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Effects of Dried Khat (*Catha edulis*) Leaves as a Natural Feed Additive on the White Leghorn Layers' Performance

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ABSTRACT

Khat contains many bioactive compounds that are beneficial for chickens' health. Most of the chemical constituents found in Khat are biologically active and are used worldwide for the treatment of many diseases. However, there are few studies on the use of Khat in poultry as a nutrition, antioxidant, and antimicrobial activity. Therefore, the present study evaluated the effects of dried Khat leave (DKL) as a natural feed additive on the White Leghorn layer's performance, serum chemistry, and hematology. A total of 180 White Leghorn chickens, aged 168 days were randomly divided into four groups consisting of 45 chickens in three replications. Thirteen layers and two cocks were assigned to each replication and reared on a deep litter system. The diets of layers in T1, T2, T3, and T4 were supplemented by DKL at proportion of 0%, 0.2%, 0.4%, and 0.6%, respectively. The layers were evaluated for feed intake, body weight gain, egg production, feed conversion ratio, and some blood parameters, such as total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, albumin, globulin, red blood cells, hemoglobin, packed cells volume, white blood cells, and total protein. Feed intake in T1 (103.5 g) was significantly higher than T4 (98.5 g) and T3 (98.9 g). Layers in T1 significantly gained higher body weight than layers in other treatments. The use of DKL in layer chickens' diet did not affect egg production, egg mass, and feed conversion ratio. An increase in the level of DKL in the layers diet significantly reduced the serum high-density lipoprotein, cholesterol, and albumin. The present study showed that chickens that received a higher proportion of dried khat leaf (0.6%) significantly recorded lower levels of low-density lipoprotein and total cholesterol in their blood.

Keywords: Blood analysis, Dried Khat leaf, Layer, White Leghorn

INTRODUCTION

Feed additives are used in small amounts in the ration to improve the productive performance of animals (Embuscado, 2015). However, conventional synthetic feed additives became public health issues because of their traceability in animal products (Wallace et al., 2010). Thus, Europe banned the use of some synthetic additives in animal feed (Nkukwana et al., 2014). These raise questions about finding natural additives (Grashorn, 2010). In Ethiopia, poultry production is mainly constrained by the quality and quantity of feed (Demeke, 2004). Hence, feed additives are used to improve quality by improving the availability of certain nutrients. Feed additives originating from herbs and spices have been also used to improve feed quality in the poultry industry (Guo et al., 2004). Natural feed additives are less toxic and residue-free as compared with synthetic antibiotics (Asfaw et al., 2022).

A dried Khat leaf is a potential natural feed because it contains many bioactive compounds such as alkaloids (cathinone, nor-pseudoephedrine, and nor-ephedrine), terpenoids, flavonoids (quercetin, hesperidin, and

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naringin), vitamin C, tannins, amino acids, minerals, glycosides, essential oils (limonene, 1-phenyl-1, 2-propanedione, camphor), and beta-carotene (Al-hebshi and Al-ak'hali, 2010) which are chemical constituents used to maintain animal health (Rama et al., 2019). Majority of Khat chemical ingredients are used as antimicrobials and antibiotics (Dai and Mumper, 2010).

The use of dried Khat leaves in animal rations not only reduces the cost of feeds, but also improves antioxidant activities and health, as a stimulant, and as coloring pigments which improve the desired egg yolk color, egg production, quality parameters, and reduced serum cholesterol. For instance, plasma cholesterol, glucose, and triglycerides in rabbits were decreased by the use of Khat leaves in the diet (Al-Habori, 2004). Khat leaf contains Alkaloids and Alkaloid is effective in lowering egg cholesterol (Santoso et al., 2015). Quercetin is among the flavonoids found in Khat leaves and its supplementation in laying hens increases the laying rate, egg production, feed efficiency and protects pathogenic metabolites (Liu et al., 2014). Moreover, Asfaw et al. (2022) noted that the use of dried Khat leaves in the diet of layers had significant effects on egg weight, albumen weight, yolk weight, shell weight, and yolk color parameters.

Despite the presence of many bioactive compounds in Khat leaves, there was no sufficient research information about the utilization of Khat (*Catha edulis*) leaves as natural additives in layers. Therefore, this study evaluated the effects of feeding layers with *Catha edulis* on feed consumption, feed conversion ratio, body weight, egg production, and some blood constituents.

MATERIALS AND METHODS

Ethical approval

All procedures related to animal handling, blood collection, and their routine manipulations were carried out according to animal care guidelines protocols approved by the Institutional Review Board of the College of Agriculture and Environmental Sciences of Haramaya University (CAES, Ethiopia) animals ethics committee with approval number CAES_HU/ECC/9/11/10/20212.

Description of the study site

This experimental study was conducted in the 2022 Gregorian calendar at the Poultry research station of Haramaya University in the eastern part of Ethiopia. The farm is located in the eastern part of Ethiopia 505 Kilometers away from the capital city, Addis Ababa. The study area is characterized by 780 millimeters of mean annual rainfall, 8℃ minimum and 24℃ maximum mean annual temperature, 9°261'N latitudes, 42°3'E longitudes, and an altitude of 1980 meters above sea level.

Sample size and chicken management

White Leghorn chickens were obtained from the Haramaya University (Ethiopia) poultry research center and randomly selected at 168 days of age. This experiment used 180 chickens consisting of one hundred fifty-six hens and twenty-four cocks. The chickens were weighed and randomly assigned into four treatments each consisting of three replications that contained fifteen chickens (thirteen hens and two cocks). The experimental chickens were acclimated for seven days to the ration and the actual period of data collection lasted seventy days. The chickens in each replication were reared in a 4 square meter pen which was covered by litter material of *teff* straw. The necessary inputs such as laying nests, watering and feeding materials, and experimental house were carefully cleaned and disinfected before utilization. The tube feeders were used to provide the experimental diets. Feed was offered to chickens twice a day at 8:00 in the morning and 14:00 in the afternoon on an *ad libtum* basis. Moreover, freshwater was offered every day using fountain drinkers on a free access basis.

Feed ingredients and chemical analysis

Chemical analyses of feeds were done at Haramaya University (Ethiopia) of Animal Nutrition and Soil laboratory. The sample was randomly collected from each feed ingredient (maize grain, noug seed cake, soybean meal, and wheat short) and evaluated for chemical composition following the AOAC (1990) procedure and then the experimental ration was formulated. The kjeldahl procedure was used to determine nitrogen (N) and then crude protein was calculated as N × 6.25. Atomic absorption spectrometry (Germany) was used to determine the calcium and total phosphorous following the AOAC (1996) procedure. The formula described by Wiseman (1987) was used to estimate the total metabolizable energy of each feed ingredient and the experimental ratio. WinFeed software (Cambridge, UK) was used to formulate the experimental diet. The experimental diet consisted of 50% maize grain, 16% wheat short, 15% noug seed cake, 10.5% soybean meal, 0.4% L-lysine HCL, 0.5% commercial vitamin premix, 0.1% Dl-methionine, 0.5% salt, and 7% limestone (Table 1). The diet was formulated to meet the nutrient requirement of layers that were iso-caloric (2800-2900Kcal ME/kg) and iso-nitrogenous (16-17%) following the NRC (1994) procedure.

Dried khat leaves preparation

A khat leaf that was used as a feed additive was purchased from Awaday town market (Ethiopia) and the leaves were separated from twigs and then dried under shade using plastic sheets. The leaves were pulverized into powder to pass through a 5-millimeter sieve then the powder was mixed with diet. Khat leave powder was added at a rate of 0%, 0.2%, 0.4%, and 0.6% of the experimental diets of T1, T2, T3, and T4, respectively following the procedure described by Asfaw et al. (2022).

Data collection

Feed intake was determined every day by subtracting feed refusal from feed offered and the average was taken for the whole experimental period. The initial and final body weight of chickens were used to determine the individual body weight change while the division of body weight change of layers to the number of days between two consecutive measurements was used to determine the body weight gain of chickens using the formula described by Tullet (1995). The daily egg production rate of each treatment was also calculated following the formula described by Tullet (1995). Qualitative and quantitative data from 144 (one hundred forty-four) eggs were evaluated. Nine eggs per treatment or three eggs per replication were randomly selected every two weeks. Each egg was individually weighed immediately after collection and average egg weight and mass were computed for each replication using the method described by North (1984). The feed intake of chickens in each replication was divided by egg mass to determine the feed conversion ratio (Tullet, 1995). At the end of the experiment, five milliliters of blood samples were collected from the bronchial wing vein of nine hen layers randomly selected from treatments or three layers from each replication. The blood sample was analyzed at Haramaya University's animal physiology and veterinary laboratory (Ethiopia). Ethylenediaminetetraacetic acid (EDTA) and plain tubes were used for blood samples. The serum chemistry (total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, albumin, and globulin) and hematology (red blood cells, hemoglobin, packed cells volume, white blood cells, and total protein) from layers were evaluated using the methods of acid hematin or Sahli's (Weiss et al., 2010) and Neubauer hemocytometer chamber (Dacie and Lewis, 1991). The difference between serum total protein and albumin was used to determine the volume of globulin (Doumas et al., 1981).

Statistical analysis

The research design used in this study was completely randomized. Analysis of variance (ANOVA) was used to analysis of data using the statistical analysis system user's guide of North Carolina, version 9.4. The least significant difference was used to identify the existence of variations among treatment means at $p < 0.05$. The model was used according to the following formula.

$Y_{jk} = \mu + tj + ejk,$

Where Y_{jk} is k^{th} observation taken under treatment j, μ is overall mean, tj is levels of dried khat leave and e_{jk} is random error.

RESULTS AND DISCUSSION

Feed ingredients and ration nutrition values

The chemical composition of feed ingredients and experimental diets are presented in Table 1 and Table 2, respectively. Khat leave was slightly higher in crude protein (CP) than maize but lower than wheat short. The crude fiber (CF) and ash content of dried Khat leave (DKL) is somewhat higher than other dietary ingredients such as maize grain, noug seed cake, soybean meal, and wheat short. DKL's metabolizable energy content is far lower than other ingredients such as maize grain, Noug Seed cake, soybean meal, and wheat short but higher in its calcium and phosphors. On the contrary, a higher CP (12.3%) value was reported by Brhanu and Gebremariam (2019) for Khat. The recorded CP value of khat in this study was almost within the range (10.7-12.6%) that was reported by Mohammed (2005). This variation in CP value might be related to the proportion of leaves maturity and species, leave harvest season, and soil fertility where the Khat plant is grown. The crude fiber content of the diet slightly increased from group T1 to group T4 as the level of DKL increased; this may be due to the slightly higher CF contents of Khat leaves. Consistently, Okorie (2006) noted that the diets formulated using tropical browse plants revealed higher crude fiber (15.56%). In the current study, the diet formulated for all treatments met the iso-caloric and iso-nitrogenous nutrient requirement of layers as recommended by NRC (1994). Layers can adjust their feed consumption to obtain adequate energy when receiving diets ranging in energy from approximately 2500 to 3300 kcal ME per kg of diet (NRC, 1994).

Table 1. Chemical composition of the feed ingredients formulated for White Leghorn layers

Chemical Component	Ingredients				
	Maize Grain	Noug Seed cake	Soybean meal	Wheat short	Khat leave
DM (%)	89.0	92.0	91.9	92.2	94.3
CP (%DM)	9.3	31.7	38.2	15.3	10.6
EE (%DM)	5.6	8.1	5.7	4.3	4.8
Ash (%DM)	3.4	7.6	6.4	6.2	10.7
CF (%DM)	4.1	11.4	4.9	5.6	19.6
Ca (%DM)	0.05	0.38	0.19	0.11	0.86
P (%DM)	0.54	0.50	0.65	0.59	1.91
ME(Kcal/kg)	3944.8	3558.9	3632.7	3550.5	2039.8
Chemical Component	**Treatments**				
	T1(0% DKL)	**T2 (0.2% DKL)**	**T3(0.4% DKL)**	**T4 (0.6% DKL)**	
DM (%)	90.9	90.9	91.4	92.1	
CP (%DM)	16.1	16.8	16.1	16.9	
EE (%DM)	3.3	3.5	3.7	4.1	
Ash (%DM)	11.2	11.2	11.3	12.2	
CF (%DM)	8.6	8.8	8.9	8.9	
Ca (%DM)	2.86	2.92	2.88	3.11	
P (%DM)	0.43	0.49	0.53	0.54	
ME (Kcal/kg)	2909.9	2904.2	2896.4	2878.9	

DM: Dry mater; CP: Crude protein; EE: Ether extract; CF: Crude fiber; Ca: Calcium; P: Phosphorus; ME: Metabolizable energy; kcal/kg: Kilo calorie per kilogram, DKL: Dried Khat leaves

Table 2. Performance of layers-fed diets containing different levels of dried Khat leaves

Treatments / Parameters	T1	T2	T3	T4	SEM	SL
Feed Intake (g/hen /day)	103.5^{a}	100.5^{ab}	98.9^{b}	98.5^{b}	1.08	*
Initial BW (g)	1197.2	1211.0	1229.7	1221.0	12.68	NS
Final BW (g)	1229.0	1225.1	1214.9	1202.1	15.95	NS
Body Wt. Change (g/hen/day)	31.8^{a}	14.1^{ab}	-14.9^{b}	-18.9^{b}	10.34	*
BW gain (g/hen/day)	0.5^{a}	0.2^{ab}	-0.2^{b}	-0.3^{b}	0.15	*
Total Egg /Bird (No)	36.8	33.5	34.5	38.2	4.65	NS
HDEP (%)	50.5	47.9	49.3	54.6	6.54	NS
EM (g/hen/day)	26.4	24.3	25.9	26.8	3.53	NS
FCR (g feed/g egg)	3.9	4.1	3.8	3.6	0.55	NS

^{ab} means within a row with different superscripts are significantly different; * Significant at $p < 0.05$; NS: non-significant at $p > 0.05$; SL: Significant level; SEM: Standard error of the mean; g: Gram; BW: Body weight; HDEP: Hen-day egg production; FCR: Feed conversion ratio; EM: Egg mass (g /hen/day), Wt: Weight

Feed intake

This study result revealed that feed intake was decreased as the dietary inclusion level of DKL increased (Table 2). This might be due to the high concentration of tannins and crude fiber content in Khat leaves. Consistently, Atlabachew et al. (2014) approved the higher tannins (70.2-153 mg tannic acid equivalent/g of dry matter) in their study and associated lower feed intake with tannins because of their bitterness. Uchegbu et al. (2013) also noted decreased feed intake in layers of chicken as the fiber and tannin content of the diet increased. Besides, Buragohain (2018) revealed that the presence of tannin in *Tithonia diversifolia* leaf was attributed to the decreased feed intake and nutrient digestibility in broiler chickens. Brhanu and Gebremariam (2019) reported decreased feed intake in sheep across treatments with an increased inclusion level of Khat. Correspondingly, Abd El-Motaal et al. (2008) reported significantly less feed intake in layer chickens fed diets supplemented with *Eucalyptus* leaves powder as compared to the control group. On the contrary, Cayan and Erener (2015) reported insignificant effects of olive leaf powder use in layers. Lower feed intake of layers in T3 and T4 could also be due to their higher crude fiber content of the ration.

Body weight change and gain

The use of dried khat leaves significantly influenced body weight change and gain ($p < 0.05$, Table 2). The lowest body weight gains were recorded for layers in treatments T3 and T4. This implied decreased body weight gain as DKL increased in layer chickens' diet. This is possibly due to decreased feed intake as DKL increased. Correspondingly, Brhanu and Gebremariam (2019) noted heavier total body weight and average body weight gain in a sheep-fed diet with Khat leave at a higher level (45%) than at a lower level (0-30%).

Egg production, egg mass, and feed conversion ratio

The use of DKL in layer chicken diet did not affect egg production ($p > 0.05$, Table 2). This is consistent with Cayan and Erener (2015) who reported non-significant effects on egg production of layers fed a diet with olive leaves. However, layers in T4 record higher egg numbers and hen-day egg production (HDEP) than T1, T3, and T2. This could be enhanced by the presence of some bioactive compounds such as alkaloids, terpenoids, and flavonoids in the Khat leaves (Atlabachew et al., 2014). Khat leaf has high antioxidant activity that could improve the health status as well as the reproductive and productive performances of chickens. Similarly, Ahmad et al. (2017) noted that egg production remained the same with the supplementation of *Moringa* leaf meals. On the contrary, Abd El-Motaall et al. (2008) noted significantly increased egg number in layers-fed diets supplemented with *Eucalyptus* leaves. The inclusion of DKL in the layers' diet did not affect egg mass and FCR (Table 2). The egg mass and FCR might be related to egg production which was not significantly affected by the use of DKL. Consistently, Paguia et al. (2014) reported that egg mass and FCR remained unchanged by using *Moringa* leaf meal. On the contrary, Liu et al. (2014) noted decreased FCR in a dose-response of quercetin supplementation which is among the bioactive compounds found in Khat leave.

Blood analysis

The results showed that the total serum cholesterol (TSC) was significantly low at a high level of DKL (Table 3, $p < 0.05$). This finding could be associated with the effects of biochemical such as alkaloids, terpenoids, and flavonoids in Khat leaves. Likewise, Mashayekhi et al. (2018) noted decreased serum total cholesterol levels of broilers due to *Eucalyptus* leaf powder supplementation in their diets. On the contrary, Zangeneh and Torki (2011) reported a non-significant effect of olive leaves on the blood cholesterol levels in layer chickens. Serum cholesterol of layers fed neem leaf meal was not significant ($p > 0.05$, Odoh et al., 2016). The chemical constituents found in Khat leaves such as alkaloids are effective in lowering egg cholesterol. This idea is supported by Santoso et al. (2015) who noted the lowered egg cholesterol due to alkaloids. The phenolic compounds (flavonoids, tannins, phenolic acids, terpenes) in Khat leaves could decrease plasma cholesterol in this study. In line with this finding, Al-Habori and Al-Mamary (2004) noted an increased c-AMP concentration due to the stimulating effect of Khat on β-adrenergic receptors by activation of adenylate cyclase and converting ATP to c-AMP. Consequently, c-AMP has a stimulatory effect on lipolysis.

Table 3. Serum chemistry and hematology of layers fed diets containing different levels of dried khat leaves

Parameters \ Treatments	T1	T2	T3	T4	SEM	SL
Total cholesterol (mg/dl)	160.7^{a}	154.3^{ab}	142.0^{bc}	139.1^{c}	4.15	*
HDL (mg/dl)	31.6^{b}	31.8^{b}	33.8^{ab}	35.7^{a}	0.70	*
LDL (mg/dl)	104.5^{a}	99.8^{ab}	95.1^{bc}	93.5^{c}	1.57	*
TP (g/dl)	4.2	4.3	4.6	4.4	0.14	NS
Albumin (g/dl)	1.5^{b}	1.5^{b}	1.6^{a}	1.7^{a}	0.03	**
Globulin (g/dl)	2.7	2.9	2.9	2.8	0.13	NS
RBC (10^6/µl)	3.1	3.2	3.3	3.1	0.05	NS
WBC (10^4/µl)	6.8^{b}	6.1^{b}	8.0^{a}	7.1^{ab}	0.35	*
Hb (g/dl)	10.6	10.4	10.7	10.6	0.24	NS
PCV (%)	32.2	31.4	32.8	32.0	0.48	NS

[abc] Means within a row with different superscripts are significantly different; * Significant at $p < 0.05$; **: Significant at $P < 0.05$; NS: Non-significant; RBC: Red Blood Cells; Hb: Hemoglobin; PCV: Packed Cells Volume; WBC: White Blood Cells; TP: Total Protein; HDL: High-Density Lipoprotein; LDL: Low-Density Lipoprotein

The study revealed that T4 was significantly higher in serum HDL cholesterol than in T1 and T2 ($p < 0.05$). This agrees with Santoso et al. (2010) who revealed significantly increased HDL cholesterol concentration in serum with supplementation of alkaloids from *Sauropus androgynuos* leaves in a layer chicken diet. Al-Habori and Al-Mamary (2004) noted increased HDL cholesterol in rabbits due to Khat leave supplementation. On the contrary, Mashayekhi et al. (2018) reported that feeding *Eucalyptus* leaf powder to broiler chickens did not influence their HDL. Significantly lower serum LDL concentration was recorded in T4 as compared with T1 and T2 ($p < 0.05$). This could be due to the phenolic compounds which can decrease the absorption of cholesterol into the blood by adhering to LDL and then inhibit free radicals. This is consistent with the study reported by Brenes and Roura (2010). Besides, decreased blood LDL in rats was reported by the use of an aqueous extract of *Eucalyptus* (Arise et al., 2009). Serum total proteins and globulin were non-significant among treatments ($p > 0.05$). Significantly higher albumin was recorded in T3 and T4 as compared to layers fed on T1 and T2 ($p < 0.05$). However, a non-significant effect of Neem leaves on layers of serum albumin was reported by Odoh et al. 2016 ($p > 0.05$). The change of those parameters in blood revealed that the bioactive components of Khat leaf could activate immune functions such as lymphocyte proliferation, phagocytosis, red blood cells, hemoglobin, and white blood cell counts.

The result showed that the inclusion of DKL in the layers diet did not influence the total red blood cell, hemoglobin, and packed cell volume ($p < 0.05$). Correspondingly, a non-significant effect of *Moringa oleifera* leaf on hemoglobin in broilers was reported by Olugbemi et al. (2010). However, Esonu et al. (2007) reported a significant effect of hematological parameters by the use of neem leaf. The values of RBC fall within the recommended range ($0.5–3.9 \times 10^6/\mu l$) which is the normal value for chickens (Gulland and Hawkey, 1990). Significantly higher white blood cells were recorded in T3 as compared to layers fed on T1 and T2 ($p < 0.05$). Likewise, Mashayekhi et al. (2018) noted increased WBC levels due to *Eucalyptus* leaf powder supplementation in broilers' diets. This might be due to the increased response of layers immune to Khat phenolic compounds which stimulate lymphocyte production.

CONCLUSION

The feed intake of layers in T1 was significantly higher than in T4 and T3. There was a significantly higher body weight change and body weight gain for layers in T1 than in T4 and T3. The study revealed that Khat leaves use in layer diet up to 0.6% significantly reduced feed intake, the body weight of hens, total serum cholesterol, and LDL and significantly increased HDL and WBC. Dried Khat leaves decreased serum LDL from 104.5 in T1 to 93.5 mg/dl in T4. The present study might be used as the ground information of dried Khat leaves effects on layer performance. Thus, further study is needed to identify active chemical compounds in khat leaves.

DECLARATIONS

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Availability of data and materials

All data analyzed and presented in this article are available at the request of the principal and corresponding authors.

Authors' contributions

Aschalew Girma Asfaw conducted experimental research, collected data, analyzed data, and drafted a research report. Meseret Girma Abebe is a major supervisor to the research who commented on the research report. Ewonetu Kebede Senbeta initiated the research idea, drafted the research proposal for a grant, supervised the student as co-advisor, prepared the manuscript, and submitted it. Kasech Mulatu assisted the student during data collection and commented on the manuscript. All authors contributed to the research proposal development and commented on the final manuscript.

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Ethical considerations

Ethical issues, such as data fabrication, double publication and submission, redundancy, plagiarism, consent to publish, and misconduct, have been checked by all the authors before submission to this journal.

Competing interests
All authors have no conflict of interest.

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