

# The Effects of Different Levels of Untreated and Treated Green Grape Leaf on Performance, Egg Traits Quality and Blood Parameters of Laying Hens

Research Article

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Received on: 12 Jun 2014

Revised on: 11 Jul 2014

Accepted on: 15 Jul 2014

Online Published on: Mar 2015

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## ABSTRACT

This experiment was conducted to evaluate the effects of different levels of untreated and treated green grape leaf with urea on performance, egg quality traits and blood parameters of laying hens. In this experiment 192 Hy-Line (w-36) laying hens, 35 to 46 wk of age were divided into 4 treatments with 4 replicates (12 birds per replicate) in a completely randomized design experiment. The treatments included: 1) control group, 2) group with 3% untreated green grape leaf, 3) group with 3% green grape leaf treated with 0.5% urea and 4) group treated with 3% green grape leaf treated with 1% urea. The best values for egg weight, egg production percentage, egg mass, feed consumption, feed conversion ratio, feed price for per kilogram of egg production, eggshell weight, yolk weight and Haugh unit were in group 3. Using green grape leaf in diets reduced the levels of blood high density lipoprotein (HDL) and the lowest amount was obtained with 3% green grape leaf treated with 0.5% urea. The lowest count of white blood cells, heterophil and highest lymphocyte percentage and the low heterophil to lymphocyte ratio were noted in group 4. The overall results indicated that in laying hens, using 3% green grape leaf treated with urea in diets significantly improved their performance, egg traits and production cost, blood parameters.

**KEY WORDS** blood parameters, egg traits, grape leaf, laying hens, performance.

## INTRODUCTION

Poultry industry is a predominant source of animal protein in both developed and developing countries. The expansion of the poultry industry depends largely on the availability of good quality feed in sufficient quantity and at prices affordable to both producers and consumers (Adejimi *et al.* 2011). This is very important for intensive enterprise especially for layers which are very sensitive to nutrition such that inadequacies in nutrient supply often lead to fall in egg production and even cessation of laying. With the present trend of rising prices of feed ingredients, there has been a search for non conventional feedstuff with potentials of

improving poultry performance at reduced cost. Such non conventional feed sources, leaf protein concentrates have been reported by Farina *et al.* (1991). Including those of cassava leaf *Leuceana leucocephala* (Bhatnagar *et al.* 1996). Leaf meals are gaining acceptance as feed stuffs in poultry diet as due to its availability and its similar nutrient content and are considered to be un-conventional feeds. Satisfactory performances despite the inherent limitations for monogasters (cell walls and plant secondary metabolites like coumarols, oestrogenic, isoflavones, tannin, etc although with antinutritional properties, are indispensable co-evolutionary principles. It has been reported of various leaf meals tested in the diet of some poultry species (Oige *et al.*

2006). Fortunately, high fiber containing feedstuffs are utilized successfully in pullets and layers nutrition. Akiba and Matsumoto (1978) found that feeding of cellulose did not affect body weight gain and feed efficiency. Vargas and Naber (1984) reported that dietary fiber (nutrients density) had no significant effect on egg production, egg weight, body weight change and energy balance. El-Deek *et al.* (1995) using experimental diets containing corn with cob, rice polishing and wheat bran at 20% or other combination of each two (40%) or three (60%) ingredients showed that Alexandria pullets growth during 8 to 23 wks of age, feed conversion ratio, egg number at 10% egg production and mortality rate were not affected by dietary composition. Moreover, El-Deek *et al.* (1988) with casuarinas branch lets; Osei *et al.* (1990) with gliricidia leaf meal; Yassein *et al.* (1998) with *Leucaena leucocephala* and Talaat (2003) with *Kochia indica* indicated that, no adverse effects were recorded on performance of pullets and laying hens. On the other hand, Estrow *et al.* (1982) reported that pectin, lignin and alginate reduced chicks growth as feeding inclusion levels of dietary folacin-containing diet.

The vine leaf is a rich source of one of the most beneficial groups of plant flavonoids, proanthocyanidins oligomers. Grape leaf hydroalcoholic extract spasmolytic effect is due to the blockade of the voltage dependent calcium channels and activation of  $Ca^{2+}$  operated potassium channels (Gharib Naseri *et al.* 2006). Grape leaves with antioxidant activity (Monagas *et al.* 2006) have been reported to treat chronic venous insufficiency in humana (Kiesewetter *et al.* 2000) and nephrotoxicosis induced by citrinin (Bilgrami and Jeswal, 1993). It has also been demonstrated that the grape leaf hydro-alcoholic extract (GLHE) induces spasmolytic effect on rat uterus precontracted by oxytocin (Gharib Naseri and Ehsani, 2006) and the same extract induces vasorelaxant effect on rat isolated aorta. The latter effect was dependent on integrity of endothelium and NO and cGMP productions (Gharib Naseri *et al.* 2005).

It was showed that inclusion green grape leaf (GGL) up to 2% in broiler diets did not have any adverse effects on broiler performance, but significantly affected the blood parameters, by feeding 1.5% green grape leaf meal, the blood level of HDL, heterophil and ratio of heterophil to lymphocyte increased, while the lymphocyte percentage decreased (Tayer *et al.* 2012). The main inhibiting factor in feeding grape leaf in poultry diets is it tannins compounds (Onyimonyi, 2009). Grape green leaves contain variable amount of tannins. Tannins in monogastric animals via protein precipitation in gut, has adverse effect on protein digestion and amino acids absorption (Mitaru *et al.* 1983). Different methods are used to improving of tannin containing feeds; of them is urea treating (Medugu *et al.* 2012). Using jackbean meal containing tannins treated with urea solution

involving young broiler chicks demonstrated that jackbeans so processed could be tolerated by broiler chicks up to 25% inclusion level in the diet without adverse effects on performance (Udedibie and Nkwocha, 1990). Jackbeans soaked in 5% urea solution had the highest true metabolizable protein (TMP) of 253.32 mg/g value and also had an appreciable true metabolizable energy (TME) of 2.761 kcal/g which become effective and cheap 2.761 kcal/g which become effective and cheap method of processing that can be conveniently practiced at farm site (Akinmutimi *et al.* 2000). Broiler chickens can tolerate dry urea treated jackbean meal containing tannins at up to 20% in their diets (Udedibie *et al.* 1994). In the present study the effects of untreated and treated green grape leaf (GGL) with different levels of urea (0%5 and 1%) on performance, production cost, egg traits, blood biochemical, and immune cells of laying hens are investigated.

## MATERIALS AND METHODS

### Birds and experimental design

In this experiment 192 Hy-Line (w-36) laying hens, (35 to 46 wk of age and  $1750 \pm 75$  g of body weight) were divided in to 4 experiment groups with 4 replicates (12 birds per replicate) in a completely randomized design experiment. The treatments included: 1) control group, 2) group with 3% untreated GGL, 3) group with 3% GGL treated with 0.5% urea and 4) group treated with 3% GGL treated with 1% urea. Sufficient amount of GGL leaves were collected from vineyards and after proper drying, completely mixed with 0.5% and 1% urea solution, inserted into nylon bags and ensiled for 21 days. The compositions of GGL were determined according to AOAC (2002) and after fine milling; they were mixed with other diets ingredients (Table 1).

### Diets preparation

The diets were formulated to meet the requirements of birds established by the NRC (1994) for laying hens by using UFFDA software as shown in Table 2. During experimental period the lighting program for laying hens was 16 h light and 8 h darkness.

House system was whole controlled and bird density was normal, so there were 3 birds in each pen. Environmental temperature was controlled by exactly thermometers and was approximately 18 °C. Humidity percent was between 65 to 70 during experimental period. Feed and water were available *ad libitum* throughout the experiment. Feed intake, feed conversion ratio, egg production percentage, egg mass and egg weight were determined weekly. Egg mass was measured by multiplying of egg production percentage to egg weight and feed conversion ratio was measured by dividing amount of feed intake to egg mass.

**Table 1** The chemical composition of green grape leaf (100% dry matter base)

Composition	Dry matter (%)	Crude protein (%)	Crude fiber (%)	Calcium (%)	Phosphorus (%)
GGL untreated	86.89	8.55	23	0.81	0.54
GGL treated with 0.5% urea	55.26	11.26	Unevaluated	Unevaluated	Unevaluated
GGL treated with 1% urea	61.44	14.40	Unevaluated	Unevaluated	Unevaluated

GGL: green grape leaf.

**Table 2** The composition of basic diets

Feeds ingredients	0%	3% GGL	3% GGL with 0.5% urea	3% GGL treated with 1% urea
Corn	55.00	55.00	55.00	55.00
Wheat	11.65	7.13	7.61	8.34
Soybean meal (42% CP)	20.17	20.02	20.58	19.91
Soybean oil	3.26	3.92	3.87	3.80
GGL	0	3.00	3.00	3.00
Oyster shell	7.40	7.45	7.44	7.45
Bone meal	1.70	1.64	1.64	1.66
Salt	0.30	0.31	0.31	0.31
Vitamin premix <sup>1</sup>	0.25	0.25	0.25	0.25
Mineral premix <sup>2</sup>	0.25	0.25	0.25	0.25
DL-methionine	0.02	0.03	0.03	0.04

## Analysis results

Feed price (toman/kg)	863	887	867	859
Metabolisable energy (kcal/kg)	2900	2900	2900	2900
Crude protein (%)	14.50	14.50	14.50	14.50
Ca (%)	3.40	3.40	3.40	3.40
Available phosphorus (%)	0.33	0.33	0.33	0.33
Sodium (%)	0.16	0.16	0.16	0.16
Crude fiber (%)	2.46	3.35	3.30	3.25
Lysine (%)	0.74	0.72	0.72	0.72
Methionine + cysteine (%)	0.54	0.55	0.55	0.55
Tryptophan (%)	0.19	0.18	0.18	0.18

<sup>1</sup> Vitamin premix per kg of diet: vitamin A (retinol): 8500000 IU; vitamin D<sub>3</sub> (cholecalciferol): 2500000 IU; vitamin E (tocopheryl acetate): 11000 IU; vitamin K<sub>3</sub>: 2200 mg; Thiamine: 1477 mg; Riboflavin: 4000 mg; Panthothenic acid: 7840 mg; Pyridoxine: 7840 mg; Cyanocobalamin: 10 mg; Folic acid: 110 mg and Choline chloride: 400000 mg.

<sup>2</sup> Mineral premix per kg of diet: Fe (FeSO<sub>4</sub>·7H<sub>2</sub>O, 20.09% Fe): 75000 mg; Mn (MnSO<sub>4</sub>·H<sub>2</sub>O, 32.49% Mn): 74.4 mg; Zn (ZnO, 80.35% Zn): 64.675 mg; Cu (CuSO<sub>4</sub>·5H<sub>2</sub>O): 6000 mg; I (KI, 58% I): 867 mg and Se (NaSeO<sub>3</sub>, 45.56% Se): 200 mg.

GGL: green grape leaf.

Mortality was recorded when it occurred. The collected eggs were classified as normal or damaged; the latter including fully cracked eggs (an egg with broken shell and destroyed membrane), hair cracked eggs (an egg with broken shell but intact membrane) and eggs without shell (an egg without shell but with intact membrane). For measuring the egg traits, at the end of the experiment, 3 eggs were collected from each replicate to measure egg traits. Egg shells were cleaned and maintained at environmental temperature for 48 h until they were dried, and then they were weighed. Then, their average was considered as final thickness of egg shell for each experimental unit. Color index of the yolk (Roche color index), yolk and white index, Haugh units were also determined (Farkhoy *et al.* 1997). The price each kilogram of eggs were measured by multiplying the price of each kilogram of feed to feed conversion ratio.

#### Hematological indices and blood biochemical and immune parameters

At the end of the experiment period, two birds from each replicate were randomly chosen for blood collection and

approximately 5 mL blood samples were collected from the right brachial vein. One mL of collected blood was transferred to tubes with ethylenediaminetetraacetic acid (EDTA) to determine of hemoral and cell mediated parameters including red blood cells, hemoglobin, white blood cells, heterophiles and lymphocytes (Gross and Sigel, 1983). The remaining 4 mL blood was centrifuged to obtain serum for determining the blood biochemical parameters which include cholesterol, triglyceride, albumen, total protein, and uric acid. Kit packages (Pars Azmoon Company; Tehran, Iran) were used for determining the blood biochemical parameters using Anision-300 auto-analyzer system (Nazifi, 1997).

#### Statistical analysis

The data were subjected to one-way analysis of variance procedures appropriate for a completely randomized design using the general linear model procedures of SAS (2005). Means were compared using the Duncan multiple range test (Valizadeh and Moghaddam, 1994). Statements of statistical significance were based on (P<0.01).

## RESULTS AND DISCUSSION

### Performance

The effects 3% of untreated and treated GGL on the performance of laying hens are shown in Table 3. The highest values for egg weight, and the lowest amount of feed cost/kg egg occurred with 3% GGL treated with 0.05% and 1% of urea, whereas the best egg production percentage, the highest amount of egg mass and the lowest feed conversion ratio were obtained in group contained GGL treated with 0.5% urea. The amount of feed intake increased in groups fed by GGL and GGL treated with 0.5% urea.

### Egg traits

The effects of using 3% of untreated and treated GGL with 0.05% and 1% of urea on egg quality traits of laying hens are shown in Table 4. Untreated and treated GGL in diets improved some of egg traits in laying hens ( $P < 0.01$ ). When compared to the control, GGL improved egg yolk color index, egg shell weight and Haugh, while did not affect the albumin weight ( $P > 0.05$ ). The high yolk color value was observed with 1% urea treated GGL. Feeding GGL reduced the eggshell thickness ( $P < 0.5$ ), but compared to control group, in feeding 0.1% urea treated GGL, the eggshell thickness did not reduce ( $P > 0.05$ ).

### Blood biochemical parameters

The effects 3% of untreated and treated GGL on laying hens blood biochemical parameters are presented in Table 5. Untreated and treated GGL significantly affected blood the level of HDL ( $P < 0.05$ ). Inclusion GGL in diets caused the blood level of HDL decrease so, the lowest level of HDL was observed in group 4, while it did not change by feeding 3% untreated GGL ( $P > 0.05$ ). Without HDL, using GGL could not change any other there blood parameters ( $P > 0.05$ ).

The effects of 3% of untreated and treated GGL on blood immune cells are shown in Table 6. Inclusion GGL in laying hens diets had positive effect on immune cells, so the lowest white blood cells count, the lowest percentage of heteropheils, the highest percentage of lymphocyte and the lowest heteropheil to lymphocyte ratio were obtained by fed 3% GGL treated with 1% urea, the highest count of white blood cells belonged to group contained 3% of untreated GGL. Inclusion GGL into laying hens diets in compared to control group, had positive effects on laying hens performance. Improve in production parameters may be related to nutritional (minerals and vitamins) and secondary substances such as antioxidants contents of GGL (Gharib Naseri *et al.* 2006). The results of another report (Tayer *et al.* 2012) in feeding GGL in broilers not in agree of these results obtained in laying hens. The difference in the results

may be related to bird strain, GGL level and composition and diets ingredients.

Between treatments, the highest amount of feed consumption was observed in group fed 3% GGL treated with 0.5% of urea in diet. Increase in the amount of daily feed consumption could be having some reasons such as palatability and high fiber content. The highest level of feed intake could have increased the nutrient availability which resulted in high egg production. In the present study, inclusion 3% GGL treated with 0.5% of urea, caused the highest value of egg weight, egg production; egg mass, feed intake, feed conversion ratio and the lowest feed / egg production price were obtained. Urea treating of GGL may be had positive effects on GGL cell wall degradation, so, the highest amounts of nutrients such as protein and energy and secondary substances such as antioxidants released, absorbed and improved of laying hens performance. Antioxidants by preserve of sensitive nutrients against oxidation in diets and digestive tract, can improve the performance (Sayiedpiran *et al.* 2011). Improve in the nutrient digestibility of urea treated beans was reported previously (Udedibie *et al.* 1994).

As has been seen in Table 4, like performance, inclusion GGL into laying hens diets had positive effects on egg quality traits. These qualification effects may be related to GGL nutrients and non nutrients contents. GGL contain higher levels of beta-carotene, calcium, phosphorus and potassium (Zargari, 1990). Improve in egg yolk color related to GGL beta-carotene content (Sayiedpiran *et al.* 2011). The main reason for eggshell increase is high level of calcium availability during egg formation (Farkhoy *et al.* 1997), this subject is highly improved by urea treating of GGL (Udedibie *et al.* 1994).

Urea treating of GGL, may be degraded the cell wall and reduced the tannins compound, so, higher amounts of nutrients and non- nutrients released and used by hens for upgrading of egg quality traits (Farkhoy *et al.* 1997), so, the best results were obtained with GGL that treated with urea. High feed consumption in this group resulted in high beta carotene, calcium and amino acid supply, and consumption of them increased egg yolk color index and calcium bioavailability.

As the heaviest eggs were obtained with GGL that treated with 0.05% of urea, also, the highest values for eggshell, albumin and yolk weight belonged to this group. By using GGL the eggshell thickness decreased, this is related to increase in the egg weight. As by inclusion GGL in hen diets, the egg size increased, so, deposition of calcium occurred in large volume, so the egg shell thickness was thinner. GGL is rich source of antioxidant and flavonoids (Zargari, 1990). It was shown that these compounds have positive effects on the amounts of blood parameters.

**Table 3** The effects of GGL on the performance of laying hens (35-46 wk)

Treatments	Egg weight (g)	Laying rate (%)	Egg mass (g/day)	Feed intake (g/day)	FCR (g/g)	Feed cost/kg egg (rials)
Control group	60.98 <sup>c</sup>	68.41 <sup>c</sup>	41.72 <sup>c</sup>	110.68 <sup>b</sup>	2.65 <sup>a</sup>	2284.07 <sup>a</sup>
3% GGL	61.64 <sup>b</sup>	73.34 <sup>b</sup>	45.20 <sup>b</sup>	111.04 <sup>a</sup>	2.46 <sup>b</sup>	2136.85 <sup>b</sup>
3% GGL with 0.5% urea	62.32 <sup>a</sup>	77.34 <sup>a</sup>	48.18 <sup>a</sup>	111.19 <sup>a</sup>	2.31 <sup>c</sup>	1986.04 <sup>c</sup>
3% GGL with 1% urea	62.31 <sup>a</sup>	74.27 <sup>b</sup>	46.26 <sup>b</sup>	110.61 <sup>b</sup>	2.39 <sup>b</sup>	2028.67 <sup>c</sup>
SEM	0.07	0.68	0.44	0.10	0.02	20.82
P-value	0.0001	0.0001	0.0001	0.0085	0.0001	0.0001

The means within the same column with at least one common letter, do not have significant difference ( $P > 0.01$ ).

GGL: grass grape leaf.

FCR: feed conversion ratio.

SEM: standard error of the means.

**Table 4** The effects of GGL on egg traits of laying hens (46 wk)

Treatments	Yolk color (Rosh)	Shell weight (g)	Albumin weight (g)	Yolk weight (g)	Haugh unit	Eggshell thickness (mm)
Control group	2.22 <sup>d</sup>	5.89 <sup>b</sup>	38.39	16.21 <sup>b</sup>	73.09 <sup>b</sup>	0.39 <sup>a</sup>
3% GGL	3.22 <sup>c</sup>	6.64 <sup>a</sup>	40.80	18.19 <sup>a</sup>	74.49 <sup>a</sup>	0.36 <sup>b</sup>
3% GGL with 0.5% urea	3.40 <sup>b</sup>	6.46 <sup>a</sup>	40.27	18.65 <sup>a</sup>	74.42 <sup>a</sup>	0.38 <sup>ab</sup>
3% GGL with 1% urea	5.33 <sup>a</sup>	6.40 <sup>a</sup>	39.89	18.30 <sup>a</sup>	74.19 <sup>a</sup>	0.37 <sup>a</sup>
SEM	0.21	0.09	0.77	0.34	0.19	0.01
P-value	0.0010	0.0020	0.2271	0.0043	0.0021	0.0175

The means within the same column with at least one common letter, do not have significant difference ( $P > 0.01$ ).

GGL: grass grape leaf.

SEM: standard error of the means.

**Table 5** The effects of GGL on blood biochemical parameters of laying hens (46 wk)

Treatments	Total cholesterol (mg/dL)	White (g/dL)	Total protein (g/dL)	Uric acid (g/dL)	HDL cholesterol (mg/dL)
Control group	181.54	3.32	7.14	5.04	8.00 <sup>a</sup>
3% GGL	177.43	3.22	6.02	4.24	6.88 <sup>ab</sup>
3% GGL with 0.5% urea	222.90	3.07	5.65	3.77	3.75 <sup>bc</sup>
3% GGL with 1% urea	144.27	3.09	5.21	2.87	3.43 <sup>c</sup>
SEM	47.12	0.12	0.52	0.67	0.97
P-value	0.7128	0.4607	0.1335	0.2187	0.0240

The means within the same column with at least one common letter, do not have significant difference ( $P > 0.05$ ).

GGL: grass grape leaf and HDL: high density lipoprotein.

SEM: standard error of the means.

**Table 6** The effects of urea untreated and treated GGL on blood immunity parameters of laying hens (46 wk)

Treatments	Hematocrit (%)	Hemoglobin (%)	WBC ( $10^6/\text{mm}^3$ )	Heterophil (%)	Lymphocyte (%)	Heterophil / lymphocyte ratio (1/1)
Control group	30.34	10.07	21.83 <sup>b</sup>	30.67 <sup>a</sup>	71.33 <sup>b</sup>	0.441 <sup>a</sup>
3% GGL	29.67	9.84	27.33 <sup>a</sup>	23.33 <sup>ab</sup>	72.33 <sup>b</sup>	0.331 <sup>b</sup>
3% GGL with 0.5% urea	29.00	9.57	22.83 <sup>ab</sup>	16.33 <sup>b</sup>	82.33 <sup>ab</sup>	0.201 <sup>b</sup>
3% GGL with 1% urea	28.23	9.37	19.50 <sup>b</sup>	13.67 <sup>b</sup>	85.33 <sup>a</sup>	0.161 <sup>b</sup>
SEM	1.56	0.48	1.56	3.73	3.26	0.07
P-value	0.8202	0.7545	0.0411	0.0472	0.0359	0.0298

The means within the same column with at least one common letter, do not have significant difference ( $P > 0.05$ ).

GGL: grass grape leaf.

WBC: white blood cells.

SEM: standard error of the means.

Contrary to this finding the HDL value is reduced by using GGL treated with 0.5% of urea, while other blood parameters did not change. Decrease in the blood HDL level in this group may be related to egg production status. As the high performance occurred in this experimental group, may be the highest amount of antioxidant and flavonoids compounds were transferred into eggs and the level of blood HDL reduced.

Decreased the white blood cell count, heterophil percentage, heterophil to lymphocyte ratio and increase in the percentage of lymphocyte could be an index in improvement of immune system function (Sturkie, 1995), this results were observed in group contained 3% GGL treated with 1% of urea. As the performance in this group compared to group contained 3% GGL treated with 0.5% of urea was low. So, high amounts of useful compounds such as fla-



vonoids, vitamin C and carotenoids not transferred into eggs, remain in blood and improved the immune index. These results were observed in the immune system generally benefits from the herbs and spices rich in flavonoids, vitamin C and carotenoids. These compositions can increase the immune status of blood in layers (Farkhoy *et al.* 1997).

In contrary with groups contained GGL, despite of low production, the adverse immune index (high heterophil percentage, high ratio of heterophil to lymphocyte and the lowest percentage of lymphocyte) were observed in control group, it may be related to low levels of effective compounds (flavonoids, vitamin C and carotenoids) those were found in GGL.

## CONCLUSION

The overall results indicated that inclusion 3% green grape leaf treated with 0.5% and 1% urea in contrast with control group and group contained untreated green grape leaf, significantly improved the performance, egg traits, production cost and immune status of laying hens.

## ACKNOWLEDGEMENT

This study has been supported by Islamic Azad University of Maragheh Branch. The author likes to appreciate Dr. Ahadi for his supporting during experimental period in poultry farm.

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