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ARTICLE

Effect of Sea Buckthorn (*Hippophae rhamnoides* L.) Seed Supplementation on Egg Quality and Cholesterol of Rhode Island Red×Fayoumi Laying Hens

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Abstract The present trial was carried out to study the effect of sea buckthorn seed supplementation on egg quality of laying birds. A total of 160 Rhode Island Red× Fayoumi layers was divided into four groups of 40 birds each, which was further replicated four times with 10 hens per replicate. Group one was kept as a control, while other three groups were supplemented with sea buckthorn seed powder at a dose rate of 1 (T1), 2 (T2) and 3 (T3) g/kg of feed. The results showed that egg production was significantly (p<0.05) higher in T3 at the end of the study. Egg weight was significantly (p<0.05) high in T2 and T3 during week 39 and 40. Egg yolk weight was significantly (p<0.05) in T3 compared to the control. Significantly (p<0.01) lower egg cholesterol was recorded in T2 and T3. From the results of the present study, we concluded that laying hens supplemented with sea buckthorn at the rate of 2 and 3 g/kg improved the egg quality parameters and egg cholesterol.

Keywords sea buckthorn, egg, hens, cholesterol

Introduction

Healthy nutrition has an essential role in reducing the cardiac disease (Khan et al., 2017). The body requires only a small amount of cholesterol and the excess of the consumed cholesterol is deposited in the arteries, including the coronary arteries, leading to serious conditions such as atherosclerosis, or hardening of the arteries and increased incidence of coronary heart disease (Ostlund, 2004; Shahid et al., 2015; Sultan et al., 2015). The average cholesterol content of eggs ranges from 195 to 230 mg per egg (Shahid et al., 2015) and is influenced by factors including hen genetics, diet and stage of production (Raza et al., 2016). Today, the focus of the researchers is to reduce the cholesterol content in egg yolk to minimize the cholesterol related cardiac

diseases (Elkin et al., 2003). Cholesterol level can be lowered through dietary changes or prescribed drugs. Different herbs are highly effective in lowering cholesterol (Alzawqari et al., 2016; Abudabos et al., 2016; 2018; Tehseen et al., 2016).

Seed of sea buckthorn (*Hippophae rhamnoides* L.) is rich in unsaturated fatty acids (17% oleic acids, 20% linolenic and 40% linoleic) and lower in saturated fatty acids (8% steric acids and 13% palmitic acid). Two essential fatty acids, namely linolenic acid (39%) and linoleic acid (42%) are present in high concentration in seed oil extracted from this subspecies (Cenkowski et al., 2006). The oil contains high amount of vitamin E and ß-carotene, therefore, could be used as an effective medicine for many diseases (Ahmad et al., 2009). The use of sea buckthorn as food supplement has been reported to increase HDL-cholesterol levels and decreases LDL-cholesterol, total cholesterol, triglycerides when compared to the diet which is free from sea buckthorn (Krejcarova et al., 2015). Sea buckthorn contains ß-carotene, α-tocopherol and unsaturated fatty acids, which help to prevent hepatocytes damage caused by hepatotoxins (Ramesbabu et al., 2011). In sea buckthorn flavonoids present is primarily responsible to prevent lipid deposition and protect liver against fattening (Li and Beveridge, 2003). In poultry, sea buckthorn supplementation increases productive performance in term of body weight and egg of laying hens (Wang, 1997). Sea buckthorn fruit, seeds, leaves and residues have been reported to increase egg weight and laying rate in poultry (Biswas et al., 2010).

At present, little is known about the positive effects of sea buckthorn in poultry. Kaushal and Sharma (2011) reported that sea buckthorn may be fed to the farm animals with no harmful effects. The aim of the present study was to evaluate the effect of the supplementation of sea buckthorn seed powder on the laying performance, egg quality and egg cholesterol content in Rhode Island Red×Fayoumi layers.

Materials and Methods

This study was conducted in compliance with the Ethics and Animal Welfare, Faculty of Animal Husbandry & Veterinary Sciences, The University of Agriculture, Peshawar, Pakistan.

Experimental design and bird husbandry

A total of 160 layers was selected for this study. They were divided into four groups of 40 birds each, which was further replicated four times with 10 hens per replicate. All birds were maintained in cages under similar environmental conditions in an open sided house. Commercial laying ration at the rate of 110 g per bird was provided to all birds. The feed composition is given in Table 1. Group one was kept as a control, while other three groups were provided with sea buckthorn seed powder at a dose rate of 1 (T1), 2 (T2) and 3 (T3) g/kg of feed respectively. Sea buckthorn was procured from a commercial store (Khyber Chemical Store, Karachi Market, Pakistan). Water was provided *ad libitum*. Sixteen hours light was provided to all birds on daily basis. The study was continued for five weeks including one week of adaptation period.

Measurement of feed intake

Measured amount of feed was offered every day in the morning and collected the remaining feed if any the next morning. Feed intake was calculated as the difference of the offered amount minus the feed refused to eat.

Measurement of egg production and quality

Eggs were collected on daily basis from each replicate and were recorded to work out hen day egg production. At the end

Table 1. Ingredient composition and calculated nutritional composition of layer ration

Ingredients	(%)	Ingredients	(%)
Corn	51.25	Dry matter	88.86
Canola meal	6	Metabolizable energy (kcal kg^{-1})	2,916
Soybean meal	8	Crude protein	17.05
Fish meal	4	Ether extract	10.03
Sunflower meal	10	Fiber	8.94
Corn gluten meal	4	Ash	10.36
Soya oil	4	Calcium	3.52
Wheat bran	0	Phosphorous available	0.59
Marble chips	7	Linoleic acid	1.39
Dicalcium phosphate	2	Lysine	0.85
Molasses	3	Methionine	0.40
Lysine	0.2	Cysteine	0.28
Methionine	0.05	Methionine+Cysteine	0.73
Salt	0.15	Salt	0.41
Soda	0.05	Threonine	0.59
Coccidiostat	0.05	Tryptophan	0.18
Zinc becitracine	0.05		
Vitamin mineral premix	0.2		

of each week, five eggs from each replicate (20 egg samples, from each treatment group) were collected at random to record the egg quality traits. Weight of individual egg was determined with the help of electric balance for all replicates. Shell thickness was determined using the standard screw gauge after removing the shell membrane from three different places and average was calculated. Yolk weight was determined by pouring it in Petri dish with the help of electric balance. For recording Haugh unit, the egg sample was broken in a Petri dish and albumen height was recorded. The Haugh unit was calculated using the following formula (Silversides et al., 1993).

$$HU=100 \log [H+7.57-1.7W^{.37}]$$

H= height of albumin in millimetres

W=weight of egg in grams.

Estimation of egg yolk cholesterol

Five eggs were collected per replicate and evaluated for cholesterol contents weekly (Shahid et al., 2015). Eggs from each of the replicate were broken, the yolks were separated and pooled. Immediately, one gram of egg yolk was mixed with Folch solution and centrifuged at 2,000 rpm for 1 h. After centrifugation, the solution was sieved and 2.2 mL distilled water was added and again centrifuged at 1,000 rpm for five min. The upper layer was discarded and the remaining contents were mixed with 1.5 mL of wash solution consisting of choloroform, methanol and water in a ratio of 8:4:3 respectively. The upper layer of the mixture was removed and the mixture in the bottom was washed three times and then the heated the mixture until evaporation of the solvent. The mixture was filtered and the egg yolk cholesterol was determined by spectrophotmeteric method using chemistry analyzer (Map Lab Plus, Japan). The wave length was set at 532. Spectrophotometeric protocol was followed as given in the pamphlet of manufacturing company.

Data analyses

The data was analyzed statistically with the standard procedure of analysis of variance using completely randomized design (CRD). The mean values were compared with least significance differences (LSD) as described by Steel and Torrie (1981). Significant differences were separated by Least Significant Difference. A *p*-value less than 0.05 was considered statistically significant or otherwise mentioned.

Results

The effect of different levels of sea buckthorn on feed intake is given in Table 2. The results shows that the supplementation of sea buckthorn had no significant effect on feed intake of laying hens. The effect of different levels of sea buckthorn seeds on the egg day production, egg weight, eggshell weight and eggshell thickness of laying hens is given in Table 3. No significant difference was found on hen day egg production during different weeks of the experiment. Significantly ($p<0.01$) higher hen day egg production was recorded in hens supplemented with buckthorn in T3 at the end of week 40. No significant change was observed in egg weight of laying hens during 36 to 38 weeks of the experiment. During week 39, significantly ($p<0.01$) higher egg weight was observed in hens supplemented with buckthorn in T2 and T3. In the following week, significantly ($p<0.05$) higher egg weight was recorded in hens in T3 at the end of week 40. No significant ($p>0.05$) difference was found in these two parameters of laying hens at different weeks of the experiment.

The effect of different levels of sea buckthorn seeds on egg yolk weight, albumin weight, albumin height and Haugh unit of laying hens is given in Table 4. Egg yolk weight was significantly ($p<0.05$) high in birds in T3 at the end of week 40. No significant difference was found in albumin weight, albumin height and Haugh unit of laying hens in different weeks of the experimental period.

The effect of different levels of sea buckthorn seeds on egg cholesterol of laying hens is presented in Table 5. Whole egg cholesterol and yolk cholesterol were significantly ($p<0.01$) low in eggs of hens obtained from hens in T3.

Discussion

In the present study, egg production was improved in the supplemented birds. It has been reported that sea buckthorn is a good source of flavonoids, which have antioxidant properties (Bal et al., 2011; Brisibe et al., 2008). Little information is available to compare the effect of sea buckthorn on the egg quality of laying hens. Wang (1997) worked on sea buckthorn plant and reported that this plant has the potential to increase laying production and body weight of hen. Eggshell thickness was not significantly ($p>0.05$) affected by sea buckthorn seed supplementation. Boltumelo (2004) reported that eggshell

Table 2. Effect of sea buckthorn seed supplementation on feed intake (g·hen^{-1}·day^{-1}) of laying hens

Age (wk)	Control	T1	T2	T3	*p*-value
36	108.56±0.55	107.99±0.66	107.90±0.25	107.58±0.32	0.56
37	109.23±0.30	108.08±0.35	107.67±0.47	107.64±0.78	0.15
38	108.63±0.38	107.74±0.20	108.93±0.48	108.72±0.41	0.19
39	108.97±0.24	108.19±0.36	108.91±0.40	108.03±0.36	0.17
40	109.07±0.58	108.31±0.45	108.30±0.24	108.57±0.51	0.35

T1–3, buckthorn seeds were supplemented at the rate of 1, 2 and 3 g/kg of feed respectively.

Table 3. Effect of sea buckthorn seed supplementation on hen day egg production, egg weight, eggshell weight and eggshell thickness of laying hens

Age (week)	Control	T1	T2	T3	*p*-value
Hen day egg production (%)					
36	70.21±0.95	71.93±0.98	73.93±0.89	73.30±1.23	0.24
37	70.24±1.19	70.00±1.19	72.38±1.19	71.76±2.27	0.56
38	68.21±0.68	71.43±0.58	72.47±0.41	72.21±0.89	0.12
39	71.43±0.58	72.86±0.58	75.35±0.68	75.00±1.01	0.32
40	70.24±1.19^{b}	75.00±2.27^{ab}	77.38±1.19^{a}	79.76±2.27^{a}	0.01
Egg weight (g)					
36	59.07±0.24	60.07±0.41	60.62±0.34	61.47±0.51	0.63
37	59.54±0.65	59.64±0.27	60.22±0.57	61.36±0.31	0.106
38	59.56±0.70	59.85±0.23	60.19±0.70	61.18±0.44	0.403
39	58.69±0.56^{b}	59.11±0.61^{b}	60.88±0.50^{a}	61.68±0.35^{a}	0.003
40	58.85±0.46^{b}	59.48±0.58^{b}	60.29±0.34^{ab}	61.10±0.38^{a}	0.03
Eggshell weight (g)					
36	5.07±0.25	4.89±0.24	4.82±0.19	4.72±0.12	0.70
37	4.92±0.21	4.88±0.17	4.72±0.25	4.52±0.17	0.53
38	4.89±0.21	4.77±0.28	4.71±0.16	4.63±0.08	0.83
39	5.04±0.37	4.93±0.27	4.80±0.24	4.56±0.21	0.67
40	5.00±0.44	4.94±0.39	4.63±0.16	4.66±0.23	0.81
Eggshell thickness (mm)					
36	0.49±0.02	0.47±0.01	0.45±0.01	0.45±0.01	0.24
37	0.48±0.01	0.47±0.02	0.47±0.01	0.45±0.02	0.52
38	0.47±0.01	0.47±0.01	0.46±0.02	0.45±0.02	0.80
39	0.49±0.02	0.48±0.02	0.46±0.01	0.46±0.01	0.53
40	0.48±0.03	0.47±0.01	0.45±0.02	0.45±0.02	0.37

Means in the same column with different superscripts are significantly different ($p<0.05$).
T1–3, buckthorn seeds were supplemented at the rate of 1, 2 and 3 g/kg of feed respectively.

weight and egg shell thickness are affected by calcium level in the diet. Biswas et al. (2010) reported that the calcium level in sea buckthorn seed is quite low and this may be a possible reason for non-significant effect of sea buckthorn seed supplementation on egg shell thickness.

Results of this study show that egg weight was significantly higher in sea buckthorn seed supplementation. Higher weight was recorded T3, which was fed with higher level of sea buckthorn seed. Sohail et al. (2003) reported that egg weight is positively correlated with protein content in the feed. Sea buckthorn seed consist of 26.4% protein and 10.2% carbohydrate which may be a possible reason for high egg weight in the treated groups (Zhong et al., 2006). The increased egg weight in treated groups may also be due to increased yolk and albumen weight in the present study.

Significantly lower egg yolk cholesterol was recorded in supplemented group as compared to all other groups. Sea buckthorn seed oil is rich source of phytosterols primarily comprised of β-sitosterol (97%) (Cenkowski et al., 2006) and these dietary plants sterols have shown anticholesterolemic action in chicken (Khan et al., 2012a). This action is considered to be due to formation of a non-absorbable complex of cholesterol and phytosterols at the absorption site of intestine (Davis, 1955). Phytosterols also blocks absorption of cholesterol by co-precipitation and crystallization (Khan et al., 2012b). Moreover, phytosterols have low hydro dissolvability than cholesterol so it loses the cholesterol from digestive tract micelles and

Table 4. Effect of sea buckthorn seed supplementation on egg yolk weight, albumin weight, albumin height and Haugh unit of laying hens

Age (wk)	SBT-0	T1	T2	T3	*p*-value
Egg yolk weight (g/egg)					
36	15.38±0.33	15.92±0.24	16.35±0.21	17.09±0.23	0.42
37	15.59±0.39	16.21±0.35	16.68±0.18	17.32±0.33	0.17
38	15.37±0.32	15.93±0.12	16.66±0.06	16.86±0.33	0.52
39	15.24±0.17	15.95±0.22	16.20±0.28	16.75±0.33	0.33
40	15.50±0.11^{c}	16.15±0.28^{b}	16.73±0.08^{ab}	16.96±0.25^{a}	0.04
Albumin weight (g/egg)					
36	38.62±0.78	39.24±0.61	39.45±0.54	39.60±0.54	0.70
37	38.02±0.97	38.71±0.17	39.02±0.76	39.15±0.72	0.69
38	38.29±0.90	39.16±0.14	38.89±0.81	39.55±0.20	0.55
39	38.51±0.44	38.11±0.27	39.25±0.46	39.57±0.15	0.12
40	38.40±0.49	38.44±0.67	38.92±0.73	39.30±0.55	0.71
Albumin height (cm)					
36	4.98±0.32	5.16±0.41	5.41±0.31	5.49±0.49	0.78
37	4.84±0.38	5.19±0.65	5.45±0.37	5.58±0.59	0.75
38	4.80±0.27	5.22±0.48	5.25±0.16	5.66±0.70	0.63
39	5.11±0.09	5.26±0.50	5.31±0.51	5.33±0.30	0.97
40	5.06±0.41	5.10±0.42	5.20±0.41	5.35±0.53	0.96
Haugh unit					
36	71.96±0.34	72.52±1.31	73.16±1.30	74.28±3.10	0.82
37	73.32±0.23	73.74±0.75	73.75±1.49	74.13±0.43	0.93
38	75.00±1.73	75.19±1.38	75.90±1.67	76.45±1.35	0.90
39	73.85±1.08	73.96±2.16	75.54±1.60	75.63±0.84	0.74
40	75.25±2.01	75.50±1.93	75.75±1.65	76.75±1.25	0.93

Means in the same column with different superscripts are significantly different ($p<0.05$).
T1–3, buckthorn seeds were supplemented at the rate of 1, 2 and 3 g/kg of feed respectively.

Table 5. Effect of sea buckthorn seed supplementation on egg yolk cholesterol

Group	Cholesterol (mg/g of yolk)	Cholesterol (mg/egg)
Control	17.54±0.69^{a}	282.85±11.60^{a}
T1	16.05 ±0.83^{b}	255.59±14.30^{b}
T2	15.15±0.65^{bc}	243.91±9.54^{bc}
T3	14.45±0.42^{c}	232.18±5.23^{c}
p-value	0.001	0.001

Means in the same column with different superscripts are significantly different ($p<0.05$).
T1–3, buckthorn seeds were supplemented at the rate of 1, 2 and 3 g/kg of feed respectively.

this action binds cholesterol absorption at intestinal site (Khan et al., 2012c). Phytosterols also helps to lower hepatic cholesterol formation by decreasing enzyme activity such as 3-hydroxy-3methyle glutaryl co enzyme A (HMGCoA) reductase that helps to control rate of cholesterol formation in the liver (Khan et al., 2012d) and delay of ACAT (Acyl-coenzyme A:cholesterol acyltransferase) activity in HepG2 cells (Ma et al., 2015). Krejcarova1 et al. (2015) reported that supplementation of sea buckthorn seed has the potential to reduce serum cholesterol level, which may also be a probable reason for the lower egg cholesterol.

Conclusion

From the results of the present study, it was concluded that sea buckthorn seeds supplementation at the rate of 2 and 3 g/kg improved egg quality and cholesterol in Rhode Island Red×Fayoumi layers.

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