

Effect of dietary Goji Berry (*lyceum barbarumL.*) leaf meal on performance, egg quality and egg yolk cholesterol levels of laying Hens

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ARTICLE INFORMATION	ABSTRACT
Received: 10-10-2019 Received in revised form: 13-11-2019 Accepted: 20-12-2019	In this study effects of dietary goji berry (<i>Lycium barbarumL.</i>) leaf (GBL) meal on egg rate, feed intake, egg yield and mass, feed conversion ratio, external and internal egg quality and egg yolk cholesterol level of laying hens were investigated. A total of 55 Lohman Brown laying hens aged 48 weeks were used. The birds were fed with basal diet supplemented with 1 g/kg (GBL1), 5 g/kg (GBL5), 10 g/kg (GBL10), 20 g/kg (GBL20) of goji berry leaves and negative control. These mentioned doses were added to per kg basal diet (166 g crude protein and 2821 kcal ME kg ⁻¹). Feed was offered limited (110 g/hen) and water was available <i>ad-libitum</i> . The trial was continued for 8 weeks. At the end of the study, egg yield, egg mass, feed intake and feed conversion ratio of laying hens were not affected by treatments with the addition of GBL (P>0.05). Whereas GBL20 treatment reduced the yolk cholesterol level (P<0.05). GBL10 and GBL20 decreased egg shell thickness compared to control group (P<0.05). GBL10 increased albumen index compared to control group (P<0.05). As a result, 20 g/kg GBL was used in layer diets as a feed additive to reduce egg yolk cholesterol levels.
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Original Research Article	Keywords: Goji berry leaves, laying hen, feed additive, yolk, cholesterol

INTRODUCTION

Poultry production is necessary animal activities for fulfilling essential amino acids of people health and human nutrition at the save time contributing to improve economy of the countries (Hanusová *et al.*, 2015; Orhan *et al.*, 2016). Food safety has become an increasingly important topic for livestock industry in the worldwide. Goji berry (or wolfberry) is a common name of *Lycium barbarumL.*, *Lycium chinenseL.* and *Lycium ruthenicumL.* species of the *Lycium species*, belongs to the family of *Solanaceae* (eggplants), is commonly found in arid and semi-arid regions of China, Tibet, Himalayas, Mongolia, Southeast Europe and the Mediterranean countries (Levin and Miller 2005; Potterat, 2010; Bucheli *et al.*, 2011). Goji berry is a perennial plant and in the form of strata. *Lycium ruthenicumL.* and *Lycium barbarumL.* types are produced commercially (Wang *et al.*, 2015). Goji berry is highly tolerant in terms of ecological requirements. Moreover goji berry is a plant resistant to inefficient soils and high altitudes.

Goji berry has the highest oxygen radical absorbance capacity values than other vegetables and fruits consumed as food (USDA, 2016). Foods with high oxygen radical absorbance capacity levels

protect cells and cell components from oxidative damage (Prior *et al.*, 1999). Goji berry fruit also includes polysaccharide, monosaccharide, essential oils, vitamins, amino acids, mineral elements, carotenoid and flavonoids (Potter at, 2010; Amagase Farnsworth, 2011; Endes *et al.*, 2015). Flavonoids are antioxidant compounds neutralize harmful impacts of formerly consumed substances in the body (Kanti Dey *et al.*, 2012; Khalili *et al.*, 2016). Goji berry is used as an auxiliary component in cosmetic products such as lotion, shower gel and cream due to its anti-aging properties. In folk medicine goji berry has been used for many benefits and life-improving effect in China (Gündüz *et al.*, 2015). In addition goji berry has been reported in studies that it can increase muscle mass, prevent fatty liver, effective in regulating blood pressure, strengthening the immune system, balancing blood sugar, anti-tumor effect, effective in alzheimer's disease, preventing loss of vision, aphrodisiac effect, anti-cancer, lowering cholesterol, anti-bacterial, anti-fungus, effective against leukemia, memory enhancer and effective in the treatment of digestive difficulty (Cheng *et al.* 2005; Zhao *et al.*, 2005; Luo *et al.*, 2006; Amagase & Hsu, 2009; Ming *et al.*, 2009; Ueland *et al.*, 2010; Mao *et al.*, 2011; Ranjbar *et al.*, 2012; Pop *et al.*, 2013; Lee

et al., 2014; Wang *et al.*, 2015; Zhang *et al.*, 2015; Kulczynski & Gramza-Michalowska, 2016).

The objective of this research was to determine the effects of goji berry (*Lycium barbarum*L.) leaf (GBL) meal on egg rate, feed intake, egg yield and mass, feed conversion ratio, egg yolk cholesterol level and external and internal egg quality of laying hens.

MATERIALS AND METHODS

Diets, birds and feeding treatments

A total number of fifty five birds of 48 week Lohman Brown laying hens obtained from a commercial enterprise were used. The laying hens were subjected to the experiment individually in laying cages with 3 compartments and 4 storeys (200 cm x 97 cm x 46 cm). The hens were exposed to 8 hours of dark and 16 hours of light illumination for 8 weeks. Temperature of the experiment unit remained at 25 °C. Two weeks before starting the experiment, daily egg yields of the hens were recorded and at the end of the second week their body weight were noted and randomly distributed to the experiment groups with similar body weight and similar egg yield in accordance with experiment model. 0 (control), 1 (GBL1), 5 (GBL5), 10 (GBL10) and 20 (GBL20) g/kg of goji berry leaves (*Lycium barbarum*L.) were added to the feed of the laying hens and 5 groups were established and therefore totally 55 hens were used as 11 hens in each group. Feed was restrictedly given (110g per bird); however, water was given as *ad-libitum*. Components of experimental layer diet formulated according to NRC (1994) (Table 1). The goji berry leaves and the goji berries used as food supplements were obtained from a commercial enterprise. After removing impurities from the collected goji berry leaves, it was dried, ground and then fed to birds. Total phenolic matter of the goji berry leaves was determined (Elzaawely & Tawata, 2012) ,total flavonoid matter was obtained through aluminum chloride colorimetric method and the antioxidant activity analyses were made according to Chang *et al.*, (2002). Villano *et al.* (2007) (Table 2 and Table 3).

Table I: Experimental layer diet (Phase I)

Feed Ingredients	gkg ⁻¹
Corn	463
Full fat soya	150
Sunflower meal	100
Corn gluten meal	80
Wheat bran	80
Vegetable oil	25
CaCO ₃	85
DCP (175 (gkg ⁻¹))	8
Methionine&Lysine	1
NaHCO ₃ &NaCl	3
Mineral and Vitamin premix*	5
Calculated composition	
ME, kcal kg ⁻¹	2821
Crude protein, gkg ⁻¹	166.5
Lysine, gkg ⁻¹	7.5
Methionine + systine, gkg ⁻¹	4
Ca, gkg ⁻¹	39
P (available), gkg ⁻¹	7
Na, gkg ⁻¹	2

*Per kg diet included 10000 IU Vitamin A, 2500 IU Vitamin D₃, 20 mg Vitamin E, 2 mg Vitamin K₃, 4 mg Vitamin B₂, 10 mg Vitamin B₁₂, 100 mg Mn, 90 mg Zn, 25 mg Fe, 5 mg Cu, 0.25 mg Co, 1 g Iodine, 0.3 mg Se

Table II: Total phenolic and total flavonoid contents of goji berry leaves

Parameters	Gojiberryleavescontents
Total Fenolic Content (mg GA EQ / 1 g)	7.76
Total Flavonoid Content (TFC mg CATECEQ/1 g)	3.47

Table III: Inhibition of goji berry leaves

Parameters	Inhibition (%)
AscorbicAcid	96.82
Gojiberryleaves	93.21

Growth parameters

Body weight change was considered as last day weight minus first day weight of the bird. Feed intake, egg number and egg mass were determined daily and feed conversion ratio was calculated according to the following equation:

FCR= Total Egg Mass / Total Feed Intake.

As Reddy *et al.* (1979) reported shape index, as Sharp & Powell (1930) reported egg yolk index, as Heiman & Carver (1936) reported albumen index and as Haugh (1937) stated Haugh unit were calculated in internal and external egg quality analysis.

Egg yolk cholesterol analysis

In order to determine the cholesterol quantity in yolk of the eggs 30 eggs as six from each group were collected. The collected eggs were boiled for 10 minutes and the yolks were taken from the boiled eggs and mashed, mixed, homogenized and made ready for the analysis. Taking 0.1 g of the sample was scaled on the precision scale, it was transferred to glass tube and 4 ml of isopropyl alcohol was added to it. The sample was mixed in vortex for 2 or 3 minutes and centrifuged at 3000 rpm for 10 minutes. Cholesterol concentration of the egg yolk was determined in spectrophotometer with a commercial kit and calculations were made according to the method stated by Biochemical Analysis and Food Analysis (Boehringer Mannheim Gmbh Biochemica, 1989)

Statistical analysis

Statistical analysis of the obtained research data was made by One-way ANOVA using SPSS package programme and Duncan multiple comparison tests was used in the comparison of experimental group averages (SPSS, 2007).

RESULTS AND DISCUSSION

The effect of goji berry leaves (GBL) supplements on initial body weights, final body weights and change body weight changes of laying hens is described in Table 4. The differences observed in initial body weights, final body weights

and change body weights of layer hens fed with GBL were not found significant ($P>0.05$).

Table IV: The effects of GBL on the initial body weight, final body weight and change body weight of laying hens

Parameters (g)	Goji berry leaves meal (g/kg)					P
	Control	1	5	10	20	
Initial Body Weight	1738.00	1734.55	1735.46	1736.50	1734.50	1.00
Final Body Weight	1681.00	1697.27	1625.91	1627.50	1608.50	0.38
Body Weight Change	-57.00	-37.28	-109.55	-109.00	-126.00	0.49

The effect of GBL added to the feed of the laying hens at different levels on feed intake, feed conversion ratio, egg yield (number) and egg rate (%) is shown in Table 5. The effect of GBL to the feed intake, feed conversion ratio, egg yield and egg rate of laying hens was not statistically found significant ($P>0.05$).

Table V: The effects of GBL on feed intake, feed conversion ratio (FCR), egg yield (number), egg rate (%) of laying hens

Parameters (g)	Goji berry leaves meal (g/kg)					P
	Control	1	5	10	20	
Feed Intake	109.97	108.41	108.59	107.22	109.47	0.19
Feed Conversion Ratio	1.75	1.74	1.87	1.71	1.76	0.57
Egg Yield (number)	41.86	41.50	43.50	39.00	45.37	0.64
Egg Rate (%)	74.74	74.11	77.68	69.24	72.19	0.39

Effect of GBL on egg yolk cholesterol concentration is indicated in Table 6. Egg yolk cholesterol concentration of GBL20 group was found significantly low when compared to GBL1 and GBL5 groups ($P<0.05$).

Table 6: Effects of GBL on egg yolk cholesterol concentration

GBL Levels, (gkg ⁻¹)	EggYolkCholesterolConcentration, mgkg ⁻¹
GBL0	51.40 ^{ab}
GBL1	57.60 ^a
GBL5	60.76 ^a
GBL10	51.51 ^{ab}
GBL20	45.71 ^b
P	0.03

^{a-b} Differences in groups with different letters in the same column are statistically significant (P<0.05).

Effect of GBL on internal and external egg quality is presented in Table 7. The differences observed in terms of GBL supplementation and egg weight, albumen weight, egg shell weight, yolk weight, albumen weight, yolk color, shape index, yolk index and Haugh Unit were not found statistically significant (P>0.05). However, egg shell thickness of control group was higher than GBL10 and GBL20 (P<0.05). Albumen index of GBL10 were found higher than control, GBL1 and GBL5 (P<0.05).

Table 7: Effects of GBL on initial and external egg quality

Parameters	GBL0	GBL1	GBL5	GBL10	GBL20	P
Egg Weight (g)	65.08	64.13	63.47	66.69	64.48	0.27
Egg Shell Weight (g)	9.44	8.86	8.97	9.46	9.16	0.15
Yolk Weight (g)	15.69	16.20	15.38	16.18	15.93	0.06
Albumen Weight (g)	32.78	32.30	33.48	31.95	32.68	0.13
Egg Shell Thickness (µm)	0.40 ^a	0.38 ^{ab}	0.38 ^{ab}	0.37 ^b	0.35 ^b	0.02
Yolk Color	12.63	13.09	12.79	12.91	12.90	0.38
Shape Index	76.86	78.19	78.72	78.87	78.13	0.24
Albumen Index	9.25 ^b	9.37 ^b	9.27 ^b	10.68 ^a	10.37 ^{ab}	0.04
Yolk Index	57.39	55.78	58.68	58.75	60.00	0.15
Haugh Unit	93.59	94.87	92.89	97.49	97.08	0.15

^{a-c} Differences in groups with different letters in the same line are statistically significant (P<0.01).

^{a-b} Differences in groups with different letters in the same line are statistically significant (P<0.05).

DISCUSSION

There are so many health benefits of goji berry leaves such as liver, kidney, immune system, longevity-related functions and activities, studies on this subject are very limited. Also, it is a powerful antibacterial and antioxidant besides its cholesterol lowering effect (Mocan *et al.*, 2014; Gong *et al.*, 2016). According to our knowledge, there is no study on the possible effect of goji berry leaves on laying hens. In this study, it was hypothesized that beneficial contents of goji berry leaves may enhance the health status of laying hens reflecting to egg yield and internal and external characteristics, egg yolk cholesterol and performance. In addition, there have been limited studies on the antioxidant activity, total phenolic and total flavonoid contents of goji berry leaves. It was also determined that GBL had a cholesterol lowering effect with 20 g/kg of supplementation to feed. No study was found on cholesterol lowering effect of GBL in livestock. However, there are studies on the use of different plant leaves in laying hens' feeding. Al-Harti *et al.* (2009) and Zhao *et al.* (2013) declared that cholesterol level of egg yolk decreased with leaf meal supplement.

No significant difference was found in initial body weight, final body weight and change body weight of laying hens with GBL supplementation in this study. Odunsi *et al.* (2002) stated that change body weight decreased; however, Tesfaye *et al.* (2014) declared that initial body weight and final body weight did not change with leaf meal supplementation to the feeds of laying hens. In our study there was no significant difference in feed intake among the groups. The laying hens in each group consumed feed in the rates (approximately 90-110 g/day) indicated in management guide. It can be said that GBL has no negative effect on feed intake. Similar to this study, Paterson *et al.* (2000), Fasuyi *et al.* (2005) and Lin *et al.* (2017) also reported that feed intake did not change with leaf meal treatment to the feeds of laying hens. The findings of this study indicated that FCR was not affected by the dietary GBL supplementation, which is similar to that indicated in laying hens (Ige *et al.* 2006; Radwan *et al.* 2007; Al-kirshi *et al.*, 2010). It

can be said that feed intake and egg rate was not affected from GBL. It is also known that energy and protein contents of the feed have an effect on feed intake and egg yield (Kakengi *et al.* 2007). Therefore, the fact that feed intake, egg yield and FCR of laying hens are not negatively affected by GBL can be explained with sufficient amount of protein and energy intake. Duru (2013) reported that egg yield and egg rate Nhan *et al.* (1997) did not change with leaf meal supplementation. However, Atawodi *et al.* (2008) determined that egg yield decreased with leaf meal supplementation to the feed of laying hens. However, in our study egg yield increased especially with GBL20, but this increase was numerical.

No significant difference between the groups in terms of egg weight and egg shell weight with GBL was found; however, eggshell thickness significantly decreased as GBL level in the feed increased. It is reported that egg weight has positive relationship with egg shell thickness and egg shell weight which is directly related with egg shell quality (Choi *et al.*, 1983; Stedalman, 1986). However, egg shell thickness was negatively affected in the study, although there was a positive relationship between egg weight and egg shell weight. This can be explained by the fact that the amount of GBL increases because calcium level of GBL in the feed is low and this decreases egg shell thickness. Udedibie and Opara (1998); Aro *et al.* (2009); Abou-Elezz *et al.* (2011), Christaki *et al.* (2011) and Gakuya *et al.* (2014) concluded that leaf meal treatment had no effect on egg weight.

The shape index of the eggs obtained from this study is close to the standard egg shape index (76.86-78.87) reported by Şenköylü (1991). Abou-Elezz *et al.* (2012), Lu *et al.* (2016) and Swain *et al.* (2017) suggested that egg shape index was not affected by the leaf meal. Egg yolk colour cause changes in the eggs of hens intaken feed with the components such as xanthophylls and caroten which are controlling the color density. However, it could not be found as mentioned in a study that the eggs obtained from the laying hens taken feed with GBL contain components that would change the color of egg yolk. This can be explained by the fact that yolk color of the eggs in this study did not lead to any change as compared to control group. Cayan & Erener (2015) reported that yolk color was between 12.20-13.00 with leaf meal supplement.

Haugh unit and albumen index are important parameters that inform about the freshness of eggs and albumen quality. Albumen index significantly increased statistically with GBL

10 and GBL20 treatment to the feed and Haugh unit also increased numerically. In addition, the fact that GBL treatment to the feed did not affect the feed intake supports this situation. Esonu *et al.* (2004) stated that leaf meal had no effect on albumen index. However, Osei *et al.* (1990), Muramatsu *et al.* (1993), Lokaewmanee *et al.* (2009) and Lu *et al.* (2016) obtained similar results in terms of Haugh Unit.

CONCLUSION

Considering the data obtained from experiment, it can be said that GBL supplementation was not affected negatively on the production performance of laying hens. 20 g/kg GBL treatment decreased egg yolk cholesterol. Also, there was no incidence of disease in experimental layers. However, further studies are needed to assess the cholesterol lowering mechanism and to be used as a feed additive of GBL.

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