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Influence of designer diet and holy basil (*Ocimum sanctum*) leaves on yolk colour, yolk carotenoid, and immunity status of layers

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ABSTRACT

A biological experiment of six weeks duration, followed by several laboratory investigations was carried out to study the effect of designer layer mash (DLM) containing full fat flaxseed, oil rich sardine fish, holy basil leaf meal (BLM), vitamin E and organic selenium (Sel-plex), on yolk colour, carotenoid and immune status of layers. Both designer diet and BLM had significantly improved the yolk colour and pigment levels. They also exhibited a synergistic effect. The DLM had significantly increased the vitamin E and selenium levels in the egg; but BLM had no such effect. The immune status of the layers, expressed as Immunoglobulin IgY and HI titre, indicated that both designer diet as well as the BLM had significantly improved the IgY levels and HI titre.

Keywords: Designer layer mash, Vitamin E, Yolk colour, Yolk carotenoid

Recently, scientists are incorporating various unconventional feedstuffs and herbs in the hens' feed; with the objective of incorporating their active principles into the egg; so that the egg will become a versatile food cum medicine. Narahari *et al.* (2003) added, 2 g/kg spirulina, 2 g/kg Basil (*Ocimum sanctum*) leaves or both to the designer egg feed, along with a control. They found that carotenoid pigment levels were 24.5, 56.1, 44.8, 65 mcg/g of yolk in the control and the three designer eggs. In this study, designer layer mash, vitamin E and selenium were added to the diet and the impact on yolk colour, carotenoid and immunity status of the layer were studied.

MATERIALS AND METHODS

This is a 2 × 3 factorial experiment consisting of two types of layer feeds namely, standard layer mash (Control) and special designer egg layer mash (DLM); each with three levels of basil leaf meal (BLM), i.e. 0, 1 and 2 g/kg levels. Four replicates were randomly assigned to each of the six dietary treatments; with six hens per replicate, involving a total of 144 hens of 30 weeks of age. These single comb White Leghorn hens (SCWL) were placed in individual cages. One week before starting the experiment, all birds were dewormed with Levamisole hydrochloride; followed by vaccination against Newcastle Disease with Lasota vaccine through drinking water; for better immunity development. Dietary treatments were T_1 - Control – standard layer mash, T2 - Standard layer mash + 1 g/ kg BLM (C-BLM 1 g), T_3 - Standard layer mash + 2 g/ kg BLM (C-BLM 2 g), T_4 - Designer egg layer mash (DLM), T_5 - DLM + 1 g/ kg BLM (DLM - BLM 1 g), T_6 - DLM + 2 g/ kg BLM (DLM - BLM 2 g).

Samples of flaxseed, sardine fish and BLM used in the experimental feeds were assayed in duplicate (AOAC, 1990) for accurate feed formulation. The flax seed, sardine and basil Leaf meal's analysed proximate compositions (g/ kg), viz. moisture (48, 125 and 131), crude protein (233, 380 and 180), ether extract (377, 220 and 70), crude fibre (130, 1.7 and 99), total ash (31.2, 207 and 101), sand and silica (8.5, 7.1 and 19.9) and calcium (1.0, 59 and 3.0). Based on these values the feeds were formulated (Table 1).

Feed analysis: Representative samples of six experimental diets were also assayed in duplicate for their proximate composition, calcium and phosphorus levels according to the methods of AOAC (1990). Based on the values of NRC (1994) and Narahari (1997) the ME, levels were calculated. The chemical composition of T_1 to T_3 feed and T_4 to T_6 feed are, Analyzed Crude protein value – (178.2 and 180.2 g/kg, Calculated metabolizable energy value - 10.82 and 11.57 MJ/kg, Analyzed Ether extract value - 21.7 and 61.0 g/kg, Analyzed Calcium value - 35.2 and 35.3 g/kg, Analyzed Total phosphorus value - 5.0 and 5.8 g/kg, Calculated Lysine value - 8.1 and 8.8 g/kg, Calculated Methionine value - 3.1 and 4.4 g/kg.

Basil leaves analysis: Random samples of basil leaves could be collected and extract the Total phenolic content were extracted, as per the method cited by Wangcharoen and Morasuk (2007). The values of Basil leaves extracted compositions are, Total Phenolic compound-3.05±0.12

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Table 1. Ingredient composition of the experimental layer feeds (g/kg)

Ingredient	Control feed (T_1 - T_3)	DLM feed (T_4 - T_6)
Corn	300	300
Pearl millet	270	220
Sunflower meal	127	130
Soya meal	200	70
Sardine fish	—	100
Flaxseed	—	100
Dicalcium phosphate	15	—
Shell grit	80	73
Salt	3	1.7
Sodium bicarbonate	2	2
Trace mineral premix[1]	1	1
Vitamin premix[2]	0.5	0.5
Choline chloride 60%	1	1
Vitamin E 50%	—	0.4
Sel-plex (organic selenium)	—	0.2
Ethoxyquin	—	0.1

[1]At the level added, the trace mineral premix supplied: Manganese: 100 mg; Zinc: 80 mg; Iron: 60 mg; Copper: 5 mg and Iodine: 1 mg/kg diet. [2]At the level added, the vitamin premix supplied: Retinol, 3.6 mg, Cholecalciferol, 62.5 mg; Menadione, 1.5 mg; µ-Tocopherol, 20 mg; Thiamine, 3 mg; Riboflavin, 5 mg; Niacin, 35 mg; Pantothenic acid, 15 mg; Pyridoxine, 10 mg; Folacin, 0.5 mg and Cyanocobalamine, 20 mg/kg of feed.

mg/mL of extract, Isothymusin - 0.14±0.09 mg/g of dry matter, Carnosic Acid - 0.19±0.04 mg/g of dry matter, Eugenol - 0.70±0.21 mg/g of dry matter, Sinapic Acid - 0.54±0.01 mg/g of dry matter, Rosmarinic Acid - 0.25±0.05 mg/g of dry matter.

In order to study the effect of functional feeds on the immune status of the hens, the HI titre, Immunoglobulin 'Ig-Y' level, Yolk carotenoid, Yolk Vitamin E, Yolk Selenium levels were measured. At the end of the third week of trial, blood samples were collected from one layer per replicate. The serum samples were separated from blood and used to find out the Haemagglutination Inhibiton (HI) titre level for Newcastle Disease, as per the techniques of Allan and Gough (1974). The log values of the titre were used for statistical analysis. At the end of the fourth week of trial, one egg collected from each replicate were utilized for the estimation of Immunoglobulin-Y (Ig-Y) level in the yolk, according to the techniques of Polson (1980). Raw yolk samples from one egg per replicate were used for the estimation of carotenoid pigments in the yolk. Yolk pigments were extracted by the modified version of the Bligh and Dyer (1959), modified by Lai *et al.* (1996). Following extraction, the total carotenoids were quantified by the standard AOAC procedure (1990), using an Ultraspec-2000 (Pharmacia biotech, USA). One egg yolk from each replicate was used for Vitamin E estimation. The Vitamin E levels in the yolk samples were measured after extraction, by using HPLC, according to the techniques of Abdollahi *et al* (1993). The selenium level in the pooled egg contents, i.e. both albumen and yolk put together was estimated for one egg from each replicate, using Atomic Absorption Spectrophotometer, according to the technique of Canter and Tarino (1982).

Statistical analysis: All the data collected were subjected to analysis of variance for significance according to the procedures of Snedecor and Cochron (1989), for a 2 × 3 factorial design. Wherever necessary, the per cent values were converted to ÖArcsin values and some numerical values were converted into their log values, before analysis of variance. The significance was tested using Duncans' multiple range test (Duncan 1955).

RESULTS AND DISCUSSION

Significant results are noticed in Roche yolk colour value and carotenoid pigments traits, than eggs from control group (Table 2). Highly significant (P<0.01) variations were noticed for these traits based on the diets, BLM levels, as well as between treatments. However, the interaction effect was insignificant. Both designer diet and the BLM have contributed for richer yolk colour with higher carotenoid pigments. Hence, the combination of designer diet with 2 g/kg BLM recorded the highest yolk colour score as well as the carotenoid pigment levels. The source of the pigments may be BLM, flaxseed as well as the sardine fish. Thus, the designer feeds with BLM proved to provide the ideal yolk colour, with greater consumer acceptability. Moreover, these two traits were positively correlated. Ross and Dominy (1990), Sikder *et al.* (1998) and Tallarico *et al.* (2001), Narahari *et al.* (2003) also observed richer yolk colour, with higher carotenoid pigments by feeding special diets rich in carotenoid pigments. The IgY values were significantly

Table 2. Effect of dietary treatments on Roche colour and carotenoid pigments of egg

	Between diets		Between BLM levels			Between treatments					
Trait	Control diet (C)	Designer diet (DLM)	0 g/kg BLM	1 g/kg BLM	2 g/kg BLM	C:0 BLM	C: 1 BLM	C:2 BLM	DLM: 0 BLM	DLM: 1 BLM	DLM: 2 BLM
Roche colour value**	7.3^{y}± 0.26	10.9^{x}± 0.36	7.8^{n}± 0.36	9.5^{mn}± 0.28	10.1^{m}± 0.12	6.3^{d}± 0.25	7.5^{c}± 0.28	8.3^{bc}± 0.25	9.3^{b}± 0.47	11.5^{a}± 0.28	12.0^{a}± 0.00
Carotenoid pigments (mcg/g yolk) **	46.9^{y}± 1.01	73.2^{x}± 3.51	54.1^{n}± 1.47	58.6^{n}± 1.44	67.4^{m}± 3.86	39.0^{c}± 0.00	45.2^{bc}± 0.45	56.6^{b}± 2.58	69.2^{a}± 2.95	72.0^{a}± 2.44	78.3^{a}± 5.14

**Means within each category for each trait, bearing different superscripts, differ significantly (P < 0.01).

Table 3. Effect of dietary treatments on IgY, HI titre, Vitamin E and Selenium parameters

Trait	Between diets		Between BLM levels			Between treatments					
	Control diet(C)	Designer diet(DLM)	0 g/kg BLM	1 g/kg BLM	2 g/kg BLM	C:0 BLM	C: 1 BLM	C:2 BLM	DLM: 0 BLM	DLM: 1 BLM	DLM: 2 BLM
IgY**	$13.3^{y}\pm$	$19.6^{x}\pm$	$14.9^{o}\pm$	$16.3^{n}\pm$	$18.2^{m}\pm$	$11.3^{e}\pm$	$13.0^{d}\pm$	$15.6^{c}\pm$	$18.5^{b}\pm$	$19.5^{b}\pm$	$20.8^{a}\pm$
(mg/ml yolk)	0.25	0.26	0.32	0.32	0.19	0.25	0.34	0.16	0.25	0.3	0.23
HI titre**	$4.1^{y}\pm$	$6.3^{x}\pm$	$4.3^{o}\pm$	$5.1^{n}\pm$	$6.1^{m}\pm$	$3.3^{e}\pm$	$4.0^{de}\pm$	$5.0^{cd}\pm$	$5.3^{bc}\pm$	$6.3^{ab}\pm$	$7.3^{a}\pm$
(log value)	0.22	0.25	0.25	0.12	0.32	0.25	0.00	0.40	0.25	0.25	0.25
Vitamin E	$82.4^{y}\pm$	$217.5^{x}\pm$	$151.1^{m}\pm$	$148.5^{m}\pm$	$150.3^{m}\pm$	$82.5^{b}\pm$	$82.7^{b}\pm$	$82.0^{b}\pm$	$219.7^{a}\pm$	$214.2^{a}\pm$	$218.7^{a}\pm$
(mcg/g yolk)**	7.0	8.1	8.2	6.6	7.8	5.5	9.3	6.0	10.9	4.0	9.5
Selenium	$200.9^{y}\pm$	$369.2^{x}\pm$	$286.3^{m}\pm$	$284.7^{m}\pm$	$284.1^{m}\pm$	$203.5^{b}\pm$	$198.7^{b}\pm$	$200.5^{b}\pm$	$369.2^{a}\pm$	$370.7^{a}\pm$	$367.7^{a}\pm$
(ng/g egg)**	9.7	8.8	7.8	7.9	11.9	6.6	7.7	14.7	9.1	8.0	9.1

**Means for each trait, within each category, bearing different superscripts, differ significantly (P<0.01)
**Highly significant (P<0.01).

(P<0.01) increased by all the factors studied, including the interactions (Table 3); the HI titre was significantly (P<0.01) increased by the dietary treatments.

The effect of DLM and BLM on immune status of the layers, expressed as Immunoglobulin IgY and HI titre, indicated that both designer diet as well as the BLM had significantly improved the immune status of the birds. Supplemental vitamin E and organic selenium 'sel-plex' might have contributed for better immune stimulation in the hens. The vitamin E, selenium and BLM might have acted synergistically, to boost the immunity in chicken. The active principles in the BLM like eugenol, flavanoids and other aromatic compounds as well as other unknown principles in BLM might have contributed for higher IgY and HI titre in the BLM groups. Moreover, there appeared to be a synergism between the designer diet and BLM; because a combination of designer diet with 2 g/kg BLM had resulted in the highest level of immunity. Based on this study, it is possible to produce hyper immunized chicken by supplementing designer diets along with BLM. Significant results are noticed in Vitamin E and selenium levels in designer egg, than eggs from control group (Table 3). Both the diets as well as the six treatment combinations are showing highly significant (P<0.01) variations for these traits. Designer egg had several times more of vitamin E and selenium, than control egg. The BLM levels and their interaction with diets are insignificant. These two nutrients are known immuno modulators as well as natural anti-oxidants. These levels in the egg had gone up significantly by several folds in the designer egg, due to the dietary supplementation. These findings are in agreement with earlier reports of Cherian *et al.* (1996) and Narahari and Sujatha (2003). Additional vitamin E and selenium levels in the designer eggs with BLM, might be due to synergistic effect of these feedstuffs as well as vitamin E with organic selenium. Galobart *et al.* (2001) and Narahari *et al.* (2003) also arrived at similar conclusions.

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