PERFORMANCE OF EXOTIC LAYER CHICKEN FED ON PROBIOTICS (*Bacillus coagulans)* TREATED MORINGA (*M. oleifera*) LEAF MEAL-BASED DIET

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DECLARATION AND RECOMMENDATION

Declaration:

This thesis is my original work and has not been submitted or presented to any institution for the award of any degree.

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DEDICATION

This work is first and foremost dedicated to Almighty GOD for HIS guidance and protection during my studies. I also dedicate this work to my late Mum Aminata Dansira, my lovely wife, Mariama Manneh Darboe, My daughter Fatima Darboe and my son Pah Sheikou Darboe Jnr,

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ABSTRACT

Moringa oleifera leaf meal is a potential novel feedstuff that can be incorporated into poultry feeds. However, the lack of appropriate knowledge and antinutritive factors in MOLM limits its use in poultry production. This study evaluated the Egg production and quality of exotic layer chicken Fed on Probiotics *(Bacillus coagulans)*-Treated Moringa (*M. oleifera*) Leaf Meal-Based Diet**.** The objectives of this study were to determine the (i) *In-vitro* dry matter digestibility and condensed tannins content of probiotics-treated MOLM. (ii) Determination of performance and sensory properties of layer chicken fed on Probiotics (*Bacillus Coagulans*)-Treated MOLM.. (iii) Effect of probiotic-treated MOLM in exotic chicken diets on egg quality and consumer acceptability. *Moringa oleifera* leaf meal was treated through different fermentation methods, using *Clostridium butyricum, Bacillus coagulans* and spontaneous fermentation in three replicates. The finding of the study showed a significant difference in IVDMD. Fermentation with *Bacillus coagulans* had the highest *invitro*-digestibility compared to the rest, and the untreated meal had the lowest IVDMD. Fermentation with *Clostridium butyricum* resulted in a higher IVDMD% than spontaneous fermentation. There was a significant improvement in the CP content compared to the untreated. Tannins and phenol content were reduced with MOLM treated with *Bacillus coagulans*. Thirty-six (36) sixteen, sixteen (16) week-old ISA Brown layers were distributed into 12 deep litter pens, each with three hens, and randomly allocated to four dietary treatments in a completely randomised design. Data analysis was conducted using the statistical analysis system's general linear model (GLM) approach. Tukey's test ($p<0.05$) was used to separate significant means. Results showed that birds fed on the diet with 20% MOLM treated with *Bacillus coagulans* recorded the highest average daily feed intake and highest Feed conversion ratio. The diet with 10% MOLM treated with *Bacillus coagulans* recorded the highest hen-day production. There was no significant difference ($p>0.05$) between the 0 and 10% MOLM treated with *Bacillus coagulans* diets on the average daily gain, Feed conversion ratio and egg weight. The diet with 20% MOLM treated with *Bacillus coagulans* recorded the Roche colour fan's most profound yolk colour value. The results showed that the 10% MOLM treated with *Bacillus coagulans* diet had higher shell weight, yolk weight, and yolk ratio, while the control group, 0% with MOLM, recorded better yolk width and yolk length. It was therefore concluded that including 10% MOLM treated with *Bacillus coagulans* layers (Isa-Brown) diet improved performance.

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LIST OF ABBREVIATIONS AND ACRONYMS

AA	Amino acid
ADFI	Average daily feed intake
ADG	Average daily gain
ANF	Anti-nutritive factor
ANOVA	Analysis of variance
CF	Crude fibre
CP	Crude protein
CRD	Completely randomized design
EAC	East African Community
EE	Ether Extract
FAO	Food Agriculture Organization of the United Nations
FAOSTAT	Food Agriculture Organisation Statistics
FCR	Feed conversion ratio
FER	Feed efficiency ratio
FI	Feed intake
GLM	Generalized linear model
HDP	Hen Day Production
HU	Haught unit
ISA Brown	"Institute de Selection Animale"
IVDMD	*In vitro* dry matter digestibility
ME	Metabolizable energy
MOLM	*Moringa oleifera* leaf meal
NCFR	Non-conventional feed resources
NRC	National Research Council
SADC	Southern Africa Development Community
SAS	Statistical Analysis System
SBM	Soybean Meal
SPSS	Statistical package of Social Science
TAP	Tatton Agriculture Park

CHAPTER ONE
INTRODUCTION

1.1 Background Information

Poultry constitutes a significant source of protein and income in low and middle-income countries; from 2000 to 2030, many African countries are expected to see a 100% growth in the demand for chicken products per person (Gilbert *et al.*, 2015). This increase in demand is driven by population expansion, shifting purchasing habits brought on by urbanisation and rising income. Demand for poultry products is rising, which fuels sector-wide structural changes. These may take the form of expansion, with more people producing, intensifying production and increased trade in products such as table eggs. In 2002, an estimated 53.4 million tons of table eggs were produced. In 2009, an estimated 62.1 million metric tons of table eggs were produced worldwide from a total laying flock of approximately 6.4 billion hens (Akanbi *et al.*, 2020). however, poultry production remains one of the key enterprises among smallholder households in Kenya. The poultry industry is categorised by dualism in Kenya. The industry comprises large-scale and small-scale poultry producers (Victor *et al.*, 2015).

Significant growth in the poultry sector has occurred due to the use of quality inputs, modern technology and improved management practices over the last few decades (Saleque *et al.*, 2020). Even though it is one of the livestock sectors with the most incredible growth rate globally, the poultry industry is constrained by a severe lack of feed components, particularly in developing nations; exploring non-traditional feed sources that could be used in chicken feed is therefore crucial. A versatile tree that grows well in both tropical and subtropical climates is *Moringa oleifera;* Moringa is among non-legume trees and shrubs and is a promising option for animal feed due to its high availability and excellent nutritive value (Beyene, 2021). Moringa is a tree belonging to the Moringaceae family, and it is an essential tree in India, Ethiopia, the Philippines and Sudan; it is grown in West, East and South Africa, tropical Asia, Latin America, the Caribbean, Florida and the Pacific Islands, tropical, subtropical and semi-arid regions of the world. It can grow under drought conditions, but its intensive irrigation and fertilisation production can increase yields to more than 100 t/ha. It is known by several common names, such as Marango, Moringa, Reseda, Radish tree, Ramrod tree, Angela, Asparagus tree, Pearl tree, Ben tree, tree of life, Drumstick tree, Horseradish tree, Ben oil tree or Benzoil tree and the tree of miracles (Rodríguez *et al.*, 2017).).

According to Gadzirayi *et al.* (2012), *Moringa oleifera* leaf meal (MOLM) has high quantities of vitamins, minerals, and crude protein. Moringa leaf meals are rich in nutrients such as protein (22.9–29.36%), ash (8.05–10.38%), carbohydrates (47.25–56.28%), crude fibre (6–9.6%), and fat (4.03–9.51%) that are good for poultry health and development (sultana *et al.,* 2020). The amino acid profile of MOLM is also impressive as most essential and nonessential ones, namely valine (1.23–1.44%), leucine (1.69–1.94%), isoleucine (1.12–1.3%), histidine (0.53–0.62%), lysine (1.24–1.39%), methionine (0.33–0.42%), phenylalanine (1.41–1.5%), tryptophan (0.88–1.02%), threonine (1.33–1.42%), glycine (8.74–9.4%), alanine (0.98–1.09%), proline (1.04–1.19%), arginine (1.43–1.55%), cysteine (0.37–0.44%), serine (0.79–0.92%), aspartic (1.29–1.46%), and glutamic (2.33–2.45%), are present (Faustin *et al.,* 2022).

This makes MOLM a suitable supplement for dietary formulations in poultry to improve growth performance through enhanced gut health (Faustin *et al.*, 2022). However, specific secondary metabolites, such as polyphenols (tannins), may negatively affect nutrient digestibility (Nkukwana *et al.,* 2014) and absorption in poultry, resulting in higher feed conversion. For instance, Cui *et al.* (2018) observed a decline in some parameters of productive performance with increasing dietary levels of MOLM in broiler chickens and recommended up to 1.56% inclusion in the diets. Regarding slow-growing birds, Sebola *et al.* (2015) suggested up to 7% of Moringa in the diet of indigenous chickens intensively grown. The possibility of including high levels of MOLM in the diets for poultry was demonstrated by the adequate growth rate, egg production, viability, health, egg quality, higher leanness in meats and good flavour of the final products (meat and eggs), with such promising attributes, Moringa can be utilised as a viable feed ingredient to satisfy the nutrient requirement and promote the health of poultry species (Taufek *et al.,* 2022). Research findings showed the hat use of probiotics in layer diets enhanced egg production (Peralta-Sánchez *et al.,* 2019). The inclusion of *Lactobacillus acidophilus* (109 cfu/kg feed) significantly enhanced the number of eggs. A combination of *Lactobacillus spp*. and *Bacillus spp*. as liquid probiotics mixed culture when fed to the ISA Brown layers showed an increase in egg production (Raka *et al.,* 2014). Abdelqader *et al.* (2013) showed that feeding of *Bacillus subtilis* (maximum 2.3×108 cfu/g) had significantly better egg production compared to control at doses of 1g/kg feed and 0.5 g/kg feed for a duration of 10 weeks during late production period starting from 64 weeks.

1.2 Statement of Problem

For poultry, the primary protein sources are fish and soybean meals. However, the costs of these protein sources have been steadily rising, which accounts for 60-80% of production costs. Their supply is frequently scarce and low quality due to adulteration and growing competition as food between humans and livestock. The soybean meals also do not contain enough β-carotene, which gives the egg yolk the deep yellow colour consumers prefer. Despite Moringa leaf being locally available, it has not been commonly used in poultry feeds due to a lack of appropriate knowledge, and antinutritive factors in MOLM limit its use in poultry production, such as polyphenols (tannins), which may negatively affect nutrient digestibility and absorption in poultry. These anti-nutrients can be reduced or neutralized through various treatments like fermentation with microorganisms.

1.3 Objectives

1.3.1 Broad Objective

To contribute to food and nutritional security through sustainable chicken production in the tropics by the use of MOLM as a locally available protein source.

1.3.2 Specific Objectives

I. To determine the *in-vitro* dry matter digestibility and condensed tannins content of probiotics-treated MOLM.

II. Determination of performance and sensory properties of layer chicken fed on Probiotics (*Bacillus Coagulans*)-Treated MOLM.

III. To determine the effect of probiotics (*Bacillus coagulans*) treated MOLM in exotic layer chicken diets on egg yolk colour, eggshell thickness, egg shape index, yolk index, egg weight and consumer acceptability.

1.3.3 Hypotheses

i. The *in-vitro* dry matter digestibility and condensed tannins content of probiotics-treated and non-treated MOLM are not significantly different.

ii. Incorporation of probiotics (*Bacillus coagulans*) treated MOLM in an exotic layer chicken diet has no significant effect on the performance and sensory properties.

iii. Incorporation of Probiotic (*Bacillus coagulans*)--treated MOLM in exotic layer chicken has no significant effect on the egg yolk colour, eggshell thickness, egg shape index, yolk index egg weight and consumer acceptability.

1.4 Justification of the Study

Poultry plays a significant role in creating job opportunities, generating income, and enhancing nutrition in developing nations. It provides affordable and high-quality protein, which contributes to Sustainable Development Goals (SDGs1-zero hunger, good health, and well-being). The fundamental aim of the poultry industry is to feed the world by ensuring a secure supply of adequate and healthy poultry products to customers worldwide. This will make a nutritious, affordable, and sustainable protein source accessible to everyone. (Henriksen, 2017). Chicken eggs contain 13 essential vitamins and minerals, protein, and about 70 calories (Egg Nutrition Center, 2016); even though it is one of the animal sectors with the greatest rate of growth globally, the poultry industry is constrained by a severe lack of feed components which accounts for 60-80% of production cost. Hence, this has led to the search for locally available alternative protein sources. Moringa leaf meal has been identified as a locally available alternative protein source for poultry feeds that may improve egg productivity and quality in the chicken diet compared to the diet without MOLM (Ebenebe *et al.,* 2013). Moringa is among non-legume trees and shrubs and is a promising option for animal feed due to its high availability and excellent nutritive value (Beyene, 2021). Moringa leaf meal can partially substitute protein sources like soybean meal at 10% on a protein basis (Swain *et al.,* 2017). Moringa is high in six major nutrients: carbohydrates, protein, vitamins, minerals, lipids and water. The leaves contain various essential amino acids and are a good source of alpha-linoleic acids (Mahfuz *et al.,* 2019). Moringa leaf meals are rich in nutrients such as protein (22.9–29.36%), ash (8.05–10.38%), carbohydrates (47.25–56.28%), crude fibre (6–9.6%), and fat (4.03–9.51%) that are good for poultry health and development (sultana *et al.,* 2020). The amino acid profile of MOLM is also impressive as most essential and nonessential ones, namely valine (1.23–1.44%), leucine (1.69–1.94%), isoleucine (1.12–1.3%), histidine (0.53–0.62%), lysine (1.24–1.39%), methionine (0.33–0.42%), phenylalanine (1.41–1.5%), tryptophan (0.88–1.02%), threonine (1.33–1.42%), glycine (8.74–9.4%), alanine (0.98–1.09%), proline (1.04–1.19%), arginine (1.43–1.55%), cysteine (0.37–0.44%), serine (0.79–0.92%), aspartic (1.29–1.46%), and glutamic (2.33–2.45%), are present (Mbailao *et al.,* 2014). This study evaluated the performance of exotic chicken fed on a probiotics-treated Moringa (*M.oleifera)* leaf meal-based diet on egg production and quality.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of the Poultry Industry in the World

The poultry industry is one of the largest food industries in the world. In 2016, there were approximately 23 billion chickens worldwide, about three birds per person. Since 2016, poultry meat production has been dominating the global meat industry. An earlier report projected that world poultry production would increase by 121% from 2005 to 2050 (Mottet & Tempio, 2017). They are kept and raised in various production systems and provide mainly meat, eggs and manure for crop fertilisation. Poultry meat and eggs are among the most common animal sources of food consumed at the global level through a wide diversity of cultures, traditions and religions, making them the key to food security and nutrition. (Foastat, 2016). Within the livestock sector, poultry emerges as the most efficient sub-sector in its use of natural resources and providing protein to supply the globally growing demand. Poultry is particularly important for smallholders and poor rural and urban communities and is mainly produced in large-scale and intensive operations, making it one of the fastest-growing agricultural sub-sectors (Nkukwana, 2018). At the same time, most of the sector's growth has been driven by private investments. The global human population is estimated to reach 9.6 billion in 2050, with about 70% living in urban areas, while incomes could increase by 2% a year (Foastat, 2016). In this context, Alexandratos and Bruinsma (2012) projected that the demand for animal-derived food could grow by 70% between 2005 and 2050. While beef and pork demand could increase by 66% and 43%, respectively, poultry meat is expected to have the highest growth, with 121%, and demand for eggs could increase by 65%.

2.2 Overview of the Poultry Industry in Kenya

Poultry keeping is one of Kenya's most popular livestock enterprises due to its low capital space requirements; most rural families in Kenya keep poultry. According to the 2019 household and livestock census, the Kenyan poultry population was estimated to be over 38 million birds, with 78% being free-ranging indigenous chicken, 21% commercial layers and broilers, and the rest being other poultry species (ducks, turkey, pigeons, ostriches, guinea fowls and quails) (KNBS, 2019). Turkeys are reared commercially in small numbers compared to chickens, ducks and quails. The main genotype of commercial layers is Isa Brown and Ross, while commercial broiler genotypes include Arbor Acres, Hybro, Cobb (United Kingdom) and Hypeco (Holland). There are two genotypes of turkeys-local small bronze and buff types and the commercial large white and buff types; ducks are of the Muscovy type, while guinea fowls are the helmeted type (Mwai *et al.*, 2021).

Kenya has a well-developed commercial poultry industry in Africa, providing a major source of animal proteins in many diets; poultry meat and eggs contribute to a well-balanced diet, as few cultural or religious taboos hinder the consumption of these products. Poultry also plays important socio-cultural roles in Kenyan society. For example, poultry is slaughtered during religious festivals such as Ramadhan, the Eid ceremony for Muslims, and Easter and Christmas for Christians. The sector is also linked with sports and culture; cockerel fighting is a big attraction in some communities in Kenya, especially the Luhya community. Part of the income derived from poultry farming is appropriated as government revenue, representing 30% of the agricultural contribution (25%) to GDP. The rest forms an important pathway out of poverty, especially among the rural population (Mwai *et al.,* 2021).

2.3 Overview of the Livestock Feed Industry in Kenya

The feed industry in Kenya largely depends on imported feed ingredients such as maize, maize germ/bran, wheat bran and pollard, soybean and its derivatives, sunflower cake, cotton seed cake, fish meal, and micro-ingredients (usual additives) from East African Community (EAC), Southern Africa the Development Community (SADC) regional market and other international markets (Ambula *et al.,* 2021). There has been an upward trend in the cost of ingredients used in feed manufacturing. This trend results from increased demand for ingredients, competition between animal and human food requirements, and other industrial needs such as biofuel production in the world market (Mwai *et al.*, 2021). Over-dependence on rain-fed agriculture exacerbates the susceptibility of the agricultural sector to climate change, thereby causing instability in the supply chain whenever there is inadequate rainfall. In the Kenya feed industry, competitive rivalry is real because the feed industry has low entry barriers that allow mediocre manufacturers/suppliers to require nutrition knowledge supported by scientific data from laboratories that determine real-time product chemical composition and quality control that meets target animal requirements, due to important of alliterated raw materials, it is challenging to make feed products that distinguish each feed millers brand, in the absence of strong regulatory framework, counterfeiting and repacking remains a major threat (Mwai *et al.,* 2021).

2.4 Nutritional Requirements of Exotic Layers

The nutritional requirements for chicks and layers, the critical nutrients required for optimum laying performance are energy and protein (amino acids); layer diets aim to optimise egg production (in terms of egg numbers, egg size or egg mass), provide the nutrition required to safeguard the health and maintain the desired body weight. As with layer pullets, different breeders recommend different feeding strategies for their birds, including the number of different diets fed during the laying stage. Calcium is increased for eggshell formation; a balanced ration will supply different nutrients in the right proportions according to the requirements for maintenance and various productive functions. The nutrients required by poultry must be supplied in rations through the ingredients available in sufficient quantity economically (NRC, 1994). Table 2.1 provides data on typical nutrient requirement levels for layer diets.

Table 2.1 Nutritional requirements of exotic layers (DM basis%)

Nutrient	Requirement
Metabolizable Energy (ME)	2,900 Kcal/kg
Crude Protein (CP)	16.50
Crude Fat	5.00
Crude Fibre (CF)	7.00
Calcium	3.60
Phosphorus	0.45
Lysine	0.87
Methionine	0.44
Methionine + Cysteine	0.70
Threonine	0.60
Tryptophan	0.20

Source: NRC. (1994).

2.5 Conventional Feed Resources for Chicken

Conventional poultry rations usually include many bowls of cereal, like maize, wheat, oat and barley, and a few cereal by-products such as wheat-bran, animal and vegetable protein sources like fish-meal, meat-meal, soybean-oil-meal and groundnut cake according to their availability. The whole ration is fortified with adequate minerals and vitamins, either chemically pure or through ingredients known to be rich in these nutrients (Steiger *et al.*, 2014). With the cost of feed soaring high and the availability of conventional ingredients becoming scarce, intensive and continuous efforts are being made to determine the nutritive value of agro-industrial by-products to replace more costly ingredients in poultry rations, some of the common feedstuffs used for making poultry rations are maize. Maize is highly digestible and contains very little fibre. It is used as an energy source and is low in protein, especially lysine and sulphur-containing amino acids. The yellow varieties are a good source of vitamin A and xanthophyll. The latter is responsible for the yellow skin in certain breeds of fowl (Dewhurst, 2013). Barley is not very palatable because of its high fibre content and should not constitute more than 15 per cent of the ration. Oat is not very palatable because of its high fibre content; it should not constitute more than 20 per cent of the ration because of its manganese content. It may help in preventing hock disorders, feather pulling and cannibalism. Wheat can be used to replace maize as an energy source; wheat bran is bulky and quite laxative because of its high fibre, manganese and phosphorus content. Pearl millet is a very useful feedstuff, similar to wheat in its nutritive value, but it is rich in protein and ash content; groundnut is palatable and is widely used as a source of protein in poultry rations. It contains about 40 per cent protein. Fish meal is one of the best poultry feedstuffs as a source of animal protein, and its composition varies widely depending upon whether it is made from whole bony fish or fish cannery scraps. Most fish meals contain 45 to 55 per cent protein. The presence of fish scales reduces its feeding value; Limestone is a source of calcium. It should not contain more than 5 per cent magnesium. Oyster-shell contains more than 38 per cent calcium and is a good substitute for limestone. It is quite palatable (Alhotan *et al.*, 2016).

2.6 Non-Conventional Feed Resources for Chicken

Non-conventional feed resources (NCFR) generally refer to all those feeds that have not been traditionally used for feeding livestock and are not commercially used to produce livestock feeds. Several known examples include palm leaf meals, palm press fibre, cassava foliage, spent brewer's grains, sugar cane bagasse, rubber seed meal and some aquatic plant forages (Amata, 2014). Non-conventional feed resources can be looked at as covering a wide diversity of feeds and their nutrient contents; a common feature about feeds is that the traditional feeds of tropical origin tend to be mainly from annual crops and feeds of animal and industrial origin. In this sense, the term NCFR could be more appropriately referred to as "new feeds", and this term is increasingly being used. Thus, the term NCFR has been frequently used to describe sources such as oil palm by-products, single-cell proteins and feed materials derived from agro-industrial by-products of plant and animal origin, poor-quality cellulosic roughages from farm residues and other agro-industrial by-products such as slaughter-house by-products and those from the processing of sugar, cereal grains, citrus fruits and vegetables from the processing of food for human consumption (Onte *et al.*, 2019).

This list can be extended to include derivatives from chemical or microbial processes, as in the production of single-cell proteins. However, it is sometimes difficult to draw a distinct line between traditional feeds and NCFR; an example is wheat straw, which is very widely used, in addition to the availability of NCFR, mainly of plant origin. These non-conventional alternative protein sources have excellent nutritive value and therapeutic properties. Their crude protein contents are excellent (Amata, 2014). It is apparent from several previous studies that the inclusion of these non-conventional protein sources in poultry diets improves the performance of chickens in terms of growth rate and egg production. However, their recommended inclusion levels should strictly adhere to avoid deleterious effects they may likely confer on poultry. For instance, up to 10% inclusion of MOLM in laying hens' diets has been considered safe. Hence, their inclusion in poultry feeds without posing any deleterious effects on the birds' performances as well as the consumers of the products, and ultimately, leading to better profitability on the part of the farmers, will be a novelty (Olarotimi *et al.*, 2017).

2.7 Moringa tree

Figure 2. 1 Moringa tree

Source*:* *https://www.google.com/search?q=moringa&client=firefox*.

Retrieved date 18Oct 2022

The Moringa tree (*M. oleifera*) has probably been the most popular plant of an underutilized tropical tree. The tree is native to India but has been planted around the world and is naturalized in many locales. Moringa goes by many names. In the Philippines, where the leaves of the Moringa are cooked and fed to babies, it is called "mother's best friend" and "malunggay."Other names for it include the benzolive tree (Haiti), horseradish tree (Florida), Nébéday (Senegal) and (Gambia) and drumstick tree (India). There are about 13 species of Moringa trees in the family Moringaceae. They are native to India, the Red Sea area and parts of Africa, including Madagascar (Tahir *et al.,* 2020).

Morphology

Moringa is a small to medium evergreen or deciduous tree that can grow to a height of 10-12 m. It has a spreading open crown, typically umbrella-shaped. The roots are deep. The bole is crooked, generally one-stemmed, but sometimes forked from the base. The bark is corky and grey. The branches are fragile and drooping, with feathery foliage. Young twigs and shoots are covered in short, dense hairs, purplish or greenish-white in colour. Moringa leaves are alternate, 7-60 cm long, tripinnately compound, with each pinnate bearing 4-6 pairs of dark green leaflets, elliptical to obovate, and 1-2 cm in length. The inflorescences are 10-20 cm long, spreading panicles bearing many fragrant flowers (Akinyeye *et al.,* 2019). Moringa flowers are pentamerous, zygomorphic, 7-14 mm long and white to cream in colour. The fruit is a typically 3-valved capsule, 10 to 60 cm in length, often referred to as a "pod" and looking like a drumstick (hence the name "drumstick tree"). The fruit is green when young and turns brown at maturity. The mature fruit splits open along each angle to expose the seeds. The capsule contains 15-20 rounded oily seeds, 1-1.5 cm in diameter, surrounded by three papery wings up to 2.5 cm long. Moringa seeds contain a lot of oil (Akpoka *et al.,* 2019).

2.7.1 Biological Role of Moringa leaf meal

The Moringa tree is globally known for its economic and therapeutic roles. It has been honoured as the "Botanical of the Year 2007" by the National Institute of Health (USA) (Mahruz, 2019). The tree is also known as "never die" or "miracle tree" to the people of Africa; now, the application of Moringa leaf in preparing foods is receiving significant attention. People from Ghana, Nigeria, Ethiopia, East Africa, and Malawi are consuming the Moringa tree leaves directly in their diets (Agbogidi *et al.,* 2012). Furthermore, Moringa leaves have been used for making soups, foods, bread, cakes, and yoghurts

(Oyeyinka & Oyeyinka, 2018). It was thought that Moringa contains different anti-nutritional factors, such as tannins, phytates, oxalates and cyanide, which may affect normal digestion and metabolism of nutrients in animals (Moreki *et al.*, 2014). In Moringa, tannins and phytates are 12 and 21 g kg^{-1} of DM, respectively, which can be neutralized by different feed processing techniques, including chopping, socking, heat steaming, and fermentation with beneficial organisms (Nouman *et al.*, 2014). Considering the health benefits effects of Moringa, it is a unique plant due to its enriching minerals with lower anti-nutritional components.

2.7.2 Nutritional Properties of MOLM

Moringa is also very popular for its nutritional value. It is reported as a good source of six major nutrients: Carbohydrates, especially dietary fibre, protein, vitamins, minerals, lipids, and water. The unique features of moringa are its richness in protein, carbohydrates, and fibre with low fat. The leaves have been reported to enclose a range of essential amino acids and are a good source of alpha-linolenic acid. Moringa leaves have been seen to exhibit high contents of vitamins A, C, and E. The relative bioavailability of folate originating from moringa leaves was about 82% in a rat model, confirming that Moringa leaf exhibits a rich source of dietary folate (Daba, 2016). The nutritional composition of MOLM (dry matter basic) showed dry matter (DM) about 93.63% to 95.0%, crude protein (CP) 17.01% to 22.23%, carbohydrate 63.11%to 69.40%, crude fibre (CF) 6.77% to 21.09%, crude fat (EE) 2.11% to 6.41%, ash (total mineral) 7.96% to 8.40%, gross energy 14.790 (MJ/kg), and fatty acid 1.69% to 2.31%.

In addition, estimated calcium (Ca) was 1.91%; potassium (K) was 0.97%; sodium (Na) was 192.95, iron was (Fe) 107.48, manganese (Mn) was 81.65, Zinc (Zn) was 60.06, and phosphorus (P) was 30.15 parts per million (ppm) (Ogbe & Affiku, 2012). The leaves of the plant are enriched with methionine, phosphorus, calcium, and iron. It is believed that the leaves of MOLM contain more calcium and twice as much protein than milk, higher vitamin C than oranges, higher potassium and iron than bananas, and higher vitamin A than carrots (Kumar *et al.*, 2016). An active component that was identified from MOLM can improve the absorption of different vitamins, minerals, and other micronutrients in the gastrointestinal tract of the host (Islam *et al.*, 2022). The nutritional composition of MOLM is presented in Table 2.2.

Table 2.2 Chemical Composition of Dry MOLM on (% basis)

Nutritive value %	**Dry leaves**
Moisture	9.533
Crude protein	30.29
Fat	6.50
Ash	7.64
Neutral detergent fibre	11.40
Acid detergent fibre	8.49
Acid detergent lignin	1.8
Acid detergent cellulose	4.01
Condensed Tannins (mg/g)	3.12
Total polyphenol (%)	2.02

Source: NRC. (1994).

Table 2.3 Mineral Contents of Dried MOLM (% DM basis)

Mineral %	**Dry Leaf**
Macro elements	
Calcium	3.65
Phosphorus	0.30
Magnesium	0.50
Potassium	1.50
Sodium	0.164
Sulphur	0.63
Microelement (mg/kg)	
Zinc	31.03
Copper	8.25
Manganese	86.8
Iron	490
Selenium	363.00
Boron	49.93

Source: NRC. (1994)

2.7.3 Utilization of Moringa Leaf Meal on the Performance of Livestock

Moyo *et al.* (2014) concluded that supplementing cross-bred Xhosa lop-eared goats with Moringa produced chevon with higher meat quality attributes, improved lightness, redness values, Warner-Bratzler shear force and higher sensory consumer scores compared with the control group. Mukumbo *et al.* (2014) concluded that the inclusion of MOLM (2.5, 5 and 7.5 per cent) in finisher pig feed had no detrimental effects on carcass characteristics or Physico-chemical meat quality, and it significantly improved the acceptability of pork colour, odour and lipid profile. Qwele *et al.* (2013) determined meat's chemical composition, fatty acid content and antioxidant capacity from goats supplemented with Moringa leaves. They indicated that their anti-oxidative potential might play a role in improving meat quality (chemical composition, colour and lipid stability). Hazra *et al.* (2012) concluded that the use of the crude extract of Moringa leaves (1, 1.5 and 2%) had significant antioxidant and antimicrobial effects and improved the organoleptic quality by enhancing the tenderness and juiciness in cooked ground buffalo meat. Muthukumar *et al.* (2014) observed that the Moringa leaves extract (0.1 per cent) was more effective than Butylated hydroxytoluene (BHT) in preventing increased Thiobarbituric acid-reactive substances (TBARS) in the number of precooked goat meat patties during storage at 4°C. Moringa (6 g/kg meat) had improved crude protein and reduced fat content. Therefore, with higher crude protein levels in Moringa-enriched products, a small quantity will be required by consumers to meet their nutrient requirement, and hence, reduce expenditure on meat and meat products. Nkukwana *et al.* (2014) stated that diets supplemented with or without MOLM (1, 3 and 5% of dry matter intake) and with the high saturated fatty acid content improved the fatty acid profile and reduced lipid oxidation in broiler breast meat.

In most of the feeding experiments in poultry, the fresh, green, and undamaged mature Moringa leaves were air-dried correctly, and then the dried leaves were ground to a fine powder in a hammer mill and considered as Moringa leaf powder or leaf meal. Similarly, fresh mature Moringa seeds were air-dried and ground and considered as Moringa seed meal. In some experiments, the ground particles were soaked in distilled water for 24 hours, and the filtered aqueous solution was considered a Moringa extract. Due to the rich nutrient content, especially the high amount of crude protein (CP), vitamins, and minerals, Moringa leaf can be used as a valuable resource of dietary supplementation for livestock as well as poultry (Nouman *et al.*, 2014). In addition, Briones *et al.* (2017) stated that Moringa leaf could be applied as a dietary supplement in layers and broilers due to high production performance and improved egg quality. However, there are still many debates on the chicken's performance with different doses of Moringa. There are also many variables on doses and parts of the plant used, such as leaves, extracts, sods, or seeds. Finally, many scientists agreed that the Moringa plant might positively improve chickens' production performance and health status. Further studies are still needed to detect the application doses for optimum chicken performance.

2.7.4 Effect of Moringa Leaf Meal on Egg Production and Quality

The egg quality parameters, including egg size, shape, colour, shell thickness, and egg yolk cholesterol, indirectly and indirectly influence egg consumers (Voemesse *et al.*, 2019). Moringa leaf meal was used in layer chickens' diets from 1 day old to 55 weeks of age to investigate the effects of Moringa leaf meal on growth performance, egg production performance, and blood parameters. Moringa leaf meal was used at three different levels (0%, 1%, and 3%). In the growing period from 1 day to 20 weeks of age, this study did not find any significant differences in feed intake, but average daily body weight gain, final body weight, and FCR were improved in MOLM-supplemented groups. In the laying period, from 21 weeks to 55 weeks, feed intake was lower in Moringa-fed groups, but the laying per cent and FCR were higher in supplemented-fed groups than in the non-supplemented group. The higher body weight gain and egg production may be related to improved digestibility in supplemented groups due to the different active components in Moringa leaf meal. The author concluded that feeding MOLM at a 1% level positively affected laying hens' growth and egg production. In addition, MOLM at 10% levels showed higher egg production in laying hens (Moreki *et al.*, 2014).

In another study, Lu *et al.* (2016) found that MOLM had no effects on egg production, egg weight, and feed intake in Hy-Line Grey commercial layers, but birds fed with MOLM at 15% levels showed deeper egg yolk colour than the non-supplemented fed group. Similarly, the albumen height and Haugh unit were higher in Moringa-supplemented groups during storage of eggs at 4 C and 28 C for four weeks; the author further stated that 5% Moringa leaf meal could be included in laying hens' ration without adverse effects on egg production and egg quality. Similarly Abousekken *et al.* (2011) found that MOLM could improve egg yolk colour scores and albumen percentage. This study further observed the lower egg-laying percentage and egg mass in laying hens fed with MOLM because of readily available proteins with their essential amino acids in the MOLM. The author stated that 10% Moringa leaf meal could be incorporated into the diets of Rhode Island Red laying hens. Feed intake, feed conversion ratio, and laying percentage were not influenced by adding Moringa leaf meal at a

10% level, which was noticed by El-Sheikh *et al.* (2015). However, including 10% Moringa leaf meal could increase the egg Roche colour score.

Interestingly, Moringa leaf meal at a 5% level increased the egg weight, but the decreased egg weight was found when the inclusion level was at 20%. The authors assumed that higher feed intake, FCR with lower egg production per cent, egg mass, and egg weight at a higher-level supplementation was due to poor digestibility of nutrients because of different anti-nutritional, phytochemical presences in Moringa leaf. Improving egg quality by increasing its anti-oxidative properties by supplementing unconventional natural resources has gained significant interest in poultry research. The synthesis antioxidants, like butylated hydroxyanisole and butylated hydroxytoluene, are commonly used in food processing. However, they are found to be carcinogenic to human health; therefore, discovering natural antioxidant products as safe and effective alternatives is a very crucial need (Zhang *et al.,* 2018).

2.7.5 Anti-Nutritional Factors in Moringa leaf meal

Anti-nutritional factors are compounds synthesized by the plant that reduce nutrient utilization from plants or plant products and determine the use of the particular plant as human food (Gemede & Ratta, 2014). They are synthesized through normal metabolism pathways in plants as secondary metabolites intended for their plant defence, and they affect the digestibility, bioavailability and utilization of nutrients, mainly proteins, minerals and vitamins in food and reduce their nutritive values; some have beneficial and some have deleterious toxic biological responses to human nutrition and health (Cockell *et al.*, 2012). Anti-nutritional factors in the plants include tannins, oxalates, phytates, saponins, lectins, trypsin and protease inhibitors, alkaloids, coumarins, gossypol and cyanogenic glycosides. Moringa leaves contain 21 g/kg phytate, 10.5 g/kg oxalates, negligible tannins, saponins, trypsin, amylase inhibitors and no detectable cyanogenic compounds. Moringa leaves have low levels of anti-nutritional factors compared to other leaf vegetables and produce no toxic effect on consumption (Stohs & Hartman, 2015). The low level of anti-nutritional factors compared to Amaranthus spp, spinach, Ethiopian mustard, and other Brassica spp vegetables contribute to the acceptability and wide use of *M. oleifera* as the leaf vegetable. However, anti-nutritional factors are affected mainly by processing methods, like blanching, drying, fermentation and de-fatting, which results in reduced or deactivated activity (Devisetti *et al.,* 2016). On the other hand, Moringa seeds contain alkaloids, phytates, tannins, oxalates and saponins approximately at 291, 175, 131, 110 and 33 mg/100g, respectively (Ijarotimi *et al.,* 2013). The roots contain 45 mg/100g tannins, 17.08 mg/100g oxalates, 4.20 mg/100g saponins, 0.07 mg/100g phytates and 2.72 mg/100g cyanogenic glycosides (Igwilo *et al.*, 2014).

2.8 Application of Probiotics in Laying Hens

Egg production and quality

Probiotics increase egg production, improve egg quality and decrease egg contamination. Further, it has been reported that probiotics increase eggshell weight, shell thickness and serum calcium in layers and found that commercial probiotic (Protexin™) supplemented diets resulted in a decreased broken egg ratio in layers (Khan *et al.*, 2013). Ray *et al.* (2022) found that egg production increased linearly with increasing levels of probiotics (*Lactobacilli spp.* + *Enterococcus faecium* + *Bifidobacterium bifidum* + *Aspergillus oryza*) during the late laying period in layers. However, increased egg production, shell weight, shell thickness and serum calcium were recorded by feeding a Lactobacillus-based of 100 mg/kg and 200 mg/kg of feed, although there was no significant difference between the two treatments. In the same experiment, the eggshell and serum calcium improved significantly (Khan *et al.*, 2013). Kozłowski & Sobczak. (2015) reported that dietary preparation of L. sporogenes at 100 mg (6 × 108 spores) per kg of diet significantly increased egg production, eggshell-breaking strength, shell weight and shell thickness in laying hens.

However, egg weight, specific gravity and Haugh unit were not influenced. Several studies with layers indicated that supplementation of probiotics had no positive effect on egg production (Youssef *et al.*, 2013). Variations in the effect of probiotics obtained from various studies have been attributed to the difference in strains, the form of bacteria, their concentration in the diet and viability in the gastrointestinal tract recorded a greater shell thickness following supplementation with *Lactobacillus*-based probiotics in Single Comb White Leghorn layers. The beneficial effects on eggshells have been attributed to the favourable environment in the intestinal tract resulting from probiotic supplementation, which may absorb more calcium from the gut, leading to thicker eggshells. However, a probiotic (*Lactobacillus acidophilus*, *Lactobacillus bifidus* and *Streptococcus faecalis*) showed no effect on chick quality in broilers produced from breeder flocks at various ages (Naz *et al.*, 2013).

CHAPTER THREE

DETERMINATION OF *IN-VITRO* DRY MATTER DIGESTIBILITY AND CONDENSED TANNINS OF PROBIOTICS-TREATED MOLM

Abstract

This study evaluated the *in-vitro* dry matter digestibility (DMD) and condensed tannins of probiotics-treated MOLM and the nutritional and anti-nutritional changes in MOLM on fermentation. Three fermentation methods were used: *Clostridium butyricum Bacillus coagulans* and spontaneous fermentation, each in three replicates. All the fermentation methods used positively affected the parameters measured. For the *in-vitro* digestibility to match the digestive system in the chicken stomach and intestines, a trial was carried out as described by Gabler *et al.* (2015). Results indicated a significant difference in *in-vitro* DMD. Fermentation with *Bacillus coagulans* had the highest digestibility (66.24±0.76%) compared to the rest, while untreated had the lowest (42.20±0.78%). *Clostridium butyricum* fermentation resulted in a higher *in-vitro* DMD (57.24±0.74%) than Spontaneous fermentation (48.23±0.57%). There was an increase in *in-vitro* DMD% after treating the leaf meal with the *Bacillus coagulans* (66.24±0.76%) relative to the untreated (42.20±0.78). There was a significant improvement in the CP content of 33.41±0.57 compared to the untreated 27.48±0.25. The anti-nutritive (tannins and phenolics) compounds were reduced in the fermented substrate; *Bacillus coagulans* fermented substrates had higher nutritional value and increased *in-vitro* DMD, improved CP and decreased CF and tannins in fermented substrates. Among the three fermentations, *Bacillus coagulans* proved best in all the parameters evaluated. Therefore, it was used for the fermentation of MOLM in the feeding trials.

3.1 Introduction

Poultry production has significant economic, social, and cultural benefits. It plays a significant role in family nutrition in the world, substantially contributing to household food security throughout developing countries (Hinsemu *et al.*, 2018)**.** Even though it is one of the animal sectors with the most remarkable growth rate globally, the poultry industry is constrained by a severe scarcity of feed components, particularly in developing nations. Evaluating locally available non-traditional feed sources that could be used in chicken feed formulas is therefore crucial (Mahfuz *et al.* 2019)**.** Moringa is a non-legume tree with the potential for use as animal feed due to its high availability and nutritive value (Beyene, 2021). Its leaves have been found to contain phytochemicals, vitamins, minerals, and amino acids (Okiki *et al.,* 2015). Mahfuz *et al.* (2019) stated that Moringa leaf could be applied as a dietary supplement in layers and broilers due to high production performance and improved egg quality, as reported in several studies.

This demonstrates the feasibility of using Moringa leaf meal in livestock and poultry diets (Rehman *et al.,* 2017). It has been shown that the dietary inclusion of 2 to 25% of moringa leaf meal in broiler and layer chicken diets improved growth rate and egg production (AbouSekken, 2015; Banjo, 2012; Gadzirayi *et al.,* 2012; Moreki & Gabanakgosi, 2014). Research has shown that the Moringa plant might positively improve chickens' production performance and health status (Mahfuz *et al.,* 2019). Previous studies have revealed that the natural fermentation of cooked moringa seeds in banana leaves significantly increased protein content, essential amino acids and polyunsaturated fatty acid profiles (Ijarotimi *et al.,* 2013). Furthermore, methanolic moringa leaves fermented by lactic acid bacteria were used to treat hyperglycemia and hepatic steatosis in obese mice (Joung *et al.*, 2017). Moringa leaf extracts fermented by *Rhizopus oligosporus* were applied in mice to treat atopic dermatitis. This objective evaluated *in-vitro* dry matter digestibility and condensed tannins content of probiotics-treated MOLM.

3.2 Material and Methods

3.2.1 Study site

An *in-vitro* experiment was conducted at Egerton University Animal Nutrition laboratory. The University is situated at 0° 23' S, 35° 55' N within Njoro Sub-County, Nakuru County. The altitude is 1800 meters above sea level with an average annual rainfall of 900- 17 1,200 mm. The area has average daily temperatures ranging from 17°C- 22°C (Egerton University Department of Agricultural Engineering, Metrological Station 2018, Personal Communication).

3.2.2 Collection and processing of MOLM

The dried Moringa leaves were sourced from Meru County in Kenya, located along the eastern side of the Mount Kenya region. Local farmers in Meru were contracted to obtain fresh leaves by cutting the tree branches, stripping leaves off the tips of the branches by hand (manually), and air drying them under a shade until they were crisp to the touch while retaining their greenish colouration. The leaves were then milled using a hammer mill with a

5 mm sieve size to produce MOLM. The MOLM was stored in airtight sacs until the time for use in laboratory analysis and compounding of feed.

3.2.3 Preparation of experimental diets

There were four experimental diets with three replicates each: T1 MOLM fermented with *Clostridium butyricum*, T2 MOLM treated *Bacillus coagulans*, T3 MOLM fermented using Spontaneous fermentation, and T4 untreated MOLM (control).

3.2.4 Fermentation of MOLM

The Moringa leaf meal samples were fermented with three fermentation set-ups according to the method described by Ogodo *et al*. (2018) with slight modifications. The MOLM was mixed with distilled water in the ratio of 1:0.5 (w/v) in a 500-ml beaker and mixed thoroughly with a hand mixer. The samples were sterilised in an autoclave at 121°C for 10 min to minimise the risk of contamination and allowed to cool for 30 min at room temperature (25 ± 2°C). The samples were inoculated with 0.0025 grams of 2.0× 10^4 CFU/g of *Clostridium butryricum* and *Bacillus coagulans* strains and allowed to ferment in a solid state for 48 hrs.

3.2.5 Moringa leaf meal treated with *Clostridium butyricum*

Clostridium butyricum powder was acquired from the Feed Biotechnology Laboratory, China Agricultural University Ninja, and its concentration was 2.0× 10^4 CFU/g. The *Clostridium butyricum* was included at 0.0025 grams mg/kg of feedstuff in the dry state as per the manufacturer's instructions and recommendations and mixed thoroughly. The inoculated MOLM were incubated in the laboratory at 37°C in tightly sealed 750ml plastic bottles for 48 h. After 48 h, samples were collected for proximate analysis. The pH of individual samples was measured and recorded using a digital hand-held pH meter (pH/ORP/Temperature Combo Tester - HI98121 HANNA instruments).

3.2.6 Fermentation of MOLM with *Bacillus coagulans*

A single strain of commercial_*Bacillus coagulans* powder was acquired from Feed Biotechnology Laboratory, China Agricultural University Ninja, and its concentration is 2.0× 10^4 CFU/g. *Bacillus coagulans* was used as the starter culture. Three samples of 600mg of MOLM were mixed with distilled water at 1:2.50 (wt. /vol), and the culture was added to the mixture. The inoculated MOLM were incubated in the laboratory at 37°C in tightly sealed 750ml plastic bottles for 48 h. After 48 h, samples were collected for proximate analysis. The pH of individual samples was measured and recorded using a digital hand-held pH meter (pH/ORP/Temperature Combo Tester - HI98121 HANNA instruments).

3.2.7 Spontaneous fermentation of MOLM

A mixture of 600 g MOLM with distilled water at a ratio of 1:2.75 (wt. /vol) in triplicate was incubated at a temperature of 22°C for seven days using 250 ml plastic bottles (Marii *et al.,* 2022). The plastic containers were sealed tightly to create anaerobic conditions. After seven days, the pH of individual samples was recorded using a portable pH meter (pH/ORP/ Temperature Combo Tester - HI98121 HANNA instruments), and a sample was obtained for proximate analysis.

3.2.8 Proximate analysis

Dry matter (DM) was determined by drying in a hot air oven at 105°C for 24 h following standard method 925.09 (AOAC, 2006), Ash by burning the samples in a muffle furnace at 550°C for eight hours following standard method 923.03 (AOAC, 2006), ether extract by the Soxhlet method (using ether) following standard methods 920.39 (AOAC, 2006). Total nitrogen for crude protein (N x 6.25) determination was obtained using the micro-Kjeldahl method following standard methods 920.87 (AOAC, 2006). Constituents of the cell wall, neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid Detergent lignin (ADL) were determined using the Van Soest method (Van Soest *et al.,* 1991). Hemicellulose was determined as a difference between the neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL).(Nurnazratul *et al.,* 2021).

3.2.9 Determination of condensed tannins and total phenolics

Extraction of tannins:

For the extraction process, aqueous acetone (70%) was used. Each dried (finely ground) sample (200 mg) was taken in a glass beaker of approximately 25 mL capacity. Ten mL of aqueous acetone (70%) was added, and the beaker was suspended in an ultrasonic water bath for 20 minutes at room temperature. The contents of the beaker were then transferred to centrifuge tubes and subjected to centrifugation for 10 min at approximately 3000 g at 4°C using a refrigerated centrifuge (Rahman & Lamara, 2023). The supernatant was collected and kept on ice. The pellet left in the tube was transferred to the beaker using two portions of 5 mL each of 70% aqueous acetone and again subjected the contents to ultrasonic treatment for 20 min. The supernatant was again collected as described above.

Determination of condensed tannins (Proanthocyanins)

Porter *et al.* (1986) described the method to determine condensed tannins in the extracts. The Butanol–HCl reagent (butanol–HCI 95:5 v/v) was prepared by mixing 950 mL of n–butanol with 50 mL concentrated HCl (37%). Ferric reagent (2% ferric ammonium sulfate in 2N HCl) was prepared by dissolving 2.0 g of ferric ammonium sulfate in 2N HCl (16.6 mL of concentrated HCl was made up to 100 mL with distilled water to make 2N HCl). The reagents were stored in dark bottles. In a 100 mm x 12 mm glass test tube, 0.5 mL of the tannin extract diluted with 70% acetone was pipetted. The quantity of acetone was large enough to prevent the Absorbance (550 nm) in the assay from exceeding 0.6. Three mL of the butanol–HCl reagent and 0.1 mL of the ferric reagent were added to the tubes. The tubes capped with a glass marble were shaken using a Vortex and then placed on a heating block adjusted at 97 to 100°C for 60 min. After cooling the tubes, Absorbance was recorded at 550 nm. The Absorbance of the unheated mixture (considered as a suitable blank) was subtracted from the Absorbance of the heated mixture, which was the actual reading at 550 nm to be used to calculate condensed tannins.

The development of pink colour without heating the sample indicates the presence of flavanols. If this happened, one heated blank for each sample, comprising 0.5 mL of the extract, 3 mL of butanol and 0.1 mL of the ferric reagent, was used. Condensed tannins (% in dry matter) as leucocyanidin equivalent was calculated by the formula: (A 550 nm x 78.26 x Dilution factor)/(% dry matter). This formula assumes that the effective El%, 1 cm, 550 nm of leucocyanidin is 460 (Porter, 1986). Here, the dilution factor equals 1 if no 70% acetone was added and the extract was made from a 200 mg sample in 10 mL solvent. Where 70% acetone was added (for example, to prevent the Absorbance from exceeding 0.6), the dilution factor was 0.5 mL/ (volume of extract taken) in the current.

3.2.10 Two-step *in-vitro* digestibility of dry matter determination (IVDMD)

For the *in-vitro* digestibility to match the digestive system in the chicken stomach and intestines, a trial was carried out as described by Gabler *et al.* (2015). Four treatments with three replicates each were used: T1: Moringa treated with *Clostridium butyricum*, T2: Moringa treated with *Bacillus coagulans*, and T3: using spontaneous fermentation, T4: Untreated MOLM (control).

Step one (poultry stomach simulation phase)

A ground feed sample (0.4g) was weighed and placed in a 100 ml conical flask. A sample of 0.4 g was weighed and placed in the flask. Sodium phosphate buffer solution, 200 ml (0.1 M, pH 6.0), was added to the flask and carefully mixed with the sample by stirring to simulate the stomach digestive process. 80 ml of 0.2 M HCl was added, and the pH was adjusted to 2.0 with 1M HCl or 1M NaOH solutions (Sharma *et al.*, 2020). This was followed by the addition of prepared 5 mL pepsin porcine grade enzyme with 4x USP activity (Pepsin from porcine gastric mucosa powder, ≥250 units/mg solid Sigma-Aldrich Corp., St. Louis, MO, USA) containing 1 mg pepsin per ml 0.02 M HCl. To each conical flask, 2ml Chloramphenicol C-0378; Sigma-Aldrich Corp., St. Louis, MO, USA (0.5g/100ml ethanol) was added to inhibit bacterial growth. The flasks were closed, incubated in a water bath at 39° C, and stirred continuously for 2 hours.

Step two: Poultry intestines simulation

This step simulated the intestinal digestion of the poultry. The mixture from step one was mixed with 80 ml of phosphate buffer (0.2M, pH 6.8) and 20 ml of 0.6 M NaOH. The pH was adjusted to 6.8 using 1M HCl or 1M NaOH to provide a stable environment for intestinal enzymes to thrive, as described by (Ramaswamy, 2001). To the mixture, 10.6 ml of artificial pancreatin P-1750 Sigma-Aldrich Corp., St. Louis, MO, USA (porcine grade enzyme with 3 x USP activities) containing 100 mg/1 litre buffer was added and incubated at 39°C with continuous stirring for 4 hours. The residues were filtered through a nylon bag (pores size of (42 µm), washed with distilled water, and then washed twice using 20 ml, 95% ethanol, and 20 ml, 99.5% acetone. The residues were dried in an oven at 70°C for 12 hours and weighed**.**

Dry matter digestibility calculations

The *in-vitro* digestibility (*IVDMD*) of dry matter (DM) was calculated using the following formulae (Boisen & Fernandez, 1997).

$$DM\ digestibility = \left(\frac{DM_{In} - DM_{RS}}{DM_{In}}\right) \times 100$$

Where:

DM_{In} and DM_{RS} are the initial (DM) and residual (DM), respectively.

3.3 Statistical analysis

Data were analysed with IBM SPSS Statistics version 22. The assumption for the data's normality and homogeneity of variance was checked using Shapiro-Wilk and Levenes test statistics, respectively, with data assumed to be normal when ($p<0.05$). The *in-vitro* DMD was analysed using the GLM model procedures. Mean separation was conducted using Tukey's HSD (Honest Significant Difference) test at a 0.05 significance level. Application of Excel solver in Microsoft Excel was used in curve fitting and in the *in-vitro* DMD%.

3.4 Results

3.4.1 pH of fermented MOLM

The fermentation method affected the pH of the treatments (Table 3.1).

Table 3.1 pH of treated and fermented MOLM

Treatments	pH	P-value
T1	3.63^{b}±.0.98	<.0001
T2	3.57^{b}±0.98	<.0001
T3	3.89^{a}±1.04	<.0002
T4	3.95^{a}±1.04	
		<.0002

The means within a column with the same letter superscript are not significantly different (at a 5% significance level). T1 was treated with *Clostridium butyricum*. T2 was treated with *Bacillus coagulans*—T3 spontaneous fermentation and T4 untreated MOLM.

3.4.2 Determination of the *in-vitro* dry matter digestibility and condensed tannins of probiotics-treated MOLM

Treatment 2 with *Bacillus coagulans* had the highest digestibility (66.24±0.76%) relative to the rest, while the untreated fermentation had the lowest (42.20±0.78%). *Clostridium butyricum* resulted in a higher *in-vitro* DMD% (57.24±0.74%) than spontaneous fermentation (48.23±0.57%). There was an increase in *in-vitro* DMD% (Fig. 1) after treating the MOLM with the *Bacillus coagulans* (66.24±0.76) relative to the untreated (T4) (42.20±0.78%).

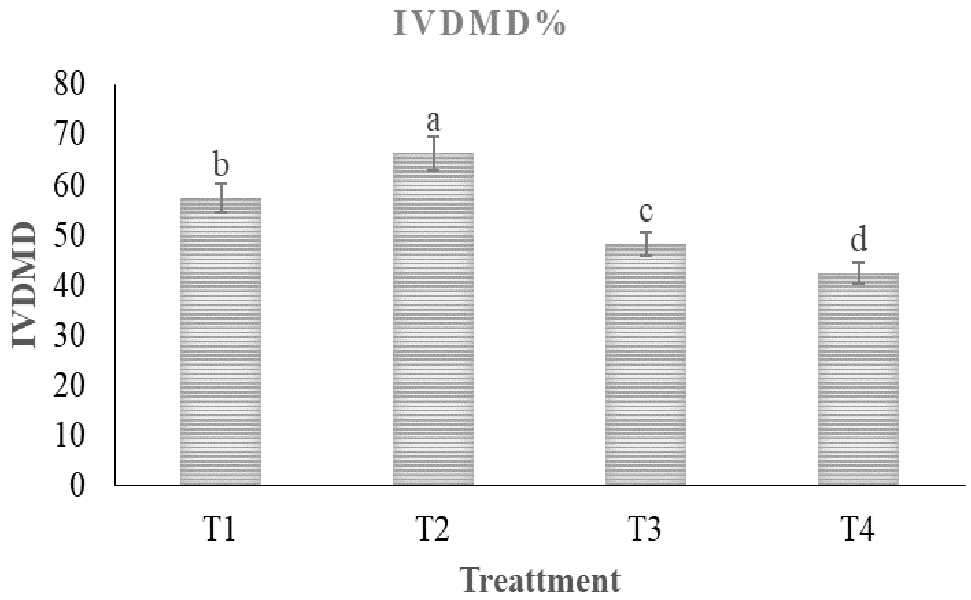

Figure 3.1 The in-vitro digestibility of probiotics, fermented and untreated moringa leaf meal. Error bars represent the mean (±SE) IVDMD of MOLM**.**

Table 3.2 Chemical composition (Mg/100g) of moringa leaf meal on (DM basis %)

	Treatments				
Parameters	**T1**	**T2**	**T3**	**T4**	**P-value**
Moisture	7.86 ^{b}±0.26	6.7^{b}±0.31	12.83^{a}±0.28	8.71^{b}±0.16	0.0128
Crude Protein	29.31^{b}±0.29	33.41^{a}±0.57	27.18^{c}±0.55	27.48^{bc}±0.25	<.0001
Crude fibre	7.90^{c}±0.01	6.61^{d}±0.16	8.61^{b}±0.08	9.54^{a}±0.09	<.0001
Ash	7.75±0.05	9.28±0.03	8.63±0.2	8.55±0.71	0.2170
Ether Extract	8.73^{b}±0.05	7.35^{c}±0.01	10.4^{a}±0.25	6.73^{c}±0.11	<.0001
ADL	1.41^{ab}±0.2	1.06^{b}±0.07	1.73^{a}±0.06	1.78^{a}±0.01	0.0052
ADF	6.63^{b}±0.37	5.01^{c}±0.28	8.05^{a}±0.25	8.45^{a}±0.34	0.0002
NDF	9.95^{ab}±0.13	9.73^{b}±0.09	10.6^{ab}±0.12	10.9^{a}±0.38	0.0160
TANNINS mg	2.33^{b}±0.14	2.09^{b}±0.14	3.10^{a}±0.21	3.10^{a}±0.16	0.0040
Phenolics mg	1.13^{b}±0.01	1.04^{c}±0.01	1.16^{b}±0.01	1.26^{a}±0.01	<.0001

^{abcd}Means within a row with different superscript letters are statistically different p<0.05. T1= *Clostridium butyricum* treated MOLM, T2= *Bacillus coagulans* treated MOLM, T3 spontaneous fermentation of MOLM, T4 untreated moringa ± represent standard error of the mean.

3.5 Discussion

Several important biochemical processes are used for solid-state fermentation (SSF), which is necessary to achieve optimal SSF outcomes (Dhiman *et al.,* 2018). Several bacterial strains can grow as cultures to improve the nutritional quality of non-conventional feed resources (Yao *et al.,* 2018). Wang *et al.* (2018) reported that the nutritional quality and digestibility of MOLM were significantly improved via SSF using lactic acid bacteria. The application of several strains of bacteria in fermentation is relatively unexplored, and SSF of MOLM using *Clostridium butyricum* and *B. coagulans* has not yet been reported. In this study, these microorganisms were used to ferment MOLM, and the success of MOLM fermentation can be explained in part by the fact that *Bacillus coagulans* has more significant cellulase, pectinase, and amylase activities; this activity facilitates the degradation of cellulose and starch, which in turn increases the available carbon. *Bacillus coagulans* can also convert non-protein nitrogen into microbial protein and secrete enzymes that accelerate cellulose and starch degradation, thereby promoting mycelial growth. They can also improve the palatability of MOLM for livestock. There was no significant difference ($p>0.05$) between *Bacillus coagulans* and *Clostridium butyricum* in the fermented substrate's pH (Table 3.1). There was a substantial decrease in the pH of the fermenting substrates in all the fermentation methods used compared to the untreated, and this was attributed to the high lactic acid concentration in the microorganism during fermenting. This contributes most to the decrease in pH during fermentation because it is about 10 to 12 times stronger than other significant acids (e.g., acetic acid and propionic acid) (Kung *et al.*, 2018). The result of pH measurement in the growth medium indicated a reduction ranging between 4.4 and 3.2 by all the strains of lactic acid bacteria (LAB) within 48 h; this could be a helpful factor in the exertion of antagonism against spoilage and pathogenic organisms that may be associated with food products.

***in-vitro dry* matter digestibility**

Treatment 2 had the highest digestibility (66.24±0.76%) relative to the rest, while untreated T4 had the lowest (42.20±0.78%). Untreated MOLM significantly differed from *Bacillus coagulans* ($p<0.05$). *Clostridium butyricum* (57.24±0.74%) resulted in a higher *in-vitro* DMD% than spontaneous fermentation (48.23±0.57%). There was an increase in *in-vitro* DMD% (Fig 3.1) after treating the leaves with the probiotics *Bacillus coagulans* (66.24±0.76%) relative to the untreated (42.20±0.78%). Therefore, the results obtained in this study pointed in the direction of a good survival of *Bacillus coagulans* in the GI tract of poultry due to its potential to aid in the digestion of protein and carbohydrates. This result agreed with the finding of Sui *et al*. (2020), who reported that *B. coagulans* showed good assimilation potential in *in-vitro* dry matter digestibility. Results revealed that the Moisture content of MOLM was higher in spontaneous fermentation (12.83±0.28%), while the lowest was recorded in *Bacillus coagulans* (6.6±0.31), respectively. The increase in the moisture content can be attributed to the addition of water to the substrate before fermentation. Moisture in feed determines the rate of feed absorption and assimilation within the body. It also determines the keeping quality of feed. Also, the increase in DM content of the fermented substrates in this study could be due to the microorganisms' biomass (Faseleh *et al.,* 2012). Also, the water-soluble carbohydrates in the leaf, a readily available substrate for the *lactobacillus* bacteria, could result in high DM losses because the fermentation goes to the ethanol pathway (Silva *et al.,* 2017).

Therefore, reduced moisture content can improve the keeping quality. This can protect the fermented MOLM from mycotoxin attack, improve the quality of fermented leaves and ensure longevity in case of use for large-scale feed manufacturing (Gillis *et al.,* 2016). The results revealed that all the fermented substrates exhibited an increase in crude protein, where *Bacillus coagulans* had the highest CP (33.41±0.57) compared to untreated (27.48±0.25). With *Bacillus coagulans* significantly differed from untreated ($p<0.05$). *Clostridium butyricum* resulted in a higher CP (29.31±0.29) than spontaneous fermentation (27.18^c±0.55). Higher CP could be attributed to proteolytic enzymes produced by the microorganisms (Jabbar *et al.,* 2021). It could also be attributed to the activities and increase in the number of microorganisms during fermentation. It can also result from the synthesis of protein by fermenting substrates, which could have resulted in increased production of amino acids. The increase in the protein content would be due to either the increase in the biomass supported in this case by conducive conditions for growth in MOLM during the fermentation, which is in the range of optimal pH of the lactic bacteria. This finding agreed with those of Léopold *et al.* (2013), who reported that there was a general increase in crude protein content of fermented Moringa leaves from 38 g/100g DM to 44 g/100g DM for the fermented MOLM leaves at 37˚C and from 33 g/100g DM to 39 g/100g DM for the fermented MOLM at 37˚C. A significant quantity of crude protein in MOLM means that Moringa leaf protein concentrates could be used as a nutritionally valuable healthy ingredient to decrease protein deficiency in the poultry diet. The improvement in food's protein value during fermentation has been reported by Shi *et al.* (2015). The results of this study showed that *Bacillus coagulans* had higher ash content while spontaneous and untreated did not differ ($p>0.05$) (Table 3.2). Thus. The increased ash content could lead to an increase in mineral content and an indication of the substrates' mineral composition level. The profile of ash content during fermentation reflects the conversion of substrates from one form to another without significant loss in the form of volatile compounds (Ntuli *et al.*, 2013). Ash in diet contributes to the residues remaining after all the moisture has been removed and the organic materials (fat, protein, carbohydrates, vitamins, organic acid) have been incinerated (Salma Sultana, 2020). The results in this study showed that crude fat (ether extract) of MOLM was high in spontaneous (10.4^{a}±0.25) compared to untreated (6.73^{c}±0.11) (Table 3.2). In contrast, *Clostridium butyricum* (8.73^{b}±0.05) significantly differed ($p<0.05$) from *Bacillus coagulans* (7.35^{c}±0.01) (Table 3.2). These values agree with the findings of Salma Sultana (2020), who reported that the fat content was in the range (4.03,9.51%) in moringa leaf meal, which is desirable. Moringa leaf meal contains more dietary polyunsaturated fatty acids (PUFAs) than saturated fatty acids (SFAs). The increase in lipids in fermented leaves may be attributed to the microbial transformation of carbohydrates to lipids. The fermentation of moringa leaf meal and grass pea seed meal with *Bacillus species*. It was reported by Bairagi *et al.* (2004) and Ramachandran *et al.* (2005). to increase the lipid level, especially free fatty acids. The crude fibre level in MOLM is primarily composed of cellulose with small amounts of lignin, which is indigestible for poultry. The low fibre content in MOLM in this study compared with most forage plants; the fibre fraction defines the extent and rate of feed digestibility in poultry. Crude fibre enhances digestibility and aids in the absorption of microelements, glucose and fat; however, its presence in high levels can cause intestinal irritation (40%), lower digestibility and decreased nutrient usage (Sultana *et al.*, 2020).

The crude fibre content of MOLM in this study shows that T2 had lower crude fibre than the rest, untreated T4 recorded the highest CF, and Treatment 2 significantly differed from T4 ($p<0.05$). Treatment1 had lower CF than T3; this finding agreed with Sultana *et al.* (2020), who reported that the crude fibre content of MOLM (6.00–9.60%) recorded was considered acceptable. The crude fibre content of MOLM of the fermented substrate with microbial inoculation was lower compared to spontaneously fermented substrate and untreated substrate. This could have been due to the microorganism's ability to degrade fibre, hence unlocking the nutrients in MOLM, allowing for rapid microbial growth and enzymatic production and, therefore, immediate and fast breakdown of crude fibre (Hu *et al.*, 2011). All fermentation methods affected the acid detergent lignin (ADL), neutral detergent fibre (NDF) and acid detergent fibre (ADF) fractions positively; plant cell substances can be divided into less digestible cell walls (made of hemicellulose, cellulose and lignin) and highly digestible cell contents (containing starch and sugars). Hemicellulose, cellulose and lignin are indigestible in non-ruminants, while hemicellulose and cellulose are partially digestible in ruminants. Neutral detergent fibre is a good indicator of the "bulk fibre and has been used to predict feed intake. The probiotic fermentation of MOLM had a significantly decreasing effect on ADL, NDF and ADF, thereby reducing the fibre contents after fermentation. *Bacillus coagulans* probiotics were reported to degrade crude fibre, cellulose and hemicellulose levels in *Leucaena leucocephala* leaf meal, wheat bran and grass pea seed meal (Ghosh *et al.,* 2017). Anti-nutritional factors (tannins, phytate, oxalates) are compounds synthesised by the plant that reduce nutrient utilisation from plants or plant products and determine the use of particular plants as animal feed (Gemede & Ratta, 2014). They are synthesised through normal metabolism pathways in the plant as secondary metabolites intended for their plant defence (Itkin *et al.,* 2013). They affect the digestibility, bioavailability and utilisation of nutrients, mainly proteins, minerals and vitamins in food and reduce their nutritive values (Akinrinde & Adisa, 2014). However, Choi *et al.* (2022) established that tannin acid at levels below 972 mg/kg could be added to layers feed without a negative effect on productive performance.

Moringa leaves contain 21 g/kg phytate, 10.5 g/kg oxalates and a negligible amount of tannins, saponins, trypsin and amylase inhibitors (Teixeira *et al.,* 2014). All fermentation methods significantly decreased tannins and phenolics contents. There were significant differences in phenolic content ($p<0.05$). Untreated MOLM meal recorded the highest phenolic content, whereas the lowest phenolic content was observed in *Bacillus coagulans* fermentation. Fermentation using *Clostridium butyricum* ($1.13^b \pm 0.01$) did not differ ($p>0.05$) from spontaneous fermentation. The condensed tannins' results showed no significant difference ($p>0.05$) between untreated MOLM meal and spontaneous fermentation. In contrast, *Bacillus coagulans* fermentation and *Clostridium butyricum* did not differ ($p>0.05$). However, the tannins level was reduced to below detectable levels in MOLM fermented with *Bacillus coagulans* and *Clostridium butyricum* compared to untreated. This agrees with the finding of Amita *et al.* (2014), who reported that tannin and phytic acid had been observed in all the fermented groups with BS *(Bacillus subtilis),* BC *(Bacillus coagulans)* and SC *(Saccharomyces cerevisiae*), and the phytic acid level was reduced to below detectable levels in pods fermented for 96 h with *Bacillus* species. From Table (3.2), the finding shows that there was a significant difference ($p<0.05$) between T2 and T4, *Clostridium butyricum* T1 and T2 did not differ from ($p<0.05$) in the phenol content of fermented MOLM. This is due to the action of cellulolytic, ligninolytic and pectinolytic enzymes produced by microorganisms (bacteria) during the fermentation to break down the plant wall components and to hydrolyse the ester bonds which link phenolics to the cell wall, contributing to the release of the individual phenolic compounds from the matrix (Ajila *et al.,* 2012; Dulf *et al.,* 2018).

3.6 Conclusion

From the results of this study, it was concluded that MOLM fermented with *Bacillus coagulans* and the untreated meal had the highest (66.24±0.76%) and lowest (42.20±0.78%) *in-vitro* digestibility, respectively. The digestibility of the meal fermented with *Clostridium butyricum* was significantly higher (57.24±0.74%) than the spontaneously (48.23±0.57%) fermented one. Condensed tannin levels in *Bacillus coagulans* (2.09mg[b]±0.14) and *Clostridium butyricum* (2.33mg[b]±0.14) fermented MOLM were similar and were significantly lower than in the untreated and spontaneous (1.26mg[a]±0.01) fermented one. Tannin levels were similar in the untreated and spontaneously treated MOLM.

CHAPTER FOUR

DETERMINATION OF PERFORMANCE AND SENSORY PROPERTIES OF LAYER CHICKEN FED ON PROBIOTICS (*Bacillus coagulans*)-TREATED MOLM

Abstract

The increasing cost of feed resources in livestock production has been identified as a severe impediment to meeting the demand for animal protein, particularly in developing countries; this challenge has necessitated research to evaluate locally available alternative feed resources that could reduce the cost of feeding without negatively influencing feed intake, average daily gain: feed conversion ratio, egg production and sensory properties the hen and sensory attributes. This study evaluated the effect of different inclusion levels of MOLM in exotic laying hen diet on average feed intake, body weight gain, feed conversion ratio, egg production and sensory attributes. The experimental diets contained 0, 10, 15, and 20% MOLM treated with *Bacillus coagulans*. Thirty-six (36) sixteen (16) week-old ISA Brown layers were distributed into 12 deep litter pens, each with three hens, and randomly allocated to four dietary treatments in a completely randomised design. Average daily feed intake, average daily weight gain, feed conversion ratio and Hen day production were determined for ten (10) weeks. The statistical analysis system's general linear model (GLM) was used in the data analysis. Tukey's test was used to separate significant means. Results from the feeding trial showed that birds fed on the diet with 20% MOLM recorded the highest average daily feed intake, and the highest hen day production (HDP) was from birds fed on the diet with 10% MOLM treated with *Bacillus coagulans*. The lowest HDP was recorded in birds fed on the diet containing 20% MOLM; there was no significant difference ($p>0.05$) in production between diet with 0% MOLM and 10% MOLM on the average daily gain. Diets with 0% MOLM and 10% did not differ in feed conversion ratio. The hens fed on the diet with 20% MOLM had the lowest FCR. Therefore, it was concluded that the inclusion of 10% MOLM treated with *Bacillus coagulans* in the diet of exotic laying hens improved performance and sensory attributes.

4.1 Introduction

In the commercial poultry production system, profit can be attained by minimising feed cost, which accounts for about 60 - 70% of the total cost of production under intensive system management (Adegbenro *et al.*, 2020). Regular feed supply above maintenance requirements is essential to improve productivity in the family of poultry production. The increasing cost of feed resources in livestock production has been identified as a severe impediment to meeting the demand for animal protein, particularly in developing countries. This challenge resulted in a research focus that could reduce the cost of feeding without negatively influencing the performance of the birds. Feed is the essential input for poultry production, and the availability of low-priced, high-quality feeds is critical for expanding the poultry industry and quality (Mengesha, 2011). The high cost of conventional feed ingredients and additives has led researchers worldwide to search for alternatives that will give an optimum performance (meat and egg) and to make these products available.

Moringa is among non-legume trees and shrubs that are promising options for animal feed due to its availability and excellent nutritive value (Beyene, 2021). Its leaves contain compounds of nutritional value, phytochemicals, vitamins, minerals, and amino acids (Al Taweel *et al.*, 2019). Briones *et al.* (2017) stated that Moringa leaf could be a dietary supplement in layers and broilers because it supports high production performance and improved egg quality. Moringa leaf meal might positively improve chickens' production performance and health status (Mahfuz *et al.*, 2019). This study determined the effects of substituting different levels of soybean meal with MOLM treated with *Bacillus coagulans* on laying hens' feed intake, average daily gain: feed conversion ratio, egg production and sensory properties.

4.2 Materials and Method

4.2.1 study site

The study was conducted at the Poultry Research Unit, Tatton Agriculture Park (TAP), at Egerton University. The University is in Nakuru County, Njoro sub-County. 0°22'11.0"S, 35°55'58.0" E (Latitude:-0.369734; Longitude:35.932779 in Kenya, and it is 1,800 m above sea level with an average temperature between 17–22^{0}C but can drop to 11^{0}C during the cold season (July-August). The average annual rainfall in two short and long seasons is 1,200±100 mm (Mulle *et al.*, 2020). The long rain starts in March and ends in July, while the short rain starts in October and ends in December.

4.2.2 Collection and processing of MOLM

As described in Chapter 3.2.2.

4.2.3 Determination of the nutrient composition of MOLM

As described in Chapter 3.2.1.

4.2.4 Management of experimental birds

The hens were kept in a deep litter system; the poultry house was thoroughly cleaned and disinfected (Kupicide®) before the start of the experiment. Forty-five (45) 16-week-old exotic pullets (ISA Brown) were purchased from a poultry farmer within Nakuru County. They were Vaccinated against Newcastle, infectious bursal disease, fowl pox, and Marek's disease and dewormed before the commencement of the experiment. The birds were acclimatised for two weeks before the feeding trial commenced. The pullets were weighed and assigned into four dietary treatments in a completely randomised design, with three replicates with three pullets per pen. Wood shavings were used as litter materials and were managed through raking to aerate and changing wet materials. Feed and fresh, clean water were provided *ad libitum*. One feeder and drinker were allocated in each pen for three birds. The daily management practices included cleaning the feeders, waterers and feeding.

4.2.5 Experimental diets

The experimental diets were formulated to meet the National Research Council (1994) requirements for exotic pullets (ISA Brown) before compounding and mixing. The diets used in the experiment are presented in (Table 4.1.) The MOLM was incorporated in the following levels: 0%, 10%, 15%, and 20%, to have a diet containing 16% crude protein and metabolisable energy of 2850 to 2900 kcal/kg, A layer premix was added to supply minerals and vitamins. The dietary treatments were**:** Treatment l, 0% MOLM, 0% probiotics (*Bacillus coagulans*)**;** Treatment 2, 10% MOLM, 0.01 probiotics; Treatment 3, 15% MOLM 0.01 probiotics; Treatment 4, 20% MOLM 0.01% probiotics.

Table 4.1 Composition of experimental diets

Ingredients kg	T1	T2	T3	T4
Maize meal	70	67	67	66
Soybean meal	18	12	7	4
Fish meal	3.1	4	4	4
MOLM	0	10	15	20
DCP	2.4	2.4	2.4	2.4
Limestone	6	4.09	4.09	3.09
Iodised salt	0.3	0.3	0.3	0.3
Layer premix	0.2	0.2	0.2	0.2
Probiotics	0	0.01	0.01	0.01
Total	100 kg	100 kg	100 kg	100 kg
Calculated analysis				
CP (kg)	16.88	16.92	16.22	16.4
ME (Kcal)	2.874.88	2.870.27	2.854.42	2.856.42
CF (kg)	2.53	3.43	3.78	4.23

4.3 Data collection

4.3.1 Feed intake, feed conversion ratio, average daily gain, hen day production

Average Daily Feed intake

The average daily feed intake was determined every 24 hours as the difference between the amount of feed offered and the amount of feed left over (refusal).

Feed conversion ratio (FCR)

Feed conversion ratio (FCR) was calculated as gram feed consumption per day per hen divided by gram egg mass:

$$FCR = \frac{\text{Feed consumption (g)}}{\text{Egg mass (g)}}$$

Average weight gain ADG

The average daily weight increase was calculated as the difference between 2 successive weighings divided by the duration between weighing. The chicken was weighed together before feeding.

$$\text{average daily gain per bird (g)} = \frac{\text{weight after 7 days g} - \text{weight at the start of 7 days}}{\text{7 days}}$$

Hen day production

Eggs were collected twice a day in the morning (8.30-9 am) and afternoon (5.00-6 pm). The number of eggs laid by each replicate was recorded daily, and the hen-day production (HDP) was calculated as the number of eggs laid divided by the number of hens present daily multiplied by 100 (Ahmed *et al.*, 2014).

$$\text{HDP} = \frac{\text{Total number of eggs produced each day}}{\text{Number of hens alive on each day}} \times 100$$

Egg weight and egg mass

Immediately after the collection of the eggs, the mean egg weight was determined by dividing the total egg weight by the total number of eggs collected from each pen. The average egg mass per day was computed by multiplying the mean egg weight by the percentage of hen-day egg production (Asrat *et al.*, 2018). The Egg Weight (EW) was measured with an electronic balance to the nearest 0.01 g (number SF- 400 equipped with a high-precision strain gauge sensor.

4.4 Experimental design

A completely randomised design (CRD) was employed. There were 12 experimental units with three layers per unit, and each was replicated thrice.

The statistical model used is as follows.

$$\boldsymbol{y_{ij}} = \mu + \boldsymbol{\alpha_i} + \boldsymbol{\beta_j} + (\boldsymbol{\alpha\beta})\ \boldsymbol{ij} + \boldsymbol{\varepsilon_{ij}}$$

where,

$\boldsymbol{y_{ij}}k$ = Response variable of interest (feed intake, feed conversion ratio, average daily gain, egg quality).

μ= overall population means.

$\boldsymbol{\alpha_i}$= effect due to the i^{th} treatment, Moringa {diet 1, 2, 3, and 4 diets).

$\boldsymbol{\beta_j}$=effect due to Probiotics {1,2,3 and 4 diets).

$(\boldsymbol{\alpha\beta})\ \boldsymbol{ij}$=interaction effect of i^{th} Moringa on j^{th} level of probiotics.

$\boldsymbol{\varepsilon_{ij}}_k$= random error term.

4.5 Descriptive sensory Attributes

Descriptive sensory analysis was conducted for three days on boiled eggs at the sensory evaluation facility at the Department of Dairy, Food Science and Technology, Egerton University. Tests of eggs were sampled on week 10 of the experiment. A sensory panel of 11 individuals, seven (7) males and four (4) females, aged 20 to 45, was set up. The panellists received training a day before the three-day sensory tests of the eggs were conducted. Questions and scales were adjusted based on the feedback received from the panel according to procedure of Sasaki *et al.* (2012). Eggs used in the sensory attributes were laid two days before each test date and stored at 4 °C. They were labelled with a number code on the shell. The marked eggs were cooked in a stainless-steel pot for 35 minutes at 75^{o}C. After that, they were immediately placed in cold water for 3 minutes to ease peeling. The shelled eggs were placed individually in small, covered plastic cups labelled by three identifiers. The eggs were then peeled, cut into two halves and placed on disposable plastic plates. The disposable plates were marked based on the treatments from which the eggs were sampled. Panellists were advised not to consume food or drinks at least an hour before the taste test sessions. They were asked not to communicate with one another and directly go to their assigned individual booths and rest for a minute before the beginning of the taste tests. Distilled water was provided for the panellists to rinse their mouths before and in between tastings, and the flavour was evaluated before conducting the same evaluations on the egg yolk. The panellists filled out the appropriate section of the questionnaire (Appendix E) step by step. The questionnaires were collected, and the tasters rested for 15 minutes before tasting the second egg in each session. Panellists used questionnaires to evaluate the sensory attributes of the four samples in appearance, flavour, taste, texture and overall acceptability. Attribute profiling was done using a scale of 10 cm long (Appendix E) with anchored words such as too light and too soft, for the extreme left-hand side of the scale to too dark and too hard; at the extreme right side and neither light nor dark, or neither soft nor hard at the centre. The scale enabled continuous data to be recorded. The values were transformed into percentages for ease of use.

The parameters were quantified by a nine-point hedonic scale (1 = dislike extremely; 9 = like extremely).

Data analysis

Data was compiled and computed to determine statistical significance based on the number of correct responses. The effects of probiotics-treated Moringa meal on sensory data were analysed using analysis of variance (ANOVA) at a 5% significance level. Where the means were significant, mean separation was done using Duncan's Multiple Range Test. Calculations were done following the statistical analysis system (SAS) version 9.3.

4.6 Results

Table 4.6 Analysed chemical composition of the diets (DM basis %)

Nutrients	Diets			
	T1	**T2**	**T3**	**T4**
MC	9.2723	9.589	8.684	9.938
ASH	13.506	12.953	11.455	9.371
EE	4.6220	5.3705	5.1710	4.770
CF	5.0200	5.4430	6.085	6.194
CP	16.17	16.186	16.240	16.293

T1 0% MOLM 0% *Bacillius coagulan* T2 10% MOLM 0.01 *Bacillius coagulan* T3 15% MOLM 0.01 *bacillius coagulan* T4 20% MOLM 0.01 *Bacillius coagulan.* MC Moisture *EE* Ether Extract (EE) CF crude fibre, CP crude protein.

4.6.1 Effect of MOLM-based diets on daily feed intake and feed conversion ratio

The results on average daily feed intake (ADFI) and feed conversion ratio (FCR) as affected by the incorporation of MOLM in layer diets are shown in Table 4.3.

Table 4.3 Effect of experimental diets on the performance of exotic layers

Parameters	T1	T2	T3	T4	p-Value
ADFI (g)	101.61 ± 1.37^{c}	108.21 ± 1.44^{b}	109.47 ± 1.44^{b}	116.96 ± 1.16^{a}	<.0001
HDP (%)	58.33 ± 2.36^{b}	68.05 ± 2.27^{a}	55.75 ± 2.43^{bc}	47.61 ± 2.62^{c}	<.0001
ADG (kg)	0.25 ± 0.03^{ab}	0.32 ± 0.03^{a}	0.14 ± 0.04^{bc}	0.11 ± 0.04^{c}	0.0007
FCR (g)	1.17 ± 0.06^{c}	1.22 ± 0.07^{c}	1.60 ± 0.08^{b}	2.13 ± 0.08^{a}	<.0001
Egg weight (g)	63.29 ± 0.3^{a}	65.63 ± 0.56^{a}	59.29 ± 0.80^{b}	59.08 ± 0.64^{b}	<.0001

Means within a row with different superscripts differ significantly (P<0.05): T1 diet containing 0% MOLM, T2 diet containing 10% MOLM, T3 diet containing 15% MOLM, T4 diet containing 20% MOLM. ADFI: Average daily feed intake, FCR: Feed conversion ratio, ADG: Average weight gain, HDP: Hen day production.

All the parameters were significantly (p<0.05) affected by the experimental diets, and There was an increase in average daily feed intake (ADFI) and FCR with increasing inclusion levels of MOLM in the diets. Average daily gain, HDP and egg weight decreased with increasing inclusion levels of MOLM in the diets.

Table 4.4 Results for descriptive sensory attributes of eggs

parameters	T1	T2	T3	T4	p-Value
Taste	5.07 ± 0.33^{b}	$5.63\pm0.42b^{ab}$	6.37 ± 0.34^{a}	6.45 ± 0.33^{a}	<.0001
Flavour	5.07 ± 0.32^{b}	5.83 ± 0.33^{ab}	6.43 ± 0.34^{a}	5.70 ± 0.38^{ab}	<.0001
Albumen texture	5.90 ± 0.35^{b}	6.70 ± 0.33^{a}	6.67 ± 0.32^{a}	6.40 ± 0.38^{ab}	0.0006
Text of yolk	5.90 ± 0.23^{c}	6.77 ± 0.31^{a}	6.73 ± 0.30^{a}	6.50 ± 0.33^{b}	0.0001
Egg size	6.10 ± 0.32^{ab}	5.53 ± 0.28^{b}	6.27 ± 0.34^{a}	6.10 ± 0.28^{ab}	<.0001
General app	5.07 ± 0.37^{b}	6.37 ± 0.33^{a}	6.23 ± 0.37^{a}	6.53 ± 0.34^{a}	<.0001

Means within a row with different superscripts differ significantly (P<0.05); T1 diet containing 0% MOLM, T2 diet containing 10% MOLM, T3 diet containing 15% MOLM, T4 diet containing 20% MOLM: the general app, General Appearance.

4.7 Discussion

The highest average daily feed intake in the diet with 20% MOLM could have been a result of the birds trying to meet their nutritional requirements due to depressed feed digestibility because of the high fibre content. High fibre content in the diet increases the viscosity of the intestinal content, making it inaccessible to digestive enzymes, resulting in a decrease in the bioavailability of dietary nutrients. (De Vries, 2015). Therefore, the high feed intake for the hens meets nutritional requirements. Higher levels of MOLM in the diet led to an increase in crude fibre content (C.F.) that consequently might have decreased dietary energy density, thereby increasing the feed intake in the birds to meet optimal energy requirements to sustain growth. As dietary fibre increases, there is also a tendency for nutrient dilution, forcing the birds to consume more feed to meet body nutrient requirements. The weight gain observed in this study showed that the bird fed on a diet with 10% MOLM had a higher weight gain. This could be due to a higher efficiency of utilisation of feed consumed by these birds; these nutrients were utilised to develop the body tissue, which translated to an increase in body weight. This could be explained as better nutrient digestibility, improved feed utilisation and increased body weight gains. This indicates that the inclusion of a 10% MOLM treated with *Bacillus coagulans* diet did not negatively affect the birds' performance.

The mechanisms through which the diet with 10% MOLM treated with *Bacillus coagulans* based diets improved the performance of the birds could be due to the moderate fibre content in the diet and the probiotic (*Bacillus coagulans*); these could have improved the gut morphology, thereby stimulating mucosa enzyme activity and eventually improving nutrient digestibility. This agreed with the findings of Chang *et al.* (2019), who reported that probiotics aid in improving performance, such as gain in body weight for growing animals and improving feed conversion efficiency for meat and egg production. Additionally, the digestion of energy and nutrients from ingested feed requires the interaction of the chicken's biochemical reactions and the microbiota present in the gastrointestinal tract (GIT) (Borda-Molina *et al.*, 2018). Therefore, the incorporation of probiotics-treated Moringa leaf meal in the diets may have led to an increase in the digestive and absorptive capacity of the birds by encouraging the proliferation of beneficial microbiota. These microorganisms play an essential role in digestion, encouraging greater flow and absorption of nutrients in the small intestines (Jha & Berrocoso, 2016). This could be attributed to the fact that the probiotics hydrolysed the CF in the diet containing 10% MOLM (Table 4.3) to improved nutrient digestibility by the birds fed on 0% MOLM and 10% diets. The FCR for these two diets (0 & 10% MOLM) is not significantly different (similar) compared to the birds on 15% and 20% MOLM diets. The higher feed conversion ratio exhibited by these birds (15 and 20% MOLM) could have been due to higher fibre content in the diets and some negligible amount of tannins, which might have diluted the diet's energy content, leading to nutrient deficiency. The similar egg production in hens on 0% and 10% MOLM diets could have been due to higher feed utilisation efficiency exhibited by these birds. These results concurred with the finding of Kakengi *et al*. (2007), who reported that HDP was moderately influenced by MOLM levels, where there was a slight decrease with an increase of MOLM proportion in the diet. Laying hens on the 20% MOLM diet were characterised by a higher feed conversion ratio (FCR), an indication of a lower feed utilisation efficiency. Egg weight, a primary criterion for grading eggs for the market, is influenced by feed, protein and energy consumption (Wang *et al.,* 2017). Dietary nutrient supply and feed characteristics (texture, composition and energy) are the most critical factors affecting egg quality. The finding in this study revealed that birds fed on a diet with 0% and 10% MOLM had heavier egg weights throughout the feeding trial period. That could have been due to the efficiency of feed utilisation, hence more nutrients available for the production of egg weights. This finding agrees with the results of Onunkwo *et al.* (2015), who observed that the 20% MOLM diet had lower egg production per cent, egg mass, and egg weight. This could also have been due to the lower digestibility of the higher dietary fibre content in the Moringa leaf meal.

4.8 Effect of probiotics(*Bacillus coagulans*);-treated MOLM on descriptive sensory attributes of exotic chicken layers eggs

In sensory studies, descriptive evaluations play a crucial role as they can yield results that are almost as accurate as objective methods. This is due to the involvement of trained panellists who participate in brainstorming sessions to determine relevant terms and validate them, selecting only those agreed upon to describe the parameters of the product being tested accurately. The Just About Right (JAR) method helps predict and explain consumer acceptance and is valuable information in supplementing product optimization (Jahnke, 2006). Sensory evaluation of food products provides an understanding and guidance on the key attributes for the consumers that enable a product to become competitive in the market (Lyon, 2012). This current study gave a descriptive hedonic quality measurement for the main attribute of ISA brown-boiled eggs as described by a semi-trained panel. Egg flavour, taste, egg size, texture and general appearance are complex attributes of boiled eggs and were affected by the inclusion of MOLM. The descriptive panel did not find any significant differences between 0% MOLM and 10% MOLM in the Taste of the eggs, treatment 3 (15%) did not differ from 20% MOLM in the same parameter, flavour 15% MOLM was described with extremely intense in flavour, there were no significant differences ($p<0.05$) between 0% 10% and 20% MOLM. Scores for the texture attributes did not differ in all the dietary treatments; scores for egg size small, medium, and big, 0%, 15%, and 20% MOLM did not differ in egg size, while 10% recorded medium intense in egg size, the score for general appearance fairy, good, and very good 10%, 15% and 20% MOLM had extremely intense appearance, while 0% MOLM had medium intense in general appearance Table (4.4).

4.9 Conclusion

From the results of this study, it was concluded that birds fed on the diet with 20% MOLM diet with *Bacillus coagulans* recorded the highest average daily feed intake and the highest feed conversion ratio. The highest egg production was recorded in a 10% MOLM diet with *Bacillus coagulans*. From the descriptive sensory attributes, the panellist scored eggs in the 15% and 20% MOLM diet as best in taste, flavour, albumin, yolk texture, egg size and general appearance. However, the albumin texture and general appearance were similar for the diet eggs containing 10, 15 and 20% MOLM. Therefore, 10% *Bacillus coagulans*-treated MOLM can substitute soybean meal as a protein source in exotics layer chicken diets to improve performance.

CHAPTER FIVE

DETERMINATION OF THE EFFECT OF PROBIOTICS (*Bacillus coagulans*)-TREATED MOLM IN EXOTIC LAYER DIETS ON EGG CHARACTERISTICS AND CONSUMER ACCEPTABILITY

Abstract

The study evaluated the effect of the incorporation of treated *Bacillus coagulan*s MOLM in exotic layer chicken diets on egg characteristics and consumer acceptability. In a completely randomised design, thirty-six (36), sixteen (16) week old ISA Brown layers were selected from a flock of sixty (60) birds and allocated to four dietary treatments with three replicates of three birds each, housed in a deep litter system; The treatments were T1 0% MOLM, T2 10% MOLM, T3 15% MOLM and T4 20% MOLM. Data was collected on external and internal egg qualities (eggshell thickness, shell weight, egg width and length, shape index) (yolk weight, yolk width and length, yolk ratio, yolk colour and egg weight), respectively. Data collected were 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 results showed significant differences in all the parameters evaluated on all the eggs from the birds fed on the different dietary treatments. Treatment 4 (20% MOLM) recorded the highest yolk colour 12 scores on the Roche colour fan scale of (1-16); the eggs from the hen fed on a diet containing 10% and 15% MOLM, recorded 8 and 10, respectively. The results showed that the eggs from the hen fed on a diet containing 10% MOLM had a better shell weight, yolk weight, and yolk ratio, while the ones from hens on a 0% MOLM diet recorded better qualities in yolk width and yolk length. It was therefore concluded that the inclusion of 10% MOLM treated with *Bacillus coagulans* in layers diet improved the egg qualities of Isa-Brown hens.

5.1 Introduction

Poultry production is the most innovative and profitable way of increasing the accessibility to high-quality protein food. Eggs provide a perfectly balanced nutrition containing all the essential amino acids, minerals, and vitamins (Schonfeldt *et al.,* 2013). Egg quality is a factor that contributes to the better economic price of fertile and table eggs. Egg quality was defined as characteristics important for consumers (Stadelman, 1977). Financial success for a production flock is measured by the total number of qualitatively produced eggs (Groothuis *et al.,* 2013). Egg quality is presented by its weight, percentage of eggshell, thickness and strength. Studies have consistently shown that the strain of the hen influences eggshell quality. Hens with coloured feathers lay larger eggs than non-coloured ones (Hanusova *et al.*, 2015). As crucial as poultry is to the economy, the poultry industry faces certain constraints, mainly the high feed cost (Alade & Demola, 2013). Feed is the essential input for poultry production, and the availability of low-priced, high-quality feeds is critical for expanding the poultry industry (Mwesigwa *et al.*, 2015). The high cost of conventional feed ingredients and additives has led this study to explore non-conventional low-level priced feed resources; Moringa leaf meal was recognised to have the potential for use as animal feed due to its high availability and excellent nutritive value (Beyene, 2021). The leaves have been found to contain phytochemicals, vitamins, minerals, and amino acids (Okiki *et al.*, 2015). Mahfuz *et al.* (2019) stated that Moringa leaf could be used as a dietary supplement in layer and broiler feeds due to the high production performance and improved egg quality recorded. This study examined the effect of different inclusion levels of *Bacillus coagulans* treated MOLM on external (egg shape, egg length, egg width and eggshell thickness) and internal egg qualities (albumen weight, yolk colour).

5.2 Materials and Methods

5.2.1 Study site

As described in chapter four 4.2.1

5.2.2 Experimental birds

Thirty-six (36) ISA Brown hens, aged 16 weeks, were randomly assigned to four dietary treatments, each with three replications. Each replication was one cage of three hens. The treatments included 0, 10, 15 and 20% MOLM diets. The four experimental diets were formulated to meet the requirement of layer chicken (NRC, 1994). The chickens were kept in 12-floor pens, each with separate feeders and drinkers. After two weeks of acclimatisation, the diets were offered in mash form, and chickens had *ad-libitum* access to feed and water. The experimental duration was ten weeks.

5.2.3 Measurement of external egg characteristics

Eggs were collected twice daily at 9.00 hrs. and 18.00 hrs., labelled by day, treatment (T) and date (D) (e.g., day 1 T1D1) and placed in egg trays. Three eggs per treatment were sampled weekly to determine the quality characteristics. External egg quality characteristics assessed included egg length, egg width, shell thickness, shape index and eggshell weight. Individual eggs were weighed on a digital balance to the nearest 0.01 g accuracy (Rath *et* *al.*, 2015). The shape index was calculated as the ratio of breadth: length multiplied by 100. All the shells were wiped dry with a paper towel and weighed with a digital balance. The thickness of four pieces of shells, one each from the two ends (broad and narrow end) and two from the body of the egg shell, egg length and egg width were measured with a digital Vernier calliper to the nearest of 0.01 mm, and the measurements averaged. (Ayim-Akonor, 2014).

Eggshell weight (g): The shells were dried after the yolk and albumen were removed from the shell. Shell weight was determined according to the procedures of Gamage *et al*. (2020).

Shell Thickness (ST, mm): Thickness was measured after removing the internal membranes of the eggshell. A precision micrometre was used to the nearest 0.01mm. The average shell thickness of each egg was computed as the Average of the three measurements of eggshell thickness.

5.2.4 Measurement of internal characteristics

The internal qualities assessed included yolk index, egg yolk ratio and yolk colour, and the length and width of the yolk were measured (in mm) with a digital Vanier calliper.

Plate 1: Roche colour fan

Yolk colour: The yolk colour from every egg sampled was determined weekly. Yolk colour was measured using a Roche yolk colour fan. Samples were taken on a piece of white paper, and the yolk colour was determined by a scale of 1-12, where 1=extremely pale colour and 12= intense yellow colour. Yolk index (YI) was calculated for three eggs produced in different treatments every week. The yolk height was measured using Vernier Callipers. The YI was calculated as YI = $(Yolk\ height\ in\ mm)/(Yolk\ diameter\ in\ mm)\ x100$.

The length and width: were measured in mm with a Vernier calliper. The internal qualities assessed included yolk index, egg yolk ratio and yolk colour.

Plate 2: Digital vernier calliper with model number SF- 400 equipped with high-precision strain gauge sensor**.**

5.3 Experimental design

A completely randomised design (CRD) was employed. There were 12 experimental units with three layers per replicate, and each was replicated three times.

Statistical model are as follows.

$$y_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)\, ij + \varepsilon_{ijk}$$

where,

$y_{ij}k$ = Response variable of interest (internal and external, egg qualities).

μ= overall population means.

α_i= effect due to the ith treatment (MOLM inclusion levels).

β_j=effect due to Probiotics in MOLM

ε_{ij}k= random error term.

5.4 Data analysis

Data were 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 ($p<0.05$).

5.5 Sensory Evaluation and Consumer Acceptability of Eggs

A total of 30 semi-trained panel members were constituted of lecturers, technicians, and final year Bachelor of Science students at the Department of Dairy, Food Science and Technology at Egerton University, Kenya, aged between 25 and 45 years, carried out the egg sensory evaluation. The panellists received training a day before the three-day sensory tests of the eggs were conducted. Questions and scales were adjusted based on the feedback received from the panel (Sasaki *et al.* 2012). A total of 18 eggs per treatment were obtained for the panel sensory evaluation. Eggs were boiled in the form of cooking and cooled by placing them in water. The boiling of eggs lasted for 75 minutes from the start of boiling at a temperature of 80oC. Then, the saucepan was removed from the stove, and the hot water was discarded and immediately replaced with cold water at room temperature. The eggs remained in the cold water for about 3 minutes. The eggs were then peeled, cut into halves and placed on plastic disposable plates. The disposable plates were labelled in relation to the treatments from which the eggs were sampled. Before the sensory analysis, panellists were asked not to consume any food within 3 hours of the actual testing. A seven-point descriptive Hedonic Scale (7- Like extremely, 6- Like very much, 5- Like moderately, 4- Neither like nor dislike, 3- Dislike moderately, 2- Dislike very much, 1- Dislike extremely) was also used to determine general acceptability. The panellists were placed in a room alone so as not to influence the outcome of the others.

5. 6 Data analysis

Data was compiled and computed to determine statistical significance based on the number of correct responses. The effects of probiotics-treated MOLM on sensory data were analysed using analysis of variance (ANOVA) at a 5% level of significance. Where the means were significant, mean separation was done using Duncan's Multiple Range Test. Calculations were done following the statistical analysis system (SAS) version 9.3

5.7 Results

Plate Yolk Colour of difference treatment

Table 5.1 Effect of MOLM on Egg Quality

Parameters	T1	T2	T3	T4	p-value
Egg width (mm)	41.93±0.82^{b}	41.96 ±0.80^{b}	42.23±0.73^{ab}	42.70±0.81^{a}	<.0001
Egg Length (mm)	54.46±0.74^{b}	54.53±0.73^{b}	54.78±0.74^{ab}	55.78±0.80^{a}	<.0001
Yolk length (mm)	40.42±0.51	40.07±0.43	41.55±0.25	40.45±0.67	0.7387
Yolk width (mm)	36.30±0.75^{a}	35.30±0.81^{ab}	33.88±0.72^{b}	33.28±0.98^{b}	<.0001
Yolk colour	1.00±0.00^{d}	8.00±0.00^{c}	10±0.00^{b}	12±0.00^{a}	<.0001
Shell thickness (mm)	0.68±0.05^{b}	0.72±0.05^{ab}	0.82±0.07^{ab}	0.89± 0.07^{a}	0.0002
Shape index (%)	77.14±0.65^{ab}	76.82±1.28^{b}	77.12±0.59^{ab}	78.30±0.49^{a}	<.0001
Yolk ratio (%)	61.44±1.46^{a}	59.77±1.28^{a}	53.61±1.12^{b}	50.73±1.45^{b}	<.0001
Yolk weight (g)	15.10±0.22^{b}	16.33±0.17^{a}	13.64±0.13^{c}	14.53±0.30^{b}	<.0001
Shell weight (g)	6.18±0.09^{b}	7.38±0.07^{a}	5.63±0.11^{c}	5.79±0.18b^{c}	<.0001

^{abcd}Means within a row with different superscripts differ significantly (P<0.05) by Tukey's studentized range (HSD); mm = millimetre: T1control 0% MOLM T2 10% MOLM; T3 15% MOLM; T4 20% MOLM.

Table 5.2 Consumer acceptability and sensory evaluation of eggs of ISA Brown layers fed on diets with probiotics-treated MOLM

Parameters	T1	T2	T3	T4	p-value
Colour	4.32±0.36^{b}	5.77±0.25^{a}	5.68±0.26^{a}	5.50±0.34^{a}	0.0008
Flavour	4.45±0.29^{d}	5.14±0.41^{b}	5.23±0.44^{a}	4.55±0.46^{c}	<.0001
Texture of yolk	5.09±0.30^{b}	5.91±0.20^{a}	5.36±0.22^{ab}	5.00±0.29^{b}	<.0001
Texture of Albumin	4.91±0.31^{b}	5.64±0.29^{a}	5.77±0.25^{a}	5.14±0.30^{ab}	<.0001
Taste	4.86±0.27^{b}	5.82±0.25^{a}	5.73±0.25^{a}	5.23±0.28^{ab}	<.0001
General Appearance	4.32±0.30^{b}	5.95±0.22^{a}	5.82±0.22^{a}	5.41±0.23^{a}	<.0001

^{abcd}Means within a row with different superscripts differ significantly (p<0.05) by Duncan's Multiple Range Test.; control 0%MOLM T2 10% MOLM; T3 15% MOLM; T4 20% MOLM.

5.8 Discussion

Dietary nutrient supply and feed characteristics (texture, composition and energy) are the most important factors that affect egg characteristics. Internal and external egg quality parameters measured in the current study were affected by the dietary inclusion of MOLM; some quality parameters were improved when MOLM was included in the diet. Moringa is rich in xanthophyll content, a good pigmenting agent for poultry products (Tesfaye *et al.,* 2013). In the present study, the yolk colour showed a higher score when MOLM was included in the diet, which is similar to the findings of different researchers with 5 and 10% inclusion of MOLM in the layer diet. The quality of eggs is mostly influenced by the supply of dietary nutrients and the characteristics of the feed, such as texture, composition, and energy. The yolk colour scored higher in the study when MOLM was included in the diet, which is consistent with the findings of Olugbemi *et al.* (2010), who used 5% and 10% inclusion of MOLM in the layer diet and observed yolk colour was higher. The colour of egg yolks plays a significant role in satisfying consumers and influencing their appetite. People generally prefer yolks that are golden yellow to orange in colour. A recent study has shown that adding *Moringa oleifera* Leaf Meal (MOLM) to chicken feed can intensify the yellowish colour of egg yolks. This makes MOLM a viable yolk-colouring agent that can make eggs more appealing to consumers. Among the different groups of chickens studied, those that were fed a diet containing 20% MOLM had the deepest yolk colour, while the control group, which had no MOLM in their diet, had a lighter yolk colour. The yolk colour of the 20% MOLM group was significantly different from the control group. Moreover, the group of chickens that were fed a diet containing 15% MOLM had higher yolk colour scores than the group fed a diet containing 10% MOLM. This is because Moringa leaves contain carotenoids and flavonoids that are natural antioxidants and can modify the levels of β-carotene and quercetin in egg yolks. According to Bidura *et al.* (2014), β-carotene in Moringa leaves ranges from 2.7 to 3.10 mg/100 g dried leaves. When added to feed, this bioactive compound and phytochemicals increase egg production and positively affect chicken health. Carotenoids play an essential role in developing different colour scores in egg yolk, especially lutein, which is an active yellow dye. Bidura *et al.* (2020) reported a similar finding that including 10-20% Moringa leaves in broiler feed or laying hens significantly increased the yellow colour of the skin and egg yolk. There were no significant differences ($p>0.05$) in egg length and width in the birds fed with 15% to 20% MOLM treatments. The egg width and length were higher in 20% and 15% MOLM diets than in other diets, while 0% and 10% MOLM did not differ ($p>0.05$). Treatment 1, T2, T3 and T4 did not differ at ($p>0.05$) in yolk length across all the dietary treatments (Table 5.1). Birds fed on 0% MOLM (T1) recorded higher yolk width, while there was no significant difference between T2, T3, and T4 at (p>0.05). The diet of 20% MOLM resulted in the highest shape index (Table 5.1) relative to rest, while T1, T2, and T3 did not differ (p>0.05). This agrees with the findings of Duman *et al.* (2013), who found that egg shape index and shell thickness did not show any significant difference (p>0.05) due to the supplementation of MOLM. Similarly, according to the results of an earlier study, no adverse effect was found on the egg quality traits due to MOLM supplementation (Kaijage *et al*., 2015). An eggshell is a biological barrier that protects internal egg contents (Portugal *et al.,* 2018). Egg shell quality is of paramount importance because it determines the marketability of the eggs (Jiang *et al.,* 2013; Maxkwee *et al.,* 2014). In this study, eggshell weight was generally higher in birds fed on 10% MOLM diets and increased as the laying period progressed. Eggshell quality is probably due to the birds' improved calcium and phosphorus metabolism (Jonchère *et al.*, 2012). Eggshell thickness in this study revealed that 20% MOLM recorded the highest shell thickness relative to the rest. In contrast, the lower eggshell thickness was recorded in the 0% MOLM (Table 5.1); there were no significant differences in shell thickness (p>0.05) between 10% MOLM and 15% MOLM (Table 5.1). It was noted that the proportion of eggshells increased with the increased level of Moringa in the diet of laying birds (Lan *et al.,* 2019). This could be due to an adequate supply of calcium and phosphorus by the MOLM at 20% and the birds' efficient calcium and phosphorus metabolism. Egg shells are a mixture of bioceramic material with 95% calcium carbonate as polymorphic calcite and 3.5% organic matrix material (Hincke *et al.*, 2021). Thus, the similarity in the overall mean of eggshell thickness implied that diets supplied adequate calcium and phosphorus that could have improved the metabolism of these minerals during eggshell calcification (Zhang *et al.*, 2019). It may be hypothesised that Moringa increased eggshell weight and thickness due to the photogenic compounds that may have the ability to improve calcium storage, uterine functions and intestinal secretions, which could lead to enhancing eggshell and egg quality (Liu *et al.,* 2020). The finding agrees with the results of Aguayo *et al*. (2017), who stated that the proportion of eggshells increased with the increased level of Moringa in the diet of laying chicken. Birds fed on the diet of 10% MOLM recorded the highest yolk weight relative to the rest, while 15% MOLM recorded the lowest yolk. weight This could be explained as better nutrient digestibility, improved feed utilisation and increased yolk weight in a diet containing 10%. The inclusion of 0% MOLM did not differ from 20% (Table 5.1); this could be due to an increase in feed intake with an increase in MOLM in the diet resulting in increased nutrient intake and hence the size of egg components increased as reported by Prasad & Ganguly, (2012). Moringa leaves are also a source of vitamin A, riboflavin, nicotinic acid, folic acid, pyridoxine, ascorbic acid, beta-carotene, calcium, iron, and α-tocopherol. They are essential for growth, development and reproduction (Gakuya *et al.,* 2014) and egg yolk bioactive compounds. Antioxidants, flavonoids, carotenoids, amino acids, proteins, and energy levels decrease egg water content and can increase nutrient density in egg yolk.

5.9 Effect of MOLM-based diet on consumer Acceptability of ISA Brown layers eggs

A group of semi-trained panellists conducted a test to evaluate the taste of eggs from hens that had been fed diets containing probiotics-treated Moringa leaf meal. The overall acceptability of a food product reflects the total sum of the sensory attributes (Murray *et al.,* 2000). In this study, hedonic scores for most of the sensory attributes, including flavour, texture, taste, colour, and general acceptability of the MOLM treatments, were not significantly different. The similarity of hedonic scores for the sensory attributes for the control and treatments was reflected in the overall acceptability scores, where treatment scores were similar to those of the controls. The panellists found no discernible difference in flavour between eggs from birds-fed diets containing 0, 10, 15, or 20% MOLM. This could be because MOLM does not affect these parameters; results are consistent with those of a previous study by Jabbar *et al.* (2010), which also found that consumers could not taste a difference in the flavour of hard-boiled eggs. Botsoglou *et al.* (2012) found that lipid oxidation did not result in any undesirable tastes in eggs from chickens fed with flax. However, in this study, the evaluators showed that the addition of MOLM to the chickens' diets caused a change in the yolk's colour. The colour changes may be due to the existence of xanthophyll in the MOLM. Xanthophyll is an oxygen-based form of carotenoids. Gebrehawariat *et al.* (2016) also reported a similar change in yolk colour with MOLM inclusion in the diet of layer chicken. The reason for the egg yolk pigmentation in MOLM is due to the presence of xanthophyll. This pigmentation is affected by how easily the carotenoids are digested, metabolised, transferred, and deposited in the yolk. The quantity and types of carotenoids present in the yolk determine the colour change, which can impact the eggs' overall appeal, as Adetoro (2021) reported. A strong positive correlation confirmed the connection between yolk colour and egg acceptability, as shown in Table (5.2). According to Zaheer (2015), consumers base their egg choices on internal quality characteristics, such as cholesterol, fatty acid levels, and yolk colour. Including MOLM in the diet probably led to a rise in carotenoid levels in the eggs, causing the yolk colour to become more vividly yellow compared to eggs from diets lacking MOLM. (Alebachew *et al.*, 2016). The colour change may have played a role in enhancing the quality and appeal of the eggs to the consumers. People increasingly acknowledge the benefits of using MOLM in yielding eggs that have a better consistency of albumen and richer yellow yolk colour.

Conclusion

The results showed that the layer diet with 10% MOLM positively influenced eggshell weight, yolk weight, and yolk ratio, while the diet with 0% MOLM resulted in better yolk width and yolk length. It was concluded that a layer diet containing 10% MOLM could improve the egg qualities of Isa-Brown chicken and influence customer acceptability of the eggs.

CHAPTER SIX

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

6.1 General Discussion

Poultry production in Kenya is constrained by an inadequate supply of good quality feed, and escalating costs and competition between humans and livestock have caused this protein source. The competition between humans and livestock for the same products worsens the situation. Therefore, alternative locally available low-cost materials would be needed to substitute conventional raw materials already in the market, especially soybean and fishmeal (Gakuya *et al.,* 2014). As a non-conventional feed resource, Despite Moringa being locally available, it has not been commonly used in poultry feeds due to a lack of appropriate knowledge and antinutritive factors in MOLM limit its use in poultry production. Moringa leaf meal has low levels of anti-nutritional factors and produces no toxic effect on consumption (Stohs &Hartman, 2015). The low level of anti-nutritional factors in comparison to *Amaranthus spp*, spinach, Ethiopian mustard and other *Brassica spp* vegetables. However, anti-nutritional factors are mostly decreased by processing methods, like blanching, drying, fermentation and de-fatting, which results in reduced or deactivated activity (Devisetti *et al.,* 2016). This study was conducted to determine the effect of probiotic treatment on digestibility and tannin content. It also evaluated the effect of incorporating MOLM treated with *Bacillus coagulans* in an exotic layer diet on performance and egg quality.

Chapter three (3) of this thesis was achieved using an *in-vitro* experiment, whose findings indicated that fermentation using *Bacillus coagulans* improved the digestibility of MOLM. Digestibility is the indicator of the nutritive value of a feed (NRC, 1994). Therefore, overcoming the limitation of poor digestibility associated with high fibre through biotechnological interventions (fermentation) can be a viable option**.** However, This experiment evaluated how treating MOLM with *Bacillus coagulans* affected the digestibility and condensed tannin content of MOLM. Treatment of MOLM by spontaneous fermentation, *Bacillus coagulans* and *Clostridium butyricum* increased crude protein content while the total fibre constituents decreased. Fermentation with *Bacillus coagulans* and *Clostridium butyricum* improved the digestibility of MOLM compared to the untreated MOLM. This study chooses *in vitro* methods over *in vivo* ones because the *in vivo* experiments are typically more complex and may involve the entire organism or specific organs or systems within a living organism, such as an animal.

Chapter four (4) described the determination of the effects of incorporating *Bacillus coagulans*-treated MOLM in the exotic layer chicken diet on the performance and descriptive sensory attributes. The inclusion of up to 20% MOLM with *Bacillus coagulans* increased feed intake, which was probably associated with increased bulkiness due to high-fibre content and some tannins. This may have resulted in the birds increasing feed intake to meet their nutritional requirements due to depressed feed digestibility. There was also a general increase in the feed conversion ratio as the level of inclusion of MOLM increased in the diet associated with the high fibre content of the feed. Better performance was observed in birds fed on the diet containing 10% MOLM, which had lower FCR, higher average daily gain, hen day production, and egg weight. This was due to a higher digestibility of the feed, hence a higher efficiency of dietary nutrient utilization (low FCR) and more nutrients available for weight gain, egg production and egg weight. From the descriptive sensory attributes, the panellists scored eggs from the hens fed on the diet containing 15% and 20% MOLM as having the best taste, flavour, albumin texture, yolk texture, egg size and general appearance.

Chapter five (5) a write-up of the effect of the inclusion of *Bacillus coagulans*-treated MOLM in exotic layer chicken diets on egg quality (yolk index, shape index, yolk colour, yolk weight, shell thickness) and consumer acceptability, diet containing 20% MOLM had the highest yolk colour score of 12, and diets with 10 and 15% MOLM recorded score of 8 and 10 respectively, on the Roche colour fan in a scale of 1 to 16 (plate 1), where 1=extremely pale colour and 16= intense yellow colour The diet with 0% MOLM had a score of 1. Better egg qualities and consumer acceptability were obtained from birds fed on the diet with 10% MOLM.

6.2 Conclusions

From the results of this study, the following conclusions were made:

i. Probiotics-treated MOLM with *Bacillus coagulans* significantly increased *in-vitro* digestibility compared to untreated; *Clostridium butryricum* fermentation resulted in a higher than *in-vitro* DMD% Spontaneous fermentation.

ii. The Inclusion of 0 and 10% *Bacillus coagulans*-treated MOLM in exotic chicken layers diets had similar effects on feed intake, feed conversion ratio, and hen day production. The highest feed intake was observed in hens fed on the diet with 15 and 20% MOLM diets.

iii. It was concluded that the inclusion of 10% *Bacillus coagulans*-treated MOLM in an exotic chicken layer diet improved egg quality and consumer acceptability.

6.3 Recommendations

From the study results, the following general recommendations were made:

i. Conduct *in vitro* digestibility trials to evaluate the digestibility of feedstuffs whose digestibility is unknown.

ii. Treat MOLM to be incorporated in poultry diets with *Bacillus coagulans* to improve digestibility.

iii. Incorporate 10% MOLM treated with *Bacillus coagulans* to replace soybean meal in layer chicken diets.

iv. The study recommends planting more Moringa trees.

6.4 Areas of further research

i. To evaluate the antimicrobial effects of *Bacillus coagulans* -treated MOLM.

ii. Evaluate the cost-benefit of feeding *Bacillus coagulans* -treated MOLM in layer chicken diets.

iii. Evaluate the effect of incorporation of *Bacillus coagulans*-treated MOLM in an exotic layer chicken diet on the vitamin A content of the egg.

iv. Determination of the actual level of inclusion of *Bacillus coagulans*-treated MOLM 10-20% to improve egg production and quality.

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APPENDICES

APPENDIX A: NACOSTI PERMIT

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **516101**

Date of Issue: **02/March/2023**

RESEARCH LICENSE

This is to Certify that Mr.. Abdou Karim Karim Darboe of Egerton University, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Nakuru on the topic: USE OF PROBIOTICS TREATED MORINGA (M. oleifera) LEAF MEAL IN EXOTIC LAYING HENS EFFECT ON PERFORMANCE EGG PRODUCTION AND QUALITY for the period ending : 02/March/2024.

License No: **NACOSTI/P/23/23775**

516101

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

APPENDIX B: Ethical Clearance

EGERTON **UNIVERSITY**

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EGERTON

EGERTON UNIVERSITY INSTITUTIONAL SCIENTIFIC AND ETHICS REVIEW COMMITTEE

EU/RE/DVC/009
Approval No. EUISERC/APP/210/2023 — ***9th January, 2023***

Abdou Karim Darboe
Egerton University
Department of Animal sciences
P.O. Box 536
Egerton
Email address: darboejula13@gmail.com
Telephone number: 0113436462

Dear Abdou,

RE: ETHICAL APPROVAL: USE OF MORINGA (M. OLEIFERA) LEAF MEAL WITH PROBIOTICS IN EXOTIC LAYING HENS: EFFECT ON EGG PRODUCTION AND QUALITY

This is to inform you that ***Egerton University Institutional Scientific and Ethics Review Committee*** has reviewed and approved your above research proposal. Your application approval number is *EUISERC/APP/210/2023.* The approval period is *9th January, 2023 –10th January, 2024.*

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 Institutional Scientific and Ethics Review 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 Institutional Scientific and Ethics Review 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 Institutional Scientific and Ethics Review 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.
viii. Submission of an executive summary report within 90 days upon completion of the study to ***Egerton University Institutional Scientific and Ethics Review Committee.***

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://oris.nacosti.go.ke and also obtain other clearances needed.

Yours sincerely.

EGERTON UNIVERSITY — Date. 09/01/2023 — RESEARCH ETHICS

Prof. Raphael M. Ngure
CHAIRMAN, EGERTON UNIVERSITY INSTITUTIONAL SCIENTIFIC AND ETHICS REVIEW CTTEE
RMN/BK/

APPENDIX C: ANOVA Statistical Output

Tukey's Studentized Range (HSD) Test for ***IN VITRO*** **DRY MATTER DIGESTIBILITY**

Note: This test controls the Type I experiment-wise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	6
Error Mean Square	2.043156
Critical Value of Studentized Range	4.89559
Minimum Significant Difference	4.0401

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRT
A	66.240	3	T2
B	57.243	3	T1
C	48.227	3	T3
D	42.283	3	T4

Dependent Variable: IVDMD

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	989.890233	197.978047	96.90	<.0001
Error	6	12.258933	2.043156		
Corrected Total	11	1002.149167			

R-Square	Coeff Var	Root MSE	IVDMD Mean
0.987767	2.671840	1.429390	53.49833

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	3	989.8253667	329.9417889	161.49	<.0001
REP	2	0.0648667	0.0324333	0.02	0.9843

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	3	989.8253667	329.9417889	161.49	<.0001
REP	2	0.0648667	0.0324333	0.02	0.9843

The GLM Procedure

Tukey's Studentized Range (HSD) Test for ADFI

Note: This test controls the Type I experiment wise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	612
Error Mean Square	236.4869
Critical Value of Studentized Range	3.64307
Minimum Significant Difference	4.3223

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRT
A	116.966	168	T4
B	109.472	168	T3
B			
B	108.214	168	T2
C	101.609	168	T1

The GLM Procedure

Dependent Variable: ADFI

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	59	82503.7837	1398.3692	5.91	<.0001
Error	612	144729.9858	236.4869		
Corrected Total	671	227233.7695			

R-Square	Coeff Var	Root MSE	ADFI Mean
0.363079	14.09991	15.37813	109.0655

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	3	19976.86608	6658.95536	28.16	<.0001
REPS	2	9217.88572	4608.94286	19.49	<.0001
DAYS	54	53309.03188	987.20429	4.17	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	3	19954.37117	6651.45706	28.13	<.0001
REPS	2	9221.30501	4610.65250	19.50	<.0001
DAYS	54	53309.03188	987.20429	4.17	<.0001

The GLM Procedure

Dependent Variable**: EGG WIDTH**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	12	1322.470427	110.205869	119.95	<.0001
Error	83	76.260556	0.918802		
Corrected Total	95	1398.730983			

R-Square	Coeff Var	Root MSE	EWIDTH Mean
0.945479	2.271179	0.958542	42.20458

Source	DF	Type I SS	Mean Square	F Value	Pr > F

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	3	9.226908	3.075636	3.35	0.0230
WEEKS	7	1312.527417	187.503917	204.07	<.0001
REPS	2	0.716102	0.358051	0.39	0.6785

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	3	9.226908	3.075636	3.35	0.0230
WEEKS	7	1312.527417	187.503917	204.07	<.0001
REPS	2	0.716102	0.358051	0.39	0.6785

The GLM Procedure

Dependent Variable: Yolk WIDTH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	12	1008.062673	84.005223	11.26	<.0001
Error	83	619.052690	7.458466		
Corrected Total	95	1627.115362			

R-Square	Coeff Var	Root MSE	YWIDTH Mean
0.619540	7.872783	2.731019	34.68938

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	3	134.4300542	44.8100181	6.01	0.0009
WEEKS	7	850.8935125	121.5562161	16.30	<.0001
REPS	2	22.7391062	11.3695531	1.52	0.2238

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	3	134.4300542	44.8100181	6.01	0.0009
WEEKS	7	850.8935125	121.5562161	16.30	<.0001
REPS	2	22.7391062	11.3695531	1.52	0.2238

The GLM Procedure

Dependent Variable: EGG WEIGHT

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Model	12	925.416667	77.118056	6.79	<.0001
Error	83	942.572917	11.356300		
Corrected Total	95	1867.989583			

R-Square	**Coeff Var**	**Root MSE**	**EWEIGHT Mean**
0.495408	5.450909	3.369911	61.82292

Source	**DF**	**Type I SS**	**Mean Square**	**F Value**	**Pr > F**
TRT	3	732.6145833	244.2048611	21.50	<.0001
WEEKS	7	189.4062500	27.0580357	2.38	0.0286
REPS	2	3.3958333	1.6979167	0.15	0.8614

Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr > F**
TRT	3	732.6145833	244.2048611	21.50	<.0001
WEEKS	7	189.4062500	27.0580357	2.38	0.0286
REPS	2	3.3958333	1.6979167	0.15	0.8614

APPENDIX D: Sensory Panel Recruitment Form

I………………………………………………… have voluntarily agreed to take part in this study. I understand that I will not directly benefit from this study. I have had the study explained to me, and I understand that it entails sensory evaluation of boiled eggs. My participation in this study involves tasting the boiled eggs and profiling the predetermined sensory properties according to my perception against the standards. I confirm that I am not allergic to the product and do not have any issue consuming any of the ingredients contained in the food product. I understand that the results will be kept for 3 months after the date of examination and will be treated confidentially. My identity will remain anonymous after the study, and this will be done by coding my details.

………………………. ……………………………

Signature of participant Date

…………………………

Signature of researcher

I believe that the participant is giving informed consent to participate in this study.

APPENDIX E: Score sheet of descriptive sensory for boiled eggs

SENSORY ANALYSIS#-005-4-2023

Name …………………… **Date**:……/………/…………

You are provided with 4 coded boiled egg samples (**RSA, SYR, CAM and ZRT)**. Kindly score your impression/judgement of the ATTRIBUTES listed on the left side of the table based on the scorecard provided for each sample.

Patched/inconsistent — Appearance — Smooth/consistent

Creamy — Flavour — Extremely Creamy

Not tasty/bitter — Taste — Extremely tasty/sweet

Sticky — Texture of Albumen — Extremely sticky

Sticky — Texture of yolk — Extremely sticky

Fairly shape — Egg size — Very good

APPENDIX F: Score Sheet for Consumer Acceptability of Boiled Eggs

Panellist code…………………………… Name of Panellist…………………………….

Date…………………….

Instruction:

You are provided with 4 coded samples **(RSA, SYR, CAM and ZRT)**. You must score and record each sample as per your judgement of the attributes listed on the left side of the table in the appropriate box. You can score 7 = like extremely, 6 = like moderately, 5 = like slightly, 4 = neither like nor dislike, 3 = dislike slightly, 2 = dislike moderately, and 1= = dislike extremely.

Attributes	**Sensory Scale**	**Sample Codes**			
		RSA	**SYR**	**CAM**	**ZRT**
Colour	(7= Like extremely to 1= Dislike extremely)				
Flavour	(7= Like extremely to 1= Dislike extremely)				
Texture of the yolk	(7= Like extremely to 1= Dislike extremely)				
Texture of the albumen	(7= Like extremely to 1= Dislike extremely)				
Taste	(7= Like extremely to 1= Dislike extremely)				
General Acceptability	(7= Like extremely to 1= Dislike extremely)				
Comments (if any					

APPENDIX G: Research Pictorial

a) *in-vitro* dry matter determination at the laboratory

b) Weighing of chicken

C). Measurement of egg characteristics

d). View of layers (ISA Brown) in different treatments

e) Sensory evaluation, panellist scoring different parameters of boiled eggs

F) different eggs display in plates with codes

APPENDIX H: Publications

World Journal of Food Science and Technology
2023; 7(2): 35-40
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Science Publishing Group

Effect of Probiotics-Treated *Moringa oleifera* Leaf Meal in Exotic Layer Diets on Egg Characteristics and Consumer Acceptability

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Abstract: The study was conducted to evaluate the effect of treated MOLM in exotic layer diets on egg characteristics and consumer acceptability. Thirty-six (36), sixteen (16) week old ISA Brown layers were selected from a flock of sixty (45) birds and allocated to four dietary treatments with three replicates of three birds each, confined in a deep litter system in a completely randomised design; The treatments consisted of T1 0% MOLM, T2 10% MOLM, T3 15% MOLM and T4 20% MOLM. Data was collected on external parameters (eggshell thickness, shell weight, egg width and length, shape index) and internal qualities (yolk weight, yolk width and length, yolk ratio, yolk colour and egg weight). Data of the parameters were 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 results showed significant differences ($p < 0.05$) in all the parameters evaluated on all the birds fed on different dietary treatments; treatment 4 (20% MOLM) recorded the highest yolk colour (12) in the Roche colour fan, in comparison 10%, 15% MOLM recorded (8, and 10) respectively on the scale of the Roche colour fa n, birds fed on 0% MOLM recorded low yolk colour (1) on the Roche colour fan; The results show that 10% MOLM have a better influence in shell weight, yolk weight, and yolk ratio, while the control group record better qualities in yolk width, yolk length, It could be concluded that 10% MOLM in layers diet could improve egg qualities of Isa-Brown birds.

Keywords: Eggs, Layers, Molm, Probiotics, Weight

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Determination of *in-vitro* dry matter digestibility and condensed tannins of probiotics-treated molm

Abdou Karim Darboe, Mary K Ambula and Anthony M Kingori

Abstract
This study investigated the impact of three fermentation methods (*Clostridium butyricum*, *Bacillus coagulan*, and spontaneous fermentation) on MOLM's nutritional and anti-nutritional changes. Three fermentation methods were used *Clostridium butyricum Bacillus coagulan* and spontaneous fermentation, each in three replicates. All the fermentation methods had a positive impact on the measured parameters. For the *in-vitro* digestibility to match the digestive system in the chicken stomach and intestines, a trial was conducted following the methodology outlined by Gabler *et al.* (2015). Results indicated a significant difference in *in-vitro* DMD%. Fermentation with *Bacillus coagulan* had the highest digestibility (66.24±0.76%) compared to the rest, while untreated had the lowest (42.20±0.78%). *Clostridium butyricum* fermentation resulted in a higher *in-vitro* DMD (57.24±0.74%) than Spontaneous fermentation (48.23±0.57%). There was an increase in *in-vitro* DMD% after treating the leaf meal with the *Bacillus coagulan* (66.24±0.76%) relative to the untreated (42.20±0.78). There was a significant improvement in the C.P. content of 33.41±0.57 compared to the untreated 27.48±0.25. The anti-nutritive (tannins and phenolics) compounds were reduced in the fermented substrate; *Bacillus coagulan* fermented substrates had higher nutritional value and increased *in-vitro* DMD, improved C.P. and decreased C.F. and tannins in fermented substrates. Among the three fermentations, *Bacillus coagulan* proved best in all the parameters evaluated. It was therefore used for the fermentation of MOLM for feeding trials.

Keywords: Anti-nutritive compounds, fermentation, *in-vitro*, Moringa, probiotics, proximate analysis