



Impact of functional diet and basil leaf (*Ocimum sanctum*) meal on layer egg's fatty acid level and layer's production performance

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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 in a 2×3 factorial design consisting of two types of layer feeds namely, standard layer mash (control) and functional diet (designer egg layer mash-DELM); each with three levels of basil leaf meal (BLM) i.e. 0, 1 and 2 g/kg diet levels, viz. T₁ to T₆. Four replicates were randomly assigned to each of the six dietary treatments. One egg yolk from each replicate was used for the fatty acid estimation. The layer's body weight gain, per cent hen-day egg production, feed/egg parameter did not show any variations between diets and basil leaf meal dosage level. The feed consumption was significantly different between diets and not between BLM levels. The diets and BLM levels had highly significant effect on Myristic acid (C_{14:0}), Palmitic acid (C_{16:0}), Stearic acid (C_{18:0}), Oleic acid (C_{18:1}); n-6 polyunsaturated fatty acids, viz. Linoleic acid (C_{18:2}), Arachidonic acid (C_{20:4}) and n-3 polyunsaturated fatty acids, viz. α -Linolenic acid (C_{18:3}), Eicosapentaenoic acid-EPA (C_{20:5}) and Docosahexaenoic acid-DHA (C_{22:6}) levels. Both DELM and BLM significantly increased the n-3 PUFA levels in egg yolk.

Keywords: Egg yolk, Layer, *Ocimum sanctum*, Omega 3 fatty acid

Researchers found that, omega 3 fatty acids are beneficial for heart patients, pregnant women and children. Hence, trials are conducted in layer birds with different raw materials, which are having high omega 3 fatty acids for the purpose of producing the functional egg or designer egg. Fernandez *et al.* (2011) stated that, omega-3 fatty acids are involved in the prevention of general cardiovascular diseases. Jiang *et al.* (1991) examined the effect of 150g/kg flaxseed in laying hens' diet. They found no effect on egg production and found an increase in the α -Linolenic acid (LNA) content of yolk by more than six fold, over the control. Sari *et al.* (2002) investigated the effect of the addition of ground flaxseed to layer diet and found a 1.80, 7.07, 8.35 and 12.20% of n-3 fatty acids in egg yolk lipids for 0, 5, 10 and 15% flaxseed incorporated diet, respectively and also they found that decreased feed consumption, egg mass and increased egg production. Narahari *et al.* (2003) assigned layers to a basal diet containing 10% flaxseed and 10% full fat sardine fish, to enrich egg yolk lipids with n-3 fatty acids. They found that, n-3 fatty acid levels were higher in designer egg, compared to the control eggs.

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Ezhilvalavan *et al.* (2003) reported that designer egg yolk had higher n-3 poly unsaturated fatty acid (n-3 PUFA) and mono unsaturated fatty acid (MUFA), and lower levels of n-6 poly unsaturated fatty (n-6 PUFA) and saturated fatty acid (SFA) compared to standard egg. Moreover, the n-6/n-3 ratio was decreased from 13.0 in standard egg to 2.6 in designer egg. Meluzzi *et al.* (2000) reported that 30g/kg fish oil in laying hens' diet, increased the n-3 fatty acids of the yolk; particularly, the eicosapentaenoic acid (EPA) level, which increased from 0.74 mg in the control to 19.53 mg/egg from hens fed fish oil. The docosahexaenoic acid (DHA) level increased from 43.6 mg to 143.7 mg. Narahari (2003) and Sujatha and Narahari (2003) reported significant increase in the yolk EPA and DHA levels, due to feeding of hens with oil rich fish or fish oil, compared to the regular egg yolks.

MATERIALS AND METHODS

A biological study of six weeks was carried out at Layer farm; followed by laboratory assay to study the effect of dietary full-fat flaxseed, oil rich sardine fish, basil leaf meal (BLM) on fatty acid composition and layer's production parameters were also recorded. 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 was a 2×3 factorial design consisting of two types of layer feeds namely, standard

Table 1. Analyzed chemical composition of flaxseed, sardine fish and basil leaf meal (BLM) (g/kg)

Component	Flaxseed	Sardine	BLM
Moisture	48	125	131
Crude protein	233	380	180
Ether extract	377	220	70
Crude fibre	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

layer mash (control) and functional diet or 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. Samples of flaxseed, sardine fish and BLM used in the experimental feeds were assayed in duplicate (AOAC 1990) for accurate feed formulation. The analyzed compositions of these three feedstuffs are shown in Table 1. Based on these values, the feeds were formulated. The ingredient composition of the control and DELM feeds are shown in Table 2.

Table 2. Ingredient composition of the experimental layer feeds (%)

Ingredient	Control feed (T ₁ - T ₃)	DELM feed (T ₄ - T ₆)
Corn	30	30
Pearl millet	27	22
Sunflower meal	12.7	13
Soya meal	20.0	7
Sardine fish		10
Flaxseed		10
Dicalcium phosphate	1.5	
Shell grit	8.0	7.3
Salt	0.3	0.17
Sodium bicarbonate	0.2	0.20
Trace mineral premix ¹	0.1	0.1
Vitamin premix ²	0.1	0.1
Choline chloride 60%	0.1	0.1
Vitamin E 50%		0.01
Sel-plex (organic selenium)		0.01
Ethoxyquin		0.01

*Basil leaf meal (T₁, 0 g/kg diet; T₂, 1 g/kg diet; T₃, 2 g/kg diet; T₄, 0 g/kg diet; T₅, 1 g/kg diet; T₆, 2 g/kg diet). ¹At 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. ²At the level added, the "vitamin premix" supplied. Retinol: 3.6 mg, Cholecalciferol: 62.5 µg, Menadione: 1.5 mg, µ-Tocopherol: 20 mg, Thiamine: 3 mg, Riboflavin: 5 mg, Niacin: 35 mg, Pantothenic acid: 15 mg, Pyridoxine: 10 mg, Folic acid: 0.5 mg and Cyanocobalamin: 20 µg/kg of feed.

Routine normal farm practices were followed throughout the experimental period. All the birds were fed *ad lib.* with the respective experimental feeds, throughout the six weeks experimental period, from 27 to 32 weeks of age. The initial and final body weight of the individual experimental birds was recorded to one gram accuracy at 27 and 32

weeks of age to find out the body weight gain. Replicate wise feed intake and daily egg production were recorded, to calculate the feed efficiency and hen-day egg production. One egg yolk from each replicate was used for the fatty acid estimation. Methyl esters of fatty acids were prepared from yolk sample from each egg, as per the method of Wang *et al.* (2000). Using a fused silica capillary column of 30 m × 0.25 mm i.d., 0.25 µm film thickness fitted to a Supelco 2380 gas chromatograph. The fatty acids were identified with reference to the standards and they were quantified as per the 'area normalization method'. They were expressed as percentage of total fatty acids. All the data collected were subjected to analysis of variance for significance according to the procedures of Snedcor and Cochran (1989), for a 2 × 3 factorial design. The significance was tested using Duncan's multiple range test (Duncan 1955).

RESULTS AND DISCUSSION

The effect of diets and basil leaf meal on production performance of layers and the yolk fatty acid levels are shown in Tables 3, 4 and 5. The effect of feeding functional diet and basil leaf meal on body weight gain, per cent hen-day egg production, feed/egg parameters did not show any variations between diets and basil leaf meal dosage level. The feed consumption was significantly different between diets and not between BLM levels. High energy level in the functional diet due to oil from flaxseed and sardine fish which significantly reduced the feed consumption compared to the control. This result concurs with earlier findings of Gonzalez and Lesson (2000). No significant interaction effect was noticed in feed consumption parameter. Lower body weight gain of layers was due to hens fed flaxseed incorporated diet. This result concurs with findings of Scheideler and Froning (1996). No significant difference was noticed in egg production between diets and BLM levels. However, Sari *et al.* (2002) noticed increased egg production. The diets and herbal levels had highly significant effect on Myristic acid (C_{14:0}), Palmitic acid (C_{16:0}), Stearic acid (C_{18:0}), Oleic acid (C_{18:1}); n-6 polyunsaturated fatty acids, viz. Linoleic acid (C_{18:2}), Arachidonic acid (C_{20:4}) and n-3 polyunsaturated fatty acids, viz. α-Linolenic acid (C_{18:3}), eicosapentaenoic acid-EPA (C_{20:5}) and docosahexaenoic acid-DHA (C_{22:6}) levels. The functional diet and basil leaf meal's interaction effect was significant in C_{20:4}, C_{18:3}, C_{20:5} and C_{22:6} level in egg yolk. Both DELM and BLM had significantly (P<0.01) increased the n-3 PUFA levels in egg yolk. The flaxseed and sardine fish, rich in n-3 PUFA, had significantly increased n-3 PUFA levels in egg yolk, with proportionate reduction in the Palmitic acid (C_{16:0}) level. The oil in flaxseed has increased the µ-linolenic acid level; whereas the oil in fish has increased the eicosapentaenoic acid-EPA (C_{20:5}) and docosahexaenoic acid-DHA (C_{22:6}) levels. Similar conclusions were drawn by Basmacioglu *et al.* (2001), Galobart *et al.* (2001), Sirri *et al.* (2001) and Narahari *et al.* (2003). Surprisingly, the BLM at low levels of inclusion (1 and 2g/kg) had also contributed significantly for elevation

Table 3. Effect of dietary treatments on production traits in WLH layers from 27 to 32 weeks of age

Production trait	Between diets		Between BLM levels			P-value		
	Control diet (C)	Designer egg layer mash (DELM)	0 g BLM per kg diet	1 g BLM per kg diet	2 g BLM per kg diet	Diet	Herb level	Diet × Herb level
Body weight gain (g)	81.5±20.5	78.3±29.4	81.3±14.6	85.9±45.0	72.6±15.2	0.148	0.151	0.286
% Hen-day egg production	84.3±20.5	82.4±2.2	82.4±3.5	83.0±1.6	84.5±1.8	0.189	0.192	0.145
Feed consumption (g/hen/day) *	99.3±0.7	92.3±0.6	95.2±0.8	96.5±0.7	95.7±0.4	0.018	0.021	0.017
Feed/egg (g)	118.1±3.8	111.9±3.4	115.8±5.9	115.9±1.9	113.2±2.9	0.511	0.489	0.469

*Means within each category for each trait bearing different superscripts, differ significantly (P<0.05). NS, Not significant (P>0.05); *, Significant (P<0.05).

Table 4. Effect of dietary treatments on saturated fatty acid (SFA) and monounsaturated fatty acid (MUFA) levels in yolk lipids (g/100 g of total fatty acids)

Fatty acid	Between diets		Between BLM levels			P-value		
	Control diet (C)	Designer egg layer mash (DELM)	0 g BLM per kg diet	1 g BLM per kg diet	2 g BLM per kg diet	Diet	Herb level	Diet × Herb level
C _{14:0} **	0.28 ^x ±0.02	0.24 ^y ±0.01	0.30 ^m ±0.018	0.25 ⁿ ±0.014	0.21 ⁿ ±0.01	0.004	0.004	0.284
C _{16:0} **	25.2 ^x ±2.19	18.9 ^y ±3.51	20.3 ⁿ ±1.47	22.9 ^m ±1.44	22.9 ^m ±3.86	0.005	0.005	0.145
C _{18:0} **	7.77 ^x ±0.12	7.21 ^y ±0.13	8.11 ^m ±0.16	7.44 ⁿ ±0.10	6.91 ^o ±0.09	<0.001	<0.001	0.017
C _{16:1} NS	2.24 ^x ±0.11	2.46 ^x ±0.13	2.82 ^m ±0.10	2.37 ^m ±0.14	2.46 ^m ±0.10	0.08	0.08	0.465
C _{18:1} **	40.7 ^x ±0.44	37.5 ^y ±0.28	40.0 ^m ±0.26	39.1 ^m ±0.56	38.15 ⁿ ±0.24	<0.001	<0.001	0.151

**Means within each category for each trait bearing different superscripts, differ significantly (P<0.01). NS, Not significant (P>0.05); **, Highly significant (P<0.01).

Table 5. Effect of dietary treatments on n-6 and n-3 PUFA levels in yolk lipids (g/100g of total fatty acids)

Fatty acid	Between diets		Between BLM levels			P-value		
	Control diet (C)	Designer egg layer mash (DELM)	0 g BLM per kg diet	1 g BLM per kg diet	2 g BLM per kg diet	Diet	Herb level	Diet × Herb level
C _{18:2} **	15.7 ^x ±0.021	15.0 ^y ±0.019	14.9 ^m ±0.014	15.4 ^m ±0.24	15.9 ^m ±0.24	<0.001	<0.001	0.180
C _{20:4} **	1.42 ^y ±0.11	2.35 ^x ±0.06	1.94 ^m ±0.07	1.88 ^m ±0.11	1.82 ⁿ ±0.06	<0.001	<0.001	<0.001
C _{18:3} **	0.20 ^y ±0.02	4.58 ^x ±0.14	1.93 ^m ±0.11	2.52 ^m ±0.08	2.71 ^m ±0.03	<0.001	<0.001	<0.001
C _{20:5} **	0.16 ^y ±0.02	0.73 ^x ±0.03	0.36 ^m ±0.02	0.46 ^m ±0.02	0.52 ^m ±0.02	<0.001	<0.001	0.002
C _{22:6} **	0.24 ^y ±0.02	4.58 ^x ±0.14	1.93 ^m ±0.11	2.52 ^m ±0.08	2.71 ^m ±0.03	<0.001	<0.001	0.005

**Means for each trait, within each category, bearing different superscripts, differ significantly (P<0.01). NS, Not significant (P>0.05); **, Highly significant (P<0.01).

in the yolk n-3 PUFA levels, especially the α -Linolenic acid (C_{18:3}). With just 70g/kg lipid level in BLM, which might have higher levels of LNA, is responsible for elevated yolk μ -LNA level. Singh *et al.* (2007) reported that basil oil contains α -linolenic acid, an omega-3 fatty acid, which on metabolism produces eicosapentaenoic acid. Hence, it is found that BLM appeared to be a good feed supplement to increase the yolk μ -LNA level and eicosapentaenoic acid and by reducing the yolk saturated fatty acid (SFA) levels proportionately. Narahari *et al.* (2003) also concluded that a combination of oil rich fish with flaxseed, spirulina and BLM had enhanced the yolk n-3 fatty acid levels, considerably. The omega 3 fatty acid rich raw materials like flaxseed, sardine fish and basil leaf meal associated together and increased the omega 3 fatty acid level and reduced the saturated fatty acid level in layer egg yolk.

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