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

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Abstract

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

Keywords: Egg, enzyme, hens, moringa, treatment

1. Introduction

The chicken industry is one of the animal sectors with the fastest rate of growth in the world, but it is constrained by a severe shortage of protein feed ingredients, particularly in developing nations^[5]. Consequently, it is important to investigate the non-traditional feed sources that are available and that can be utilized to formulate chicken feed^[13]. This is crucial for layer hens, which are highly sensitive to nutrition, often decreased egg production and even cessation of lay as a result of inadequate nutrient supply^[3]. According to^[28], the nutritional content of eggs is influenced by geography and the composition of the diet, which affect factors like egg size, weight, albumen to yolk and shell ratios. The current trend of increasing feed ingredient prices has led to a quest for unconventional feedstuffs that have the potential to improve chicken performance at a lower cost^[4]. The primary protein source that is frequently utilized is soybean meal due to its adequate balance of essential fatty acids and amino acids to meet the nutritional requirements of laying hens^[10]. Due to soybean meal's remarkable performance, the demand for it in the animal feed sector has increased, which has resulted in both shortages of it and an increase in its price^[1]. Research and industry are becoming more interested in the

utilization of non-conventional protein sources to partially or entirely supplement soybean meal in layer diets in the goal of improving egg quality, lower feeding costs, and increase profit margins [18]. The non-traditional protein source selected to substitute soybean meal in layer diets must also provide sufficient nutrients that can enhance egg quality and consumer health. The Moringa (*M. oleifera*) plant has tremendous potential and might be grown as an economically successful crop to help reduce poverty [15]. Its outstanding qualities make Moringa a suitable soybean meal substitute. It is a perennial plant that may be harvested multiple times within a single growing season, is easily planted in the field, has good coppicing ability, and has the potential to lower the cost of feed [25]. Since Moringa leaf meal (MOLM) comprises a considerable amount of nutrients, including proteins, vitamins, minerals, flavonoids, phenols, and carotenoids, it is expected to be a viable resource for commercial egg production in developing countries. The range of MOLM's crude protein is 23.0 to 30.3% [32]. For growth and development, it has a much higher mineral content than soybean or corn meal, containing calcium and phosphorus up to 12.0% [29]. There have been numerous initiatives to improve the egg quality of laying hens. The improvement in yolk colour will help the poultry sector and the general public because it is a crucial organoleptic factor for the acceptance of products that consumers associate with quality. Since Moringa leaves contain 16.3 mg of β -carotene per 100g of dry matter, they are excellent for increasing egg yolks colour [27]. The addition of MOLM to the ration can enhance egg quality and have a good impact on chicken health and performance due to the bioactive components and other

phytochemicals it contains. Based on this, this study was done to ascertain the impact of varying inclusion levels of enzyme-treated MOLM on the quality of eggs laid by improved indigenous laying hens.

2. Materials and Methods

2.1 Study site

The feeding trial took place at the Naivasha Poultry Research Unit of the Kenya Agricultural and Livestock Research Organization (KALRO). The Institute is located in the Naivasha sub-county in Nakuru County. The Research Center receives 1,100 millimeters of rain annually on average and is situated at a height of around 1,700 meters above sea level. The lowest temperature is 8°C in July and August, while the maximum is 25°C in January and February [16].

2.2 Collection and preparation of moringa leaf meal

The Moringa leaves were purchased from Emuka Moringa Farmers' Cooperative Society, comprising of farmers from Emali, Mulala and Tutini Wards in Makueni County. The Farmers' Cooperative Society is supported both by Child Fund International Organization (a non-profit organization) funded by local partner cooperation and the County government of Makueni. The leaves were harvested by cutting off young branches of the trees and stripping off from the tips by hand (manually), and then washed with warm water; air dried under a shade for 3-4 days until they were crispy to touch, and retained their greenish colour. The leaf meal (MOLM) was prepared by milling the leaves using a BS-180 hammer mill® through a 3 mm filter then stored in airtight sacs until it was required for feed formulation.

Table 1: Composition of experimental diets

Ingredients (g/kg)	T1	T2	T3	T4	T5	T6
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
CP	16.28	16.25	16.85	16.80	16.66	16.76
CF	3.21	3.17	5.03	5.05	5.67	5.72
Recommended analysis						
ME (KJ/kg)	2600	2600	2600	2600	2600	2600
CP	16	16	16	16	16	16
CF	4	4	4	4	4	4

The experimental diets were compounded by inclusion of enzyme-treated MOLM which was collected from Makueni County at 0, 20 and 40 kg of the total diet. A premix containing: vitamin A 750,600IU/kg, Vitamin E 30.61IU/kg, vitamin B 24000 mg, biotin 30 mg, 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

In accordance with the procedures of AOAC [6] 15th Edition, proximate analysis of the feed samples was carried out at Egerton University's Animal Nutrition Laboratory: dry matter

[6], ash [6], ether extract [6]. Total nitrogen was determined by Kjeldahl method multiplied by 6.25 for the crude protein content [6]. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) components of the cell wall were determined [31]

2.4 Management of experimental birds

The layers were kept in a deep litter system with wood shavings as litter material, which was managed through periodic raking and replacing damp portions whenever necessary. Before the experiment began, the house was properly washed and disinfected. One hundred (100), twelve week-old improved indigenous layer chicken were purchased from Kenya Agricultural Livestock Research Organization

(KALRO), from which ninety (90) birds were randomly sampled. The birds were weighed and assigned to six dietary treatments in a completely randomized design with a factorial layout. There were 5 birds per treatment, and each treatment had three replicates. The birds were acclimatized for one week, before feeding trial commenced. The routine management practices included provision of feed and water daily, cleaning of the feeders and drinkers, collection of eggs and periodic maintenance of bedding in good condition. The experiment lasted 45 weeks.

2.5 Data collection

To evaluate the external and internal egg quality, seventy-two eggs (four eggs per replicate) were randomly selected from each group and weighed. Before the eggs were broken, their weights were recorded. Manual separation of the egg's components (yolk, albumen, and shell) was done. With a digital Vernier caliper, four sections of egg shells one from each of the two ends (wide and narrow end) and two from the body were measured for thickness to the nearest 0.01 mm, and the measurements were averaged. Yolk colour was determined using a Roche yolk colour fan [24]

2.6 External egg quality

Egg and shell weight was determined using a 0.001 g sensitive electronic weighing device. Egg length and width was determined using a Vernier caliper. Egg width per egg length multiplied by 100 was used to compute the shape index. Eggshell weight divided by egg weight multiplied by 100 was used to calculate egg shell ratio.

2.7 Internal egg quality

Albumen and yolk weight, height and width were recorded separately [9]. Yolk weight per egg weight multiplied by 100 was used to compute the yolk ratio. Yolk height divided by yolk diameter multiplied by 100 was used to compute the yolk index. Yolk weight divided by albumen weight multiplied by 100 was used to compute yolk/albumen ratio. Yolk diameter was determined by digital Vernier caliper.

2.8 Experimental design

A completely randomised design with a factorial layout where the initial weight was fitted as a covariate was used. There

were 18 experimental units with 5 growers 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

2.9 Statistical analysis

Data was analyzed using ANOVA of the general linear model (GLM) procedure of the statistical analysis system version 9.0. Significant means were separated using Turkey's test at $p < 0.05$.

3. 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
MOLM	89.7	11.59	30.24	7.36

Table 3: Chemical composition of experimental diets

Parameters (%)	T1	T2	T3	T4	T5	T6
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
NDF	30.18	30.13	32.03	32.16	35.34	35.10
ADF	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: Effect of MOLM-based diets on external and internal egg quality of improved indigenous laying hens.

Parameters (mm)	T1	T2	T3	T4	T5	T6	p value	SEM
Egg weight (g)	57.14 ^a	57.63 ^a	60.29 ^b	60.73 ^b	64.97 ^c	64.44 ^c	<.0001	0.26
Egg length	60.23 ^a	60.45 ^a	59.06 ^b	59.74 ^b	55.75 ^c	55.40 ^c	<.0001	0.15
Egg width	43.26	43.07	44.09	42.99	43.12	42.23	0.59	0.13
Egg index	77.55	76.40	75.91	74.97	75.63	75.92	0.66	0.34
Albumin length	98.29 ^a	97.82 ^a	95.51 ^b	94.12 ^b	90.01 ^c	91.41 ^c	<.0001	0.52
Albumin width	62.07	62.24	62.45	63.79	61.28	62.55	0.87	0.66
Albumin height	5.16 ^a	5.17 ^a	6.38 ^b	6.28 ^b	6.96 ^c	6.87 ^c	0.03	0.13
Yolk weight (g)	15.43 ^a	15.33 ^a	15.61 ^b	15.73 ^c	16.24 ^d	16.8 ^e	<.0001	0.08
Yolk diameter	41.36 ^a	42.34 ^a	38.18 ^b	38.72 ^b	36.72 ^c	36.92 ^c	<.0001	0.15
Yolk height	14.25	14.24	13.88	13.79	12.96	12.93	0.17	0.06
Yolk ratio%	24.18	24.15	24.37	24.11	23.98	22.15	0.49	0.26
Yolk index%	33.39	35.93	37.17	37.13	37.14	33.19	0.09	0.21
Yolk/Albumin ratio %	48.39	46.83	43.19	43.94	44.22	41.64	0.12	0.35
Yolk colour	6.71 ^a	6.93 ^a	13.62 ^b	13.31 ^b	14.62 ^c	14.56 ^c	<.0001	0.13
Shell weight (g)	5.36 ^a	5.48 ^a	6.25 ^b	6.28 ^b	6.81 ^c	6.71 ^c	0.02	0.12
Shell thickness	0.43 ^a	0.44 ^a	0.58 ^b	0.56 ^b	0.79 ^c	0.74 ^c	<.0001	0.07
Shell ratio%	11.84	11.46	11.39	11.79	12.04	10.71	0.95	0.21

Means in the same row without common superscripts are differ at $p < 0.05$

3.1 Egg characteristics

Consumer preference is influenced both directly and indirectly by the egg quality characteristics such as size, shape, colour, and shell thickness.

Egg weight, shape index, shell thickness

Increased egg weight in hens fed with MOLM-based diets compared to control diet was caused by the presence of nutrients e.g. vitamins and minerals in Moringa leaf meal. This is in agreement with [23] who reported that Moringa leaves are also a source of vitamin A, riboflavin, nicotinic acid, folic acid, pyridoxine, ascorbic acid, beta-carotene, calcium, iron, and α -tocopherol.

In response to dietary *M. oleifera* leaf meal, there was no difference in egg shape index across the groups ($p>0.05$). This is consistent with [12] finding that adding MOLM had no impact on the egg shape index, which is correlated to egg quality and eggshell strength. Egg shape index increases significantly with age within chicken breed [30]. The egg shape index measured in this study ranged from 74 g to 77 g. This is in line with what [7] reported in commercial layers. It is possible that the higher calcium content of Moringa leaves (870 mg/100 g) [20] as compared to soybean meal (300.36 mg/100 g) [14] is what caused the laying hens fed a diet with 40% MOLM inclusion to have higher ($p<0.05$) shell thickness and weight.

Yolk weight and colour

A desirable attribute for consumers is the colour of the yolk, and MOLM has a high content of xanthophyll's (167.1 6.1 lg/g; [22]), which are associated with the coloration of body parts. Inclusion of MOLM in the diet increased the intensity of yellow colour in egg yolk ($p<0.05$) in comparison to the control diet. Significantly ($p<0.05$), the eggs from chicken fed a diet containing 40 percent MOLM inclusion had the highest Roche colour score of 14.62. The yolk colour of eggs from hens fed MOLM-containing diets showed that the increased yolk colour was caused by the diet's high xanthophyll content. Yolk colour value increased linearly with the increasing level of inclusion of MOLM (figure 1), which is in line with the finding of [2]. According to [11], broilers' combs, beaks, and legs developed more intense colouring after MOLM inclusion. This is consistent with [17]. In this study, the increase in egg yolk colour intensity demonstrated that chicken can effectively absorb and use the carotenoid pigments found in Moringa leaves, which are rich in vitamin A. The carotenoid content of MOLM is responsible for the increase in yellow colour intensity. The development of various colour scores in egg yolks is significantly influenced by carotenoids. This finding is supported by research by [21], who found that adding 10-20 percent MOLM to laying chicken or broiler diet can significantly enhance the yellowness of the skin and egg yolk, respectively.

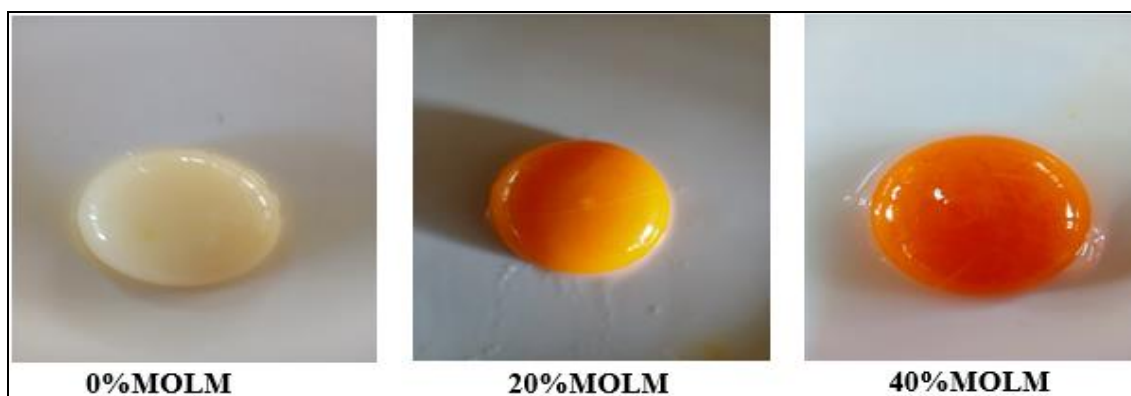


Fig 1: Yolk colour variation of eggs from hens fed different levels of MOLM

When MOLM-based diets were compared with control, there were significant variations in yolk weight ($p<0.05$). This pattern could be explained by the presence of lysine and methionine in MOLM and a variety of other amino acids, which could provide the required amount of important nutrients for improved egg production and yolk weight [26].

Egg length, albumin length, height, and yolk diameter

When evaluating albumen quality and egg freshness, the albumen height is a crucial parameter. The albumen height of eggs increased as dietary MOLM inclusion increased; this could be attributable to the availability of lysine and methionine in Moringa leaves, as reported by [8]. The egg width, albumin width, yolk height, ratio, index and yolk/albumin ratio, shell weight and ratio were similar ($p>0.05$) for all the dietary treatments. As the amount of MOLM in the diet increased, the egg length, albumin length, and yolk diameter were all significantly reduced ($p<0.05$) in comparison to the control. This is because reducing dietary protein from the diet lowers egg quality traits like albumen size because the protein content of soybean meal (40-49%) is higher than that of MOLM (30.3%) [19].

4. Conclusion and recommendation

Dietary inclusion of 20-40 percent enzyme-treated MOLM improved egg weight, yolk weight, albumin height, yolk colour and shell thickness of eggs with no negative effects on egg quality of laying hens. Inclusion of MOLM at levels above 20 percent also increased the intensity of yellow-orange colour of the egg yolk compared with the control diet. Therefore, MOLM can be used both as a source of protein and also to replace synthetic carotenoids which are used by commercial feed millers to improve egg yolk colour.

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6. References

1. Abbas TE. The use of *Moringa oleifera* in poultry diets. Turkish Journal of Veterinary & Animal Sciences. 2013;37(5):492-496.
2. Abou-Elezz FMK, Sarmiento-Franco, L, Santos-Ricalde R, Solorio-Sanchez F. Nutritional effects of dietary inclusion of *Leucaena leucocephala* and *Moringa oleifera* leaf meal on Rhode Island Red hens' performance. Cuban Journal of Agricultural Science. 2011;45(2):163-169.
3. Adejinmi OO, Hamzat RA, Raji AM, Owosibo AO. Performance, nutrient digestibility and carcass characteristics of broilers fed cocoa pod husk-based diets. Nigerian Journal of Animal Science. 2011;13:51-58.
4. Adeosun SL, Ogundipe SO, Sekoni AA, Omege JJ. Effects of synthetic ascorbic acid and baobab fruit pulp meal supplementation as sources of ascorbic acid for layer diets during cool wet season (Sub Theme Livestock Improvement, Health and Management). Journal of Advances in Agricultural Science and Technology. 2013;1(2):24-27.
5. Al-Harhi MA, El Deek AA, Attia YA, Bovera F, Qota EM. Effect of different dietary levels of mangrove (*Laguncularia racemosa*) leaves and spices supplementations on productive performance, egg quality, lipids metabolism and metabolic profiles in laying hens. British Poultry Science Journal. 2009;50:700-708.
6. AOAC. Official Methods of Analysis. 15-th Edition. Association of Official Analytical Chemists, Washington, USA; c1990.
7. Ayanwale BA, Kpe M, Ayanwale VA. The effect of supplementing *Saccharomyces cerevisiae* in the diets on egg laying and egg quality characteristics of pullets. International Journal of Poultry Science. 2006;5(8):759-763.
8. Bunchasak C, Silapasorn T. Effects of adding methionine in low-protein diet on production performance, reproductive organs and chemical liver composition of laying hens under tropical conditions. International Journal of Poultry Science. 2005;4(5):301-308.
9. Dasari S, Manthani GP, Mallam M, Rudra NC. Genetic analysis of egg quality traits in White Leghorn chicken. Veterinary World. Egg Production and Egg Shell Quality of Japanese quail. International Journal of Poultry, c2013, p.263-266.
10. De Moraes Oliveira VR, de Arruda AMV, Silva LNS, de Souza Jr JBF, de Queiroz JPAF, et al., Sunflower meal as a nutritional and economically viable substitute for soybean meal in diets for free-range laying hens. Animal Feed Science and Technology. 2016;220:103-108.
11. Donkor AM, Glover RLK, Addae D, Kubi KA. Estimating the nutritional value of the leaves of *Moringa oleifera* on poultry; c2013.
12. Ebenebe CI, Anigbogu CC, Anizoba MA, Ufele AN. Effect of various levels of *Moringa* leaf meal on the egg quality of ISA Brown breed of layers. Advances in Life Science and Technology. 2013;14(1):35-49.
13. El-Deek AA, Attia YA, Al-Harhi MA. Including whole inedible date in grower-finisher broiler diets and the impact on productive performance, nutrient digestibility and meat quality. Animal. 2010;4:1647-1652.
14. Etiosa OR, Nnadozie BC, Anuge. Mineral and Proximate Composition of soya bean. Asian Journal of Physical and Chemical Sciences. 2017;4(3):1-6.
15. Fatima GH, Muna AI. *Moringa oleifera*: Nature is most nutritious and Multipurpose Tree, International Journal of Scientific and Research Publication. 2013;3(4):1-5.
16. Herrero M. Climate Variability and Climate Change and their Impacts on Kenya's Agricultural sector, Nairobi, Kenya: International Livestock Research Institute (ILRI); c2010.
17. Kaijage JT. Effect of substituting sunflower seed meal with *Moringa oleifera* leaf meal on the performance of commercial egg strain chickens and egg quality. Unpublished MSc Dissertation, Sokoine University of Agriculture, Tanzania; c2003.
18. Moreki JC, Gabanagosi K. Potential use of *Moringa oleifera* in poultry diets. Global Journal of Animal Scientific Research. 2014;2(2):109-115.
19. Moyo B, Masika P, Hugo A, Muchenje V. Nutritional characterization of *Moringa (Moringa oleifera Lam.)* leaves. African Journal of Biotechnology. 2011;10(60):1292-1293.
20. Ming-Chih S, Cheng-Ming C, Sue-Ming K, Min-Lang T. Effect of different parts (leaf, stem and stalk) and seasons (summer and winter) on the Chemical Compositions and Antioxidant Activity of *Moringa oleifera*. International Journal of Molecular Science. 2011;12(5):6077-6088.
21. Olugbemi TS, Mutayoba SK, Lekule FP. Evaluation of *Moringa oleifera* leaf meal inclusion in cassava chip based diets fed to laying birds. Livestock Research for Rural Development. 2010;22(6):1-6.
22. Pasaporte MS, Rabaya FJR, Toleco MM, Flores DM. Xanthophyll content of selected vegetables commonly consumed in the Philippines and the effect of boiling. Food Chemistry. 2014;158:35-40.
23. Prasad A, Ganguly S. Promising medicinal role of *Moringa oleifera*: A review. Journal of Immunology and Immunopathology. 2012;14(1):1-5.
24. Saeed M, Abd El-Hack ME, Arif M, El-Hindawy MM, Attia AI, Mahrose KM, Noreldin AE. Impacts of distiller's dried grains with solubles as replacement of soybean meal plus vitamin E supplementation on production, egg quality and blood chemistry of laying hens. Annals of Animal Science. 2017;17(3):849-862.
25. Sarwatt SV, Milang'ha MS, Lekule FP, Madalla N. *Moringa oleifera* and cottonseed cake as supplements for smallholder dairy cows fed Napier grass. Livestock Research for Rural Development. 2004;16(6):12-18.
26. Sohail S, Bryant M, Roland D. Partial Explanation for Difference in Response of Hens Fed Diets Formulated Based on Lysine vs. Protein. Journal of poultry Science. 2003;2(5):345-350.
27. Singh G, Kawatra A, Sehgal S. Nutritional composition of selected green leafy vegetables, herbs and carrots. Plant Foods for Human Nutrition. 2001;56(4), 359-364.
28. Sparks NHC. The hen's egg-is its role in human nutrition changing? World's Poultry Science Journal. 2006;62(2):308-315.
29. Teixeira EMB, Carvalho MRB, Neves VA, Silva MA, Arantes-Pereira L. Chemical characteristics and fractionation of proteins from *Moringa oleifera* leaves. Food Chemistry. 2014;147:51-54.
30. Usman M, Bashir AM, Akram I, Zahoor, Mahmud A. Effect of age on production performance, egg geometry

- and quality traits of lakha variety of Aseel chicken in Pakistan. *Journal of Basic and Applied Sciences*. 2014;10:384-386.
31. Van Soest PV, Robertson JB, Lewis B. Methods for dietary fibre, neutral detergent fibre, and non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*. 1991;74(10):3583-3597.
 32. Wu D, Cai Z, Wei Y, Zhang C, Liang G, Guo Q. Research advances in Moringa as a new plant protein feed. *Chinese Journal of Animal Nutrition*. 2013;25(3):503-511.