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TRIALS ON METAL ENRICHED *SPIRULINA PLATENSIS* SUPPLEMENTATION ON POULTRY GROWTH

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ABSTRACT
Zinc and iron are the key minerals in the development of broiler poultry birds. The study was undertaken to check the applicability of *Spirulina platensis* enriched with iron and zinc as a feed supplement for broilers. *Spirulina platensis* an isolate from Lonar lake was mass cultivated in race way open ponds. The biomass on dewatering exposed to metal solutions (Zn and/or Fe, 100 mg/lit) for enriching through biosorption and accumulation. The dewatered biomass was pelletized and sun dried. Metal enrichment in *Spirulina* was confirmed by metal analysis of biomass and feeds under trial. *Spirulina* pellets were used in the formulation of poultry diets as starter, grower and finisher feed. The trials were conducted with 1000 Indbro fast white broiler in five dietary treatments groups consisting of 200 chicks per group. The birds were inspected daily for their body weight, feed consumed and mortality. Feed Conversion Ratio (FCR) and Specific Growth Rate (SGR) were estimated from the experimental data. Rise is body weight with simultaneous decrease in feed consumption was noticed with *Spirulina* supplemented feeds. The variation in the values was statistically nonsignificant at $p < 0.05$. No adverse effects on growth performance of broilers were seen during the trials. The trials on broiler poultry have shown the promise to make poultry feed cost effective especially if it is formulated with locally available cheap and low nutritive feed ingredients. Inclusion of metal enriched *Spirulina* biomass in poultry feed not only provides essential micronutrients and minerals in bioavailable form but also can reduce the overall cost and dependency on commercially available feeds.

KEYWORDS: Iron, Zinc, Poultry feed, Metal enrichment, *Spirulina*.

INTRODUCTION

Poultry industry is one of the most profitable businesses in the agriculture sector, providing meat and eggs that add to the nutritional values of human diet. Broiler industry has become an attractive business due to its quick returns and ever increasing market. The poultry meat is rich in protein and provides all essential nutrients. However, the quality of meat, concentration of nutrients and fatty acid composition depends on the diet fed to the poultry. Availability of quality feed at a reasonable cost is one of the limiting factors in the success of business. The feed cost incurred is about 60-65% of the total cost of broiler production (Basak *et al*., 2002). Recently, the feed business is directed towards the use of natural ingredients as an alternative to antibiotics, synthetic colours and other chemicals. *Spirulina,* the blue green alga has been used as human food supplement for over 20 years. Use of *Spirulina* as high quality natural feed additive in animal and poultry nutrition is relatively recent (Belay *et al*., 1996). *Spirulina* contains higher amounts of protein (55-65%) and all essential amino acids, vitamins and minerals (Doreau *et al*., 2010). Also, it is a rich source of carotenoids and fatty acids, especially gamma-linolenic acid (GLA) which infers health benefits (Guroy *et al*., 2012) and has been used throughout the world as a feed component in broiler and layer diets to enhance yolk colour, flesh and egg fertility (Ross and Dominy, 1990). It also improves both, cell mediated and mononuclear phagocytic system potential in chickens allowing them to resist diseases. The higher carotenoid content of *Spirulina* helps in supplementation of vitamin A, provides antioxidant activity and enhances immunity (Qureshi *et al*., 1996) helps in hormonal regulation and plays additional roles in growth, reproduction and maturation (Nikodémusz *et al*., 2010). Trace minerals, such as zinc (Zn) and iron (Fe) are essential for broiler growth and are involved in many digestive, physiological, and biosynthetic processes within the body. Zinc participates as a cofactor or component of more than 240 enzymes, being important for protein and carbohydrate metabolism, growth, and reproduction. In poultry, zinc serves not only as a nutrient but can also be used as a dietary supplement to manipulate the reproductive system of the birds (Park *et al*., 2004). Though zinc is widely distributed throughout the body, animals have a limited capacity for storing it in a form that can be mobilized rapidly to prevent deficiency (Fraker *et al.,* 1987). Iron is the another essential mineral involved in a varied number of metabolic processes, but with a major function in oxygen transport as a component of haemoglobin and myoglobin molecules (Bess *et al*., 2012).Traditionally, these trace minerals are supplemented in the form of inorganic salts, such as sulfates, oxides, and carbonates, to provide the level of minerals for preventing clinical deficiencies and allowing the birds to reach their genetic growth potential, or both. There are dual benefits from the iron and zinc supplementation of broilers; health improvement and improvement in quality of product for human consumption. The content of microelements in livestock feed is currently regulated by feeding standards. Since the content of microelements in conventional feeds frequently does not meet these requirements, they are supplemented as mineral feed additives. In innovative bio-supplementation, a natural biomass can be enriched with microelements by biosorption/bioaccumulation, and then used as the feed additive for providing specific element/nutrient (Chojnacka, 2007; Zielinska and Chojnacka, 2009; Michalak *et al*., 2011).

The aim of the present study was to investigate the applicability of *Spirulina platensis* enriched with iron and zinc as a feed supplement for the broilers. The study was undertaken to test the feasibility of zinc and/or iron enriched biomass as the bio-supplementation of broiler feed to achieve a balanced diet.

MATERIALS & METHODS

Preparation of *Spirulina platensis* meal

The cyanobacterial culture *Spirulina platensis* used in the present study was isolated from alkaline water body (Lonar Lake, MS). *Spirulina platensis* was cultivated in an open race way pond (dimensions 1.8 m × 4.8 m) of 2000 liter capacity, covered with a green net sheet (Fig. 1). The pond was equipped with a paddle for mixing the biomass and the culture was harvested by 300 mesh cloth. *Spirulina platensis* was cultivated in the formulated medium containing 1 gm/liter, commercial fertilizer (Sujala 19:19:19, Make: Rashtriya Chemicals & Fertilizers Ltd, India); 16.6 gm/liter, commercial sodium bicarbonate; 2.67 gm/liter, sodium chloride and 0.05 gm/liter, magnesium sulphate. The harvested biomass of *Spirulina platensis* was enriched with iron and zinc by biosorption in batch mode. The enrichment process was performed in 10 liter vessel containing 6 liter metal solution (100 mg/liter each of zinc or iron at pH 5.0) and 500 g of *Spirulina* paste (containing ≅25% dry weight) at room temperature for 30 min. A mixture of zinc and iron (500 mg/liter each) was used to enrich the biomass with both the metals. After metal biosorption, the biomass was refiltered, extruded into pellets (size diameter ≤1 mm × length, 2-3 mm) using locally available mould and sun dried. The pellets were pooled up and stored in plastic bags until used for feeding.

FIGURE 1: Cultivation to trials on poultry feed supplementation of *Spirulina platensis*

a, Mass cultivation of *Spirulina platensis;* b, Dewatering of biomass; c, Pellets for feed supplementation; d, One day old broiler chicks; e, 42 days old birds

Feed formulation

The experimental diet formulations were prepared based on the nutritional requirements of poultry in accordance with the Bureau of Indian Standards BIS (1992). The composition of formulated feeds was as shown in Table 1.

Feed Production

Mash feed was produced in the form of coarse powder using small feed mill at Yashwant Agritech Pvt. Ltd. Hatcheries and Breeding Farms, Paldhi, Dist. Jalgaon, MS, India. Five feed diets were prepared supplemented with *Spirulina platensis* biomass; consisted of starter (0-1 week), grower (2-3 week) and finisher (4-6 week).

Poultry trials

The trials were conducted with 1000 Indbro fast white broiler day old chicks up to 42 days of age at Rahul poultry farm, Ghumaval, Dist. Jalgaon, MS. Broiler chicks were equally divided and distributed in five dietary treatments groups consisting of 200 chicks per group (Table 1). From each dietary treatment group 10 chicks were weighed for taking measuring the poultry growth in response to the feeding treatments. The experimental birds were managed properly including housing environment, providing floor space, feeder-waterer space, litter bed, lighting and medication.

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TABLE 1: Design of experimental treatments varying composition of formulated feeds and their nutritional values as per the

Feed ingredient (%)	Treatment and poultry diet														
	Starter diet					Grower diet					Finisher diet				
	T1	T2	T3	T4	T5	T1	T2	T3	T4	T5	T1	T2	T3	T4	T5
Maize	53	53	53	53	53	53.7	53.7	53.7	53.7	53.7	58	58	58	58	58
Spirulina platensis	0	1[a]	1[b]	1[c]	1[d]	0	1[a]	1[b]	1[c]	1[d]	0	1[a]	1[b]	1[c]	1[d]
Soybean meal (DOC)	41.35	40.23	40.33	40.33	40.33	39	37.88	37.98	37.98	37.98	33.7	32.38	32.48	32.48	32.48
Edible oil	2	2.22	2.22	2.22	2.22	3.55	3.87	3.87	3.87	3.87	4.60	4.97	4.97	4.97	4.97
Dicalcium phosphate (DCP)	1.4	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.45	1.4	1.4	1.4	1.4
Lime Stone Powder (LSP)	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6
Common Salt	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
DL-Methionine	0.2	0	0	0	0	0.2	0	0	0	0	0.2	0.2	0.2	0.2	0.2
Vitamin + Trace minerals	0.15	0.15	0.05	0.05	0.05	0.15	0.15	0.05	0.05	0.05	0.15	0.15	0.05	0.05	0.05
Nutritional estimations															
ME (kcal/kg)	3000	3000	3000	3000	3000	3100	3100	3100	3100	3100	3200	3200	3200	3200	3200
% Crude Protein	23	23	23	23	23	22	22	22	22	22	20	20	20	20	20
% Calcium	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
% P	0.5	0.5	0.5	0.5	0.5	0.6	0.6	0.6	0.6	0.6	0.7	0.7	0.7	0.7	0.7

a, Native *Spirulina;* b, Zn enriched *Spirulina*; c, Fe enriched *Spirulina*; d, Zn-Fe enriched *Spirulina*; ME, Metabolizable Energy; CP, Crude protein

The medications were given according to suggested vaccination program from Indbro Company. The birds were inspected daily for their body weight, feed consumed and mortality. Feed Conversion Ratio (FCR) was calculated as ratio of total feed consumed and body weight. Appropriate controls were run wherein, the feed with no addition of *Spirulina* pellets was fed to birds. Specific growth rate (%SGR) was estimated as using following equation.

% SGR = [*ln* (Final Body Weight)-*ln* (Initial Body Weight)/Age of birds (days)] 100

Metal analysis

In order to ascertain and quantify the metal enrichment the pelleted *Spirulina* biomass as well as formulated feed was digested with 5 ml of 70% perchloric acid and 10 ml of 15 M nitric acid in a COD digester (Model: 2015M, Make: Spectralab Inst. Pvt. Ltd., India) at 150°C for 2 hr. The digested product was reconstituted to 10 ml with 0.1 M hydrochloric acid and used for metal analysis using atomic absorption spectrophotometry (Model: S2, Make: Thermo, USA) using the 'SOLAAR' software. Analyses were performed using hollow cathode lamps using wavelength of 248.3 nm and 213.9 nm iron and zinc respectively. Air-acetylene flame was generated using a fuel flow rate of 0.8 to 1.1 lit/min. All analyses were replicated three times.

Statistical analysis

All the data presented in the paper are the mean values of minimum 10 observations ± SD. Data were statistically assessed using one-way ANOVA to identify differences between the mean values of different treatments at the statistical significance, $p < 0.05$.

RESULTS

Metal enrichment of *Spirulina platensis*

Data of metal analyses of pelleted *Spirulina platensis* biomass and various feed formulations are shown in Table 2. Enrichment of biomass with essential metals under study was confirmed by metal analysis of *Spirulina* pellets. Biosorption could lead to loading the biomass with zinc and iron to the tune of 5.22 and 24.08 g/kg, respectively. The concentration of zinc and iron in *Spirulina* was increased to 179 and 32 folds as compared to native biomass. Decrease of 20% and 41% in the amount of zinc and iron adsorption, respectively, could be noticed when biomass was exposed to mixed metal solution. Supplementation of *Spirulina* biomass in feeds resulted in to the rise of overall concentration of zinc and iron.

TABLE 2: Metal analysis of *Spirulina* biomass and feeds under trial

Sample	Amount of metal (mg/kg)	
	Zinc	Iron
Native *Spirulina* pellets	29.12 ± 01.87	754.56 ± 08.22
Zn enriched *Spirulina* pellets	5217.62 ± 30.44	790.22 ± 09.13
Fe enriched *Spirulina* pellets	31.45 ± 02.11	24080.65 ± 32.87
Fe-Zn enriched *Spirulina* pellets	4175.55 ± 40.05	14160.26 ± 39.02
Formulated Feed (T1)	58.29 ± 06.34	254.24 ± 07.53
Formulated Feed (T2)	62.88 ± 04.63	272.61 ± 06.50
Formulated Feed (T3)	138.36 ± 09.06	268.49 ± 08.09
Formulated Feed (T4)	61.46 ± 03.33	482.56 ± 07.37
Formulated Feed (T5)	104.75 ± 08.87	380.02 ± 11.14

All the values are the mean of 10 observations ± standard deviation

Growth performance parameters

Body weight

The data on effect of different diets supplemented with iron-zinc enriched *Spirulina platensis* biomass on body weight of broilers are presented in Table 3. There was no significant difference in the body weight of broilers, which was measured at the beginning of the experiment. All the values when statistically assessed using one-way did not show significant difference at $p < 0.05$. Highest body weight (1910g) was obtained for the Treatment-5 (T5). Higher gains in body weight of birds was seen when the feed was supplemented with zinc and/or iron in comparison with control feed (T1) and feed supplemented with native *Spirulina*.

TABLE 3: Body weights of broiler poultry fed with *Spirulina* supplemented feed

Age of birds	Body weight (gm)				
	T1	T2	T3	T4	T5
One day old	42.9 ± 3.43	41.21 ± 2.68	41.44 ± 3.22	42.43 ± 2.28	42.42 ± 2.34
1st week	167.74 ± 2.49	166.08 ± 4.06	172.21 ± 2.71	165.13 ± 3.74	175.04 ± 3.43
2nd week	412.72 ± 3.77	430.21 ± 2.21	451.36 ± 2.83	440.82 ± 3.13	445.24 ± 3.92
3rd week	814.24 ± 2.83	848.07 ± 2.49	864.87 ± 3.89	852.02 ± 3.40	886.08 ± 4.62
4th week	1106.64 ± 4.00	1107.26 ± 3.40	1148.45 ± 2.91	1127.47 ± 4.52	1190.07 ± 4.24
5th week	1593.22 ± 2.36	1617.30 ± 3.77	1700.61 ± 3.33	1641.52 ± 4.14	1780.43 ± 3.65
6th week	1810.24 ± 3.53	1852.64 ± 2.16	1890.89 ± 3.27	1867.26 ± 3.92	1910.11 ± 4.71

All the values are the mean of 10 observations ± standard deviation

Feed consumption

Data on effect of zinc and iron enriched *Spirulina platensis* on broiler feed consumption are presented in Table 4. Feed consumption was almost similar in different dietary treatments and the differences were non-significant at all ages of experiment periods. The minimum feed consumption (3430g) was seen for feed containing *Spirulina* pellets enriched with zinc and iron. Decrease in feed consumption was obtained for treatment T2-T5 in comparison with Control Treatment (T1).

TABLE 4: Feed consumption of broiler in the trials with metal enriched *Spirulina*

Age of birds	Cumulative feed consumption (gm)				
	T1	T2	T3	T4	T5
1st week	186.45 ± 3.40	180.31 ± 3.89	182.36 ± 2.83	176.04 ± 3.13	168.81 ± 3.40
2nd week	540.22 ± 2.49	530.05 ± 3.92	532.02 ± 3.40	528.35 ± 3.27	488.08 ± 3.77
3rd week	1189.56 ± 4.24	1154.44 ± 4.11	1160.53 ± 3.27	1142.81 ± 4.90	1108.34 ± 3.42
4th week	1892.71 ± 4.32	1794.37 ± 4.55	1803.26 ± 4.52	1785.97 ± 3.68	1775.62 ± 4.14
5th week	2931.09 ± 4.35	2862.23 ± 3.43	2867.71 ± 4.81	2855.07 ± 3.80	2794.81 ± 4.89
6th week	3686.26 ± 4.42	3481.21 ± 3.30	3489.92 ± 4.55	3473.28 ± 3.16	3430.44 ± 4.81

All the values are the mean of 10 observations ± standard deviation

Feed conversion ratio

Table 5 shows the feed conversion ratios obtained for the trials on poultry growth fed with various diets. It can be seen that the use of *Spirulina* pellets in the poultry diet resulted in the improvement of FCR. The treatments could be placed in an order on the basis of decreased FCR as T1 < T2 < T4 < T3 < T5. The variation among the FCR calculated for all the treatments did not find significant at $p < 0.05$.

TABLE 5: Feed Conversion of Ratio of broilers in the trials on *Spirulina* supplemented feed

Age of birds	**Feed Conversion Ratio (FCR)**				
	T1	**T2**	**T3**	**T4**	**T5**
1st week	1.11	1.09	1.06	1.07	0.96
2nd week	1.31	1.23	1.18	1.20	1.10
3rd week	1.46	1.36	1.34	1.34	1.25
4th week	1.71	1.62	1.57	1.58	1.49
5th week	1.84	1.77	1.69	1.74	1.57
6th week	2.04	1.88	1.85	1.86	1.80

Specific growth rate

The specific growth rate (%SGR) of the broiler fed with metal enriched *Spirulina* as presented in Table 6 were not significantly different compared to birds fed control diets (T1 and T2). Also, % mortality was also not varied significantly among all the treatments. However, marginal increase in SGR could be seen when poultry feeds are supplemented with *Spirulina* pellets.

TABLE 6: Specific growth rate of broiler fed with *Spirulina* supplemented feed

Age of birds	Specific growth rate (%)				
	T1	T2	T3	T4	T5
1st day to 7th day	19.81	20.06	19.98	19.55	20.39
7th day to 21st day	11.27	11.62	11.65	11.72	11.58
21st day to 42nd day	3.81	3.71	3.72	3.74	3.60
Day 1 to 42nd day	8.96 (2)*	9.07 (3)	9.07 (2)	9.04 (2)	9.06 (2)

[a]Values in parentheses are of % cumulative mortality

DISCUSSION

The overall economy of a broiler is determined by its growth performance. Poor management and feed contribute to reduced growth performance (Salim *et al.*, 2012). The primary role of feed is not only to provide enough nutrients to fulfill metabolic requirements of the performance of broilers. Numerous studies shown that dietary supplementation of *Spirulina* can improve the growth performance of poultry (Ross and Dominy, 1990; Qureshi *et al.*, 1996; Al-Batshan *et al.*, 2001; Raju *et al.*, 2005; Kharde *et al.*, 2012; Kaoud, 2013; Zahroojian *et al.*, 2013; Mariey *et al.*, 2014). The present study is an attempt to assess the effect of supplementation of broiler feed with *Spirulina* enriched with zinc and iron. Use of inorganic salts of minerals can results in the poor availability of minerals, hence increasing the levels may not be useful. In order to increase the bioavailability and accessibility metals can be chelated with biomolecules. Biosorption of metals can be the one of the modes to achieve this. Binding of metals to charges sites on the cells provides shielding effect to the minerals, thus help in maintaining bioavailability. With these views in current study the mineral rich feeds were formulated using biosorptively enriched *Spirulina platensis* biomass. A marginal but statistically nonsignificant rise in body weight, specific growth rate with improvement of feed conversion ratio was seen when broilers fed with zinc and/or iron enriched *Spirulina* biomass. Various researchers have reported that body weight increased with inclusion of *Spirulina platensis* (Saxena *et al.*, 1983; Ross and Dominy 1990; Ross *et al*., 1994, Venkataraman *et al*., 1994; Qureshi *et al*., 1996; Toyomizu *et al*., 2001; Raju *et al*., 2005; Kaoud, 2013). Ross and Dominy (1990) replaced dehulled soybean meal with either 10% or 20% of dry matter of *Spirulina* in the poultry diets fed to White Leghorn cockerel chicks and male broiler chicks and recorded no significant differences on growth, egg production, egg quality, fertility, hatchability through trial period. Similarly, no various in growth parameters were also reported for replacement of fishmeal in a commercial diet with *Spirulina* at isonitrogenous concentrations of 140 gm/kg and 170 gm/kg in the broiler diets by Venkataraman *et al*. (1994). Mariey *et al*. (2014) fed different dietary *Spirulina* levels 0.1, 0.2 and 0.3g *Spirulina*/kg to commercial broiler chickens and recorded feed intake during the starter period, chicks consumed significantly less feeds. Thereafter during finisher period, dietary treatments had no significant effects on feed intake of birds. Generally, total feed intake for the whole Zahid *et al.* (1995) conducted feeding experiments of laying hens with enriched macroalgae with concomitant increase in weight of hens and reported that the experimental period was significantly higher for birds fed with 0.3 gm and 0.2 gm *Spirulina*/kg diet. Kaoud (2013) and Kharde *et al.* (2012) demonstrated that dietary inclusion of *Spirulina platensis* can significantly improve feed conversion ratio of broilers in comparison with the control diets. Bao *et al.* (2007) found that supplementation of iron and zinc (40 mg) improved broiler performance. Saenmahayak *et al.* (2010) found that supplementation of zinc influenced both growth and processing performance of broiler chicks irrespective of sources and levels. Michalak *et al.* (2011) examined the effect of supplementation of feed for laying hens with macroalgae enriched with Cu(II), Co(II), Mn(II), Zn(II) and Cr(III). They found that the eggshell thickness increased in all experimental groups in comparison to the control group, in which the birds were fed with feed supplemented with inorganic salts. The non-significant differences found in the overall growth performance of broilers when fed with native and Zn/Fe enriched *Spirulina* has certainly pointed out that there were no adverse effects of formulated diets. Also, the experimental results have shown promise to make poultry diet cost effective especially if it is formulated with locally available cheap and less nutritive feed ingredients. Inclusion of metal enriched *Spirulina* biomass in poultry feed not only provided essential micronutrients and minerals in bioavailable form. It also given lead to reduce the overall cost and dependency on commercially available feeds.

CONCLUSION

Spirulina platensis has an excellent nutritional profile and could be safely used as feed resource to support broiler production. This study confirmed that *Spirulina platensis* enriched with microelements; iron and zinc can be used to improve the value of feed and to increase the productivity of broiler. Bio-metallic feed additives could constitute an alternative to inorganic mineral salts, which are commonly used in poultry feed industry. The feeding trials with broiler showed that body weight was increased, feed consumption and feed conversion ratio were decreased using enriched iron and zinc in *Spirulina platensis* as feed additives and did not have adverse effects on broiler's health.

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REFERENCES

Al-Batshan, H. A., Al-Mufarrej, S. I., Al-Homaidan, A. A. and Qureshi, M. A. (2001) Enhancement of chicken macrophage phagocytic function and nitrite production by dietary *Spirulina platensis*. *Immunopharma Immunotoxicol.* **23**, 281-307.

Basak, B., Pramanik, Md. A. H., Rahman, M. S., Tarafdar, S. U. and Roy, B. C. (2002) Azolla (*Azolla pinnata*) as a feed ingredient in broiler ration. *Int J Poult Sci.* **1**, 29-34.

Belay, A., Kato, T. and Ota, Y. (1996) *Spirulina (Arthrospira)*: potential application as an animal feed supplement. *J. Appl. Phycol.* **8**, 303-311.

Bess, F., Vieira, S. L., Favero, A., Cruz, R. A. and Nascimento, P. C. (2012) Dietary iron effects on broiler breeder performance and egg iron contents. *Anim Feed Sci Technol.* **178**, 67-73.

Bureau of Indian Standards BIS (1992) Indian Standard Nutrient Requirements for Poultry. BIS, New Delhi.

Chojnacka, K. (2007) Using biosorption to enrich the biomass of *Chlorella vulgaris* with microelements to be used as mineral feed supplement. *World J Microbiol Biotechnol.* **23**, 1139-1147.

Doreau, M., Bauchart, D. and Chilliard, Y. (2010) Enhancing fatty acid composition of milk and meat through animal feeding. *Anim Prod Sci.* **51**, 19-29.

Fraker, J., Jardieu, P. and Cook, J. (1987) Zinc deficiency and immune function. *Arch Dermatol.* **123**, 1699-1701.

Guroy, B., Sahin, I., Mantoglu, S. and Kayali, S. (2012) *Spirulina* as a natural carotenoid source on growth, pigmentation and reproductive performance of yellow tail cichlid *Pseudotropheus acei*. *Aquacult Int.* **20**, 869-878.

Kaoud, H. A. (2013) Effect of *Spirulina platensis* as a dietary supplement on broiler performance in comparison with prebiotics. *Sci J App Res.* **2**, 42-46.

Kharde, S. D., Shribhate, R. N., Bahiram, K. B. and Nipane, S. F. (2012) Effect of *Spirulina* supplementation on growth performance of broilers. *Ind J Vet Res.* **21**, 66-69.

Mariey, Y. A., Samak, H. R., Abou-Khashba, H. A., Sayed, M. A. M. and Abou-Zeid, A. E. (2014) Effect of using *Spirulina platensis* algae as a feed additives for poultry diets: 2-productive performance of broiler. *Egypt Poult Sci.* **34**, 245-258.

Michalak, I., Chojnacka, K., Dobrzanski, Z., Gorecki, H., Zielinska, A., Korczynski, M. and Opalinski, S. (2011) Effect of macroalgae enriched with microelements on egg quality parameters and mineral content of eggs, eggshell, blood, feathers and droppings. *J Anim Physiol Anim Nutr*. **95**, 374-387.

Nikodémusz, E., Páskai, P., Tóth, L. and Kozák , J. (2010) Effect of dietary *Spirulina* supplementation on the reproductive performance of farmed pheasents. *Technical Article-Poultry Industry* 1-2.

Park, S. Y., Birkhold, S. G., Kubena, L. F., Nisbet, D. J. and Ricke, S. C. (2004) Review on the role of dietary zinc in poultry nutrition, immunity and reproduction. *Biol Trace Element Res.* **101**, 147-163.

Qureshi, M. A., Garlich, J. D. and Kidd, M. T. (1996) Dietary *Spirulina platensis* enhances humoral and cell-mediated immune functions in chickens. *Immunopharmacol Immunotoxicol.* **18**, 465-476.

Raju, M. V. L. N., Rao, S. V. R., Radhika, K. and Chawak, M. M. (2005) Dietary supplementation of *Spirulina* and its effects on broiler chicken exposed to aflatoxicosis. *Ind J Poult Sci.* **40**, 36-40.

Ross, E. and Dominy, W. (1990) The nutritional value of dehydrated, blue-green algae (*Spirulina plantensis*) for poultry. *Poult Sci.* **69**, 794-800.

Ross, E., Puapong, D. P., Cepeda, F. P. and Patterson, P. H. (1994) Comparison of freeze-dried and extruded *Spirulina platensis* as yolk pigmenting agents. *Poult Sci.* **73**, 1282-1289.

Saenmahayak, B., Bilgili, S. F., Hess, J. B. and Singh, M. (2010) Live and processing performance of broiler chickens fed diets supplemented with complexed zinc. *J Appl Poult Res.* **19**, 334-340.

Salim, H. M., Lee, H. R., C. Jo, S. K. Lee and Lee,B. D. (2012) Effect of dietary zinc proteinate supplementation on growth performance, and skin and meat quality of male and female broiler chicks. *Br Poult Sci.* **53**, 116-124.

Saxena, P. N., Ahmad, M. R., Shyam, R. and Amla, D. V. (1983) Cultivation of *Spirulina* in sewage for poultry feed. *Experientia.* **39**, 1077-1083.

Toyomizu, M., Sato, K., Taroda, H., Kato, T. and Akiba, Y. (2001) Effects of dietary *Spirulina* on meat colour in muscle of broiler chickens. *Br Poult Sci.* **42**, 197-202.

Venkataraman, L. V., Somasekaran, T. and Becker, E.W. (1994) Replacement value of blue-green alga (*Spirulina platensis*) for fishmeal and a vitamin-mineral premix for broiler chicks. *Br Poult Sci.* **35**, 373–381.

Zahid, P. B., Aisha, K. and Ali, A. (1995) Green seaweeds as component of poultry feed. *Bangladesh J Bot*. **24**, 146-153.

Zahroojian, N., Moravej, H. and Shivazad, M. (2013) Effects of dietary marine algae (*Spirulina platensis*) on egg quality and production performance of laying hens. *J Agr Sci Tech.* **15**, 1353-1360.

Zielinska, A. and Chojnacka, K. (2009) The comparison of biosorption of nutritionally significant minerals in single- and multi-mineral systems by the edible microalga *Spirulina* sp. *J Sci Food Agric.* **89**, 2292-2301.