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Fertility, hatchability and chick quality of improved indigenous laying hens in Kenya fed different dietary levels of enzyme-treated Moringa (*M. oleifera*) leaf meal based diets

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Abstract
Fertility and hatchability are vital economic traits in the poultry sector because they have a significant impact on chick output. The impact of substituting soybean meal in layers feed with Moringa oleifera leaf meal (MOLM) on fertility, hatchability, and chick quality of improved indigenous laying hens was investigated. Ninety hens and 18 cocks were randomly assigned to six dietary treatments with three replications. MOLM was included in treatment rations: T_1 (0 percent MOLM, 0 percent enzyme), T_2 (0 percent MOLM, 0.035 percent), T_3 (20 percent MOLM, 0 percent enzyme) T_4 (20 percent MOLM, 0.035percent), T_5 (40 percent MOLM, 0 percent enzyme), T_6 (40 percent MOLM, 0.035 percent enzyme). Data on egg fertility and hatchability, chick quality, and embryonic mortality in viable eggs were also recorded during the period of incubation. The highest ($p<0.05$) hatchability was seen in the eggs from hens fed MOLM-based diets, while the lowest hatchability was seen in the eggs from hens fed control diet. Eggs from hens fed diets based on MOLM had considerably ($p<0.05$) higher fertility rates than those from the control group. High early embryonic mortality was observed in control diet and was statistically different ($p>0.05$) with the MOLM-based diets. Hens fed MOLM-based diets had significantly ($p<0.05$) lower mid embryonic mortality than the others. Mid embryonic mortality was high in control diet compared to the MOLM-based diets. There was no significance difference in the late embryonic death, pips and culls in the MOLM-based diets compared to control diet. Day-old chicks from hens fed MOLM-based diets were heavier than those from hens fed a control diet. When compared to the control diet, the length of the chicks on MOLM-based diets was not significantly different ($p>0.05$). Hens fed MOLM diets improved statistically ($p<0.05$) in terms of visual scoring which involved checking chicks that were free from deformities, clean and had bright eyes.

Keywords: Chick quality, embryonic mortality, fertility and hatchability

1. Introduction
Animal breeding is dependent on nutrition. The appropriate diet, both in terms of quality and quantity, is crucial for chicken since it gives them the energy they need to complete the mating process and provide nutrients to the egg. Production of poultry at all levels of operation completely relies on consistent day-old chick supply. The availability of day old chicks for poultry production can be greatly influenced by the fertility and hatchability of these eggs. The percentage of eggs that survive to the end of incubation and hatch into chicks is known as hatchability. Hatchability has a significant economic impact on the poultry sector since it significantly affects the number of chicks produced [24]. Eggs were first incubated beneath broody chickens in the early days. For the large-scale production of chicks, this hatching technique is highly unsatisfactory therefore incubators that offer settings comparable to those of broody hens are utilized. Despite all these advancements, there are still certain elements which promote energy loss to the chicken which laid down the eggs and those who incubated them due to the inability of the eggs to hatch. Individuals, breeds, and varieties within a breed differ in terms of the heritable qualities of fertility and hatchability [22]. Egg age, storage conditions, flock age, mating system, incubation, relative humidity, egg turning, husbandry system, and rearing technology are a few of the factors that can have an impact on these [45].

The amount of micro and macronutrients that the hen places in the egg, as well as the developing embryo, have an impact on how easily the egg will hatch [14]. These nutrients are provided by the various parts, including the albumen, yolk, and shell. Strong correlations between the weight of hatched chicks and the weights of the yolk and albumen have been reported [46]. According to [22], the features of the egg that have the greatest influence on hatchability include weight, shell thickness and porosity, shape index, and homogeneity of the contents. Heat stress lowers egg hatching and reduces both the internal and external egg quality. Locally bred chickens in rural communities in Africa offer the general public with micronutrients [27]. However, a decline of the chicken raised locally, primarily as a result of their poor reproductive and productive abilities [8]. It is well known that under natural mating circumstances, a low hatchability and fertility rate arise from poor sperm quality brought by inadequate nutrition [9]. However, [34] stated that enhanced management, particularly focusing on nutrition through supplementation, can raise the productivity of chicken bred locally. Due to the strong correlation between overall fertility and nutrition, diet modification has been recommended in order to improve the quality of cockerel semen [19, 37] reported that nutrition can have a significant impact on the quality of sperm, an indicator of sperm's ability to effectively fertilize an egg, which is crucial for the production of eggs that hatch. Amino acids as methionine, cysteine, and arginine are necessary for spermatogenesis process in order to produce semen of high-quality [17], a fatty acid α-linoleic, vitamin A, C and E as well as zinc and selenium [12]. The nutritional and therapeutic benefits of leaf meals have recently led to their usage in animal nutrition. One of the plants containing all of these compounds is *Moringa oleifera*. The most notable characteristics of MOLM include its high protein content in leaves (27%) and sufficient amino acid profile. It also contains high levels of vitamins A and E, low levels of anti-nutritional compounds, fatty acids, phenol (8 g/ml), flavonoids (27 g/ml), an alkaloid (0.07%), ferulic acid (46.8 mg/g), and chlorogenic acid (18.0 mg/g) [40]. The in cooperation of MOLM in the feeds of chickens raised locally was predicted to have no adverse impact on the fertility, hatchability, and chick quality characteristics. Therefore, the purpose of this research was to determine how MOLM affected improved indigenous laying chickens' fertility, hatchability, and chick quality characteristics.

2. Materials and Methods

2.1 Study site

The feeding study was held at the Naivasha Poultry Research Unit of the Kenya Agricultural and Livestock Research Organization (KALRO). The Institute is in the Naivasha sub-county of Nakuru County [20].

2.2 Collection and preparation of Moringa leaf meal

The Moringa leaves were obtained from the Emuka Moringa Farmers' Cooperative Society, which is made up of farmers from Makueni County's Emali, Mulala, and Tutini Wards. The leaves were gathered by cutting off young branches of the trees and ripping them off from the tips by hand (manually), then cleaning them with warm water and air drying them under a shade for 3-4 days until they were crisp to the touch and maintained their greenish colour. The leaf meal (MOLM) was produced by milling the leaves through a 3 mm filter using a BS-180 hammer mill® and was stored in airtight sacs until it was needed for feed formulation.

Table 1: Composition of experimental diets

Ingredients (g/kg)	T_1	T_2	T_3	T_4	T_5	T_6
Whole maize	61.00	61.00	51.00	51.00	39.00	39.00
Soybean meal	20.00	20.00	10.00	10.00	5.00	5.00
MOLM	0.00	0.00	20.00	20.00	40.00	40.00
Fishmeal	10.00	10.00	10.00	10.00	10.00	10.00
DCP	0.50	0.50	0.50	0.50	0.50	0.50
Limestone	7.50	7.50	7.50	7.50	5.00	5.00
Iodized salt	0.30	0.30	0.30	0.30	0.30	0.30
Premix	0.25	0.25	0.25	0.25	0.25	0.25
Vegetable oil	0.50	0.50	0.50	0.50	0.50	1.00
Enzyme	0	0.035	0	0.035	0	0.035
Calculated analysis						
ME (KJ//kg)	2607.60	2609.50	2605.50	2603.60	2600.02	2606.60
Crude protein	16.28	16.25	16.85	16.80	16.66	16.76
Crude fibre	3.21	3.17	5.03	5.05	5.67	5.72
Recommended analysis						
ME (KJ/kg)	2600	2600	2600	2600	2600	2600
Crude protein	16	16	16	16	16	16
Crude fibre	4	4	4	4	4	4

ME: Metabolizable energy, DCP: Di-calcium phosphate, MOLM*: Moringa oleifera* leaf meal. A premix containing: vitamin A 750,600IU/kg, vitamin E 30.61IU/kg, vitamin B 24000 mg, biotin 30mg, copper 5000 mg, lysine 0.42%, methionine 0.5%, alanine 0.84, Arginine 0.93% and Cysteine 0.32% was added at 0.25% of diet to supply minerals, vitamins, trace elements and amino acids.

2.3 Proximate Analysis

Analysis of the dry matter, ash, ether extract, and crude protein in feed samples was done. according to AOAC [5] methods. The cell wall components, neutral detergent fibre, and acid detergent fibre were identified. by the procedure of [44].

2.4 Management of experimental chicken

The layers were kept in a system of deep litter with wood shavings as litter material, which was monitored by periodic raking and replacing moist areas as necessary. The house was thoroughly cleaned and disinfected before the trial began. In a completely randomized design using a factorial layout, 90 chickens were randomly selected, weighed, and assigned to six dietary treatments. Five chicken were replicated three times for each treatment. Before feeding trial, birds were acclimatized for one week. A 5 hen to 1 rooster mating ratio was established at week 31, and egg collecting was carried out over a period of four weeks. Regular management practices included providing feed and water on a daily basis, cleaning the feeders and drinkers, and collecting eggs on a regular basis.

2.5 Data collection

At the end of the study, incubation eggs were collected (11 weeks of lay) and were kept at 18 °C for a week prior to setting. Fifty medium-sized eggs (50 eggs per replication) were randomly picked, disinfected, and incubated for 18 days with a broad end sticking up in an incubator with temperature set at 37.8 °C and 55% relative humidity (RH). Fertility was checked with an egg candler on the 9th and 21st day of incubation in the dark room, and the eggs were then transferred to a hatcher at 37.4 °C and RH 70% until the eggs hatch. The chicks were tallied after hatching, and the eggs that did not hatch was taken into account to determine the fertility rate using the formula proposed by [3]. Percentages on average fertility was calculated by dividing the total number of viable eggs found during candling by the total number of eggs set. Fertility = number of fertile eggs /number of eggs × 100. Hatchability was determined through the number of chicks produced by viable eggs employing [39] formula. Hatchability = number of hatchlings / number of viable eggs × 100.Distinct stages of the embryonic mortality of incubated eggs was measured by breaking eggs that appeared mortal at the end of incubation to assess early, mid, and late embryonic mortalities. The assessment of chick quality was carried out using chick weight, chick length, and visual scoring. Stretching the chick from the beak to the end of the middle toe along a ruler, we were able to determine its length. At hatch, a chick was weighed to determine its weight [29]. The chick's cleanliness, dryness, lack of diseases or abnormalities, and presence or absence of brilliant eyes were taken into account while determining the visual score [38].

2.6 Experimental design
The initial weight was fitted as a covariate in a completely randomised design with a factorial layout. There were 18 experimental units with 5 hens and 1 rooster per treatment, each replicated 3 times. The statistical model was as follows:

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

Assumptions were that x_{ij} is not affected by treatment
x_{ij} is deviated from the mean of the covariate, x bar

$Y_{ijk=}$Response variable of interest
μ= Overall mean
$A_{i=}$= Effect associated with the i^{th} level of MOLM
$B_{j=}$= Effect associated with the j^{th} level of enzyme
$AB_{ij=}$ Effect associated with the i^{th} level of MOLM and j^{th} level of enzyme
X_{ij} = Initial body weight of an individual bird (covariate)
$\bar{x}$ = Overall mean for initial body weight
ε_{ijk}= Random error

2.7 Statistical analysis
The general linear model (GLM) approach of the statistical analysis system version 9.0 was used to analyze the data. Tukey's test at *p*<0.05 was used to differentiate significant means.

Results and Discussions

Table 2: Chemical composition of ingredients

Ingredients (%)	Dry matter	Ash	Crude protein	Crude fibre
Maize	90.7	3.09	9.65	3.42
Fish meal	91.7	3.21	58.21	3.16
Soybean meal	91.4	7.99	47.87	6.56
Moringa oleifera leaf meal	89.7	11.59	30.24	7.36

Table 3: Chemical composition of experimental diets

Parameters (%)	T_1	T_2	T_3	T_4	T_5	T_6
Gross energy (MJ/kg)	11.2	11.2	11.13	11.13	11.10	11.10
Dry matter	92.3	91.6	92.1	91.7	92.6	92
Ash	24.59	24.89	22.58	22.57	29.7	22.61
Crude protein	18.21	18.15	19.9	19.95	20.68	20.62
Crude fibre	2.71	2.95	3.15	3.05	3.56	3.48
Ether extract	5.46	4.66	6.69	6.56	6.82	6.44
Nutrient detergent fibre	30.18	30.13	32.03	32.16	35.34	35.10
Acid detergent fibre	11.22	11.32	12.1	11.86	14.37	14.74
Hemicellulose	18.86	18.71	19.83	20.13	20.87	20.29

Table 4: Fertility and hatchability of hens fed different dietary levels of MOLM

Parameters (%)	T_1	T_2	T_3	T_4	T_5	T_6	SEM	*p* value
Fertility	76.21^a	76.76^a	81.95^b	81.36^b	94.56^c	95.05^c	2.18	<.0001
Hatchability	71.83^a	71.56^a	84.09^b	84.88^b	95.98^c	94.67^c	6.18	0.02
Embryonic Mortality								
Early embryonic mortality	0.16^c	0.19^c	0.06^a	0.04^a	0.14^b	0.12^b	0.13	<.0001
Mid embryonic mortality	0.28^c	0.24^c	0.00^a	0.00^a	0.16^b	0.14^b	0.09	0.04
Late embryonic mortality	2.15	2.24	1.87	1.90	1.68	1.62	0.38	0.22
PIPS	0.74	0.79	0.57	0.52	0.62	0.63	0.18	0.42
Culls	0.05	0.05	0.00	0.00	0.05	0.05	0.04	0.85

Fig 1: Pictorial of embryonic Mortality of chicks from hens fed different levels of MOLM

According to Table 4, eggs from hens fed MOLM-based diets showed the highest hatchability ($p<0.05$), while eggs from hens fed a control diet showed the lowest hatchability. Eggs from hens on MOLM-based diets had significantly ($p<0.05$) greater fertility rates than those from control groups in terms of fertility rate. High early embryonic mortality was observed in control diet and was statistically different ($p>0.05$) with the MOLM- based diets. Hens fed MOLM-based diets had significantly ($p<0.05$) lower mid embryonic mortality than the others. Mid embryonic mortality was high in control diet compared to the MOLM-based diets. There was no significance difference in the late embryonic death, pips and culls in the MOLM-based diets compared to control diet

Table 5: Effects of feeding different levels of MOLM on chick quality of improved indigenous laying hens

Parameters	T_1	T_2	T_3	T_4	T_5	T_6	SEM	*p* value
Chick weight (g)	42.09[a]	42.27[a]	44.40[b]	44.14[b]	48.79[c]	48.12[c]	1.43	0.01
Chick length(mm)	18.64	18.27	18.40	18.41	18.34	18.33	0.16	0.65
Visual scoring	0.19[c]	0.18[c]	0.00[a]	0.00[a]	0.14[b]	0.14[b]	0.07	<.0001

Day-old chicks from hens fed MOLM-based diets were heavier than those from hens fed the control diet. They were no significant ($p>0.05$) different in the chick length on MOLM-based diets compared to the control diet (Table 5). Hens fed MOLM diets improved statistically ($p<0.05$) in terms of visual scoring which involved checking chicks that were free from deformities, clean and had bright eyes.

3. Discussion

Fertility and hatchability

Fertility and hatchability are attributes that are most influenced by environmental and genetic variables [37] reported that bird's strain, nutrition, egg quality, egg storage conditions, and mating ratio are some of the factors that affect hatchability and fertility. Selenium-containing plant leaves enhanced hatchability and fertility percentages [35]. When compared to chickens fed a control diet, chickens fed MOLM-based diets actually displayed better fertility and hatchability of viable eggs. Iron, calcium, phosphorus, and moderately high levels of Vitamin C are all contained in substantial amounts in Moringa [4]. The current study is also consistent with [2], who observed that adding ascorbic acid to the diets of indigenous venda hens increased hatchability [30] showed that Zinc and vitamin E are abundant in MOLM which is in agreement with [36] and [6] who reported that egg hatchability may be enhanced by zinc and vitamin E. Zinc aids in maintaining the integrity of the sperm nucleus' deoxyribonucleic acid (DNA) chromatin, which protects the genetic material structure necessary for successful fertility. Zinc helps protect the DNA chromatin structure in the sperm nucleus, or the structure of the genetic material, which is necessary for successful fertilization, according to research by [10]. Increased hatchability was shown by [13] with higher levels of zinc in the diet of brown parent stock layers. The environment of the oviduct's sperm storage tubules was improved by adding organically bound selenium to the diets of laying hens. This prolonged the sperms' lifespans and increased a count of sperm pores in the yolk layer [18, 35] reported that addition of selenium-rich plant leaves enhanced fertility and hatchability [32] stated that the physical features of the egg, such as shape index, weight, length, and width, all play a crucial role in embryo growth and hatching success. This could explain why the group fed MOLM-based diets had higher fertility and hatchability in the current study. However, the control group's comparatively poor fertility and hatchability may be due to a lack of essential elements such as zinc and Vitamin E, which are required for better hatchability [26]. The proportion of viable eggs that hatched in this study was higher than the 77% reported by [41] for layer hens fed a diet containing 5% MOLM, but it was similar to the 84% reported by [31] for PK chickens that had been artificially inseminated with sperm from excellent performing cocks. The fertility and hatchability rates in the current investigation were comparable to those reported by [47], who indicated that the addition of MOLM at different levels enhanced egg fertility and hatchability. However, this research's results were inconsistent with the findings of [16], who used MOLM as an alternative feed ingredient in the layer ration and found that MOLM had no effect on fertility, hatchability, or embryonic mortality.

Chick quality

The length, width, weight, shell thickness, and form of the egg are all indices that have a significant impact on how well the egg hatches and how well the embryo develops [32]. These may account for the improvement in chick quality observed in the groups offered MOLM-enriched diets in the current research. In in comparison to the control group, the weight of one-day-old chicks was found to be positively improved in diets containing MOLM. The current study's improved chick weight is consistent with the findings of [16], who discovered that hens fed 5% MOLM had greater chick weight. The addition of protein to chicken feed, according to [11], increases the weight of chicks at hatching. According to [1] and [28], who discovered a correlation between egg size and chick weight at hatching, the current study's findings relating egg size and chick weight at hatching were in agreement with their findings [25] observed that more protein in the chicken's diet could increase chick weight. The high protein content of MOLM is well known [7]. In the current research, adding MOLM to the layers improved early and mid-embryonic mortalities but had no effect on late embryonic mortality. This is consistent with study by [16], who described the use of M. oleifera leaf meal and cassava root chips as an alternate feed component for layer rations. Early embryonic mortality has been associated to poor parent stock feeding, fumigation during the initial days of incubation, handling hatched eggs before setting, trembling and shocking, and insufficient turning, according to studies by [21, 43]. On the other hand, mid and late embryonic mortality is significantly influenced by turning and caring for hatching eggs. However, temperature, humidity, and ventilation are all elements that influence distinct phases of embryonic mortality [21].

Hatchability and chick quality are closely correlated with egg quality parameters, with the best hatchability and chick quality being produced by eggs with the better egg size, yolk, albumen, and yolk quality [22, 42] have shown that the primary factor influencing weight of the chick at hatch is egg weight. The incorporation of MOLM in the present research improved quality of the chick by enhancing both interior and exterior egg quality indicators. According to [23] the nutrients deposited in the eggs are required for the development and growth of the developing embryo and the chick after hatch.

4. Conclusion
The results of this research indicate that adding enzyme-treated MOLM significantly enhanced features related to fertility, hatchability, and chick quality of improved indigenous laying hens.

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