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Effect of supplementing herbal feed mixture in combination with linseed oil in the diet of white leghorn layers on egg quality parameters

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Abstract
The study was conducted to evaluate the effects of dietary supplementation of a mixture of herbal feed additives along with various levels of linseed oil on egg quality parameters in White Leghorn layers. A feeding trial was conducted for a period of 12 weeks on 120 numbers of 28-weeks old Leghorn layers and were randomly distributed into four treatment groups *i.e*. control T_1: fed basal diet; T_2: fed basal diet incorporated with 1 percent herbal feed mixture along with 1.5 percent linseed oil; T_3: fed basal diet incorporated with 1 percent herbal feed mixture along with 2 percent linseed oil and T_4: basal diet incorporated with 1 percent herbal feed mixture along with 2.5 percent linseed oil. Results showed no significant effect on egg shell quality parameters in terms of shape index, shell weight, shell weight percentage and shell thickness among treatment groups. Significantly (p<0.05) higher albumen height (mm) was observed in groups T_2 (7.07±0.14), T_3 (7.19±0.13) and T_4 (7.26±0.16) compared to group T_1 (6.54±0.13). Significantly (p<0.05) higher albumen weight (gm) was observed in groups T_2 (38.76±0.43) and T_3 (37.64±0.54) compared to group T_1 (34.44±0.48). Significantly (p<0.05) higher haugh unit was observed in groups T_3 (84.89±0.61) and T_4 (85.64±0.67) compared to group T_1 (81.16±0.70). The egg yolk weight, yolk index and yolk colour did not differ significantly among treatment groups. However, egg yolk cholesterol (mg/gm) was significantly (p<0.05) reduced in groups T_3 (12.48±0.29) and T_4 (12.45±0.61) compared to group T_1 (14.53±0.28). The egg yolk triglycerides (mg/g) also reduced significantly (p<0.05) in group T_3 (172.73±2.19) and group T_4 (169.25±1.56) compared to other two groups. In fatty acid profile *i.e*. linoleic, linolenic and arachidonic acid significantly (p<0.05) improved in groups T_2, T_3 and T_4 compared to group T_1. From the results obtained from the study it can be concluded that dietary supplementation of 1 percent herbal feed mixture in combination with 2 percent linseed oil improved egg quality with low cholesterol and triglycerides and improved egg fatty acid profile in White Leghorn laying chickens.

Keywords: Herbal feed additive, linseed oil, white leghorn layers, egg quality

Introduction
The Poultry production becomes the fastest growing, most dynamic and one of the most profitable segment in the animal husbandry sector in India. In poultry industries feed cost consist of 70-75 percent of total cost of production, thus minimizing the cost of feed has a significant and beneficial impact on poultry production. As a results, various feed additives are added in ration of poultry for improvement of production performance in terms of growth rate, egg production and quality of eggs. Feed additives such as antibiotic growth promoter causes antimicrobial resistance in humans, as a results use of antibiotic growth promoter is not preferable and banned in many countries. Thus considerable attention has recently been paid to herbal feed additives as an alternative to traditional antibacterial feed additives.

Black cumin (*Nigella sativa*), garlic (*Allium sativum*) and Turmeric (*Curcuma longa*) are herbal feed additives used in poultry ration to improve the performance due to presence of several bioactive molecules/ phytochemicals such as thymoquinone, thymol, alliine, ajoene, diallylpolysulfides, curcumin, curcuminoids etc. (Amagase *et al*., 2001) [39, 1]. These phytochemicals have anti-bacterial, antifungal, antiparasitic, anti-inflammatory, antioxidant properties as well as hypocholesterolemic, appetite stimulant and digestive enzyme secretion properties. Linseed oil or flaxseed oil, is a colourless to yellowish oil obtained from the flax plant's (*Linum usitatissimum*) dried mature seeds. Flaxseed is one of the most concentrated PUFA sources available for poultry in natural feedstuff (Caston and Leeson, 1990; Jiang *et al*., 1991) [9, 21], because it contains high levels of Linolenic acid (an omega-3 fatty acid) (Genser, 1994) [16].

When hens are fed with this oil, some of alpha-linolenic acid breaks into two desirable fatty acids which makes their eggs excellent source of EPA and DHA. Consuming omega-3 fatty acids has many beneficial effect on health include reducing heart disease, reducing circulating cholesterol level and reducing inflammation in humans (Klatt. 1986) [24]. Thus keeping in view the benefits of black cumin, garlic, turmeric and linseed oil, the present study was conducted to investigate the effect of supplementing herbal feed mixture (black cumin, garlic and turmeric in equal proportion) in combination with various levels of linseed oil on egg quality parameters in white leghorn layers.

Materials and Methods

The study was conducted at IPF, G.B.P.U.A.T., Pantnagar, Uttrakhand, India. The experiment was conducted on White Leghorn layers of 28 weeks of age for a period of 12 weeks (28-40 weeks) and randomly divided into four treatments groups. Each treatment group had thirty numbers of hens which again divided into three replicates had ten hens each. Four treatments include control T_1: fed basal diet; T_2: fed basal diet incorporated with 1 percent herbal feed mixture along with 1.5 percent linseed oil; T3: fed basal diet incorporated with 1 percent herbal feed mixture along with 2 percent linseed oil and T4: basal diet incorporated with 1 percent herbal feed mixture along with 2.5 percent linseed oil. All the managemental practices were done according to the standard protocol. Hens were kept in individually in cages (California battery type) which fitted with feeder and Waterer. Basal diets were prepared according to the standard of BIS (2007) [6].

Egg quality traits

Internal and external egg quality was analysed at the end of feeding trial by randomly selecting 9 eggs randomly (3 eggs from each replicate) from each treatment groups (Novak *et al*., 2004; Karaman *et al*., 2006) [29, 22] which is described below:

Egg weight

The egg weight (gm) was measured on electronic balance.

Shape index

The maximum egg length and maximum egg width was measured by Vernier calliper and shape index was express as ratio of maximum egg width and maximum egg length and expressed as percentage.

Shape index (%) = (Egg width in mm ÷ Egg length in mm) × 100

Shell weight

After gently breaking the eggs by using a spatula, the egg contents were kept in a glass plate and the shell was dried in the oven for 72 hours at 60 °C. The shell weight was measured in grams on electronic balance after proper drying of egg shell. Shell weight percentage was calculated as percentile ratio of shell weight and egg weight.

Shell thickness

The thickness of shell was measured by screw gauge at air cell point, equator and sharp end point and expressed in millimetres.

Albumen height

After shell removal, the egg content was transferred on a plate of plane surface to makes egg immobile. Then albumen height was taken with the help of Spherometer from three different sites of thick albumin and expressed in millimetres.

Albumen weight

The egg albumen weight was measured by subtracting the shell weight and yolk weight from the whole egg weight and expressed in percentage.

Haugh unit

Haugh unit (HU) was measured for egg quality in terms of egg protein and calculated from egg albumen height and egg weight by Raymond Haugh (1937).

Haugh unit= $100 \log (H + 7.57 - 1.7 W^{0.37})$

Where, H= Albumen height (mm); W= Egg weight (gm)

Yolk weight

The yolk weight (gm) was measured on electronic balance after separating the egg yolk from albumen and yolk weight percentage was calculated by dividing yolk weight by whole egg weight and multiplies the value by 100.

Yolk index

The maximum yolk height was taken and the average yolk diameter was taken from different points for calculation of yolk index described by Card and Nesheim, 1972 [8].

Yolk index (%) = (Yolk height in mm ÷ Yolk width in mm) × 100

Yolk colour

The yolk colour was measured by DSM yolk colour fan strip. This colour fan was matched with the yolk colour and this strip had standard colour code from 1 to 15 which stand for extremely pale to dark orange yellow colour according to which the number was given to yolk colour.

Egg cholesterol

Completely separated egg yolks from albumen was taken and yolk lipids were extracted by a method described by Folch *et al.* (1957) [14]. Egg cholesterol was estimated by using Erba diagnostic kit in spectrophotometer as described by Zlatkis *et al.* (1953) [41] and expressed in mg/dl.

Egg triglycerides

Egg triglycerides was estimated by using GPO-Trinder with the help of Erba diagnostic kit in spectrophotometer as described by Fossati and Prencipe (1969) [15] and McGowan *et al*. (1983) [28]. Concentration of triglycerides was express in mg/dl.

Egg fatty acid profile

Three eggs from each treatment group were selected and yolks were separated and the separation of fat from egg yolk was done by the standard method (Angelo *et al.*, 1987) [2]. Preparation of methyl ester was done by the standard method (Luddy *et al.*, 1968) [26] and fractionation of methyl ester was done by using gas chromatography.

Results and Discussion

Egg quality parameters

Egg shell quality

Average values of egg shell quality in terms of shape index,

shell weight, shell weight percentage and shell thickness of the present study are presented in Table 1.

The average shape index for groups T_1, T_2, T_3 and T_4 were 77.15±1.09, 76.38±1.45, 74.52±0.27 and 74.13±0.61 percent, respectively and did not differ significantly (p>0.05) among the treatment groups. The average values of shell weight for groups T_1, T_2, T_3 and T_4 were 5.98±0.08, 5.96±0.11, 6.02±0.13 and 5.74±0.37 gm, respectively. The average shell percentage in groups T_1, T_2, T_3 and T_4 were 10.20±0.24, 9.34±0.37, 9.83±0.14 and 9.67±0.43 percent, respectively. The average shell thickness (0.01 mm) in different groups T_1, T_2, T_3 and T_4 were 36.89±0.86, 35.65±0.60, 36.76±0.89 and 35.82±0.64, respectively.

The average value of shell weight, shell weight percentage and shell thickness did not differ significantly (p>0.05) among the treatment groups. The present findings are in agreement with the findings of Singh (2016) [38] who reported non-significant effect of black cumin, garlic, and turmeric powder supplementation in the layer ration. Promila *et al.* (2016) [35] also reported non-significant effect on shape index due to linseed oil supplementation which is in agreement with this study.

Egg albumin quality

The average values of albumin quality in terms of albumin height, albumin weight, albumin percentage and haugh unit are presented in Table 2. Statistically significant (p<0.05) difference was observed in albumen height, albumin weight and haugh unit among the groups. Significantly higher albumin height (mm) was observed in groups T_2 (7.07±0.14), T_3 (7.19±0.13) and T_4 (7.26±0.16) compared to group T_1 (6.54±0.13). Whereas, significantly higher albumin weight was observed in groups T_2 (38.76±0.43) and T_3 (37.64±0.54) compared to the group T_1 (34.44±0.48). Statistically (p<0.05) higher haugh unit was observed in groups T_3 (84.89±0.61) and T_4 (85.64±0.67) compared to control group T_1 (81.16±0.70). The findings are in accordance with the study of Singh (2016) [38] who reported significant (p<0.05) effect on albumin height, albumin weight and haugh unit due to black cumin, garlic and turmeric incorporation in the layer ration. Significant improvement in haugh unit due to black cumin supplementation was also reported by Khan *et al.* (2013) [23] and Chongtham *et al.* (2015) [11] in laying hens. Canogullari *et al.* (2010) [7] and Mahmoud *et al*. (2010) [27] reported significant (p<0.05) effect on albumin height and haugh unit due to garlic supplementation in layer ration. Hazim *et al*. (2010) [18] reported addition of linseed in the layer ration resulted in significant increase (p<0.05) in albumin height, albumin weight, albumin percentage and haugh unit which is in agreement with this present finding. Improvement in albumen height and Haugh unit might be attributed to antioxidant property of turmeric powder (Tilak *et al.*, 2004) [40]. Garlic is rich in selenium and amino acids known as selenomethinine (Arnaulta and Augreb, 2006) [4]. In poultry, inactive form *i.e*. selenomethionine is converted to active form selenocysteine which might improve the albumen quality (Edens *et al.*, 2002) [13].

Egg yolk quality

The average values of egg yolk quality in terms of yolk weight, yolk percentage, yolk index and yolk colour are presented in Table 3. No significant (p>0.05) effect was observed due to dietary supplementation of herbal feed mixture and linseed oil on yolk weight, yolk percentage, yolk index and yolk colour among treatment groups. In agreement with the study, Singh (2016) [38] did not find any significant effect of black cumin, garlic and turmeric powder supplementation on yolk weight, yolk percentage and yolk index. Olobatoke and Mulugeta (2011) [31] and Deko *et al.* (2018) [12] reported non-significant (p>0.05) effect of garlic powder supplementation on yolk weight, yolk percentage and yolk colour. Phuoc *et al.* (2019) [34] and Lagana *et al.* (2019) did not find any significant effect of turmeric supplementation on yolk index and yolk colour which is similar to the present findings.

Egg yolk cholesterol and triglycerides

Average values of yolk cholesterol and triglycerides due to incorporation of herbal feed additive mixture with various levels of linseed oil in layer diet are presented in Table 3. The average yolk cholesterol differed significantly among different treatment groups. Significantly (p<0.05) lower yolk cholesterol (mg/g) was observed in groups T_3 (12.48±0.29) and T_4 (12.45±0.61) compared to group T_1 (14.53±0.28). Egg yolk triglycerides (mg/g) of the treatment groups T_2 (186.81±2.29), T_3 (172.73±2.19) and T_4 (169.25±1.56) supplemented with mixture of herbal feed and linseed oil were significantly (p<0.05) lower as compared with control group T_1 (202.65±2.45). These findings are in concordance with Singh (2016) [38], who reported significant (p<0.05) reduction in yolk cholesterol and yolk triglycerides due to supplementation of black cumin, garlic and turmeric in various combinations in layers. Similarly, Islam *et al.*, 2011 [20] and Hossain *et al.*, 2016 [19] reported significant (p<0.05) reduction in cholesterol and triglycerides contents of egg due to black cumin supplementation in layers. Ayed *et al.*, 2018 [5] and Omer *et al.*, 2019 [33] also reported significant (p<0.05) reduction in yolk cholesterol concentration due to garlic supplementation in layer ration. Decreased level of egg cholesterol and egg triglycerides might be due to supplementation of garlic, black cumin seed and turmeric rhizome powder, as they have hypolipidemic, and hypocholesterolaemic action due to phytochemicals which act as an active principles present in herbs. These herbal mixture depresses the enzymatic action of glucose-6-phosphatase dehydrogenase, malic enzyme and fatty acid synthase which helps in reducing the levels of lipids and cholesterols (Chi *et al.*, 1982; Qureshi *et al.*, 1983) [10, 36].

Fatty acid profile

Average values of fatty acid profile (%) of egg due to incorporation of herbal feed mixture along with various levels of linseed oil in the layer diet have presented in Table 4. The fatty acid profile of the eggs which including linoleic acid, linolenic acid and arachidonic acid, showed significant differences among treatment groups.

Significantly (p<0.05) higher linoleic acid was observed in groups T_3 (14.55±0.28) and T_4 (14.67±0.12) followed by group T_2 (13.89±0.11) compared to group T_1 (12.15±0.16). Similarly, significantly (p<0.05) highest linolenic acid was observed in group T_4 (5.31±0.21) followed by group T_3 (4.07±0.05) and group T_2 (3.15±0.40) and significantly (p<0.05) lowest linolenic acid was observed in group T_1 (1.55±0.13). In case of arachidonic acid, significantly (p<0.05) higher content was observed in groups T_2 (0.91±0.01), T_3 (0.93±0.04) and T_4 (0.97±0.07) compared to group T_1 (0.71±0.04). These findings are in concordance with Promila *et al.* (2016) [35] who reported significant (p<0.05)

increased in the level of linoleic acid, linolenic acid and arachidonic acid with the increasing level of linseed oil in the diet compared to control group. Similarly, Ansari *et al*., (2006) [3], Oliveira *et al*., (2010) [30] and Omar *et al*., (2014) [32] reported significant improvement in PUFA concentration and decreases saturated fatty acid in egg due to linseed oil supplementation in layer ration. In plant origin linseed oil is the richest source of linolenic acid which could be used as a feed for poultry (Scheideler *et al*., 1998) [37]. Increased fatty acid profiles in terms of linoleic acid, linolenic acid and arachidonic acid might be due to incorporation of linseed oil in the layer ration.

Table 1: Average values of shape index and shell quality of White Leghorn layers fed diet supplemented with herbal feed mixture in combination with linseed oil

Parameters	Groups				SEm	CD at 5%
	T_1	T_2	T_3	T_4		
	Basal diet (control)	1% herbal feed mixture + 1.5% linseed oil	1% herbal feed mixture + 2.0% linseed oil	1% herbal feed mixture + 2.5% linseed oil		
Shape index	77.15±1.09	76.38±1.45	74.52±0.27	74.13±0.61	0.969	3.162
Shell weight (gm)	5.98±0.08	5.96±0.11	6.02±0.13	5.74±0.37	0.21	0.686
Shell weight (%)	10.20±0.24	9.34±0.37	9.83±0.14	9.67±0.43	0.319	1.041
Shell thickness (0.01 mm)	36.89±0.86	35.65±0.60	36.76±0.89	35.82±0.64	0.665	2.171

^{a,b,c} Mean values bearing different superscripts within a row differ significantly from each other, $^{*}p<0.05$

Table 2: Average values of albumen quality and high unit of White Leghorn layers fed diet supplemented with herbal feed mixture in combination with linseed oil

Parameters	Groups				SEm	CD at 5%
	T_1	T_2	T_3	T_4		
	Basal diet (control)	1% herbal feed mixture + 1.5% linseed oil	1% herbal feed mixture + 2.0% linseed oil	1% herbal feed mixture + 2.5% linseed oil		
Albumen height* (mm)	6.54^{a}±0.13	7.07^{b}±0.14	7.19^{b}±0.13	7.26^{b}±0.16	0.143	0.468
Albumen weight* (gm)	34.44^{a}±0.48	38.76^{c}±0.43	37.64^{bc}±0.54	35.97^{ab}±0.74	0.565	1.845
Albumen weight (%)	61.35^{a}±0.52	63.15^{b}±0.70	62.79^{ab}±0.28	61.51^{ab}±0.43	0.511	1.668
Haugh unit*	81.16^{a}±0.70	83.28^{ab}±0.66	84.89^{bc}±0.61	85.64^{c}±0.67	0.667	2.175

^{a,b,c} Mean values bearing different superscripts within a row differ significantly from each other, $^{*}p<0.05$

Table 3: Average values of egg yolk quality of White Leghorn layers fed diet supplemented with herbal feed mixture in combination with linseed oil

Parameters	Groups				SEm	CD at 5%
	T_1	T_2	T_3	T_4		
	Basal diet (control)	1% herbal feed mixture + 1.5% linseed oil	1% herbal feed mixture + 2.0% linseed oil	1% herbal feed mixture + 2.5% linseed oil		
Yolk weight (gm)	16.41±0.19	17.10±0.94	16.37±0.29	16.42±0.38	0.539	1.758
Yolk weight (%)	28.44±0.47	27.49±0.56	27.37±0.85	28.78±0.53	0.624	2.037
Yolk index (%)	44.68±0.58	44.05±0.16	44.14±0.35	44.54±1.22	0.704	2.297
Yolk colour	5.01±0.57	7.16±0.60	7.03±0.97	7.18±0.61	0.707	2.307
Yolk Cholesterol* (mg/g)	14.53^{b}±0.28	13.61^{ab}±0.33	12.48^{a}±0.29	12.45^{a}±0.61	0.403	1.315
Yolk Triglycerides* (mg/g)	202.65^{c}±2.45	186.81^{b}±2.29	172.73^{a}±2.19	169.25^{a}±1.56	2.151	7.015

^{a,b,c} Mean values bearing different superscripts within a row differ significantly from each other, $^{*}p<0.05$

Table 4: Fatty acid profile (%) of eggs of White Leghorn layers fed diet supplemented with herbal feed mixture in combination with linseed oil

Parameters	Groups				SE_m	P-value	CD at 5%
	T_1	T_2	T_3	T_4			
	Basal diet (control)	1% herbal feed mixture + 1.5% linseed oil	1% herbal feed mixture + 2.0% linseed oil	1% herbal feed mixture + 2.5% linseed oil			
Linoleic acid	12.15^{a}±0.16	13.89^{b}±0.11	14.55^{c}±0.28	14.67^{c}±0.12	0.184	0.001	0.601
Linolenic acid	1.55^{a}±0.13	3.15^{b}±0.40	4.07^{c}±0.05	5.31^{d}±0.21	0.241	0.001	0.787
Arachidonic acid	0.71^{a}±0.04	0.91^{b}±0.01	0.93^{b}±0.04	0.97^{b}±0.07	0.031	0.002	0.103

^{a,b,c} Mean values bearing different superscripts within a row differ significantly from each other, $^{*}p<0.05$

Conclusion

From the above findings, it could be concluded that the dietary supplementation of herbal feed mixture (garlic, black cumin and turmeric) along with 2% linseed oil on white leghorn layers had beneficial effects on egg quality parameters such as albumen height, albumen weight, high unit, yolk cholesterol, yolk triglycerides and egg fatty acid profile. Therefore, considering all these beneficial effect, black cumin, garlic, turmeric and linseed oil can be used in layer's ration to get maximized results.

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