

Evaluation of dried *Khat (Catha Edulis)* leaf as natural additives on egg quality, embryonic mortality, and chick quality of white leghorn layers

G. Asfaw, M. G. Abebe, and E. K. Senbeta*

School of Animal and Range Sciences, College of Agriculture and Environmental Sciences,
Haramaya University; P.O. Box 138, Dire Dawa, Ethiopia

*Corresponding e-mail: ewonetu2011@gmail.com

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ABSTRACT

An experiment was conducted to evaluate the effects of supplementation of *Khat* leave as a natural feed additive on egg quality, fertility, embryonic mortality, hatchability, and chick quality of White Leghorn (WL) layers. One hundred fifty-six hens and twenty-four cocks of WL at twenty-four weeks of age were randomly distributed to four treatments, each replicated three times with thirteen layers and two cocks per replication and kept on a deep litter system. Treatment rations were formulated by the addition of *Khat* leave on layers ration with the proportion of 0% (T1), 0.2% (T2), 0.4% (T3), and 0.6% (T4). All data were subjected to analysis of variance. The egg weight of layers in T3 (54.1) was higher ($P < 0.05$) than that of layers in T4 (51.5) and T1 (51.8). Roche color reading was significantly higher for T4 (4.7 ± 0.19) than T3 (3.4 ± 0.19), T2 (2.2 ± 0.19), and T1 (1.7 ± 0.19). There were no significant differences ($P > 0.05$) among treatments in yolk height, yolk index, yolk diameter, albumen height, Haugh unit, fertility, hatchability, chick weight, and visual score. Embryonic mortality percentage was significantly ($P < 0.05$) higher for T1 than for other treatments. Chick length was significantly ($p < 0.05$) higher in T2, T4, and T3 than in T1. Finally, this study can be concluded that the use of dried *Khat* leaves as an additive up to 0.6% in ration of White Leghorn layers increased eggshell weight and yolk color and reduced embryonic mortality.

Keywords: Chick quality, Dried Khat leaves, Egg, Fertility, Hatchability, Layers

INTRODUCTION

Natural medicinal products originating from herbs and spices have been used as feed additives for poultry (Guo *et al.*, 2004). Compared with synthetic antibiotics or inorganic chemicals, these plant-derived products have proven to be natural, less toxic, residue-free, and are thought to be ideal feed additives in feeds for animal production (Wang *et al.*, 1998). Any attempt to im-

prove poultry production and increase its efficiency, therefore, needs to focus on the utilization of locally available and affordable new ingredients that are not in direct competition with human food. In this context, dried *Khat* leave is a potential feed additive in the poultry ration.

Khat in most western countries, 'chat' in Ethiopia are common names for *Catha edulis*, an evergreen plant of the family *Celastraceae* that endemically grows in South-West Arabia and

East Africa. It is a cash crop widely grown by the majority of smallholder farmers in Eastern and Southern Ethiopia (Mulatu and Kassa, 2001). Despite the daily use and consumption by millions of people and its leftover is in use as supplementation by livestock in Ethiopia and other countries (Yoseph *et al.*, 2008; Misganaw, 2009). It possesses many bioactive ingredients (Cox and Rampes, 2003), antioxidant activity (Hailu *et al.*, 2017), antimicrobial activity (Fabio *et al.*, 2003), and for its good nutritive values (Yoseph *et al.*, 2008; Getinet and Yoseph, 2014; Woldu *et al.*, 2015). *Khat* contains many bioactive compounds such as alkaloids, terpenoids, flavonoids, sterols, glycosides, tannins, amino acids, vitamin C, minerals, and essential oils that are beneficial for the health of animals (Rama *et al.*, 2019). According to Ting *et al.* (2011), the performance and egg quality were shown to increase with hesperidin, naringin, and quercetin which is a bioactive compound found in *Khat* leaves. However, there is limited information on the utilization of *Khat* (*Catha edulis*) leave in layers feeding. Therefore, this study was designed to evaluate the effects of feeding different levels of dried *Khat* (*Catha edulis*) leave as an additive on egg quality, fertility, hatchability, embryonic mortality, and chick quality.

MATERIALS AND METHODS

Study Area

The study was conducted at Haramaya University Poultry farm which is located 505 km East of Addis Ababa. The site is placed at an altitude of 1980 meters above sea level, 9° 26'N latitudes, and 42° 3'E longitudes. The mean annual rainfall is 780 mm. The mean annual minimum and maximum temperatures are 8°C and 24°C, respectively.

Management of Experimental Chickens

A total of 180 White Leghorn chickens comprising 156 layers and 24 cocks of twenty-four (24) weeks of age were randomly selected from the Poultry Farm. The layers were randomly distributed into four treatments; each replicate

three times. Each replication contained 15 chickens (13 hens and 2 cocks). The chickens were acclimatized to experimental rations for 7 days before actual data collection and the experiment lasted for 70 days. Layers in each replicate were kept in 2 by 2- meter pens partitioned by wire mesh and covered with *teff* straw. The chickens were provided experimental ration on *ad libitum* basis at 8:00 and 14:00 hours and also offered water on free access basis.

Feed Ingredients and Experimental Rations

The proportion of feed ingredients used in ration formulation were maize grain (50%), wheat short (16%), noug seed cake (15%), soybean meal (10.5%), L-lysine HCL (0.4%), vitamin premix (0.5%), DL-methionine (0.1%), salt (0.5%), and limestone (7%). The ingredients were obtained from the Haramaya University feed processing unit. The treatment ration was formulated to be iso-caloric and iso-nitrogenous with 2800-2900 kcal ME/kg DM and 16-17% CP to meet the nutrient requirements of layers (NRC, 1994).

Preparation of Khat Leaves

Khat leaves were purchased from Awaday town (the most *Khat* farming area and marketing center). The leaves were air-dried by spreading on plastic sheets under shade. The leaves were separated from the twigs and are finely pulverized by a hammer mill to pass through 5 mm then the powder was added to a commercial layer ration. The powder *Khat* leaf was added at a rate of 0%, 0.2%, 0.4%, and 0.6% of the experimental ration of T1, T2, T3, and T4, respectively.

Data Collection

Egg qualities were assessed in terms of albumen height and weight, shell thickness and shell weight, yolk height, yolk diameter, weight, yolk color, yolk index, and Haugh Unit (HU). All parameters were measured for each replicate and average was taken. A total of 144 eggs were used for quality analysis which means 9 eggs per treatment or 3 eggs per replication were randomly taken every two weeks. Eggs were individual-

ly weighed, marked, and broken on a flat tray and the height and weight of the thick albumen of each egg were measured with a tripod millimeter and sensitive balance respectively. Haugh Unit value for each egg was calculated by using the formula given by Haugh (1937). Shell weight was measured by sensitive balance after removing the shell membrane whereas shell thickness was measured with a micrometer gauge having a sensitivity of 0.001 mm, at three points of the egg broader end, equator, and sharp end, and the average value was considered. The Yolk was separated from the other components of the egg and measured for height, diameter, and weight using a tripod millimeter, ruler, and sensitive balance respectively. The yolk was thoroughly mixed and two sample droplets from each egg yolk were placed on a piece of white paper and compared with a Roche color fan which consisted of a series of fifteen colored plastic strips (1 rated as very pale yellow and 15 as deep intense reddish-orange) as a reference to determine yolk color. The yolk index was computed as a proportion of yolk height to yolk diameter. A total of 504 medium-sized eggs were purposefully selected by their size using an egg grader and incubated to evaluate fertility, hatchability, embryonic mortality, and chick quality. Evaluation of fertility and hatchability were done twice and at a time 252 eggs (63 eggs from each treatment or 21 from each replicate) were used. Fertility was determined by candling the incubated eggs on the 7th day of incubation and computed following the procedure described by Bonnier and Kasper (1990). Embryonic mortality was determined by candling eggs at the 7th, 14th, and 18th days of incubation according to the procedure described by Rashed (2004). The percentage hatchability for each replicate was computed according to the formula given by Fayeye *et al.* (2005). Chick quality was measured for all hatched chicks for visual scoring, weight, and length according to the methods of North (1984). Day-old chick's weight and length were taken for all chicks of each replicate and then the average was taken. Chick length was determined by measuring the length of a stretched chick from the tip of the

beak to the tip of the middle toe in centimeters using a ruler and recorded for the same chick from which weight measurement was taken.

Experimental Design and Data Analysis

A completely randomized design (CRD) with four treatments; each replicates three times and each replication contained fifteen chickens was used. Data were subjected to analysis of variance using SAS version 9.4. The existence of a significant difference among treatment means was tested by the least significant difference (LSD) at $p < 0.05$. The used model was: $Y_{ij} = \mu + t_i + e_{ij}$. Where, Y_{ij} : represents the j^{th} observation taken under treatment i , μ : overall mean, t_i : levels of feed additive effect, and e_{ij} : random error.

RESULTS AND DISCUSSION

Albumen, Yolk, and Shell Weight

The use of dried *Khat* leaves (DKL) in the diet of layers had significant effects on egg weight, albumen weight, yolk weight, and shell weight parameters (Table 1). Heavier ($p < 0.05$) egg weight and yolk weight were recorded in T3 than in T4 and T1 but the result in T3 was similar ($p > 0.05$) to T2. Similarly, the substitution of *Moringa oleifera* leaf meal (MOLM) at 5% had significantly improved egg weight from 48.66 to 54.51 and yolk weight from 15.33 to 20.66 egg quality parameters (Wubalem *et al.*, 2016). On contrary, Cayan and Erener (2015) did not find any effect of olive leaf on egg weight and yolk weight. This might be the positive association between egg weight and yolk weight (Suk and Park 2001). Albumen weight was higher ($p < 0.05$) for T2 than for T4 however, T2, T3, and T1 had similar values. Likewise, the substitution of MOLM at 5% had significantly improved albumen weight from 21.67 to 29.66 (Wubalem *et al.*, 2016). This study result is in line with the work of Suk and Park (2001) who noted a positive association between yolk weight and albumen weight. Significantly higher egg-shell weight was recorded for T3 and T4 than for T2 and T1. This might be due to the phenolic

compounds (Al Hebshi and Skaug, 2005; Atlabachew *et al.*, 2014) found in the *Khat* which contribute to the significant variation in egg weight, albumen weight, yolk weight, and shell weight. On contrary, Cayan and Erener (2015) did not find any effect of olive leaf on shell weight. The lightest egg weight, albumen weight, and yolk weight in T4 could be associated with the high concentration of tannins in *Khat* which reduces feed intake of layers due to the bitterness of the *Khat* leaves with high content of tannins which intern decreased egg weight and its components except for shell weight. This is confirmed by Atlabachew *et al.* (2014) who reported the high concentration of tannins (70.2–153 mg TAE/g of dry matter) in *Khat* that resulted in the bitterness. The significantly higher eggshell weight recorded for T3 and T4 could be associated with the increased levels of *Khat* additive that has bioactive ingredients which contributed to enhancing the availability of minerals and their efficiency of absorption.

Albumen Height and Haugh Unit

The result showed that there was no significant ($p > 0.05$) difference among treatments in terms of albumen height and haugh unit (HU). This could be attributable to the freshness of eggs and the proper age of hens. This means the experimental eggs used for evaluation of albu-

men height were fresh and the experimental layers used in all treatments were the same age. The HU is a measure of egg quality based on the height of its egg white (inner thick albumen). Thus, the reason for non-significance in HU was due to the non-significant effect of *Khat* leave additive on albumen height. In line with this study finding, Abd El - Motaal *et al.* (2008) noted the non-significant effect of *Eucalyptus* leaves on albumen height and HU. Likewise, Cayan and Erener (2015) reported that the dietary inclusion of olive leaf powder at the rate of 1% to 3% did not affect both traits. On contrary, Abbas (2013) and Nobakht and Moghaddam (2013) noted that plants containing bioactive compounds, such as essential oils, flavonoids, and carotenoids improved HU and albumen height. The HU value for all treatments was within the recommended range of good egg quality (70-100) that was specified by Lewko and Omowicz (2009).

Yolk Height, Diameter, and Index

There was a non-significant ($p > 0.05$) difference in yolk height, index, and yolk diameter among treatments. This is consistent with Cayan and Erener (2015) who noted that the dietary inclusion of olive leaf powder at a rate of 1-3% did not affect yolk height, diameter, and index. On contrary, Wubalem *et al.* (2016) reported that MOLM substitution at different levels had a sig-

Table 1. Effects of dried *Khat* leave on egg quality parameters of White Leghorn Layers

Parameter	Treatments				SEM	SL
	T1	T2	T3	T4		
Egg weight (g)	51.8 ^b	52.7 ^{ab}	54.1 ^a	51.5 ^b	0.59	*
Albumen weight (g)	31.2 ^{ab}	31.5 ^a	31.4 ^{ab}	29.9 ^b	0.48	*
Albumen height (mm)	7.8	6.9	7.4	6.9	0.38	NS
Haugh unit	96.7	92.8	94.7	92.9	1.68	NS
Yolk weight (g)	15.3 ^b	15.9 ^{ab}	16.5 ^a	15.5 ^b	0.26	*
Yolk height (mm)	13.5	13.9	14.1	14.1	0.35	NS
Yolk diameter (mm)	39.9	40.4	40.4	40.5	0.03	NS
Yolk index (mm)	0.3	0.3	0.4	0.4	0.003	NS
Yolk color (*RSP)	1.7 ^c	2.2 ^c	3.4 ^b	4.6 ^a	0.19	**
Shell thickness (mm)	0.3	0.3	0.3	0.3	0.005	NS
Shell weight (g)	5.4 ^b	5.4 ^b	6.2 ^a	6.1 ^a	0.15	**

^{a,b,c} Means marked with different superscript letters in the same row are significantly different ($P < 0.05$), T1: Layers received no *Khat* leaf additive, T2: Layers received 0.2% *Khat* leaf additive, T3: Layers received 0.4% *Khat* leaf additive, T4: Layers received 0.6% *Khat* leaf additive, *: significant at ($p < 0.05$); **: significant at ($p < 0.01$); NS: non-significant at $p > 0.05$, SL: significant level, RSP: Roche scale point, SEM: standard error.

nificant effect on the yolk height, yolk diameter, and yolk index. The yolk index value for all treatments was within the recommended range of good internal egg quality (0.3-0.5) for leaf meal-based diets as reported by Oluyemi and Robert (2000). The non-significant difference in yolk height, index, and yolk diameter could be due to the freshness of the eggs used in the study. The yolk index is utilized to determine the shape changes in the yolk, primarily during storage. During storage of eggs, the yolk index value (an indicator of the spherical nature of egg yolk) declines as a result of a progressive weakening of the vitelline membranes, reduction of the total solid, and liquefaction of the yolk caused mainly by the osmotic diffusion of water from the albumen and gives the yolk a somewhat flattened shape on top and a general "out-of-round" shape. This is consistent with Karoui *et al.* (2006).

Yolk Color

There was a significant difference in yolk color among the treatments (Table 1). The Roche color fan reading recorded ranges from 1 to 7 but the majority of the egg's yolk color score falls on the 3rd Roche color fan number (Table 2). However, the accepted color of yolk by consumers in most areas is 7 to 8 or deeper yolk color (Leeson and Summers, 2001). As the level of DKL inclusion increased, the yolk color increased and the highest value was noted in T4. This can be attributed to the carotenoid contents of DKL. Carotenoids found in *Khat* leaf (Cox and Rampes, 2003) play an important role in the development of different color scores in egg yolk. The result was in line with Cayan and Erener (2015) who noted a linear increase in egg yolk color in proportion to the amount of olive leaf powder inclusion in the layer diet. Besides, Atlabachew *et al.*

Table 2. Frequency of yolk color scores of eggs from different experimental diets

Treatments	Frequency Roche color fan scores							Total (No)
	1	2	3	4	5	6	7	
T1	16	10	10	0	0	0	0	36
T2	8	12	16	0	0	0	0	36
T3	0	2	20	8	4	2	0	36
T4	0	0	2	14	10	8	2	36
Total	24	24	48	22	14	10	2	144

T1: Layers received no *Khat* leaf additive, T2: Layers received 0.2% *Khat* leaf additive, T3: Layers received 0.4% *Khat* leaf additive, T4: Layers received 0.6% *Khat* leaf additive.

Table 3. Effects of dried *Khat* leave on fertility, hatchability, embryo mortality, and chick quality

Parameters	Treatments				SEM	SL
	T1	T2	T3	T4		
Fertility (%)	90.5	84.1	87.3	91.3	3.6	NS
Hatchability ^f (%)	75.4	82.9	80.8	82.7	2.4	NS
Hatchability ^t (%)	68.3	69.8	70.6	75.4	4.0	NS
Embryonic mortality (%)	15.9 ^a	8.5 ^b	10.8 ^b	10.5 ^b	1.3	*
Chick weight (g)	33.8	33.4	32.3	31.9	0.94	NS
Chick length (cm)	15.7 ^b	16.0 ^a	15.9 ^a	15.9 ^a	0.06	*
Chick visual score (%)	94.0	91.6	95.6	93.7	3.11	NS

^{a,b,c} Means marked with different superscript letters in the same row are significantly different ($P < 0.05$), T1: Layers received no *Khat* leaf additive, T2: Layers received 0.2% *Khat* leaf additive, T3: Layers received 0.4% *Khat* leaf additive, T4: Layers received 0.6% *Khat* leaf additive, f: Hatchability on fertile egg bases, t: Hatchability on total egg bases; *: Significant at $p < 0.05$, NS: non-significant at $p > 0.05$, SEM: Standard error of mean, SL: Significant level, %: percentage.

(2014) confirmed that *Khat* leaves are enriched with carotenoids which are strong natural antioxidants that can increase egg yolk color.

Eggshell Thickness

The study result indicated that the use of DKL as additives at different levels had no significant ($p>0.05$) effects on eggshell thickness. This could be associated with immature shell glands of layers used in the experiment. Because two-thirds of calcium deposited in the uterus is directly supplied by the hen's diet and one-third (10-30%) by mobilization of bone calcium. Thus, the use of the same age group of birds might be contributed to the non-significant difference in shell thickness. Besides, the amount of DKL level used was very small. Corresponding to this study result, Cayan and Erener (2015) also reported that the dietary inclusion of olive leaf powder at 1-3% did not affect eggshell thickness. On contrary, Wubalem *et al.* (2016) reported a significant effect of MOLM supplementation in layers ration on eggshell thickness.

Fertility, Embryonic Mortality, Hatchability, and Chick Quality

The result revealed that the fertility and hatchability percentage did not show a significant ($P>0.05$) difference among the treatments (Table 3). This result was similar to the findings of Etalem *et al.* (2014) who noted a non-significant effect of MOLM on fertility and hatchability. On contrary, Wubalem *et al.* (2016) reported a significant effect of MOLM supplementation in layers ration on fertility and hatchability. This study result indicated that supplementation of DKL up to the level of 0.6% did not influence the fertility and hatchability of eggs. The non-significant effect of fertility and hatchability among the treatments could be associated with different factors. The eggs used in this study were from layers of the same age, fresh eggs or eggs with the same storage period, eggs with similar shell thickness, and the same female to male sex ratio. The non-significant fertility could also contribute to the non-significant effect of hatchability because fertility

and hatchability are interrelated heritable traits (King'ori, 2011). Besides, *Khat* contains bioactive compounds such as alkaloids that influence fertility by decreasing semen quality. This is confirmed by Lwow *et al.* (2017) who noted that a diet with a high concentration of alcohol seems to exert an especially harmful effect on male fertility due to its decreasing semen quality.

The DKL had a significant ($p<0.05$) effect on embryonic mortality. The embryonic mortality of T1 was significantly higher than DKL treated groups. This could be associated with the bioactive compounds found in *Khat* that are beneficial for the health of chickens which could protect chickens against pathogenic metabolites and reduce the susceptibility of embryonic tissue to lipid peroxidation (Liu *et al.*, 2014). Moreover, Dai and Mumper (2010) reported that most of the chemical constituents found in *Khat* are biologically active that are used worldwide for the treatment of many diseases such as antimicrobial and antibiotics. For instance, quercetin supplementation could protect chickens against pathogenic metabolites (Liu *et al.*, 2014). This might be the reason for the higher embryonic mortality recorded for the control group as compared to other groups fed on a diet with DKL. This study result is contrary to the findings reported by Etalem *et al.* (2014) who reported cassava root chips and MOLM had no significant effect on embryonic mortality.

The study result showed that there was no significant ($p>0.05$) difference in chick weight and visual score. However, there was a significant ($p<0.05$) difference in chick length where the lowest result was recorded in T1. This could be related to the lower egg weight recorded for layers in T1. Egg weight is the dominant factor that affects chick length (Wilson, 2000; Tona *et al.*, 2002). However, there was no statistical ($p>0.05$) difference among T2, T4, and T3 in terms of chick length. This is consistent with Wubalem *et al.* (2016) who noted that MOLM in layers ration had a significant effect on chick length and a significant effect on chick weight and visual score. The chick length in all treatments ranges from 15.73 to 16.02 cm which falls within the short

group (<17.8cm) according to the classification made by Petek *et al.* (2009).

CONCLUSION

It was concluded that the use of dried *Khat* leaves as additive up to 0.6% in ration of White Leghorn layers increased eggshell weight and yolk color and reduced embryonic mortality. Further in-depth research is needed to assess the identification of active chemical compounds in dried *Khat* leaves as well as their effect at higher proportions on the performance of layers.

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Competing interests

The authors declare no conflict of interest.

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