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

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Abstract: The study was conducted to evaluate the effect of treated MOLM in exotic layer diets on egg characteristics and consumer acceptability. Thirty-six (36), sixteen (16) week old ISA Brown layers were selected from a flock of sixty (45) birds and allocated to four dietary treatments with three replicates of three birds each, confined in a deep litter system in a completely randomised design; The treatments consisted of T1 0% MOLM, T2 10% MOLM, T3 15% MOLM and T4 20% MOLM. Data was collected on external parameters (eggshell thickness, shell weight, egg width and length, shape index) and internal qualities (yolk weight, yolk width and length, yolk ratio, yolk colour and egg weight). Data of the parameters were subjected to analysis of variance (ANOVA) using the general linear model (GLM) procedure of the statistical analysis system (SAS, 2009). Significant means were separated using Tukey's test at ($p < 0.05$). The results showed significant differences ($p < 0.05$) in all the parameters evaluated on all the birds fed on different dietary treatments; treatment 4 (20% MOLM) recorded the highest yolk colour (12) in the Roche colour fan, in comparison 10%, 15% MOLM recorded (8, and 10) respectively on the scale of the Roche colour fa n, birds fed on 0% MOLM recorded low yolk colour (1) on the Roche colour fan; The results show that 10% MOLM have a better influence in shell weight, yolk weight, and yolk ratio, while the control group record better qualities in yolk width, yolk length, It could be concluded that 10% MOLM in layers diet could improve egg qualities of Isa-Brown birds.

Keywords: Eggs, Layers, Molm, Probiotics, Weight

1. Introduction

Poultry production is the most innovative and profitable way of increasing the accessibility to high-quality protein food, as eggs provide a perfectly balanced nutrition containing all the essential amino acids, minerals, and vitamins [33]. Egg quality is a factor which contributes to the better economic price of fertile and table eggs. Egg quality was defined by Stadelman [28] as characteristics important for consumers. Economic success for a production flock is measured by the total number of qualitatively produced eggs [6]. Egg quality is presented by its weight, percentage of eggshell, thickness and strength. Mainly, eggshell quality differences depend on the hen strain; many studies showed that hens with coloured feathers lay bigger eggs than white ones. [12] As crucial as poultry is to the economy, the poultry industry faces certain constraints, including the high feed cost [25]. Feed is the essential input for poultry production, and the availability of low-priced, high-quality feeds is critical for expanding the poultry industry [23]. The high cost of conventional feed ingredients and additives has led this study to explore non-conventional feed resources; Moringa was recognised to have the potential for use as animal feed due to its high availability and excellent nutritive value [4]. The leaves have been found to contain phytochemicals, vitamins, minerals, and amino acids [26]. Mahfuz et al., [19] stated that Moringa leaf could be a dietary supplement in layer and broiler feeds due to high production performance and improved egg quality. This study determined the effect of different inclusion levels of MOLM on egg qualities (albumen weight, yolk colour, eggshell thickness).

2. Materials and Methods

2.1. Study Site

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

2.2. Collection and Processing of Moringa Leaves

The Moringa leaves were sourced from Meru County, Kenya; local farmers in Meru to obtained fresh leaves by cutting the tree branches and stripping the leaves off the tips of the branches by hand (manually). The leaves were air-dried under a shade until they were crisp to the touch while retaining their greenish colouration. The leaves were then milled using a hammer mill with a sieve size of 5 mm to ensure adequate particle size for feed intake, yielding a product known as Moringa leaf meal (MOLM), which was stored in airtight sacs until needed.

2.3. Determination of the Nutrient Composition of Moringa Leaf Meal

The nutrient composition of dried Moringa leaf meal was determined at the Egerton University Animal Nutrition laboratory in the Department of animal sciences according to Official Methods of Analysis, Association of Official Analytical Chemists AOAC 2006).

2.4. Experimental Birds

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

2.5. Experimental Diets

The experimental diets were formulated to meet the National Research Council (1994) requirements for exotic pullets (ISA Brown) before compounding and mixing. The diets used in the experiment are presented in (Table 1). The MOLM was incorporated in the following levels: 0% and 10%. 15%, and 20%, to have a diet containing 16% crude protein and metabolisable energy of 2850 to 2900 kcal/kg, A layer premix was added to supply minerals and vitamins. The dietary treatments were**:** Treatment 1 l 0% MOLM 0% probiotics (*Bacillus coagulans*)**;** Treatment 2 10% MOLM, 0.01 probiotics; Treatment 3 15% MOLM 0.01 probiotics; Treatment 4 20% MOLM 0.01% probiotics.

Table 1. *Composition of experimental diets.*

Ingredients	T1	T2	T3	T4
Maize meal	70	67	67	66
Soybean meal	18	12	7	4
Fish meal	3.1	4	4	4
MOLM	0%	10%	15%	20%
DCP	2.4	2.4	2.4	2.4
Limestone	6	4.09	4.10	3.09
Iodised salt	0.3	0.3	0.3	0.3
Layer premix	0.2	0.2	0.2	0.2
Probiotics	0	0.01	0.01	0.01
Total Calculated analysis	100 kg	100 kg	100 kg	100 kg
CP (%)	16.88	16.92	16.22	16.4
ME (Kcal)	2.874.88	2.870.27	2.854.42	2.856.42
CF (%)	2.53	3.43	3.78	4.23

2.6. Measurement of External Egg Characteristics

Eggs were collected twice daily at 9.00 hrs. and 18.00 hrs., labelled by day, treatment (T), date (D), and the day (e.g., day 1 T1D1) and placed in egg trays. Three eggs per treatment were sampled weekly to determine the quality characteristics. External egg quality characteristics assessed included egg length, egg width, shell thickness, shape index and eggshell weight. Individual eggs were weighed on a digital balance to the nearest 0.01 g accuracy [31]. The shape index was calculated as the ratio of breadth: length multiplied by 100. All the shells were wiped dry with a paper towel and weighed with a digital balance. The thickness of four pieces of shells, one each from the two ends (broad and narrow end) and two from the body of the egg shell, egg length and egg width were measured with a digital Vernier calliper to the nearest of 0.01 mm, and the measurements averaged. [3].

Eggshell weight: The shells were dried after removing the yolk and albumen from the shell. Shell weight was determined according to the procedures Gamage et al, [9]. described.

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

2.7. Measurement of Internal Characteristics

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

$$YI = (\text{Yolk height in mm})/(\text{Yolk diameter in mm}) \times 100.$$

Albumen weight (AW) This was calculated as the difference between the weight of the whole egg and the weight of the yolk and eggshell. The length and width of the eggs were measured in mm with a Vernier calliper. The internal qualities assessed included yolk index, egg yolk ratio and yolk colour. The length and width of the yolk were measured (in mm) with a digital Vernier calliper.

3. Experimental Design

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

Statistical model are as follows.

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

where,

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

μ= overall population mean.

α_i= effect due to the treatment, [MOLM inclusion levels].

β_j=effect due to Probiotics in MOLM.

$\alpha\beta$ interaction between MOLM and Probiotics

ε_{ijk}= random error term.

4. Data Analysis

Data on egg characteristics were subjected to analysis of variance (ANOVA) using the general linear model (GLM) procedure of the statistical analysis system (SAS, 2009). Significant means were separated using Tukey's test at (p < 0.05). Data on consumer acceptability was compiled and computed to determine statistical significance based on the number of correct responses. The effects of probiotics-treated Moringa on sensory data were analysed using analysis of variance (ANOVA) at a 5% level of significance. Where the means were significant, mean separation was done using Duncan's Multiple Range Test. Calculations were done following the statistical analysis system (SAS) version 9.3.

5. Sensory Evaluation and Consumer Acceptability of Eggs of Exotics Layers

A trained panellist (12-13) aged between 25 and 45 years carried out the egg sensory evaluation. A total of 18 eggs per treatment were obtained for the panel sensory evaluation. Eggs were boiled, cooked and cooled by placing eggs in water. The cooking of the eggs lasted for 8 minutes from the start of boiling. Then, the saucepan was removed from the stove, and the hot water was discarded and immediately replaced with cold water at room temperature. The eggs stayed in the cold water for about 3 minutes. The eggs were then peeled, cut into halves and placed on plastic disposable plates. The disposable plates were labelled in relation to the treatments from which the eggs came. Before the sensory analysis, panellists were asked not to consume any food within 3 hrs of the actual testing. Sensory evaluation parameters evaluated were appearance, yolk colour, general aroma, general flavour, and texture [14]. The panellists were instructed on the process of evaluating the parameters like appearance, odour, texture, taste, and acceptance of the sensory quality of eggs [14]. The parameters were quantified by a seven-point hedonic scale (1 = dislike extremely; 7 = like extremely). The panellists were placed in a room alone so as not to influence the outcome of the others.

6. Results

Table 2. *Analysed chemical composition of the diets.*

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

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

Table 3. *Effect of MOLM on egg Characteristics.*

Parameters	T1	T2	T3	T4	p-value
Egg width (mm)	41.93±0.82^{b}	41.96 ±0.80^{b}	42.23±0.73^{ab}	42.70±0.81^{a}	<.0001
Egg Length (mm)	54.46±0.74^{b}	54.53±0.73^{b}	54.78±0.74^{ab}	55.78±0.80^{a}	<.0001
Yolk length (mm)	40.42±0.51	40.07±0.43	41.55±0.25	40.45±0.67	0.7387
Yolk width (mm)	36.30±0.75^{a}	35.30±0.81^{ab}	33.88±0.72^{b}	33.28±0.98^{b}	<.0001
Yolk colour	1.00±0.00^{d}	8.00±0.00^{c}	10±0.00^{b}	12±0.00^{a}	<.0001
Shell thickness (mm)	0.68±0.05^{b}	0.72±0.05^{ab}	0.82±0.07^{ab}	0.89± 0.07^{a}	0.0002
Shape index (%)	77.14±0.65^{ab}	76.82±1.28^{b}	77.12±0.59^{ab}	78.30±0.49^{a}	<.0001
Yolk ratio (%)	61.44±1.46^{a}	59.77±1.28^{a}	53.61±1.12^{b}	50.73±1.45^{b}	<.0001
Yolk weight (g)	15.10±0.22^{b}	16.33±0.17^{a}	13.64±0.13^{c}	14.53±0.30^{b}	<.0001
Shell weight (g)	6.18±0.09^{b}	7.38±0.07^{a}	5.63±0.11^{c}	5.79±0.18b^{c}	<.0001
Egg weight (g)	63.29±0.3^{a}	65.63±0.56^{a}	59.29±0.80^{b}	59.08±0.64^{b}	<.0001

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

Table 4. *Effect of consumer acceptability of eggs of exotic layers fed on probiotics-treated MOLM.*

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

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

7. Discussion

Dietary nutrient supply and feed characteristics (texture, composition and energy) are the most important factors affecting egg quality. Internal and external egg quality parameters measured in the current study were affected by the dietary inclusion of MOLM. Some quality parameters were improved when MOLM was included in the diet. Moringa is a good pigmenting agent for poultry products due to its rich xanthophyll content [34]. In the present study, the yolk colour showed a higher value when MOLM was included in the ration, which is supported by the findings of different researchers with 5 and 10% inclusion of MOLM in the layer ration [26]. Egg yolk colour is a significant factor in consumer satisfaction and influences human appetite, with a preference for golden yellow to orange yolk colour [13]. The intense yellowish yolk colour recorded in this study for eggs produced from birds on diets containing MOLM confirms its viability as a yolk-colouring agent, which can enhance the marketability of the eggs. In this study, birds fed on 20% MOLM had the deepest yolk colour compared to the rest, while the 0% MOLM control group recorded lighter yolk colour; birds fed on 20% MOLM differed from 0% MOLM ($p < 0.05$). Birds fed on 15% MOLM resulted in higher yolk colour than 10% MOLM (Table 3). This is attributed to the fact that Moringa leaves are enriched with carotenoids and flavonoids, powerful natural antioxidants that can modify the levels of β-carotene and quercetin egg yolk [9]. According to Bidura *et al.* [32]. β-carotene in Moringa leaves ranges from 2.7 to 3.10 mg/100 g dried leaves. When added to feed, these bioactive and phytochemicals increase egg production and positively affect chicken health. Carotenoids play an essential role in developing different colour scores in egg yolk, especially lutein, which is an active yellow dye; a similar finding was reported by Bidura *et al.* [5], who claim that the inclusion of 10-20% Moringa leaves in broiler feed or laying have significantly increased the yellow colour of the skin and egg yolk. No significant differences ($p>0.05$) in egg length and width in birds fed with 15% to 20% MOLM treatments. The egg width and length were higher in 20% and 15% MOLM in comparison to other diets. While 0% and 10% MOLM did not differ (p > 0.05).

Treatment 1, T2, T3 and T4 did not differ at ($p > 0.05$) in yolk length across all the dietary treatments (Table 3). Birds fed on 0% MOLM (T1) recorded higher yolk width, while there was no significant difference between T2, T3, and T4 ($p > 0.05$). The diet of 20% MOLM resulted in the highest shape index (Table 3). relative to rest, while T1, T2, and T3 did not differ ($p> 0.05$). This agrees with the findings of Duman *et al.* [7]. who found that egg shape index and shell thickness did not show any significant difference ($p>0.05$) due to the supplementation of MOLM. Similarly, according to the results of an earlier study, no adverse effect was found on the egg quality traits due to MOLM supplementation [1]. An eggshell is a biological barrier that protects internal egg contents [29]. The shell contains various minerals, with calcium. Egg shell quality is of paramount importance because it determines the marketability of the eggs [15, 21]. In this study, eggshell weight was generally higher in birds fed on 10% MOLM diets and increased as the laying period progressed. Eggshell quality is probably due to the birds' improved calcium and phosphorus metabolism [16]. Eggshell thickness in this study revealed that 20% MOLM recorded the highest shell thickness relative to the rest. In contrast, the lower eggshell thickness was recorded in the 0% MOLM (Table 3); there were no significant differences ($p>0.05$) between 10% MOLM and 15% MOLM (Table 3). It was stated that the proportion of eggshells increased with the increased level of Moringa in the diet of laying birds [17]. This could be due to an adequate supply of calcium and phosphorus by the MOLM at 20% and efficient calcium metabolism by the birds. Egg shells are a mixture of bio ceramic material with 95% calcium carbonate as polymorphic calcite and 3.5% organic matrix material [18]. Thus, the similarity in the overall mean of eggshell thickness implied that diets supplied adequate calcium and phosphorus that could have improved the metabolism of these minerals during eggshell calcification [35]. It may be hypothesised that Moringa increased eggshell weight and thickness due to the photogenic compounds that may have the ability to improve calcium storage, uterine functions and intestinal secretions, which could lead to improving eggshell and egg [35]. The finding agrees with Abdel-Wareth, A. A., & Lohakare, J. [2], who stated that the proportion of eggshells increased with the increased level of Moringa in the diet of laying chicken. Birds fed on 10% MOLM recorded the highest yolk weight relative to the rest, while 15% MOLM recorded the lowest yolk weight. A diet with 0% MOLM did not differ from T4 ($p>0.05$) (Table 3); this could be due to an increase in feed intake with an increase in MOLM in the diet resulting in increased nutrient intake and hence the size of egg components increased as reported by Prasad & Ganguly, [30]. Moringa leaves are also a source of vitamin A, riboflavin, nicotinic acid, folic acid, pyridoxine, ascorbic acid, beta-carotene, calcium, iron, and α-tocopherol. Supplementation of Moringa leaves affects egg production and mass [9]. and egg yolk bioactive compounds. Antioxidants, flavonoids, carotenoids, amino acids, proteins, and energy levels decrease egg water content and can increase nutrient density in egg yolk.

8. Effect of Consumer Acceptability

The consumer acceptance test was judged by a semi-trained panellist on sensory evaluation of eggs from hens fed on probiotics-treated moringa leaf meal-based diets. The sensory evaluation of hard-boiled eggs by semi-trained panellists revealed that no difference in flavour could be identified in 0% MOLM, 10% MOLM, 15% MOLM, and 20% MOLM eggs. The results of the present study agree with the findings of [14], who reported that consumers could not detect a flavour difference in hard-boiled eggs fed hens with flax seed. This is supported by the conclusion of [20]. that lipid oxidation did not contribute to detectable off-flavours in flax-fed eggs. In this study, there was a change in yolk colour with the use of MOLM in the diets as scored by the panellists. The yolk colour changes could have been due to xanthophyll content in the diets of MOLM incorporation. Xanthophyll (oxygen derivative of carotenoid) The change in colour of the egg yolk with the use of MOLM in the diets was also reported by Gebrehawariat *et al.* [10]. Xanthophyll in the MOLM is responsible for egg yolk pigmentation (Santos-Ricalde *et al.,* 2011), which in turn depends on digestibility, metabolism, transfer and deposition of carotenoid within the yolk. The content and profiles of carotenoids are responsible for the yolk colour change. Yolk colour change with the use of MOLM in the diets was reported to influence the acceptability of the eggs [8]. This was further revealed by the strong positive correlation between yolk colour and egg acceptability (Table 4). [8]. revealed that the choice of consumers for eggs is no longer based on internal quality characteristics like level of cholesterol and fatty acid profile but on yolk colour too. Incorporation of MOLM in the diets must have led to increased levels of carotenoids which were digested and deposited in the egg, and hence the change in the yellow yolk as compared to the yolk colour of eggs from diets without MOLM. Increased egg acceptability could have been due to the improved quality of the eggs with yellow yolk colour. In diets with MOLM compared to 0% MOLM, Consumers are increasingly becoming aware of the quality and get more attracted to better quality eggs with firm albumen and dense yellow yolk colour [24]. Feeding layers on MOLM had an influence on the taste of the eggs compared to the 0% MOLM.

9. Conclusion

The results show that 10% MOLM have a better influence on shell weight, yolk weight, and yolk ratio, while the control group recorded better qualities in yolk width and yolk length. It can be concluded that a 10% MOLM in layers diet could improve the egg qualities of Isa-Brown birds and influence customer acceptability of the eggs.

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