and digesta retention time in the GIT (Ravindran, 2013). Therefore, nutritional strategies to overcome limitations could help in effective utilization of unconventional feed ingredients to produce cost-effective feed for broiler chicken.

2.4.2 Use of Fermentation Treatment

Fermentation degrades the substrate prior to feeding and potentially renders especially the fibrous fraction more available for digestion in the pig and ultimately improves the energy digestibility. Soaking compound feed with water is means of achieving a fermented diet. These diets are characterized by a pH between 3.5 and 4.5, high levels of lactic acid, and, to a lesser extent, acetic acid and alcohol. Fermented diets seem to improve growth performance

of pigs, compared with non-fermented diets. Fermentation of feed prior to feeding, which is the conversion of carbohydrate to alcohol, carbon dioxide, organic acids, has been largely explored in swine nutrition. A wide variety of feeds and fermentation techniques are used for fermented-wet feeding for pigs. Fermented-wet feed can reduce gastric pH and the number of coliform bacteria in the gastrointestinal tract this is due to increase of lactic acid bacteria in the stomach and small intensines that produces lactic acid that lowers gastric PH (Canibe & Jensen, 2003).

Pedersen and Lindberg (2003) found that *in vitro* fermentation of wet feed improved digestibility of organic matter (OM) and crude protein. Both lactic acid bacteria and yeast, which are normally found in fermented feed are capable of *myo*-inositol hexakisphosphate (IP6) a microbial degradation that deals with nutritional problems in monogastrics animals as well as to prevent environmental phosphate pollution thus increasing availability of phosphorus in cereal based-diets (Reale *et al*., 2004). The effects of fermentation on the properties of wet feed depend on the activity and nature of the microbial populations present which are in turn affected by the use of yeast cultures e.g. *Saccharomyces cerevisiae* or other innocula. Fermentation of feeds offered to broilers could be a better means of enhancing nutrient utilization in feed and improving productivity above that of wet feeding which has been proposed by earlier researchers.

2.4.3 Use of Yeast (*Saccharomyces cerevisiae)* in Poultry

The yeast *Saccharomyces cerevisiae* has received considerable attention in the last decade. Feed supplementation with live yeast cells improve feed efficiency, enhance feed digestibility, increase animal performance, reduce the number of pathogenic bacteria, improve animal health and reduce the negative environmental impacts of livestock production (Haldar *et al*., 2011).

Yeast supplementation also reduces the negative environmental impacts of livestock production (Ogbuewu *et al*., 2019). Studies have shown that the addition of live yeast may improve fibre digestibility, inhibit the growth of pathogens, produce antibacterial compounds, stimulate the immune system and improve gut morphological structure. Supplementation of S. *cerevisiae* in the diets of non-ruminants has the potentials to improve feed intake, enhance digestibility by improving of fibre digestibility, reduce pathogenic microbes, improves animal health and performance. Gut villi stimulation in the jejunum is the mechanism by which S. *cerevisiae* improve growth rate (Cheng *et al*., 2014).

2.5 Background Information on Treated Plant Animal Feeds

Protein sources are the second most important component in poultry diets. Due to the fluctuation in price of soybean meal (SBM) and persistent increase in feed prices, nutritionists have been exploring alternative protein sources. Replacement of SBM with alternative protein sources in poultry diets could reduce human-livestock competition for soybean and support the production of more animal protein. However, the use of alternative protein sources is limited by low inclusion due to the presence of anti-nutritional factors (ANF) such as glucosinolates (rapeseed meal), gossypol (cottonseed meal), non-starch polysaccharides (NSP) in lupin flour, high fibre (palm kernel cake), total phenolic contents and phytic acid (canola meal) known to impair animal performance, nutrient digestibility and feed utilization .Solid-state fermentation (SSF) has been researched for a long time in the food industry since it leads to production of enzymes, organic acids and other metabolites of economic importance's has been employed to enhance nutrient bioavailability, inhibit gut pathogenic bacteria and reduce ANF in plant protein sources resulting in improved nutrient digestibility, thereby improving performance and gut health of broiler chicken.

Table 2.4 Effect of Solid-State Fermentation and Synthetic multi-enzyme on Plant Animal Feeds

Substrate	Microorganism/Enzyme	Results	References
Rape seed meal	*Saccharomyces cerevisiae, Lactobacillus fermentum, Bacillus subtilis Bj-1*	Decreased ANFS, isothiocyanates, increased CP, Improved body weight gain, feed conversion ratio compared to unfermented rape seed Improved body weight gain and feed conversion ratio compared to broilers fed unfermented rapeseed meal	Chiang *et al*. (2010)
Cotton seed cake	*Bacillus subtilis BJ-1, Saccharomyces cerevisiae N5-CAIR*	Increased crude protein, reduced crude fibre, increased ash, decreased free gossypol, reduced crude fat and increased phosphorus Improved the body weight gain and feed intake of broilers at 8% dietary inclusion	Tang *et al*. (2012)
Corn	Xylanase, Cellulase	Decreased NSP	Jakobsen *et al.* (2015a)
Wheat	Phytase	Almost complete reduction of phytate bound phosphorous	Jakobsen *et al*. (2015b)
	Protease	Increased protein solubility	
Rough rice diet	Xylanase, Cellulase, β-glucanase,	Increased weight gain, feed efficiency	Wang *et al*. (2008)

2.6 Research Gaps on Use of MOLM as a Poultry Feed Ingredient

Moringa leaf meal has quality attributes that makes it a potential substitute for soybean meal or fishmeal in non-ruminant diet (Okosun & Oyedeji, 2016). It has a high biological protein, vitamins and minerals. Although Moringa leaves have been widely applied to feed all types of animals, some challenges still need to be solved for large-scale feed production. The presence of endogenous anti-nutrients in plant leaf meals is one of the dominant limiting factors. Inclusion of Moringa leaf meal in diets at high levels has a negative impact on animal growth performance. This is due to Moringa leaves' high fibre content, tannins, phytic acid, and saponin content, which reduce palatability, protein digestibility, and mineral bioavailability, limiting the biological value and acceptance of Moringa leaves as a regular food source (Shi *et al.*, 2018). Thus, the leaves should be appropriately treated before large-scale consumption. Physical, chemical, and biological methods including soaking, cooking, fermentation, selective extraction, irradiation, and enzymatic treatment can be employed to reduce or remove antinutrients e.g. phytates and cellulose that have a negative effect on the availability of iron and other minerals in leaves. Fermentation reduces phytate content by 66.9% and increases digestible protein content in Moringa leaves (Thierry *et al*., 2013).

It was concluded that Moringa leaf meal was well tolerated and can only be included in the feed to levels of up to 7.5%, as higher levels affected weight gain, feed intake and digestibility (Gakuya *et al*., 2014). Moringa leaf meal at 5% level increased the egg weight, but decreased egg weight when the inclusion level was at 20%. The authors assumed that higher feed intake; FCR with lower egg production percent, egg mass, and egg weight at a higher-level supplementation was due to poor digestibility of nutrients because of different anti-nutritional phytochemical present in Moringa leaves (Kakengi *et al*., 2007). However, a study by Gadzirayi *et al*. (2012) reported that the addition of Moringa leaf meal as a protein supplement in broiler diets at 25% promoted more growth than commercial diets. Kakengi *et al.* (2007) reported that Moringa leaf meal could be incorporated up to 10 -15 % in laying hen rations. The study using layers showed that MOLM could be used as a source of plant protein since it was highly accepted even at high inclusion levels in the diet. It showed highest performance in egg production in comparison with other leaf meals already studied. However, for optimum utilization 10% inclusion was recommended.

There are many variables on doses and part of the plant used, such as leaves, pods, or seeds. Many scientists agreed that Moringa plant might have a positive role in improving the

production performance and health status in grower IC. Further studies are still needed to determine the actual doses of inclusion for optimum performance in chicken and also methods of treatment of Moringa leaf meal in order to improve digestibility and nutrient utilization.

CHAPTER THREE

EFFECT OF FERMENTATION AND ENZYME TREATMENT ON THE CHEMICAL COMPOSITION, IN-VITRO DRY MATTER AND CRUDE PROTEIN DIGESTIBILITY OF MORINGA (*M. oleifera*) LEAF MEAL

Abstract

Moringa *(Moringa oleifera)* leaf meal has a high nutritional value, however the presence of anti-nutritional factors, poor palatability, and low digestibility restrict its use as animal feed. This study determined the chemical composition, *in-vitro* dry matter and crude protein digestibility of untreated, fermented and enzyme-treated *M. oleifera* leaf meal (MOLM). There were four treatments: T1- untreated MOLM, T2- MOLM treated with enzyme Natuzyme®, T3- MOLM treated using natural fermentation and T4- MOLM treated using *Saccharomyces cerevisiae* (NCYC 125®) for four days. A three-step *in-vitro* digestibility procedure to simulate avian stomach and intestines was conducted for 3 hours and 15 minutes using pepsin-pancreatin hydrolysis method. The crude protein (CP) cotent was similar for the enzyme-treated and S*accharomyces cerevisiae*-fermented meals but the dry matter content for all the treatments were significantly different ($p<0.05$) compared to the control. The crude fibre, nutrient detergent fibre, acid detergent fibre, acid detergent lignin, cellulose and hemi-cellulose content were significantly lower ($p<0.05$) in enzyme-treated MOLM compared to the control. The *in-vitro* DM digestibility of fermented and enzyme-treated MOLM was 54.5% and 60.3%, respectively while the CP digestibility was 50.9% and 63.1%, respectively compared to the control which was 42.45%. The treatment with multi-enzyme improved CP digestibility by 25% compared to untreated MOLM. The enzyme-treated MOLM had the highest DM and CP digestibility compared to the control. It was concluded that enzyme treatment is the best method to improve the nutritional quality and digestibility of MOLM.

3.1 Introduction

The animal feed industry has been confronted with increased costs of cereals and oilseeds mainly which is attributed to competition with humans for energy sources and the high cost of protein rich ingredients, most of which are imported. Poultry industry in developing countries is facing some challenges due to high costs of conventional feed ingredients e.g. fishmeal and soybean meal which are mainly used in poultry rations (Abd El-Hack *et al*., 2015). There are locally available feed resources that contain anti-nutritional factors (ANFs) that limit the efficiency of their utilization. The inclusion of feed ingredients containing ANFs may adversely affect poultry performance however use of enzymes and fermentation can reduce the negative effects of ANFs. Supplementation of commercial enzymes can enhance the nutritional value of crops containing high contents of soluble non-starch polysaccharides since enzymes improve nutrient digestibility (Rehman *et al*., 2017).

To develop feeding standards for animals, knowledge and understanding of nutrients in the feed and their utilization by animals is needed. Animal and plant ingredients are the main sources of protein used in poultry diets and they vary in digestibility and amino acid composition (Parsons *et al*., 1997). Digestibility is used in practice as an estimator of the amino acid bioavailability in poultry diets. Protein quality assessment of feed ingredients for poultry is often achieved using *in-vitro* or *in-vivo* testing. The *in-vivo* methods can be expensive and time consuming to conduct. Protein quality can be evaluated using *in-vitro* chemical methods (Boisen & Eggum, 1991). The *in-vitro* assays are less expensive, can evaluate more ingredients, and are less time consuming than in vivo assays. Therefore, the degradation kinetics and bioavailability of proteins are both important factors, which could be considered when trying to maximize yield in poultry. Moringa leaf meal (MOLM) is highly nutritious, containing high levels of protein, vitamins, minerals and phytochemicals (Leone *et al*., 2015).

These nutritional traits together with its high production of leaf mass and adaptability to dry climatic conditions and dry soils make MOLM a potential high quality feed source for livestock (He *et al*., 2020). Dietary inclusion of MOLM in broiler diets has been shown to enhance nutritional status and growth performance (Cui *et al*., 2018). Such characteristics show that MOLM is a rich source of nutrients and biological activities for livestock, which could help to relieve the shortage of feed resources. Despite the clear benefits of MOLM

supplementation, its use is limited due to the presence of anti-nutritional factors, low palatability and digestibility.

The anti-nutritional factors (ANFs) interfere with the digestion and absorption of other important nutrients such as zinc, iron, calcium and magnesium when consumed in large quantities (Nouman *et al*., 2014). The ANFs in MOLM, such as tannins, phytic acid and glucosinolates, could affect the palatability, digestion and absorption, limiting nutrient availability (Stevens *et al*., 2016). Moreover, most of the proteins are insoluble despite MOLM having relatively high protein content (Teixeira *et al*., 2014). A process for improving MOLM foliage quality is therefore imperative. Fermentation breaks down the substrate prior to feeding and renders the fibrous fraction available for digestion and ultimately improves the digestibility. Solid-state fermentation (SSF) involves the growth of microorganisms on substrates with limited water content (Dulf *et al*., 2017).

Solid state fermentation systems have been effectively applied to MOLM to increase its protein content, while reducing undesirable substances such as fibre, tannin and phytic acid (Zhang *et al*., 2017). Numerous studies have demonstrated that the functionalities of various agricultural by-products can be enhanced by SSF. Indeed, many beneficial compounds have been produced through fermentation, such as organic acids, enzymes, aromatic and flavor compounds, as well as bioactive compounds (Bennett & Yang, 2012). Solid-state fermentation has been widely used in the feedstock industry and has shown good prospects for promoting nutrient utilization and decreasing ANF levels (Chi & Cho, 2016). In this study chemical composition, i*n-vitro* dry matter and crude protein digestibility of fermented and enzyme-treated MOLM was determined.

3.2 Materials and Methods

3.2.1 Study Site

An *in-vitro* experiment was conducted at Egerton University, Animal Nutrition laboratory. The University is situated within Njoro Sub-County, Nakuru County. The altitude is 1800 meters above sea level with an average annual rainfall of 900-1,200 mm. The area has average daily temperatures ranging from 17°C- 22°C (Egerton University Weather Station-personal communication, 2020).

3.2.2 Collection and Preparation of Moringa Leaf Meal

The Moringa leaves were purchased from Emuka Moringa Farmers' Cooperative Society, which comprises of farmers from Emali, Mulala and Tutini Wards in Makueni County. This Farmers' Cooperative Society is supported both by Child Fund International

organization (non-profit organization) funded by local partner cooperation and the county government of Makueni. The leaves were harvested by cutting off young branches of the trees and stripping off the leaves from the tips by hand (manually), and then washed with warm water; air dried under a shade for 3-4 days until they were crispy to touch, and retained their greenish colour. The leaves were then milled using a BS-180 hammer mill® through a 3 mm sieve to produce the leaf meal (MOLM) which was stored in air tight sacs until needed for feed formulation.

3.2.3 Preparation of Experimental Treatments

There were four treatments with 3 replicates each. The treatments were:

T1: Untreated Moringa leaf meal (control)

T2: Moringa leaf meal treated using natural (spontaneous) fermentation

T3*:* Moringa leaf meal treated with *Saccharomyces cerevisiae*

T4: Moringa leaf meal treated with an enzyme (Natuzyme®) -(12,000 units/g of xylanase, 6,000 units/g of cellulase, 1,500 units/g of phytase, 700 units/g of beta-glucanase, 700 unit/g protease and 400 unit/g of alpha-amylase presented in powder form)

3.2.4 Preparation of Enzyme-Treated MOLM

The Natuzyme® enzyme was purchased from Coopers Kenya brand limited. It was added in the dry form at a rate of 350mg/kg of MOLM as per the manufacturer's instructions and recommendations.

3.2.5 Preparation of Naturally Fermented MOLM

Moringa leaf meal (MOLM) was sterilized at 121 ^{o}C for 20 min and then cooled to room temperature (18-22 ^{o}C). A mixture of 40g MOLM with water at a ratio of 1:1 (1-part dry MOLM to 1-part water) was incubated in triplicate at 30 ^{o}C under anaerobic conditions in sealed plastic containers for 4 days (Zhang *et al.*, 2017). A sample for proximate analysis was obtained from the individual samples while the rest was oven-dried at 50 ^{o}C for 12 hr for determination of *in-vitro* digestibility.

3.2.6 Preparation of Fermented MOLM using *Saccharomyces cerevisiae*

A mixture of 40g Moringa leaf meal was prepared by mixing with water in a ratio 1:1 (1-part dry MOLM to 1-part water) in triplicate. The yeast powder *S. cerevisiae* (NCYC 125®), purchased from the Agro-chemical and Food Company Ltd (ACFC, Kenya) was added to the dry MOLM at 5% level incubated in plastic containers at 30 ^{o}C and fermented for 4 days (Zhang *et al*., 2017).

3.2.7 Determination of Nutrient Composition

Moringa leaf meal was analyzed for proximate composition (AOAC, 1990) 15th Edition at the Egerton University Animal Nutrition laboratory. Proximate analysis: dry matter (method 934.01; AOAC, 1990), ash (method 942.05; AOAC, 1990), ether extract (using ether) (method 920.39; AOAC, 1990). Crude protein (N X 6.25) (method 984.13; AOAC, 1990) and crude fibre (method 978.10).

3.2.8 Three-way Determination of *In-vitro* Digestibility

To simulate the digestion process in the avian stomach, an *in-vitro* digestibility was conducted according to the procedure by Latorre *et al*. (2015). All incubations were done in a water bath at 42 ± 1 °C with constant stirring of 70 rpm. There were four treatments, each with 3 replicates. T1: MOLM (control), T2: MOLM naturally fermented, T3: MOLM fermented with *S. cerevisiae*, T4: enzyme-treated MOLM

Step One (Simulation of the Crop Phase)

A ground sample of 2.0g was weighed and placed in a 100 ml conical flask. Ten milliliters (10ml) of 0.1 M hydrochloric acid (0.1M, pH 5.2) was added to the flask, agitated vigorously and incubated in a water bath at 39 ^{o}C for 30 minutes.

Step Two (Simulation of the Proventiculus Phase)

The mixture from step 1 was mixed with 3000 U pepsin/gm of MOLM in 2.5 ml of 1.5 M HCl (pH 1.4–2) was incubated in a water bath at 39 ^{o}C for 45 minutes.

Step Three (Simulation of the Small Intestines Phase)

The mixture from step 2 was mixed with 6.84 mg /ml of pancreatin (Porcine grade enzyme with 3 x USP activities) in 6.5 ml of 1.0M sodium bicarbonate (1.0M, pH 6.4-6.8) and incubated in a water bath at 39 ^{o}C for 2 h. Hence, the completion of *in vitro* digestion process took 3 h and 15 min. The residues were filtered through a nylon bag (pore size of (42 μm) washed with distilled water and dried in an oven at 70 °C for 24 hours then weighed.

3.2.9 Calculation of Crude Protein and Dry Matter Digestibility

Dry matter digestibility (DDM) and crude protein digestibility (DCP) were computed using the following formulae (Furuya *et al*., 1979):

$$\text{DDM digestibility} = \frac{\text{DM feed} - \text{DM undigested feed}}{\text{DM feed}} \text{x } 100$$

$$\text{DCP digestibility} = \frac{\text{CP feed} - \text{CP undigested feed}}{\text{CP feed}} \text{ x } 100$$

where:

DM feed: grams of dry matter in 2g of sample

DM undigested feed: grams of DM precipitate

CP feed: grams of CP in 2g of sample

CP undigested: grams of CP in precipitate

3.3 Statistical Analysis

Data was subjected to analysis of variance (ANOVA) using the general linear model (GLM) procedure of the statistical analysis system (SAS, 2009). Significant means were separated using Tukey's test at $p<0.05$. The statistical model was as follows

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

Where;

Y_{ij}= effect of the response variable (*in-vitro* dry matter digestibility)

μ = overall mean

T_i = effect due to the ith treatments (fermentation, untreated and enzyme treatment)

ε_{ij} = the random error

3.4 Results

3.4.1 Chemical Analysis of Treated and Untreated MOLM

The chemical composition for different treatments of MOLM before and after fermentation process is presented on Table 3.1

Table 3.1 Chemical composition of treated and untreated MOLM

Nutrient (%)	*Saccharomyces cerevisiae* Fermented	Naturally Fermented	Enzyme Treated	Control	*p* value
Dry matter	92.86^{a}±0.02	93.45^{c}±0.04	93.6^{d}±0.04	93.26^{b} ±0.07	<.0001
Metabolisable energy (kcal)	4211.2^{c}±0.01	4041.5^{b}±0.07	4225.5^{c}±0.01	3888.5^{a}±0.01	<.0001
Crude protein	27.89^{b}±0.86	26.25^{a}±0.17	27.95^{b}±0.71	26.8^{a}±0.04	<.0001
Crude fibre	14.66^{b}±0.06	14.78^{c}±0.36	12.39^{a}±0.33	17.77^{d}±0.12	<.0001
Ether extract	4.17^{a}±1.61	4.19^{a}±0.06	4.17^{a}±0.26	4.47^{b}±0.17	0.03
Neutral detergent fibre	55.18^{b}±1.61	57.56^{c}±0.38	51.65^{a}±0.26	58.92^{d}±0.17	0.002
Acid detergent fibre	33.70^{c}±0.05	31.60^{b}±0.1	29.20^{a}±0.18	37.30^{d}±0.03	<.0001
Acid detergent lignin	10.48^{b}±0.16	11.36^{c}±0.13	9.78^{a}±0.11	12.13^{d}±0.13	<.0001
Hemicellulose	21.48^{a}±0.03	21.67^{b}±0.04	22.45^{c}±0.04	25.99^{d}±0.32	<.0001
Cellulose	$23.22\pm^{c}0.16$	20.21^{b}±0.02	$19.42\pm^{a}0.03$	$25.12\pm^{d}0.09$	<.0001

$^{abc,\ d}$ means within a row with different superscript letters are significantly different at p <0:05.

The enzyme-treated and *Saccharomyces cerevisiae* fermented MOLM had higher levels of crude protein compared to the untreated (control). The content of crude fibre, neutral detergent fibre, acid detergent fibre, cellulose and hemicellulose were significantly lower (p<0.05) in enzyme-treated MOLM compared to the control. The crude protein was similar for the enzyme-treated and *Saccharomyces cerevisiae* fermented meals but the dry matter content for all the treatments were significantly different (p<0.05) compared to the control. The ether extract (EE) was highest in the control but was similar in the *Saccharomyces cerevisiae* and naturally fermented meals.

3.4.2: Crude Protein and Dry Matter Digestibility

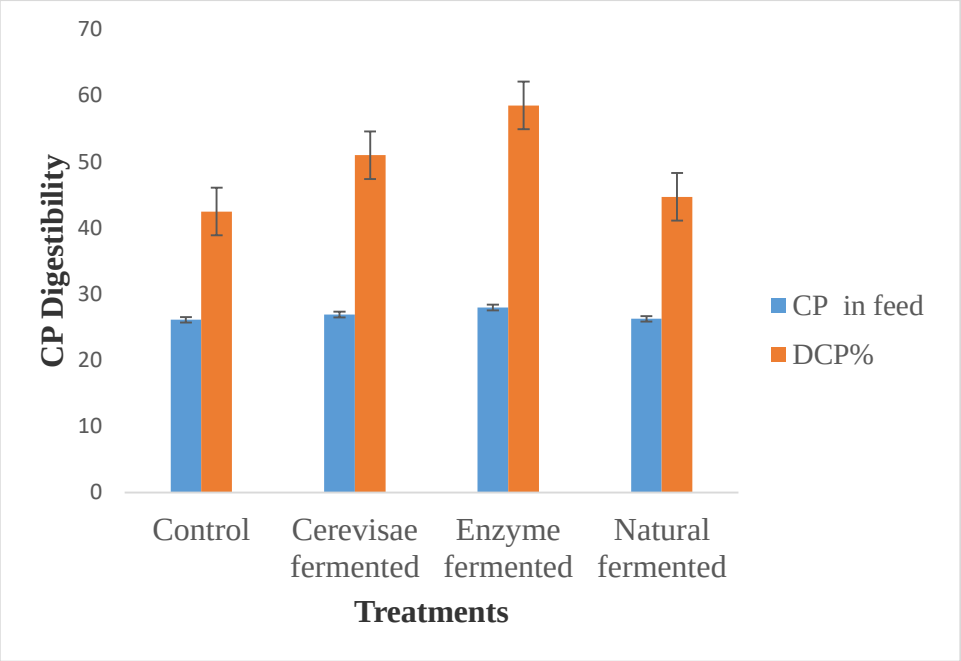

Figure 3.1 Crude protein digestibility of MOLM treatments

The crude protein digestibility was 63.07% in enzyme-treated MOLM, which represented a significant increase (p<0.05) relative to the control. The R^2 (correction coefficient) was 0.84, thus 84% of the variance in crude protein digestibility could be explained by the model. These analyses demonstrated good correlation between the experimental and predicted values. In this study, the crude protein digestibility of fermented MOLM was significantly higher than that of control.

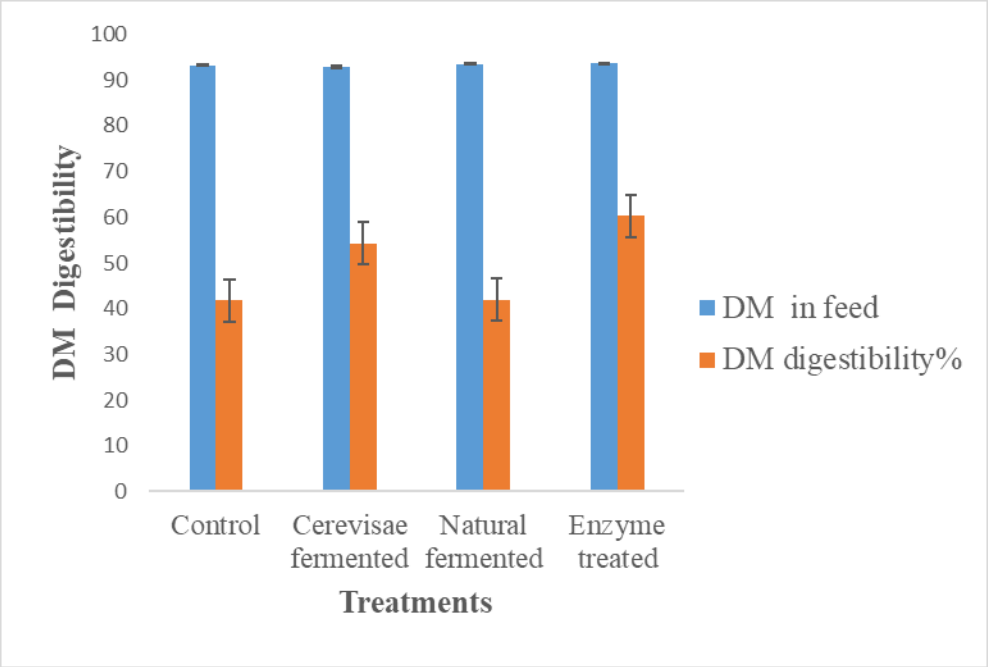

Figure 3.2 DM digestibility of MOLM treatments

The enzyme-treated MOLM had the highest DM digestibility (60.3%) followed by *S. cerevisiae* fermented (54.4%), which indicates that they were significantly different compared to control. The R^2 was 0.98, thus 98% of the variance in dry matter digestibility could be explained by the model hence they were a good correlation.

3.5 Discussion

3.5.1 Chemical Composition of Treated and Untreated MOLM

The enrichment of CP in the *S. cerevisiae* treated MOLM could be the result of increased microbial biomass, suggesting that the treated substrate could act as a good protein source for livestock. However, it could also be due to the degradation of ANFS, an increase in the amount of small-sized peptides, increased content of free amino acids and bioavailability of nutrients. An increase in CP levels following fermented Moringa leaf meal (FMOLM) can also be ascribed to the loss of DM (mainly carbohydrates) and the synthesis of microbial biomass proteins (Hong *et al*., 2004). Furthermore, FMOLM contained more CP, which is in agreement with the results obtained with fermentation of soybean meal (Chen *et al*., 2013).

The content of CF, EE, NDF, ADF, cellulose and hemicellulose were significantly lower in enzyme-treated MOLM compared to the control. This is because enzymes cause the disruption of the plant cell wall integrity and consequently release of nutrients encapsulated

by the cell wall (Ravindran, 2013). Other studies indicated that supplementation of commercial enzymes in poultry diets containing sunflower stimulated digestion of fibre and decreased their harmful effects (Alagawany *et al.*, 2017). The decrease in CF in this study suggested that cellulose was broken down into monosaccharide by enzymatic hydrolysis. It is likely that *S. cerevisiae* utilized these simple sugars to proliferate, thereby avoiding the suppressive effects of metabolites. There was a reduction of ether extract (fat) in the FMOLM compared to the control. This suggests that the microbial fermentation process mobilized fats. Several microbes are able to grow synergistically, and are widely applied as co-cultures to improve the nutritional quality of non-conventional feed resources (Yao *et al*., 2018).

3.5.2 Crude Protein and Dry Matter Digestibility of MOLM Treatments

The use of enzymes in animal feed is of great importance. Research work has shown that the negative effects of nonstarch polysaccharides (NSPs) can be overcome by dietary modifications including supplementation of diets with suitable exogenous enzyme preparations (Creswell, 1994). The crude protein digestibility in the enzyme-treated MOLM represented a significant increase relative to the control. This is because enzymes break down the NSPs, decrease intestinal viscosity and eventually improve the digestibility of nutrients. Enzymes e.g. pentosanase, protease, cellulase, beta-glucanase, phytase, pectinase and amylase are capable of degrading pentosans, protein, cellulose, starch and phytate, subsequently improving the nutrient digestibility and absorption in the avian intestine (Ramesh & Devegowda, 2004).

Enzyme supplementation caused positive effect in energy and protein digestibility in broiler chicken (Pourreza *et al*., 2007). Sherif (2009) noticed that the addition of Avizyme, Sicozyme, Natuzyme, or phytase in the broiler diet led to significant improvements in digestibility of DM, EE and CP and nitrogen retention rate compared to the control group. In Japanese quail, digestibility of nutrients (DM, OM, CF, EE, CP, and NFE) were influenced positively by addition of exogenous enzyme (Bio-Feed® Pro) which contained amylase, protease, betaglucanase and xylanase to diets (Rabie &Abo El-Maaty, 2015). Cowieson *et al*. (2017) concluded that phytase is effective in enhancing the digestibility of amino acids and that these impacts originate from the removal of the anti-nutritional impacts of phytic acid. Other studies have shown that beta-glucanase in barley-based diets aided in disrupting the cell wall structure of the endosperm, allowing more rapid access of the chickens' endogenous amylases and proteases to the cell contents (Hesselman & Aman, 1986).

The crude protein digestibility of FMOLM was significantly higher than that of the control. This was likely because active proteases secreted by the microorganisms during fermentation were able to break down the large proteins (Chi & Cho, 2016). This reduction of protein sizes is important to increase the digestibility. Higher digestibility of FMOLM may be due to degradation of the structure of lignocellulose biomass by solid state fermentation, thereby increasing the accessibility of nutrients. It has been reported that feed supplementation with live yeast cells improved feed efficiency, feed utilization and enhanced feed digestibility (Haldar *et al.*, 2011). Studies have shown that addition of live yeast may increase nutrient digestibility by improving fibre degradability and inhibiting pathogens (Borda-molina *et al*., 2018).

3.6 Conclusion

The enzyme-treated MOLM had the highest *in-vitro* DM digestibility (60.3%) followed by *S. cerevisiae* fermented (54.4%). The treatment with multi-enzyme improved *in-vitro* CP digestibility by 25% compared to untreated MOLM .The content of crude fibre, neutral detergent fibre, acid detergent fibre, cellulose and hemicellulose were lower in enzyme-treated MOLM compared to the control.

CHAPTER FOUR

EFFECT OF FEEDING ENZYME-TREATED MORINGA *(M. oleifera*) LEAF MEAL-BASED DIETS ON PERFORMANCE OF IMPROVED INDIGENOUS GROWER CHICKEN IN KENYA

Abstract

The high cost of conventional protein feed ingredients in poultry diets has necessitated the investigation into non-conventional readily available feedstuffs. A feeding trial was conducted to investigate the effect of inclusion of enzyme-treated Moringa *(M. oleifera)* leaf meal on feed intake, feed conversion ratio, average daily gain and point of lay of improved indigenous grower chicken. Ninety chicken were assigned to six treatment diets in a completely randomised design (CRD) with a factorial arrangement with each treatment having 5 chicken per cage, replicated three times. The dietary treatments were: T1 - containing 0kg MOLM and 0g enzyme, T2 - containing 0kg MOLM and 0.035g enzyme, T3 - containing 20kg MOLM and 0g enzyme, and T4 - containing 20kg MOLM and 0.035g enzyme, T5- diet containing 40kg MOLM and 0g enzyme, T6-diet containing 40kg MOLM and 0.035g enzyme of the diet, respectively. The feed intake, feed conversion ratio, ADG and point of lay were determined. Results from proximate analysis showed that the level of CF and ash increased as the level of inclusion of MOLM in the diet increased. The feed intake decreased significantly (p<0.05) in diets T5 andT6 (40% MOLM) compared to control. There was a significant (p<0.05) difference in the FCR of the chicken among the treatments. The ADG was significantly (p<0.05) lower for the MOLM-based diets in comparison to the control diets. There was a significant (p<0.05) reduction in the point of lay of the chicken fed MOLM-based diets in comparison to control. The study concluded that inclusion levels of up to 20% MOLM improved feed intake however there was a reduction in average daily gain, feed conversion ratio and point of lay as the inclusion level increased to 40%, therefore inclusion levels of enzyme-treated MOLM at 40% and above should not be incorporated in improved indigenous grower diets as they have detrimental effects in the performance of improved grower indigenous chicken**.**

4.1 Introduction

Poultry industry in developing countries faces challenges due to high cost of conventional protein feed ingredients e. g fishmeal and soybean meal which are mainly used in poultry rations (Abd El-Hack *et al*., 2015). Consistent increase in the price of feed ingredients has been a major constraint in most of the developing countries. As a consequence, cheaper non-conventional feed ingredients which contain higher percentage of non-starch polysaccharides (soluble and insoluble/crude fibre) along with starch are being evaluated. Non starch polysaccharides (NSPs) are polymeric carbohydrates, which differ in composition and structure from starch (Morgan *et al*., 1995).

The inclusion of feed ingredients containing anti-nutritional factors may adversely affect poultry performance. Anti-nutritional factors which include β-glucans in barley, pentosans in wheat, and certain oligosaccharides in soybean meal reduce the utilization of nutrients, leading to depressed chicken performance (Annison & Choct, 1991). The use of commercial enzymes can enhance the nutritional value of crops containing high contents of soluble non-starch polysaccharides (Rehman *et al*., 2017). Several reports have indicated that utilization of such commercial enzyme preparations can improve the productive performance of chicken (Cowieson *et al*., 2000). Feed biotechnology can enhance the utilization of high fibre containing feed ingredients (Attia *et al*., 1998). Using enzyme technology is the principle rationale to improve the nutritive value of feedstuffs **(**Bedford & Partridge, 2001).

Therefore, development of commercially available exogenous enzyme preparations to target specific substrates in the feeds and ameliorate their anti-nutritive effects has received increased attention in the last decade and is of great importance. The impact of indigenous chicken in improving the nutritional status, income, food security and livelihood of smallholders is significant owing to their low cost of production (FAO, 1997). Indigenous chicken contributes to the overall well-being of the households through employment creation and income generation (Moreki *et al*., 2010). Indigenous chicken constitutes a vital pillar of food security improvement, socio-cultural and economic development for most rural people.

The chicken are hardy and survive under harsh conditions with minimal inputs. Supplementation of high protein feed, provision of housing and disease control were found to improve productivity of chicken (King'ori *et al*., 2007). The major limiting factor of indigenous chicken production is feed in terms of both quantity and quality (Mohamed & Abate, 1995). Feed ingredients of plant origin contain components that are refractive to monogastrics digestive enzymes because of lack or insufficiency of endogenous enzyme

secretions (Ravindran *et al*., 1999). Therefore, this study was conducted to investigate the response of improved grower indigenous chicken to feeding with diets with different inclusion levels of *Moringa oleifera* leaf meal along with dietary supplementation with commercial enzyme preparation (Natuzyme®). The response was evaluated in terms of feed intake, feed conversion ratio, average daily gain and point of lay of the chicken.

4.2 Materials and Methods

4.2.1 Study Site

The feeding trial was conducted at the Poultry Research Unit at Kenya Agricultural and Livestock Research Organization (KALRO), Naivasha. The Institute is located at Naivasha sub-county, Nakuru County. It is about 100 km west of Nairobi along the Nairobi-Nakuru highway. The Research Centre is about 1,700 m above sea level and has average annual rainfall of 1100 mm with bimodal peaks recorded from March to May and October to December. Minimum temperature is 8°C in July and August; the maximum is 25° C in January and February (KALRO Naivasha Weather Station- personal communication, 2018).

4.2.2 Experimental Diets

The composition of the experimental diets is presented on Table 4.1

Table 4.1 Composition of experimental diets

Ingredients (%)	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**
Whole maize	60.95	60.92	51.45	51.42	39.95	39.92
Soybean meal	20.00	20.00	10.00	10.00	5.00	5.00
MOLM	0.00	0.00	20.00	20.00	40.00	40.00
Fishmeal	10.00	10.00	10.00	10.00	10.00	10.00
DCP	0.50	0.50	0.50	0.50	0.50	0.50
Limestone	7.50	7.50	7.50	7.50	3.00	3.00
Iodized salt	0.30	0.30	0.30	0.30	0.30	0.30
Premix	0.25	0.25	0.25	0.25	0.25	0.25
Vegetable oil	0.50	0.50	0.00	0.00	1.00	1.00
Enzyme	0	0.035	0	0.035	0	0.035
Calculated analysis						
ME (KJ//kg)	**2607.60**	**2609.50**	**2605.50**	**2603.60**	**2600.02**	**2606.60**
CP	**16.40**	**16.65**	**16.95**	**16.80**	**16.76**	**16.90**
CF	**3.21**	**3.17**	**5.03**	**5.05**	**5.67**	**5.72**
Recommended analysis						
ME (KJ/kg)	**2600**	**2600**	**2600**	**2600**	**2600**	**2600**
CP	**16**	**16**	**16**	**16**	**16**	**16**
CF	**4**	**4**	**4**	**4**	**4**	**4**

The experimental diets were formulated to meet the nutrient requirement for improved IC, 2600 KJ/kg ME, 120 g/kg CP (King'ori *et al*., 2014). The enzyme was added in the dry form at a rate of 350mg/kg of MOLM as per the manufacturer's instructions and recommendations. The dietary treatments were: T1 - containing 0kg MOLM and 0g enzyme, T2 - containing 0kg MOLM and 0.035g enzyme, T3 - containing 20kg MOLM and 0g enzyme, and T4 - containing 20kg MOLM and 0.035g enzyme, T5- diet containing 40kg MOLM and 0g enzyme, T6-diet containing 40kg MOLM and 0.035g enzyme of the diet, respectively. Dietary ingredients for the study (whole maize, soybean meal, ground fishmeal, Dicalcium phosphate, limestone, growers premix) were purchased from feed millers in Nakuru city.Canola oil contained 900 kcal, protein-0.1g, carbohydrate- 0.1g, fat-91.2g was added to the diets to enhance the energy cotent;*A Premix containing: vitamin A 750,600IU/kg, Vitamin E 30.61IU/kg, vitamin B 24000mg,biotin 30mg, copper 5000 mg, Iron 40000 mg, manganese 80000 mg, zinc 50000 mg, selenium100 mg, lysine 0.42%, Methionine 0.5%, alanine 0.84,Arginine 0.93% and Cysteine 0.32% was added at 0.5% of diet to supply minerals, vitamins, trace elements and amino acids to improve feed conversion ratio and performance.Results from the *in-vitro* digestibility trial indicated that enzyme treatment was the best method to improve nutrient utilisation of the meal.

4.2.3 Proximate Analysis

Proximate analysis of the feed samples was done at the Egerton University, Animal Nutrition laboratory following the procedures of AOAC (1990) 15th Edition: dry matter (method 934.01; AOAC, 1990), ash (method 942.05; AOAC, 1990), ether extract (method 920.39; AOAC, 1990). Total nitrogen was determined by Kjeldahl method (method 954.01; AOAC, 1990) multiplied by 6.25 for the crude protein content. Constituents of the cell wall, neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined (Van Soest *et al*., 1991). Proximate analysis for each diet was performed in duplicates. Gross energy was determined using a bomb calorimeter based on the complete combustion of an accurately weighed amount of feed in an enclosed space. The heat that is generated during combustion is accurately measured. Based on these results, the gross energy of the sample was determined (Carpenter & Clegg ,1956).

4.2.4 Management of Experimental Chicken (housing, feeding and disease control)

The house was thoroughly cleaned and disinfected using kupacide® disinfectant before the start of the experiment. The growers were kept in a deep litter system with wood shavings as litter material which was managed through aeration and changing whenever it got wet. One hundred (100), twelve weeks old improved indigenous chicken were purchased from Kenya Agricultural Livestock Research Organization (KALRO) from which ninety (90) chicken were randomly sampled. The chicken were weighed and assigned to six dietary treatments in a completely randomized design (CRD) with a factorial arrangement. There were 5 chicken per treatment, and each treatment had three replicates.

One rectangular feeder (99.06 cm by 22.86 cm) and a round drinker (30.5cm by 28cm) were allocated to a pen of 5 chicken. Feed and fresh clean water were provided *ad libitum*. Feed was offered at 0800 hrs while refusals were collected during the next day prior to feeding. The refusals were weighed using a digital weight balance then used to compute daily feed intake. Chicken body weight for each pen was recorded on a weekly basis using a digital weighing scale with 5 grams' accuracy. The weekly weights recorded were used to compute the body weight gains. Body weight gains and daily feed intake were used to compute the feed conversion ratio. The pullets were vaccinated against fowl typhoid at 8 weeks, 3rd dose Newcastle disease vaccine at 18 weeks and deworming done using piperazine® at 19 weeks respectively. The routine management practices included cleaning of the feeders and drinkers daily, provision of fresh feed and maintenance of bedding in good condition. The experiment was for 12 weeks' period.

4.3 Data Collection

Feed intake (FI)

Feed intake was calculated as the difference between feed offered and leftover (refusal) after 24 hours.

$$\text{Feed Intake (FI) per grower (g)} = \frac{\text{Feed offered (g)} - \text{Feed left over(g)}}{\text{Number of growers}}$$

Average daily gain (ADG)

The chicken within a cage were weighed together every week before feeding. Average daily gain was calculated as the difference between the weight after 7 days and weight of the chicken at the start of the 7 days divided by 7 days

$$\text{Average daily gain per grower(g)} = \frac{\text{Weight after 7 days (g)} - \text{Weight at start of 7 days (g)}}{\text{7 days}}$$

Feed conversion ratio (FCR)

Feed conversion ratio was calculated as average feed intake (g) consumed by the chicken divided by average weight gain per grower (g) during each week).

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Feed consumed per grower (g)}}{\text{Average weight gain per grower(g)}}$$

4.3.1 Experimental Design

A completely randomised design (CRD) with a factorial arrangement with the initial weight fitted as a covariate. There were 18 experimental units with 5 growers per treatment, each replicated 3 times.

$$Y_{ijk} = \mu + A_i + B_j + AB_{ij} + \beta(X_{ij} - \bar{x}) + \varepsilon_{ijk}$$

Assumptions were that x_{ij} is not affected by treatment

x_{ij} is deviated from the mean of the covariate, x bar

$Y_{ijk=}$Response variable of interest (feed intake, feed conversion ratio, weight gain, point of lay)

μ= Overall mean

$A_{i=}$= Effect associated with the i^{th} level of MOLM

$B_{j=}$= Effect associated with the j^{th} level of enzyme

$AB_{ij=}$ Effect associated with the i^{th} level of MOLM and j^{th} level of enzyme

X_{ij} = Initial body weight of an individual chicken (covariate)

$\bar{x}$ = Overall mean for initial body weight

ε_{ijk}= Random error

4.4 Statistical Analysis

Data was subjected to analysis of variance (ANOVA) using the general linear model (GLM) procedure of the statistical analysis system (SAS, 2009). Significant means were separated using Tukey's test at $p<0.05$.

4.5 Results

4.5.1 Chemical Composition of the Experimental Diets and MOLM

The diets were formulated to be isonitrogenous and isocalorific. The chemical composition of the diets and MOLM were determined in duplicate. Gross energy (MJ, measured by bomb calorimeter). The results of DM, Ash, CP, CF, EE, NDF, ADF, cellulose and hemi-cellulose of the diets and Moringa leaf meal are presented on Table 4.2 and 4.3

Table 4.2 Chemical composition of MOLM

Nutrient component (%)	**MOLM**
Dry matter	93.29
Ash	10.26
Gross energy(MJ/kg)	17.14
Crude protein	26.80
Ether extract	7.58
Nitrogen free Extract	36.13
Crude fibre	12.23
Crude fibre components	
Neutral detergent fibre	4.75
Acid detergent fibre	2.69
Acid detergent lignin	0.89
Hemicellulose	1.87
Cellulose	2.03

Table 4.3 Chemical composition of the experimental diets

Nutrients (%)	T1	T2	T3	T4	T5	T6	SEM	*p* value
Dry matter	90.39[c]	90.05[a]	90.06[a]	91.1[e]	90.89[d]	90.21[b]	0.043	<.0001
Gross energy (MJ/kg)	11.40[c]	11.40[c]	11.10[b]	11.10[b]	10.90[a]	10.90[a]	0.002	<.0001
Ash	11.13[b]	9.22[a]	11.72[c]	14.12[d]	14.31[e]	14.41[f]	0.043	<.0001
Crude protein	16.42	16.38	16.49	16.3	16.18	16.23	0.198	0.8648
Crude fibre	3.30[a]	3.07[a]	3.42[a]	3.39[a]	5.45[b]	5.25[b]	0.122	<.0001
Ether extract	4.77[a]	4.63[a]	6.52[b]	6.32[b]	7.28[c]	7.17[c]	0.076	<.0001

[abc, d] means in the same row without common superscripts are different at $p<0.05$

The proximate analysis showed a significant increase ($p<0.05$) of crude fibre as the level of inclusion of MOLM in the diet increased. Diets with 40% Moringa leaf meal (T5 and T6) had the highest 5.45% and 5.25% respectively. The ash content also increased as the level of inclusion of MOLM increased.

4.5.2 Performance of the Chicken

The feed intake in diets T5 and T6 (40% MOLM) was significantly different ($p<0.05$) compared to control. The ADG was significantly ($p<0.05$) lower for the MOLM-based diets in comparison to the control diets.

Table 4.4 Effect of diet and enzyme interaction on feed intake, feed conversion ratio and average daily gain of improved grower IC

Parameters	T1	T2	T3	T4	T5	T6	SEM	p value
Feed intake (g)	83.51[c]	88.92[d]	83.99[c]	88.91[d]	76.62[b]	73.72[a]	0.57	<.0001
Feed conversion ratio	1.58[b]	1.49[a]	3.89[d]	3.82[c]	4.62[f]	4.59[e]	0.05	<.0001
Average daily gain (g)	70.14[d]	74.34[d]	60.95[c]	69.37[c]	50.09[b]	40.43[a]	4.14	0.007

[abc, d] means in the same row without common superscripts are different at $p<0.05$; SEM=Standard error of means

4.5.3 Feed Intake (FI)

Chicken fed on T1 and T3, T2 and T4 diets had similar daily feed intake in comparison to T5 and T6 that had higher inclusion level of MOLM. The feed intake decreased significantly ($p<0.05$) with the increasing level of MOLM in comparison to control. However, there was a marked reduction in the feed consumption in chicken fed on

T5 and T6 (40%) MOLM in the diet. This reduction could be due to high CF and reduced palatability of the diet (Kakengi *et al*., 2003). The R^2 (correlation coefficient) was 0.92, thus 92% of the variance in feed intake could be explained by the model hence there was a good correlation.

4.5.4 Feed Conversion Ratio (FCR)

There was a significant (p<0.05) difference in the FCR of the chicken among the treatments.

4.5.5 Average Daily Gain (ADG)

The ADG was lower for T5 and T6 (40% MOLM) diets in comparison to the control diet.

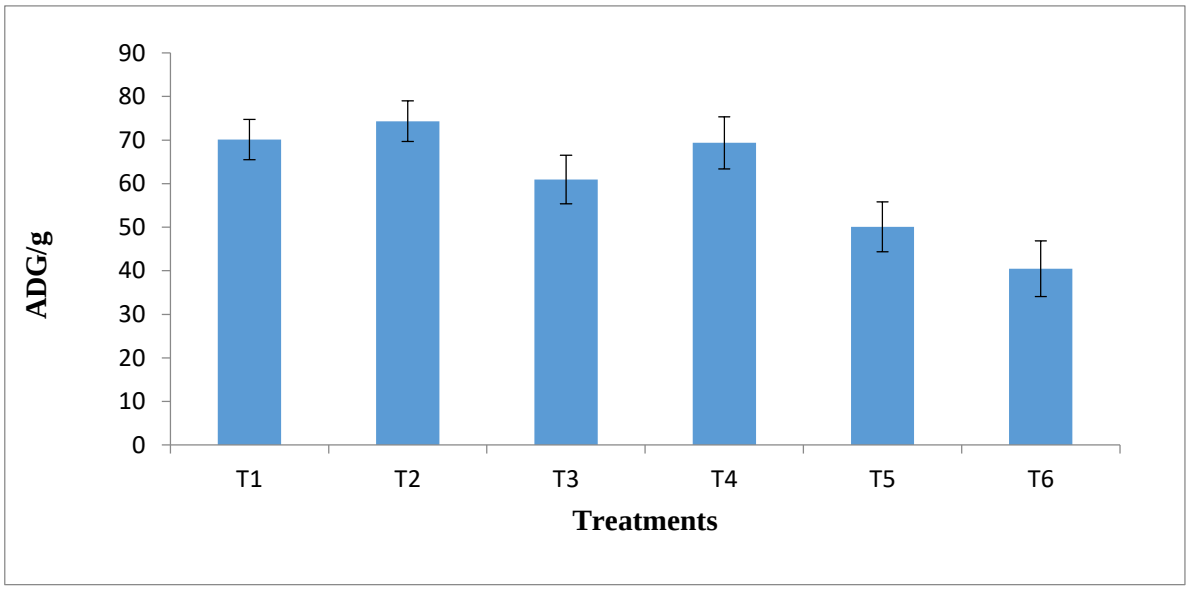

Figure 4.1 Weight gain of chicken fed different levels of enzyme-treated and untreated MOLM

4.5.6 Point of Lay

There was a significant (p<0.05) difference in the point of lay of chicken fed enzyme-treated MOLM-based diets in comparison to control. Chicken fed on 0% MOLM (T1 and T2) started laying at 149 and 139 days, respectively, compared to MOLM-based diets (Table 4.5). Diets containing 20% MOLM inclusion level (T3 and T4) laid at 156 and 152 days, respectively, compared to diets containing 40% MOLM (T5 and T6) which started laying at 172 days.

Table 4.5 Effect of diet on body weight, point of lay and egg weight of improved indigenous grower chicken

Parameters	T1	T2	T3	T4	T5	T6	*p* value
Body weight (kg)	2.04±0.09	2.07±0.16	1.87±0.05	1.84±0.01	1.86±0.08	1.86±0.04	0.106
Point of lay (days)	149^{b}±0.00	139^{a}±0.00	156^{d}±0.00	152^{c}±0.00	172^{e}±0.00	172^{e}±0.00	<.0001
Egg weight (g)	47.6±1.59	43.03±2.26	44.6±1.53	47.5±3.21	46.8±4.5	46.6±0.81	0.614

^{abcde}Means in the same row without common superscripts are different at $p<0.05$

4.6 Discussion

4.6.1 Feed Intake (FI)

Feed intake was similar in T1 and T3 and in T2 and T4 in comparison to T5 and T6 treatments. Daily feed intake for treatments T3 and T4 (20% MOLM) were similar since they fell within the same range. That agreed with Kakengi *et al*. (2007) who in related studies observed no difference in feed intake in layer hens fed diets containing 20% levels of *M. oleifera* leaf meal, but rather increased feed intake was noted in both inclusion levels. There was, however, increased feed intake for all treatments as from week five which could be due to acclimatization of chicken to the diets.

Inclusion of *Moringa oleifera* leaf meal at 40% levels led to decrease in feed intake. Chicken fed on T1 and T2 diets (0% MOLM) had the highest feed intake compared to T5 and T6 diets (40% MOLM). There was a general decline in daily feed intake as MOLM in the diet increased, this is because of the high fibre content in MOLM-based diets. Results showed that chicken fed with T1 and T2, T3 and T4 gained significantly ($p<0.05$) higher weight than chicken fed T5 and T6 diets. This confirmed the observations made by Ash and Petaia (1992) and Olugbemi *et al*. (2010) that increasing inclusion level of leaf meals in broiler diets resulted in depressed growth performance. This observation could be generally attributed to increasing fibre content of the diet, which may have impaired nutrient digestibility and absorption (Ige *et al*., 2006). It could also be attributed to the crude protein content or

palatability of the control diet, which enhances its acceptability and utilization. The negative effect of the anti-nutritional factors and phytochemical compounds present in *M. oleifera* leaf meal on the chicken could be responsible for the decrease in performance in terms of feed intake, feed conversion ratio and average daily gain (Onu, 2010). Tannins are contained in several feed ingredients commonly used in chicken diets, such as sorghum and barley. Tannins are produced by green plants in different levels and qualities. In broiler chicken, the inclusion of up to 3% dietary tannins can improve gut health and digestive performance. Tannins levels are considered high when >10% and low when <10%. The use of tannin up to 15 mg/kg feed is safe for all animal species (Huang *et al*., 2011). The content of tannins present in Moringa leaves ranges from 12.0 to 20.6 mg g^{-1} (Teixeira *et al*., 2014). Tannin is a phenolic compound that interacts with trypsin and amylase or with the substrates of these enzymes to form complexes that are not readily digestible, resulting in decreasing palatability and reducing feed intake. *M. oleifera* leaf contain saponins, which provide a bitter taste, their amounts in dry matter are 4.7-5g/kg-1(Moyo *et al.*, 2011). Leaf meals are generally bitter in taste, therefore, the inclusion of MOLM in the diets could have resulted in reduced palatability and thus reduction in feed intake of the chicken. Omekam (1994) observed that unpalatability of a feedstuff consequently prevents chicken from consuming adequate quantity of the feed.

4.6.2 Feed Conversion Ratio (FCR)

The FCR among the treatments was significant. The T5 and T6 diets resulted in high FCR due to high inclusion level of MOLM.This indicated low efficiency of utilization of the nutrients. Poor performance of these chicken could be attributed to the presence of some anti-nutritional factors which resulted to poor feed digestibility and utilization (D'Mello, 1995). Moringa leaf meal based diets have antinutritional factors that give a slight bitter taste with increased leaf meal inclusion and, therefore, reduce palatability and subsequent voluntary feed intake .The results of this trial tended to agree with earlier observations that dietary inclusion of leaf meals of *Leucocephala, Gliricidia sepium, Cajanus cajan, Sesbania sesban and Manihot esculenta* depressed growth, feed intake, FCR and growth rates of chicks at levels ranging from 75-100g/kg (D'Mello *et al.,* 1987; Raharjo *et al.,* 1988). That could be attributed to reduced voluntary feed intake with increasing inclusion levels of leaf meals that tends to contain high energy levels. That is in line with Makkar and Becker (1997) who indicated that voluntary feed intake is explained by ME content and by the palatability of diets.

4.6.3 Average Daily Gain (ADG)

When the levels of inclusion of MOLM increased, the ADG of the chicken decreased significantly. This is due to the low feed intake and low efficiency of utilization of the nutrients due to high fibre cotent in the feed. This result was in contrast with the finding of Kakengi *et al.* (2003), Olugbemi *et al*. (2010) and Banjo (2012) who reported that the inclusion of MOLM in the diet of the broilers significantly (p<0.05) enhanced their weight gain at 1% MOLM level which was significantly higher than the control. The chicken fed on the diet that contained 20% MOLM obtained significantly (p<0.05) higher weight gain as compared to those fed on the diet that contained 40% MOLM. This result may be attributed to higher crude fibre content which may have impaired nutrient digestion and absorption (Aderemi, 2003). The lower weight gains of chicken fed on 40% MOLM diet was due to low utilization of the high crude protein content in MOLM and the negative effect of the anti-nutritional factors present in MOLM on the chicken (Onu & Aniebo, 2011).

The Moringa leaves contain 1-23g of tannin in every 1 kilogram of leaves (Kakengi *et al*., 2003). Tannin has been reported to interfere with the biological utilization of protein and to a less extent available carbohydrate and lipids (Esonu, 2001). Tannins depressed growth rate and feed utilization by forming complexes with proteins and carbohydrates or inhibition of digestive enzymes (Abeke *et al*., 2003).The enzymatic oxidation of tannins enhances their enzyme inhibitory effect and toxicity (Awad *et al*., 2001).Unlike ruminant animals, poultry do not have microbes in their gastrointestinal tract to detoxify or reduce the effect of tannins ,negative effects on feed intake, nutrient digestibility and production performance (Redondo *et al*., 2014).Tannins bind proteins, thus impairing protein digestion (Olomu,1995). Tannins are responsible for an astringent taste of the feed that induces a lower feed intake due to reduced palatability (Butler *et al*., 1984). Hassan *et al*. (2003) and Ravindran *et al*. (2006) all reported that tannins in poultry diets reduced dry matter intake, body weight gain, feed efficiency and nutrient digestibility.

4.6.4 Point of Lay

The energy requirement for animals is partitioned into maintenance and production. Energy and protein content have an influence on growth, maturation and egg production (Kakengi *et al*., 2007). Sohail *et al.* (2003) reported that methionine and lysine levels in poultry diets have positive correlation with egg production and egg weight. Layers require a completely balanced ration to sustain maximum egg production. The average point of lay of improved IC is 143 days of age (Kamau *et al*., 2018). Inclusion of T5 and T6 diets (40%

MOLM) negatively affected point of lay of the chicken in this study (Table 4.5). The delay in laying could be explained by the impaired palatability reflected by lower feed intake due to the existence of antinutritional factors e.g. saponins and high crude fibre content that affects digestibility resulting to inadequate levels of energy, protein lowering availability and utilization of nutrients when MOLM was supplemented at 40% as compared to control. This is in agreement with studies by Kakengi *et al.* (2007) who reported a decrease in egg mass production, egg production percentage and egg weight at higher level (>15% MOLM) which was attributed to low digestibility of energy and protein.

The high levels of dietary fibre may increase digestible energy loss, reduce mineral availability, and influence negatively the bio productive indicators (Mateos *et al*., 2012). The fibrous components in the Moringa leaf meal lowered the efficiency of digestible energy and metabolizable energy in non-ruminant species such as poultry and pigs because structural carbohydrates are poorly digested and absorbed in the stomach and small intestine. They can also reduce digestion and absorption of other feeds that make up the diet due to their physical and chemical properties (Yu *et al*., 2016). The delayed point of lay observed in this study was associated to low digestibility of energy and CP, bulkiness at 40% inclusion level of MOLM in the diet that contributed to low energy and CP availability to layers. Low energy availability is associated with poor digestibility of energy in the CF component of MOLM as in other plant leaves (Tangendjaja *et al*., 1990).

The factors that affect egg size include age of the hen, breed, weight of the hen, management factors e.g. lighting, heat, stress and nutrition. The ideal weight of pullets varies according to breed or strain which range from 1.6-1.8kg at mature body weight. Pullets significantly underweight at sexual maturity will produce small eggs. Zita *et al.* (2009) reported that the weight of eggs increased with the age of chicken. However, egg weight at 72-74 weeks of age does not differ indicating that early period there was gain in egg weight but towards later part the egg weight remains static. Similar observation was reported by suk and park (2001).

The nutrients that control egg size are linoleic acid, protein and some specific amino acids e.g. lysine, methionine, cysteine. Reducing the level of one, or a combination of these nutrients in the diet will reduce egg size. Moringa leaf meal did not significantly $p>0.05$ influence egg weight and body weight in the present study. The egg weight had lower values which could have been due to fact that laying chicken used in the present study were within the first phase of egg production. Eggs from white leghorn pullets in first phase are usually smaller than in 2nd and 3rd phase (Kakengi, 2007).

4.7 Conclusion

It is concluded that feeding enzyme-treated MOLM-based diets in improved indigenous chicken grower diets improved feed intake up to 20% inclusion level however there was a decrease in feed conversion ratio, average daily gain, and point of lay as the levels of MOLM in the diets increased up to 40% inclusion level.

CHAPTER FIVE

ECONOMIC IMPLICATION OF FEEDING ENZYME -TREATED MORINGA *(M. oleifera*) LEAF MEAL TO IMPROVED INDIGENOUS GROWER CHICKEN IN KENYA

Abstract

Livestock feed prices in Kenya have been rising over the last decade with the increase in price being attributed to the cost of protein ingredients especially soybean meal and fishmeal in the feed. The main goal of poultry farmers is to minimize production costs and to increase farm profits. Feed costs amount to a considerable proportion of production cost in any intensive livestock production system. The use of non-convectional feed materials that are cheaper can be a viable option to counteract the high cost of feed. The high cost of feed is a result of the competition between man and livestock for these feed ingredients. An experiment was conducted to assess the effect of supplementation of *Moringa oleifera* leaf meal (MOLM) with multi-enzyme on the economics of production in improved indigenous chicken. The objective of this study was to determine cost of poultry production with inclusion of treated Moringa leaf meal in growing chicken's diets. Total feed cost was calculated as the product of feed intake per chicken and the cost per kg of each dietary treatment. Total feed cost per kilogram of gain of each treatment was calculated as the total feed cost divided by the body weight gain of each chicken per dietary treatment. Data were analysed using the general linear model (GLM) procedure of the statistical analysis system (SAS, 2009). Mean separation was conducted using Tukey's HSD. From the results, control diet (T1) significantly (p<0.05) resulted in the lowest feed cost per kilogram gain compared to MOLM-based diets. Inclusion of MOLM with Natuzyme® multi-enzyme complex at 40% (T5, T6) resulted in the highest cost (Kes 165.32) of production compared to the cost of control diet (Kes 70.47). The inclusion of enzyme-treated MOLM at high levels (20-40%) led to increase of cost per kg weight gain, therefore, lower levels should be used to improve profit margins.

5.1 Introduction

The chicken subsector has experienced several challenges with the high cost of feed driving some farmers out of production. The poultry production in Kenya is constrained by inadequate supply of good quality feed and escalating costs. A major constraint is the very high cost of conventional feed ingredient especially protein sources. Research into the use of non-conventional feed ingredients poultry diets is being intensified with the view to bringing down cost of feed and hence poultry products (Mengesha, 2012). Leaf meals have been incorporated in the diets of poultry as a means of reducing the high cost of conventional protein sources. This is due to poor availability and expensive raw materials especially the proteins. The competition of humans and livestock for the same products further worsens the situation and therefore the need for sourcing for other available low cost materials that would substitute the raw materials already in the market especially the soybean meal and fishmeal. Nworgu *et al*. (2003) and D'Mello *et al*. (1987) observed that leaf meals do not only serve as protein source but also provide some necessary vitamins, minerals and oxycarotenoids which cause yellow colour of broiler skin, shank and egg yolk. Indigenous chicken farming is a pillar of food security improvement, socio-cultural and economic development (Missohou, 2002).

The main objective of any chicken enterprise is to ensure chicken welfare, structure viability, job security and consumers' food safety. Therefore, poultry industries have been concerned with improvement and optimum profitability. In developing countries, economic conditions and the quest for profit maximization have led farmers to compromise the use of most factors of production, especially poultry feed. The importance of poultry feed is based on the fact that it accounts for approximately 60-70% of the production cost (Ravindran, 2013). The high cost of feed is a result of the competition between man and livestock for these feed ingredients (Madubuike *et al*., 2006). Poultry farmers can formulate ration in such a way to fit animal nutritional requirements and improve performance while minimizing costs. Moringa is a non-leguminous multi- purpose tree widely available in the tropical zone and is used in feeding livestock. Leaves of this plant are known to contain 26% of crude protein, 9.5 MJ/kg of metabolizable energy, high quantities of saponins, carotene, ascorbic acid, iron, methionine and cysteine (Sultana *et al*., 2014). This study, therefore, was undertaken to evaluate the financial impact of varying inclusion levels of treated *M. oleifera* leaf meal in diets of improved grower indigenous layer chicken.

5.2 Materials and Methods

5.2.1 Economics of Production

Economic analysis was conducted to compare the feed cost for 1 kg weight gain. The feed cost per weight gain was calculated based on price of raw materials during the time of the experiment (March, 2021). The feed cost per diet was computed by multiplying the price per kilogram of each ingredient by the proportion of each ingredient in the diet.

Total feed cost was then calculated as the product of total feed consumed during the experimental period and the cost per kg of each diet. Thus, the total feed cost per kilogram of gain (Kes/kg) was equal to total feed cost divided by total body weight gain. This methodology compares the feed cost for 1 kg weight gain (Choi *et al*., 2015).The cost per kg of MOLM was calculated based on the collection fee paid, transport cost and the cost of milling and mixing with other ingredients.

$$\text{Feed cost per weight gain} = \frac{\text{Feed cost (Kes/kg)} \times \text{Feed intake per head(kg)}}{\text{Weight gain per head (kg)}}$$

5.2.2 Statistical Analysis

Data was subjected to analysis of variance (ANOVA) using the general linear model (GLM) procedure of the statistical analysis system version 9.1. Significant means were separated using Tukey's test at $p<0.05$.

The model used was:

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

Where;

Y_{ijk} = response variable of interest (**Feed cost per weight gain**)

μ = population mean

T_i = fixed i_{th} treatment effect (T1, T2, T3, T4, T5 and T6)

ε_{ijk} = random error

5.3 Results

5.3.1 Feed Cost (Kes) Per Kg Gain

Control diet (T1) resulted in lower feed cost per kilogram gain compared to MOLM-based diets that had a significantly ($p<0.05$) higher feed cost per kilogram gain. Inclusion of

MOLM at 40% level (T5 and T6) had the highest feed cost per kilogram gain when compared to control ($p< 0.05$).

Table 5.1 Effect of diet and enzyme interaction on feed cost per weight gain

Parameters	T1	T2	T3	T4	T5	T6	SEM	p value
Feed intake (g)	83.51^{c}	88.92^{d}	83.99^{c}	88.91^{d}	76.62^{b}	73.72^{a}	0.57	<.0001
Feed cost/daily gain	70.47^{a}	75.22^{b}	111.78^{d}	109.11^{c}	165.32^{f}	142.86^{e}	4.86	<.0001
Cost/kg of feed (Kes)	62.30^{a}	62.48^{b}	81.48^{d}	81.19^{c}	91.13^{f}	90.95^{e}	4.75	<.0001
Cost/feed consumed	5311.70^{a}	5716.00^{b}	7141.00^{d}	7541.70^{f}	7345.00^{e}	6865.30^{c}	52.92	<.0001
Profit	2188.16	1783.4	358.72	634.45	57.80	154.53		

Cost of feed consumed (Kes)= product of feed intake/chicken and cost/kg of feed. Selling price=product of the final body weight of chicken and N500 (selling price per kg of live chicken in Naivasha market). Profit=selling price minus cost of feed consumed (all other costs were assumed constant). Means in the same row without common superscripts are different at $p<0.05$.

5.4 Discussion

5.4.1 Economic Benefit of Including Treated MOLM in Improved Grower IC Diets

The purpose of modern poultry production systems is to obtain maximum profit at minimum production cost, of which 60-70 % of this production cost consists of the feed cost (Tesfaye *et al.*, 2013). The high cost of feed is attributed to competition with humans for energy and protein sources and the high cost of protein ingredients, most of which are imported. Differences observed in accrued revenue and yield can be due to differences in weight gain. These differences in weight gain can be explained by the effect of MOLM on feed conversion ratio. Feed conversion ratio is known to be the key element in livestock production and it is preferred to be as low as possible by every farmer. Concerning revenue and profit maximization, the results revealed that chicken offered T4 (20% MOLM), had the

best feed conversion ratio, which resulted in low feed intake when compared with the chicken on T6 (40% MOLM).

Moringa leaf meal incorporation into feed at 40% resulted in low feed efficiency. This adverse effect can be attributed to the high content of anti-nutritional factors such as tannins, non-starch polysaccharides, saponins, oxalates, glucosinolates and phytic acid in the diet containing 40% MOLM (T5 and T6). Study conducted by Teteh *et al*. (2017) confirmed this adverse effect where hens on higher amount of MOLM (2%) had the higher feed transit due to the relative high amount of anti-nutritional factors in his diet. The net return in this study decreased as the level of MOLM inclusion in the diet increased. This is due to the decreasing body weight gain in relation to increasing MOLM level beyond 20%.

The cost of feeding the chicken was significantly ($p<0.05$) higher in MOLM-based diets compared to control diets. The feed cost per average daily gain and feed cost per kilogram feed was higher in MOLM-based diets compared to control diets (Table 5.1). Diets T5 and T6 had the highest inclusion of MOLM (40%) resulted in increased feed cost. This is in line with the findings of the studies by Onibi *et al*. (2008) and Tendonkeng *et al*. (2011) in which feed cost/kg live body weight of broiler finishers increased with Moringa leaf meal (15%) inclusion in the diets. This is comparable to the findings of Zanu *et al*. (2012) who observed that partial replacement of fishmeal with MOLM at 5, 10 and 15% decreased the net revenue from broilers according to their reduction in weight gain. Ayssiwede *et al*. (2010) reported that the lowest feed cost/kg carcass weight was achieved when 8% and 16% of MOLM was introduced into the diets of the chicken. This is in line with this study which observed that it was not profitable to include 20-40 % enzyme-treated MOLM in the diets in comparison to the control diets. The increasing feed costs along with reduction of economic margins can be explained by the influence of the high price of the opportunity cost of harvesting, processing leaves and transportation of Moringa leaves meal.

5.5 Conclusion

It is not economical to incorporate enzyme-treated MOLM at 20-40% inclusion level in improved indigenous grower chicken diets as it does not improve economics of production in Kenya.

CHAPTER SIX

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

6.1 General Discussion

Poultry production performance depends on nutrition, genetics and environmental factors. Protein supplementation is very important for production and performance with the main sources being the soybean meal and fishmeal in Kenya. These two sources are however not always available to farmers because of the high demand from rapidly growing human population coupled with their escalating costs. It has therefore become necessary to look for alternative feed sources that need to be identified and evaluated (Nuhu, 2010). There is need to use other feed resources that can make chicken perform at the same level as when fed on conventional feeds. MOLM has great potential in replacing soybean meal through sustainable feed availability, quality and lower feed cost. It has high biological value protein, vitamins and minerals. Utilization of this feed by chicken is limited by high fibre content which lowers digestibility. Research work has shown that the negative effects of non- starch polysaccharides (NSPs) e.g. cellulose, hemicellulose, pectin which are part of the dietary fibre can be overcome by dietary modifications including supplementation of diets with suitable exogenous enzyme preparations (Creswell, 1994).Studies on the growth performance of broiler chicken demonstrated that *M. oleifera* leaf meal can significantly improve bowel health by balancing intestinal microflora, thus promoting weight gain (Nkukwana *et al*., 2014). In a study conducted by Onunkwo and George (2015), there was no difference in the feed intake and body weight gain of broiler chicken fed with soybean meal with *M. oleifera* leaf meal at the rate of 0.0, 5.0, 7.5, and 10%, demonstrating that *M. oleifera* leaf meal can replace soybean and ground nut cake (partial protein source) in poultry diets without causing any deleterious effects on growth performance. Among all dietary treatments, 8 and 16% *M. oleifera* leaf in the diets were the most economically profitable formulas, which significantly increased the growth rate of chicken (Ayssiwede *et al.*, 2011). Gadzirayi *et al*. (2012) investigated the effects of *M. oleifera* leaf at a rate of 0%, 25%, 50%, 75% and 100% as a protein source substitute for soybean meal in poultry feeding. Although the feed intake under different treatments was not significantly different, the feed conversion ratio significantly differed as evidenced by the variation in weight gain.

Fermentation and enzyme are methods used in treatment of feeds to improve digestibility and utilization. Fermentation degrades the substrate prior to feeding and potentially renders especially the fibrous fraction more available for digestion in chicken and ultimately improves the energy digestibility. Pedersen and Lindberg (2003) found that *in vitro* fermentation of wet feed improved digestibility of organic matter (OM) and crude protein. Enzymes are specialized proteins that catalyse or accelerate the chemical reaction. Commonly used exogenous enzymes in poultry diets are β-glucanase, xylanase, amylase, α-galactosidase, protease, lipase, and phytase (Adeola *et al*., 2011). The role of exogenous enzymes is to fulfil the absence of endogenous enzymes, to counter the anti-nutritional factors present in conventional and unconventional poultry diet. These exogenous enzymes, in combination with non-conventional ingredients, are used to reduce the cost of feeding and to utilize the non-conventional feed ingredients efficiently as non-conventional feedstuffs are typically high in fibre and are not degraded by endogenous enzymes of poultry (Costa *et al*., 2008). Results from this study showed a significant (p<0.05) increase in CP levels of enzyme-treated (27.95%) and Fermented MOLM (27.89%) compared to untreated MOLM (26.8%). This can also be ascribed to the loss of DM (mainly carbohydrates) and the synthesis of fungal biomass proteins (Hong *et al*., 2004). Increase in CP in FMOLM is in agreement with the results obtained for fermented soybean meal (Chen *et al*., 2013). The content of CF, NDF, ADF, Cellulose and hemicellulose were significantly (p<0.05) lower in the enzyme- treated and fermented MOLM compared to the control. This is because enzymes cause the disruption of the plant cell wall integrity and consequently release nutrients encapsulated by the cell wall (Ravindran, 2013). The crude protein digestibility in the enzyme-treated was significantly higher relative to control, this is because enzymes break down the NSPs, decrease intestinal viscosity and eventually improve the digestibility of nutrients.

Feed intake ensures an adequate and balanced nutrient intake and has been suggested as the single-most important factor determining the growth rate of broilers (Ferket & Gernat, 2006).A wide variety of both nutritional and non-nutritional factors affect feed intake in chicken (Applegate, 2012).The factors that affect feed intake, and hence nutrient intake, of the chicken include dietary factors (feed form, nutrient density and composition anti-nutritional factors, feed formulation and feed stuff inclusion levels), management factors (stocking density, temperature, lighting, feed and water availability to the chicken, environmental management, disease control stress and water supply) and chicken (genotype, sex, age and capacity of digestive tract) factors (Abdollahi *et al.*, 2013c ; Applegate, 2012;

Brickett *et al.*, 2007; Latshaw & Moritz, 2009; Sklan, 2001).In this study the feed intake decreased significantly (p<0.05) with the increasing level of MOLM in comparison to control, this is attributed to the high fibre content in MOLM. The difference could be due to high fibre levels that were in treatment five and six with 40%MOLM in the diet as protein source. The findings agree with literature that monogastrics cannot utilise high crude fibre diets efficiently Results showed that chicken fed diet with 0% MOLM had significantly (p<0.05) higher weight gain than chicken fed diets with MOLM. Chicken fed on 20-40% MOLM had significantly (p<0.05) lower weight gains compared to control diets. This confirmed the observations made by Ash and Petaia (1992) and Olugbemi *et al*. (2010) that increasing inclusion level of leaf meals 20-30% cassava chips with similar chemical composition as MOLM in broiler diets results in depressed growth performance. This observation could be generally attributed to increasing fibre content of the diet, which impaired nutrient digestion and absorption (Ige *et al*., 2006). It could also be attributed to the crude protein content of the control diet, which enhances its acceptability and utilization. The negative effect of the anti-nutritional factors (ANFs) e.g. tannins, phytates, oxalates, saponins, high fibre cotent and phytochemical compounds e.g. glucosinolates, flavonoids, phenolic acid, carotenoids and tocopherols present in *Moringa oleifera* leaf meal could be responsible for decreasing performance on the chicken in terms of feed intake, feed conversion ratio and weight gain (Onu, 2010). The tannins interact with trypsin and amylase or with substrates of enzymes to form complexes that are not readily digestible, saponins provide bitter taste resulting to decreasing palatability and feed intake while increasing fibre content of the diet may have impaired nutrient digestibility and absorption (Ige *et al*., 2006). There was a significant (p<0.05) decrease in the feed conversion ratio of the chicken fed on MOLM based diets as compared to the control group. This may be attributed to low digestibility of feeds consumed by chicken fed MOLM based diets due to its bioactive compounds, including antioxidants and phytoestrogens which reduce nutrient utilization. The results are in contrary with the finding of Ebenebe *et al*. (2012) who reported that, chicks fed on Moringa based diets had higher weight gain and better feed conversion ratio (p<0.05) than the chicken of control group. This improvement in body weight gain and feed conversion ratio may be attributed to the nutrient content Sarwatt *et al*. (2004) and antimicrobial properties of MOLM (Fahey *et al*., 2001).

Proper nutrition, supply of available and digestible nutrients are necessary to maintain adequate egg production. In this study inclusion of 40% MOLM negatively affected the point of lay of the chicken in this study (Table 4.5). The delay in laying could be explained by the

impaired palatability reflected by lower feed intake due to the existence of antinutritional factors e.g. saponins and high crude fibre content that affects digestibility when MOLM was supplemented at 40% as compared to control. The high levels of dietary fibre may increase digestible energy loss, reduce mineral availability, and influence negatively the bio productive indicators (Mateos *et al*., 2012). The delayed point of lay observed in this study was associated to low digestibility of energy and CP, bulkiness when MOLM was higher in the diet that contributed to low energy and CP availability to layers. The net return in this study decreased as the level of MOLM inclusion in the diet increased. This is due to the decreasing body weight gain in relation to increasing MOLM level beyond 20%. This is in line with the findings of the studies by Onibi *et al*. (2008) and Tendonkeng *et al*. (2011) in which feed cost/kg live body weight of broiler finishers increased with Moringa leaf meal (15%) inclusion in the diets. This is comparable to the findings of Zanu *et al*. (2012) who observed that partial replacement of fishmeal with MOLM at 5,10 and 15% decreased the net revenue from broilers according to their reduction in weight gain.

6.2 Conclusions

i. The enzyme-treated MOLM had the highest DM digestibility (60.3%) followed by *S. cerevisiae* fermented (54.4%) respectively. The treatment with multi-enzyme improved CP digestibility by 25% compared to untreated MOLM. The content of crude fibre, neutral detergent fibre, acid detergent fibre, cellulose and hemicellulose were lower in enzyme-treated MOLM compared to the control therefore, enzyme treatment was beneficial in improving the chemical composition, *in-vitro* dry matter and crude protein digestibility of MOLM compared to all other treatments tested

ii. Inclusion of enzyme-treated MOLM-based diets in improved indigenous chicken grower diets improved feed intake up to 20% inclusion level however there was a decrease in feed conversion ratio, average daily gain, and point of lay as the levels of MOLM in the diets increased up to 40% inclusion level.

iii. Inclusion of enzyme-treated MOLM at 20-40% level did not improve the economics of production of improved grower IC compared to control diet.

6.3 Recommendations

i. I recommend use of enzyme treatment which improved the nutritional quality of MOLM in terms of chemical composition, DM and CP digestibility.

ii.I recommend use of enzyme-treated MOLM up to at 20% inclusion level which improved performance, however higher levels above that have detrimental effect.

iii.It is not economical to incorporate enzyme-treated MOLM at 20-40% inclusion level in IC grower diets.

6.4 Areas for Further Research

i. Conduct a study to determine the level of inclusion between 10-20% that will give a performance better or equal to the control regarding the parameters assessed in this study.

ii. Conduct a study comparing the chemical composition of MOLM from different sites in Kenya iii. Conduct a study to determine the effect of incorporation of MOLM on egg quality

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APPENDICES

APPENDIX 1: Research Ethics Clearance

EGERTON UNIVERSITY
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EGERTON

EGERTON UNIVERSITY RESEARCH ETHICS COMMITTEE

EU/RE/DVC/009
Approval No. EUREC/APP/141/2021 **21st October, 2021**

Caroline Nkirote Muremera
P.O. Box 536-20115
Egerton
Telephone: 07133449915
E-mail: carolinemuremera@gmail.com

Dear Caroline,

RE: ETHICAL APPROVAL: EVALUATION OF INCORPORATION OF TREATED MORINGA (MORINGA OLEIFERA) LEAF MEAL IN THE DIETS OF IMPROVED INDIGENOUS GROWER CHICKEN ON GROWTH PERFORMANCE IN KENYA

This is to inform you that ***Egerton University Research Ethics Committee*** has reviewed and approved your above research proposal. Your application approval number is ***EUREC/APP/141/2021.*** The approval period is ***21st October, 2021 –22nd October, 2022.***

This approval is subject to compliance with the following requirements;

i. Only approved documents including (informed consents, study instruments, MTA) will be used.
ii. You are required to adhere Institutional Experimental Animals use and Care policy.
iii. All changes including (amendments, deviations, and violations) are submitted for review and approval by ***Egerton University Research Ethics Committee***.
iv. Death and life-threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to ***Egerton University Research Ethics Committee*** within 72 hours of notification
v. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to ***Egerton University Research Ethics Committee*** within 72 hours
vi. Clearance for Material Transfer of biological specimens must be obtained from relevant institutions.
vii. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.

APPENDIX 2: NACOSTI Permit

THE SCIENCE, TECHNOLOGY AND INNOVATION ACT, 2013

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY & INNOVATION

Ref No: 847560

Date of Issue: 10/November/2021

RESEARCH LICENSE

This is to Certify that Miss. Caroline Nkirote Muremera of Egerton University, has been licensed to conduct research in Nakuru on the topic: EVALUATION OF INCORPORATION OF TREATED MORINGA (Moringa oleifera) LEAF MEAL IN THE DIETS OF IMPROVED INDIGENOUS GROWER CHICKEN ON GROWTH PERFORMANCE IN KENYA for the period ending: 10/November/2022.

License No: NACOSTI/P/21/14052

847560

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY & INNOVATION

Verification QR Code

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APPENDIX 3: ANOVA Outputs

DM digestibility

The SAS System 19:01 Saturday, April 17, 2021 2

The ANOVA Procedure

Dependent Variable: output

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	3077.677373	1025.892458	782.03	<.0001
Error	44	57.720675	1.311834		
Corrected Total	47	3135.398048			

R-Square	Coeff Var	Root MSE	output Mean
0.981591	2.309131	1.145353	49.60104

Source	DF	Anova SS	Mean Square	F Value	Pr > F
trt	3	3077.677373	1025.892458	782.03	<.0001

The ANOVA Procedure

Tukey's Studentized Range (HSD) Test for output

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	trt

A	60.2742	12	Enzyme
B	54.3933	12	Cerevisiae
C	41.8992	12	Natural
C	41.8375	12	control

CP digestibility results

The SAS System 22:16 Saturday, April 17, 2021 2

The ANOVA Procedure

Dependent Variable: output

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	1057.164092	352.388031	14.38	0.0014
Error	8	196.095200	24.511900		
Corrected Total	11	1253.259292			

R-Square	Coeff Var	Root MSE	output Mean
0.843532	10.24281	4.950949	48.33583

Source	DF	Anova SS	Mean Square	F Value	Pr > F
trt	3	1057.164092	352.388031	14.38	0.0014

The ANOVA Procedure

Tukey's Studentized Range (HSD) Test for output

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	trt
A	63.067	3	Enzyme
B	48.803	3	Cerevisiae
B			
B	43.873	3	natural
B			
B	37.600	3	control

Data for second objective

Experiment two

Feed intake

The SAS System 14:54 Friday, September 28, 2001 54

The GLM Procedure

Dependent Variable: FI

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	711744.7346	142348.9469	30.92	<.0001
Error	12	55246.1883	4603.8490		
Corrected Total	17	766990.9228			

R-Square	Coeff Var	Root MSE	FW Mean
0.927970	3.720255	67.85167	1823.844

The GLM Procedure

Least Squares Means

Adjustment for Multiple Comparisons: Tukey

Least Squares Means for Effect Treatment*enzyme

t for H0: LSMean(i)=LSMean(j) / Pr > |t|

Dependent Variable: FI

i/j	1	2	3	4	5	6
1		1.527058	1.761351	3.081793	9.197244	8.725649
		0.6553	0.5216	0.0792	<.0001	<.0001
2	-1.52706		0.234293	1.554735	7.670186	7.198591
	0.6553		0.9999	0.6395	<.0001	0.0001
3	-1.76135	-0.23429		1.320442	7.435893	6.964298
	0.5216	0.9999		0.7693	<.0001	0.0002
4	-3.08179	-1.55473	-1.32044		6.115451	5.643856
	0.0792	0.6395	0.7693		0.0006	0.0012
5	-9.19724	-7.67019	-7.43589	-6.11545		-0.47159
	<.0001	<.0001	<.0001	0.0006		0.9963
6	-8.72565	-7.19859	-6.9643	-5.64386	0.471595	
	<.0001	0.0001	0.0002	0.0012	0.9963	

Feed conversion ratio

The GLM Procedure

Class Level Information

Class	Levels	Values
Treatment	6	1 2 3 4 5 6
Replicate	3	1 2 3
enzyme	2	with without

Number of observations 18

The GLM Procedure

Dependent Variable: FCR

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	28.41089444	5.68217889	642.86	<.0001
Error	12	0.10606667	0.00883889		
Corrected Total	17	28.51696111			

R-Square	Coeff Var	Root MSE	fcr Mean
0.996281	2.804104	0.094015	3.352778

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treatment	5	28.41089444	5.68217889	642.86	<.0001
Treatment*enzyme	0	0.00000000	.	.	

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	5	28.41089444	5.68217889	642.86	<.0001
Treatment*enzyme	0	0.00000000	.	.	

The GLM Procedure

Least Squares Means

Adjustment for Multiple Comparisons: Tukey

Treatment	fcr LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
1	1.59000000	0.05427980	<.0001	1
2	1.68000000	0.05427980	<.0001	2
3	3.82000000	0.05427980	<.0001	3
4	3.82000000	0.05427980	<.0001	4
5	4.61666667	0.05427980	<.0001	5
6	4.59000000	0.05427980	<.0001	6

Least Squares Means for effect Treatment

Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: fcr

i/j	1	2	3	4	5	6
1		0.8414	<.0001	<.0001	<.0001	<.0001
2	0.8414		<.0001	<.0001	<.0001	<.0001
3	<.0001	<.0001		1.0000	<.0001	<.0001
4	<.0001	<.0001	1.0000		<.0001	<.0001
5	<.0001	<.0001	<.0001	<.0001		0.9991
6	<.0001	<.0001	<.0001	<.0001	0.9991	

Average daily gain

The GLM Procedure

Dependent Variable: ADG

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	16	139489.3422	8718.0839	14.12	<.0001
Error	199	122849.8417	617.3359		
Corrected Total	215	262339.1839			

R-Square	Coeff Var	Root MSE	ADG Mean
0.531714	40.80687	24.84624	60.88741

The GLM Procedure

Least Squares Means

Adjustment for Multiple Comparisons: Tukey

Least Squares Means for Effect Treatment*Enzyme

t for H0: LSMean(i)=LSMean(j) / Pr > |t|

Dependent Variable: ADG

i/j	1	2	3	4	5	6
1		0.716964	0.848365	2.285885	5.789022	4.138999
		0.9797	0.9579	0.2047	<.0001	0.0007
2	-0.71696		0.131401	1.568921	5.072057	3.422035
	0.9797		1.0000	0.6200	<.0001	0.0097
3	-0.84837	-0.1314		1.43752	4.940657	3.290634
	0.9579	1.0000		0.7041	<.0001	0.0148
4	-2.28588	-1.56892	-1.43752		3.503137	1.853114
	0.2047	0.6200	0.7041		0.0074	0.4343
5	-5.78902	-5.07206	-4.94066	-3.50314		-1.65002
	<.0001	<.0001	<.0001	0.0074		0.5665
6	-4.139	-3.42203	-3.29063	-1.85311	1.650023	
	0.0007	0.0097	0.0148	0.4343	0.5665	

Feed cost

The SAS System 14:54 Friday, September 28, 2001 69

The GLM Procedure

Dependent Variable: cost

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	20638.19105	4127.63821	58.10	<.0001

Error	12	852.56280	71.04690
Corrected Total	17	21490.75386	

R-Square	Coeff Var	Root MSE	cost Mean
0.960329	7.495304	8.428932	112.4562

The GLM Procedure

Least Squares Means

Adjustment for Multiple Comparisons: Tukey

Least Squares Means for Effect Treatment*enzyme

t for H0: LSMean(i)=LSMean(j) / Pr > |t|

Dependent Variable: cost

i/j	1	2	3	4	5	6
1		0.689955	-4.92443	-5.31255	-13.0921	-9.82659
		0.9797	0.0037	0.0020	<.0001	<.0001
2	-0.68995		-5.61439	-6.00251	-13.782	-10.5165
	0.9797		0.0012	0.0007	<.0001	<.0001
3	4.92443	5.614385		-0.38812	-8.16765	-4.90216
	0.0037	0.0012		0.9985	<.0001	0.0038
4	5.312553	6.002508	0.388122		-7.77953	-4.51403
	0.0020	0.0007	0.9985		<.0001	0.0072
5	13.09208	13.78204	8.167652	7.779529		3.265497
	<.0001	<.0001	<.0001	<.0001		0.0584
6	9.826586	10.51654	4.902155	4.514033	-3.2655	
	<.0001	<.0001	0.0038	0.0072	0.0584	

APPENDIX 4: Research Pictorial

Moringa tree

. Moringa leaves

Moringa leaf meal

Milling of MOLM

Fermentation of treated MOLM

***In-vitro* digestibility** **Feeding experimental chicken**

Chicken feeding on MOLM

APPENDIX 5:Publications

International Journal of Veterinary Sciences and Animal Husbandry 2022; 7(3): 48-52

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Performance of improved indigenous grower chicken in Kenya fed enzyme-treated Moringa *(M. oleifera)* leaf meal-based diets

CN Muremera, MK Ambula and AM King'ori

DOI: https://doi.org/10.22271/veterinary.2022.v7.i3a.423

Abstract
A feeding trial was done to investigate the impact of inclusion of enzyme-treated *Moringa oleifera* leaf meal (MOLM) on feed conversion ratio, average daily gain, feed intake and point of lay of improved indigenous grower chicken. Ninety chicken were randomly allocated to six treatment diets in a completely randomised design with a factorial layout, with each treatment having five chickens per cage, replicated three times. The following were the dietary treatments used: T1 – comprised of 0kg MOLM and 0g enzyme, T2 – comprised of 0kg MOLM and 0.035g enzyme, T3 – comprised of 20kg MOLM and 0g enzyme, and T4 – comprised of 20kg MOLM and 0.035g enzyme, T5- diet comprised of 40kg MOLM and 0g enzyme, T6-diet comprised of 40kg MOLM and 0.035g enzyme of the diet, respectively. The statistical analysis system's general linear model (GLM) approach was used in the data analysis. Tukey's test (p<0.05) was used to differentiate significant means. When comparing diets T5&T6 to the control, the results showed that feed intake differed significantly (p<0.05). The ADG of the MOLM-based diets was significantly (p<0.05) lower in comparison to the control diet. The point of lay of the chicken fed MOLM-based diets differed significantly (p<0.05) from the control. The study recommended inclusion levels of enzyme-treated MOLM at 10-20% which would improve performance.

Keywords: Enzyme, improved indigenous chicken, *Moringa oleifera* leaf meal, performance

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Effect of feeding enzyme-treated Moringa (*M. oleifera)* leaf meal based-diets on egg quality of improved indigenous layer chicken in Kenya

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DOI: https://doi.org/10.22271/veterinary.2022.v7.i5a.443

Abstract
The egg quality of indigenous chicken in Kenya is low due to inadequate supply of quality feed which can only be achieved by nutritional improvement. *Moringa oleifera* leaf meal (MOLM) provides proteins, vitamins, minerals and ox carotenoids that have a positive effect on the quality of eggs. The objective of this research was to determine the influence of MOLM on egg quality traits of improved indigenous laying hens. Ninety chicken (90) were assigned to six treatment diets in a completely randomised design with a factorial layout, each treatment having 5 birds per cage, replicated three times. The dietary treatments were: T1 – 0 kg MOLM and 0 g enzyme, T2 – 0 kg MOLM and 0.035 g enzyme, T3-20 kg MOLM and 0 g enzyme, and T4 – 20 kg MOLM and 0.035 g enzyme, T5-40 kg MOLM and 0 g enzyme, T6-40 kg MOLM and 0.035 g enzyme of the diet, respectively. The egg weight in hens fed with MOLM-based diets increased significantly (p<0.05) compared to control diet. In response to dietary *M. oleifera* leaf meal, there was no difference in egg shape index across the groups (p> 0.05). The laying hens fed a diet with 40 percent MOLM inclusion significantly had a higher (p<0.05) shell thickness and weight. Inclusion of MOLM in the diet increased the intensity of yellow colour in egg yolk (p<0.05) in comparison to the control diet. Significantly (p<0.05), the eggs from chicken fed a diet containing 40 percent MOLM inclusion had the highest Roche colour score of 14.62. When MOLM-based diets were compared with control, there were significant variations in yolk weight (p<0.05). The albumen height of eggs increased significantly (p<0.05) as dietary MOLM inclusion increased compared to control. The egg width, albumin width, yolk height, ratio, index and yolk/albumin ratio and shell ratio were similar (p>0.05) for all the dietary treatments. As the amount of MOLM in the diet increased, the egg length, albumin length, and yolk diameter were all significantly reduced (p<0.05) in comparison to the control. This study concluded that inclusion of 20-40 percent enzyme-treated MOLM in diets of laying hens improved egg weight, yolk weight, albumin height, yolk colour and shell thickness (p<0.05) compared to control diet.

Keywords: Egg, enzyme, hens, moringa, treatment