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Effects of Supplementing Layers, With Varying Levels of Alfalfa and Moringa Fresh Leaves on Egg Production and Quality

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ABSTRACT

This feeding trial was conducted to evaluate and demonstrate the effects of supplementing intensively managed commercial layer chickens with varying levels of fresh-leaves of *Medicago sativa* (Al) and *Moringa stanophetela* (Ms) on the characteristics of eggs. A 3x3 factorial arrangement was used Levels of Al fresh leaves were 0, 200, or 400 g, and levels of Ms fresh leaves were also 0, 200, or 400 g. Each treatment was replicated 4 times and 11 laying birds were allocated per replicate. Treatment arrangements/combinations were as follows, such as T_1 (0 g of Ms & 0 g of Al), T_2 (0 g of Ms &200 g of Al), T_3 (0 g of Ms & 400 g of Al), T_4 (200 g of Ms & 0 g of Al), T_5 (200 g Ms & 200 g of Al), T_6 (200 g Ms & 400 g of Al), T_7 (400 g Ms & 0 g of Al), T_8 (400 g of Ms & 200 g of Al) and T_9 (400 g of Ms & 400 g of Al). The actual investigation was started in the 30th week and then ended after 10 weeks. To evaluate egg quality traits, 9 eggs (3 eggs per pen per month), with a total of 36 eggs per treatment were randomly selected and analyzed. The result revealed that yolk color was strongly influenced ($P<0.05$) by an interaction effect of that of the leveled Al and Ms supplementations. However, egg weight, shape index, shell thickness, albumen weight, Haugh unit, and yolk weight weren't significantly ($P>0.05$) influenced by such supplementations. The total egg production, egg production per pen, egg weight, egg mass, the hen-housed egg production and a hen day egg production were not ($P < 0.05$) influenced. The trend showed that the yellowness of the yolk-color became more deepened when increasing the amounts of either Al or Ms supplementation, as the main factors in the diets.

Keywords: Egg quality, Egg production, Yolk-pigmentation, Internal egg quality, and Egg characteristics.

INTRODUCTION:

Table egg quality refers to various standards that define both external and internal qualities. The internal quality is focused on the yolk height, yolk color, albumin viscosity, and Haugh unit. In contrast, the external quality refers to the eggshell thickness, egg width, and height and cleanliness. An egg with yellowish yolk is always preferred by most Ethiopian consumers. This consumer's habit is supported by Grashorn, (2016) who reported that in the most countries of the world, consumers prefer pigmented egg yolks, whereas pigmented poultry tissues are less desired. However, in contrast to the consumers' preference, as aforementioned, most of the eggs harvested from an intensively managed commercial layers have very low yolk pigmentation (less yellowness). On the other hand, Grashorn, (2016) suggested that pigmentation of egg yolks and poultry tissues (mainly skin and fat) directly

reflects the contents of carotenoids in the feed of birds. In this case, alfalfa leaves are mostly added to poultry diets as a source of xanthophylls for pigmentation. Dehydrated alfalfa is usually added at a very low level in poultry diets, due to its high crude fiber and low metabolic energy contents (Dansky, 1971) however, it is a rich source of vitamins, carotenoids and saponins (Whitehead *et al*., 198; Sen *et al*., 1998). Diarra *et al*. (2017) added that when layers were supplemented with dried moringa leaves, the performance of laying hens in terms of egg production and yolk pigmentation were significantly improved. Moreover Lu *et al*. (2016) reported also that layers supplemented with moringa leaves had a deeper yolk color and the height unit of albumen and Haugh were also increased. Kaijage *et al*. (2015) suggested that supplementation of moringa leaves increased the following parameters, such as yolk color score values, consumers' egg aroma score index and egg taste score index (Rahman *et al*., 2019).

A large number of reports on the nutritional quality of Moringa have earned it the name "a tree of life". Jed, (2005) suggested also that the protein quality of Moringa leaves comparable to that of milk and eggs. Additionally, scholars (Turk and Barnett, 1972; Mc-Naughton, 1978) reported that the presence of alfalfa, in the diet decreased the cholesterol level of the egg yolk. Similarly Güçlü *et al*. (2004) showed that adding 90 g/kg alfalfa into poultry diets decreased the serum lipids and cholesterol levels of the egg yolk, without adversely affecting performance. In contrast Mourao *et al*. (2006) reported that alfalfa meal had no effect on egg yolk cholesterol.

This current study used leaves of *M. stanophetela*. This preference is due to its abundancy, accessibility, and availability of this material around the vicinity areas. Melesse, (2011) and Melesse *et al.* (2012) reported also that leaves of *Moringa stenopetala* and Moringa oleifera have almost similar nutrient composition. Melesse *et al*. (2009) and Negesse *et al*. (2009) added also that leaves of M. stenopetala are rich in protein (28.2-36.2%) and contain considerable amounts of essential amino acids. Although the effects of leaves of alfalfa or moringa were individually investigated by previous research scholars, there is still limited information on the synergic effects of these two supplements on the egg characteristics of intensively managed layers.

Objectives of the study

To improve the yolk color of eggs which are mostly collected from an intensively managed commercial chickens through supplementation, with selected green leaves.

MATERIALS AND METHODS:

Study areas

The study was undertaken at Debre-Zeit Agricultural Research center (DZARC), which is located 45 km south east of Addis Ababa, at an altitude of 1900 meters above sea level and at 8.44^{o}N latitude and 39.02^{o} E longitude. The area has a bimodal rainfall pattern with a long rainy season from June to October and a short rainy season from March to May. The average annual rainfall and average maximum and minimum temperature for the area are 1100 mm 28.3 ^{0}C and 8.9 ^{0}C, respectively (DZARC, 2003).

Management of Experimental Chickens

Three hundred ninety-six pullets were purchased from Alema farm PLC and were housed in DZARC's National Poultry Farm. These chickens were vaccinated against Newcastle disease, Fowl Typhoid, and Fowl Pox diseases as per recommended date. The chickens were kept in experimental pens, with concrete floor and deep-litter system. *Teff-straw* was used as a bedding material. Based on the requirements of the chickens, the diets were formulated in DZARC (**Table 1**). The "feed-win software" was used while formulating the diet. When the age of the chickens was 30th weeks, the experimental birds were randomly allocated onto the experimental pens, with that of 11 birds per replicate. The bird's allocation was based on the equivalency of their grouped body weights. Eggs were collected every day (three times a day from each pen: at 8:30 pm and 10: 30 pm). These collected eggs were also weighted immediately after collection for each pen and the average egg weight was computed by dividing the total egg weight by the number of eggs. To evaluate egg quality traits, 9 eggs (3 eggs per pen per month), with a total of 36 eggs per treatment were randomly selected and analyzed. Collected eggs were recorded and weighed using electronic weighing balance to the nearest 0.01g.

Table 1: Feed ingredients and nutrient composition of the basal diet used in the experiment.

Ingredients	%
Maize	56.5
Wheat Middling	7.75
Soybean meal	10.4
Noug seedcake	10.0
Meat and bone meal	4.4
Salt	1.0
Limestone	9.0
Premix	0.5
DL-Lysine	0.3
DL-methionine	0.15
Total %	**100**
Nutrient composition	
CP %	16
EE %	3.71
CF %	5.14
Ca %	4.13
P %	0.56
ME kcal/kg	2800

CP: Crude Protein, EE: ether extract, CF: crude fiber, Ca: Calcium, P: phosphorus, ME: Metabolizable energy

The external egg quality parameters were assessed in terms of egg weight and egg shape index. After breaking the egg, near to the sharpen end, and carefully separating and dropping the contents, internal egg quality parameters were measured, in terms of shell weight, shell thickness, yolk weight, yolk height and yolk color, albumen weight, albumin height and Haugh Unit Score (HUS). Shell thickness was measured by the digital caliper while removing the internal membranes. While measuring this thickness, the average value was taken from blunt, middle, and sharp points of the egg (Aberra, 2010) Height of the thick albumen was measured with the micrometer and the Haugh Unit Score was also calculated using the formula (Haugh, 1937).

$HU = 100 \log (AH-1.7EW^{0.37}+7.6)$ (Haugh, 1937).

Where, HU = Haugh unit,
AH = observed albumen height (mm) and
EW = weight of egg (g).

The yolk color determined by comparing the color of a properly mixed yolk sample placed on a colorless glass with the color strips of Roche color fan measurement, which consists of 1 to 15 strips ranging from pale to orange-yellow. Shape index was computed using the following formula.
Egg shape index = (Width of egg/Length of egg) x100

Preparation of experimental diets

The fresh leaves of *Moringa stenopetala* were harvested from 7 years old trees that were planted in the Dibandiba and Adama integrated farm. Whereas leaves of Alfalfa (*Medicago sativa*) were also collected from DZARC, and both Ms and Al leaves were washed, weighted, and then hanged (in an upside-down position) in the middle of the experimental pens that to being reached by every bird that to pick/forage the fresh leaves. Samples of leaves of both Ms and Al were collected and subjected to chemical analysis at JIJE Analytical Testing Service Laboratory, Addis Abeba, which enabled us to know the nutrient composition (**Table 2**).

Table 2: Chemical composition of the leaves of *Moringa Stenopetala* and Alfalfa (*Medicago sativa*) (%).

	Moisture	Crude Fiber	Crude Fat	Crude Protein	Ash	Total Phosphorus	Calcium
Al	9.83	13.34	3.69	24.98	12.01	0.34	1.55
Ms	9.20	8.77	3.64	21.48	13.29	0.19	2.30

MS: Moringa (*Moringa stanophetela*), Al: Alfalfa *(Medicago sativa)*

Experimental design

A 3 x 3 factorial arrangement, involving 3 levels of Al (0, 200 or 400 g) and 3 levels of Ms (0, 200 or 400 g) was used in this feeding trial. A completely randomized design (CRD) was used. Each of the nine treatments was replicated four times and 11 laying birds were also allocated per replicate. The treatments' layout was as follows: T_1 (0 g of Ms & 0 g of Al), T_2 (0 g of Ms & 200 g Al), T_3 (0 g of Ms & 400 g of Al), T_4 (200 g of Ms & 0 g of Al), T_5 (200 g Ms & 200 g of Al), T_6 (200 g Ms & 400 g Al), T_7 (400 g Ms & o g of Al), T_8 (400 g Ms & 200 g of Al) and T_9 (400 g of Ms & 400 g of Al). The actual investigation was finished within 10 weeks (it was started on the 30th week and then ended on 40th week of the laying birds' age). Eggs were collected every day (three times a day from each pen: at 8:30 am, 1:30 pm, and 10:30 pm). These collected eggs were also weighed immediately

after collection for each pen and the average egg weight was computed by dividing the total egg weight by the number of eggs.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using the General Linear Models (GLM) Procedure of Statistical Analysis System (SAS, 2002). When significant differences were observed, treatment means were compared with Duncan's Multiple Range Test. All statements of statistical differences were based on p<0.05.

RESULTS AND DISCUSSION:

External and internal egg quality

The results of the effects of various levels of Moringa (*Moringa stelopetala)* (Ms) and Alfalfa (*Medicago sativa*) (Al) on internal and external egg quality characteristics are presented in **Table 3 & 4**. The results showed that the yolk color was significantly (P<0.05) affected by an interaction effect. This yolk color was also significantly (P<0.05) influenced by both the main effects. The yolk index and Albumen height were also influenced by an increasing level of leaves of Ms in diets as supplement. Whenever, increasing the amounts of an individual testing materials (Ms or Al), in the diets, the yellowness of the yolk color became significantly (P<0.05) deeper and deeper. Similarly Wubalem *et al.* (2016) reported also that yolk color was higher when higher amounts of moringa leaf added into the diets. Birds supplemented with all levels of Ms (0, 200 or 400 g), in their diets had significant (P<0.05) differences on pH values of the egg contents, albumen height and yolk index as compared to the other birds fed on diets supplemented with all levels of Al (0, 200 or 400 g). Albumen weight, Haugh unit, and yolk weight weren't significantly (P>0.05) influenced by the supplement-ation of leaves of Ms or Al. Moreover, these supplementations had no interaction effects on afore-mentioned parameters. The external egg characteristics, such as egg shape, egg weight, shape index and shell thicken had no significant (P>0.05) differences among the treatments. However, birds supplemented with all levels of Ms (0, 200 or 400 g), in their diets had significant (P<0.05) differences on egg weights.

Table 3: Effects of supplementing leveled fresh leaves of Moringa (*Moringa stanophetela*) and Alfalfa (*Medicago sativa*) on the internal egg quality.

MS	AL	Shell thickness	Albumen weight	Albumen height	Hough unit	Yolk weight	Yolk Index	Yolk color	Egg PH
0.00	0.00	0.40	35.15	8.75^{ab}	89.40	15.75	39.73	1.70^{d}	7.78
	200.00	0.40	38.93	9.53^{a}	90.05	16.55	42.38	6.40^{bc}	7.63
	400.00	0.38	36.23	8.83^{ab}	89.15	15.65	40.58	7.93^{abc}	7.63
200.00	0.00	0.40	36.65	8.23^{ab}	89.58	17.53	41.95	5.83^{c}	8.00
	200.00	0.40	34.55	8.20^{ab}	89.45	15.90	40.88	7.35^{abc}	7.78
	400.00	0.40	36.68	8.90^{ab}	89.48	15.30	42.13	8.15^{ab}	7.73
400.00	0.00	0.40	38.03	8.25^{ab}	89.90	17.30	40.48	6.20^{bc}	8.10
	200.00	0.40	36.63	8.08^{b}	89.73	15.98	38.70	7.23^{abc}	7.93
	400.00	0.40	37.35	8.05^{b}	89.93	16.00	38.55	9.13^{a}	8.13
Pooled SEM		**0.00**	**0.51**	**0.12**	**0.12**	**0.26**	**0.41**	**0.36**	**0.06**
Main Effect									
0.00		0.39	36.77	9.03^{a}	89.53	15.98	40.89^{ab}	5.34^{b}	7.68^{ab}
200.00		0.40	35.96	8.44^{ab}	89.50	16.24	41.65^{a}	7.11^{ab}	7.83^{ab}
400.00		0.40	37.33	8.13^{b}	89.85	16.43	39.24^{b}	7.52^{a}	8.05^{a}
	0.00	0.40	36.61	8.41	89.63	16.86	40.72	4.58^{c}	7.96
	200.00	0.40	36.70	8.60	89.74	16.14	40.65	6.99^{b}	7.78
	400.00	0.39	36.75	8.59	89.52	15.65	40.42	8.40^{a}	7.83
Source Variations (P- values)									
Ms		0.73	0.56	0.00	0.46	0.80	0.05	0.03	0.05
Al		0.73	0.99	0.76	0.77	0.17	0.95	0.00	0.48
Ms X Al		0.89	0.36	0.14	0.65	0.53	0.33	0.00	0.94

a,b,c Means between treatments, within a column with different superscript letters are significantly different p<0.05), Ms: *Moringa stanophetela*, Al: Alfalfa (*Medicago sativa*)

Table 4: Effects of supplementing leveled fresh leaves of moringa (*moringa stanophetela*) and Alfalfa (*medicago sativa*) on the external egg quality.

Ms	Al	Egg Weight	Shape index	Shell thickness	Shell Weight
0.00	0.00	59.63	76.25	0.40	5.85^{ba}
	200.00	64.80	78.23	0.40	3.87^{c}
	400.00	61.33	77.18	0.38	4.94^{bc}
200.00	0.00	62.78	76.45	0.40	5.71^{abc}
	200.00	62.40	77.98	0.40	5.74^{abc}
	400.00	62.50	78.13	0.40	5.58^{abc}
400.00	0.00	64.10	78.40	0.40	7.37^{a}
	200.00	63.48	77.98	0.40	5.59^{abc}
	400.00	64.30	78.05	0.40	5.93^{ba}
Pooled SEM		0.45	0.38	0.00	0.19
Main Effect					
0.00		61.92	77.22	0.39	4.89^{c}
200.00		62.56	77.52	0.40	5.68^{b}
400.00		63.96	78.14	0.40	6.29^{a}
	0.00	62.17	77.03	0.40	6.31^{a}
	200.00	63.56	78.06	0.40	5.07^{ab}
	400.00	62.71	77.78	0.39	5.48^{ab}
Source Variation (P-value)					
Ms		0.17	0.61	0.73	0.01
Al		0.46	0.53	0.73	0.02
Ms X Al		0.17	0.84	0.89	0.13

a,b,c Means between treatment within a column with different superscript letters are significantly different ($p<0.05$) Ms: *Moringa stanophetela*, Al: Alfalfa (*Medicago sativa*)

Results of egg production parameters are shown in **Table 5**. Egg production parameters, such as total egg production, egg production per pen, egg mass, hen housed egg production (HHEP) and hen day egg production (HDEP) were not ($P>0.05$) influenced by an interaction effect of supplementing, with an increased levels of fresh-leaves of Ms and Al. These parameters were not also influenced by supplementation of main effects (Ms or Al). Additionally, there were no significant differences among the treatment groups. The finding was in line with Etalem *et al*. (2014) who observed a non-significant effect of a diet containing leaves moringa in layer rations at 5% on HDEP. Also Kwari *et al*. (2011) and Olabode and Okelola, (2014) noted non-significant results on egg weight and egg production when fed M. oleifera leaf and twig meals at different levels ranging from 0.2 to 0.8%. On the contrasty Wubale *et al.* (2016) egg production parameters (total egg weight and HDEP) were significantly higher for birds fed diets containing 5% MOLM whereas HHEP was showed a lower value for birds at 10% MOLM added diets than 5 MOLM and similar with the rest treatments 0% and 15% MOLM added diets.

Table 5: Effects of supplementing leveled fresh of Moringa (Moringa *stanophetela*) and Alfalfa (*Medicago sativa*) on the egg production.

Ms	Al	Total egg Production	Egg production/pen	Egg weight (gm)	Egg mass	HHEP (%)	HDEP (%)
0.00	0.0	583.00	8.21	59.63	45.19	74.65	74.65
	200.0	520.75	7.34	64.80	43.29	66.68	69.79
	400.0	583.50	8.22	61.33	46.10	74.71	76.71
200.00	0.0	581.00	8.18	62.78	45.30	74.39	74.39
	200.0	574.50	8.09	62.40	48.28	73.56	78.89
	400.0	572.25	8.06	62.50	46.17	73.27	75.15
400.00	0.0	553.75	7.80	64.10	45.22	70.90	74.45
	200.0	552.00	7.78	63.48	45.27	70.68	74.08

	400.0	580.00	8.17	64.30	47.43	74.27	77.86
Pooled SEM		8.10	0.11	0.45	0.75	1.04	1.09
Main Effect							
0.00		562.42	7.92	61.92	44.86	72.01	73.72
200.00		575.92	8.11	62.56	46.58	73.74	76.14
400.00		561.92	7.92	63.81	45.97	71.95	75.46
	0.00	572.58	8.06	62.17	45.23	73.31	74.49
	200.00	549.08	7.73	63.56	45.61	70,30	74.25
	400.00	566.75	8.15	62.71	46.57	74.08	76.57
Source Variation (P-values)							
Ms		0.75	0.75	0.15	0.68	0.75	0.68
Al		0.33	0.33	0.41	0.79	0.33	0.67
Ms xAl		0.63	0.63	0.17	0.82	0.63	0.57

Ms: Moringa (*Moringa stanophetela*), Al: Alfalfa (*Medicago sativa*), HHEP: hen housed egg production, HDEP: hen day egg production.

CONCLUSION AND RECOMMENDATIONS:

Egg yolk-color is one of the most important aspects for the consumers. Since consumers are trying to relate the yolk color to the eggs' quality, it is therefore important to know the demand of the consumers. Eggs that are collected from those intensively managed commercial chickens are mostly lacking yellowness of the yolk. On the other hand, this color is mostly related to the feed that layers are consuming. The current study showed that due to supplementation of fresh leaves of Ms or Al, on diets of layers, the yolk color became yellowish. This yolk color was also influenced by an interaction effect of supplementing graded levels of Ms and Al. This indicates that yolk color can be improved by supplementation of either Ms or Al on the diets of commercial layers. Therefore, commercial egg producers are advised to add these both fresh leaves up 400g that to improve the egg yolk color and thereby to satisfy the consumers.

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CONFLICTS OF INTEREST:

There is no conflict of interest from the authors' end.

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