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YA Rather
Ph.D., (Scholar), Department of Poultry Science, College of Veterinary Science & A.H., Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh, India

G Goyal
Assistant Professor, Department of Poultry Science, College of Veterinary Science & A.H., Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh, India

S Nayak
Professor and Head, Department of Animal Nutrition, College of Veterinary Science & A.H., Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh, India

A Shinde
Assistant Professor, Department of Poultry Science, College of Veterinary Science & A.H., Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh, India

A Gattani
Associate Professor, Department of Veterinary Physiology and Biochemistry, College of Veterinary Science & A.H., Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh, India

S Gupta
Associate Professor, Department of Livestock Products Technology, College of Veterinary Science & A.H., Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh, India

L Chouhan
Assistant Professor, Department of Poultry Science, College of Veterinary Science & A.H., Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh, India

K Shrivastav
Assistant Professor, Department of Animal Biotechnology Centre, College of Veterinary Science & A.H., Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh, India

S Singour
Ph.D. (Scholar), Department of Animal Nutrition, College of Veterinary Science & A.H., Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh, India

Corresponding Author:
YA Rather
Ph.D., (Scholar), Department of Poultry Science, College of Veterinary Science & A.H., Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh, India

Effect of omega-3 PUFA, antioxidant and multienzymes on egg quality parameters and fatty acid profile of Kadaknath eggs

YA Rather, G Goyal, S Nayak, A Shinde, A Gattani, S Gupta, L Chouhan, K Shrivastav and S Singour

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Abstract
The study was conducted to investigate the effects of different designer diets on egg quality and fatty acid profile of kadaknath eggs. A total of one hundred eighty, 30 weeks age laying hens (Kadaknath birds) were selected. These layers were randomly distributed to 5 replicates having 6 birds each and were allotted to 6 dietary treatments. T_0 (basal diet), T_1 (basal diet having 10% Flaxseed replace), T_2(basal diet having 1.5% fish oil), T_3 (basal diet + Vitamin E 1000 mg/kg feed), T_4 (basal diet + multi-enzymes 2 g/kg feed) and T_5 (basal diet having 5% flaxseed, 0.75% fish oil, 500 mg Vitamin E/kg feed and 1 g multi-enzymes/kg feed). Experiment was conducted for 12 weeks period from 31st to 42nd weeks. Egg quality parameters and fatty acid profile of egg collected at 4 weeks intervals were determined.
The egg quality parameters like shape index, albumin index, yolk index, Haugh index and shell thickness were not affected by designer diet. PUFA content in egg yolk were significantly (p<0.05) increased in groups supplemented with 10% flaxseed, 1.5% fish oil and combination supplemented group while the MUFA and SFA content in egg yolk were decreased due to designer diets. Omega6: omega3 fatty acid ratio was also improved in T_2, T_1 and T_5 groups (1.5% fish oil, 10% flaxseed and combination supplemented groups).

Keywords: Flaxseed, fish oil, vitamin E, multi-enzymes, Kadaknath, egg quality, fatty acid

Introduction
Poultry farming is one of the fastest growing sectors among the agriculture and allied sectors and play vital role in Indian economy. India is ranked second with a compound annual growth rate (CAGR) of 6.87%, also the annual egg production in Indiahas been increased from 129.60 billion in 2021-22 to 142.77 billion in 2023-24 (BAHS, 2023-24) [1]. Since egg has been described as rich source of nutrients including high quality protein, essential fatty acids, minerals and vitamins; the demand of egg is increasing day by day to fulfil the rising demand of quality nutrients at affordable prices, resulting in rapid growth of the Indian poultry sector.
Kadaknath a native breed of India, is known for its black coloured meat. Although the meat of this breed has an unattractive appearance, it has a delicious flavour. The meat and eggs are considered rich sources of protein and iron. The meat of Kadaknath breed contains a high percentage (25.47%) of protein and is believed to have aphrodisiac properties. Although the Kadaknath breed has many unique characteristics, it has been neglected because of its poor production potential but in recent years, interest among consumers and farmers in native germ plasm is increased because of its unique hardiness of breeds, their ability to thrive under adverse climatic conditions, and the desirable taste and flavour of eggs and meat (Valavan *et al*., 2016) [19].
In the current competitive world, people are prone to more stress which caused by free radical-associated diseases (Sen and Chakraborty, 2011) [17]. The risk of diseases due to oxidative stress is also associated with unhealthy lifestyles, like exposure to chemicals, pollution, drugs, cigarette smoking, etc.

Materials and Methods

Location of work: The proposed work was carried out at Department of Poultry Science, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh. The required facilities for experiment were available under All India Coordinated Research Project (AICRP) on Poultry Breeding, Department of Poultry Science, College of Veterinary Science and Animal Husbandry, N.D.V.S.U., Jabalpur.

Source of animals: A total of one hundred eighty, 30 weeks old laying hens (Kadaknath birds) were selected from the Poultry farm, All India Coordinated Research Project (AICRP) on Poultry Breeding, Department of Poultry Science, College of Veterinary Science and Animal Husbandry, N.D.V.S.U., Jabalpur.

Duration of work

Experiment was conducted for a period of twelve weeks from Jan 2024 to April 2024. A metabolic trial of 7 days was also conducted at the end of feeding trial to determine the digestibility and retention of nutrients.

Research methodology and experimental design

Experimental design

The design of the experiment was completely randomized design. All the layers of 30 weeks age were individually weighed at the start of the experiment. The birds were randomly assigned to various groups so that weight of the layers among the groups did not differ significantly. Overall, there were six dietary treatments. Each treatment consisted having five replicates of six layers each.

Table 1: Distribution of birds as per experimental design

Treatment group		T_0 Control (Basal diet)	T_1 Basal diet + Flaxseed (10%)	T_2 Basal diet + Fish oil (1.5%)	T_3 Basal diet + Vitamin E (1000 mg/kg feed)	T_4 Basal diet + Multienzyme (2 g/kg feed)	T_5 Basal diet + Flaxseed + fish oil + VitE + Multienzyme
No. of birds per replicate	R_1	6	6	6	6	6	6
	R_2	6	6	6	6	6	6
	R_3	6	6	6	6	6	6
	R_4	6	6	6	6	6	6
	R_5	6	6	6	6	6	6
No. of birds per Treatment		30	30	30	30	30	30
Total no. of birds		180					

(T_5 group-5% flaxseed + 0.75% fish oil + 500 mgvit E/kg feed and 1 g multi-enzymes/kg feed)
(Multi-enzymes= Each 1 kg contains xylanase-50000 U, pectinase 40000 U, cellulose 20000 U, phytase 5000 U, protease 20000 U amylase 20000 U, mannanase 10000 U, beta glucanase 10000 U and lipase 1000 U)

Formulation of Ration

The feed ingredients were procured from feed store, AICRP on poultry breeding Department of Poultry Science, College of Veterinary Science and Animal Husbandry, N.D.V.S.U., Jabalpur and analyzed for proximate composition before formulation of diets. The analysed protein and ME values of feed ingredients were used for computation of rations.

The basal diets were formulated with 16.5% CP and 2600 kcal ME/kg feed as per (ICAR, 2013) [8] feeding standards for kadaknath laying birds. Designer diets were formulated by incorporation of flaxseed @ 10.0% of the basal diet, fish oil @ 1.5% of the basal diet, Vitamin E @ 1000 mg/kg feed, multi-enzymes @ 1 g/kg feed and flaxseed @ 5% + fish oil @ 0.75% + Vitamin E @ 500 mg and multi-enzymes @ 1 g for T_1, T_2, T_3, T_4 and T_5 groups, respectively. All the diets fed to different treatment groups during experimental period were isocaloric and isonitrogenous. Composition of different experimental diets used in the study is given in table 02.

Table 2: Ingredient composition of layer experimental diets

Feed ingredient	Experimental diets					
	T_0	T_1	T_2	T_3	T_4	T_5
Maize (kg)	450	440	445	450	450	445
Deoiled Rice bran (kg)	250	256	255	250	250	253
Soyabean meal (kg)	221	215	219.5	221	221	217.25
Flaxseed	-	10	-	-	-	5
Fish oil	-	-	1.5	-	-	0.75
Shell grit (kg)	28	28	28	28	28	28
Calcite/LSP(kg)	14	14	14	14	14	14
Dicalcium phosphate (kg)	2.5	2.5	2.5	2.5	2.5	2.5
mineral premix(Kg)	25	25	25	25	25	25
vitamin premix (kg)	2.5	2.5	2.5	2.5	2.5	2.5
Salt (kg)	6.0	6.0	6.0	6.0	6.0	6.0
Total	1000	1000	1000	1000	1000	1000
Nutrient composition analysed (%):						
Energy (kcal ME/kg diet)	2600	2604	2605	2600	2600	2602
Crude protein	16.5	16.53	16.54	16.5	16.5	16.52
Ca	3.26	3.26	3.26	3.26	3.26	3.26
Total P	0.44	0.44	0.44	0.44	0.44	0.44

Calculated composition of diet-16.5% CP and 2600 K cal ME/Kg in layer ration.

Feeding and Watering
The feed was offered according to standard requirement of Kadaknath birds. All-mash system of feeding was practiced during the experiment. Fresh and clean drinking water was available to birds all the time. During the entire study period uniform condition of housing, feeding and watering was maintained for all the groups of the experiment.

Egg quality parameter and nutrient composition of egg:
Following egg quality parameter were recorded and nutrient composition was analyzed from the eggs collected (2 eggs from each replicate) at 4 weeks intervals from 31st to 42nd weeks of age (at 30th, 34th, 38th and 42nd weeks age).

a. Egg quality parameter:

- **Egg weight:** Individual egg weight was recorded replicate wise.
- **Shape index:** The egg shape index was derived as 100 times the ratio of maximum breadth to maximum length. The length and breadth of an individual egg were measured with Vernier Caliper and the shape index was calculated as per Schultz (1953) [18].

$$\text{Shape Index} = \frac{\text{Maximum width of egg (cm)}}{\text{Maximum length of egg (cm)}} \times 100$$

- Albumin index (Heiman and Carver, 1936) [7].
  After breaking the egg on smooth surface, the height (mm) of thick albumen was measured at two different points with the help of Spherometer. The average of the measured height was calculated. Similarly, the width of thick albumen from two different places was measured by the help of Vernier Caliper and the average width was calculated. The albumen index was calculated according to Heiman and Carver (1936) [7] as shown hereunder:

$$\text{Albumen Index} = \frac{\text{Average Albumen Height (mm)}}{\text{Average Albumen width (mm)}}$$

- Yolk index (Funk, 1948) [4]
  The height of yolk was taken at the highest point, the diameter was measured at two different points and the average of which was taken for its width. The yolk index was calculated as follow:

$$\text{Yolk Index} = \frac{\text{Height of yolk (mm)}}{\text{Average width of yolk (mm)}}$$

- Haugh Unit (Haugh, 1937) [6].
  It is a measure of internal egg quality in relation to egg weight and calculated as per formula:

H.U. = 100 log (H + 4.18-0.8939 W0.6674)

Where: H= Albumen height in mm; W= Egg weight in g

- Shell thickness will be estimated using Screw-gauge.
  The egg shell thickness was measured with screw gauge at three points, the two narrow ends and in the middle of the egg.

Procedure of fatty acid profile estimation
GC conditions
GLC 5700 supplied by Nucon Engineers and equipped with column SGE Forte GC Capillary Column (column description: 60 m*0.25 mm*70 ìm-BPX70) was used for fatty acid analysis with the following conditions.

Detector: 270 °C
Injector: 260 °C
Oven temperature: initial 120 °C and final 240 °C
Attenuation: 1
Split ratio: 1:10

Helium was used as a carrier gas at constant inlet pressure (205 kPa). Individual Fatty acid was identified by comparing the area and retention time with that of standard mixture of 37 components of FAME Mix (Supelco, Bellefonte, PA) and concentration of individual fatty acids were calculated from their peak area.

Results and Discussion
Egg quality parameters
The overall means and standard error for egg quality parameters (egg weight, shape index, albumin index, yolk index, haugh unit and shell thickness) in all treatment groups have been presented in table 3, 4, 5, 6, 7 and 8. Weekly egg weight was recorded replicate wise whereas, shape index, albumin index, yolk index, haugh unit and shell thickness were estimated at 4 weeks interval from 31st to 42nd weeks of age.

Table 3: Average egg weight (g) of Kadaknath birds in different treatment groups

Age in Weeks	T_0	T_1	T_2	T_3	T_4	T_5
31st	45.51±0.87	43.80±1.24	44.60±0.81	43.52±0.86	44.38±0.77	44.96±0.84
32nd	43.80±1.24	43.81±1.10	44.42±0.96	44.50±0.90	44.58±1.09	43.52±0.67
33rd	44.20±1.28	44.40±1.28	45.00±1.14	43.46±0.97	44.30±0.86	44.88±0.70
34th	45.00±1.48	45.10±1.14	45.00±1.19	44.64±1.12	45.62±1.34	45.06±0.98
35th	44.60±1.07	45.44±0.54	45.00±0.68	43.34±0.75	44.60±1.03	45.50±0.85
36th	42.60±1.07	44.00±1.17	43.20±0.80	43.12±0.64	43.16±1.02	44.04±0.43
37th	43.00±1.41	44.30±0.96	44.40±1.24	43.42±0.82	43.40±1.36	44.88±0.78
38th	43.40±0.87	44.20±1.01	43.40±1.12	42.69±1.02	44.18±1.42	43.56±0.76
39th	43.20±0.87	43.00±0.70	43.90±0.64	43.32±0.74	44.00±1.41	45.68±0.61
40th	45.20±1.49	43.50±0.83	45.40±1.06	44.16±1.24	44.72±1.09	43.58±0.83
41st	44.00±1.09	43.80±0.80	45.20±0.96	45.14±0.79	44.42±1.04	44.62±0.33
42nd	44.40±1.16	44.60±0.67	44.00±1.22	44.00±1.07	46.14±0.62	44.38±0.82
Avg	44.07±0.45	44.16±0.39	44.46±0.25	43.80±0.16	44.46±0.34	44.55±0.31

Mean egg weight (g/bird) of Kadaknath birds in different treatment groups differed non-significantly in all the groups.

Average egg weight (g) in group T_0, T_1, T_2, T_3, T_4 and T_5 was 44.07±0.45, 44.16±0.39, 44.46±0.25, 43.80±0.16, 44.46±0.34 and 44.55±0.31, respectively. Our results were in agreement with Petrovic *et al*. (2012) [14] as they reported non-significant change in egg weight on diets containing 1%, 2%, 3% or 4% of linseed oil. Similarly, Chaurasiya *et al*. (2021) [3] reported non-significant change in egg weight with designer diet (3% Linseed oil + 200 mg Vit E + 0.2 mg Organic Se + 80 mg Organic Zn +100 mg Organic Fe+ 0.2 mg Organic Cr per kg feed fed to Jabalpur colour bird. However, contrary to our result, Omar *et al*. (2014) [12] reported significant increase in egg weight on supplementation of fish oil, linseed oil and its combination in the layer diet.

Table 4: Average shape index of eggs of Kadaknath birds in different treatment groups

Age in Weeks	T_0	T_1	T_2	T_3	T_4	T_5
30th	72.00±1.38	73.80±1.24	73.40±0.98	71.80±1.02	71.20±0.58	71.40±1.08
34th	71.20±1.02	73.20±0.86	73.60±0.93	71.40±1.17	70.40±0.93	70.60±1.08
38th	71.80±0.58	73.60±1.03	73.40±0.75	72.00±0.55	71.80±0.66	71.00±0.71
42nd	71.80±0.58	73.80±1.24	73.60±1.17	71.80±0.37	72.60±1.17	70.80±1.16

The mean shape index of eggs laid by birds of the Kadaknath did not differ significantly in all treatments. At the beginning of the experiment average shape index of egg in group T_0, T_1, T_2, T_3, T_4 and T_5 was 72.00±1.38, 73.80±1.24, 73.40±0.98, 71.80±1.02, 71.20±0.58 and 71.40±1.08, respectively. The average shape index of egg within a group did not change significantly (p>0.05) throughout the experimental period and at the end of experiment it was 71.80±0.58, 73.80±1.24, 73.60±1.17, 71.80±0.37, 72.60±1.17 and 70.80±1.16 in respective groups; T_0, T_1, T_2, T_3, T_4 and T_5.
Our results were in agreement with Petrovic *et al*. (2012) [14] as they reported non-significant change in egg weight on diets containing 1%, 2%, 3% or 4% of linseed oil. Contrary to our findings, Radwan *et al*. (2008) [16] postulated that the supplementation of 200 mg vitamin E/kg diet to hen's diets numerically increased (p≤0.05) the percentage of egg shape index.

Table 5: Average albumin index of eggs of Kadaknath birds in different treatment groups

Age in Week	T_0	T_1	T_2	T_3	T_4	T_5
30th	11.56±0.33	10.24±0.29	10.58±0.43	12.16±0.73	12.38±0.87	10.70±0.25
34th	11.76±0.30	10.60±0.54	10.86±0.37	11.42±0.61	10.58±0.24	11.26±0.58
38th	11.54±0.36	11.60±0.83	10.54±0.49	10.78±0.46	10.38±0.41	12.98±0.72
42nd	11.58±0.26	10.40±0.39	10.70±0.29	12.36±1.03	11.64±0.95	11.52±0.51

At the beginning of the experiment average albumin index of eggs in group T_0, T_1, T_2, T_3, T_4 and T_5 was 11.56±0.33, 10.24±0.29, 10.58±0.43, 12.16±0.73, 12.38±0.87and 10.70±0.25, respectively. The average albumin index of egg did not change significantly within a group (p>0.05) throughout the experiment period and at the end of experiment it was 11.58±0.26, 10.40±0.39, 10.70±0.29, 12.36±1.03, 11.64±0.95and 11.52±0.51in respective groups; T_0, T_1, T_2, T_3, T_4 and T_5.
Average albumin index of eggs of Kadaknath birds in different treatment groups did not differ significantly. Consistent with our results, Chaurasiya *et al*. (2021) [3] did not observe significant effect by supplementing designer diet.
Our results were in agreement with Petrovic *et al*. (2012) [14] as they reported non-significant change in albumin index on diets containing 1%, 2%, 3% or 4% of linseed oil. Contrary to our findings, Radwan *et al*. (2008) [16] postulated that the supplementation of 200 mg vitamin E/kg diet to hen's diets numerically increased (p≤0.05) the percentage of egg albumin index.

Table 6: Average yolk index of eggs of Kadaknath birds in different treatment groups

Age in Weeks	T_0	T_1	T_2	T_3	T_4	T_5
30th	44.04±0.94	41.16±0.76	41.34±0.74	43.72±1.16	43.26±1.19	42.68±0.79
34th	43.08±1.08	43.78±0.48	43.38±0.83	45.64±0.49	44.62±1.06	43.22±0.69
38th	44.48±0.85	42.72±1.12	42.46±0.89	45.38±0.47	44.30±0.41	42.99±1.07
42nd	42.46±0.70	43.40±1.25	40.70±0.24	44.36±1.33	44.74±1.52	41.88±1.08

At the beginning of the experiment average yolk index of egg in group T_0, T_1, T_2, T_3, T_4 and T_5 was 44.04±0.94, 41.16±0.76, 41.34±0.74, 43.72±1.16, 43.26±1.19 and 42.68±0.79, respectively. The average yolk index of egg did not change significantly within a group (p>0.05) throughout the experiment period and at the end of experiment it was 42.46±0.70, 43.40±1.25, 40.70±0.24, 44.36±133, 44.74±1.52 and 41.88±1.08 in respective groups; T_0, T_1, T_2, T_3, T_4 and T_5.

Mean yolk index of eggs of Kadaknath birds in different treatment groups did not differ significantly due to designer diet supplementation. Our results were in agreement with Petrovic *et al*. (2012) [14] as they reported non-significant change in egg weight on diets containing 1%, 2%, 3% or 4% of linseed oil. Contrary to our findings, Radwan *et al*. (2008) [16] postulated that the supplementation of 200 mg vitamin E/kg diet to hen's diets numerically increased (p≤0.05) the percentage of yolk index.

Table 7: Average Haugh index of eggs of Kadaknath birds in different treatment groups

Age in Weeks	T_0	T_1	T_2	T_3	T_4	T_5
30th	85.40±0.58	83.04±0.88	83.62±0.83	82.92±0.79	83.36±0.74	83.50±1.29
34th	84.92±0.92	83.50±0.51	83.80±0.79	82.00±0.24	84.18±1.24	83.84±1.23
38th	85.80±1.04	82.52±1.00	82.00±0.89	84.38±0.09	83.94±0.69	83.24±0.61
42nd	85.10±1.43	84.34±2.53	81.86±0.67	87.32±0.36	82.96±0.51	82.10±0.21

At the beginning of the experiment average Haugh index of egg in group T_0, T_1, T_2, T_3, T_4 and T_5 was 85.40±0.58, 83.04±0.88, 83.62±0.83, 82.92±0.79, 83.36±0.74and 83.50±1.29, respectively. The average Haugh index of egg did not change significantly within a group ($p>0.05$) throughout the experiment period and at the end of experiment it was 85.10±1.43, 84.34±2.53, 81.86±0.67, 87.32±0.36, 82.96±0.51and 82.10±0.21in respective groups; T_0, T_1, T_2, T_3, T_4 and T_5.

Mean Haugh unit of eggs of Kadaknath birds in different treatment groups did not differ significantly. Similar to our findings, Petrovic *et al.* (2012) [14] as they reported non-significant change in egg weight on diets containing 1%, 2%, 3% or 4% of linseed oil.

Table 8: Average shell thickness (mm) of eggs of Kadaknath birds in different treatment groups

Age in Weeks	T_0	T_1	T_2	T_3	T_4	T_5
30th	0.36±0.01	0.33±0.01	0.34±0.01	0.33±0.00	0.34±0.00	0.33±0.00
34th	0.37±0.01	0.34±0.01	0.33±0.01	0.34±0.01	0.35±0.01	0.33±0.01
38th	0.35±0.00	0.34±0.00	0.33±0.01	0.35±0.01	0.34±0.00	0.34±0.01
42nd	0.37±0.01	0.34±0.01	0.33±0.01	0.35±0.01	0.33±0.00	0.33±0.01

At the beginning of the experiment average shell thickness of egg in group T_0, T_1, T_2, T_3, T_4 and T_5 was 0.36±0.01, 0.33±0.01, 0.34±0.01, 0.33±0.00, 0.34±0.00 and 0.33±0.00, respectively. The average shell thickness of egg did not change significantly within a group ($p>0.05$) throughout the experiment period and at the end of experiment it was 0.37±0.01, 0.34±0.01, 0.33±0.01, 0.35±0.01, 0.33±0.00 and 0.33±0.01 in respective groups; T_0, T_1, T_2, T_3, T_4 and T_5.

Mean shell thickness of eggs of Kadaknath birds in different treatment groups did not differ significantly due to designer diet. Similar to our findings, Petrovic *et al.* (2012) [14] as they reported non-significant change in egg weight on diets containing 1%, 2%, 3% or 4% of linseed oil.

Fatty acid profile of eggs

The result pertaining to the effect of designer diets on fatty acid profile of kadaknath eggs have been presented in table 9, 10, 11 and 12. Result revealed prominent changes between the treatment and control groups.

Table 9: Mean saturated fatty acid (SFA) content in egg yolk (% of total fatty acid) of Kadaknath birds in different treatment groups

Age of Bird (Wks)	T_0	T_1	T_2	T_3	T_4	T_5
30th week	34.11^{d}±0.02	33.17^{abc}±0.46	32.42^{a}±0.18	33.74^{cd}±0.10	33.53^{bcd}±0.11	32.89^{ab}±0.33
34th week	34.58^{c}±0.23	32.61^{a}±0.21	32.76^{a}±0.31	33.58^{b}±0.14	33.44^{b}±0.10	32.64^{a}±0.32
38th week	34.42^{d}±0.13	32.53^{a}±0.17	32.40^{a}±0.15	33.87^{cd}±0.19	33.33^{bc}±0.08	32.89^{ab}±0.33
42nd week	34.41^{c}±0.20	32.58^{a}±0.29	32.78^{a}±0.24	33.81^{b}±0.07	34.00^{bc}±0.20	32.23^{a}±0.09

Means bearing different superscript with in row showing significant difference ($p<0.05$)

At the 42nd week of age mean saturated fatty acid (SFA) content in egg yolk (% of total fatty acid) was significantly decreased in T_5 group (32.23±0.09), T_1 group (32.61±0.21) and T_2 group (32.78±0.24) compared to control group. In conformity with our results Kralik *et al.* (2008) [10] stated that the increased concentration of linseed oil in layer diets resulted in less SFA ($p<0.001$) and more n-3 PUFA in egg yolks. Similarly, Batkowska *et al.* (2021) [2] reported the total content of SFA was reduced in the linseed oil supplemented diet.

Table 10: Average mono unsaturated fatty acid (MUFA) content in egg yolk (% of total fatty acid) of Kadaknath birds in different treatment groups

Age of Bird (Wks)	T_0	T_1	T_2	T_3	T_4	T_5
30th week	43.46^{a}±0.02	43.60^{ab}±0.07	43.62^{ab}±0.10	43.67^{b}±0.02	43.75^{b}±0.03	43.61^{ab}±0.02
34th week	43.48±0.01	43.60±0.03	43.58±0.08	43.72±0.01	43.69±0.04	43.63±0.07
38th week	43.46^{a}±0.01	43.59^{ab}±0.08	43.62^{ab}±0.02	43.74^{b}±0.02	43.75^{b}±0.04	43.74^{b}±0.09
42nd week	43.47^{a}±0.02	43.57^{ab}±0.03	43.63^{abc}±0.05	43.74^{bc}±0.05	43.73^{bc}±0.03	43.88^{c}±0.17

Means bearing different superscript with in row showing significant difference ($p<0.05$)

At the 34th week of age MUFA content of egg yolk was statistically ($p>0.05$) similar in all the groups and it was 43.48±0.01, 43.60±0.03, 43.58±0.08, 43.72±0.01, 43.69±0.04 and 43.63±0.07 in group T_0, T_1, T_2, T_3, T_4 and T_5, respectively. But from 38th week onward the MUFA content of egg yolk changed with supplementation and it significantly ($p<0.05$) increased in the supplemented groups. At the 42nd week of age significantly ($p<0.05$) highest MUFA content in egg yolk was found in the group T_5 (43.88±0.17). Promila *et al.* (2017) [15] corroborate our results as they reported that the layers fed basal diet supplemented with linseed oil at levels of 1, 2, 2.5, 3, 3.5 and 4% had significant ($p<0.05$) decrease in palmitic (C: 16), stearic (C: 18) and oleic acid (18:1) content being lowest values of MUFA in 4% linseed oil supplemented group.

Table 11: Mean poly unsaturated fatty acid (PUFA) content in egg yolk (% of total fatty acid) of Kadaknath birds in different treatment groups

Age of Bird (Wks)	T_0	T_1	T_2	T_3	T_4	T_5
30th week	22.32ᵃ±0.03	23.82ᵈ±0.19	23.43ᶜ±0.02	22.82ᵇ±0.19	22.89ᵇ±0.02	23.57ᶜᵈ±0.05
34th week	22.36ᵃ±0.05	23.52ᶜ±0.18	23.41ᶜ±0.07	23.02ᵇ±0.10	22.94ᵇ±0.10	23.45ᶜ±0.12
38th week	22.52±0.05	23.55±0.03	23.48±0.02	23.29±0.68	22.69±0.04	23.25±0.15
42nd week	22.49ᵃ±0.06	23.53ᶜ±0.04	23.56ᶜ±0.04	22.89ᵇ±0.02	22.79ᵇ±0.04	23.49ᶜ±0.15

Means bearing different superscript with in row showing significant difference ($p<0.05$)

At 34th week of age the PUFA content in egg yolk changed due to supplementation and it was significantly ($p<0.05$) increased in the supplemented groups. The significantly ($p<0.05$) higher PUFA content in egg yolk was reported in the group T_1 (23.52±0.18), T_5 (23.45±0.12) and T_2 (23.41±0.07) compared to control group (22.49±0.06). Further, at 42nd week of age PUFA content was significantly ($p<0.05$) increased in the group T_2, T_1 and T_5 (23.56±0.04, 23.53±0.04 and 23.49±0.15, respectively) compared to control group (22.49±0.06).

Mean poly unsaturated fatty acid (PUFA) content in egg yolk (% of total fatty acid) in different treatment groups was significantly improved in the designer diets groups. Our findings were in accordance with Omar *et al*. (2014) [12] as they stated that the diets containing linseed oil resulted in a significant increase of polyunsaturated fatty acid concentrations in eggs compared to the control group. Consistent to our findings, Panaite *et al*. (2024) [13] reported significant increase in concentration of omega-3 polyunsaturated fatty acids after feeding flaxseed meal and different antioxidant sources to laying hens. Promila *et al.* (2017) [15] also corroborate our results as they reported that the layers fed basal diet supplemented with linseed oil at levels of 1, 2, 2.5, 3, 3.5 and 4% had significant ($p<0.05$) increase in linoleic acid (C18:2) linolenic acid (18:3) and arachidonic acid (C20:4) content being highest values of PUFA in 4% linseed oil supplemented group. Likewise, Batkowska *et al*., (2021) [2] stated that the content of the PUFA, largely determines the dietary value of eggs, as they concluded that the inclusion of oils (soybean or linseed) in the diet contributed to an increase in PUFA concentration in the yolk, which was an undoubted benefit in terms of health-promoting value.

Table 12: Mean values of ω 6: ω 3 ratio in egg yolk of Kadaknath birds in different treatment groups

Age (Wks)	T_0	T_1	T_2	T_3	T_4	T_5
30th	14.67	14.29	14.3	13.50	14.21	14.29
34th	12.50ᵇ	6.25ᵃ	7.71ᵃ	11.19ᵇ	11.13ᵇ	7.69ᵃ
38th	12.48ᵇ	5.92ᵃ	7.68ᵃ	11.14ᵇ	11.11ᵇ	6.67ᵃ
42nd	11.11ᵇ	5.88ᵃ	6.67ᵃ	11.13ᵇ	11.12ᵇ	6.25ᵃ

Means bearing different superscript with in row showing significant difference ($p<0.05$)

Initially at 34th week of age the ω 6: ω 3 ratio in egg yolk was statistically ($p>0.05$) similar in all the groups and it was 14.67, 14.29, 14.3, 13.50, 14.21and 14.29 in groups T_0, T_1, T_2, T_3, T_4 and T_5, respectively. But after 34th week of age the ω6:ω3 ratio in egg yolk changed due to designer diets and it was significantly ($p<0.05$) improved in the flaxseed, fish oil and combined supplemented groups. At the end of experiment similar pattern of ω6:ω3 ratio of egg yolk was noticed as the ω 6: ω 3 ratio in egg yolk was significantly ($p<0.05$) improved in flaxseed @ 10% per kg diet supplemented group (T_1), combined designed diet supplemented group (T_5) and fish oil @ 1.5% group (T_2) as valued at 5.88, 6.25 and 6.67, respectively. However, supplementation of vitamin E 1000 mg/kg feed(T_3), muti-enzymes 2 g/kg feed(T_4) and control group (T_0) valued at 11.13, 11.12 and 11.11, respectively and did not show any significant ($p>0.05$) impact on ω6:ω3 ratio.

Being aware of n-3 PUFA benefits and health promoting effects, the nutritionists recommend a diet rich in n-3 fatty acids, with a lowered n-6/n-3 ratio (2-4:1) in eggs from the currently commonly found n-6/n-3 ratio in eggs (15-20:1). According to Kralik *et al.* (2008) [10], the ratio of n-6/n-3 PUFA in egg yolk lipids less than 4:1 is considered to be beneficial for human health. The present findings were in conformity with Gordana *et al*., (2021) [5] reported decreased P n-6 PUFA/P n-3 PUFA ratio from 8.69 to 4.54 in laying hens fed diets containing different shares of fish oil as (FO) in combination with soybean oil (SO). Similarly, Jinyi *et al.* (2023) [9] reported the significant reduction in n-6:n-3 ratio in the egg yolk under influence of flaxseed-based diet in alone and in combination with protease and carbohydrase enzymes. Likewise, Mattioli *et al*. (2016) [11] also reported positive effect of 10% extruded flaxseed supplementation on the n-3 long-chain polyunsaturated fatty acids content in eggs (3.25 vs. 0.92 mg/g in control diet eggs), mainly DHA content in the eggs.

Conclusion

It may be concluded that the egg quality parameters like shape index, albumen index, yolk index, haugh index and shell thickness were not affected by designer diets. Incorporation of combination supplemented group, 1.5% fish oil and 10% flaxseed (T_5, T_1 and T_2) significantly decreases saturated fatty acid and Increase Mono-unsaturated fatty acid and polyunsaturated fatty acid content of egg. The ω:3 PUFA was increased by 2-2.5 times by using designer diet.

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