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Impact of incorporation of flax seed, sardine fish and basil leaf (*Ocimum sanctum*) meal in layer diet on egg weight and its components and egg quality traits

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Abstract

A total of 144 single comb 'Forsgate' strain White Leghorn (SCWL) pullets of 27 weeks of age, belonging to the same hatch, and of uniform body size were randomized into 24 groups of 6 hens each. This experiment is a 2 X 3 factorial design consisting of two types of layer feeds namely, standard layer mash (control) and special designer egg layer mash (DELM); each with three levels of Basil (*Ocimum sanctum*) leaf meal (BLM) i.e. 0, 1 and 2 g/kg levels, viz., T₁ to T₆. Four replicates were randomly assigned to each of the six dietary treatments. The egg weight was significantly higher in birds fed DELM compared to the control. The BLM level had no effect on egg size.

Keywords: Ocimum sanctum, layer, egg weight, egg yolk

Introduction

Market value of egg is determined based on egg weight. In general, size of egg is positive proportion to nutrient level of egg. Twenty years ago, only a small proportion of the laying flock would have been capable of achieving egg weights within the range of 53 to 67 g (Nys et al., 2008) [24]. The layer strains available now are producing 60 g weight of egg during 25-26 weeks of its age. Hence, researchers tried to increase the egg weight with different raw materials. Eder et al. (1998) [5] observed that the effect of diets containing 0, 5, 10, or 15 per cent full fat flaxseed or ground flaxseed to enrich eggs. They also observed that height of air chamber, yolk index and Haugh units were not influenced by dietary flaxseed. Novak and Scheideler (2001) [13] concluded that the flaxseed feeding to the hens had significantly increased the egg albumen percentage. Sari et al. (2002) [15] and Sujatha (2002) [20] reported increased yolk weights by feeding hens with ground flaxseed in basal diet. Jiang et al. (1992) [10] investigated the effect of 150 g/kg full-fat flaxseed in layer ration. They found no adverse effect on egg internal quality in terms of Haugh unit. They also observed a darker yolk colour in those eggs. Cherian et al. (1996) [3] studied the effect of 35 g/kg flax oil in laying hens' diet on internal egg qualities, such as Haugh unit; which was affected during storage; but not in fresh eggs. Basmacioglu et al. (2001) [2] concluded that feeding of dietary flaxseed oil at 1.5 g/kg level to laying hens did not significantly affect the egg quality. Tallarico et al. (2001) [22] observed no differences in albumen index, Haugh unit and yolk index between the control and groups receiving flaxseed in the layer diet. Sujatha (2002) [20] reported that 150 g/kg flaxseed in laying hens' diet, had slightly increased the albumen index, yolk index and Haugh unit, compared with control egg. Sujatha (2002) [20] investigate the effect of 20 g/kg fish oil in laying hens' diet and noticed reduced egg and yolk weight and increased albumen and shell weight, compared with the control eggs. Cherian et al. (1996) [3] reported that 35 g/kg fish oil in laying hens' diet has significantly affected the internal qualities, such as Haugh unit during storage, but not in fresh eggs. Basmacioglu et al. (2001) [2] observed that feeding fish oil at 15 g/kg level did not significantly affect the egg quality. Sujatha (2002) [20] studied the effect of 20 g/kg fish oil in laying hens' diet, and noticed increase in the Albumen index, Haugh unit and decrease in the yolk index, compared with the control eggs. In this trial, flax seed, sardine fish, basil leaf are incorporated into layer diet and analyze the egg weight and its components and egg quality parameters.

Materials and Methods

A biological study of six weeks duration was carried out and followed by laboratory assay to study the effect of dietary full-fat flaxseed (FFFS), oil rich sardine fish, basil (tulasi) leaf meal (BLM) on egg weight and it's components and egg quality traits.

A total of 144 single comb 'Forsgate' strain White Leghorn (SCWL) pullets of 27 weeks of age, belonging to the same hatch, and of uniform body size were randomized into 24 groups of 6 hens each. This experiment is a 2 x 3 factorial design consisting of two types of layer feeds namely, standard layer mash (control) and special designer egg layer mash (DELM); each with three levels of BLM i.e. 0, 1 and 2 g/kg levels. Four replicates were randomly assigned to each of the six dietary treatments. Details of the six dietary treatments are

shown in Table 1. Samples of flaxseed, sardine fish and BLM used in the experimental feeds were assayed in duplicate (AOAC, 1990) [1], for accurate feed formulation. The analyzed composition of these three feedstuffs are shown in Table 2. Based on these values the feeds were formulated. The ingredient composition of the control and DELM feeds are show in Table 3. The BLM was added to each feed at three levels; as indicated in Table 1. The experimental feeds were prepared once in three weeks.

Table 1: Experimental design and treatments

Treatment	Treatment code	Treatment details	No. of replicates	No. of birds/ replicates	Total no. of birds
T_1	Control	Control - standard layer mash	4	6	24
T_2	C-BLM 1 g	Standard layer mash + 1 g/ kg BLM	4	6	24
T ₃	C-BLM 2 g	Standard layer mash + 2 g/ kg BLM	4	6	24
T_4	DELM	Designer egg layer mash	4	6	24
T ₅	DELM - BLM 1 g	DELM + 1 g/ kg BLM	4	6	24
T ₆	DELM - BLM 2 g	DELM + 2 g/ kg BLM	4	6	24

Table 2: Analyzed chemical composition of flaxseed, sardine fish and BLM (g/kg)

Component	Flaxseed	Sardine	BLM
Moisture	48	125	131
Crude Protein	233	380	180
Ether Extract	377	220	70
Crude Fiber	130	1.7	99
Total Ash	31.2	207.2	100.8
Sand and Silica	8.45	71.4	29.9
Calcium	10	59	30

Table 3: Ingredient composition of the experimental layer feeds (g/kg)

Ingredient	Control feed (T ₁ - T ₃)	DELM 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	2.0
Trace mineral premix ¹	1	1.0
Vitamin premix ²	1	1.0
Choline chloride 60%	1	1.0
Vitamin E 50%		0.1
Sel-plex (organic selenium)		0.1
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 μg, Menadione: 1.5 mg, ∞-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 μg/kg of feed.

Representative samples of six experimental diets were assayed in duplicate for their proximate composition, calcium and phosphorus levels according to the methods of AOAC (1990) [1]. Based on the values of NRC (1994) [14] and

Narahari (1997) [12] the ME, levels were calculated. The chemical composition of six experimental feeds are reported in Table 4.

Table 4: Chemical composition of the experimental diets

Nutrient	Regular layer feed (T ₁ - T ₃)	Enriched layer feed (T ₄ - T ₆)	
CP* (g/kg)	178.2	180.2	
ME** (MJ/kg)	10.82	11.57	
EE* (g/kg)	21.7	61.0	
Calcium* (g/kg)	35.2	35.3	
Total phosphorus* (g/kg)	5.0	5.8	
Lysine** (g/kg)	8.1	8.8	
Methionine** (g/kg)	3.1	4.4	

^{*} Analysed values

^{**} Calculated values

Management of the experimental birds

Each experimental bird was housed in individual cage, with a cage space of 930 cm² per hen, located in a well ventilated open sided house. 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. A 16 hour photo period and 8 hour darkness per day was provided. Routine normal farm practices were followed throughout the experimental period. All the birds were fed *ad libitum* with the respective experimental feeds, throughout the six weeks experimental period, from 27 to 32 weeks of age. Two eggs from each replicate were collected randomly during 30 weeks of age, labeled properly and weighed individually to 0.1 g accuracy.

Egg quality traits Shape Index

After taking the individual weights of these 48 eggs, the long axis and short axis of each egg were measured to calculate the shape index according to the formula of Shuttz (1953) [18].

Specific gravity

Specific gravity of the egg was calculated by dividing the egg weight in grams with egg volume in milliliter.

Specific gravity =
$$\frac{\text{Egg weight (g)}}{\text{Egg volume (ml)}}$$

Albumen Index

The eggs were gently broke open at the equatorial region and the contents were carefully released on an egg breaking stand. The length and width of thick albumen were measured to 1mm accuracy by using a dial vernier caliper. The thick albumen height was measured to 0.1mm accuracy by using an "Ames tripod stand micrometer". The albumen index was calculated according to the formula of Heiman and Carver (1936) [8].

Haugh units

Based on the egg weight and thick albumen height, the Haugh unit values of the individual eggs were calculated, using the formula of Haugh (1937)^[7].

Yolk Index

The yolk width was measured at two places to 1mm accuracy, using a dial vernier caliper. The yolk height was measured to 0.1mm accuracy, using "Ames tripod stand micrometer". The

yolk index was calculated as per the formula of Sharp and Powel (1931)^[17].

Shell Thickness

The shell membranes were peeled, the shells were washed and dried in a hot air oven at $110^{\circ} \pm 5$ °C overnight. After cooling the shell thickness were measured at broad, narrow and equatorial regions, using a "shell thickness gauge" to 0.01 mm accuracy. Then, the average shell thickness was calculated for each egg.

All the data collected were subjected to analysis of variance for significance according to the procedures of Snedcor and Cohorn (1989) [19], for a 2×3 factorial design. Wherever necessary, the per cent values were converted to $\sqrt{\text{Arcsin}}$ values, before analysis of variance. The significance was tested using Duncans' multiple range test (Duncan, 1955) [4].

Results and Discussion

Egg weight

The data on egg weight, are shown in Table 5 and their 'F' values in Table 6. Highly significant (p<0.01) variations in egg weight, was noticed between the control and designer eggs. No significant difference was noticed due to BLM levels. The egg weight was significantly higher in birds fed designer diet compared to the control; irrespective of the dietary BLM level. The BLM level had no effect on egg size. Increased egg size in the designer diet group, might be due to higher oil content in the feeds, resulting in better yolk size and thereby the egg size. Gonzalez and Leeson (2000) [6] also noticed better egg weight due to fish oil in the feed. On the contrary, Jiang et al. (1991) [9], Scheideler and Froning (1996) [16] and Sari et al. (2002) [15] reported decreased egg size, with designer feeds. Eder et al. (1998) [5], Maier et al. (1998) [11] and Novak and Scheideler (2001) [13] did not notice any variation in egg size between treatments.

Egg components

The percentages of Albumen, yolk and shell as influenced, by dietary treatments and their Anova are shown in Tables 5 and 6, respectively. None of these traits showed any significant variation between treatments. The percentages of albumen, yolk and shell in egg did not vary between dietary treatments. This finding agrees with the earlier observations of Van Elswyk *et al.* (1992) [23]. However, Novak and Scheideler (2001) [13], Sari *et al.* (2002) [15] and Sujatha (2002) [20] noticed variations in the albumen and yolk percentages, due to feeding of flaxseed and fish oil.

Table 5: Effect of Dietary treatments on egg weight and egg components in WLH hens

Trait	В	Between BLM levels			
	Standard layer mash (control)	Special designer egg layer mash (DELM)	0 g/Kg BLM	1 g/Kg BLM	2 g/Kg BLM
Egg weight **	48.9 ^x ±0.7	$51.8^{y}\pm1.2$	50.3 ^m ±1.3	50.3 ^m ±0.9	50.4 ^m ±0.7
% Albumen NS		60.2±0.4	60.7±0.5	60.3±0.5	60.0±0.3
% Yolk ^{NS}	28.5±0.3	28.6±0.4	27.9±0.3	28.5±0.4	28.9±0.3
% Shell NS	11.3±0.2	11.2±0.2	11.4±0.2	11.2±0.1	11.1±0.1

^{**} Mean egg weights, bearing different superscripts differ significantly (p<0.01)

Table 6: 'F' Values for egg weight, percentage of albumen, yolk and shell

Trait	Diet	Herb level	Diet X Herb level
Egg weight	10.5 **	0.001 ^{NS}	0.0005 ^{NS}
% Albumen	0.733 ^{NS}	2.34 ^{NS}	0.224 ^{NS}
% Yolk	1.64 ^{NS}	3.98 ^{NS}	0.73 ^{NS}
% Shell	0.04 ^{NS}	1.91 ^{NS}	2.26 ^{NS}

NS Not significant (p>0.05)

^{**} Highly significant (p<0.01)

Egg quality traits

The influence of diets on shape index, specific gravity, shell thickness, albumen index, yolk index and Haugh unit and their F-values are presented in Tables 7 and 8, respectively. All these traits did not show any variation between treatments, as well as their interactions. None of the egg quality traits were influenced by the dietary treatments; suggesting the all

experimental diets had no adverse effect on egg quality. Similar observations were made by Jiang *et al.* (1992) [10] and Basmacioglu *et al.* (2001) [2]. However, Cherian *et al.* (1996) [3], Sujatha (2002) [20] and Sujatha and Narahari (2003) [21] noticed variations in egg quality, based on the dietary flaxseed and fish oil.

Trait	Between diets		Between BLM levels		
	Standard layer mash (control)	Special designer egg layer mash (DELM)	0 g/Kg BLM	1 g/Kg BLM	2 g/Kg BLM
Shape Index NS	73.9±1.15	73.9±0.55	74.0±0.49	74.0±1.10	73.7±0.94
Specific gravity NS	1.05±0.001	1.04±0.001	1.05±0.002	1.04±0.002	1.05±0.002
Shell thickness (mm) NS	0.33±0.00	0.33±0.00	0.33±0.00	0.33±0.00	0.33±0.00
Yolk Index NS	0.48±0.01	0.47±0.00	0.47±0.00	0.47±0.00	0.47±0.00
Albumen Index NS	0.11±0.10	0.11±0.00	0.11±0.00	0.11±0.00	0.10±0.00
Haugh Unit ^{NS}	94.9±0.97	95.8±0.71	95.6±0.92	95.6±0.92	94.8±0.77

Table 7: Effect of Dietary treatments on egg quality traits in WLH hens

Table 8: 'F' Values for egg quality traits

Trait	Diet	Herb level	Diet X Herb level
Shape Index	0.003 ^{NS}	0.06 ^{NS}	0.07 ^{NS}
Specific gravity	1.09 ^{NS}	0.79 ^{NS}	1.86 ^{NS}
Shell thickness	0 NS	0 NS	0^{NS}
Yolk Index	0.86 ^{NS}	0.37 ^{NS}	0.12 ^{NS}
Albumen Index	0.03 ^{NS}	1.00 ^{NS}	2.35 NS
Haugh Unit	1.30 NS	0.49 ^{NS}	2.54 ^{NS}

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