ISSN: 0976-3031

Available Online at http://www.recentscientific.com

CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research
Vol. 9, Issue, 7(E), pp. 28050-28056, July, 2018

International Journal of Recent Scientific Research

DOI: 10.24327/IJRSR

Research Article

NUTRITIONAL FORTIFICATION OF POULTRY PRODUCTS BY DIETARY SUPPLEMENTATION OF OMEGA-3 FATTY ACIDS FROM *NANNOCHLOROPSIS GADITANA L.M. LUBIAN*

Abirami, S[1]., Murugesan, S[1*] and Narender Sivaswamy, S[2]

[1]Department of Botany, Division of Algal Biotechnology and Bionano Technology, Pachaiyappa's College, Chennai-600 030, India
[2]SynkroMax Biotech Pvt Ltd, Chennai. Tamil Nadu, India

DOI: http://dx.doi.org/10.24327/ijrsr.2018.0907.2383

ARTICLE INFO

Article History:
Received 4th April, 2018
Received in revised form 25th May, 2018
Accepted 23rd June, 2018
Published online 28th July, 2018

Key Words:
Feed supplement, *Nannocholorpsis gaditana,* DHA, omega 3 fatty acids, fortification

ABSTRACT

Access to affordable and quality food is one of the key challenges of our time to feed a growing world population, to feed it adequately and to feed it using sustainable production practices. Many of the world's key environmental problems today are related to agricultural practices. Agriculture and food industries are also uniquely positioned to make a constructive contribution towards efforts designed to address these problems. The new category of food products named functional foods have extended further interest which has been fuelled by increased media attention and an increasing number of consumers determined to take greater responsibility for their own health. The main objective of the present study is to explore *Nannochloropsis gaditana* as an effective dietary feed supplement for the production of fortified poultry eggs and meat with essential nutrients especially omega-3 fatty acids, antioxidants and pigments. The results shown that the retention of docosohexaenoic acid (DHA) in the albumin of experimental group hens fed with basal di*et al*ong with 10% processed *N. gaditana* was 699.67 mg whereas 430 mg (per 100 g of egg mass) in the control group hens fed only with the basal diet. Similarly, the quantity of DHA in the experimental group egg yolk was 599 mg while the same in the control group was 419.67 mg per 100 g of egg mass.

INTRODUCTION

Recent days consumers fascinate towards safe, natural and environmentally friendly products with additional health benefits, those foods are termed as functional foods. Functional foods consist of vitamins, omega-3 fatty acids and antioxidants. Poultry meat and eggs were considered as one of the effective functional food as they were the perfect package of all necessary nutrients (Peric L *et al*,2011). Marine environment has always proved to be a wide supply of important food and feed nutrients (Evans F and Critchley A, 2014). Habitually marine animals and plants were utilized, directly or indirectly in the form of nutrient supplements for human beings (Hallsson S V,1964). They produce different beneficial secondary metabolites which include essential fatty acids, flavonoids, alkaloids, pigments and antioxidants, etc. With wide range of biodiversity the exploration of bioactive compounds from the marine source remains a great challenge for the researchers (Rasmussen R S and Morrissey M T, 2007). Currently, research in algae is promising due to the usefulness in the improvement of existing food stuffs and introducing new products (Choi YJ *et al*, 2012). The theory of fortified foods is introduced to minimize the health risks and enhance the nutrient profiling of a wellbeing. Fortification of foods can be achieved by adding novel supplements or by changing the conventional active compounds. Microalgae such as *Chlorella* sp., *Scenedesmus* sp., *Nannochloropsis* sp., *Spirulina* sp., and *Aphanizomenon flos-aquae* have been utilized as the source of nutritional compounds (Gantar M and Svircev Z. 2008)

MATERIALS AND METHODS

Sample preparation

Nannochloropsis gaditana L.M. Lubian was isolated from the offshore water of the Pondicherry Coast of Southeast India and were undergone optimization of different culture parameters (Abirami S *et al,* 2017). Crude *N. gaditana* concentrate was

Corresponding author:* **Murugesan, S
Department of Botany, Division of Algal Biotechnology and Bionano Technology, Pachaiyappa's College, Chennai-600 030, India

harvested carefully such that to prevent any loss of the bioactive compounds after the mass cultivation.

Feeding experimental design and formulation

In the present study the feeding trial was carried out in Sri Venkateshwara farm, Namakkal, Tamil Nadu. A total number of 1400 Lohmann breed laying hens were selected and grouped into control and experimental, each with replicate of 350 birds. The layers were housed in a wire cage, and were subjected to acclimatization of the environmental conditions. The layers were fed mash diets; designed into two groups control and experimental where, the experimental feed was included with 10% dosage of *N. gaditana* supplement along with the basal diet. The protocol for the experiment was followed by Bureau of Indian standard methods of tests for animal feeds and feeding stuffs, Part 1: General methods (IS: 7874: R.2004) and parameters were tested in the Omegaa analytical testing and research center (Recognized by OHSAS 18001:2007), Namakkal, Tamil Nadu

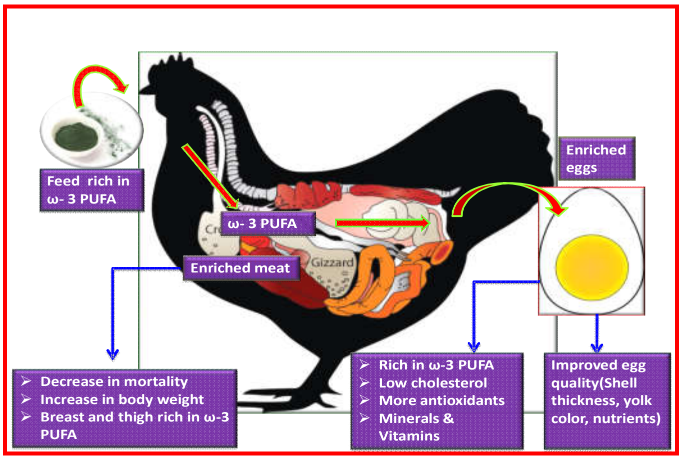

Figure 1 Pictographic overview of enrichment of eggs and meat by dietary supplementation of N.gaditana

Dietary composition

The ingredients of the two diet group control and experimental were shown in the tables 1 and 2 below:

Table 1 Basal diet composition – Control group (kg)

Maize	**350.00**
Broken rice	160.00
Jower	25.00
Deoiled rice bran	117.00
Soya	110.00
Distiller dried grains	50.00
Rapeseed	30.00
Groundnut cake	25.00
Soy flour	75.00
Shell grit	75.00
Calcite Powder	30.00
Dicalcium phosphate	3.00
Total	**1050.00**
Lysine	0.25
Methionine	1.00
N. gaditana supplement	0.00
Phytase 2500IU	0.10
Trace mineral	1.00
Sodium bicarbonate	0.50
Salt	3.00
Liver powder	0.25
Choline chloride	0.25
Toxin binder	1.00
Premix	0.25
Vitamin-C	0.10

Table 2 Basal diet composition- Experimental group (Kg)

Maize	**340.00**
Broken rice	160.00
Jower	35.00
Deoiled rice bran	37.00
Soya	100.00
Distiller dried grains	50.00
Rapeseed	25.00
Groundnut cake	30.00
Soy flour	65.00
Shell grit	75.00
Calcite Powder	30.00
Dicalcium phosphate	3.00
Total	**1050.00**
Lysine	0.25
Methionine	1.00
*N. gaditana*supplement	0.10
Phytase 2500IU	0.10
Trace mineral	1.00
Sodium bicarbonate	0.50
Salt	3.00
Liver powder	0.25
Choline chloride	0.25
Toxin binder	1.00
Premix	0.25
Vitamin-C	0.10

Growth performance

The growth performance and feed utilization of experimental hens were evaluated by standard methods (Siddhuraju P and Becker K, 2003) in terms of final body weight (g), weight gain (WG, %), feed conversion ratio (FCR) and protein efficiency ratio (PER). Feed consumption and mortality were recorded on a daily basis and all birds were individually weighed at weekly intervals. The major parameters used in measuring productivity from the chicken were weight gain, mortality, egg production rates (hen day) and efficiency of feed utilization. Feed intake and body weight were measured every week and feed conversion (feed/gain) was calculated (Roy S S *et al*, 2011).

Egg quality parameters

The egg quality refers to several standards which define both internal as well as external qualities. While the external quality is characterized by shell cleanliness, texture and shape, the internal quality are taken care of the egg white (albumin), viscosity and yolk strength. Feed intake (g/hen/d) was recorded weekly by replicate. Eggs were collected daily, and egg production and egg mass (grams of egg produced per day) were determined weekly. The egg weight and shell weight were measured with an electronic balance to the nearest 0.01 g, by excluding cracked and soft-shell eggs.

Eggshell thickness (without shell membrane) of the eggs was measured by a dial pipe gauge. The breaking strength of uncracked eggs was measured with an eggshell strength tester (FHK, Fujihira Ltd., Tokyo, Japan). The eggshell color and yolk color were measured by using the egg multitester provided by TSS (Technical services and supplies Ltd, York, England). The individual Haugh Unit (HU) score was calculated using the egg weight and albumen height. The Haugh Unit value was calculated for individual eggs using the following formula (Oke O E *et al*,2014). $HU = 100 \log_{10} (H + 7.5 - 1.7W^{0.37})$ Where, H = Observed height of the albumen in mmW = Weight of egg in grams. The egg yolk color was determined using the Roche yolk color fan. The shell thickness was measured by a micrometer screw gauge after air drying at room temperature (Ogunwole O A *et al*, 2015).

Biochemical analysis

The chemical analysis for moisture, proximate and minerals were determined according to the procedures outlined by the Association of Official Analytical Chemists (AOAC International, 2000). Calcium and phosphorus were measured by atomic absorption spectrophotometer.

Omega-3 fatty acid composition of egg yolk

At the end of the experiment of fourteen weeks, the eggs were randomly selected from each treatment (4 eggs/cage), and the fatty acid composition of egg yolk was determined according to the modified method (Kim Y *et al*, 2003). The lipid from the egg yolk was extracted with hexane/isopropanol (3:2 v/v). The extracted lipids were mixed with 0.5 mL of toluene and 2 mL of 5% KOH-MeOH, and heated at 70°C for 8 min; and then 2 mL of 14% NaOH-BF_3-MeOH was added to the above mixture, and heated at 70°C for 2 mins. The fatty acid methyl esters (FAME) were extracted with 3 mL of 5% NaCl and 1 mL of hexane. The samples were analyzed for total fatty acids, using gas chromatography with a flame ionization detector (Hewlett Packard 5890 Series II, Palo Alto, CA, USA). The fatty acid methyl esters (FAME) was separated using a Supelcowax-10 fused silica capillary column (100 m×0.32 mm×0.25 µm; Supelco, Inc., Bellefonte, PA, USA), with 1.2 mL/min of helium flow. The oven temperature was increased from 220 to 240°C, at the rate of 2°C/min. Temperatures of the injector and detector were 240 and 250°C, respectively. The peak of fatty acids was identified by comparing the retention time and peak area of each fatty acid standard. The content of each fatty acid was expressed as a percentage of the sum of all the fatty acids analyzed.

RESULTS AND DISCUSSION

The mortality rate of the control group was in the range of 0.15 to 0.72%, whereas, it was in the range of 0.14 to 0.29% in the experimental group supplemented with *N. gaditana,* and no mortality was found during the 5th week in the experimental group (Table 3). Hen's diet supplemented with 10% N. gaditana was found to be better in overall egg production, egg weight and feed efficiency.

In the present study, supplementation of 10% *N. gaditana* in layer mash seemed to have advantages in improving the egg production. A part of increase in egg production might due to the improvement of *N. gaditana* availability. The inclusion of 10% *N. gaditana* diet decreased hen feed intake by 1.75% and increased the egg production rate by 1.1% (Table.4). A similar study on inclusions of algae in diets for poultry has shown that algae can be used safely at dietary levels of 5 to 10% (Halle I *et al*, 2009). Zheng *et al*, 2012 reported that the inclusion of microalgae (*Chlorella vulgaris*) in the laying hen feed had positive effects on the egg production (Table.5).

Table 3 Effect of 10% *N. gaditana* feed supplement on survival rate of laying hens

Period (Week)	No. of hens: Control Group	No. of hens: Experimental Group	Mortality: Control Group	Mortality: Experimental Group	Mortality (%): Control Group	Mortality (%): Experimental Group
1	700	700	3	2	0.43	0.29
2	697	698	2	2	0.29	0.29
3	695	696	4	1	0.58	0.14
4	691	695	5	1	0.72	0.14
5	686	694	3	0	0.44	0.00
6	683	694	3	1	0.44	0.14
7	680	693	2	2	0.29	0.29
8	678	691	1	1	0.15	0.14
9	677	690	2	1	0.30	0.14
10	675	689	3	2	0.44	0.29
11	672	687	3	2	0.45	0.29
12	669	685	2	2	0.30	0.29
13	667	683	1	1	0.15	0.15
14	666	682	1	1	0.15	0.15
	Mortality		**35**	**19**	**0.37**	**0.20**

Physico-chemical parameters

The physico-chemical parameters of an egg include egg weight, shell thickness, yolk color, egg quality and nutrient concentration which were discussed in the present study (Table 6). The results from the current study indicated that supplementation of *N. gaditana* had a significant effect on laying rate, egg weight; egg mass and feed conversion were observed. The amount of albumin in an egg differs between genetic strains and changes with the age of the hen (Silversides F G and Villeneuve P,1994)

Yolk color

The effect of supplementation of *N. gaditana* on yolk color in comparison with basal diet is shown in Table.7. The supplementation of 10% dosage of *N. gaditana* in layer mash significantly improved yolk color during the 5th and 10th weeks in comparison with the basal diet. The average yolk color score of the control group eggs was 6 and that of the experimental group eggs was 9. Yolk weight and color have shown a significant difference in the experimental group compared to control group due to the availability of excess pigments in the *N. gaditana* fed hens. Lipstein *et al*, 1980 also observed more intensive yellow color of the yolk when the laying hens were fed with given microalgae supplemented diet. It was also postulated that the carotenes and xanthophylls from the microalgae influence the egg yolk color (Arakawa S *et al*, 1960)

Nutritional composition of hen eggs

The moisture, energy, protein, carbohydrates, lipids, fatty acids, cholesterol, calcium, magnesium, phosphorus, potassium, selenium, iron, chromium, iodine, zinc, thiamine, riboflavin, niacin, folic acid, cyanocobalamine, pyridoxine, tocopherol, ascorbic acid and total antioxidant content of the designer eggs were found to be marginally higher than the control group. Of concern were the lowest levels of tocopherol, thiamine, cyanocobalamine content found in both the eggs. In the present study, protein content of experimental group eggs was found to be 15.33 g, whereas it was 13.32 g in control group eggs (Table.8).The *Arthrospira platensis* and *Chlorella* contain up to 70% dry wt protein; these microalgae, also have an amino acid profile that compares well with egg, notably containing all of the essential amino acids (EAA) that humans cannot synthesize and must obtain from foods (Eyster K M 2007). Microalgae contain 8-71% crude protein, and their amino acid profiles are superior to those of many feed proteins (Becker E W 2004)

Table 4 Effect of dietary supplementation of *N. gaditana* on feed intake, feed conversion ratio, protein efficiency and body weight in laying hens

Trial Week	Feed intake (g/day)			Feed conversion ratio (kg/d/hen)			Protein efficiency (g/d/hen)			Body Weight (g)		
	Control Group	Experimental Group	t	Control group	Experimental Group	t	Control Group	Experimental Group	t	Control Goup	Experimental Group	t
1	110 ± 1.00	107 ± 1.00**	3.674	0.80 ± 0.10	1.00 ± 0.10 NS	2.449	0.12 ± 0.01	0.17 ± 0.01**	6.124	1531 ± 1.00	1529 ± 1.00 NS	2.449
2	108 ± 1.00	108 ± 1.00 NS	0.000	1.20 ± 0.10	1.00 ± 0.15 NS	1.265	0.13 ± 0.01	0.18 ± 0.01**	6.124	1533 ± 1.00	1533 ± 1.00 NS	0.000
3	109 ± 1.00	106 ± 1.00**	3.674	1.20 ± 0.10	1.20 ± 0.10 NS	0.000	0.16 ± 0.01	0.23 ± 0.01**	8.573	1536 ± 1.00	1537 ± 1.00 NS	1.225
4	109.± 1.52	108 ± 1.00 NS	1.265	1.30 ± 0.10	1.20 ± 0.10 NS	1.225	0.17 ± 0.01	0.24 ± 0.01**	8.573	1534 ± 1.00	1536 ± 1.00 NS	2.449
5	108 ± 1.00	106 ± 1.00 NS	2.449	1.30 ± 0.10	1.23 ± 0.15 NS	0.632	0.17 ± 0.01	0.23 ± 0.01**	5.060	1539 ± 1.00	1540 ± 1.00 NS	1.225
6	107 ± 1.00	104 ± 1.00**	3.674	1.40 ± 0.10	1.26 ± 0.20 NS	1.000	0.16 ± 0.01	0.27 ± 0.01**	13.472	1543 ± 1.00	1547 ± 1.00**	4.999
7	110 ± 1.00	107 ± 1.00**	3.674	1.30 ± 0.10	1.20 ± 0.10 NS	1.225	0.17 ± 0.01	0.26 ± 0.01**	8.854	1547 ± 1.00	1549 ± 1.00 NS	2.449
8	111 ± 1.00	108 ± 1.00**	3.674	1.40 ± 0.20	1.20 ± 0.10 NS	1.549	0.18 ± 0.01	0.40 ± 0.10**	3.792	1550 ± 1.00	1553 ± 1.00**	3.674
9	107 ± 1.00	108 ± 1.00 NS	1.225	1.40 ± 0.10	1.20 ± 0.10 NS	2.449	0.30 ± 0.01	0.32 ± 0.01 NS	0.345	1553 ± 1.00	1557 ± 1.00**	4.899
10	108 ± 1.00	105 ± 1.00**	3.674	1.40 ± 0.10	1.23 ± 0.15 NS	1.581	0.20 ± 0.10	0.33 ± 0.01 NS	2.283	1556 ± 1.00	1560 ± 1.00 NS	4.899
11	109 ± 1.00	106 ± 1.00**	3.674	1.50 ± 0.10	1.20 ± 0.10**	3.674	0.20 ± 0.10	0.40 ± 0.10 NS	2.449	1560 ± 1.00	1563 ± 1.00**	3.674
12	106 ± 1.00	106 ± 1.00 NS	0.000	1.46 ± 0.15	1.26 ± 0.20 NS	1.342	0.22 ± 0.01	0.30 ± 0.10 NS	1.379	1564 ± 1.00	1565 ± 1.00 NS	1.225
13	107 ± 1.00	107 ± 1.00 NS	0.000	1.50 ± 0.10	1.23 ± 0.15 NS	2.530	0.24 ± 0.01	0.35 ± 0.00**	17.000	1567 ± 1.00	1569 ± 1.00 NS	2.449
14	111 ± 1.00	106 ± 1.00**	6.124	1.46 ± 0.20	1.20 ± 0.10 NS	2.000	0.22 ± 0.01	0.36 ± 0.02**	9.168	1570.33 ± 1.52	1571 ± 1.00 NS	0.632

* Values are expressed as Mean ± SEM, n=3, as t- test. Significant at 1% level, ** Significant at 5% level (<0.05), NS Non significant

Table 5 Effect of dietary supplementation of *N. gaditana* on egg production, egg productivity, egg weight and egg mass in laying hens

Week	Total no. of eggs			Egg productivity			Egg Weight			Egg mass (g/egg/hen/day)		
	Control Group	Experimental Group	t	Control Group	Experimental Group	t	Control Group	Experimental Group	t	Control Group	Experimental Group	t
1	4621 ± 1.00	4651 ± 1.00**	36.742	94.20 ± 0.10	94.80 ± 0.10**	7.348	55.30 ± 0.10	56.40 ± 0.10**	13.472	540.94 ± 0.02	553.69 ± 0.01**	877.745
2	4620 ± 33.77	4636 ± 1.0**	0.820	94.33 ± 0.15	95.03 ± 0.41	2.734	55.50 ± 0.10	57.50 ± 0.10**	24.495	543.80 ± 0.01	563.60 ± 0.10**	242.999
3	4594 ± 1.00	4610 ± 0.57**	24.500	94.47 ± 0.15	94.60 ± 0.10	1.265	55.80 ± 0.10	58.50 ± 0.10**	33.068	546.84 ± 0.10	573.29 ± 0.01**	323.90
4	4584 ± 1.00	4611 ± 1.00**	33.068	94.80 ± 0.10	94.67 ± 0.15	1.265	56.00 ± 1.00	58.73 ± 1.58	2.522	548.70 ± 0.10	557.61 ± 0.01**	152.499
5	4570 ± 1.00	4601 ± 1.00**	37.967	95.20 ± 0.10	97.60 ± 0.10**	29.394	56.63 ± 0.15	57.50 ± 0.10**	8.222	553.60 ± 0.10	563.40 ± 0.10**	120.025
6	4555 ± 1.00	4606 ± 1.00**	62.462	95.30 ± 0.10	94.80 ± 0.10**	6.124	57.00 ± 1.00	58.50 ± 0.10	2.585	548.70 ± 0.58	573.40 ± 0.10**	302.512
7	4544 ± 1.00	4621 ± 1.00**	94.305	95.50 ± 0.10	95.20 ± 0.10**	3.674	57.23 ± 0.15	56.40 ± 0.10**	7.906	560.89 ± 0.10	553.60 ± 0.10**	21.249
8	4501 ± 1.00	4601 ± 1.00**	122.474	94.67 ± 0.15	95.23 ± 0.15**	4.543	58.00 ± 1.00	56.53 ± 0.15	2.511	558.50 ± 0.01	553.60 ± 0.10**	60.012
9	4430 ± 1.00	4603 ± 1.00**	211.881	93.60 ± 0.10	95.30 ± 0.10**	20.821	57.30 ± 0.10	57.70 ± 0.10**	4.899	560.55 ± 0.01	566.42 ± 0.01**	557.193
10	4410 ± 1.00	4586 ± 1.00**	215.555	93.50 ± 0.10	95.20 ± 0.10**	20.821	58.00 ± 1.00	57.53 ± 0.15	0.799	558.50 ± 0.10	563.54 ± 0.02**	85.600
11	4350 ± 1.00	4576 ± 1.00**	276.792	92.50 ± 0.10	95.23 ± 0.15**	25.931	57.50 ± 0.10	58.40 ± 0.10**	11.023	563.49 ± 0.01	573.30 ± 0.10**	169.071
12	4310 ± 16.46	4536 ± 1.00**	23.735	91.80 ± 0.10	94.60 ± 0.10**	34.293	57.60 ± 0.10	60.00 ± 1.00**	4.136	563.52 ± 0.02	578.23 ± 0.15**	164.386
13	4250 ± 1.00	4501 ± 1.00**	307.411	92.00 ± 1.00	94.23 ± 0.15**	3.824	58.00 ± 1.00	59.20 ± 0.10	2.068	558.60 ± 0.10	580.17 ± 0.01**	369.377
14	4201 ± 1.00	4411 ± 1.00**	257.196	90.23 ± 0.15	92.50 ± 0.10**	21.503	57.50 ± 0.10	58.36 ± 0.37**	3.833	563.57 ± 0.15	570.46 ± 0.18**	50.260

Values are expressed as Mean ± SEM, n=3, as t- test. Significant at 1% level, ** Significant at 5% level (<0.05), NS Non-significant

Table 6 Effects of dietary supplementation of *N. gaditana* on egg quality

At the end of 6th week	Control Group	Experimental Group	t	Sig.
Egg weight (g)	56.0 ± 1.00	58.40 ± 0.10	4.136	0.014
Albumin weight (g)	38.0 ± 1.00	39.60 ± 0.10	2.758	0.051 NS
Yolk weight (g)	17.0 ± 1.00	18.80 ± 0.10	3.102	0.036
Haugh unit	83.0 ± 1.00	85.40 ± 0.10	4.136	0.014
Shell weight (g)	5.30 ± 0.10	5.54 ± 0.01	4.136	0.014
Shell thickness (mm)	0.32 ± 0.01	0.37 ± 0.01	6.124	0.004
At the end of 12th week				
Egg weight (g)	57.63 ± 0.15	59.10 ± 0.10	13.914	0.000
Albumin weight (g)	39.26 ± 0.15	39.70 ± 0.10	4.111	0.015
Yolk weight (g)	18.23 ± 0.15	19.53 ± 0.15	10.423	0.000
Haugh unit	82.50 ± 0.10	85.20 ± 0.10	33.068	0.000
Shell weight (g)	5.10 ± 0.10	5.36 ± 0.01	4.481	0.011
Shell thickness (mm)	0.32 ± 0.01	0.36 ± 0.01	4.111	0.015

Values are expressed as Mean ± SEM, n=3, as t- test. Significant at 1% level,
** Significant at 5% level (<0.05), NS Non significant

Table7 Yolk color values of eggs laid by supplemented diets with microalga *N. gaditana*

Period (Week)	Yolk color values		t	Sig.
	Control Group	Experimental Group		
1	6 ± 1.00	7 ± 1.00	1.225	0.22 NS
5	5 ± 1.00	9 ± 1.00	4.899	0.00
10	6 ± 1.00	9 ± 1.00	3.674	0.02
14	7 ± 1.00	9 ± 1.00	2.449	0.07 NS

Values are expressed as Mean ± SEM, n=3, as t- test. Significant at 1% level** Significant at 5% level (<0.05), NS Non significant

Effect of supplementation of N. gaditanaon DHA content in egg yolk and eggs

Chen *et al.*, (2011) reported that the fish oils were the prominent sources of omega-3 fatty acids, for various functional food fortifications. Also number of studies has been conducted to enrich eggs with n-3 fatty acids through diet manipulations (Abril R and Barclay W, 1998; Jia, W 2008).

Table 8 Nutritive content of normal and designer eggs

S.No	Composition (Unit)	Basal diet	Basal diet with 10% *N. gaditana*	t	Sig.
1	Moisture (g)	74.26 ± 0.01	72.37 ± 0.01	178.669	0.000
2	Energy (Calories)	143.37 ± 0.15	148.80 ± 0.10	51.545	0.000
3	Protein (g)	13.32 ± 0.01	15.33 ± 0.01	161.42	0.000
4	Carbohydrates (g)	0.833 ± 0.00	2.02 ± 0.01	203.12	0.000
5	Total lipids (g)	9.63 ± 0.01	8.52 ± 0.01	88.99	0.000
6	Cholesterol (g)	0.133 ± 0.00	0.205 ± 0.00	80.97	0.000
7	Calcium (mg)	78.20 ± 0.01	91.26 ± 0.15	123.96	0.000
8	Magnesium (mg)	5.92 ± 0.01	5.95 ± 0.01	3.479	0.025
9	Phosphorus (mg)	389.27 ± 020	385.57 ± 0.15	24.820	0.000
10	Potassium (mg)	106.37 ± 0.05	105.53 ± 0.15	8.839	0.001
11	Selenium (mg)	0.294 ± 0.00	0.943 ± 0.01	73.224	0.000
12	Iron (mg)	4.86 ± 0.01	6.34 ± 0.02	99.281	0.000
13	Chromium (mg)	0.03 ± 0.00	1.22 ± 0.01	134.03	0.000
14	Iodine (mg)	0.02 ± 0.00	0.95 ± 0.01	159.12	0.000
15	Zinc (mg)	1.36 ± 0.02	1.42 ± 0.02	3.674	0.021
16	Thiamine (Vitamin-B_1) (mg)	0.186 ± 0.00	0.206 ± 0.00	13.416	0.000
17	Riboflavin (mg) (Vitamin- B_2)	0.725 ± 0.01	0.536 ± 0.00	19.731	0.000

Quantities represent an edible portion of about 100 g.Values are expressed as Mean ± SEM, n=3, as t- test.
Significant at 1% level, ** Significant at 5% level (<0.05), NS Non significant

The supplementation of 10% *N. gaditana* feed resulted in an average of 250 ± 1.00 mg of DHA in control group albumin whereas 499.67± 1.52 mg in the experimental group, and 399.67 ± 1.00 mg was observed in control yolk, whereas, in the experimental group yolk it was found to be 599± 1.00 mg per 100 g of egg in the sixth week of the trial (Table.9). Similarly, during eleventh week, the average of albumin was 240 ± 1.00 mg in control group eggs and 520.33± 0.57 mg in the experimental group. The yolk DHA in the experimental group (449.67 ± 1.52 mg) was found to be higher than that of the control group (240.33± 1.52 mg).

Incorporation of DHA in poultry meat fed with experimental diet

Fatty acid profile of poultry meat can be altered depending on the fatty acid profile of food used in poultry nutrition (Qi K K *et al*,2010). The DHA content was very less in poultry meat of thigh and breast muscles of the control group in the sixth week. Whereas, in the experimental group, the DHA content of poultry meat of thigh and breast muscle got increased significantly to 0.10%, when supplemented with 10% *N. gaditana* (Table.10). Similarly, Taulescu *et al.,* (2010) established that the content of PUFA in broiler muscle tissue increased significantly when flax seed was introduced into broiler nutrition.

Economic efficiency

In the present study, the economic efficiency of birds fed with *N. gaditana* was superior to that of the basal diet. From the table it was found that total profit per bird when supplementing 10% dose *N.gaditana* would be Rs 1.14 (Table.11).

Along with the enrichment of meat with polyunsaturated fatty acids, the strategy of supplementing of food using antioxidants must be developed, in order to prevent oxidation of lipids in meat and subsequently improve the sensory quality of meat (Grau A *et al*, 2001; Bou R *et al*,2004; Kotrbacek V *et al,* 2013). Omega-3 fatty acids were reported earlier that they may easily be incorporated into the tissues of meat-type poultry (Cortinas L *et al*, 2004). The beneficial effect of dietary supplementation of *N. gaditana* on egg production might be related to the positive effect on intestinal micro flora as it provides beneficial prebiotic sources utilized by gut and intestinal microflora. Those cecal lactic acid bacteria might affect pathogenic micro flora such as *S. enteritidis* (Surachon P *et al* 2011), thus improving the host's health status and productivity. In the present study, the infectious micro flora population got reduced by the supplementation of *N. gaditana.*

Table 9 Effect of *N. gaditana* supplement on the retention of DHA in the yolk and albumin

Weeks	Study-I						Study-II					
	Albumin (mg)			Yolk (mg)			Albumin (mg)			Yolk (mg)		
	Control Group	Experimental Group	t	Control Group	Experimental Group	t	Control Group	Experimental Group	t	Control Group	Experimental Group	t
6	250 ± 1.00	300 ± 1.00**	61.237	399.00 ± 1.00	599.00 ± 1.00**	24.494	430.00 ± 1.00	699.67 ± 1.52**	255.82	260.00 ± 1.00	341.00 ± 1.00**	92.204
11	240 ± 1.00	280 ± 1.00**	48.990	419.67 ± 1.52	449.67 ± 1.52**	24.054	399.67 ± 1.52	520.33 ± 0.57**	127.98	240.33 ± 1.52	300.33 ± 1.52**	48.107

Values are expressed as Mean ± SEM, n=3, as t- test. Significant at 1% level, ** Significant at 5% level (<0.05), NS Non significant.

Table 10 DHA content (%) of poultry meat

S.No	Sample	Thigh muscle				Breast muscle		
		Control Group	Experimental Group	t	Sign.	Control Group	Experimental Group	t
1	Study 1	0.00 ± 0.00	0.10 ± 0.00	28.000	0.000	0.00 ± 0.00	0.10 ± 0.00	28.000
2	Study 2	0.00 ± 0.00	0.10 ± 0.00	28.000	0.000	0.00 ± 0.00	0.10 ± 0.00	28.000

Values are expressed as Mean ± SEM, n=3, as t- test. Significant at 1% level,
** Significant at 5% level (<0.05), NS Non significant.

Table 11 Economic efficiency of basal diet and supplementation of *N. gaditana* on laying hens

Items	Basal diet	Basal diet supplemented with 10% *N.gaditana*	Return on investment	Return on investment/Bird
No. of layer hens	350	350	700	-
Total feed consumption (kg)	7224	7200	-	-
Feed cost (1 kg)	20	19.75	-	-
Feed consumption for raising layer hens (kg)	144480	142200	2280	3.26
100g *N.gaditina* cost (Rs.3,000/kg)	0	Rs.300/-	-	-
Mortality	35	19	4000	5.71
No. of eggs /hen/day	62510	64137		
Egg price (Rs.)	3.15	3.15	5125.05	7.32
Body weight of layer chicken (g)	1548	1550	2	-
1 kg slaguther weight price (Rs.)	52	52	72.8	0.104
Total revenue/hen/day	0.17	0.17	-	-
Total profit for experimental group up to 14 weeks/Bird	-	-	-	16.4
Total profit for experimental group up to 1 week/Bird	-	-	-	1.17

CONCLUSION

In the present study, the n-3 fatty acid concentration in eggs was found to be significantly increased by supplementation of 10% of *N. gaditana*. Considering that *N. gaditana* contains high EPA, the dietary nutrients were directly incorporated into the eggs. It can be proposed that *N. gaditana* fed to layers are the best feeding practice that is able to decrease the n-6 to n-3 fatty acid ratio in the eggs. According to the combined results of nutritional composition, yolk color and consumer acceptability of egg-derived products supplemented with *N. gaditana* fed seem to be the effective fortification of poultry products. Additionally, clinical investigations of the liver, trachea and intestines of the experimental group, observed normal without any infections, may be due to the uptake of omega-3 fatty acids, antioxidants and prebiotic polysaccharides from *N. gaditana.* It may also reduce the use of synthetic antibiotics, thus reducing the potential for developing antibiotic resistance. The improvement in feed formulation and limiting exposure to infectious agents through biosecurity, supportive therapy, cleaning and disinfection were also recommended for the commercial production of enriched products

Acknowledgement

The authors sincerely thank PG and Research Department of Botany, Unit of Algal Biotechnology and BionanoTechnology, Pachaiyappa's College, Chennai for permitting the field work, the Management of Synkro Max Biotech Pvt Ltd, Chennai for laboratory assistance and technical support. Also would thank Dr V Karthikeyan from Sri Venkateshwara farm,Namakkal for the immense guidance throughout the poultry study.

References

1. Peric, L., Rodic, V and Milosevic, N. 2011. Production of poultry meat and eggs as functional food - challenges and opportunities. *Biotechnology in Animal Husbandry.* 27 (3): 511-520.
2. Evans, F and Critchley, A. 2014. Seaweeds for animal production use. *J. Appl. Phycol*. 26: 891–899.
3. Hallsson, S.V. 1964. The uses of seaweed in Iceland. *Paper presented at the Proc. of the fourth international seaweed symposium.,* Biarritz, France.
4. Rasmussen, R.S and Morrissey, M.T. 2007. Marine biotechnology for production of food ingredients. *Adv. Food Nutr. Res.* 52: 237–292.
5. Choi, Y.J., Lee, S.R and Oh, J.W. 2012. Effects of dietary fermented seaweed fusiforme on growth performance, carcass parameters and immunoglobulin concentration in broiler chicks. *Asian Austral. J. Anim. Sci.* 27: 862-870.
6. Gantar, M and Svircev, Z. 2008. Microalgae and cyanobacteria: food for thought. *J. Phycol.* 44: 60-68.
7. Abirami, S. *et al*2017. Screening and optimization of culture conditions of *Nannochloropsisgaditana*for omega 3 fatty acid production. *Journal of Applied Biology & Biotechnology*. 5 (03): 013-017
8. Siddhuraju, P and Becker, K. 2003. Comparative nutritional evaluation of differentially processed mucuna seeds [*Mucunapruriens* (L.) DC. var. utilis (Wall ex Wight) Barker ex Burk] on growth performance, feed utilisation and body composition in *Niletilapia* (*Oreochromisniloticus*). *Aquaculture.* 34:487-500
9. Roy, S.S., Chaudhuri, A., Mukherjee, S., HomeChauduri, S and Pal, R. 2011. Composite algal supplementation in nutrition of *Oreochromismossambicus*. *J. Algal Biomass Utln.* 2 (1): 10 – 20.
10. Oke, O.E., Ladokun, A.O and Onagbesan, O.M. 2014. Quality parameters of eggs from chickens reared in deep litter system with or without access to grass or legume pasture. *Livestock Research for Rural Development*. 26 (11).
11. Ogunwole, O.A., Ojelade, A.Y.P., Oyewo, M.O and Essien, E.A. 2015. Proximate Composition and Physical Characteristics of Eggs from Laying Chickens Fed Different Proprietary Vitamin-Mineral Premixes Under Two Rearing Systems During Storage.*International Journal of Food Science and Nutrition Engineering*. 5(1): 59-67

12. AOAC International, AOAC. 2000. Official Methods of Analysis. Vol. 17th Ed. Association of Official Analytical Chemists, Arlington, VA, USA.
13. Kim, Y. J., Lee, K. W., Lee, S., Kim, H and Lee, H.J. 2003. The production of high-purity conjugated linoleic acid (CLA) using two-step urea-inclusion crystallization and hydrophilic arginine-CLA complex. *J. Food Sci.* 68:1948-1951
14. Halle, I., Janczyk, P., Freyer, G and Souffrant, W.B. 2009. Effect of Microalgae *Chlorella Vulgaris* on Laying Hen Performance. *Arch. Zootech*. 12: 5-13.
15. Zheng, L., Oh, S.T., Jeon, J. Y., Moon, B.H., Kwon, H.S., Lim, S U., An, B.K and Kang, C.W. 2012. The dietary effects of fermented *Chlorella vulgaris* (CBT) on production performance, liver lipids and intestinal microflora in laying hens. *Asian Australas. J. Anim. Sci*. 25: 261-266
16. Silversides, F.G and Villeneuve. P. 1994. Is the Haugh unit correction for egg weight valid for eggs stored at room temperature.*Poult. Sci.* 73: 50-55
17. Lipstein, B., Hurwitz, S and Bornstein, S. 1980. The nutritional value of algae for poultry. Dried *Chlorella* in layer diets. *Br. Poult. Sci.* 21: 23-27
18. Arakawa, S., Tsurumi, N., Murakami, K., Muto, S., Hoshino, J and Yagi, T. 1960. Experimental breeding of White leghorn with the *Chlorella*-added combined feed. *Jpn. J. Exp. Med*. 30: 185-192
19. Eyster, K.M. 2007. The membrane and lipids as integral participants in signal transduction: lipid signal transduction for the non-lipid biochemist. *AdvPhysiol Educ.* 31:5–16.
20. Becker, E.W. 2004. Microalgae in human and animal nutrition. In: Richmond A, editor. *Handbook of microalgal culture*. Oxford: Blackwell. p 312–51.
21. Chen, J., Jiang, Y., Ma, K. Y., Chen, F and Chen, Z. Y. 2011. Microalga decreases plasma cholesterol by down-regulation of intestinal NPC1L1, hepatic LDL receptor, and HMG-CoA reductase. *J. Agric. Food Chem*. 59: 6790-6797
22. Abril, R and Barclay, W. 1998. Production of docosahexaenoic acid-enriched poultry eggs and meat using an algae-based feed ingredient.*World Rev Nutr Diet*. 83:77-88
23. Jia, W., Slominski, B.A., Guenter, W., Humphreys, A and Jones, O. 2008. The effect of enzyme supplementation on egg production parameters and omega-3 fatty acid deposition in laying hens fed flaxseed and canola seed.*Poult. Sci.* 87 (10): 2005- 2014.
24. Qi, K.K., Chen, J.L., Zhao, G.P., Zheng, M.Q and Wen, J. 2010. Effect of dietary x6/x3 on growth performance, carcass traits, meat quality and fatty acid profiles of Beijing-you chicken. *Journal of Animal Physiology and Animal Nutrition*. 94:474-485
25. Taulescu, C., Mihaiu, M., Bele, C., Matea, C., Dan, S.D., Mihaiu, R., Lapusan, A and Ciupa, A. 2010. Manipulating the fatty acid composition of poultry meat for improving. Consumer's Health Bulletin UASVM. *Veterinary Medicine*, 67: 2-12
26. Grau, A., Codony, R., Grimpa, S., Baucells, M.D and Guardiola, F. 2001. Cholesterol oxidation in frozen dark chicken meat: Influence of dietary fat source, and *α*-tocopherol and ascorbic acid supplementation. *Meat Science*. 57: 197-208.
27. Bou, R., Guardiola, F., Tres, A., Barroeta, A. C and Codony, R. 2004. Effect of dietary fish oil, *α*-tocopheryl acetate, and zinc supplementation on the composition and consumer acceptability of chicken meat. *Poultry Science,* 83: 282- 292.
28. Kotrbacek, V., Skrivan, M., Kopecky, J., Penkava, O., Hudeckova, P., Uhríkova, I and Doubek, J. 2013. Retention of carotenoids in egg yolks of laying hens supplemented with heterotrophic *Chlorella*. *Czech J. Anim. Sci*. 58:193-200
29. Cortinas, L., Villaverde, C., Galobart, J., Baucells, M.D., Codony, R and Barroeta A.C. 2004. Fatty acid content in chicken thigh and breast as affected by dietary polyunsaturation level.*Poult. Sci.* 83: 1155-1164
30. Surachon, P., Sukon, P., Chaveerach, P., Waewdee, P and Soikum, C. 2011. Screening of lactic acid bacteria isolated from chicken ceca for in vitro growth inhibition of *Salmonella enteritica*serovarEnteritidis. *J. Anim. Vet. Advan.* 10: 939-944

How to cite this article:

Abirami, S *et al.* 2018, Nutritional Fortification of Poultry Products by Dietary Supplementation of Omega-3 Fatty Acids from Nannochloropsis Gaditana l.m. Lubian. *Int J Recent Sci Res.* 9(7), pp. 28050-28056.
DOI: http://dx.doi.org/10.24327/ijrsr.2018.0907.2383

****