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Effect of Dietary Supplementation of Herbal Feed Additives in Combination with Linseed Oil on Nutrient Utilization and Haemato-biochemical Parameters in White Leghorn Layers

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ABSTRACT

The study was carried out to discern the effects of dietary incorporation of herbal feed additives (black cumin, garlic and turmeric) in combination with linseed oil on nutrient utilization and haemato-biochemical parameters in White Leghorn layers for a period of 12 weeks. Birds were randomly distributed into four groups *i.e.* control T_1: fed basal diet; T_2, T_3 and T_4: fed basal diet incorporated with 1% herbal feed additives and 1, 1.5 and 2.5% linseed oil, respectively. Results showed no significant effect among groups in terms of DM and OM utilization. However, CP utilization (%) was found to be significantly highest in T_2 (87.74 ± ± 1.22) followed by T_3 (86.65 ± 0.97) compared to T_1 (82.42 ± 0.74). The EE utilization (%) also improved significantly in T_2 (90.59 ± 1.02), T_3 (91.12 ± 0.58) and T_4 (90.77 ± 0.33) compared to the T_1 (86.92 ± 0.80). The average values of haematological parameters in terms of haemoglobin, PCV, TEC and TLC did not differ significantly among groups. Serum glucose concentration (mg/dl) was significantly lower in T_2 (189.22 ± 2.75), T_3 (186.40 ± 4.19) and T_4 (186.80 ± 1.77) compared to T_1 (204.03 ± 3.50). The average values of serum total protein did not differ significantly among groups. Significantly lower serum cholesterol (mg/dl) was observed in T_3 (115.23 ± 3.37) and T_4 (111.28 ± 5.25) compared T_1 (142.83 ± 4.53) and T_2 (133.33 ± 3.93). Significantly lowest serum triglycerides (mg/dl) were observed in T_4 (212.86 ± 3.50) and T_3 (219.02 ± 4.96) followed by T_2 (230.30 ± 5.76) and highest observed in T_1 (248.96 ± 5.31). Therefore, considering all these beneficial effect, 1% herbal feed additives along with 2% linseed oil could be used in layer's ration to get maximized results.

HIGHLIGHTS

- Dietary incorporation of additives significantly improves CP and EE utilization in layers.
- Significantly reduced serum glucose, serum cholesterol and serum triglycerides in layers.

Keywords: Herbal feed additives, Linseed oil, Nutrient utilization, White Leghorn layers

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

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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. (McGrowder *et al*., 2020; Wani*et al*., 2022). 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 because it contains high levels of linolenic acid (an omega-3 fatty acid) (Alagawany *et al*., 2019; Irawan *et al*., 2022). 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 (Tortosa-Caparros *et al*., 2017). 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, GBPUAT, 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). 120 numbers of White Leghorn laying hens were randomly distributed into four treatments groups with 30 hens per treatment having three replicates in each. Four treatments include T_1 (control): fed basal diet; T_2: fed basal diet incorporation of 1% herbal feed additives (black cumin, garlic and turmeric powder; 1:1:1 ratio) and 1.5% linseed oil; T_3: fed basal diet incorporation of 1% herbal feed additives and 2% linseed oil and T_4: fed basal diet incorporation of 1% herbal feed additives along with 2.5% linseed oil. Basal diets were prepared as per BIS (2007) specification and proximate analysis were performed as per standard methods (AOAC, 2012).

To investigate the nutrient utilization, metabolic trial was conducted for a period of 7 days, on two birds from each replicate were randomly selected and housed in metabolic cages. During collection period, weighed amount of feed i.e. 150 gm was offered daily to layers and residue left was weighed in the next morning at the same time. Simultaneously, faecal trays covered with polythene sheets were placed for collection of excreta and weighted individually daily. Excreta were dried in hot air oven at 70º C for a period of 48 hours and polled for dry matter estimation and thereafter, ground and stored for further analysis for proximate principles (AOAC, 2012). Representative sample of feed was drawn for chemical analysis. Fresh excreta sample were also preserved in 5% sulphuric acid (V/V) for nitrogen estimation. Nutrient digestibility was calculated by applying the following formula:

$$\text{Nutrient Digestibility (\%)} = \frac{\text{Nutrient intake in feed} - \text{Nutrient loss in faeces}}{\text{Nutrient intake in feed}} \times 100$$

The blood samples were collected at the end of feeding trial by randomly selecting six birds from each treatment groups. 3 ml of blood samples were collected aseptically from wing vain with sterile syringe and needle. Half of the collected blood was transferred into EDTA vial for analysis of haematological parameters and rest of blood was transferred into serum vial (without anticoagulant) for serum separation. For serum separation sample vials were allowed to stand for three to four hours at room temperature in slanting position and centrifuging at 3000 rpm for 10-15 minute. Then the serum was collected in Eppendorf tube and was kept at -20ºC in a deep freeze with date and sample number for further analysis of biochemical parameters.

Haematological parameters like haemoglobin, Packed cell volume (PCV), Total erythrocyte count (TEC) and Total leukocyte count (TLC) were estimated by standard procedure. Haemoglobin (g/dl) were estimated by the

method described by Higgins *et al*., 2008. To estimate PCV, micro-haematocrit method was used as described by Sharma and Singh (2000). The total erythrocyte count (TEC) and total leucocytes count (TLC) were performed with Neubauer's counting chamber as described by Jain, 1986. Serum biochemical parameters such as serum glucose, serum cholesterol, serum total protein, serum albumin, serum globulin, serum calcium and serum phosphorus were estimated as per the standard procedure by using Erba diagnostic kit.

STATISTICAL METHODS

The experimental data obtained in this study were analysed statistically by using SPSS package.

RESULTS AND DISCUSSION

Nutrient utilization

The data on average values of nutrient utilization of White Leghorn layers during the metabolic trial period, in terms of dry matter, crude protein, ether extract and organic matter fed diet incorporated with mixture of black cumin, garlic and turmeric in combination with different levels of linseed oil of different treatment groups are presented in Table 1. The DM utilization in T_1, T_2, T_3 and T_4 groups were 70.03 ± 0.96, 72.67 ± 1.00, 69.01 ± 1.28 and 70.00 ± 1.11 per cent, respectively. There was no significant difference (P>0.05) in dry matter utilization among treatment groups. The crude protein utilization (%) was found to be significantly (P<0.05) highest in T_2 group (87.74 ± 1.22) followed by T_3 (86.65 ± 0.97) group compared to T_1 (82.42 ± 0.74) group. The ether extract utilization (%) improved significantly (P<0.05) in T_2 (90.59 ± 1.02), T_3 (91.12 ± 0.58) and T_4 (90.77 ± 0.33) groups compared to the T_1 (86.92 ± 0.80) group. The organic matter utilization in T_1, T_2, T_3 and T_4 groups were 75.08 ± 0.81, 77.94 ± 0.84, 75.26 ± 1.18 and 75.09 ± 0.64 per cent, respectively and did not show any significant (P>0.05) difference among treatment groups. Improvement in nutrient utilization in terms of CP utilization and EE utilization might be due to the antibacterial and antioxidant property of herbal feed additives which might have helped in reduction of harmful pathogens and improved beneficial bacterial in the digestive tract and thus improved the digestibility of nutrients (Issa and Omar, 2012). Improved EE utilization might be due to the increased linseed oil content in the diet of layers other than control group. These results are in agreement with the findings of Singh (2016) who reported significant (P<0.05) improvement in crude protein utilization and ether extract utilization due to various combinations of black cumin, garlic, and turmeric supplementation in the diet of layers. Aswal *et al.* (2017) also reported significant improvement (P<0.05) in crude protein and ether extract retention due to garlic powder supplementation in the diet of layers. Similarly, Promila *et al.* (2017) reported significant (P<0.05) improvement in nitrogen retention in laying hen

Table 1: Average values of nutrient utilization (%) of White Leghorn layers fed diet supplemented with herbal feed additives in combination with linseed oil

Parameters	Treatments/Groups				SE_m	P-value	CD at 5%
	T_1	T_2	T_3	T_4			
	Basal diet (control)	Diet with 1% herbal feed additives + 1.5% linseed oil	Diet with 1% herbal feed additives + 2.0% linseed oil	Diet with 1% herbal feed additives + 2.5% linseed oil			
Dry matter utilization (%)	70.03 ± 0.96	72.67 ± 1.00	69.01 ± 1.28	70.00 ± 1.11	1.099	0.188	Ns
Crude protein utilization* (%)	82.42^{a} ± 0.74	87.74^{c} ± 1.22	86.65^{bc} ± 0.97	84.25^{ab} ± 0.72	0.937	0.015	3.058
Ether extract utilization* (%)	86.92^{a} ± 0.80	90.59^{b} ± 1.02	91.12^{b} ± 0.58	90.77^{b} ± 0.34	0.735	0.012	2.399
Organic matter utilization (%)	75.08 ± 0.81	77.94 ± 0.84	75.26 ± 1.18	75.09 ± 0.64	0.895	0.138	Ns

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

due to linseed oil supplementation in the layer ration. In contrary, Singh (2016) reported significant improvement (P<0.05) in dry matter utilization. Aswal *et al*. (2017) reported significant (P<0.05) improvement in organic matter retention due to garlic powder incorporation in the diet of layers.

Haematological parameters

The average values of haematological parameters in terms of haemoglobin, packed cell volume, total erythrocytes count and total leucocytes count have been presented in Table 2. The average haemoglobin content of layers for groups T_1, T_2, T_3 and T_4 were 10.72 ± 0.21, 11.17 ± 0.46, 10.44 ± 0.31, 10.89 ± 0.45 g/dl, respectively. Average values of packed cell volume for groups T_1, T_2, T_3 and T_4 were 30.83 ± 0.46, 32.40 ± 0.33, 31.36 ± 0.43 and 32.19 ± 0.60 per cent, respectively. Total erythrocytes count for various groups T_1, T_2, T_3 and T_4 were 2.96 ± 0.03, 3.16 ± 0.13, 3.08 ± 0.07 and 3.10 ± 0.09 × 10^6/µl, respectively. The total leukocytes count in groups T_1, T_2, T_3 and T_4 were 28.57 ± 0.75, 28.25 ± 0.55, 28.65 ± 0.51 and 27.54 ± 0.30 ×10^3/µl, respectively. The average values of haematological parameters in terms of haemoglobin, packed cell volume, total erythrocytes count and total leucocytes count did not differ significantly (P>0.05) among treatment groups. These results are in agreement with the findings of Singh (2016) who reported no significant (P>0.05) effect of black cumin, garlic and turmeric supplementation on packed cell volume and Promila *et al.* (2017) who reported non-significant effect on total erythrocytes count due to linseed oil supplementation.

Serum biochemical parameters

The average values of serum biochemical parameters in terms of serum glucose, serum total protein, serum albumin, serum globulin, serum cholesterol, serum triglycerides, serum calcium and phosphorus have been presented in Table 3. The average values of serum glucose for treatment groups T_1, T_2, T_3 and T_4 were 204.03 ± *3.50*, 189.22 ± *2.75*, 186.40 ± 4.19 and 186.80 ± 1.77 mg/dl, respectively. The average values of serum glucose concentration differed significantly (P<0.05) and decreased with dietary supplementation of herbal feed additive mixture in combination with linseed oil compare to control group. Serum glucose concentration was significantly lower in treatment group T_2, T_3 and T_4 compared to T_1. There was no significant difference among T_2, T_3 and T_4 groups. Decreased in serum glucose concentration might be due to the phytochemicals in the herbal feed additives such as allicin, s-allyl cysteine sulfoxide, allyl propyl etc. which increased the insulin level in blood by blocking liver inactivation of insulin and thus reduced in serum glucose (Banerjee and Maulik, 2002; Sher *et al.*, 2012).The present findings are concordance with those of Singh (2016), who reported significant reduction in serum glucose content due to supplementation of black cumin, garlic, turmeric powder in layer ration in various combinations. Aswal *et al.* (2017) also reported reduction in glucose concentration due to garlic supplementation in laying hens. In contrary, Canogullari *et al.* (2009) reported non-significant effect of garlic supplementation on glucose concentration.

The average values of serum total protein in White Leghorn layers were 4.92 ± 0.29, 5.85 ± 0.30, 5.11 ± 0.07 and 5.19 ± 0.17 g/dl for groups T_1, T_2, T_3 and T_4, respectively. The

Table 2: Average values of haematological parameters of White Leghorn layers fed diet supplemented with herbal feed mixture in combination with linseed oil

Parameters	Treatments/Groups				SE_m	P- value
	T_1	T_2	T_3	T_4		
	Basal diet (control)	Diet with 1% herbal feed additives + 1.5% linseed oil	Diet with 1% herbal feed additives + 2.0% linseed oil	Diet with 1% herbal feed additives + 2.5% linseed oil		
Haemoglobin (g/dl)	10.72 ± 0.21	11.17 ± 0.46	10.44 ± 0.31	10.89 ± 0.45	0.379	0.606
Packed cell volume (%)	30.83 ± 0.46	32.40 ± 0.33	31.36 ± 0.43	32.19 ± 0.60	0.470	0.143
Total erythrocytes count (×10^6 / µl)	2.96 ± 0.03	3.16 ± 0.13	3.08 ± 0.07	3.10 ± 0.09	0.093	0.500
Total leukocytes count (×10^3 / µl)	28.57 ± 0.75	28.25 ± 0.55	28.65 ± 0.51	27.54 ± 0.30	0.556	0.516

Table 3: Average values of serum biochemical constituents in White Leghorn layers fed diet supplemented with herbal feed mixture in combination with linseed oil in various combination

Parameters	Treatments/Groups				SE_m	P- value	CD at 5%
	T_1	T_2	T_3	T_4			
	Basal diet (control)	Diet with 1% herbal feed additives + 1.5% linseed oil	Diet with 1% herbal feed additives +2.0% linseed oil	Diet with 1% herbal feed additives +2.5% linseed oil			
Glucose* (mg/dl)	$204.03^{b} \pm 3.50$	$189.22^{a} \pm 2.75$	$186.40^{a} \pm 4.19$	$186.80^{a} \pm 1.77$	3.187	0.013	10.394
Total protein (g/dl)	4.92 ± 0.29	5.85 ± 0.30	5.11 ± 0.07	5.19 ± 0.17	0.228	0.085	Ns
Albumin (g/dl)	1.65 ± 0.26	1.86 ± 0.27	1.45 ± 0.19	1.44 ± 0.27	0.254	0.624	Ns
Globulin* (g/dl)	$3.26^{a} \pm 0.02$	$3.99^{c} \pm 0.08$	$3.66^{b} \pm 0.11$	$3.75^{bc} \pm 0.10$	0.089	0.003	0.291
Cholesterol* (mg/dl)	$142.83^{b} \pm 4.53$	$133.33^{b} \pm 3.93$	$115.23^{a} \pm 3.37$	$111.28^{a} \pm 5.25$	4.333	0.003	14.13
Triglycerides*(mg/dl)	$248.96^{c} \pm 5.31$	$230.30^{b} \pm 5.76$	$219.02^{ab} \pm 4.96$	$212.86^{a} \pm 3.50$	4.958	0.004	16.17
Calcium (mg/dl)	20.23 ± 0.80	21.12 ± 0.84	20.31 ± 1.02	20.33 ± 0.76	0.866	0.872	Ns
Phosphorus (mg/dl)	4.90 ± 0.09	4.81 ± 0.09	5.08 ± 0.12	5.10 ± 0.16	0.122	0.325	Ns

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

average values of serum albumin were 1.65 ± 0.26, 1.86 ± 0.27, 1.45 ± 0.19 and 1.44 ± 0.27 g/dl for groups T_1, T_2, T_3 and T_4, respectively. The average values of serum total protein and serum albumin did not differ significantly (P>0.05) among treatment groups. These results are in agreement with the findings of Singh (2016), who reported non-significant (P>0.05) effect of black cumin, garlic, turmeric supplementation in laying ration on serum total protein concentration. Similarly, Yalcin *et al.* (2012) reported that black cumin supplementation did not have any significant effect on serum total protein.

The average serum globulin in groups T_1, T_2, T_3 and T_4 were 3.26 ± 0.02, 3.99 ± 0.08, 3.66 ± 0.11 and 3.75 ± 0.10 g/dl, respectively. Significant (P<0.05) effect of dietary supplementation on serum globulin was observed in T_2, T_3 and T_4 groups compared to T_1 group. The results are in agreement with the findings of Omer *et al.* (2019) who reported significant (P<0.05) effect of garlic supplementation on serum globulin in layers. Similarly, Yassein *et al.* (2015) reported significant (P<0.05) effect on serum globulin due to flaxseed supplementation in layer ration.

The average concentration of serum cholesterol for treatment groups T_1, T_2, T_3 and T_4 were 142.83 ± 4.53, 133.33 ± 3.93, 115.23 ± 3.37 and 111.28 ± 5.25 mg/dl, respectively. The average serum cholesterol differed significantly (P<0.05) among treatment groups. Significantly lower serum cholesterol was observed in groups T_3 and T_4 compared to other two group *i.e.* T_1 and T_2. The average values of serum triglycerides concentration for treatment groups T_1, T_2, T_3 and T_4 were 248.96 ± 5.31, 230.30 ± 5.76, 219.02 ± 4.96 and 212.86 ± 3.50 mg/dl, respectively. The average serum triglycerides differed significantly (P<0.05) among treatment groups. Significantly lowest serum triglycerides were observed in groups T_4 and T_3 followed by group T_2 and highest observed in group T_1. Decreased level of serum cholesterol and triglycerides might be due to synergistic effect of garlic, black cumin seed and turmeric rhizome powder along with linseed oil as they have hypolipidemic, and hypocholesterolemic 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). Linseed oil also might have hypolipidemic, and hypocholesterolemic effect on birds which leads to reduce in serum cholesterol and serum triglycerides. The present findings are in agreement with the findings of Singh (2016), who reported significant reduction in serum cholesterol and serum triglyceride due to black cumin and garlic supplementation in various combination in layers. Similarly, Yalcin *et al.* (2012) reported significant decreased in serum cholesterol and triglycerides in White

Leghorn layers due to various levels of black cumin seed supplementation in the layer ration. Canogullari *et al.* (2009), Aswal *et al.* (2017) and Omer *et al.* (2019) also reported significant decreased in serum cholesterol concentration due to garlic supplementation in layer ration. Yassein *et al.* (2015) reported significant decreased in serum cholesterol and triglycerides level due to flaxseed supplementation in layer ration.

The average values of serum calcium and phosphorus has been presented in Table 3. Serum calcium concentration was 20.23 ± 0.80, 21.12 ± 0.84, 20.31 ± 1.02 and 20.33 ± 0.76 mg/dl for groups T_1, T_2, T_3 and T_4, respectively and serum phosphorus concentration was 4.90 ± 0.09, 4.81 ± 0.09, 5.08 ± 0.12 and 5.10 ± 0.16 mg/dl for groups T_1, T_2, T_3 and T_4, respectively. These values are within normal range (Undrewood and Suttle, 1999) which indicated that dietary herbal feed additives did not affect normal mineral metabolism in laying hens. These results are consistent with the findings of Singh (2016), who reported no significant effect of black cumin, garlic and turmeric supplementation on serum calcium and phosphorus concentration.

CONCLUSION

From the above findings, it could be concluded that dietary supplementation of herbal feed additives along with 2 per cent linseed oil on white leghorn layers had beneficial effects on nutrient utilization in terms of CP and EE utilization and on blood biochemical parameters such as serum glucose, serum globulin, serum cholesterol and triglycerides. Therefore, considering all these beneficial effect 1 per cent herbal feed additives along with 2 per cent linseed oil could be used in layer's ration to get maximized results.

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