UNIVERSITY OF EDUCATION, WINNEBA

EFFECT OF GRADED LEVELS OF UNDESHELLED DEFATTED *Moringa oleifera* SEED CAKE (UDMOSC) ON GROWTH AND LAYING PERFORMANCE OF LOHMANN BROWN LAYERS

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2020

DECLARATION

STUDENT'S DECLARATION

I, Yakubu Anthony prosper, declare that this thesis, with the exception of quotations and references contained in published works which have all been identified and duly acknowledged, is entirely my own original work, and it has not been submitted, either in part or whole, for another degree elsewhere.

Signature:…….

Date:…….

SUPERVISOR(S)' DECLARATION

I / We hereby declare that the preparation and presentation of this work was supervised in accordance with the guidelines for supervision of thesis/dissertation/project as laid down by the University of Education, Winneba.

Professor James K. Kagya - Agyemang (Co-Supervisor)

Signature :……

Date:……

Mr. Fritz R. K. Bonsu (Principal Supervisor)

Signature :……

Date:……

DEDICATION

I dedicate this work to the Almighty God and my loving family, Elizabeth, Andy, Andrea and Kendra.

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LIST OF ABBREVIATIONS

Hb	-	Haemoglobin
HCT	-	Hematocrite
HDLC	-	High Density Lipoprotein Cholesterol
HDEP	-	Hen-day Egg Production
HHEP	-	Hen-house Egg Production
LDLC	-	Low Density Lipoprotein Cholesterol
MCH	-	Mean Corpuscular Haemoglobin
MCHC	-	Mean Corpuscular Haemoglobin Concentration
MCV	-	Mean Corpuscular Volume
RBCs		Red Blood Cells
TC	-	Total Cholesterol
TRG	-	Triglyceride
UDMOSC	-	Undeshelled Defatted *Moringa Oleifera* Seed Cake
vLDLC	-	Very Low-Density Lipoprotein Cholesterol
WBCs	-	White Blood Cells

ABSTRACT

The effect of undeshelled defatted *Moringa oleifera* seed cake (UDMOSC) on layer chicken was investigated in an experiment with 3 sequential phases for 9 months. Phases 1 and 2 determined the effect of UDMOSC on growth performance and economic efficiency in production of layer chicks (0 – 8 weeks) and growers (9 – 22 weeks). Phase 3 determined the effect of UDMOSC on productive performance, blood profile, organoleptic characteristics of eggs and economic efficiency of feed in egg production. In the starter and grower phases, a total of 150 day old chicks and 8 weeks old growers were randomly allocated to five dietary treatments, replicated three times with each replicate having 10 birds. In the layer phase, a total of 120 (22 weeks old) hens were allocated to the five dietary treatments, replicated three times with each replicate having 8 hens. A completely randomized design was used. The birds were fed starter, grower and layer diets respectively containing 0%, 5%, 10%, 15% and 15% UDMOSC with enzyme designated as $UDMOSC^{0}$, $UDMOSC^{5}$, $UDMOSC^{10}$, $UDMOSC^{15}$ and $UDMOSC^{15E}$ respectively for each of the three phases. The results showed that, in the starter phase, chicks fed the test diets had reduced feed intake but final body weight did not vary significantly (P = 0.33). However, chicks on the control diet and $UDMOSC^{5}$ had significantly higher (P = 0.02) body weight gain. Feed conversion ratio was significantly lower (P = 0.03) in chicks fed $UDMOSC^{5}$. Mortality rate was significantly higher (P = 0.01) in chicks fed $UDMOSC^{10}$. In the grower phase, feed intake was significantly higher (P = 0.01) in growers fed the control diet and $UDMOSC^{15E}$ whilst final body weight and body weight gain were significantly higher (P = 0.00 and <.00 respectively) in growers fed the control diet. However, feed conversion ratio and mortality rate did not vary significantly (P = 0.45 and 0.60 respectively) among

treatments. In the layer phase, final body weight, hen day egg production and number of eggs were significantly higher ($P < 0.05$) in hens fed the control diet. Hen-house egg production and body weight gain were significantly higher ($P < 0.05$) in hens fed the control diet and $UDMOSC^{5}$. Age of first egg, age of 50% lay and egg circumference were significantly higher ($P < 0.05$) in hens fed $UDMOSC^{15}$ and $UDOSC^{15E}$ whereas feed intake, yolk colour, WBCs, lymphocytes and monocytes were all significantly lower ($P < 0.05$) in hens fed $UDMOSC^{15}$ and $UDMOSC^{15E}$. MCV and HDL were significantly higher ($P = 0.02$) in hens fed $UDMOSC^{15}$ and $UDMOSC^{10}$ respectively. Hens fed $UDMOSC^{10}$ prduced significantly lower number of grade 2 eggs while hens fed $UDMOSC^{5}$ produced significantly higher number of grade 3 eggs ($P = 0.03$ and 0.04 respectively). Egg and albumen length were significantly lower ($P = 0.03$ and <.00 respectively) in hens fed the control diet whereas egg height, shell thickness, albumen weight and yolk height were all significantly lower ($P < 0.05$) in hens fed $UDMOSC^{10}$. Haugh unit score of eggs were all excellent whereas sensory evaluation of eggs did not show significant difference ($P > 0.05$) between eggs of the control and the test diets. Mortality rate was significantly lower ($P = 0.01$) in hens fed $UDMOSC^{15E}$.UDMOSC reduced feed cost per kilogram body weight gain of starters and growers but negatively affected egg production. This study highlights the fact that, though inclusion of UDMOSC at these levels showed a reduction in feed cost hence economically efficient in producing starters and pullets, it is undesirable for egg production and therefore, including UDMOSC in layer diets should be done with caution.

CHAPTER ONE

1.0 INTRODUCTION

1.1. Background to the Study

Poultry plays very important role for mankind allover the world through food supply in the form of meat and eggs, income and employment generation, provision of raw materials for industries and facilitating research works. However, the prospects of the poultry industry today is undermined by high cost of production due to high prices of feed ingredients especially protein and energy sources (Akinmutimi, 2004; Puran *et al*., 2014). This is as a result of lack of cereal and legume surpluses in low income and food deficit countries making it almost impossible for cereals and legumes to be included in poultry diets (Gueye and Branckaert, 2002). Feed resources for the poultry sector is also limited due to high demand of cereals and legumes from rapidly growing human population coupled with uncontrolled escalating prices (Branckaert, 2002). This calls for the use of non-conventional plants and plant resources that are available and have the potential of improving poultry performance at a reduced cost. One of such plants is *Moringa oleifera*.

Moringa oleifera is considered as one of the most useful plants because almost all parts of the plant has either been used as medicinal (Caceres *et al.,*1992) or nutritional purpose (Siddhuraju and Becker, 2003; Anhwange *et al.,* 2004). *Moringa oleifera* has also been found to be a very good indigenous source of highly digestible protein, calcium, iron, vitamin C and carotenoids (Fugile, 1999). Foid and Paul (2008) reported that the leaf of *Moringa oleifera* has significant quantities of all the essential amino acids and provides minerals whether eaten raw, cooked or dried. 50 – 70kg of pods per year may be harvested

from each moringa tree given favorable environmental and climatic conditions (McCabe *et al*., 2005). Fully matured, dried seeds are round or triangular in shape where the kernel is surrounded by light wooded shell with three papery wings. When matured, the seeds of the pods can be extracted and treated like green peas and can be fried, or roasted and eaten like peanuts. Up to 40% oil can be extracted from the seeds. Its fatty acid profile indicates about 70% oleic acid, hence moringa oil can be used as an edible vegetable or cooking oil with very little tendency to deteriorate and become rancid (Von Carlowtz *et al*., 1991).

In the past and recent times, there has been a great interest in the use of *Moringa oleifera* as a source of protein for livestock allover the world (Makker and Becker, 1997) mainly because moringa is a good source of protein and many vital minerals. Alagbemide *et al.* (2017), in evaluating the nutrient content and mineral composition of *Moringa oleifera* seed reported a crude protein content of 35.97%. This high crude protein content, suggests that *Moringa oleifera* seeds could be a suitable replacement or addition to soya bean meal or groundnut cake used as plant protein sources in poultry feeds.

1.2. Problem Statement

Protein ingredients are very important for growth, production and profitability of the poultry industry. The conventional ingredients used as sources of protein for poultry are groundnut cake, soybean cake and fish meal. These sources are however not readily available to farmers as a result of high demand and high cost from increased population growth. According to Abdullah *et al.* (2011), human population growth, urbanization and income improvement will cause an increase in demand for meat in developing countries. This will inturn cause an increase in demand for feed ingrediets.

The contribution of protein and energy source ingredients is more than 90% of all required nutrients for poultry rations. However, most of these ingredients for poultry feed are also used for human nutrition in Africa which promotes competition (Mengasha, 2011; John and Njenga, 1992). Gura (2008) also reported that the competition between feed and food is likely to aggravate prices of poultry feed and this will compel producers to look for alternative and locally available feed sources such as *Moringa oleifera* seed cake.

Although, moringa seeds have high crude protein, there is not enough information on how it can be used as an alternative ingredient for layer chicken feed. Moreover, lot of work has been done regarding the use of *Moringa oleifera* leaves as animal feed with very little on the use of *Moringa oleifera* seed hence the need for a research into the use *of Moringa oleifera* seed cake as a protein source ingredient for poultry. It is against this background that this study has been designed to investigate the use of *Moringa oleifera* seed cake as a protein source ingredient in layer chicken feed.

1.3. Objectives of the Study

The main objective of the study was to determine the effect of undeshelled defatted *Moringa oleifera* seed cake (UDMOSC) on growth and laying performance of Lohmann Brown layers.

Specific objectives

The specific objectives were to determine the effect of undeshelled deffated *Moringa oleifera* seed cake on Lohmann Brown layers in terms of;

1. growth performance.
2. egg production and egg characteristics.
3. Organoleptic egg characteristics.
4. haematological and biochemical characteristics.
5. economic efficiency of production.

1.4. Justification

Large quantities of moringa seeds can be obtained from easily established plots in the field without expensive inputs. Moringa is also a perennial plant that can be harvested several times in a year. The rapid growth of moringa trees in subtropical and tropical areas even under conditions of prolonged drought makes this plant a reliable resource to enhance the nutritional status of local population. This can also be a reliable source of animal feed that is not in direct competition with human food if rationalized cultivation practices are exploited.

Oil is the main component of moringa seed and represents 36.7% of the seed weight. Apart from the oil, the seed has high protein content with an average of 31.4%, whereas carbohydrates, fibre and ash contents are 18.4%, 7.3% and 6.2% respectively (Abbas, 2013). Moringa seed cake is free from most plant secondary metabolites such as tannins, saponins, alkaloids, inhibitors of trypsin and amylase, lectin and cynogenicglucosides (Makkar and Becker, 1997). Thus, the defatted seeds of moringa can be an economic source of protein if used as a feed ingredient (Alessandro *et al*., 2016).

Despite the naissance of *Moringa oleifera* as a feed resource, it is important to take cognizance of the fact that there is a dearth of information on the use of *Moringa oleifera* seed meal (MOSM), in contrast to *Moringa oleifera* leaf meal (MOLM) where extensive research has been done (Juniar *et al*., 2008; Olugbemi *et al*., 2010; Etalem *et al*., 2012; Kana *et al*., 2015; Hassan *et al*., 2016). Other research works such as the influence of *Moringa oleifera* leaf meal (MOLM) on the performance and blood chemistry of broiler chicken has been documented by researchers such as Onu and Aniebo (2011), Rafiu *et al.* (2013) and Voemesse *et al.* (2018). This therefore suggests that there is knowledge gab on the use of *Moringa oleifera* seed meal that needs to be filled. Hither to, knowledge and use of *Moringa oleifera* seed meal will go a long way to reduce cost of feed for farmers in commercial layer production.

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1. Chicken Nutrition

2.1.1. Nutrient requirement of chicken

Feeding poultry for optimum growth requires that the birds consume appropriate balance diets. According to Agriculture UK (2013), it is important that birds are fed diets that meet their nutritional requirement for good health and productivity. Beutler (2007) also reported that if poultry birds are expected to remain healthy and productive, adequate amount of all the necessary nutrients must be consumed.

Nutrient recommendations of birds are different for each species and the purpose of producing the birds (meat or eggs) as well as the stage of growth and production of the birds (Beutler, 2007). As reported by National Research Council (NRC) (1994), the quantity of each required nutrient varies depending on many variables like specie of bird, age, productive state, environmental conditions and disease status.

Poultry feed may be referred to as “complete feed” or “incomplete feed” (NRC, 1994). A complete feed contains all the protein, energy, vitamins, minerals and other nutrients necessary for proper growth, egg production and good health. Complete feed that meets all the nutritional requirements (energy, protein and amino acids, fats, vitamins, macro and micro-minerals etc.) of a particular class of birds can be purchased. However, in case of

mixing feed, these nutrient requirements must be taken into consideration (Agriculture UK, 2013).

Cross feeding, such as feeding laying hens with diet that is intended for broilers is not advisable. Therefore, raising mixed flocks should be done with caution (Beutler, 2007). According to Agriculture UK (2013), it is important to feed the right feed to a particular flock. This is because the nutrient requirements differ depending on the species, level of production and age of the bird.

No single ingredient will meet the nutrient requirement of a bird so a mixture is required. The concentration of nutrients in the diet determines the level of energy in the diet. Diets that are higher in energy will need higher concentration of different nutrients (referred to as nutrient dense diets) because the birds will eat less of a high energy diet but need to be able to obtain the total amount of each nutrient required daily. Diets that are low in energy can have lower concentrations of different nutrients because the birds can eat more of such diets and thus get the total amount of the nutrients needed each day (NRC, 1994).

2.1.2. Nutrient requirement of laying hens

The mature body weight of a laying hen can greatly influence the subsequent reproductive performance of that hen and therefore must be considered in the assessment of the nutritional requirement (Leesson and Summers, 1978a). In this direction, the diet of an immature laying hen should provide the nutrients needed for rapid growth rate, feather development and enable the hen to attain the required body weight

(Leesson and Summers, 1978a). Layer birds require relatively higher levels of energy, proteins, minerals and vitamins. However, the energy levels can be reduced once the chicks are fully feathered.

At the pullet stage, the diet should be such that it maintains rapid growth rate that will lead the pullets to reaching sexual maturity at the desired age while avoiding obesity. On a percentage basis, pullet's diet should have low energy and protein levels than chicks' diet (NRC, 1994).

The level of production of a layer hen determines it nutrient requirement. Four different diets can be fed at four different levels of production namely, peak production, high peak production, post peak production and near the end of laying period. This is done because the nutrient requirement of the hen changes with age and to ensure maximum diet efficiency (Jacquie and Pescatore, 2014). Hens in full production needs at least 14% protein, however layer diets that contain 16% protein are more common. Table 2.1 shows the nutrients required by laying hens.

Table 2.1: Nutrient specification for laying hens

Nutrient	minimum Daily intake per bird			
	Peaking (50% prod. – 32 weeks)	32 – 44 weeks	44 – 58 weeks	58 weeks +
Protein, g/bird	16.0 – 17.0	15.5 – 16.0	15.0 – 15.5	14.5 – 15.0
Methionine, mg/bird	412	400	375	350
Methionine and cystine, mg/bird	680	660	620	580
Lysine, mg/bird	800	780	740	720
Tryptophan, mg/bird	175	170	165	160
Calcium, g/bird	3.40	3.55	3.65	3.85
Phosphorus (total), g/bird	0.65	0,60	0.55	0.45
Phosphorus (available), g/bird	0,45	0.42	0.38	0.35
Sodium, mg/bird	180	180	180	180
Chloride, mg/bird	160	160	160	160
Linoleic, g/bird	1.5	1.0	1.0	1.0

Source: Richard and Jacqueline (2000)

2.2. Feed Intake of Laying Hens

Pullets need to be given appropriate diet throughout the rearing period to enable the pullets meet the recommended adult pullet target weight by 14-16 weeks of age and have the correct body composition to sustain egg production beyond 90 weeks. Any deviation from the target pullet weight will influence the mean age weight during the early laying period and total egg output for the entire period of egg production (Bouvarel *et al*., 2011). Attention must therefore be paid to the energy/protein ratio between 11 to 16 weeks, as any increase in the energy content of the diet will increase the fattening score (Cheng *et al*., 1991).

The feed particle size must be suitable for the beak size to promote feed intake and therefore increase weight gain during the rearing phase (Frinkha *et al*., 2011). However, too many changes or rapid changes of diets in the rearing phase should be avoided.

The energy and protein content of the pullet's feed needs to be adjusted to ensure that the pullet consumes sufficient feed to cope with growth and the onset of egg production at 16 weeks of age. The use of whole cereals and coarse-insoluble fibre is one way by which feed intake of pullets can be promoted (Hetland *et al*., 2005). It is also important that feed is formulated to be appetizing and available as medullar bone reserves are being formed as well as the ovary and oviduct developing at this stage (Bain *et al*., 2016).

The energy and protein requirement needs to be adjusted to optimize egg output and carefully control body weight during the laying period. At the onset of egg production, growth requirement is only needed for the first few weeks (NRC, 1994). The energy requirement for maintenance thereafter depends on body weight and feather coverage and as a result increases with age (NRC, 1994). There is always a negative correlation between feed intake and dietary energy concentration (Bouvarel *et al*., 2011). High energy diets can be used during the first part of the laying period to satisfy the continued requirement for growth and to promote heavier and early egg weight without the risk of overfeeding and producing "fat hens" because this adaptation is only partial (Perez-Bonilla *et al.*, 2012). The hen's energy requirement therefore decreases as egg production becomes established.

Fat deposition can be minimized at this stage by lowering energy levels as the birds will be able to partially compensate by increasing their feed intake. Also, laying hens are able to adjust their feed intake according to the relative size of the feed particles in relation to the

beak size (Joly, 2004; Safaa *et al.,* 2009). Balancing of the energy intake can be made possible by varying feed particle size.

The crude protein concentrations and for that matter, amino acids in the layer diet is also important with methionine being the first limiting amino acid. An average increase in egg weight of 1.4g can be achieved by an extra consumption of 1g of protein per day (Bouvrel *et al*., 2011). However, the dietary energy concentrations and the form of the ration will determine the amount of protein consumed (NRC, 1994). Ideally, the protein and amino acid concentration in the diet needs to be estimated relative to the egg weight (mg/g of egg for amino acids) and adjusted to optimize egg production throughout the laying cycle.

"Gap" feeding where there is a 3 to 4h gap between feeding allowing the birds to consume fine particles, is a useful management tool that can be used to improve feed intake efficiency and flock body weight uniformity during the laying period (Hyline International, 2015).

2.2.1. Feed intake and body weight of laying hens

A chicken's daily consumption of feed depends on the composition of the diet. Birds will typically adjust their feed intake to meet their energy requirements (NRC, 1994). As the energy content of a diet increases feed intake decreases, and vice versa (NRC, 1994). Environmental temperature plays a significant role in determining how much feed a bird will consume. During hot weather, feed intake decreases and increases in cold weather as

the birds will consume more to supply the extra energy needed to regulate their body temperature (NRC, 1994).

NRC (1994) asserted that *ad libitum* feeding is important for the leghorn laying birds reared in hot climates due to their inherently low appetite. The council further recommended *ad libitum* feeding for both the brown and the white leghorn laying hens in hot climates.

Voluntary feed intake of chicken is influenced by a lot of factors such as ambient temperature, lighting and noise, physical form of feed, feed flavour, anti-nutritional factors, feed wastage and water supply (NRC, 1994). It also includes body factors such as taste buds, genes, hormones and feather cover. Other factors such as breed or strain, age, nutrient balance of the diet, health and welfare status of the birds, accessibility of feed, flock density, feed ingredients or feed contamination can have adverse effect on voluntary feed intake (McDonald, 1987).

According to Lacin *et al.* (2008), differences in body weight of laying hens resulted in a significant difference in feed intake and feed conversion ratio. Connie *et al.* (1985) also observed that heavy birds consumed more feed per hen per day and per dozen of eggs than medium and light birds. Depending on hen size and breed, a hen at lay will consume between 4 and 8 ounces (0.14g and 0.28g) of feed daily. In that regard, laying hens be supplied with the required quantity of feed to enable the hens meet their nutrient requirement for a good lay. Table 2.2 shows feed consumption and body weight of both brown and white laying birds.

Table 2.2: Body Weight and Feed Consumption of Immature Leghorn-Type Chickens

Age (weeks)	White-Egg-Laying Strains		Brown-Egg-Laying Strains	
	Body Weight (g)	Feed Consumption (g/week)	Body Weight (g)	Feed Consumption (g/week)
0	35	50	37	70
2	100	140	120	160
4	260	260	325	280
6	450	340	500	350
8	660	360	750	380
10	750	380	900	400
12	980	400	1,100	420
14	1,100	420	1,240	450
16	1,220	430	1,380	470
18	1,375	450	1,500	500
20	1,475	500	1,600	550

Source: **NRC (1994)**

2.3. Effect of Feed on the Onset of Puberty of Chicken

Prior to and after the onset of egg laying, growth hormones (GH) participate in the growth, maturation and hormonal activity of ovarian follicles in the hen, through the regulation of steroidogenesis, proliferation and apoptosis processes (Hrabia *et al.*, 2011). A study was conducted by Bruggeman *et al.* (1998), to examine the effect of feed intake from 2 to 24 weeks of age on chicken Luteinizing Hormone-Releasing Hormone (cLHRH-I) content in the median eminence (ME) and gonadotrophin levels in pituitary and plasma of female broiler breeder hens. In the experiment, one group of the birds received *ad libitum* feeding; a second group received restricted quantity of feed; and a third group fed a restricted quantity of feed but was intended to obtain an intermediate body weight between groups one and two. The Restricted group had significantly lower levels of cLHRH-I in the median eminence (ME) as compared to the *ad libitum* group suggesting that feed has influence on the levels of cLHRH-I. In all groups, there was a major increase in cLHRH-I

in the median eminence about 3 weeks before onset of lay. The age of first oviposition was delayed by 2 and 6 weeks in the Intermediate and Restricted groups respectively, as compared to the *ad libitum* group, indicating that feeding regimes are directly related to the appearance of first egg. The researchers further indicated that, the attainment of sexual maturity may also be associated with a threshold value of cLHRH-I stored in the ME in the *ad libitum* and intermediate groups of birds. The pituitary LH and FSH contents after week 16 were positively related to the amount of cLHRH-I in the ME. Also, plasma FSH concentration in the *ad libitum* and intermediate groups peaked about 3 weeks before the first oviposition and this prepubertal peak was associated with increased pituitary FSH and cLHRH-I amount in the ME. In this regard, a conclusion was drawn that the delayed sexual maturation was caused by a chronic feed restriction, which may be associated with delayed ovarian and oviductal growth, decreased cLHRH-I stored in ME and lowered levels of LH and FSH in the pituitary.

A similar report by Onagbesan *et al.* (2006), suggested that differences in laying performance among different genotypes of laying birds, fed different nutritional levels may be partly due to difference in processes associated with follicular maturation moderated by gonadotropins and growth factors. Based on this findings, it was concluded that the age at puberty is determined mainly by feed allowance, irrespective of genotype, and that differences in laying performance may be due to a combination of factors that include changes in the levels of gonadotropins or ovarian hormones and growth factors, body weight, and the condition of different genotypes under different feeding allowances.

According to Renema *et al.* (1999), following photostimulation (PS), plasma LH and FSH concentration of birds on *ad libitum* had increased levels of nearly double that of birds on restricted feeding, indicating the role of nutrient intake with rate of reproductive development. An observation was also made that, plasma LH and FSH concentration remained elevated for a greater time period in birds on restricted feeding, possibly relating to the development of processes limiting large yellow follicles (LYF) recruitment. These findings demonstrate a modulation of reproductive hormone concentrations during sexual maturation by feeding level in conjunction with a sensitivity of the ovary to nutritional effects.

In a similar study by Soller *et al.* (1984) to evaluate the effect of diet and early quantitative feed restriction on the minimum weight requirement for onset of sexual maturity in white rock broiler breeders, concluded that fat content alone is not sufficient to initiate sexual maturity, but there may be a body mass requirement for the onset of sexual maturity that is not affected by diet. Contrary to this, Melnychuk *et al.* (2004) discovered that feed restriction affects sexual development by reducing the number of large yellow follicles (LYF) but can be modulated by photostimulation (PS).

The findings from interrelationship between feeding level and the metabolic hormones leptin, ghrelin and obstatin in control of chicken egg laying and release of ovarian hormones by Sirotkin and Grossmann (2015), confirmed an inhibitory effect of food restriction on chicken ovulation rate. This shows that food restriction- induced- reduction in egg laying is associated with a decrease in ovarian testosterone, estradiol (E) and

arginine-vasotocin (AVT) (Sirotkin and Grossmann, 2015). However, an increase in ovarian progesterone (P) release; confirms the involvement of metabolic hormone leptin, ghrelin and obestatin in the control of chicken ovarian hormones output (Sirotkin and Grossmann, 2015). The ability of metabolic hormones to mimic/antagonize or prevent/promote the effects of food restriction on both egg laying and ovarian hormones demonstrate that nutritional status can influence ovulation and endocrine functions via changes in metabolic hormones (Sirotkin and Grossmann, 2015).

Arginine (ARG) is known for its role to enhance the ovulation process of hens through increasing release of luteinizing hormone (LH) which is necessary for ovulation in chicken. Basiouni (2009) observed that addition of an extra amount of arginine into the diet of local Saudi hens increased mean LH levels before the time of oviposition. Similarly, mean LH surge amplitude levels were found to be higher in arginine treated groups. The onset of the preovulatory LH surge (minutes before the time of oviposition) was also found to occur earlier in arginine treated groups. However, the duration of the preovulatory LH surge was not found to be significantly different between the arginine treated and the control group.

On the contrary, a study to investigate the effect of increasing level(s) of arginine (ARG) and/or lysine (LYS) over the normal requirement level recommended by NRC (1994) for leghorn breeds and their interaction on performance, ovarian activity and humoral immune response of local Saudi chicken by Basiouni *et al.* (2006), revealed that feed intake and specific gravity of the eggs were the only traits significantly affected by the interaction between ARG and LYS. Hen-day egg production did not significantly increase; however,

feed conversion improved with the higher levels of ARG and LYS. Albumen height was better in the control group in both treatments which supported the higher egg size of the control group. No significant differences were found among treatment or their interaction in all ovarian and oviductal measurements. However there was a tendency for an increase in the weight of F1 follicles (largest follicles) as a result of ARG supplementation at a 2.04% level. The researchers concluded that extra ARG, LYS and their interaction did not significantly affect performance and ovarian activity of the local birds.

2.4. Protein Requirement of Laying Hens

Protein makes up 15-20% of the body weight of a bird and thus, next to water; the body's second most abundant substance. It is still a fact that protein, and only protein, is the raw material from which muscles and many other bodily tissues are built. Proteins are made up of amino acids, comprising both essential and non essential amino acids. Non essential amino acid can be produced by the liver. However, essential amino acids need to be made available through the diet because birds are unable to synthesize these amino acids at rates sufficient to meet their needs. The amino acid requirement of a bird represents the requirement for the indispensable or essential amino acids plus sufficient nitrogen in an appropriate chemical form for synthesis of the dispensable or nonessential amino acids.

Amino acid requirement of a bird may be classified as those for maintenance, growth, egg production and feather growth on the basis of their respective amino acid profiles (Hurwitz *et al.,* 1978). A protein consumed by a laying hen is overwhelmingly oriented to

yolk and albumen formation. Yolk proteins are almost exclusively synthesized in the liver, and then transported to the developing follicles (Edwin and Moran, 1987).

Proteins exist in the diets as crude proteins but birds do not have a requirement for crude protein per se. However, there should be sufficient crude protein to supply essential amino acids and to ensure an adequate nitrogen supply for synthesis of nonessential amino acids. Suggested requirements for crude protein are typical of those derived with corn-soybean meal diets, and levels can be reduced somewhat when synthetic amino acids are used (Sergio *et al*., 1994; Baker, 2009).

Layers on self selecting diets seem to voluntarily consume much less crude protein in their early life and more crude protein as maturity approaches (Summers and Leeson, 1978). Low- crude protein or low- lysine starter diets invariably depress the growth rate of both white and brown laying chicks (Douglass and Harms, 1982; Kwakkel *et al*., 1991; Maurice *et al*., 1982) because of decreased muscle synthesis. Early growth depression however affects mature body weight and thereby reduce adult performance (Milby and Sherwood, 1953; Leeson and Summers, 1987a, 1989).

According to Timson *et al.* (1983), low crude protein diets have a transitory effect on muscle fibre size rather than muscle fibre numbers. Reduction in growth of white leghorn pullets is often seen when total protein intake is less than 1kg by 140 days (Keshavarz, 1984; Leeson and Summers, 1984). An intake of 1kg of balanced protein during the same period is reported to result in maximum growth (NRC, 1994).

2.4.1. Effect of crude protein (CP) on body weight of chicken

According to Adeyemo *et al.* (2012), the best live body weight was scored by layers fed the highest CP level of 17%. Besides, a study by Keshavarz (1984) indicated a lower body weight at 20 weeks of age and decreased productive performance through the first stage of the egg production cycle when birds were fed low-protein diet throughout the rearing phase. Many researchers have also observed similar trends in low-protein diets (Alagawany *et al*., 2011; Alagawany, 2012). However, Calderon and Jensen (1990) found that body weight gain increased along with increased CP levels from 13 to 16 or 19% throughout the production period. Yakout (2010) found that the best value of body weight gain was recorded by layers fed diet containing higher percenatge CP levels. The body weight of Hy-Line layers was significantly influenced by dietary protein concentrations, since the highest level of CP (14%) resulted in the highest value of body weight as compared to the lowest CP level (13%) during egg production cycle (Bouyeh and Gevorgian, 2011).

On the contrary, Hassan *et al*. (2000) and Yakout (2000) reported that there were no significant ($P > 0.05$) differences between the overall means of body weight with different percentage CP levels in the diets of laying hens**,** while final body weight slightly increased ($P < 0.05$) with low percentage CP (16%) diet as compared to other levels. Meluzzi *et al*. (2001) reported that live body weight at 40 weeks-old of Hy-Line Brown hens was not affected by different CP levels (170 control, 150 and 130 g kg^{-1} diet). Final body weight and body weight gain of commercial laying hens (Babcock B-308) were also not significantly ($P > 0.05$) affected by dietary percentage CP levels (14, 16, 18 and 20%)

(Bunchasak *et al*., 2005; Junqueira *et al*., 2006). On the other hand, Grobas *et al*. (1999), Keshavarz and Nakajima (1995) and Sohail *et al*. (2003) reported that there was no significant impact of reducing dietary CP levels on body weight, and that this effect might be attributed to the balance and availability of amino acids provided in the tested diets.

2.4.2. Effect of (CP) on feed utilization by laying hens

According to Adeyemo *et al.* (2012), layers fed the highest percentage CP level (17%) recorded the best feed conversion ratio compared to the lowest percentage CP levels. However, feed intake and feed conversion ratio were not significantly affected by different CP (170, 150, 140 and 130 g kg^{-1}) levels of commercial leghorn hens through the first stage of production cycle (Hu *et al*., 1998; Meluzzi *et al*., 2001). Also, during the first stage of egg production cycle, Moustafa *et al*. (2005) found that feed intake was insignificantly affected by dietary CP levels in diets of laying hens**.** Moreover, Bunchasak *et al.* (2005) studied the effect of dietary CP levels (14, 16 and 18%) on the performance of laying hens and reported that feed intake was not significantly affected by these levels. But, the ratio of feed intake to egg mass (feed conversion ratio) and protein conversion ratio were significantly ($P < 0.05$) influenced by the different levels of CP, since the best values of these traits were achieved with 18% as compared to the other levels (14 or 16%).

Junqueira *et al*. (2006) observed that increases in dietary CP levels up to 20%, significantly improved feed intake through the second phase of production, while feed conversion was not affected by the increase in dietary CP level. Abd El-Maksoud *et al*. (2011) reported an increase in feed intake of laying hens when fed with low CP (12 and 16%) between 32 and

44 weeks. But, the best feed conversion (2.62g feeding/g egg) was at 16% CP diet as compared to low percentage CP diet. Also, Bouyeh and Gevorgian (2011), reported that feed consumption and feed conversion ratios in HY-line layers was significantly affected by dietary CP levels (13 and 14%), with the CP of 14% recording the highest value of feed consumption (111.95g day $^{-1}$). Moreover, protein utilization was affected by the higher levels of CP (14.6%) throughout the experiment.

Feed conversion ratio was improved from 1.680 to 1.645g feed/egg mass with decreasing dietary CP, when laying hens (Hy-line W36) were fed different CP diets (15.5, 17.8, 18.5, and 19.9 %) through the first stage of production (Novak *et al*., 2006). On the other hand, reducing dietary CP lowered feed conversion ratio in laying hens during 18 to 60 weeks of age (Novak *et al*., 2008). Additionaly, Hassan *et al.* (2000), Moustafa *et al*. (2005) and Yakout *et al*. (2004) reported that feed conversion ratio was improved when CP level of layer diet increased. The impact of feeding various levels of CP (12, 14 and 16) on productive performance of Baheij laying hens from (28 to 48 weeks of age) was reported by (Zeweil *et al.*, 2011). The authers showed that feed intake and feed conversion ratios were influenced by dietary CP levels. Murakami (1991) reported improved feed intake to egg mass ratio in hens fed diets containing higher percentage CP (19-20%) whereas Pinto (1998) found an improvement in feed conversion ratio in laying hens fed diet up to 22% CP. According to Khajali *et al*. (2008) feed conversion ratio was maintained well on low-CP diet through the first 8 months of production but tended to be impaired thereafter. In months 10 and 11 of the laying period, feed conversion ratio was significantly ($P < 0.05$)

influenced. On the other hand, Van Emous *et al*. (2015b) suggested that birds fed low-CP diet had a higher feed intake (12.8%) as compared to birds fed higher CP level.

2.4.3. Effect of (CP) on laying performance

According to Saxena *et al.* (1986), 15% CP is the optimum dietary protein level for layers in winter season. Egg number and egg weight were highly improved by increasing dietary protein level from 13 to 19% in the diet of white leghorn (Calderon and Jensen, 1990). Harms and Russell (1996) discovered that birds that consumed 13.8g of CP per day had reduced egg weight as compared to birds that consumed 14.6 or 16.3g of CP through 44 to 63 weeks old. To that end, egg production percentage and egg output (g/hen/day) were significantly ($P < 0.05$) influenced by CP levels (130, 150 and 170g/ kg $^{-1}$ CP) in layers (Hy-line brown hens). Meluzzi *et al*. (2001) reported that high CP level (170g/kg $^{-1}$) achieved the best values of egg production and egg mass as compared to low levels. Bunchasak *et al*. (2005) in a similar work stated that birds receiving 14% CP had poor egg production, egg weight and egg mass than those that received 16% CP through peak period.

A study by Abd El-Maksoud *et al*. (2011) also reported the significant effect of different CP levels on layer performance when egg production and egg mass increased with increasing CP levels from 12 to 16%. On the contrary, Bouyeh and gevorgian (2011) observed that hen-day egg production was significantly ($P < 0.05$) affected by the different CP levels, when a lower percentage CP level (14%) achieved the highest value of egg production and egg mass of Hy-line laying hens after peak production period. Moreover,

Hassan *et al*. (2000); Yakout *et al*. (2004); Novak *et al.* (2006), stated that egg production and egg weight were improved by increasing dietary CP levels.

Hu *et al*. (1998) examined the effect of low-level protein (14%) and normal protein (17%) in a 5-week experiment and reported a similar response of low egg production in the low CP diet (14%). Additionally, Junqueira *et al*. (2006) stated that layer hen performance in terms of egg numbers, egg weight and egg mass were comparable between diets containing 16 and 20% CP. Zeweil *et al*. (2011) also noted that egg production and egg mass of Bhaheij laying hens were not affected by dietary CP levels (12, 14 and 16%). However, the percentage of egg production, egg weight and egg mass maintained well with low CP diet through the first eight months of production but tended to be impaired thereafter. This suggests that the productive performance of a hen can be maintained satisfactorily on a low CP diet for only a short period of time but not on a long-term period especially during the late stages of production (Khajali *et al*., 2008; Alagawany *et al*., 2014b). Saki *et al*. (2015) also indicated egg production increased by diet that included 15% of CP level.

2.4.4. Effect (CP) on egg quality characteristics

According to Zimmermann and Andrews (1987), 14.6 and 15.5% CP in layer diets did not show any positive or negative effect on egg shell thickness and Haugh unit score during the second production phase. De Mendonca and Lima (1999), did not find any impact of dietary CP levels on egg albumen but during the second phase of production egg shell quality was improved when layers were fed 14.5% compared to those fed 16.5% CP diet.

These findings confirms the findings of Novak *et al*. (2006) that the percentages of dry and wet albumen, albumen solids as well as percentages of yolk and albumen protein were decreased with feeding low CP diets. According to Meluzzi *et al.* (2001), the treatment of CP 150g/kg $^{-1}$ diet produced the highest egg size as compared to 170 and 130g/kg $^{-1}$. This contradicts with the findings of Summer *et al.* (1995) and Lopez and Leeson (1995) who reported that the egg weight is strongly related to the CP content of the diet. However, Summer and Leeson (1983) discovered that early egg size was not affected by increases in CP content. Thus, the increases in CP did not improve Haugh unit score, egg shell weight and egg shell thickness (Junqueira *et al*, 2006; Alagawany *et al*., 2012). Lowering CP in laying hens' diets reduced egg weight through out the experimental period (18 to 60 weeks of age) (Novak *et al*., 2008) while the internal and external egg quality characteristics were not influenced by low CP diets. The ideal value for yolk weight was achieved with 14% CP (Abd El-Maksoud *et al*., 2011).

The percentages of dry and wet shell weight and dry yolk weight as well as shell thickness were significantly ($P < 0.05$) increased by decreasing CP levels in layer diets (Abd El-Maksoud *et al*., 2011). On the contrary, the percentage of wet and dry albumen, wet yolk weight as well as egg yolk index and egg shape were not affected by dietary CP (12, 14 and 16%) levels (Zeweil *et al*., 2011). However, yolk colour index was higher in eggs of layers which were fed diets containing 12 and 10.5% CP as compared with those fed the control diets (Torki *et al*., 2015).

2.4.5. Effect of (CP) on blood and immunity of laying hens

Blood parameters are related to the health conditions of a bird and hence are used as vital indices to determine the physiological and nutritional status of the bird. According to Glick *et al.* (1983), diets that have CP levels below 33% of the recommended levels could reduce the count of lymphocytes in the chicken thymus. However, this response varies with some factors such as strain, environment, stress, health status and production state of the bird (Cheema *et al*., 2003; Humphrey and Klasing, 2004).

Hu *et al.* (1998) suggested that the levels of plasma uric acid and excreta nitrogen of the high level protein (170g/kg $^{-1}$) diet increased significantly compared to the other low protein diets for commercial laying hens. All serum fractions of protein and total protein as well as triglycerides, serum non-esterified fatty acid and Newcastle Diseases (ND) titre of hens tended to decrease as CP levels increased from 14 to 16 and to 18% (Bunchasak *et al*., 2005). Additionally, uric acid in plasma and excreta increased with increasing CP level (Donshough *et al*., 2010). In this context, plasma total protein and globulin increased by increasing the CP levels from 12 to 14 and 14 to 16 weeks in layer hens.

On the contrary, albumin in plasma was significantly affected by the different CP levels (Zeweil *et al*., 2011). Also, there were no significant ($P > 0.05$) effect of the levels of CP diet on plasma uric acid, creatine and globulin but plasma total protein, albumin and urea of Lohmann layers fed 20 and 18% CP levels were significantly ($P < 0.05$) increased compared to those fed 16% from 30 to 34 weeks (Alagawany *et al*., 2011). Groups that were fed 13.9% CP had their uric acid in plasma decreased significantly ($P < 0.05$); while

the plasma triglyceride concentration increased significantly ($P < 0.05$) as compared to groups fed 15% CP in Lohmann laying hens (Ghasemi *et al*., 2014).

Blood ammonia and uric acid concentrations were not significantly affected by dietary treatment varying in protein content (16, 16.5, 17, 17.5 and 18%) (Ji *et al*., 2014). Blood triglyceride concentration was significantly ($P < 0.05$) higher in layers receiving 12 and 10.5% CP diets compared to control group (16.5% CP). The ratio of heterophil to lymphocyte was significantly ($P < 0.05$) declined by diets containing 15, 13.5 and 12% CP (Torki *et al*., 2015).

Blood constituents including total protein, glucose, high density lipoprotein and tridothyronine, were increased by layer diets containing 15% of CP levels. But, blood total cholesterol, thyroxine and low-density lipoprotein were not significantly ($P > 0.05$) influenced by different concentrations of CP (Saki *et al*., 2015).

2.5. Egg and Egg Characteristics

2.5.1. Egg formation

The simultaneous development of a series of follicles in the left ovary makes the almost daily egg production possible. This follows a defined hierarchy with only one follicle reaching maturation within 24 hours period (Bain *et al*., 2016). There are over 12000 oocytes in the ovary of a layer chicken at hatch but only a small percentage of these will reach maturity (Bain *et al*., 2016). During ovulation, the yolk mass from the largest follicle is deposited at the funnel shaped open end of the proximal oviduct, the infundibulum. The

yolk mass then travels down the oviduct and undergoes successive deposition of the different components of the egg (Gilbert, 1979; Sauveur and De Reviers, 1988; Romanoff and Romanoff, 1949).

The various components of the egg such as the albumen, membranes and egg shell are secreted by different parts of the oviduct according to a predetermined sequence of events (Bain *et al*., 2016). The albumen is formed in the longest and most glandular region of the oviduct (magnum) during the first 4 hours. As the forming egg mass passes through the isthmus, the shell membranes are deposited. After five hours of ovulation, the egg enters the shell gland, where it spends the next 19 hours (Bain *et al*., 2016). The egg shell then forms during this time. The formation of egg shell occurs in three different phases (Nys *et al*., 2004) and is regulated by the precise temporal and spatial secretions of a complex array of organic matrix proteins (Gautron *et al*.,1979; Mann *et al*., 2006). Some of the matrix proteins subsequently become incorporated into the calcified structures thereby modifying its biochemical properties (Hincke *et al*., 2012) and/or participate in its antimicrobial defenses (Rehault-Godbert *et al*., 2011).

The mammilla and palisade layer of the shell then forms from the resulting interwoven fabric of organic and inorganic constituents. The shell pigment and finally the cuticle (a non-calcified organic layer of variable thickness) are deposited in the 1 and half hours prior to oviposition. At this time, the egg is ready for oviposition. Neurohypophyseal hormones and prostaglandins secreted by the uterus and lesser extent the shell gland regulate the timing and process of oviposition (Nys and Guyot, 2011). Hens will normally

ovulate and oviposite 1 to 7 hours after dawn when on a standard 14 L: 10 D lighting programme; therefore, hens lay their eggs in the first hours of light period. The next ovulation takes place after expulsion of the egg but can also occur just prior to this in some cases (Nys and Guyot, 2011).

2.5.2. Hen-day and hen-housed egg production

The estimated total number of eggs produced per average number of hens present in a pen on daily basis is hen-day egg production (North, 1984). The hen-day egg production can be used to estimate the egg laying rate of hens. It is also a good measure of productivity (Gietema, 2006).

Hen-housed egg production on the other hand, is an estimate of the total number of eggs produced by the hens. It is determined by dividing the total number of eggs produced by the total number of hens housed at the start of the laying period (North, 1984). The laying cycle of chicken usually covers a period of 12 months. Egg production normally begins when the birds are about 18 – 22 weeks of age depending on the season or breed (Jacob *et al*., 1998).

Egg production rises sharply and reaches a peak of about 90%, 6 – 8 weeks later and gradually declines to about 65% after 12 months of lay (Jacob *et al*., 1998). Egg production is a function of feed consumed, age at point of lay, peak percentage lay, percentage hen–day egg production, laying period, rearing environment, health care and overall

management of the birds. Thus, any variation in these factors will result in a wide variability (Ali *et al*., 2003).

2.5.3. Egg quality characteristics

According to Ahmadi and Rahimi (2011), egg shell and internal egg quality characteristics are critical to the economics of the egg industry. Egg quality in general is determined by both external and internal egg characteristics. Egg quality is the characteristics of an egg that affects its acceptability by the consumer (Stadelman, 1977). Examination of egg quality characteristics is important mainly in terms of production economy. Thus it is important to evaluate the egg quality characteristics and factors that affect egg quality (Zita *et al*., 2009).

2.5.4. External egg quality characteristics

External egg quality characteristics include egg weight (g), egg length (mm), egg width (mm), shell thickness (mm) and shell weight (g) (Hussain *et al*., 2013).

2.5.5. Egg weight

According to Zhang *et al.* (2005), egg weight influences the weight of its components. The average weight of chicken egg is 58g and consists of averagely 12.3% shell including shell membrane, 55.8% egg white (albumen) and 31.9% yolk (Campbell and Lasley, 2001). Pullet weight at the point of lay, energy intake, protein intake and linoleic acid are factors that affect egg weight (Tiller, 2003). Other factors are environment, feed, chicken ecotype, age, genetic make up and number of eggs laid (Yakubu *et al*., 2008).

The preferred egg weights are medium (M) and large (L) grades which attract the most favorable prices. Smaller (S) size eggs attract lower prices while extra-large (EX) sized eggs tend to have inferior shell strength, especially with older hens (Zhang *et al.,* 2005).

Underweight pullets achieve their target egg weight late. Overwhelming pullets lay their preferred weight classes; medium and large eggs earlier in their cycle, especially birds kept in cages (Tiler, 2003).

2.5.6. Shell quality

Shell quality is defined in terms of strength, colour, shape and texture. Strength, colour and shape have moderate to high heritability and so respond readily to selection. Good quality egg shells from commercial layers contain approximately 2.2 grams of calcium in the form of calcium carbonate (Zhang *et al.,* 2005). Though shell thickness is the main factor, it is not the only factor that determines egg shell strength.

Currently, dietary manipulation such as increasing or decreasing calcium levels is the primary means of correcting egg shell quality problems (Butcher and Miles, 2012). As the hen's age increase, egg weight increases while shell strength, shell thickness, albumen height and Haugh unit decreases (Roberts, 2010). Egg shell quality may be affected by the strain and age of hen, induced moulting, nutritional factors, enzymes, contamination of feed, general and heat stress, diseases, production system or addition of proprietary products to the diets (Roberts, 2004).

2.5.7. Internal egg quality characteristics

The internal egg traits refers to albumen weight (g), albumen height (mm), albumen width (mm), yolk weight (g), yolk height (mm), yolk width (mm) and yolk colour (Hussain *et al*., 2013). Internal egg qualities may be affected by storage, hen strain and age, induced moulting, nutrition and diseases. An understanding of the range of factors that affect egg internal qualities is essential for the production of eggs of high quality (Ahmadi and Rahimi, 2011).

2.5.8. Yolk quality

Yolk quality is measured by the colour, texture, firmness, smell of the yolk (Jacob *et al*., 2011) and yolk index (Ayorinde, 1987). In most consumer survey, colour is the key factor relating to egg quality. However, consumers' preference for yolk colour are highly subjective and vary from country to country (Benton and Brake, 2000; Jacob *et al*., 2000; Jacob *et al*., 2011). Manipulation of the yolk colour by special feeding can be an advantage in meeting market demand (Ahmadi and Rahimi, 2011; Jones, 2006). Yolk index can be used to measure the desirability of the egg. Thus the higher the yolk index, the more desirable the egg (Ayorinde, 1987).

2.5.9. Albumen quality

A hen's egg consist of the yolk (30- 33%), albumen (approximately 60%) and the shell (9-12%) (Stadelman, 1995). The albumen has a major influence on overall interior egg

quality (Jacob *et al*., 2011). Albumen quality is influenced by strain or breed (Johnson and Merritt, 1955).

In healthy flocks, hen's age is the most important factor affecting albumen quality of freshly laid eggs. Albumen quality decreases rapidly with advancement of age (Samli *et al*., 2005). Albumen quality is important for the egg industry because albumen and yolk have different market demands (Ahn *et al*., 1997). Storage time and temperature appear to be the most crucial factors affecting albumen quality or Haugh unit (HU) (Samli *et al*., 2005).

According to Keener *et al.* (2006), Haugh unit is a measure of albumen quality and therefore freshness of the egg. Among many methods of egg albumen quality assessment, the determination of Haugh unit is the main criterion of egg quality being used in the international trade. The Haugh unit from 79 to 100 determines the best egg albumen quality (Haugh, 1937).

2.6. Haematology

2.6.1. Avian haematology

Avian haematology demonstrates a high intra and inters specific variability under physiological conditions which is caused by the richness in species and the high diversity of metabolic activity due to gender, age, reproductive status and season (Elagib and Ahmed, 2011). A blood panel represents the actual status of circulating cells in the peripheral blood stream. Many influencing factors cause conspicuous variations which makes haematological analysis a very sensitive diagnostic tool (Pendi, 2010). Analysis of

normal haematological parameters of poultry is essential for the diagnosis of various pathological and metabolic disorders (Elagib and Ahmed, 2011). It can be used as a diagnostic tool in order to assess the impact of environmental, nutritional and/or pathological stresses (Elagib and Ahmed, 2011). Haematological parameters provide valuable information in the immune status of animals (Kral and Suchy, 2000). Such information, apart from being useful for diagnostic and management purposes, could equally be incorporated into breeding programs for the genetic improvement of poultry (Elagib and Ahmed, 2011). It is important to know the normal physiological values, under different conditions for proper management, feeding, breeding, prevention and treatment of diseases (Elagib and Ahmed, 2011).

Blood can be collected from a variety of sites of a bird. The choice of a blood collection site is influenced by the species of bird, preference of the collector, physical condition of the bird and the volume of blood needed (Terry *et al*., 2007). For best results, venous blood should be collected for haematological studies. Blood collected from capillaries (e.g. blood from clipped nails) often results in abnormal cell distributions and contains cellular artifacts such as macrophages and materials not normally found in peripheral blood (Campbell and Ellis, 2007).

Blood to be used for haematological studies should be collected into a collection tube containing EDTA (ethylene diamine tetraacetic acid) as anticoagulant. Other anticoagulants, such as heparin, interfere with cell staining and create excessive cell clumping, resulting in erroneous cell counts and evaluations (Campbell and Ellis, 2007).

Valuation of the avian hemogram (complete blood count) involves counting the various blood cells per micro litre of blood as well as cytological evaluation of the cells.

Because avian blood does not store well (e.g. during transport), hematological results obtained soon after collection are preferred over those performed several hours after collection (Campbell, 2013). Blood volume in birds depends on the species and varies from 5ml/100g in the ring-necked pigeon to 16.3 and to 20.3ml/100g in the racing pigeon. In general, birds are better able to tolerate severe blood loss than mammals, which is due to greater capacity for extracellular fluid mobilization. However, there is a marked variation among avian species in response to blood loss, which may be a reflection of differences in blood volume or extracellular fluid depots (Campbell, 2013). According to Forbes (2008), the average blood volume of most birds is approximately 10% of their body weight and 1% may be removed for testing.

In comparison to mammals, avian blood cells show unique morphological characteristics e.g. erythrocyte and thrombocyte contain nucleus (Pendi, 2010). According to Terry *et al*. (2007) avian red cells are nucleated which is why manual white cell counts are typically not possible. White cells are similar to mammalian lines, except that the mammalian neutrophils are replaced with heterophils and mammalian platelets are replaced with thrombocytes. Important differentials for leukocytosis with profound heterophilia and monocytosis include chlamydophilosis, aspergilosis and tuberculosis. Mitchell and Johns (2008), reported that the interpretation of avian blood cells provide many challenges. However, Forbes (2008), interpreted avian blood as shown in Table 2.3:

Table 2.3: CBC (complete blood count) findings

CBC Results	Interpretation
Leuokocytosis	Infection, inflammation, necrosis, neoplasia, heavy metal toxicosis and stress (particularly in macaws, there should be no toxic changes in white cells.
Severe heterophilia	Chlamydophilosis, aspergilosis, tuberculosis (often with toxic changes in white cells.
Moderate heterophilia	Infection, cellular necrosis
Lymphocytosis	Viral infection, certain stages of chlamydophilosis
Monocytosis	Chronic infection with extensive necrosis and phagocyte activity (typically aspergillus, chlamydophilosis and tuberculosis)
Eosinophilia	Of inconsistent and unproven significance
Basophilia	Uncommon results most often associated with respiratory infections, resulting in tissue damage, parasitism and some stages of chlamydophilosis
Lepeniauco	Overwhelming bacterial or severe viral infection (particularly circovirus), leucopenia may also be associated with reduction of cells or increased use, which is demonstrated by the presence of immature or toxic white cells.

Source: **Adapted from Forbes. (2008)**

The normal PCV/HCT of birds ranges between 35 and 55%. A PCV less than 35% is indicative of anaemia and a PCV greater than 55% is suggestive of dehydration or polycythemia. An increase in red cell polychromasia is indicative of red blood cell regeneration. In normal birds, the number of polychromatic erythrocytes (or reticulocytes) found in the peripheral blood ranges between one and five percent of erythrocytes (Campbell, 2013). An anaemic bird with a 5% or less degree of polychromasia (or reticulocytosis) is responding poorly to the anaemia or there has not been enough time for the bird to demonstrate a significant response. Hypochromasia can be associated with certain nutritional deficiencies in birds, especially iron deficiency. There is a wide variation

in the normal leukograms among birds of the same species. In general, total leukocyte count greater than 10,000/µl are considered suggestive of leukocytosis in tame, adult psittacoses birds. A normal thrombocyte count ranging between 20,000 and 30,000/µl of blood or 10 to 15/1000 erythrocytes can be used as a general reference for most birds (Campbell, 2013). The various haematologic type, units, normal ranges and average values of chicken are shown in Table 2. 4:

Table 2.4: range of values for haematological parameters of chicken

Haematologic type	**Units-interpretation standards (SI)**	**Normal ranges**	**Average values**
Hb	g/dl	7.00 - 13.00	9.00
RBC	$X10^{-6}$/ml	2.50 - 3.50	3.00
WBC	$X10^{-6}$/ml	12000 - 30000	12000
PCV	%	22.00 - 35.00	30.00
MCH	Pg	33.00 - 47.00	41.00
MCV	FI	90.00 - 140.00	115.00
MCHC	%	26.00 - 35.00	29.00
HET (band)	X10/L	Rare	-
HET (mature)	X10/L	3000 - 6000	4500
LYM	X10/L	7000 - 17500	14000
MON	X10/L	150 - 2000	1500
ESIN	X10/L	0.00 - 1000	400
BAS	X10/L	Rare	-
RET	%	0.00 – 0.60	0.00
ESR*	Mm	3.00 – 12.00	7.00

Acronyms: Hb – Haemoglobin, RBC – Red Blood Cell, WBC – White Blood Cell, PCV – Pack Cell Volume, MCH – Mean Corpuscular Haemoglobin, MCV –Mean Corpuscular Volume, MCHC – Mean Corpuscular Haemoglobin Concentration, HET = Heterophils *LYM = Lymphocytes, MON* = Monocytes, *ESIN* = Esinophils *BAS* = Basophils, *RET* = Reticulocytes, *ESR – Erythrocyte Sedimentation Rate.*
Source: Nemi (1986)

2.6.2. Nutrition and haematology

Haematology refers to the study of the numbers and morphology of the cellular elements of the blood thus the red blood cells (erythrocytes), white blood cells (leukocytes), platelets

(thrombocytes) and the use of these results in the diagnosis and monitoring of disease (Merck Manual, 2012).

Blood transports or conveys nutrients and other substances to different parts of the body. Therefore, whatever affects the blood; drugs, pathogenic organism or nutrition will certainly affect the entire body adversely or moderately in terms of health, growth, maintenance and reproduction (Oke *et al*., 2012). A readily available and fast means of assessing clinical and nutritional health status of animals on feeding trials may be the use of blood analysis, because dietary components have measurable effects on blood composition (Church *et al*., 2012; Maxwell *et al*., 1990) and may be considered as appropriate measure of long term nutritional status (Olabanji, 2007).

According to Togun and Osein (2000), haematological studies have been found to be useful for disease prognosis, therapeutic and feed stress monitoring. Adamu *et al.* (2006) observed that nutrition had a significant effect ($P < 0.05$) on haematological values like PCV, Hb and RBC. Togun *et al.* (2007) reported that when the haematological values fall within the normal range, it is an indication that the diets did not show any adverse effect on haematological parameters during the experimental period but when the values fall below the normal range, it is an indication of anaemia. Physiological and nutritional status of animals could cause differences in values observed for PCV and MCV. Immune status is a function of leucocytes; neutrophils and lymphocytes (Nse Abasi *et al.,* 2014). Lymphocytes are known to play key roles in immune defense system of both man and animals (Ameen *et al*., 2007). When WBCs, (neutrophils and lymphocytes) fall within the

normal range, it indicates the feeding patterns do not affect the immune system of the animal. Most immunological abnormalities observed in malnutrition are usually corrected after nutritional rehabilitation (Ameen *et al.,* 2007).

According to Adenkola *et al.* (2008), increase in neutrophils: lymphocyte ratio is a good indicator of stress which could be nutritional stress. Blood cells arising in the bone marrow from stem cells are able to undergo processes of proliferation and differentiation in the haematopoietic microenvironment (Mensez-Ferrer *et al*., 2010; Bianco, 2011). Feeding birds with protein deficient diets decreases the production of blood cells, leading to bone marrow hypoplasia and inducing structural alterations that interfere with both innate and adaptive immunity (Xavier *et al*., 2007; Fock *et al*., 2007; Borelli *et al*., 2009; Fock *et al*., 2009). Cunha *et al*. (2013) reported that protein malnutrition (PM), as a result of feeding birds with protein deficient diets, results in pathological changes that are associated with leucopenia, bone marrow (BM) hypoplasia and alterations in BM microenvironment leading to haematopoietic failure.

2.6.3. Haematological and biochemical profile of laying hens

Blood is responsible for the transportation of nutrients, metabolic waste products and gases around the body (Zhou *et al.,* 1999). It is the means by which clinical and nutritional health status of animals are assessed (Olorode and Longe, 2000). The haemato-biochemical profiles are most commonly used in nutritional studies for chicken (Adegemi *et al.,* 2000). Biochemical testing focuses on its chemical components whereas full blood count assesses the cellular components of blood. Also, blood parameters help in diagnosing specific

poultry such as hen pathology and can serve as a basic knowledge for studies in immunology and comparative avian pathology (Bonadiman, 2009).

Dutta *et al.* (2013), in a comparative account of haematological and biochemical parameters, declared no significantly correlated ($P > 0.05$) haematological and biochemical parameters of the indigenous and five other chicken breeds as shown in Table 2.5:

Table 2.5: Haematological profile of one indigenous and five exotic male chickens from Rajshahi Bangladesh

Breeds	RBC($X10^6$ cell, μl^{-1})	WBC ($X10^6$ cell, μl^{-1})	PLATLETS ($X10^6$ cell, μl^{-1})	Hb (%)	ESR ($mm.hr^{-1}$)
IND	2.74	2.33	2.67	11.2	8.60
COB	2.12	2.27	2.14	7.20	8.20
COC	2.54	2.21	2.24	6.40	8.40
FAY	2.62	2.23	2.48	9.80	8.80
RIR	2.32	2.17	2.22	9.40	7.80
SON	2.48	2.15	2.48	9.80	8.80

Acronyms : IND – Indigenous, COB – Cob 500, COC – Cockerel, FAY – Fayoumi, RIR – Rhode Island Red, SON – Crossed Sonali, RBC – Red Blood Cell, WBC – White Blood Cell, Hb – Haemoglobin, ESR – Erythrocyte Sedimentation Rate.

Source: **Dutta *et al.*(2013)**

Table 2.6: Biochemical profile of one indigenous and five exoticmale chickens from Rajshahi Bangladesh

Breed	CAL	CHO	CRE	GLU	URE
IND	10.6	75.0	0.63	76.0	1.7
COB	9.60	83.0	0.60	154	12.2
COC	11.6	78.0	0.64	147	11.0
FAY	11.5	76.0	0.56	160	11.4
RIR	12.9	95.2	0.90	159	16.2
SON	8.88	93.4	0.54	127	13.8

Acronyms : IND – Indigenous, COB – Cob 500, COC – Cockerel, FAY – Fayoumi, RIR – Rhode Island Red, SON – Crossed Sonali, CAL – Calcium, CHO – Cholesterol, CRE – Creatinine, GLU – Glucose and URE – Urea.

Source: **Dutta *et al.* (2013)**

2.7. Moringa

2.7.1. Description of Moringa oleifera

Moringa oleifera is a multipurpose tree. *Moringa oleifera* originated from Agra and Oudah in the Northwestern region of India (Nielsen, 1994; Mughal *et al.*, 1999). Among the thirteen known species, *Moringa oleifera* is a high valued plant which is adaptable, easy to cultivate and self-propagating with a very fast growth rate (Mayde, 1986). This plant is widely cultivated in Africa, India, Mexico, Malaysia, Indonasia, Central and South America (FAO, 1999).

Moringa oleifera is mainly used for food and has many industrial, medicinal and agricultural uses including animal feeding. The plant is a nutritious, fast-growing and drought-tolerant plant which was rediscovered in the 1990s. According to Fahey (2005), *Moringa oleifera* tree is being cultivated in West, East and South Africa where it is among the most economically valuable plants. Moringa has been described as the ‘’miracle tree’’ or “tree of life” (Bosch, 2004; Radovich, 2009; Orwa *et al*., 2009) due to it nutritional and medicinal properties. The life span of moringa tree is about 20 years on the average (Seth *et al*., 2007).

2.7.2 Morphology of Moringa oleifera

Moringa is a small to medium evergreen or deciduous tree that can grow to a height of 10-12 metres. 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 a feathery foliage. Young twigs and shoots are covered in short dense hairs, purplish or greenish white in

colour. Moringa leaves are alternate, 7 to 60 cm long, tripinnately compound with each pinnate bearing 46 pairs of leaflets that are dark green, elliptical to obovate, and 1-2cm in length. The inflorescence is 10 to 20 cm long, spreading panicles bearing many fragrant flowers (Makker and Becker, 1997; Foidl *et al*., 2001).

Moringa flowers are pentamerous, zygomorphic, 7-14mm long and white to cream in colour. The fruit is a typically 3-valved capsule, 10 to 60cm in length, often referred to as a "pod" and looking like a drumstick hence the name "drumstick tree". The fruit is green when young but turns brown at maturity. The mature fruit splits open along each angle to expose the seeds. The capsule contains 15-20 rounded oily seeds, 1-1.5cm in diameter surrounded by 3 papery wings, up to 2.5cm long. Moringa seeds contain a large amount of oil (Foidl *et al*., 2001).

2.7.3 .Chemical composition of Moringa oleifera seeds

Proximate analysis by Alagbemide *et al*. (2014) indicated significantly higher contents of moisture, protein, crude fat, ash and nitrogen free extracts except crude fibre. The results are presented in Table 2.7:

Table 2.7: Chemical composition of (mg/100g) of raw and deffated Moringa oleifera seeds

Nutrient	Raw samples	Deffated samples
Moisture content	9.9 ± 0.09^{a}	9.40 ± 0.10^{b}
Crude protein	35.97 ± 0.19^{a}	17.13 ± 0.13^{b}
Crude fat	38.67 ± 0.03^{a}	8.57 ± 0.18^{b}
Ash	3.87 ± 0.09^{a}	3.47 ± 0.07^{b}
Crude fibre	2.87 ± 0.03^{a}	3.33 ± 0.09^{a}
Carbohydrate (by difference)	8.87 ± 0.12^{a}	57.77 ± 0.12^{b}

Values are means (± SEM) of triplicate samples. Means with different superscripts in the same row show significant difference ($P < 0.05$)

Source: Alagbemide *et al*. (2014)

2.7.4. Nutrient composition of Moringa oleifera

Moringa is highly nutritious due to the presence of a variety of essential nutrients in the leaves, pods and seeds. Moringa is said to provide 7 times more vitamin C than oranges, 10 times more vitamin A than carrots, 17 times more calcium than milk, 9 times more protein than yoghurt, 15 times more potassium than bananas and 25 times more irons than spinach.

Moringa is rich in phytosterols like stigmasterols, sitosterols and kampesterol which are precursors for hormones. These compounds increase estrogen production (Lakshmipriya, *et al*., 2016). The leaves for instance, contain minerals such as calcium, potassium, zinc, magnesium iron and copper (Kasolo *et al*., 2010). Vitamins such as beta carotene of vitamin A, vitamin B complex such as folic acid, pyridoxine and nicotinic acid, vitamin C, D and E are all present in the leaves of *Moringa oleifera* (Mbikay, 2012). However, phytochemicals such as tannins, sterols, terpenoids, flavonoids, saponins, anthraquinones, alkaloids and reducing sugars are also present in the leaves of *Moringa oleifera* (Berkovich *et al*., 2013).

The pods are fibrous and nutritious. Studies have shown that immature pods contain about 46.78% fibre and about 20.66% protein (Sanchez *et al*., 2010). *Moringa oleifera* pods have 30% amino acid content, the leaves have 44% and flowers have 31%. The immature pods and flowers have similar levels of palmitic, linolenic, linoleic and oleic acids (Sanchez *et al*., 2010).

Moringa has lot of minerals such as calcium which is very essential for growth and development. Moringa leaves can provide 1000 mg with moringa powder providing more than 4000 mg of calcium (Lakshmipriya *et al.,* 2016). It has been reported that moringa contains more iron than spinach (Fuglie, 2005). It also contains substantial amount of zinc and a good dietary intake of zinc is essential for proper growth of sperm cells and synthesis of DNA and RNA (Lakshmipriya *et al*., 2016). There is 25.5 to 31.03 mg of zinc/kg in *Moringa oleifera* leaves (Barminas *et al*., 1998).

A report by Raghavendra *et al.* (2015), indicated that *Moringa oleifera* leaves and pods have moderate antioxidant activity as compared to the standard antioxidant, and the leaf extract was superior to the pod in terms of antioxidant potential. The report also unraveled a high concentration of iron and calcium followed by sodium, potassium and magnesium. Based on their findings, a conclusion was drawn that, the leaves and pods contain appreciable amounts of nutrients and can be included in diets to supplement daily nutrient requirement of animals. PUFAs are linoleic acid and linolenic acids; these PUFAs have the ability to control cholesterol. Studies have shown that *Moringa oleifera* seed oil contains about 76% PUFAs making it ideal to be used as a substitute of olive oil (Thurber *et al*., 2010).

It must be noted however that nutrient composition varies according to location. According to Yang *et al.* (2006), vitamin A is abundant in hot –wet seasons, while vitamin C and iron are more in cool dry seasons. These differences can be as a result of the location, climate and environmental factors that significantly influence the nutrient content of *Moringa*

oleifera. A complete list of nutrients available in leaves, pods and seeds are shown in Table 2.8:

Table 2.8: The nutrient composition of leaves, leaf powder, seeds and pods

Nutrients	Fresh leaves	Dry leaves	Leaf powder	Seed	Pods
Calories (cal)	92	329	205	–	26
Protein (g)	6.7	29.4	27.1	35.97 ± 0.19	2.5
Fat (g)	1.7	5.2	2.3	38.67 ± 0.03	0.1
Carbohydrate (g)	12.5	41.2	38.2	8.67 ± 0.12	3.7
Fibre (g)	0.9	12.5	19.2	2.87 ± 0.03	4.8
Vitamin B1 (mg)	0.06	2.02	2.64	0.05	0.05
Vitamin B2 (mg)	0.05	21.3	20.5	0.06	0.07
Vitamin B3 (mg)	0.8	7.6	8.2	0.2	0.2
Vitamin C (mg)	220	15.8	17.3	4.5 ± 0.17	120
Vitamin E (mg)	448	10.8	113	751.67 ± 4.41	–
Calcium (mg)	440	2185	2003	45	30
Magnesium (mg)	42	448	368	635 ± 8.66	24
Phosphorus (mg)	70	252	204	75	110
Potassium (mg)	259	1236	1324	–	259
Copper (mg)	0.07	0.49	0.57	5.20 ± 0.15	3.1
Iron (mg)	0.85	25.6	28.2	–	5.3
Sulphur (mg)	–	–	870	0.05	137

All values are in 100 g per plant material

***Source*: Fuglie (2005); Olagbemide (2014)**

2.7.5. Amino acid profile of Moringa oleifera seed

Oluwole *et al.* (2013) posited that total amino acids in fermented *Moringa oleifera* (FMOF) and germinated *Moringa oleifera* (GMOF) were higher than that in raw *Moringa oleifera* (RMOF). Table 2.9 shows the amino acid profile of raw, germinated and fermented *Moringa oleifera* seed flour whereas Table 2.10 shows the chemical scores of essential amino acids of raw, germinated and fermented *Moringa oleifera* seeds.

Table 2.9: Amino acid (g/100g crude protein) profile of raw, germinated and fermented Moringa olefeira seed flour

Amino acids	RMOF	GMOF	FMOF	FAOa
Nonessential amino acids				
Alanine	5.16 ± 0.01^{c}	5.42 ± 0.00^{b}	6.29 ± 0.00^{a}	
Aspartic acid	15.70 ± 0.06^{c}	18.13 ± 0.01^{b}	21.37 ± 0.01^{a}	
Serine	3.06 ± 0.01^{c}	3.17 ± 0.00^{b}	3.53 ± 0.01^{a}	
Glutamic acid	17.87 ± 0.03^{c}	20.23 ± 0.03^{b}	22.46 ± 0.06^{a}	
Total NEAAs	41.79	46.95	53.65	
Conditionally essential amino acids				
Proline	2.18 ± 0.00^{c}	2.68 ± 0.00^{b}	3.75 ± 0.00^{a}	
Glycine	2.37 ± 0.01^{c}	2.63 ± 0.00^{b}	3.02 ± 0.00^{a}	
Arginine	8.28 ± 0.00^{c}	8.66 ± 0.01^{b}	9.66 ± 0.01^{a}	
Cysteine	1.68 ± 0.00^{c}	1.79 ± 0.00^{b}	2.02 ± 0.00^{a}	
Tyrosine	1.97 ± 0.01^{c}	2.09 ± 0.00^{b}	2.34 ± 0.01^{a}	
Histidine	1.93 ± 0.01^{c}	2.50 ± 0.06^{b}	2.94 ± 0.00^{a}	2.1
Total	18.41	20.35	23.73	
Essential amino acids				
Lysine	0.312 ± 0.01^{c}	0.363 ± 0.02^{b}	0.405 ± 0.01^{a}	4.2
Threonine	3.02 ± 0.01^{c}	3.35 ± 0.00^{b}	3.93 ± 0.00^{a}	2.8
Valine	1.08 ± 0.01^{c}	1.25 ± 0.00^{b}	1.64 ± 0.00^{a}	4.2
Methionine	0.31 ± 0.00^{c}	0.35 ± 0.01^{b}	0.41 ± 0.01^{a}	2.2
Isoleucine	4.23 ± 0.00^{c}	4.69 ± 0.00^{b}	5.14 ± 0.01^{a}	4.2
Leucine	3.83 ± 0.01^{c}	4.08 ± 0.00^{b}	5.04 ± 0.00^{a}	4.2
Phenylalanine	3.27 ± 0.01^{c}	3.57 ± 0.00^{b}	4.25 ± 0.00^{a}	2.8
Tryptophan	ND	ND	ND	
Total	16.052	17.653	20.815	33.9

Values are means (±SEM) of triplicate samples; means with different superscripts in the same row show significant difference ($P < 0.05$). ND = not detected; RMOF = raw M. oleifera flour, GMOF = germinated M. oleifera flour, FMOF = fermented M. oleifera flour.

***Source*: FAO/WHO (1990); Oluwole *et al.* (2013)**

Table 2.10: Chemical scores of essential amino acids of raw, germinated and fermented Moringa oleifera seeds

Amino acid scores	FAO/WHO ref.	RMOF	GMOF	FMOF
Lysine	5.8	5.34	6.21	7.07
Threonine	3.4	88.82	98.53	115.59
Valine	3.5	30.86	35.71	46.86
Methionine	2.2	14.09	15.91	18.64
Isoleucine	2.8	151.07	167.50	183.57
Leucine	6.6	58.03	61.82	76.36
Phenylalanine	2.8	116.79	127.50	151.79
Histidine	1.9	101.58	131.58	154.74
Tryptophan	1.1	0.00	0.00	0.00
Arginine	2	414.00	433.00	483.00
Total	33.9	88.23	96.43	111.45
First limiting amino acid		Lysine	Lysine	Lysine
Second limiting amino acid		Methionine	Methionine	Methionine

Values are means (±SEM) of triplicate samples; means with different superscripts in the same row show significant difference (P < 0.05). RMOF = raw M. oleifera flour, GMOF = germinated M. oleifera flour, FMOF = fermented M. oleifera flour.

***Source*: FAO/WHO (1990); Oluwole *et al.*(2013)**

2.7.6. Mineral composition of Moringa oleifera seeds

According to Oluwole *et al.* (2013), mineral contents in GMOF were significantly higher in iron, sodium, potassium, magnesium, and copper while FMOF were higher in calcium, phosphorus and magnesium, and both were significantly lower than those in RMOF ($P < 0.05$). Table 2.11 shows the mineral composition of raw, germinated and fermented *Moringa oleifera* seed flour.

Table 2.11: Mineral composition (mg/100g) of raw, germinated and fermented Moringa oleifera seed flour

Minerals	RMOF	GMOF	FMOF
Calcium	128.33 ± 1.67^{a}	116.67 ± 1.67^{b}	121.67 ± 1.67^{b}
Phosphorus	103.33 ± 1.67^{a}	86.67 ± 1.67^{b}	91.67 ± 1.67^{b}
Iron	7.33 ± 0.09^{a}	6.63 ± 0.09^{b}	5.63 ± 0.09^{c}
Sodium	295.10 ± 0.10^{a}	285.13 ± 0.13^{b}	280.30 ± 0.03^{c}
Potassium	52.33 ± 1.45^{a}	45.00 ± 0.00^{b}	43.67 ± 0.88^{b}
Magnesium	26.33 ± 0.33^{a}	25.10 ± 0.06^{b}	25.13 ± 0.09^{b}
Copper	0.63 ± 0.03^{a}	0.60 ± 0.00^{a}	0.57 ± 0.03^{a}
Iodine	0.11 ± 0.01^{a}	0.11 ± 0.01^{a}	0.10 ± 0.00^{a}
Na/K	5.65 ± 0.15^{b}	6.33 ± 0.00^{a}	6.42 ± 0.13^{a}
Ca/P	1.24 ± 0.03^{b}	1.35 ± 0.04^{a}	1.33 ± 0.00^{ab}
Ca/K	2.46 ± 0.09^{b}	2.59 ± 0.04^{ab}	2.79 ± 0.04^{a}
Na/Mg	11.21 ± 0.14^{a}	11.36 ± 0.03^{a}	11.14 ± 0.04^{a}
Ca/Mg	4.87 ± 0.06^{a}	4.65 ± 0.07^{a}	4.84 ± 0.08^{a}
Fe/Cu	11.65 ± 0.69^{a}	11.05 ± 0.15^{a}	10.01 ± 0.61^{a}

Values are means (±SEM) of triplicate samples; means with different superscripts in the same row show significant difference ($P < 0.05$). RMOF = raw M. oleifera flour, GMOF = germinated M. oleifera flour, FMOF = fermented M. oleifera flour.

***Source*: Oluwole *et al.*(2013)**

2.7.7. Fatty acids of Moringa oleifera seeds

Oluwole *et al.* (2013) identified linoleic acid and behenic acid as the predominant and least fatty acids respectively in *Moringa oleifera* seed. Table 2.12 shows the fatty acids composition of *Moringa oleifera* seed.

Table 2.12: Fatty acid (% fatty acid) of raw, germinated and fermented moringa oleifera seed flour

Fatty acids	RMOF	GMOF	FMOF
Saturated fatty acids			
Myristic acid	0.57 ± 0.00^{a}	0.42 ± 0.01^{b}	0.44 ± 0.01^{b}
Palmitic acid	13.48 ± 0.00^{c}	14.01 ± 0.01^{b}	14.50 ± 0.00^{a}
Stearic acid	12.62 ± 0.01^{b}	13.57 ± 0.03^{a}	13.52 ± 0.01^{a}
Behenic acid	0.16 ± 0.00^{a}	0.13 ± 0.00^{a}	0.20 ± 0.06^{a}
Arachidic acid	ND	ND	ND
Total	26.83 ± 0.01	28.13 ± 0.05	28.66 ± 0.08
Poly unsaturated fatty acids			
Linolenic acid	0.18 ± 0.00^{b}	0.13 ± 0.01^{c}	0.32 ± 0.01^{a}
Linoleic acid	58.79 ± 0.02^{c}	60.70 ± 0.01^{b}	62.05 ± 0.01^{a}
Arachidonic acid	ND	ND	ND
Total	58.97 ± 0.00	60.83 ± 0.02	62.37 ± 0.02
Mono unsaturated fatty acids			
Palmitoleic acid	0.36 ± 0.00^{a}	0.26 ± 0.00^{b}	0.22 ± 0.00^{c}
Oleic acid	13.18 ± 0.00^{a}	10.25 ± 0.03^{b}	8.32 ± 0.02^{c}
Total	13.54 ± 0.00^{a}	10.51 ± 0.03^{b}	8.54 ± 0.02^{c}
P:S	2.19	2.16	2.18

Values are means (±SEM) of triplicate samples; means with different superscripts in the same row show significant difference ($P < 0.05$). ND = not detected, RMOF = raw M. oleifera flour, GMOF = germinated M. oleifera flour, FMOF = fermented M. oleiferaflour, P = phosphorus, S = sulfur.

Source: **Oluwole *et al*.(2013)**

2.7.8. Anti-nutritional factors in Moringa oleifera seeds

Tijani *et al.* (2015) reported that anti-nutritional factors present in *Moringa oleifera* showed higher tannin, phytic acid, saponin and oxalate contents in dry leaf meal than fresh leaves. A report by Stevens *et al.* (2015) also indicates that, there are lower antinutrients in the leaves of *Moringa oleifera* than the seeds and that, the leaves will offer a better bioavailability of nutrients it contains and therefore should be preferred for consumption than the seeds.

Oluwole *et al.* (2013), in their comparative study on nutrient composition, phytochemical and functional characteristics of raw, germinated and fermented *Moringa oleifera* seed flour reported a significantly lower phytochemical/antinutrient compositions in FMOF samples than GMOF, and both were significantly lower when compared with RMOF samples. Table 2.13 shows the antinutrient and phytochemical component of *Moringa oleifera* seed.

Table 2.13: Phytochemical/antinutritional factor (mg/100 g) of raw, germinated, and fermented Moringa oleifera seed flour

Phytochemicals	RMOF	GMOF	FMOF
Tannins	241.67 ± 1.67^{a}	181.67 ± 1.67^{b}	146.67 ± 3.33^{c}
Phytate	78.33 ± 1.67^{a}	40.00 ± 0.00^{b}	28.33 ± 1.67^{c}
Phenolics	40.00 ± 0.00^{a}	34.33 ± 0.67^{b}	23.00 ± 1.00^{c}
Alkaloids	17.33 ± 0.17^{a}	15.33 ± 0.33^{b}	12.33 ± 0.17^{c}
Flavonoids	5.50 ± 0.01^{a}	5.50 ± 0.00^{a}	5.00 ± 0.02^{b}
Saponins	9.83 ± 0.17^{a}	8.00 ± 0.29^{b}	7.50 ± 0.00^{b}
Terpenoids	20.00 ± 0.11^{c}	27.50 ± 0.21^{a}	25.00 ± 0.13^{b}

Values are means (±SEM) of triplicate samples; means with different superscripts in the same row show significant difference ($P < 0.05$). RMOF - raw M. oleifera flour, GMOF - germinated M. oleifera flour, FMOF - fermented M. oleifera flour.
Source: **Oluwole *et al.* (2013)**

2.7.9. Processing of moringa

Methods of processing plants can lead to a loss of valuable nutrients. There is higher phytochemical content in raw moringa seed flour with amino acid content reaching its peak in fermented and germinated seed flour (Ijarotimi *et al*., 2013). This can be attributed to biochemical activities during fermentation. A research conducted to determine the effect of

boiling, simmering and blanching on nutrient retention of moringa leaves showed boiling as the most effective technique for reducing cyanide, oxalate and phytate contents more significantly. Phytate and antinutrients can reduce bioavailability of certain nutrients in moringa seed and leaves hence appropriate methods should be used to enhance maximum utilization of nutrients (Kachik *et al*., 1992; Sllau *et al*., 2012). Yang *et al.* (2006) also reported that boiling increases the availability of iron and antioxidant content.

2.7.10. Preservation of moringa

Moringa seeds can be preserved for longer periods without loss of nutrients. Drying or freezing can be done to store the leaves (Lakshmipriya *et al*., 2016). A report by Yang *et al.* (2006) showed that a low temperature oven used to dehydrate the leaves of *Moringa oleifera* retained more nutrients except vitamin C than freeze-dried leaves. Therefore, preservation by dehydration improves the shelf life of *Moringa oleifera* without any change in nutrient composition.

2.8. Moringa Nutrition

2.8.1. Effect of Moringa oleifera leaf meal (MOLM) on growth and mortality of layers

According to Voemesse *et al.* (2018), birds fed *Moringa oleifera* leaf meal had no significant effect on feed intake ($P > 0.05$). On the other hand, average daily body weight gain, final body weight and feed conversion ratio were improved in *Moringa oleifera* supplemented groups in the growing period from 1 day to 20 weeks old. In the laying period, from 21 weeks to 55 weeks, feed intake was lower in moringa fed groups at 1% and 3%, but the laying percentage and feed conversion ratio were higher in the supplemented

groups than the non supplemented group. The researchers concluded that feeding moringa leaf meal at 1% level had positive effect on the growth and egg laying hens.

A report by Hassan *et al.* (2016) indicated that, body weight gain and feed intake of broiler chicken increased significantly ($P < 0.05$) with increasing levels of *Moringa oleifera* leaf meal (0.1, 0.2, and 0.3%).

Olugbemi *et al.* (2010), found that addition of 5% *Moringa oleifera* leaf meal to cassava – based broilers' diet (20% and 30%) had no significant ($P > 0.05$) effect on weight gain, feed conversion ratio and final body weight. However, levels above 5% decreased broiler performance. Contrary to this finding, Juniar *et al.* (2008) reported that the inclusion of *Moringa oleifera* leaf meal up to 10% did not produce significant ($P > 0.05$) effect on feed consumption, body weight, feed conversion ratio and carcass weight. The differences shown could be as a result of the effect of the cassava in the previous study.

Estalem *et al.* (2012), in a study to evaluate the effect of replacing *Moringa oleifera* leaf meal for soybean meal in broiler ration, reported that the daily and total dry matter (DM) and total CP intake of birds fed a control diet were greater than the treatment groups (5%, 10%, 15% and 20%). There was also a significant ($P < 0.05$) difference in the final body weight with the final body weight increasing with an increase in *Moringa oleifera* leaf meal. The average daily weight gain followed a similar trend like that of the total DM intake. However, in terms of feed conversion efficiency, values for those fed 5 and 10% were similar to that of the control ($P > 0.05$), and values for those fed 15 and 20% were lower ($P < 0.05$) than the control and the 5%.

According to Onu and Aniebo (2011), birds fed *Moringa oleifera* leaf meal (2.5%, 5% and 7.5%) inclusion levels gained significantly ($P < 0.05$) higher weight and superior feed conversion ratio than birds fed the control diet. However, birds fed 2.5% and 5% diets recorded significantly ($P < 0.05$) the highest body weight gain.

Kana *et al.* (2015), studying the effects of substituting soyabean with *Moringa oleifera* leaf meal on laying and egg quality characteristics of KABIR chickens recorded mortality of 21, 30, and 37.5% for 0, 5, and 10% of *Moringa oleifera* leaf meal respectively which was above 12%, recommended by the International Practices Hatchery (IPH). The high mortality rate was however attributed to fatal diseases but not the *Moringa oleifera* leaf meal.

2.8.2. Effect of Moringa oleifera seed on growth and mortality of laying chicks

Abbas and Ahmed (2012), studied the effect of *Moringa oleifera* undecorticated seed powder (MOUSP) in broilers' diet (0%, 0.37%, 0.75% and 1.5%), and observed that during the starter phase (8 – 21 days), use of 1.5% MOUSP significantly ($P < 0.05$) reduced weight gain, body weight and feed efficiency. However, during finisher (22 – 35 days), supplementation of 1.5% MOUSP did not produce significant ($P > 0.05$) effect on weight gain, final live body weight and feed efficiency. These findings lead to a conclusion that, the use of MOUSP at an amount of 1.5% during the finisher period overcomes its deleterious effect during the starter period and therefore, it is only better to use this level during the finisher period.

A report by Molepo (2014), indicated that dietary Moringa seed meal (5%, 10%, and 15%) showed no effect ($P > 0.05$) on feed intake, metabolizable energy intake, nitrogen retention, feed conversion ratio and live body weight of unsexed Ross 308 broiler chicken. However, growth rate improved ($P < 0.05$) between age one to twenty – one days. According to the researcher, dietary moringa seed meal of 13.3 g/kg DM feed optimized growth rate of Ross 308 broiler chicken age one to twenty-one days. Whereas, moringa seed meal levels of 5%, 10%, 15% and 20% per kg DM had no effect ($P > 0.05$) on feed intake, growth rate, feed conversion ratio, live weight, metabolizable energy intake and carcass weight of female Ross 308 broiler chicken.

Ochi *et al.* (2015), in a study to evaluate the effect of *Moringa oleifera* seed powder (0.5%, 1.0% and 2.0%) revealed that addition of *Moringa oleifera* seed powder up to 2.0% in broilers' chick diet significantly ($P < 0.05$) reduced weight gain, feed conversion ratio and body weight during starter period. During finisher, supplying broiler chicks with diet 2.0% *Moringa oleifera* seed powder resulted in significant ($P < 0.05$) increase in feed conversion ratio, but showed no significant effect ($P > 0.05$) on weight gain and final live body weight. Therefore, a suggestion was made that, reduction in weight gain, feed conversion ratio and body weight due to the addition of 2.0% moringa seed powder during the starter period may be due to the presence of phytate which acts as anti-nutritional factor.

2.8.3. Effect of Moringa oleifera leaf meal (MOLM) on laying performance

According to Kana *et al.* (2015), inclusion of 5% MOLM induced the highest egg production (81%) as compared to 10% (71.42%) and the control diet without MOLM (62.85%). In relation to egg characteristics, the researchers reported that egg weight, egg width, yolk weight and yolk diameter significantly ($P < 0.05$) decreased with increasing levels of MOLM in the diet whereas, egg length, yolk length, yolk index and shell weight and thickness were not significantly affected by MOLM in the diet. Egg weight significantly ($P < 0.05$) decreased with increasing levels of MOLM.

Kakengi *et al.* (2007), indicated that inclusion of 10% and 20% MOLM in the diet of laying hens as a substitute for sunflower seed meal significantly ($P < 0.05$) increased feed intake and dry matter feed intake but decreased egg mass production. Egg production percentage decreased with an increase in the levels of MOLM. Feed conversion ratio (kg/feed/kg egg) increased when 20% MOLM was added. However, an addition of 5% MOLM significantly ($P < 0.05$) increased egg weight, but lower egg weight was observed at a level of 20%. The increase in feed intake and feed conversion ratio, and decrease in egg mass production, egg production percentage and egg weight at higher levels of MOLM, are due to the low digestibility of energy and protein (Kakengi *et al.,* 2007). Therefore, MOLM can completely replace sunflower seed meal up to 20% without any detrimental effect on laying hens. On the contrary, Ebenebe *et al.* (2013) recommended a lower level of about 2.5% to be ideal for laying performance and egg quality characteristics.

Olugbemi *et al.* (2010) also observed that supplementation of MOLM at levels up to 10% in a cassava chip-based diet had no significant effect ($P > 0.05$) on feed intake, feed conversion ratio and laying percentage. Egg weight significantly increased as a result of the supplementation of MOLM with cassava chip when compared to the control diet (free of MOLM and cassava chip).

Abou-Elezz *et al.* (2011), reported that the inclusion of different levels of MOLM (0%, 5%, 10% and 15%) in the laying hen's diet linearly decreased egg laying percentage and egg mass, while egg weight and feed intake showed a quadratic trend with the increasing levels but had no significant effect on feed conversion ratio.

These three groups of writers; Kakengi *et al.* (2007), Olugbemi *et al.* (2010) and Abou-Elezz *et al.* (2011) all agreed that the use of MOLM up to a level of 10% had no negative effect on the productive performance of laying hens, but levels above that (15% and 20%) are expected to produce adverse effect on productive performance such as laying rate.

2.8.4. Effect of Moringa oleifera seed meal on laying performance

According to Mabusela *et al.* (2018), the inclusion of *Moringa oleifera* whole seed meal (MOWSM) in layer diet reduced feed intake, body weight, rate of lay, egg weight, yolk weight and egg mass. Yolk colour significantly improved at 1, 3 and 5% inclusion levels while the albumen height decreased. Also, the albumen weight, yolk weight, egg shell weight, egg shell thickness and egg shape index showed no significant difference across all treatment groups. Based on this, the researchers concluded that, although, MOWSM

inclusion improved yolk colour, maintained external egg quality, and improved the fatty acid profile, the deleterious effect on layer performance indicates that it may not be suitable for early-laying hens at these respective levels. In addition, Ahmad *et al*. (2017) reported a decrease in production performance of layers fed *Moring oleifera* pod meal and attributed this to high fibre and different anti – nutrientional factors presence in moringa pods.

2.8.5. Effect of Moringa oleifera leaf meal (MOLM) on haematological profile of laying hens

A feeding trial was carried out by Rafiu *et al.* (2013) to evaluate the blood parameters of broiler chicken fed *Moringa oleifera* leaf meal (MOLM). MOLM was incorporated into the experimental diets at varying replacement levels of 0, 5, 10 and 15% for soybean meal. The RBC (red blood cell), Hb (haemoglobin), MCV (mean corpuscular volume), MCH (mean corpuscular haemoglobin) and MCHC (mean corpuscular haemoglobin concentration) counts all showed significant differences. PCV (packed cell volume) and Hb levels decreased as MOLM inclusion increased. Hence, it was concluded that MOLM could be included especially when the meat quality is targeted.

Tijani *et al.* (2015), in their studies on haematological and serum biochemical profile of broiler chicken fed diets containing moringa leaf meal, indicated that the birds fed 5% and 10% MOLM showed a higher ($P < 0.05$) PCV but lower in those fed 20% . The Hb values were similar ($P > 0.05$) among the birds fed 5%, 10% and 15% MOLM based diets but reduced significantly ($P < 0.05$) in birds fed 20% MOLM. The researchers further indicated that the WBC (white blood cell) count was significantly ($P < 0.05$) higher in birds fed 15%

MOLM while the lowest was observed in those fed 20% MOLM. The serum biochemical indices showed significant ($P < 0.05$) reduction in albumen total protein, uric acid, aspartate amino transferase and alanine amino transferase in birds fed 20% MOLM. The creatinine content was significantly ($P < 0.05$) higher in birds fed 20% MOLM based diet. Therefore, MOLM can be incorporated into broiler diets at 15% level without adverse effects on the haematological and serum biochemical properties of the birds (Tijani *et al.*, 2015).

According to Voemesse *et al.* (2018), total protein and albumin levels significantly ($p < 0.05$) increased in chicks fed moringa leaf (1%, and 3%) as compared to control (0%). The same trend was observed with calcium, magnesium and iron concentrations while phosphorus concentration was comparable between the three groups. In contrast, uric acid concentration was significantly lower in the birds offered moringa leaf meal than those in the control group.

Hassan *et al.* (2016), also observed that haemoglobin (Hb) increased with increasing levels of *Moringa oleifera* leaf meal (0.1, 0.2 and 0.3%). However, white haematocrit (Ht) and albumen values were not affected. Heterophil/lymphocyte (H/L) ratio on the other hand decreased with increasing levels of *Moringa oleifera* leaf meal. On the contrary, plasma total protein and globulin increased significantly ($P < 0.05$) with increasing levels of MOLM. They however concluded that addition of MOLM up to 0.3% improved broiler performance, physiological parameters and enhanced the ability to resist heat stress conditions.

A report by Onu and Aniebo (2011) indicated a significant difference ($P < 0.05$) among groups in packed cell volume (PCV) and red blood cell (RBCs) of birds fed 2.5%, 5% and 7.5% MOLM. The haemoglobin (Hb), MCV, MCH and MCHC counts showed no significant difference ($P > 0.05$) among the treatment groups. In a similar direction, there was no significant ($P > 0.05$) difference in the values of serum albumen and globulin. However, there was a significant ($P < 0.05$) difference in the values of total protein. According to these findings, MOLM can be included at 7.5% without any deleterious effect on performance and blood characteristics of broiler starters.

2.9. Enzyme and Digestion

2.9.1. Effect of enzymes on protein digestion

Many feed ingredients in use today in monogastric diets contain significant quantities of antinutritional factors (ANF) which limit both feed value and use. Treatment of the diet or individual ingredients with enzyme may aid in increasing overall diet digestibility and increasing the availability of nutrients by disrupting cell walls to allow better access of digestive enzymes to the encapsulated nutrients, destroying antinutritional factors (ANFs) and supplementing birds' own digestive enzyme array in situations where they are overwhelmed (Campbell *et al.*, 1992; Jeroch *et al.*, 1995). Almost all the enzymes currently used address this challenge to a larger extent and also, by allowing for more economic utilization of raw materials.

Factors such as enzyme source, ingredient variety and environment under which the ingredient was grown, stored and processed into animal feed, age of animal, interaction

with other dietary ingredients, and health status are shown to affect significantly the response obtained from enzyme supplementations (Bedford and Schulze, 1998).

According to Bedford (1996), exogenous enzymes often markedly influence ingredients and diets digestibility. In many cases, the digestive capacity of the bird is overwhelmed, which is certainly the case in younger birds for which output of endogenous enzymes may be deficient with high intestinal viscosity compromising the digestive efficiency of the gastrointestinal tract. Augmentation of the host's own enzyme systems and hydrolysis of viscous polymers can certainly improve nutrient digestibility in younger birds.

Specific anti-nutrients such as phytate can be catabolized from an anti-nutrient to a nutrient (*i.e.* phosphorus), providing a benefit not only in phosphorus digestion but also in removing the substrate's ability to chelate minerals and complex digestive enzymes. Enzymatic destruction of lectins and trypsin inhibitors reduces endogenous losses, thereby improving apparent digestibilities, and reduces the total energy expenditure in digestion, further benefiting performance (Bedford, 1996). Alteration of the fermentation profiles in the bird can significantly benefit performance by more effective partitioning of ileal nutrients between the bird and resident flora, providing nutrients in the caeca from fiber digestion, and by reduction in immunological challenge.

A report by Choct *et al.* (2010) showed that xylanase reduced significantly ($P < 0.01$) duodenal (2.9 vs 1. 7), jejunal (4.6 vs 2.3) and ileal (14.0 vs 3.9) digesta viscosities (mPas) and increased apparent metabolisable energy (AME) significantly ($P < 0. 01$) of the wheat and starch digestibility ($P < 0.5$) in the jejunum and ileum. Digestibility of starch was also

reduced. The enzyme supplementation reduced ($P < 0.05$) fermentation in the ileum, but increased ($P < 0.05$) it in the caeca. According to the researchers, the anti-nutritive effect of soluble non-starch polysaccharides (NSP) is related to their ability to increase digesta viscosity along the gut; this in turn causes changes in gut microflora and efficiency of nutrient utilisation by the chicken. Use of appropriate enzymes is an effective way of dealing with grains with high non-starch polysaccharides (NSP) content in poultry diets.

According to Zanella *et al.* (1999), whereas enzyme supplementation improved overall crude protein (CP) digestibility by 2.9%, this improvement was not equal for all amino acids (AA). Of the amino acids (AA) most important for broilers fed corn-soybean diets, the digestibility of lysine, methionine, and arginine were not improved or not improved significantly by the enzyme supplementation; however, that of valine was improved by 2.3% and that of thrionine was improved by 3.0%.

Zhang (2014), also indicated that xylanase significantly ($P < 0.05$) increased ileal digestibility of crude protein (CP) by 3.5%, starch by 9.3%, soluble non – starch polysaccharides (NSP) by 43.9% and insoluble NSP by 42.2% relative to the control group respectively. Also, xylanase addition increased significantly ($P < 0.05$) total tract digestibility of dry matter by 5.7%, CP by 4.1%, starch by 6.3%, soluble NSP by 50.8%, insoluble NSP by 19.9% and also had the tendency to increase CP significantly ($P < 0.03$).

2.9.2 Application of exogenous enzymes on chicken diet

The digestive process is highly dependent on endogenous enzyme activity (Osman, 1982; Pubols, 1991). However, enzyme supplementation might be more effective

to improve the activity of digestive enzymes and the absorptive capacity of the small intestine (Alagawany *et al*., 2018). Application of exogenous enzymes in chicken diets achieves the following;

1. The chicken is often compromised in its digestive capacity such that addition of exogenous enzymes can improve productive performance.
2. Exogenous enzyme can improve digestion by augmenting the chicks own capacity for protein, starch and fat digestion by removing antinutritional factors (ANFs) which interfere with the normal processes of digestion or by digestion of fibre components that would otherwise pass undigested throughout the gastrointestinal tract.
3. Interaction of the microflora in both the small intestine and caecum with the digesta makes determination of the accurate feeding value of a fibre degrading enzyme particularly difficult to access by classical digestibility techniques (Bedford, 1996)

2.10. Sensory Evaluation of Eggs

Conventional sensory studies on eggs are often conducted either with trained or untrained panelist to score eggs with respect to organoleptic characteristics. Sensory attributes such as aroma, flavour, aftertaste, texture and overall acceptability are important and often assessed (Hayat *et al*., 2010a).

A study conducted by Hayat *et al*. (2010) to evaluate sensory attributes and consumer acceptance of eggs from flaxed seed-fed hens indicated a significant difference ($P < 0.01$) between flax, flax + δ- tocopherol and flax + BHT (a phenolic antioxidant) eggs by trained

panelist. In a second study, sensory attributes were tested by untrained panelist. In this study, majority (75 to 80%) of the panelist could not distinguish flax seed - fed versus control eggs for aroma and flavour. A consumer preference test was also conducted to guage end-user response to flax seed fed eggs. The consumer acceptance testing did not find any significant difference ($P > 0.05$) between control and flax seed fed eggs. A similar study by Parpinello *et al.* (2008) suggests that vegetable lipids (palm butter, grape seed, flax seed), n-3 PUFA (flax seed and marine algae) and rosemary can be used in hens' diet without affecting the sensory properties of eggs.

Organoleptic test of regular and nutrient enriched eggs by Hayat *et al*. (2014) showed no considerable difference. The researchers based on their findings to conclude that eggs could be enriched with different micronutrients by nutritional manipulation without deteriorating egg internal and sensory qualities. Leke *et al*. (2015) also reported a highest rating of egg colour from hens fed 10% head entrails (HE), 10% filleting waste and 10% archon when the researchers undertook a study to investigate the organoleptic characters of eggs laid by local hens (Gallus domesticus) fed different proportions of Skipjack fish waste (SFW) as a source of omega-3 fatty acids. The industrial waste was processed in three forms namely (HE), filleting waste and arachon. For egg aroma, the highest score was at level 10% HE, 10% filleting waste and 5% arachon in the hen's diet. The highest rating for both egg texture and flavour were from the hens fed diets containing 10% HE, 5% filleting waste and 10% arachon. It was concluded that the use of 10% level of HE, filleting waste and arachon of Skipjack fish industrial waste in the local hen diets achieved the best products results on characteristics of colour, aroma, texture and flavour of eggs.

A study by Mahfuz *et al*. (2019) indicated that *Moringa oleifera* leaf powder at 0%, 0.2%, 0.4%, 0.6% and 0.8% inclusion levels had no significant effect ($P > 0.05$) on sensory evaluation of egg quality.

2.11. Economic Efficiency of Feed in Production of Layers

Commercial layer production is an important income generating venture in the livestock sector in Ghana. Economic efficiency of feed plays a vital role and is a prime consideration in the establishment and success of any commercial layer production. Feed is the major cost item in commercial layer production and the efficient the feed, the better the cost of production whereas eggs are the major products in commercial layers and the higher the egg production the better the profit (Farooq *et al*., 2001; 2002). A commercial layer production enterprise can be made more profitable if attention is given to cost of feed. Higher cost of feed is associated with lower profitability of laying hens, while higher length of production cycle, higher sale of eggs and higher laying percentage are associated with higher profitability (Altahat *et al*., 2012). Critical limits for various cost components should be used as a guide line to adjust budget in commercial egg production to ensure higher net profit per bird (Altahat *et al*., 2012).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1. Study Area and Period of Study

The study was conducted at the Poultry Section of the Department of Animal Science Education, College of Agriculture Education, University of Education; Winneba (UEW), Mampong – Ashanti. The study started in January, 2019 and ended in September, 2019.

Geographically, Mampong-Ashanti lies in the transitional zone between the Guinea Savana zone to the north and the tropical rain forest zone to the South of Ghana along the Kumasi-Ejura trunk road. Mampong-Ashanti is 57 km, north of the Ashanti regional capital, Kumasi. It is located within longitude 0.05° and 1.30° west and latitude 6.55° and 7.30° north with an altitude of 547m above sea level, covering a total land area of 2346 km^2. The rainfall pattern of Mampong-Ashanti is bimodal; with the peak rainfall period occurring from April to July designated as major rainy season and September to October (minor rainy season). The annual average rainfall is 1300 mm with dry periods between July – August and November - February. The mean daily temperature is between 25°C and 30°C with a high relative humidity of 91.5% (MSD, 2013).

3.2. Number and Source of Experimental Birds

Two hundred day-old layer chicks (Lohmann Brown Strain) were procured from Akate Farms in Kumasi for the experiment.

3.3. Source of UDMOSC and Processing

Undeshelled defatted *Moringa oleifera* seed Cake (UDMOSC) was obtained from Ghana Permaculture Institute, Techiman – Ghana. The moringa seed cake was milled at the feed room of the Department of Animal Science Education, College of Agriculture Education, University of Education Winneba (UEW).

3.4. Proximate Analysis of UDMOSC and Experimental Diets

A sample of the UDMOSC and the experimental diets were taken to nutrition laboratory for proximate analysis. Determination of the chemical composition was done according to the procedure outlined by Association of Official Analytic Chemists (AOAC) (2002). The metabolisable energy (ME) (kcal/kg) was calculated according to the formula derived by Pauzenga (1985):

ME (Kcal/kg) = (37 x %CP) + (81.8 x %EE) + (35 x %NFE).

3.5. Experimental Procedure and Housing

The experiment was conducted in three phases comprising starter phase, grower phase and layer phase. The birds were randomly allotted to five treatment groups with three replicates in a completely randomized design (CRD) using the deep litter system of management from 0- 8 weeks (starter phase), 8 – 22 weeks (grower phase) and 22 – 36weeks (layer phase).

In the starter phase, each of the five treatments had thirty (30) chicks with 10 chicks in each of the three replicates.In the grower phase, each of the five treatments had 30 pullets with

10 pullets in each of the three replicates. In the final phase (layer phase), each of the five treatments had 24 hens with 8 hens in each of the three replicates.

3.6. Weight of Birds

In the starter phase, the chicks were individually weighed with an electronic digital balance, Zhejiang, (China), with a 0.01 precision to get the initial and final starter body weights. The weights recorded for each treatment was used to determine the initial and final average starter body weight. The initial and final average body weights were used to determine body weight gain by differences.

In the grower phase, the average final body weights of the starters served as initial average body weights of the growers. The growers (pullets) were individually weighed at the end of phase 2 using an electronic balance to get the final body weight which was subsequently used to determine the grower (pullets) average body weight gain.

In the final phase (layer phase), the final average body weight of the pullets which also served as the initial average body weight of the hens and the final average body weight of the hens were used to determine the layer average body weight gain.

3.7. Experimental Diets

Five experimental diets were formulated and designated as UDMOSC^{0}, UDMOSC^{5}, UDMOSC^{10}, UDMOSC^{15}, and UDMOSC^{15E} such that UDMOSC^{0}(control), contained no UDMOSC whereas UDMOSC^{5}, UDMOSC^{10}, UDMOSC^{15} and UDMOSC^{15E} contained 5%,

10%, 15% and 15% with enzyme respectively as percentage inclusion of UDMOSC in the diets for each of the three phases. Crude protein (CP) and metabolisable energy (ME) were generated by the formula whereas crude fibre (CF) and ether extract (EE) were calculated based on per 100kg content of 2.20%, 11.00%, 7.00% and 1.00% for maize, wheat bran, soyabean and fish (anchovy and Tuna) respectively for crude fibre and 3.80%, 3.00%, 0.80% and 5.00% for maize, wheat bran, soyabean and fish (Anchovy and Tuna) respectively for ether extract. A crude fibre content of 20.87% and an ether extract content of 4.30% of the test sample from proximate analysis were also used in calculating crude fibre and ether extract respectively.

3.7.1. Starter diets

The starter diets were formulated to contain 21.03% CP and 2800 ME Kcal/Kg to meet NRC (1994) nutrient requirement for layer starters. The formula used in formulating the starter diet is shown in Table 3.1.

Table 3.1: Composition of starter diets (week 1 – 8)

Ingredients	UDMOSC^{0}	UDMOSC^{5}	UDMOSC^{10}	UDMOSC^{15}	UDMOSC^{15E}
Maize	56.5	55	53.5	53	53
Wheat bran	13	12.5	12	10.5	10.5
Soybean meal	9	6	2	0	0
UDMOSC	0	5	10	15	15
Anchovy	8.5	8.5	8.5	8.5	8.5
Tuna fish	10	10	11	10	10
Dicalcium	0.5	0.5	0.5	0.5	0.5
Vit/premix	0.5	0.5	0.5	0.5	0.5
Oyster shell	1.5	1.5	1.5	1.5	0.5
Salt	0.5	0.5	0.5	0.5	0.5
Total	**100 kg**	**100kg**	**100kg**	**100kg**	**100kg**
Generated/ calculated analysis					
CP (%)	21.09	21.11	21.16	21.08	21.08
CF (%)	3.49	4.23	4.92	5.64	5.64
EE (%)	3.53	3.65	3.81	3.90	3.90
ME (%)	2717.55	2713.90	2711.50	2727.10	2727.10

Acronyms: UDMOSC – Undeselled defatted Moringa oleifera seed cake, CP – Crude protein, CF - Crude Fibre, EE – Ether Extract, ME – Metabolisable Energy, Vit/premix – All Essential Vitamins

3.7.2. Grower (pullets) diets

The grower diets were formulated to contain (15% CP and 2850 ME Kcal/Kg) to meet NRC (1994) nutrient requirement for pullets. The formula used in formulating the pullets' diet is shown in Table 3.2.

Table 3.2: Composition of pullet diets (week 9 – 22)

Ingredients	UDMOSC^{0}	UDMOSC^{5}	UDMOSC^{10}	UDMOSC^{15}	UDMOSC^{15E}
Maize	60	60	59.6	60	60
Wheat bran	20	18	16	13	13
Soybean	7.5	5.5	3.5	2.3	2.3
UDMOSC	0	5	10	15	15
Anchovy	0	0	0	0	0
Tuna fish	7.4	6.8	6.3	5	5
Dicalcium	0.5	0.5	0.5	0.5	0.5
Vit/premix	0.5	0.5	0.5	0.5	0.5
Oyster shell	3.6	3.2	3.2	3.2	3.2
Salt	0.5	0.5	0.5	0.5	0.5
Total	**100**	**100**	**100**	**100**	**100**
Generated/ calculated analysis					
CP (%)	15.23	15.30	15.39	15.37	15.37
CF (%)	4.12	4.80	5.47	6.09	6.09
EE (%)	3.25	2.90	3.51	3.58	3.58
ME (%)	2611.74	2526.99	2427.85	2348.19	2348.19

Acronyms: UDMOSC – Undeselled defatted Moringa oleifera seed cake, CP – Crude protein, CF - Crude Fibre, EE – Ether Extract, ME – Metabolisable Energy, Vit/premix – All Essential Vitamins

3.7.3. Layer diets

The layer diets were formulated to contain 16% CP and 2850 ME Kcal/Kg to meet NRC (1994) nutrient requirement for layer chicken. The formula used in formulating the layer diets is shown in Table 3.3.

Table 3.3: Composition of layer diets (week 23 – 36)

Ingredients	UDMOSC^{0}	UDMOSC^{5}	UDMOSC^{10}	UDMOSC^{15}	UDMOSC^{15E}
Maize	57	54.50	53.00	52.00	52.00
Wheat bran	16	16.00	15.00	14.00	14.00
Soybean	5.0	3.00	2.00	0.00	0.00
UDMOSC	0.0	5	10.00	15	15
Anchovy	6.5	6.50	5.50	6.00	6.00
Tuna fish	7.0	6.50	6.00	4.50	4.50
Dicalcium	0.5	0.5	0.50	0.50	0.50
Vit/premix	0.5	0.5	0.50	0.50	0.50
Oyster shell	7.0	7.00	7.00	7.00	7.00
Salt	0.5	0.5	0.50	0.50	0.50
Total	**100**	**100**	**100**	**100**	**100**
Generated/ calculated analysis					
CP (%)	17.11	15.56	15.86	15.77	15.77
CF (%)	3.44	4.34	5.16	5.92	5.92
EE (%)	3.30	3.44	3.49	2.73	2.73
ME (%)	2561.67	2563.70	2582.64	2611.15	2611.15

Acronyms: UDMOSC – Undeselled defatted Moringa oleifera seed cake, CP – Crude protein, CF - Crude Fibre, EE – Ether Extracts, ME – Metabolisable Energy, Vit/premix – All Essential vitamins

3.8. Feeding

Feed was prepared using the feed formula for the respective dietary treatments (Tables 3.1, 3.2 and 3.3) and labeled as UDMOSC^{0}, UDMOSC^{5}, UDMOSC^{10}, UDMOSC^{15} and UDMOSC^{15E}. The feed was weighed with the aid of a weighing balance and supplied to the birds *ad libitum* daily. Left over feed was also weighed and recorded daily.

3.9. Health Care and Disease Control

The poultry house was scrubbed thoroughly and disinfected one week before the arrival of the experimental birds. Pens were kept clean by changing the litter every four weeks. Strict

vaccination schedule was followed throughout the experimental period. The following vaccines were administered as shown in Table 3.4**:**

Table 3.4: Vaccination schedule for the experimental birds

Vaccine	Age of Bird	Route of Administration
1st Gumboro	Week 1	Through drinking water
Hb1(1st Newcastle)	Week 2	Through drinking water
2nd Gumboro	Week 3	Through drinking water
Lasota(2ndnewcastle)	Week 4	Through drinking water
Fowl pox	Week 8	Through drinking water
Newcavac (3rd newcastle)	Week 16	Through injection

3.10. Data Collection

In the starter and grower phases, data were collected on body weight, feed intake and mortality. In the layer phase, data was collected on body weight, feed intake, egg production, internal and external egg characteristics, haematological and biochemical indices and sensory analysis of eggs.

3.10.1 Initial body weight

At the start of the experiment, number of birds in each replicate was weighed with the aid of an electronic balance and the values obtained divided by their respective numbers to get the mean initial body weight (grams per bird per replicate). The final weights of the starter phase served as the initial weights of the grower phase and the final weights of the grower phase served as the initial weights of the layer phase.

3.10.2. Feed intake (FI)

At the start and through to the end of each phase, daily feed intake for each replicate was determined by subtracting daily left over feed from daily feed supplied to each replicate.

Total feed intake for each replicate was subsequently calculated by summing up the daily feed intake of the respective replicates. Total feed intake per replicate was divided by number of birds in each replicate to get feed intake per bird per replicate.

3.10.3. Final body weight

At the end of each phase, number of birds in each replicate was weighed with the aid of an electronic balance and the values obtained divided by their respective numbers to get the mean final body weight (grams per bird per replicate).

3.10.4. Body weight gain

Body weight gain was determined by subtracting the initial body weights from the final body weights of each phase to get the body weight gain for each phase.

3.10.5. Feed conversion ratio (FCR) of starters and growers

Feed conversion ratio for the starter and grower phases were calculated by dividing the total feed intake per bird (grams per bird) by total weight gain per bird (grams per bird) as illustrated below:

$$\text{FCR} = \frac{\text{Total feed Intake per bird (g)}}{\text{Total weight gain per bird (g)}}$$

3.10.6. Feed conversion ratio (FCR) of layers

Feed conversion ratio (FCR) for the layer phase was calculated by dividing the total feed intake per bird for the laying period (grams per bird) by the total egg mass per bird for the laying period according to the formula:

$$\text{FCR} = \frac{\text{Total feed intake per bird (g)}}{\text{Total egg mass per bird (g)}}$$

3.10.7. Age at point of Lay, weight of first egg and age of 50% lay

The age (in days) from day old to the date on which the first egg in each replicate was laid was recorded. The weight of the first egg of each replicate was also recorded. The age at which the eggs collected from a replicate corresponded to half the number of hens in that replicate was recorded. The sum of values of the respective replicates in each treatment were subsequently divided by 3(number of replicates in that treatment) to get age at point of lay, weight of first egg and age of 50% lay.

3.10.8. Egg production

Hen-day and hen-housed egg production were determined for the experimental period. Hen-day egg production (HDEP) was determined daily by dividing number of eggs in a pen or replicate by the number hens in the pen that day. Hen-housed egg production (HHEP) was determined by dividing number of eggs in a pen on daily basis by the total number of hens housed in that pen at the start of the experiment. Percentage hen-day and hen-housed egg production were computed respectively using the formula given by North (1984):

$$\text{HDEP (\%)} = \frac{\text{Total number of eggs laid on daily basis}}{\text{Total number of hens available that day}} \; x\; 100\%$$

$$\text{HHEP (\%)} = \frac{\text{Total number of eggs laid on daily basis}}{\text{Total number of hens housed at the start of the experiment}} \; x\; 100\%$$

Average percentage values of hen-day and hen-house egg production were determined to be percentage hen-day and hen-house egg production for the experimental period.

3.10.9. Egg quality traits

Egg quality traits were measured bi-weekly. A total of 30 eggs (6 from each treatment) were collected fortnightly and examined for external and internal egg quality characteristics.

3.10.10. External egg quality characteristics

The following external egg quality characteristics: egg weight (EW), egg length (EL), egg circumference (EC), shell weight (SW), and shell thickness (ST) were measured.

Egg weight measurement was taken daily from the first egg produced till the end of the laying phase. The eggs were individually weighed with an electronic digital balance, Zheijiang, (China), with a range of 0.01g precision. This was used to determine the average weight of the eggs.

Egg length and circumference measurements were determined fortnightly. A digital venier caliper calibrated in mm was used to determine egg length in milimetres. A thread and a ruler were used to determine egg circumference. Egg shell weight was determined using an electronic digital balance, Zhejiang, (China) with 0.01g precision.

Shell thickness was determined with a digital micrometer screw gauge. The shell with the inner membrane was first removed with a tissue paper and air-dried at room temperature for 24 hours according to Hagan *et al*. (2013). To achieve accuracy, the shell thickness was taken from three points: the narrow, broad and middle of the shell as described by Kabir *et al*. (2014).

3.10.11. Internal egg quality characteristics

Internal egg quality characteristics: albumen weight (AW), yolk weight (YW), albumen height (AH), yolk height (YH), albumen length (AL), yolk length (YL) and yolk colour (YC) were determined. Six eggs from each treatment were selected for internal quality traits determination forthnightly. Individual eggs were broken out on a petri dish for internal egg quality characteristic measurements as outlined by Sola – Ojo *et al.* (2013). Fresh eggs laid within 2 hours were collected and measured according to Samli *et al.* (2005).

Albumen and yolk weight were determined using an electronic digital balance, Zhejiang, (China), with a range of 0.01g precision. The yolk was carefully separated from the albumen and placed in a petri dish. The weight of the albumen and the yolk were determined as the difference between the weight of the petri dish together with the albumen or the yolk and the weight of the petri dish.

The albumen and yolk height were measured with a thin broom and a vernier caliper after carefully separating the albumen from the yolk manually with a tea spoon. Extra care was taken to avoid breaking the viteline membrane that encloses the yolk as outlined by Raji *et al.* (2009).

Albumen and yolk length were also determined using the digital venier caliper.

Yolk colour score was determined using the Roche yolk colour fan. This was done by breaking the egg on a petri dish and the colour of the yolk matched with that of the Roche yolk colour fan and the number recorded.

3.10.12. Mortality

Mortality was recorded as and when it occurred throughout the experimental period. Post – mortem examination was carried out on some of the dead birds to ascertain the cause of death.

3.11. The Haugh Unit

The Haugh unit was calculated from the values obtained for albumen height and egg weight as derived by Haugh (1937):

$Hu = 100 \log (H + 7.5 - 1.7W^{0.37})$

Where:

Hu = Haugh unit

H = height of albumen (mm)

W = egg weight (grams)

The Haugh unit values were ranked from 0 – 130 according to Bhhale *et al.* (2003) as:

AA = 72 –130% (Excellent), A = 60 – 71% (good), B = 31 – 59% (average) and C = 0 – 30% (poor).

3.12. Haematological and Biochemical Parameters

Three hens from each treatment (one from each replicate) were randomly selected at the end of the experiment for haematological and biochemical component analysis. Blood

samples were collected from the wing vein into tubes containing Ethyldiamine tetra acetic acid (EDTA) as anti – coagulant for haematological components while blood for biochemical components were collected into coagulated tubes using 5ml syringes. The blood samples were taken to laboratory for analysis. For the haematological components, the blood samples were mixed and inserted into an automated haematolgy analyzer (Mindray BC – 3000 plus) to determine White Blood Cell (WBC total count), types of White Blood Cells (WBCs individual counts), Red Blood Cell (RBCs), Haemoglobin (HB), Hematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemaglobin (MCH) and Mean Corpuscular Haemaglobin Concentration (MCHC). Biochemical components analyzed were Total cholesterol (TC), Triglyceride (TRG), High Density Lipoprotein (HDLC), Low Density Lipoprotein (LDLC), and Very Low Density Lipoprotein (vDLC).

3.13. Economic Efficiency of UDMOSC in Layer Production

The economic efficiency of UDMOSC in production was determined using the prevailing price per kilogram of conventional feed, price per kilogram of UDMOSC, kilogram body weight gain, price per crate of eggs and uniform distribution of all other cost at the time of the experiment. The price per kilogram of conventional feed was multiplied by the total kilograms of conventional feed consumed per bird to get the cost of conventional feed per bird. The price per kilogram UDMOSC was multiplied by the total kilograms of UDMOSC consumed per bird to get the cost of UDMOSC per bird. Cost of conventional feed per bird and cost of UDMOSC per bird were summed up to get total cost of feed per bird. In the starter and grower phases, cost per kilogram body weight gain was determined by dividing

cost of feed per bird by kilogram body weight gain per bird. In the layer phase, average price per crate of eggs was determined and multiplied by the crates of eggs per bird to get total revenue per bird.

Net revenue was then computed as;

Net revenue (GHC) per bird = Total revenue per bird – Total cost of feed per bird.

3.14. Sensory Evaluation of Eggs

Twelve adult panelists were selected from University of Education Winneba – Mampong campus by invitation and willingness to consume prepared eggs. All the participants declared no allergy to eggs and egg products. The panelists were given a preliminary training and protocols well explained before the actual testing session. For each sensory characteristic, panalists were instructed to score the intensity of evaluation on a 5 points hedonic scale developed for this purpose; thus 1 = not intense, 2 = slightly intense. 3 = moderately intense, 4 = largely intense and 5 = extremely intense.

Egg samples were prepared as described by Hayat *et al*. (2010b). Three eggs from each treatment were boiled in a stainless steel saucepan which contained 24.9 oz (700ml) of tap water at $100C^0$ for five minutes. After boiling, the water was drained from the saucepan and the strained eggs cooled in cold tap water. The eggs were peeled and then cut into quarters (length – wise) for delivery into sample plates. Each egg was quartered and then ¼ eggs wrapped in a 15 x 12cm aluminium foil identified with a 3-digit code. Care was taken to ensure that each panelist received a quarter of egg from each of the treatments.

3.15. Statistical Analysis

The data obtained were subjected to analysis of variance (ANOVA) using Genstat statistical package version 11.1(2008) and means separated by the Least Significant Difference (LSD) test at 5% ($p<0.05$) significant level.

CHAPTER FOUR

4.0. RESULTS

4.1. Proximate Composition of UDMOSC

The proximate composition of the UDMOSC sample is presented in Table 4.1.

Table 4.1: Proximate composition of UDMOSC

Fractions on as fed basis	Composition
Moisture (%)	9.00
Crude protein (%)	31.67
Crude fibre (%)	20.87
Crude fat (%)	4.30
Ash (%)	5.50
Nitrogen free extracts (NFE) (%)	28.66
Metabolizable energy (ME) Kcal/kg	2841.63

Undeshelled defatted *Moringa oleifera* seed cake (UDMOSC) sample had a crude protein content of 31.67%. Moisture, crude fibre, ether extract, ash and nitrogen free extracts were 9.00%, 20.87%, 4.30%, 5.50% and 28.66% respectively. Metabolisable energy calculated for the test sample was 2841.63 Kcal/kg (Table 4.1).

4.2. Results of Starter Phase of Lohmann Brown Layers

4.2.1. Proximate composition of layer chicken starter diets

Table 4.2 represents the proximate composition of the starter diets.

Table 4.2: Proximate composition of the starter diets

Fraction (%)	UDMOSC^{0}	UDMOSC^{5}	UDMOSC^{10}	UDMOSC^{15}	UDMOSC^{15E}
MC	12.30	11.00	14.50	12.00	12.00
CP	15.37	18.04	18.17	17.60	17.60
EE	3.50	5.50	5.50	4.50	4.50
CF	3.00	4.90	6.86	5.88	5.88
Ash	6.00	7.00	6.50	5.75	5.75
NFE	72.13	64.56	62.97	66.27	66.27
DM	87.70	89.00	85.50	88.00	88.00

Acronyms: CP – Crude Protein, EE – Ether Extract, CF – Crude Fibre, MC – Moisture Content, NFE – Nitrogen Free Extract, DM – Dry Matter.

Results of proximate analysis on the starter diets in Table 4.2 showed that dry matter (DM) ranged between 85.50% in UDMOSC^{10} to 89.00% in UDMOSC^{5}. Crude protein (CP), ether extract (EE) and crude fibre (CF), were lowest in UDMOSC^{0} at 15.37%, 3.50% and 3.00% respectively. However, crude protein was highest in both UDMOSC^{15} and UDMOSC^{15E} at 17.60%. Ether extract and crude fibre were higher in UDMOSC^{10} diet (5.50% and 6.86% respectively). Nitrogen free extracts was higher in diet UDMOSC^{0} (72.13%) and lower in diet UDMOSC^{10} (62.97%). Ash was similarly lowest in UDMOSC^{15} and UDNOSC^{15E} diets (5.75%). Moisture content was highest (12.30%) in UDMOSC^{10} but lowest (11.00) in UDMOSC^{5}.

4.2.2. Effect of UDMOSC on growth performance of starter chicks

Table 4.3 represents the effect of UDMOSC on growth performance of the starter layer chicks.

Table 4.3: Effect of UDMOSC on growth performance of starter chicks

Parameters	$UDMOSC^{0}$	$UDMOSC^{5}$	$UDMOSC^{10}$	$UDMOSC^{15}$	$UDMOSC^{15E}$	LSD	PV
MIBW (g/bird)	34.53	33.77	33.93	33.63	33.77	1.09	0.06
MFI (g/bird)	2781^{a}	2316^{b}	2242^{b}	2326^{b}	2187^{b}	303.80	0.01
MFBW (g/bird)	416	348	401	399	357	82.70	0.33
MBWG (g/bird)	440.30^{a}	392.60^{ab}	368.40^{bc}	368.40^{bc}	324.50^{c}	64.61	0.02
FCR	6.30^{ab}	5.57^{b}	6.13^{ab}	6.33^{ab}	6.70^{a}	1.01	0.03
MM	0.00^{b}	1.67^{ab}	3.00^{a}	0.67^{b}	1.33^{ab}	1.99	0.01

Means within rows with different superscripts are significantly (P < 0.05) different. UDMOSC= undeshelled defatted Moringa oleifera seed cake, MIBW= mean initial body weight, MFI= mean feed intake, MFBW= mean final body weight, MBWG= mean body weight gain, FCR= feed conversion ratio, MM= mean mortality rate, LSD= least significant difference, PV= probability value.

The results showed that the starters responded differently to different inclusion levels of UDMOSC. Dietary treatment had a significant (P = 0.01) effect on total feed intake. The chicks that received UDMOSC treatment consumed similar lower (P = 0.01) feed with the control group consuming the highest feed (Table 4.3). Final body weight was not affected by the treatment diet (P = 0.33). However, body weight gain was significantly affected (P = 0.02) with the chicks on $UDMOSC^{0}$ and $UDMOSC^{5}$ diets gaining similar (P > 0.05) highest body weight whilst those on $UDMOSC^{10}$, $UDMOSC^{15}$ and $UDMOSC^{15E}$ gaining a similar (P > 0.05) lower body weight. Feed conversion ratio was also significantly (P = 0.01) affected with the chicks on $UDMOSC^{5}$ having a better conversion of feed. A significant difference (P = 0.01) was also observed in mortality rate with starter chicks on $UDMOSC^{5}$, $UDMOSC^{10}$ and $UDMOSC^{15}$ diets having the highest (P > 0.05) mortality rate. The lowest mortality rate was recorded by starter chicks fed $UDMOSC^{0}$ and $UDMOSC^{15}$ (P > 0.05).

4.2.3. Economic efficiency of UDMOSC in production of starter layer chicks

Table 4.4 represents the economic efficiency of UDMOSC in the starter layer production.

Table 4.4: Economic efficiency of UDMOSC in production of starter layer chicks

Parameters	**UDMOSC^{0}**	**UDMOSC^{5}**	**UDMOSC^{10}**	**UDMOSC^{15}**	**UDMOSC^{15E}**
MCCI (GHC)	5.00	3.63	3.21	2.93	2.75
MCUDMOSC (GHC)	0.00	0.06	0.11	0.18	0.17
Total cost of feed (GHC)	5.00	3.69	3.32	3.11	3.17
MBWG (kg)/bird	0.44	0.39	0.37	0.37	0.32
Cost/kg BWG	11.36	9.46	8.97	8.41	9.91

GHC5.40=1$ at the time of the experiment, UDMOSC= undeshelled defatted Moringa oleifera seed cake, MCCI= mean cost of conventional ingredients, MCUDMOSC= mean cost of undeshelled defatted Moringa Oleifera seed cake, MBWG = mean body weight gain, E = enzyme.

Analysis of economic efficiency of UDMOSC (Table 4.4) showed that, feed cost of producing layer chicks on the control diet was higher (GHC5.00) as compared to the test diets (GHC3.69, GHC3.32, GHC3.11 and GHC3.17 for UDMOSC^{5}, UDMOSC^{10}, UDMOSC^{15} and UDMOSC^{15E} respectively). Total cost of feed decreased with increasing levels of UDMOSC except UDMOSC^{15E} which came as a result of the addition of enzyme.The analysis further showed that it would cost less to gain per kilogram body weight with the inclusion of UDMOSC as compared to the control diet.

4.3. Results of Grower Phase of Lohmann Brown Layers

4.3.1. Proximate composition of the grower diets

Table 4.5 represents the proximate composition of the grower diets.

Table 4.5: Proximate composition of the grower diets

Fraction (%)	UDMOSC^{0}	UDMOSC^{5}	UDMOSC^{10}	UDMOSC^{15}	UDMOSC^{15E}
MC	15.00	14.00	14.10	11.50	11.50
CP	14.00	15.60	17.00	18.65	18.65
EE	5.50	4.50	5.50	5.70	5.70
CF	6.16	6.64	6.89	7.11	7.11
Ash	10.00	11.50	5.60	12.00	12.00
NFE	64.34	61.76	65.01	56.54	56.54
DM	85.00	86.00	86.00	88.50	88.50

Acronyms: CP – Crude Protein, EE – Ether Extract, CF – Crude Fibre, MC – Moisture Content, NFE – Nitrogen Free Extract, DM – Dry Matter.

Resuls of proximate analysis of the grower diets in Table 4.5 shows that dry matter content ranged from 85.00% in UDMOSC^{0} to 88.50% in both UDMOSC^{15} and UDMOSC^{15E}. Crude protein and crude fibre were lowest in UDMOSC^{0} at 14.10% and 6.16% respectively but higher in UDMOSC^{15} and UDMOSC^{15E} at 18.65% and 7.11% respectively. Nitrogen free extracts was higher in diet UDMOSC^{5} at 67.01% but lower in UDMOSC^{15} and UDMOSC^{15E} at 56.54%. Higher fraction of ether extract was observed in UDMOSC^{15} and UDMOSC^{15E} at 5.7% but lower in UDMOSC^{10} at 5.5%. Ash content was lower in UAMOSC^{10} at 5.6% but higher in UDMOSC^{15} and UDMOSC^{15E} at 12.00%. Diets UDMOSC^{15} and UDMOSC^{15E} showed a lower content of moisture at 11.50% with diet UDMOSC^{0} showing a higher content at 15.00%.

4.3.1. Effect of UDMOSC on growth performance of growers

Table 4.6 represents the effect of UDMOSC on growth performance of growers.

Table 4.6: Effect of UDMOSC on growth performance of growers

Parameters	UDMOSC^{0}	UDMOSC^{5}	UDMOSC^{10}	UDMOSC^{15}	UDMOSC^{15E}	LSD	PV
MIBW (g/bird)	416	348	401	399	357	82.70	0.07
MFI (g/bird)	8337^{a}	6536^{b}	6222^{b}	5874^{b}	7370^{a}	1333.50	0.01
MFBW (g/bird)	1758^{a}	1477^{b}	1349^{bc}	1194^{bc}	1113^{c}	252.5	0.00
MBWG (g/bird)	1342^{a}	1129^{b}	948^{c}	915^{c}	756^{d}	155.10	0.001
FCR	6.20	5.83	6.53	6.00	6.17	0.82	0.45
MM	1.67	2.33	2.33	1.33	2.33	1.76	0.60

Means within rows with different superscripts are significantly (P < 0.05) different. UDMOSC= undeshelled defatted Moringa oleifera seed cake, MIBW= mean initial body weight, MFI= mean feed intake, MFBW= mean final body weight, MBWG= mean body weight gain, FCR= feed conversion ratio, MM= mean mortality rate, LSD= least significant difference, PV= probability value.

At the pullet stage, the final weight for phase 1 did not vary significantly (P = 0.07) (Tables 4.3 and 4.6). Feed conversion ratio and mortality rate of the pullets were not also significantly affected (P = 0.45 and 0.60 respectively). However, feed intake, final body weight and body weight gain of the pullets were significantly affected (P = 0.01, 0.00 and <.00 respectively). The pullets fed UDMOSC^{0} inclusion level performed better in terms of feed intake, final body weight and body weight gain (Table 4.6). Pullets fed UDMOSC^{15E} also performed better (P = 0.01) in terms of feed intake with those that received UDMOSC^{5}, UDMOSC^{10} and UDMOSC^{15} consuming less feed (P = 0.01). For final body weight, the lowest performance was observed by pullets fed UDMOSC^{10}, UDMOSC^{15} and UDMOSC^{15E} (P = 0.001). The highest final body weight gain was observed by pullets fed UDMOSC^{0} followed by pullets fed UDMOSC^{5} (P < 0.001). Pullets on UDMOSC^{15E} had the lowest final body weight gain (P < 0.001).

4.3.2. Economic efficiency of UDMOSC in production of growers

Table 4.7 represents the economic efficiency of UDMOSC in production of growers.

Table 4.7: Economic efficiency of UDMOSC in production of growers

Parameters	**UDMOSC^{0}**	**UDMOSC^{5}**	**UDMOSC^{10}**	**UDMOSC^{15}**	**UDMOSC^{15E}**
MCCI (GHC)	12.26	8.88	7.17	5.84	7.34
MCUDMOSC (GHC)	0.00	0.17	0.31	0.44	0.56
Total cost of feed (GHC)	12.26	9.05	7.48	6.28	8.15
MBWG (kg)/bird	1.34	1.13	0.95	0.92	0.76
Cost/kg BWG	9.15	8.00	7.87	6.83	10.72

GHC5.40=1$ at the time of the experiment, UDMOSC= undeshelled defatted Moringa oleifera seed cake, MCCI= mean cost of conventional ingredients, MCUDMOSC= mean cost of undeshelled defatted moringa Oleifera seed cake, MBWG = mean body weight gain, E = enzyme

Analysis of economic efficiency of UDMOSC in producing pullets showed that it would cost more to produce pullets on the control diet (GHC12.26) as compared to the test diets (GHC9.05, GHC7.48, GHC6.28 and GHC8.15 for UDMOSC^{5}, UDMOSC^{10}, UDMOSC^{15} and UDMOSC^{15E} respectively). Total cost of feed decreased with increasing levels of UDMOSC except the UDMOSC^{15E} treatment which came as a result of the addition of enzyme. Pullets fed UDMOSC^{15E} had the highest cost per kilogram body weight gain followed by pullets on UDMOSC^{0} with pullets on UDMOSC^{15} having the least cost per kilogram weight gain (Table 4.7).

4.4. Results of Layer Phase of Lohmann Brown Layers

4.4.1. *Proximate composition of the layer diets*

Table 4.8 represents the proximate composition of the layer diets.

Table 4.8: Proximate composition of the layer diets

Fraction (%)	UDMOSC^{0}	UDMOSC^{5}	UDMOSC^{10}	UDMOSC^{15}	UDMOSC^{15E}
MC	15.05	14.20	14.02	12.00	12.00
CP	14.10	15.00	16.02	18.64	18.64
EE	5.40	4.60	5.60	5.80	5.80
CF	6.18	6.84	6.90	7.16	7.16
Ash	10.12	11.15	4.90	12.80	12.80
NFE	64.20	62.41	66.58	55.60	55.60
DM	84.95	85.80	85.98	88.00	88.00

Acronyms: CP – Crude Protein, EE – Ether Extract, CF – Crude Fibre, MC – Moisture Content, NFE – Nitrogen Free Extract, DM – Dry Matter.

There was a gradual increase in the dry matter content of the layer diet starting from 84.95% in UDMOSC^{0} to 88.00% in UDMOSC^{15} and UDMOSC^{15E}. Crude protein, ether extract, crude fibre and ash were all higher in diets UDMOSC^{15} and UDMOSC^{15E} at 5.8%, 7.16% and 12.80% respectively. Both crude protein and ether extract were lower in UDMOSC^{5} at 11.10% and 4.60% respectively. However, crude fibre was lower at 6.18% in diet UDMOSC^{0} with ash being lower at 4.90% inUDMOSC^{10}. Nitrogen free extracts was lower in both diet UDMOSC^{15} and UDMOSC^{15E} at 5.80% but higher in UDMOSC^{10} at 67.58%. Moisture content was also lower in both UDMOSC^{15} and UDMOSC^{15E} at 12.00% but higher in UDMOSC^{0} at 15.05% (Table 4.8).

4.4.1. Effect of UDMOSC on productive performance of layers

Table 4.9 represents the effect of UDMOSC on productive performance of layers.

Table 4.9: Effect of UDMOSC on productive performance of layers

Parameters	$UDMOSC^{0}$	$UDMOSC^{5}$	$UDMOSC^{10}$	$UDMOSC^{15}$	$UDMOSC^{15E}$	LSD	PV
MIBW (g/bird)	1758^{a}	1477^{b}	1349^{bc}	1194^{bc}	1113^{c}	252.50	<.001
MFI (g/bird)	8755	8397	7845	5768	5912	3027.90	0.15
MFBW (g/bird)	1968.30^{a}	1642.10^{b}	1631.80^{b}	1678.30^{b}	1507.00^{c}	63.63	<.00
MBWG (g/bird)	210^{b}	165^{b}	283^{ab}	484^{a}	394^{ab}	243.90	0.02
FCR	2.30	2.70	3.03	2.47	2.90	0.74	0.23
HHEP (%)	58.70^{a}	48.50^{ab}	36.80^{b}	36.40^{b}	35.90^{b}	12.95	0.01
HDEP (%)	68.00^{a}	55.40^{b}	42.70^{c}	41.00^{c}	35.90^{c}	9.26	<.001
MEW (g)	62.30	58.60	53.10	52.30	53.90	16.43	0.63
MAFE (days)	169.00^{c}	174.70^{b}	178.30^{b}	211.30^{ab}	221.30^{a}	34.60	0.02
MWFE (g)	47.70	47.30	52.70	50.00	52.70	9.67	0.60
MAA50 (days)	179.30^{c}	191.30^{c}	208.30^{bc}	227.30^{ab}	236.70^{a}	29.09	0.001
Grades							
1^{st}	15.40	8.10	8.20	13.90	7.00	8.60	0.19
2^{nd}	42.20^{a}	25.90^{ab}	16.70^{b}	42.00^{a}	43.70^{a}	21.49	0.03
3^{rd}	42.40^{b}	66.10^{ab}	75.20^{a}	44.20^{b}	49.60^{b}	24.46	0.04
MNE	454^{a}	224^{b}	83^{c}	77^{c}	71^{c}	162.80	0.001
MM	1.33^{bc}	3.67^{a}	3.00^{a}	2.33^{ab}	0.00^{c}	1.41	0.01

Means within rows with different superscripts are significantly ($P < 0.05$) different. UDMOSC= undeshelled defatted Moringa oleifera seed cake, MIBW= mean initial body weight, MFI= mean feed intake, MFBW= mean final body weight, MBWG= mean body weight gain, FCR= feed conversion ratio, HHEP= hen-house egg production, HDEP= hen-day egg production, MEW= mean egg weight, MAFE= mean age of first egg, MWFE= mean weight of first egg, MAA= mean age at 50% lay, MNE= mean number of eggs, MM= mean mortality rate, LSD= least significant difference, PV= probability value.

The final weight of the grower phase serving as initial weight for the layer phase was significantly different ($P < 0.001$) (Tables 4.6 and 4.9). The results of the productive performance of layers (Table 4.9) also showed significant ($P < 0.05$) difference in all performance parameters except feed intake, feed conversion ratio, weigth of eggs, weight of first egg and grade one eggs ($P > 0.05$). Hens fed the control diet had the highest initial body weight with those fed $UDMOSC^{10}$, $UDMOSC^{15}$ and $UDMOSC^{15E}$ having the lowest

body weight ($P < 0.001$). The highest final body weight was measured by hens fed $UDMOSC^{0}$ with those fed $UDMOSC^{15}$ measuring the lowest body weight ($P < 0.001$). There was no significant difference between hens fed $UDMOSC^{5}$, $UDMOSC^{10}$ and $UDMOSC^{15}$ ($P > 0.05$) in terms of body weight measurement at the end of the experiment (Table 4.9). This however did not translate into body weight gain since the lowest body weight was recorded by hens fed the control diet and $UDMOSC^{5}$ ($P = 0.02$) with those on $UDMOSC^{10}$, $UDMOSC^{15}$, and $UDMOSC^{15E}$ recording similar higher body weight gain (Table 4.9). The highest hen-house egg production was recorded by hens fed $UDMOSC^{0}$ with hens fed $UDMOSC^{10}$, $UDMOSC^{15}$ and $UDMOSC^{15E}$ recording the lowest hen-house egg production ($P = 0.01$). Hen-day egg production was higher in hens fed $UDMOSC^{0}$ followed by hens fed $UDMOSC^{5}$ ($P < 0.001$). Hens fed $UDMOSC^{10}$, $UDMOSC^{15}$ and $UDMOSC^{15E}$ recorded the lowest hen-day egg production ($P < 0.001$). Age of first egg was earliest in hens fed $UDMOSC^{0}$ and latest in hens fed $UDMOSC^{15E}$ ($P = 0.02$) whereas age at 50% lay was significantly earlier in hens fed $UDMOSC^{0}$ and $UDMOSC^{5}$ and latest in hens fed $UDMOSC^{15E}$ ($P = 0.001$).

In terms of egg grading, there was no significant ($P = 0.19$) difference between grade 1 eggs. However, more medium size (grade 2) eggs were laid by hens fed $UDMOSC^{0}$, $UDMOSC^{5}$, $UDMOSC^{15}$, and $UDMOSC^{15E}$ ($P = 0.03$) with those fed $UDMOSC^{10}$ laying less medium size eggs. Many smaller size (grade 3) eggs were produced by hens fed $UDMOSC^{10}$ and $UDMOSC^{5}$ with hens fed $UDMOSC^{0}$, $UDMOSC^{15}$and $UDMOSC^{15E}$ laying the least small sized eggs ($P = 0.04$). The highest number of eggs was produced by hens fed the control diet ($UDMOSC^{0}$) followed by hens fed $UDMOSC^{5}$ ($P = 0.001$). The least number of eggs was produced by hens fed $UDMOSC^{15E}$.

Mortality rate on the other hand, was higher in hens fed UDMOSC^{5} and UDMOSC^{10} followed by hens fed UDMOSC^{0} and UDMOSC^{15} (P = 0.01). Hens fed UDMOSC^{15E} diet recorded zero or the least mortality rate.

4.4.2. Effect of UDMOSC on external and internal egg characteristics

Table 4.10 represents the effect of UDMOSC on external and internal egg characteristics.

Table 4.10: Effect of UDMOSC on external and internal egg characteristics

Parameters	UDMOSC^{0}	UDMOSC^{5}	UDMOSC^{10}	UDMOSC^{15}	UDMOSC^{15E}	LSD	PV
MEL (mm)	53.37^{b}	55.70^{ab}	56.22^{a}	55.68^{ab}	55.93^{a}	2.45	0.03
MEH (mm)	42.43^{a}	42.26^{a}	41.69^{b}	42.43^{a}	42.47^{a}	0.45	0.02
MEC (cm)	15.24^{b}	15.70^{ab}	15.72^{ab}	15.79^{a}	15.84^{a}	0.51	0.02
MSW (g)	5.62	5.47	5.45	5.64	5.64	0.49	0.82
MST (mm)	0.38^{a}	0.38^{a}	0.34^{b}	0.38^{a}	0.39^{a}	0.03	0.02
MAL (mm)	67.95^{c}	74.82^{ab}	75.16^{b}	77.95^{b}	83.48^{a}	4.27	<.001
MAH (mm)	10.38^{a}	8.36^{b}	8.74^{b}	8.50^{b}	8.76^{b}	0.88	0.001
MAW (g)	34.31^{a}	32.42^{ab}	31.75^{b}	34.37^{a}	34.48^{a}	2.04	0.04
MYL (mm)	37.22	38.87	39.35	40.51	41.41	4.31	0.31
MYH (mm)	17.20^{a}	15.82^{b}	16.62^{b}	17.46^{a}	17.51^{a}	0.82	0.001
MYW (g)	13.02	13.51	14.01	14.29	14.41	1.55	0.31
MYC	6.30^{a}	6.23^{a}	7.27^{a}	7.26^{a}	4.32^{b}	0.99	<.001
HU	100.00	91.35	94.67	93.70	94.58		

Means within rows with different superscripts are significantly (P < 0.05) different. UDMOSC= undeshelled defatted Moringa oleifera seed cake, MEL= mean egg length, MEH= mean egg height, MEC= mean egg circumference, MSW= mean shell weight, MST= mean shell thickness, MAL= mean albumen length, MAH= mean albumen height, MAW= mean albumen weight, MYL= mean yolk length, MYH= mean yolk height, MYW= mean yolk weight, MYC= mean yolk colour, HU= haugh unit, LSD= least significant difference, PV= probability value.

All the external egg characteristics assessed showed significant difference (P < 0.05) except shell weight (Table 4.10). Egg length was significantly high (P = 0.03) in eggs produced by hens fed UDMOSC^{15E} at 55.93mm but lower in eggs produced by hens fed UDMOSC^{0} at 53.37cm. Egg height was similarly high (P < 0.05) in eggs produced by hens fed UDMOSC^{0}, UDMOSC^{5}, UDMOSC^{15} and UDMOSC^{15E} at 42.43mm, 42.26mm,

42.43mm and 42.47mm respectively. Egg circumference was lower in eggs produced by hens fed $UDMOSC^{0}$ but significantly high (P = 0.02) in eggs produced by hens fed $UDMOSC^{15}$ and $UDMOSC^{15E}$ at 15.79cm and 15.84cm respectively (P > 0.05). Shell thickness was significantly (P = 0.02) lower in eggs produced by hens fed $UDMOSC^{10}$ but high in eggs produced by hens fed $UDMOSC^{0}$, $UDMOSC^{5}$,$UDMOSC^{15}$ and $UDMOSC^{15E}$ at 0.38mm, 0.38mm, 0.38mm and 0.39mm respectively.

Assessment of internal egg characteristics did not show any significant difference in yolk length and yolk weight (P = 0.31) (Table 4.10). Albumen was significantly longer (P < 0.001) in eggs produced by hens fed $UDMOSC^{5}$ and $UDMOSC^{15E}$ at 74.82mm and 83.48mm respectively but shorter in eggs produced by hens fed $UDMOSC^{0}$ at 67.95mm. Albumen height was significantly higher (P = 0.001) in eggs produced by hens fed $UDMOSC^{0}$ at 10.38mm but lower in eggs produced by hens fed $UDMOSC^{5}$, $UDMOSC^{10}$, $UDMOSC^{15}$ and $UDMOSC^{15E}$ at 8.36mm, 8.74mm, 8.50mm and 8.76mm respectively. Hens fed $UDMOSC^{10}$ produced eggs with lower albumen weight at 31.75g (P = 0.04) with hens fed $UDMOSC^{0}$, $UDMOSC^{15}$ and $UDMOSC^{15E}$ producing eggs with higher albumen weight at 34.31g, 34.37g and 34.48g respectively.

Eggs produced by hens fed $UDMOSC^{0}$, $UDMOSC^{15}$ and $UDMOSC^{15E}$ had higher (P = 0.001) yolk height at 17.20mm, 17.46mm and 17.51 mm respectively whilst eggs produced by hens fed $UDMOSC^{5}$ and $UDMOSC^{10}$ had lower egg yolk height at 15.82mm and 16.82mm respectively. Yolk colour score was generally higher (P < 0.001) for eggs produced by hens fed $UDMOSC^{0}$, $UDMOSC^{5}$, $UDMOSC^{10}$ and $UDMOSC^{15}$ at 6.30, 6.23,

7.27 and 7.26 respectively with hens fed UDMOSC^{15} having the lowest score at 4.32 yolk colour intensity. Haugh unit calculated in this study all fell within AA = 72 – 130% an excellent score for eggs of the control as well as the test diets.

4.4.3. Effect of UDMOSC on haematological profile of layers

Table 4. 11 represents the effect of UDMOSC on haematological profile of layers.

Table 4.11: Effect of UDMOSC on haematological profile of layers

Parameters	UDMOSC^{0}	UDMOSC^{5}	UDMOSC^{10}	UDMOSC^{15}	UDMOSC^{15E}	LSD	PV
RBC (x10^{12}/L)	2.51	2.33	2.19	2.09	2.19	0.46	0.36
Hb (g/dl)	10.83	10.00	9.60	9.27	9.47	9.87	0.41
HCT (%)	30.60	29.80	27.10	29.30	28.50	8.81	0.92
MCV (Fl)	122.50^{b}	128.20^{ab}	124.00^{ab}	139.20^{a}	123.00^{ab}	16.28	0.02
MCH (pg)	43.07	42.83	43.93	47.60	45.57	4.75	0.22
MCHC (g/dl)	16.33	14.33	12.67	12.33	13.33	5.68	0.55
WBC (x10^{12}/L)	6.07^{ab}	7.60^{a}	8.37^{a}	4.30^{b}	4.50^{b}	2.93	0.04

Means within rows with different superscripts are significantly (P < 0.05) different. UDMOSC= undeshelled defatted Moringa oleifera seed cake, RBC= red blood cell, Hb= haemoglobin, HCT= hematocrite, MCV= mean corpuscular volume, MCH= mean corpuscular haemoglobin, MCHC= mean corpuscular haemoglobin concentration, WBC= white blood cell, LSD= least significant difference, PV= probability value.

Total RBC, HB, HCT, MCH and MCHC counts were not significantly affected (P > 0.05). However, total MCV and WBC counts were significantly (P < 0.05) affected (Table 4.11). MCV was high (P = 0.02) in hens fed UDMOSC^{15} but low in hens fed UDMOSC^{0}. Total WBC count was higher in hens fed UDMOSC^{5} and UDMOSC^{10} (P = 0.04) but lower in hens fed UDMOSC^{15} and UDMOSC^{15E}.

4.4.4. Effect of UDMOSC on biochemical profile of layers

Table 4.12 represents the effect of UDMOSC on biochemical profile of layers.

Table 4.12: Effect of UDMOSC on biochemical profile of layers

Parameters	$UDMOSC^{0}$	$UDMOSC^{5}$	$UDMOSC^{10}$	$UDMOSC^{15}$	$UDMOSC^{15E}$	LSD	PV
TC (mmol/L)	3.97	3.87	3.67	2.80	2.97	2.34	0.72
TRG (mmol/L)	1.17	1.57	1.35	0.91	1.12	1.04	0.70
HDLC (mmol/L)	1.30^{b}	1.63^{ab}	1.77^{a}	1.33^{b}	1.20^{b}	0.33	0.02
LDLC (mmol/L)	2.14	1.52	1.29	1.06	1.25	1.96	0.77
vLDLC (mmol/L)	0.53	0.71	0.61	0.41	0.51	0.47	0.69

Means within rows with different superscripts are significantly (P < 0.05) different. UDMOSC= undeshelled defatted Moringa oleifera seed cake, TC= total cholesterol, TRG= triglyceride, HDLC= high density lipoprotein cholesterol, LDLC= low density lipoprotein cholesterol, vLDLC= very low density lipoprotein cholesterol, LSD= least significant different, PV= probability value.

In terms of biochemical indices, total cholesterol (TC), triglyceride (TRG), low density lipoprotein cholesterol (LDLC) and very low density lipoprotein (vLDLC) did not vary significantly (P > 0.05). Only high density lipoprotein cholesterol was affected by the dietary treatment (P = 0.02). High density lipoprotein cholesterol was higher in hens fed $UDMOSC^{10}$ but lower in hens fed $UDMOSC^{0}$, $UDMOSC^{15}$ and $UDMOSC^{15E}$ (P = 0.02) (Table 4.12).

4.4.5. Effect of UDMOSC on WBC profile of layers

Table 4.13 represents the effect of UDMOSC on WBC profile of layers.

Table 4.13: Effect of UDMOSC on WBC profile of layers

Parameters	$UDMOSC^{0}$	$UDMOSC^{5}$	$UDMOSC^{10}$	$UDMOSC^{15}$	$UDMOSC^{15E}$	LSD	PV
LYM ($x10^{12}$/L)	5.13^{ab}	6.70^{a}	7.67^{a}	3.77^{b}	3.83^{b}	2.85	0.04
HET ($x10^{12}$/L)	0.40	0.43	0.37	0.27	0.30	0.29	0.70
MON ($x10^{12}$/L)	0.23^{a}	0.23^{a}	0.20^{ab}	0.07^{c}	0.13^{bc}	0.10	0.01
BAS ($x10^{12}$/L)	0.10	0.10	0.07	0.03	0.07	0.08	0.38
ESIN ($x10^{12}$/L)	0.17	0.17	0.10	0.10	0.13	0.16	0.79

Means within rows with different superscripts are significantly (P<0.05) different, LYM = Lymphocytes, HET = Heterophils, *MON* = Monocytes, *BAS* = Basophils, *ESIN* = Esinophils,

UDMOSC= undeshelled deffated Moringa oleifera seed cake, LSD= least significance different, PV= probability value.

In terms of WBC profile, number of heterophils, basophils and esinophils were not significantly affected (P > 0.05). Lymphocytes and monocytes were significantly affected (P < 0.05). Lymphocytes were significantly higher (P = 0.04) in hens fed $UDMOSC^{5}$ and $UDMOSC^{10}$ but lower in hens fed $UDMOSC^{15}$ and $UDMOSC^{15E}$. Monocytes were significantly higher in hens fed $UDMOSC^{0}$ and $UDMOSC^{5}$ but lower in hens fed $UDMOSC^{15}$ (P = 0.01) (Table 4.13).

4.4.6. Effect of UDMOSC on organoleptic characteristics of eggs

Table 4.14 represents the effect of UDMOSC on organoleptic characteristics of eggs.

Table 4.14: Effect of UDMOSC on organoleptic characteristics of eggs

Parameters	$UDMOSC^{0}$	$UDMOSC^{5}$	$UDMOSC^{10}$	$UDMOSC^{15}$	$UDMOSC^{15E}$	LSD	PV
AC	3.00	3.58	2.42	2.83	2.42	1.28	0.29
AA	2.25	2.33	2.75	2.75	2.58	0.99	0.70
AT	2.50	2.33	2.92	2.92	2.33	0.96	0.65
AF	2.17	2.42	2.33	2.58	2.58	0.88	0.80
AMF	2.58	2.75	2.75	2.42	2.50	1.33	0.97
AOQ	3.00	2.25	3.25	3.50	3.25	1.51	0.45
YC	2.00	2.33	1.92	2.25	2.58	1.04	0.63
YA	1.50	1.50	1.50	1.50	1.50	*	0.97
YT	2.58	2.17	2.42	2.83	2.58	1.05	0.71
YF	2.50	2.25	2.42	2.50	2.50	0.98	0.97
YMF	2.33	3.08	2.50	2.75	2.75	0.86	0.41
YOQ	2.83	3.00	2.92	2.50	3.25	1.18	0.72

UDMOSC= undeshelled defatted Moringa oleifera seed cake, AC= albumen colour, AA= albumen aroma, AT= albumen taste, AF= albumen flavour, AMF= albumen mouth feel, AOQ= albumen overall quality, YC= yolk colour, YA= yolk aroma, YT= yolk taste, YF= yolk flavour, YMF= yolk mouth feel, YOQ= yolk overall quality, LSD= least significant difference, PV= probability value.

The dietary treatments did not affect egg organoleptic attributes; colour, aroma, taste, flavour, mouth feel and overall quality ($P > 0.05$) (Table 4.14).

4.4.7. Economic efficiency of UDMOSC inproduction of eggs

Table 4.15 represents the economic efficiency of UDMOSC in production of eggs.

Table 4.15: Economic efficiency of UDMOSC in production of eggs

Parameters	**UDMOSC^{0}**	**UDMOSC^{5}**	**UDMOSC^{10}**	**UDMOSC^{15}**	**UDMOSC^{15E}**
MCCI (GHC)	12.87	7.89	8.96	5.73	5.88
MCUDMOSC (GHC)	0.00	0.21	0.39	0.43	0.45
Total cost of feed (GHC)	12.87	8.10	9.35	6.16	6.58
No. of eggs/bird	69.40	54.51	17.69	13.94	8.88
No. of crates/bird	2.31	1.82	0.59	0.46	0.30
Av.price/crate (GHC)	13	13	13	13	13
Total revenue (GHC)	30.03	23.66	7.67	5.98	3.9
Net revenue (GHC)	17.16	15.66	-1.68	-0.18	-2.68

GHC5.40 =1$ at the time of the experiment, UDMOSC= undeshelled defaatted Moringa oleifera seed cake, MCCI= mean cost of conventional ingredients, MCUDMOSC= mean cost of undeshelled defatted Moringa oleifera seed cake.

Analysis of economic efficiency of feed (Table 4.15) showed that total cost of feed was higher (GHC12.87) for hens on the control diet as compared to the test diet (GHC8.10, GHC9.35, GHC6.16 and GHC6.58 for UDMOSC^{5}, UDMOSC^{10}, UDMOSC^{15} and UDMOSC^{15E} respectively). However, there was no trend in the decrease in total cost of feed though the increase in total cost of feed in UDMOSC^{15E} came as a result of the addition of enzyme. The results further showed that hens on UDMOSC^{0} performed better with net revenue of GHC 17.16 with hens on UDMOSC^{15E} performing poorly with net revenue of GHC -2.68.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Proximate Composition of UDMOSC

The UDMOSC used in this study had a crude protein content of 31.67% (Table 4.6). This is similar to 30.06%, 31.4% and 32.1% reported by Madubuike *et al.* (2015), Abas (2013) and Boromi *et al*. (2018) respectively in *Moringa oleifera* seeds. It was however, lower than 35.97% reported by Alagbemide *et al*. (2014) but higher than 17.94% reported by Mgbemena *et al.* (2016) in *Moringa oleifera* seeds. The difference in crude protein observed in this study may be as a result of the processes of oil extraction as Alagbemide *et al*. (2014) reported 35.79% in raw samples but 17.13% in defatted samples. Soybean which is the conventional plant protein in poultry feed has a protein content of 13.3% (NRC, 1994). Therefore, the crude protein of 31.67% observed in this study suggests that UDMOSC can be used as a source of protein for laying chicks.

The UDMOSC'S crude fibre content of 20.87% was higher than 2.98%, 7.3% and 2.87% reported by Boromi *et al.* (2018), Alagbemide *et al*. (2014), and Abas (2013) respectively in *Moringa oleifera* seeds and this could be attributed to the presence of shells in the UDMOSC since the seeds were not deshelled before the oil was extracted. The high fibre content could interfere with digestibility since Hassan *et al.* (2007) reported on the contrary that low fibre content could enhance digestibility.

The ether extract value of 4.30% in the UDMOSC sample was lower than 38.67% and 8.57% in raw and defatted samples of *Moringa oleifera* seeds respectively reported by

Alagbemide *et al*. (2014). The lower ether extract content of the UDMOSC observed in this study could reduce the acceptability of it since ether extract adds taste to feed and make it acceptable or attractive to animals.

The moisture content of 9.00% compares favourably with 9.57% reported by Mgbemena *et al.* (2016) and 9.9% in raw samples and 9.40% in defatted samples of *Moringa oleifera* seeds by Alagbemide *et al*. (2014) but higher than 1.26% reported by Boromi *et al.* (2018).

The ash content of 5.50% was higher than 2.50%, 2.34%, 2.80% and 4.43% reported by many researchers (Ijatimi and Keshinro 2012; Omafuvbe *et al*., 2004 and Obun, 2007). The high ash content recorded in this study could lead to mineral toxicity since the major component of ash is minerals. Ash content in plant material to be used in compounding feed for monogastrics should be < 2.50% (Akintayo, 2004).

The carbohydrates content of 28.66% was similar to 28.88 reported by Boromi *et al.* (2018) but higher than 8.87% reported by Alagbemide *et al*. (2014). The differences in the proximate composition of UDMOSC reported in this study and those reported by other researchers could be as a result of geographical location, micro and macro environmental factors, or to the different processing methods which determine the composition of ingredients used as feedstuff (Ali *et al*., 2011).

According to Yang *et al.* (2006), vitamin A is abundant in *Moringa oleifera* leaves in hot –wet seasons, while vitamin C and iron are more in cool dry seasons. These differences

could be as a result of location, climate and environmental factors that might have significantly influenced the nutrient content of *Moringa oleifera* leaves. Since the seed is a part of the seed plant just like the leaves, the seeds could have also been influenced by these factors. The proximate composition of UDMOSC observed in this study suggests that it is suitable for animal feed.

5.2. Starter Phase of Lohmann Brown Layers

5.2.1. Proximate composition of the starter diets

There was no trend observed in the values of dry matter. Adequate quantity of crude protein in chicken feed is required for protein synthesis. Proteins are also required for growth, egg production and feather development (Mbajiogu *et al*., 2011). Increase in crude protein in the diet of chicks results in improvement of egg size and weight (Kuashalendra *et al*., 2016). The minimum requirement of crude protein by Ghana Standard Authority (2007) for layer starters is 18%. The lowest crude protein in the experimental diets was found in $UDMOSC^{0}$ at 15.37% which is below the recommended crude protein level by Ghana Standard Authority (2007) for poultry feed. The highest crude protein was found in $UDMOSC^{10}$ at 18.17% followed by $UDMOSC^{5}$ at 18.04% which were all slightly above the recommended level by Ghana Standard Authority. The crude protein levels in $UDMOSC^{15}$ and $UDMOSC^{15E}$ were also below the recommended level. All the test diets had levels above the control diet suggesting that UDMOSC has the potential of increasing crude protein in poultry feed. However, all the crude protein levels observed were all below the recommended level of 20.03% cited by NRC (1994) for starter

layer diet, an average value of 18.51% in poultry feed reported by Ofori *et al.* (2019) as well as the generated contents in the formula (Table 3.1).

Ether extract in poultry feed improves the adsorption of fat soluble vitamins and increases palatability of feed (Velmurugu, 2012; Baiao and Lara, 2005). The lowest ether extract value was recoded by UDMOSC^{0} at 3.50% which was similar to the ether extract content calculated in the formular (Table 3.1). UDMOSC^{5} and UDMOSC^{10} recorded the highest value at 5.50% which were also above the calculated ether extract content in the formular (Table 3.1). The ether extract content of 4.50% in UDMOSC^{15} and UDMOSC^{15E} were also above what was calculated. All the values were also higher than the recommended level by the Ghana Standad Authority at 3.00% and the average value of 2.47% reported by Ofori *et al*. (2019). The high ether extract content of the test diets suggests that there was some adequate amount of oil left in the UDMOSC which further suggests that the method of oil extraction was inefficient.

The lowest crude fibre content was recorded by the control diet which was also slightly lower than what was calculated in the formular. All the crude fibre content of the test diets were above the levels calculated in the formular suggesting that the diets were fibrous. The high crude fibre content of the test diets observed in this study could be attributed to the fibre in the UDMOSC as the seeds were not deshelled before the oil extraction.

The ash component of a feed describes the inorganic content of the feed and is mainly minerals. A low ash content of a feed predisposes birds to diseases and poor egg formation (Ofori *et al*., 2019). UDMOSC^{5} had the highest ash content suggesting a higher mineral

content as compared to UDMOSC^{15} and UDMOSC^{15E} with the lowest ash content. The ash content of UDMOSC^{5} and UDMOSC^{10} were above the average value in poultry feed reported by Ofori *et al*. (2019) with UDMOSC^{0}, UDMOSC^{15}and UDMOSC^{15E} all having levels below the reported average value.

Moisture content of a feed determines the amount of water in the feed. According to Saiful *et al*. (2015), moisture content of a feed is an indicater of quality and a key to safe storage. According to National Resources Institutes (NRI) (1995), high moisture, high temperature and poor aeration of feed during storage predispose the feed to mycotoxins and spoilage. UDMOSC^{10} had the highest moisture content which was above the recommended value of 12% by Ghana Standrd Authority. UDMOSC^{5} had the lowest moisture content which was also below the recommended value by the Ghana Standard Authority for poultry feed. The rest of the diets had values similar to the recommended value by the Ghana Standard Authority. It could therefore be inferred that UDMOSC^{10} could have been exposed to mycotxins and spoilage if not properly stored.

The analysis uncovered a high nitrogen free extract in UDMOSC^{0} than the test diets. This implies that UDMOSC^{0} supplied more energy to the birds than the test diets. However, all values obtained were above the avrege value of 61.81% in poultry feed reported by Ofori *et al*. (2019).

5.2.2. Effect of UDMOSC on the performance of starters

The performance of starter layer chicks fed graded levels of UDMOSC shown in Table 4.3 indicated that chicks on UDMOSC^{15} had the lowest initial body weight though the difference was not significantly different ($p = 0.06$). The similar but slightly different initial body weight of chicks used for this experiment could be attributed to differences in their weight at hatch. Chicks on the control diet consumed significantly higher feed than their counterparts on the test diets. The marked reduction in feed intake of the starters on the test diets could be attributed to the hard and sticky nature of the UDMOSC which resulted in visible vent sticking of starters that received higher levels of the UDMOSC. The reduced feed intake of chicks on the test diet could further be attributed to reduced palatability of the test diet (Kakengi *et al*., 2003). Onu and Otuma (2008) reported that unpalatable nature of a feed will eventually prevent chicks from consuming adequate quantity of the feed.

Final body weight did not vary significantly ($P = 0.33$) between chicks. However, body weight gain varied significantly ($P = 0.02$) with chicks on the control diet and UDMOSC^{5} having the highest body weight gain. Body weight gain of chicks on UDMOSC^{10}, UDMOSC^{15} and UDMOSC^{15E} did not vary significantly ($P > 0.05$) suggesting that the enzyme (burgazyme) supplementation could not improve digestibility in order to make nutrients, particularly proteins that could have been bonded available to the birds. According to Zanella *et al.* (1999), enzyme supplementation could not improved overall crude protein digestibility and this could have happened to the chicks that received the enzyme supplementation. The reduced weight gain of chicks on UDMOSC^{10}, UDMOSC^{15} and UDMOSC^{15E} could be partly attributed to the high crude fibre content of UDMOSC

which might have impaired feed digestion and nutrient absorption (Aderemi, 2003; Onu and Otuma, 2008; Onu, 2010) as in (Onu, 2011). The relatively higher body weight gained by chicks on $UDMOSC^{5}$ could be attributed to reduced fibre in the $UDMOSC^{5}$ as compared to $UDMOSC^{10}$, $UDMOSC^{15}$ and $UDMOSC^{15E}$. Chicks on $UDMOSC^{0}$, $UDMOSC^{5}$, $UDMOSC^{10}$ and $UDMOSC^{15}$ had the best feed conversion ratio suggesting that the chicks adequately utilized the nutrients from the feed regardless of them consuming slightly less amount of feed.

Mortality rate was significantly high ($P = 0.01$) in chicks fed $UDMOSC^{10}$ and this did not vary significantly with chicks on $UDMOSC^{5}$ and $UDMOSC^{15E}$. The high mortality rate could however be attributed to neonatal infections that might have predisposed the chicks to fatal disease conditions.

5.3. Grower phase of Lohmann Brown Layers

5.3.1. Proximate composition of the grower diets

In terms of the pullet diets, the dry matter content did not also show any trend. The crude protein content of the control diet was below the 15.00% recommendation of NRC (1994). However, the crude protein content of the test diets were all above the NRC (1994) recommended level (Table 4.5). The high crude protein of the test diets observed in this study confirms the acertion that UDMOSC has the potential of increasing the crude protein content of poultry feed. The crude protein content of $UDMOSC^{0}$ and $UDMOSC^{5}$ were however, below the average value of 16.76% in poultry feed reported by Ofori *et al*. (2019) whereas $UDMOSC^{10}$, $UDMOSC^{15}$ and $UDMOSC^{15E}$ had values above the reported value. The low crude protein (14.00%) in the control diet as compared to 16.76% in poultry feed

reported by Ofori *et al*. (2019) could however be attributed to geographical location, micro and macro environmental factors, or to different processing methods which determine the composition of ingredients used as feedstuff (Ali *et al*., 2011). On the other hand, the crude protein content of UDMOSC^{5} (15.60%) which is also below the 16.76% in poultry feed reported by Ofori *et al*. (2019) could be attributed to lower concentration of UDMOSC in that diet. Apart from the control diet which had a crude protein content below the values generated by the formular, all the test diets had crude protein contents above the values generated by the formular in Table 3.2, and this increase could be related to the inclusion of UDMOSC.

The ether extracts content calculated (Table 3.2) and what was analysed (Table 4.5) were all lower in UDMOSC^{5}. The calculated values of ether extracts in UDMOSC^{10} to UDMOSC^{15E} were all above the control. On the other, the analysed values of ether extract in UDMOSC^{15} and UDMOSC^{15E} (Table 4.5) were slightly ahead of UDMOSC^{0} and UDMOSC^{10}. The fact that the calculated values of ether exteract from UDMOSC^{10} to UDMSC^{15E} (Table 3.2) and the analysed values of ether extracts in UDMOSC^{15} and UDMOSC^{15E} (Table 4.5) were all higher than the control suggests that higher levels of UDMOSC will lead to higher levels of ether extracts. High ether extracts also suggest higher supply of fatty acids and for that matter higher supply of energy to the birds receiving higher levels of UDMOSC. The crude fibre content calculated (Table 3.2) and analysed (Table 4.5) in the test diets were all above what was in the control. However, all the grower diets had crude fibre content above 3.00% to 4.00% stated by Varastegani and Dahlan (2018) suggesting that the pullet diets were generally fibrous. The high ash in

UDMOSC^{15} and UDMOSC^{15E} (Table 4.5) could be associated with high minerals in the diets whereas the low nitrogen free extract indicates relatively low carbohydrates and low energy supply.

5.3.2. Effect of UDMOSC on the performance of growers

The final body weight of the chicks which served as the initial body weight of the growers or pullets did not vary significantly though the chicks on the control diet had slightly higher final body weight. This can be attributed to the fact that chicks on the control diet cosumed feed better than those on the test diets. Feed intake was significantly higher in pullets fed UDMOSC^{0} and UDMOSC^{15E} suggesting that the pullets fed UDMOSC^{15E} at this stage accepted the feed. The fact that only growers fed UDMOSC^{15E} performed better in terms of feed intake further suggest that, the hard and sticky nature of the UDMOSC impeded feed intake.

According to Ginindza *et al*. (2017) lower dietary crude fibre levels optimized growth rate whereas higher dietary crude fibre levels resulted in lower feed intake and digestibility of unsexed Venda chickens. The relatively lower body weight gained by pullets on the test diets could therefore be attributed to the high crude fibre content in the test diet which could have impaired digestibility and nutrient absorption. Feed conversion ratio did not vary significantly between birds on the control diet and their counterparts on the test diets and this was in line with the results of Molepo (2014) who found no significant effect on

feed conversion ratio when he investigated the effects of *Moringa oleifera* whole seed meal in broilers. Similarly, there was no significant difference in mortality rate between growers on the control and those on the test diets suggesting an equal health status of all the growers at this stage.

5.4. Layer phase of Lohmann Brown Layers

5.4.1. Proximate composition of the layer diets

The crude protein level of $UDMOSC^{0}$ and $UDMOSC^{5}$ were all below the recommended level of 16.00% by NRC (1994), an average value of 16.16 reported by Ofori *et al*. (2019) in poultry feed and the minimum requirement of 17.00% by the Ghana Standard Authority (2007). The crude protein content of $UDMOSC^{10}$ was similar to both the NRC (1994) recommended value as well as the reported value by Ofori *et al.* (2019) but lower than the minimum requirement by the Ghana Standard Authority (2007). $UDMOSC^{15}$ and $UDMOSC^{15E}$ had levels above the recommended level of NRC (1994) and 16.16% by Ofori *et al*. (2019) and the minimum requirement by the Ghana Standard Authority (2007). $UDMOSC^{0}$ and $UDMOSC^{5}$ had crude protein content below the calculated content in the formular (Table 3.3) whereas $UDMOSC^{15}$ and $UDMOSC^{15E}$ had crude protein content above the calculated contents in the formular. The high crude protein in $UDMOSC^{15}$ and $UDMOSC^{15E}$ could be associated with the high concentration of UDMOSC in these diets.

The lowest ether extract content was recorded by $UDMOSC^{5}$diet at 4.60% with the rest of the diets recording similar level of 5.40%, 5.60% 5.80% and 5.80% for $UDMOSC^{0}$,

UDMOSC^{10}, UDMOSC^{15} and UDMOSC^{15E} respectively. However, all the ether extract content of both the control and the test diets were above 2.75% in poultry feed reported by Ofori *et al.* (2019) and what was calculated to be in the diets (Table 3.3) suggesting that the layer diets were generally fatty. Crude fibre content of the layer diets were similar in UDMOSC^{0}, UDMOSC^{5} and UDMOSC^{10} at 6.18%, 6.84% and 6.90% respectively but higher in both UDMOSC^{15} and UDMOSC^{15E} at 7.16%. They were all higher than what was calculated in the diets (Table 3.3) suggesting that the layer diets were generally fibrous. The moisture content and nitrogen free extracts were all above the respective average values of 9.41% and 56.84% in poultry feed reported by Ofori *et al.* (2019). However, the moisture content of UDMOSC^{15} and UDMOSC^{15E} were similar to the bench mark of 12.00% by the Ghana Standard Authority (2007) whereas the moisture content of UDMOSC^{0}, UDMOSC^{5} and UDMOSC^{10} were all above the bench mark by the Ghana Standard Authority. The high moisture could predispose these feeds to mycotoxins and spoilage as indicated by (NRI, 1995).

5.4.2. Effect of UDMOSC on the productive performance of layers

The final body weight of the growers which served as the initial body weight of the layers varied significantly (P = 0.001). Growers on the control diet had significantly higher final body weight (P = 0.001) as compared to the pullets on the test diets. This could be due to the fact that, the pullets on the control diet consumed higher feed (P = 0.01) (Table 4.6). There was no significant difference in feed intake between hens on the control diet and hens on the test diet (P = 0.15). This agrees with the results of Molepo, (2014) who reported non-significant difference in feed intake in an experiment to determine the effect of supplementing *Moringa oleifera* whole seed meal on productivity and carcass

characteristics of Ross 308 broiler chicken. Birds on the control diet gained more body weight than their counterparts on the test diets. The difference in body weight gain can be attributed to the fact that these hens consumed more feed and were able to efficiently metabolize the feed for body weight gain. Feed conversion ratio did not show significant difference (P = 0.23) and this is in line with the results of Molepo (2014) who found no significant difference in feed conversion efficiency of broilers fed *Moringa oleifera* whole seed meal.

Hen-day, hen-house and total number of eggs produced in this study were significantly different with hens on the control diet having the highest hen-day, hen-house and total number of eggs produced. The lower egg production of hens on the test diets could be ascribed to concentration of UDMOSC in their diets since higher concentration of moringa can cause high accumulation of iron which can also lead to gastrointestinal distress and hemochromatosis (Gyekye *et al*., 2014). The lower egg production observed in this study is in conformity with a report by Mabusela *et al*. (2018) that inclusion of *Moringa oleifera* whole seed meal reduced rate of lay. Weight of eggs and weight of first egg produced in this study did not show any significant difference suggesting that UDMOSC did not influence egg weight and weight of first egg.

Hens reared on the control diet laid earlier than those fed the test diets. According to Bruggeman *et al*. (1998) reduction in feed consumption could cause delay in sexual maturation which might have been associated with delayed ovarian and oviductal growth, decreased cLHRH-I stored in medium eminence and lowered levels of LH and FSH in the pituitary. This might have happened to the hens on the test diet at the time of the

experiment. There was a direct relationship between onset of lay and age of 50% lay suggesting that age at 50% is directly related to onset of lay.

Larger sized eggs produced in this study did not vary significantly between hens on the control diet and their counterparts on the test diet. Medium sized eggs produced did not vary between all groups of hens except those on UDMOSC^{10}. The fact that only hens on UDMOSC^{10} laid fewer medium sized eggs suggests that this observation cannot be associated with the inclusion of UDMOSC but due to favourable gastro intestinal environment and differences in body weight (Summer and Leeson, 1983). Production of smaller sized eggs showed a similar trend. Smaller sized eggs produced by hens on the control diet did not vary significantly with hens on UDMOSC^{15} and UDMOSC^{15E}. Hens on UDMOSC^{5} and UDMOSC^{10} produced significantly smaller sized eggs also indicating that this observation is not as a result of UDMOSC.

5.4.3. Effect of UDMOSC on external and internal egg characteristics

Results on external egg characteristics in Table 4.8 showed that egg length and egg circumference varied significantly with eggs of hens on the control diet having the lowest egg length and egg circumference. The fact that eggs from the control diet were significantly lower in terms of egg length and egg circumference than eggs from the test diets (P = 0.03 and 0.02 respectively) indicates that UDMOSC has the tendency of improving egg length and egg circumference. Egg height and shell thickness of eggs produced by hens on the control diet did not vary significantly with eggs of hens on the test diets except eggs of hens on UDMOSC^{10} suggesting that the low egg height and shell thickness recorded by eggs of hens on UDMOSC^{10} was not as a result of the influence of

UDMOSC. The low egg shell thickness recorded by this group of birds can however be linked to a statement by Gerber (2012) that as egg shells are made up of 95% calcium caronate ($CaCO_3$), a decrease in the level of the hens' blood carbon dioxide (CO_2) combined with an increase in blood pH and a subsequent decrease in Ca^{2+} ions for shell formation leads to an increase in the number of thin or soft shelled egg production. The seemingly low egg length, egg circumference and egg height recorded by hens on $UDMOSC^5$ and $UDMOSC^{10}$ could be attributed to low and unavailability of proteins that impaired the development of the egg hence its length and circumference whereas the thin shell thickness could further be ascribed to the inability of the hens to efficiently metabolize calcium in their diet (Jacob *et al*., 2015). However, there was no significant effect of UDMOSC on shell weight.

Albumen length varied significantly with eggs of the control diet scoring the lowest albumen length. On the contrary, albumen height was high in hens fed the control diet. The reduced albumen height of eggs on the test diet could be attributed to their inability to consume adequate amounts of crude protein to enhance albumen height development. Albumen weight was low in eggs produced by hens fed $UDMOSC^5$. Albumen weight did not vary significantly between eggs produced by hens on $UDMOSC^0$, $UDMOSC^{10}$, $UDMOSC^{15}$ and $UDMOSC^{15E}$ suggesting that the reduction in albumin weight of eggs from hens fed $UDMOSC^5$ can be attributed to low digestibility and absorption of protein which eventually affected albumen development.

Yolk length and yolk weight did not vary significantly. However, yolk height and yolk colour varied significantly but without trend. Yolk height was high in eggs produced by hens on $UDMOSC^{0}$, $UDMOSC^{15}$ and $UDMOSC^{15E}$ whereas yolk colour was intense in eggs from hens fed $UDMOSC^{0}$ to $UDMOSC^{15}$. The reduced yolk height in eggs of $UDMOSC^{5}$ and $UDMOSC^{10}$ as well as yolk colour in eggs of $UDMOSC^{15}$could also be ascribed to low digestibility and absorption of protein which eventually affected egg formation and consequently yolk height. Hens are unable to synthesise xanthophylls and as a result the maintenance of a uniform yolk colour is dependent on the quality, colouring capacity and stability of the dietary carotenoids (Nys, 2000). It could therefore be inferred that the less intense yolk colour could be associated with low carotenoids in the seeds of moringa.

The excellent score of Haugh unit for eggs from the control and the test diets suggests that UDMOSC did not impact negatively on Haugh unit though values of eggs from the control group were slightly higher. According to Haugh, (1937) haugh unit is a measure of albumen quality and from these scores, it could be inferred that all the eggs produced in this study were of good quality in terms of egg albumen.

5.4.4. Effect of UDMOSC on haematological profile of layers

RBCs in the layers on the control diet was within the normal range of 2.40 to 3.90 x 10^{12}/L stated by Ahmed (2018) with the RBCs of layers on the test diets falling below the lower limit suggesting that the layers on the test diets could have reduced or unhealthy RBCs (Table 4.11). The Hb values obtained in this study were all within the normal range of 5.58

– 15.14 g/dl stated by Ahmed (2018) and this indicates that UDMOSC did not largely affect the Hb of the layers. MCV was significantly higher in hens on UDMOSC^{15} but did not vary significantly with hens on UDMOSC^{5}, UDMOSC^{10}, and UDMOSC^{15E}. MCV was low in hens on the control (UDMOSC^{0}) but that did not also vary significantly between what was recorded in hens on UDMOSC^{5}, UDMOSC^{10} and UDMOSC^{15E} suggesting that the difference in MCV might have been caused by anaemia but not UDMOSC. There was a significant difference in the WBCs count with the hens fed UDMOSC^{0}, UDMOSC^{5} and UDMOSC^{10} having the highest count. Interestingly, the hens fed the higher concentrations of UDMOSC had the lowest count suggesting that the hens with the highest WBCs count could be associated with infections since it is well kown that a high WBCs count is related to an infection caused by bacteria in the host or stress or ingestion of contaminated feed (Terry, 2013).

There was no trend in MCH and HCT with no significant difference indicating that UDMOSC had no impact on MCH and HCT. However, all the values for MCH and HCT obtained in this study were within the normal values of 33.00 – 43.00 pg and 22.00 - 35.00% respectively stated by Jain (1986) indicating that UDMOSC did not affect MCH and HCT. MCHC values for hens on the control diet and those on the test diets were all below the normal range of 26.00 – 35.00 g/dl stated by Jain (1986) suggesting that the decline of MCHC values was not as a result of the inclusion of UDMOSC. The low MCHC values recorded for both the treatment and control groups of layers observed in this study could be attributed to reduced vitamins C or E, or vitamins C + E since Ajakaiye *et al*. (2010) found higher MCHC in birds fed vitamins C or E, or vitamins C + E

than those in the control group. Togun *et al*. (2007) reported that when haematological values fall within the normal range, it is an indication that diets did not show any adverse effect on haematological parameters during the experimental period. It could therefore be inferred that the inclusion of UDMOSC in the diet of the birds largely did not affect the blood parameters and health of the birds.

5.4.5. Effect of UDMOSC on biochemical profile of layers

From Table 4.12, it could be seen that total cholesterol in the layers decreased with increasing UDMOSC from UDMOSC^{0}, UDMOSC^{5}, UDMOC^{10} and UDMOSC^{15} except UDMOSC^{15E} at 3.97mmol/L, 3.87mmol/L, 3.67mmol/L and 2.80mmol/L respectively. UDMOSC^{15E} was 2.97mmol/L. Even though, the difference was not significant, there was an indication that suggests that UDMOSC has the potential of reducing cholesterol in birds. This result is in line with the findings of Abdel-Aseem *et al.* (2017) who reported a significant increase in serum total cholesterol of Lohmann chicken on a control diet as compared to those fed moringa leaf powder. The researchers attributed this finding to the presence of phytochemical compounds such as alkaioids and saponins which have anti–cholesterolemic activities resulting in a decrease in total cholesterol. It can however, be inferred that these phytochemical compounds are also present in *Moringa oleifera* seeds which might have caused the slight decrease in total cholesterol of the layers.

From same Table 4.12, there was no trend for triglyceride. The triglyceride levels in layers on the control diet were above the levels in layers on UDMOSC^{15E} and UDMOSC^{15} but below the levels in layers reared on UDMOSC0^{10} and UDMOSC^{5} diets respectively

suggesting that the difference in triglyceride levels cannot be attributed to the inclusion of UDMOSC. Long *et al*. (2020) found that Broilers offered basal diet + 1% microalgae + 1% linseed oil + 1% soybean oil or basal diet + 2% fish oil + 1% soybean oil diets had significantly lower the relative weight of abdominal fat and total cholesterol content and increased PUFAs. The decrease in triglyceride level in hens on $UDMOSC^{15}$ and $UDMOSC^{15E}$ could therefore be associated with higher levels of PUFAs (linoleic and linolenic acids) in *Moringa oleifera* seeds which have the ability to control cholesterol.

Results on serum HDL in the layers showed an increasing trend from layers on $UDMOSC^{0}$, $UDMOSC^{5}$ and $UDMOC^{10}$ and declined in layers on $UDMOSC^{15}$ and $UDMOSC^{15E}$. Layers on $UDMOSC^{5}$ and $UDMOSC^{10}$ had significantly higher values. However, there was no significant difference between the HDL in the control layers and the other layers on the test diets indicating that the difference was not caused by the inclusion of the UDMOSC.

The inclusion of UDMOSC caused a decline in the serum LDLC in layers on the control diet at 2.14mmol/L to 1.52mmol/L, 1.29mmol/L, 1.06mmol/L and 1.25mmol/L in layers on $UDMOSC^{5}$, $UDMOSC^{10}$, $UDMOSC^{15}$ and $UDMOSC^{15E}$ respectively though the difference was not significant. According to Iskender *et al*. (2016), flavonoids (heseridin, naringin and quercetin) reduced lipid peroxidation levels in laying hens. The low LDLC could therefore be linked to the presence of antioxidants (flavonoids and carotenoids) in moringa seeds.

On the contrary the inclusion of the UDMOSC caused a slight increase in vLDLC from 0.53mmol/L in layers on the control diet to 0.61mmol/L and 0.71mmol/L in layers on $UDMOSC^{5}$ and $UDMOSC^{10}$ respectively. It however declined in layers on $UDMOSC^{15}$ and $UDMOSC^{15E}$ at 0.41mmol/L and 0.51mmol/L respectively. The fact that there was no significant effect and no trend in the values of vLDLC suggests that UDMOSC had no impact on vLDLC. However, the lower levels of vLDLC in layers on $UDMOSC^{15}$ and $UDMOSC^{15E}$ could be attributed to high fibre content which bind to cholesterol and prevent its initial absorption as a result of higher levels of UDMOSC. Insoluble fibres have the ability to bind to bile acids reducing the absorption of fats and cholesterol (Aseem *et al.,* 2017). Dietary cholesterol has been suggested to increase the risk of cardiovascular diseases (CVD) (Berger *et al*., 2015). It could therefore be inferred from the results of this study that UDMOSC did not negatively affect the cardiovascular health of the birds since the inclusion of UDMOSC did not negatively affect the lipid profile of the birds.

5.4.6. Effect of UDMOSC on WBC profile of layers

There was no trend in the WBC profile. Lymphocytes were significantly higher in layers fed $UDMOSC^{0}$, $UDMOSC^{5}$ and $UDMOSC^{10}$ at 5.13 $x10^{12}$/L, 6.70 x 10^{12}/L and 7 .67 x 10^{12}/L respectively but lower in layers fed $UDMOSC^{15}$ and $UDMOSC^{15E}$ at 3.77 x 10^{12}/Land 3.83 x 10^{12}/L respectively. Values for lymphocytes of hens fed $UDMOSC^{0}$, $UDMOSC^{5}$ and $UDMOSC^{10}$ were higher than the normal range reported by Samour (2013) at 1.2 – 4.2 x 10^{12}/L. However, hens fed $UDMOSC^{15}$ and $UDMOSC^{15E}$ had values within the normal range. The presence of many reactive lymphocytes is suggestive of antigenic stimulation. However, occasional reactive lymphocytes may be found in the blood film of

normal birds (Terry, 2013). The increase in lymphocyte count beyoud the upper limit observed in this study could also be associated with infection or stress. It must also be noted however, that lymphocytes are vital for producing antibodies that help the body to defend itself (Smith, 2018).

Heterophils have the primary function against bacteria in the respiratory system of birds and migrate there in the moment of infection (Ruminska *et al*., 2008). Heterophil count indicated a slight increase in the hens fed UDMOSC^{5} though the difference was not significant. The heterophil count of layers on UDMOSC^{10} to UDMOSC^{15E} diets were lower than those on the control diet indicating that the increase in the heterophil count in the layers on UDMOSC^{5} could be associated with remarkable tissue damage of these hens since heterophils are chemically attracted to the site of infection by products released from the damaged tissues. All the values for heterophils recorded in this study were below the lower limit of 0.5 x 10^{12}/L stated by Samour (2013). Jain (1986) stated that immature heterphils are rare in the blood stream. It could therefore be inferred that the heterophils could still be immature at the time of sample taking.

Monocytes are responsible for attacking and breaking down germs or bacteria that enter the body (Smith, 2018). Increase in moncytes can be associated with certain diseases that produce chemotactic agents for monocytes. These conditions include avian chlamydiosis, mycotic and bacteria granulomas and massive tissue necrosis. Monocytosis can also occur in birds on zinc-deficient diets (Terry, 2013). Monocytes in this study were significantly higher in the control layers (UDMOSC^{0}) and UDMOSC^{5} but lower in layers on UDMOSC^{15}

indicating that the increase in monocytes count in the layers on $UDMOSC^0$ and $UDMOSC^5$ could be as a result of infections picked up by these groups of hens. However, all monocyte values recorded in this study were within the normal range of 0.00 – 1.00 x 10^{12}/L stated by Samour (2013).

The exact function of esinophils and basophils in birds is unknown (Terry, 2013). However, research has shown that avian eosinophils may participate in delayed hypersensitivity reactions. Avian basophils are similar to mammalian basophils in their ability to produce, store and release histamine. Basophils appear to participate in the initial phase of acute inflammatory response in birds (Terry, 2013). All the values for basophils and esionophils were also within the normal range of 0.00 – 1.00 x 10^{12}/L and 0.00 – 1.80 x 10^{12}/L respectively indicating that the birds were not hypersensitive and had no inflammations. The normal ranges of eosinophils and basophils further suggest that inclusion of UDMOSC did not nagatively affect esinophils and basophils count in this study.

5.4.7. Effect of UDMOSC on organoleptic characteristics of eggs

Non-significant effect of UDMOSC was observed for all important sensory attributes tested in this study and was in line with earlier reports (Valavan *et al*., 2013; Hayat *et al*., 2014; Mahfuz *et al*., 2019). The results suggested that the inclusion of UDMOSC did not affect egg colour, aroma, taste, flavour, mouth feel and overall quality.

5.4.8. Effect of UDMOSC on economic efficiency in production starters, growers and eggs

The results on economic efficiency of UDMOSC in production showed that, it would cost less to gain one kilogram (1kg) body weight of starters and pullets with UDMOSC inclusion diets (Tables 4.4 and 4.7). This suggests that UDMOSC has the potential of reducing feed cost of producing starters and growers or pullets. The consistent decrease in feed cost per kilogram body weight of growers fed $UDMOSC^{5}$, $UDMOSC^{10}$ and $UDMOSC^{15}$ at GHC 8.00, GHC 7.87 and GHC 6.83 respectively as compared to growers on $UDMOSC^{0}$ at GHC 9.15 suggest that the jump in feed cost per kilogram weight gain of growers on $UDMOSC^{15E}$ at GHC 10.72 cannot be attributed to cost of UDMOSC. It could however be attributed to the inability of the pullets to make efficient use of feed as well as cost of the enzyme supplemented.

On the contrary, net revenue in terms of egg production from hens on the control diet was slightly ahead of hens on $UDMOSC^{5}$. In terms feed intake, hens on $UDMOSC^{0}$ and $UDMOSC^{5}$ consumed similar amount of feed at 8.76 kg and 8.40 kg per bird. The quantities of feed consumed by hens on $UDMOSC^{0}$ and $UDMOSC^{5}$ might have been enough to enhance the development of reproductive structures and the overall egg production efficiency. The levels of feed cosumed by hens on $UDMOSC^{10}$, $UDMOSC^{15}$ and $UDMOSC^{15E}$ at 7.85 kg, 5.77 kg and 5.91 kg per bird respectively might have not met the requirement of the hens for development of reproductive structures and egg production efficiency which impeded egg production rate resulting in negative net revenue.

The reduction in feed consumption of hens on the test diets can be attributed to the hard sticky nature of the UDMOSC which resulted in visible vent sticking of some of the starters. The fact that UDMOSC^{5} could produce net revenue close to UDMOSC^{0} suggests that *Moringa oleifera* seed cake of 5% and above without fibre and processed in manner that does not leave the cake too hard and sticky can enhance profit.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

1. UDMOSC from 5% up to 15% depressed growth of starters and growers.
2. Inclusion of UDMOSC depressed egg production but did not affect external and internal egg characteristics.
3. UDMOSC had no effect on organoleptic characteristics of eggs.
4. UDMOSC had no adverse effect on haematological and biochemical characteristics of layers.
5. Inclusion of UDMOSC reduced feed cost per kilogram body weight gain of starters and growers as well as net revenue of layers.

6.2. Recommendation

1. UDMOSC levels below 5% should be investigated to ascertain the optimum level of UDMOSC that promotes growth and reproductive performance of layer chicken.
2. The use of protinase with carbohydrase should be investigated to determine the possibility of breaking down the seeming strong protein bonds in UDMOSC to improve nutrient availability and utilization.
3. Further research work should be conducted on the use of deshelled defatted *Moringa oleifera* seed cake (DMOSC) in layer diet.

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