Journal of Current Veterinary Research

Journal of Current Research Manager Ma

ISSN: 2636-4026

Journal homepage: http://www.jcvr.journals.ekb.eg

Biochemistry

Enhancement effect of *Spirulina platensis* extract on broiler chicks' growth performance and immunity

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ABSTRACT

The current study was conducted to assess the consequences of *Spirulina platensis* extract based on growth performance and immunological response of broiler chicks. There was a total of a hundred and Eighty-one-day-old chicks were assigned at random into three dietary groups undergoing treatment with three replicates of 20 each bird. Control group was given ration without additives. The second and third groups were supplemented with *Spirulina platensis* extract at doses of 30 and 50 gm./kg. of ration, respectively. Supplementation of broiler chicks with *Spirulina platensis* extract enhanced their growth performance represented by increasing final body weight, body weight gain, phagocytic activity, and phagocytic index while significantly decreased feed intake and food conversion ratio. Moreover, it increased serum levels of Ig-M, and Ig-G and obviously change in serum level of Insulin like growth factor-1 (IGF-1) but not lead to a significant change as compared to control.

This study indicated that *Spirulina platensis* extract in ration enhanced the growth performance and immunological response of broiler chicks through increasing serum levels of immunoglobulins.

Key words: Spirulina platensis extract, insulin like growth factor-1, Immunoglobulins IgM & IgG.

INTRODUCTION

The industry of poultry is regarded as one of the most crucial revenue generators in the world. Because of the constant and broad rise in human population necessitates the provision of safe and protein-rich food. -abundant poultry or animal sources has become increasingly important. Antibiotics in the diet resulted in the emergence of antibiotic-resistant microorganisms (Sorum and Sound, 2001), residues of antibiotics in goods (Burgat, 1999) and reduce the number of microbes in the intestine (Andremont, 2000). Antimicrobial use in the poultry industry is harmful to both birds and people, posing a hazard to the industry. Several dietary strategies have been used to maximize the genetic potential for development in broiler chickens, one of which is the use of antibiotics as a feed

additive (in-feed antibiotics). Sugiharto, (2016) found that in-feed antibiotics have been related to improved growth and feed efficiency in broiler production, in addition, lower mortality and morbidity. However, most nations have outlawed the use of such compounds due to the rise of antibiotic-resistant germs in humans and animals. The removal of synthetic antibiotics from broiler feeds did, in fact, cause broiler chicken performance and health problems (Pourhossein et al., 2015; Sugiharto, 2016). It was consequently vital for food safety and long-term broiler productivity to use alternatives to replace in-feed antibiotics. As a result, for long-term broiler production and food safety, finding alternatives to in-feed antibiotics has been critical. Microalgae, such as Spirulina platensis and Chlorella vulgaris, due to their

excellent nutritional and functional features, which can aid broiler chicks, they have lately piqued the interest of poultry nutritionists. (Jamil *et al.*, 2015; Sugiharto & Lauridsen, 2016).

Microalgae, on the other hand, were regarded as a key component of aquatic biodiversity, and they were produced in a variety of habitats (sea, freshwater, and desert) and in a variety of shapes (single cells, colonies, and laments) (Stengel et al., 2011). Algae has recently been employed as a food supplement to boost nutritional value, as an animal feed addition, and even as a pharmaceutical ingredient (Shahidi, 2009; Navacchi et al., 2012). The nutritional benefits of microalgae have been widely marketed around the world during the last 10 years, and algal enterprises have started to gain traction among producers. Furthermore, specialists recently completed various research to investigate the benefits of spirulina in chicken feed (Zahroojian et al., 2013; Mariey et al., 2014; Światkiewicz et al., 2015; Evans et al., 2015; Danny et al., 2016; and Kanagaraju and Omprakash, 2016).

Spirulina is the common term for a collection of filamentous, multicellular, blue-green micro algae divided into two genera: Spirulina and Arthrospira, each with 15 species. Spirulina platensis is the most frequently available and utilized genus, having been studied extensively in a variety of sectors, including food and medical (Beheshtipour *et al.*, 2012). Blue-green algae, or cyanobacteria, are the evolutionary connection between bacteria and green plants. It held everything that life needed to evolve. For billions of years, this immortal plant has been regenerating itself and has only recently exposed itself to mankind (Edis Koru, 2012).

Spirulina is a rich a source of beneficial substances containing protein, carbohydrates, lipids, fibers, minerals, vitamins, and certain natural pigments (Becker, (2007). The Food and Drug Administration (FDA) and the National Health Authority have legally licensed spirulina as a healthy supplement (GRAS) with no toxicological impact. Because such massive ingredients give physiological potential and functional value, the food business is promising in terms of tackling malnutrition and illness in food applications (Gershwin and Belay, 2009). When it comes to nutrition, spirulina is a highquality source of protein, gamma-linolenic acid, vitamins, minerals, iron. sulfated polysaccharides, and phycocyanin (El-Baky et *al.*, 2008; Chu *et al.*, 2010). As a result, Spirulina is of significant interest since it has the potential to be employed as a functional food (Ambrosi *et al.*, 2008). Beyond their nutritional value, this term refers to foods that have been found to improve a variety of physiological functions, provide health-promoting properties, and/or lower illness risk (Hasler, 1996).

In the diets of dairy cows, the effects of *spirulina* supplementation on animal performance and product quality have recently been investigated. (Simkus *et al.*, 2007; Christaki *et al.*, 2012), rabbits (Colla *et al.*, 2008; Peiretti & Meineri, 2008; Gerencser *et al.*, 2014), fattening lambs (EL Sabagh *et al.*, 2014), pigs (Grinsteal *et al.*, 2000), common carp (Abdulrahman & Hamad Ameed, 2014), laying hens (Carrillo *et al.*, 2008; Maries *et al.*, 2012; Zahroojian *et al.*, 2013) and broilers (Alvarenga *et al.*, 2011; Bellof & Alarcon, 2013).

Broiler diet containing Spirulina platensis was linked to increased live body weight, body weight growth, and feed conversion rate (Kharde et al., 2012; Shanmugapriya and Babu, 2014; Shanmugapriya et al., 2015; Park et al., 2018). In previous studies, Rajuu et al., (2004) found that Spirulina platensis boosted humoral and cellular immunological responses, as well as lymphoid organ development, in chicks. Lokapirnasari al.. et (2016)recently demonstrated that, Spirulina platensis therapy enhanced the number of leukocytes and reduced broiler chick mortality.

The main aim of this study was designed to evaluate growth performance and immunological response of broiler chicks of dietary treatment by using *Spirulina platensis* extract through monitoring their effects on biochemical and molecular immunological response and growth performance parameters in broilers chicks.

MATERIALS AND METHODS <u>1. Chemicals and reagent:</u>

measuring serum levels of Assaying kits for cholesterol (CAT. NO.230004), triacylglycerols (TAG) (CAT. NO.314004) were obtained from Spectrum Company. (Cairo, Egypt). Malondialdehyde (MDA) detection kits (CAT. NO. MD 2529) were obtained from Bio Diagnostics Ltd. (Giza, Egypt). Kits for assaying serum levels of immunoglobulins G (CAT. NO. MBS260043) immunoglobulins & Μ (CAT. NO. MBS706158) from my BioSource Company. (Cairo, Egypt). Assay kits for serum levels of IGF-1 (CAT. NO. In-Ch0104), IF- γ (CAT. NO. In-Ch0031) by ELISA were obtained from Bio nova Co., Ltd (Beijing, China). The other compounds employed in this experiment were analytic grade.

2. Spirulina platensis extract:

Physical Properties: *Spirulina* is a free-flowing, dark blue-green powder with a mild seaweed smell, produced by spray drying the biomass of the cyanobacterium, *Spirulina platensis* (**Dillon et al., 1995).** It is not readily soluble in water or solvents, but it forms a suspension when mixed with water. Polysaccharide *Spirulina* (purity ~99%) was manufactured by national research center, Al-Doky, Giza, Egypt.

3. Experimental chicks and housing:

This experiment was conducted in accordance with the ethical guidelines of the animal care and Use committee of university of al Sadat, Egypt.

A total of 180 unsexed avian broiler chicks were allocated into three groups of 60

chicks each at random. Each group was separated into three sub-groups and kept in three separate cages, each with 20 chicks. the first group is control group was given ration without additives, second, which received *Spirulina platensis* extract at dosages of 30 gm./kg. of ration, and third, which received *Spirulina platensis* extract at doses of 50 gm./kg. of ration.

4. Ration and additives:

Broilers were fed broiler starter for 12 days, broiler grower from 12 to 22 days, and broiler finisher from 22 to the conclusion of the trial (37 days). Feed and water were freely available (Table1).

The experiment was carried out at room temperature with a 14-hour light/ten-hour dark cycle. Throughout the experiment, the room temperature was kept at 23-25°C with a relative humidity of 50:70%. The birds were kept under consistent climatic and nutritional settings throughout the duration of the experiment.

	0		
Ingredient and composition %	Starter	Grower	Finisher
Corn	57.89	61.26	62.4
Soybean meal 48%	35.00	31.00	29.00
Soybean oil	2.40	3.20	4.50
Monocalcium phosphate	1.30	1.15	1.00
Limestone	1.50	1.50	1.45
DL-Methionine	0.35	0.33	0.30
L-Lysine (%)	0.44	0.37	0.28
Threonine	0.18	0.23	0.23
Tryptophan	0.00	0.01	0.17
L-Valine	0.10	0.11	0.08
NaCl	0.23	0.24	0.23
Choline Chloride (%)	0.10	0.10	0.10
Trace mineral permix ¹	0.10	0.10	0.10
Vitamin permix ²	0.10	0.10	0.10
Sodium sulphate	0.26	0.25	0.23
Ronozyme NP ³	0.02	0.02	0.02
Rovabio Excel AP ⁴	0.01	0.01	0.01
Ronozyme ProAct ⁵	0.02	0.02	0.02
Antioxidant	0.01	0.01	0.01

¹Trace mineral premix per kilogram of diet: Iron carbonate 50 mg, Manganese oxide 100 mg, Copper sulphate 12 mg, Zinc 100 mg, Calcium iodide 1.6 mg, Sodium selenite 3 mg, Cobalt sulphate 0.4 mg; Calcium iodide 1.6 mg; Sodium selenite 3 mg; Cobalt sulphate 0.4 mg Vitamin A, 13000 IU; Vitamin D3, 4000 IU; Vitamin E, 100 mg; Vitamin B1, 3 mg; Vitamin B2, 9 mg; B6, 6 mg; B12, 0.4 mg; Folic acid, 2 mg; Biotin, 0.25 mg.² Vitamin premix per kilogram of diet: Vitamin A, 13000 IU; Vitamin D3, 4000 IU; Vitamin E, 100 mg; Vitamin B1, 3 mg; Vitamin B2, 9 mg; B6, 6 mg; B12, , 0.4 mg; Folic acid, 2 mg; Biotin, 0.25 mg.³ Ronozyme NP contains a minimum of 10000 units of Phytase per gramme.⁴ Rovabio Excel AP contains at least 22000 units of B-Xylanase and 2000 units of B-gluconase per gramme.⁵ Ronozyme ProAct has a minimum of 75000 units of protease per gramme. commercial mixture (LumanceTM, Innovad NV, Belgium) (Taha *et al.*, 2014).

Chicks were vaccinated by IBD and ND vaccines during its suitable schedule.

5. Methods: 5.1. Performance parameter: 5.1.1. Feed intake (g):

The difference between the left over and the initial quantity of feed delivered was used to compute the daily feeds eaten. The feed given to the birds in each treatment was weighed on a regular basis (weekly).

5.1.2. Body weight gain (g):

By subtracting the starting body weight at the start of the experiment from the final body weight on day 37, The weight of individual birds in each replicate was added together and then divided by the number of birds in each replicate to get the average body weight per replicate.

5.1. Feed conversion ratio:

The average weight gained, and average feed consumed by the birds in each treatment were used to calculate the feed conversion ratios (FCR).

Feed conversion ratio = Average feed intake (g) Average body weight

gain (g)

5.2. Sampling:

At the end of the experimental period, blood samples were taken from all groups. Blood samples, they were incubated for 1 hour at room temperature for coagulation. Blood samples were centrifugated for 15 minutes at 3500 rpm then clear sera were separated for analysis.

5.3. Biochemical analysis:

Serum total cholesterol and triacylglycerol levels were assayed according to Ellefson and Caraway (1976); Bucolo and David (1973) respectively. Malonaldehyde (MDA) was determined in serum according to Ohkawa *et al.*, (1979) and Koracevic *et al.*, (2001) respectively. Serum IgG & IgM concentration using ELISA was determined according to Mallery et al., (2010).

5.4. ELISA assay for determination of serum insulin like growth factor 1 (IGF-1) and Interferon γ (IFN-γ):

Serum levels of IGF-1 and IFN- γ were measured using commercially available ELISA kits, following instruction protocol of the manufacturer (Bio nova Co., Ltd, Beijing, China). Both kits based on Sandwich-ELISA technique. Briefly, 50 µl of each standard and/or sample diluted with dilution buffer were inserted into the proper well of the microtiter plate precoated with the corresponding specific antibody (against IGF-1 or IFN-y respectively). After 30 min incubation at 37°C, the solution in the wells was aspirated and discarded and wells were washed 5 times with wash buffer. Then, 50 µl of a Horseradish Peroxidase (HRP)conjugated antibody specific for IGF-1 or IFN- γ respectively was added and incubated for 30 min at 37°C. Then, the plate was washed 5 times in wash buffer, the color was developed by adding 100 µl substrate reagent and incubation for 15 min at 37°C. Finally, reaction was terminated by adding 50 µl of stop solution which turns the color from blue to yellow. The optical densitv (OD) was measured spectrophotometrically at a wavelength of 450 nm. The OD value is proportional to the concentration of IGF-1/or IFN-y. You can calculate the concentration of IGF-1/or IFN-y was calculated in the samples by comparing the OD of the samples to the standard curve (Jansen et al., 1983; Digby and Lowenthal, 1995)

5.5. Statistical analysis:

Statistical Analysis System was used to evaluate the data acquired using one-way analysis of variance (ANOVA) based on the Completely Randomized Design model (SAS, 2012). Duncan's Multiple Range Test (SAS, 2012) was used to establish the significance of the differences between groups, with differences of 5% (p0.05) being significant.

RESULTS

<u>Effect of Spirulina platensis extract (SPE) on</u> growth performance parameters:

To investigate the effect of Spirulina platensis extract (SPE) on growth performance parameters, the changes in body weight gain, feed intake and food conversion ratio in response to Spirulina platensis extract (SPE) at a dose of 30 gm/kg. (SPE1) and 50 gm/kg. (SPE2) from day 0 till the end of experiment were estimated. The results showed that, supplementation of broiler chicks with SPE increased body weight gain and decreased both feed intake and food conversion ratio compared to those of control group ($p \le 0.01$). On the other hand, there was no significant differences concerning feed intake and food conversion ratio between the second and third groups (Table 2).

• **Table (2):** The effect of *Spirulina platensis* extract on body weight gain, feed intake and food conversion ratio of broiler chicks.

	Control group	SPE at dose	SPE at dose 50		
Parameters		30gm./k. g	gm./k. g		
Initial weight (g)	39.3±.2	40.8±.3	40.5±.4		
Final body weight (g)	1985.16±61.11 ^c	2401.66±31.87 ^b	2583.8±31.4 ^a		
Body weight gain (g)	1952.5 ± 62.8^{c}	2360.8±35.08 ^b	2543.3 ± 34.43^{a}		
Daily feed intake (g)	4316.7 ± 60.8^{a}	4155 ± 46.02^{b}	4104.2± 42.6 ^b		
Food conversion ratio 2.219 ± 0.08^{a}		1.76 ± 0.04^{b}	1.61 ± 0.03^{b}		

• Data are presented as means ± SE (standard errors of means).

 The mean difference is significant at *p* <0.05. The values carrying different letters in the same ROW were statistically different.

• SPE, *Spirulina platensis* extract.

Effect of Spirulina platensis extract on biochemical parameters (Total cholesterol, triacyl glycerol's and malonaldehyde):

As shown in Table 3, supplementation of broiler chicks with *Spirulina platensis* extract (SPE) at either used doses (SPE1 or SPE2) from day zero till the end of experiment on caused no significant changes in serum level of total cholesterol, triacylglycerols or malonaldehyde among different groups. **Table (3):** The effect of *Spirulina platensis* extract on Total cholesterol (mg/dl), triacyl glycerol's (mg./dl) and malonaldehyde (nmol/ml) of broiler chicks.

	Control group	SPE at dose	SPE at dose 50		
Parameters		30gm./k. g	gm./k. g		
Total cholesterol(mg/dl)	75.02 ± 1.32	75.9 ± 0.9	78.24 ± 1.97		
Tri-acyl glycerol's (mg./dl)	50.25 ± 11.16	65.67 ± 20.4	53.8 ± 17.5		
Malonaldehyde (nmol/ml)	9.8 ± 0.18	10.2 ± 0.16	10.07 ± 0.16		

• Data are presented as means ± SE (standard errors of means).

- The mean difference is significant at p < 0.05. The values carrying different letters in the same ROW were statistically different.
- SPE, *Spirulina platensis* extract.

Effect of Spirulina platensis extract on Phagocytic activity % and Phagocytic index:

Effects of administrating the broiler chicks with *Spirulina platensis* extract (SPE) at a dose of 30 gm/kg. (SPE1) and 50 gm/kg. (SPE2) on phagocytic activity % and phagocytic index from day 0 till the end of experiment were estimated. As presented in Table 4, both used doses of SPE increased phagocytic activity % and phagocytic index as compared to that of control group ($p \le 0.01$). Moreover, this stimulatory effect of SPE on either phagocytic activity % or phagocytic index was significantly higher in second group treated with lower dose (SPE1) than in third group treated with higher dose (SPE2) ($p \le 0.01$).

• **Table (4):** The effect of *Spirulina platensis* extract on Phagocytic activity % and phagocytic index of broiler chicks.

	Control group	SPE	at	dose	SPE	at	dose	50
Parameters		30gm./	'k. g		gm./k	. g		
Phagocytic activity %	$56.35\pm0.7^{\rm c}$	64.	5 ± 0.3	35 ^a	62	2.03	$\pm 0.75^{b}$	
Phagocytic index	$1.98\pm0.05^{\rm c}$	2.4	460.03	9 ^a		2.19	0 ± 0.03	4 ^b

- Data are presented as means ± SE (standard errors of means).
- The mean difference is significant at *p* <0.05. The values carrying different letters in the same ROW were statistically different.
- SPE, *Spirulina platensis* extract.

Effect of Spirulina platensis extract on immunoglobulin IgG and Immunoglobulin IgM:

Treatment of broiler chicks with SPE at dose of 30 gm/kg. (SPE1) and 50 gm/kg. (SPE2) resulted in a significant increase in IgG and IgM in SPE1 and SPE2 as compared to that of control group ($p \le 0.01$). On the other hand, there was no significant differences between SPE1 and SPE2 groups.

		U		0		-		
	Control group	SPE	at	dose	SPE	at	dose	50
Parameters		30gm./	k. g		gm./k	. g		
IgG (mg/dl)	101.67 ± 4.18^{b}	121.	$.4 \pm 3.$	09 ^a	12	21.5	$\pm 5.42^{a}$	
IgM (mg/dl)	7.25 ± 0.48^{b}	12.2	25 ± 0.5	75 ^a	1	1.25	$\pm 0.25^{a}$	

Table (5): The effect of *Spirulina platensis* extract on Ig-G and Ig-M of broiler chicks.

• Data are presented as means ± SE (standard errors of means).

• The mean difference is significant at *p* <0.05. The values carrying different letters in the same ROW were statistically different.

• SPE, *Spirulina platensis* extract.

Effect of Spirulina platensis extract on interferon- γ and Insulin like growth factor-1:

As shown in Table 3, supplementation of broiler chicks with *Spirulina platensis* extract (SPE) at either used doses (SPE1 or SPE2) from day zero till the end of experiment on caused no significant changes in serum levels of interferon- γ and Insulin like growth factor-1 among different groups. **Table (6):** The effect of *Spirulina platensis* extract on plasma levels of Interferon- γ of broiler chicks.

Parameters	Control group	SPE at dose 30gm./k. g	SPE at dose 50 gm./k. g
INF- γ (ng/ml)	27.25 ± 7.25	36.63 ± 2.23	26.31 ± 3.125
IGF-1 (ng/ml)	53.18± 16.44	64.9 ± 48.18181	144.32 ± 28.3

• Data are presented as means ± SE (standard errors of means).

• The mean difference is significant at *p* <0.05. The values carrying different letters in the same ROW were statistically different.

• SPE, *Spirulina platensis* extract.

DISCUSSION:

Microalgae has gained a lot of interest, attention, and application from a range of producers all over the world since it is regarded as a wonderful supply of vital and naturally efficient nutrients. They've been studied as immunomodulators, anti-inflammatory, antioxidants, antimicrobials, and antivirals in experimental animals for hundreds of years, and their efficacy has been demonstrated (Ashgan et al., 2015). Microalgae are also Cosmetics and animal feed sectors both utilise it (Huntley et al., 2015; Ariede et al., 2017). Microalgae could potentially be regarded a promising basic component of animal and poultry feed. The most efficient and extensively distributed edible feed additive microalgae in people, animals, and poultry are Arthrospira (Spirulina species) as Spirulina platensis (S. platensis) and Spirulina maxima (Kanagaraju and Omprakash, 2016). Spirulina platensis is a high-quality protein, sulfated polysaccharides, gamma-linolenic acid, vitamins, minerals, iron, and phycocyanin source in nutrition terms (ElBaky et al., 2008; Chu et al., 2010). As a result, Spirulina platensis is of particular interest since it has the potential to be employed as a functional food (Ambrosi et al., 2008). Spirulina platensis has been frequently used in broiler diets as an alternative to in-feed antibiotics from the day of hatch through slaughter age (Jamil et al., 2015; Bonos et al.,

2016). Poultry meat consumption has risen dramatically in recent decades, because many believe chicken meat is "healthy" and less expensive than red meat. It's also crucial that there are no cultural or religious restrictions on eating poultry meat (Cavani et al., 2010; Petracci et al., 2013). Incorporating spirulina into broiler feed may have several benefits, including improved performance and health (Ravi et al., 2010; Kharde et al., 2012; Holman & Malau-Aduli, 2013; Shanmugapriya & Saravana Babu, 2014), Introducing its bioactive elements into meat and producing functional goods in response to customer demand for could healthy natural meals be а straightforward and convenient technique (Jimenez-Colmenero et al., 2001). The growth performance of a broiler determines its overall economy. Reduced growth performance is a result of poor management and feed (Salim et al., 2012). The primary purpose of feed is to supply sufficient nutrients to fulfil the metabolic demands of broiler performance. In the present research, inclusion of Spirulina platensis extract in ration of broiler chickens performance enhanced the growth characteristics through increasing the final body weight and body weight gain and increasing serum levels of insulin like growth factor-1 (IGF-1) and resulted in decreasing food conversion ratio and feed intake. Our results in agreement with Raju et al., (2005) who concluded that Spirulina at a concentration of 0.05 percent in the feed can somewhat offset the harmful effects of aflatoxin on broiler chicken development rate. Kharde et al., (2012); Shanmugapriya & Saravana Babu, (2014) reported that in comparison with the control group, dietary Spirulina significantly boosted weight gain and feed efficiency in chickens. Furthermore, Bellof & Alarcon (2013) reported that Dietary supplementation dramatically Spirulina improved broiler growth carcass and performance metrics in organic farming. The large increase in feed conversion ratio seen in birds fed spirulina diets could be attributed, at least in part, to an increase in live body weight gain or a higher viability %. These results are confirmed by Kaoud (2012), Mariey et al., (2012) and Mariey et al., (2014), who reported that dietary addition of Spirulina platensis boosted feed conversion ratio considerably. According to researchers, supplementing broilers with Spirulina platensis reduced mortality considerably in comparison to control broilers. Also, Evans et al., (2015) showed that the energy value of dried full-fat Spirulina algae was 90 percent that of corn (2839 kcal TMEn/ kg), with a high crude protein (76%)and necessary amino acid content They also observed that adding up to 16 percent dried seaweed to a broiler starter diet had no detrimental influence on chick performance. Shanmugapriya et al., (2015a) recently observed broilers fed a meal containing Spirulina biomass had better body weight growth (BWG), FCR, and villus length. In this regard Gružauskas et al., (2004) reported that Spirulina enhanced nutrition digestion, boosted mineral absorption, and protected against diarrhoea. Diets rich in spirulina may help to increase lactobacillus populations and enhance nutrient absorption (Tsuchihashi et al., 1987; Mariey et al., 2012). Furthermore, Spirulina has a greater protein content (55-65%), as well as all the important amino acids, vitamins, and minerals (Doreau et al., 2010). Also, it's high in carotenoids and fatty acids, notably gammalinolenic acid (GLA), which means it's good source in nutrition terms (Guroy et al., 2012) and has been used in broiler and layer diets over the world to increase yolk colour, meat quality, and egg fertility (Ross and Dominy, 1990). Bao et al., (2007) found that broiler performance was increased by employing enriched iron and zinc in Spirulina platensis as feed supplements. Also, Saenmahayak et al., (2010) found that

zinc supplementation affects broiler chick growth and processing performance, regardless of source or dose. However, Shanmugapriya *et al.*, (2015b), reported that when compared to feeding 0.5 or 1 percent Spirulina platensis, eating 1.5 percent *Spirulina* resulted in a lower end body weight. Excessive *Spirulina platensis* consumption may have caused metabolic abnormalities and harmed liver function, resulting in broiler growth retardation.

In the present research, inclusion of Spirulina platensis extract in ration of broiler chickens resulted in obvious change or numerical change in insulin growth factor -1 of third group (SPE at dose 50 gm./k.g) but not reach a significant change in comparison with control. Insulin-like growth factor 1 (IGF-1) is a polypeptide hormone of 70 amino acids generated primarily by the liver (which accounts for about 75% of circulating IGF-1) because the liver's endocrine system is stimulated by GH and insulin. IGF-1, on the other hand, stimulates somatostatin production in the pituitary, which acts as an inhibitory feedback signal on GH release in the hypothalamus (Ohlsson et al., 2009). The numerical increase of insulin-like growth factor-1 (IGF-1) serum level in our study is considered a good marker on enhancement growth performance parameters and is in accordance with those of Guobin et al., (2011). who reported that in mammals and chickens, IGFs were major positive body and muscle growth modulators. Growth hormones and IGF-1 are required to support normal growth (Scanes, 2009).

In our study, supplementation of broiler chicks with Spirulina platensis extract (SPE) at either used doses (SPE1 or SPE2) caused no significant changes in serum level of total cholesterol, triacylglycerols or malonaldehyde among different groups. Various tests on laboratory animals have demonstrated the hypolipidemic impact of Spirulina or its preparations. (Mariey et al., 2012; Deng and Chow, 2010). Our result in agreement with, Canogullari Dogan et al., (2016) who reported that Supplementation with Spirulina platensis reduced plasma total cholesterol and triglyceride levels in both groups numerically but not statistically. In a study by Hosseini-Vashan et al., (2012), The total cholesterol content in broiler chickens' blood might be reduced by feeding them a phytogenic substance before or after heat stress. Mariey et al., (2012) reported that in a study of the effects

of Spirulina platensis on local laying hens, it was discovered that plasma cholesterol levels were considerably lower in the Spirulinacontaining diets than in the control diets. Spirulina's hypolipidemic impact has been attributed to C-phycocyanin, which inhibits pancreatic lipase activity in a dose-dependent way (Deng and Chow, 2010). Furthermore, organic substances that interfere with free radicals may help to restore the oxidant/antioxidant equilibrium, resulting in improved health and development. Also, Akbarian et al., (2016) Some antioxidant phytochemicals, such as flavonoids and similar substances, have been demonstrated to lower MDA levels and increase SOD in heat-stressed chickens while having little or no impact in non-stressed birds.

In our study, addition of *Spirulina platensis* extract in ration also resulted in improvement immunity indices in broilers such as phagocytic activity, phagocytic index, IgM, IgG.

According to, spirulina has been demonstrated improve immunological to function, reproduction, and growth (Khan et al., 2005). It also boosts the ability of hens' cell-mediated and mononuclear phagocytic systems to resist illness. The higher carotenoid content of Spirulina helps in supplementation of vitamin A. provides antioxidant activity, and enhances immunity (Qureshi et al., 1996). In addition, helps in hormonal regulation and plays additional roles in growth, reproduction, and (Nikodémusz maturation et al.. 2010). Immunological function, reproduction, and growth have all been demonstrated to benefit from spirulina. Microbiological killing, antigen processing, and T-cell activation have all been observed to improve when spirulina is added to chicken diets at a concentration of less than 1% (Qureshi, et al., 1994). This might be since Spirulina contains colors (carotenoids like carotene and zeaxanthin, for example) (Maoka, phycobiliproteins 2011). (for example. phycocyanin, a cyanobacteria-specific protein) (Eriksen, 2008), vitamins (Becker, 1994), macro and micro mineral elements (Becker, 1994; Spolaore et al., 2006) and antioxidants (Christaki et al., 2013). Antibacterial, antioxidant, anti-cancer, and anti-inflammatory properties are all present in these compounds, which also serve as immune boosters and colorants (Freitas et al., 2012; Batista et al., 2013; Christaki et al., 2013).

CONCLUSION

Spirulina platensis has a high nutritional profile and may be utilized as a safe feed source for broiler production. The feeding trials with broiler showed that improved broiler performance by improving BW, BWG, serum levels of IGF-1 and FCR as well as immunological response through increasing serum levels of IgM, IgG, phagocytic activity, and phagocytic index as well as using *Spirulina platensis* was used as a feed addition with no negative impact on broiler health.

REFERENCES

- Abdulrahman, N.M. and Hamad Ameed, H.J. (2014). Replacement of fishmeal with microalgae spirulina on common carp weight gain, meat and sensitive composition and survival. Pakistan J. Nutr. 13, 93-98.
- Akbarian, A.; Michiels, J., Degroote, J.; Majdeddin, M.; Golian, A.; De Smet, S. (2016). Association between heat stress and oxidative stress in poultry; mitochondrial dysfunction and dietary interventions with phytochemicals. J Anim Sci Biotechnol, 7:37.
- Alvarenga, R.R.; Rodrigues, P.B.; Cantarelli,
 V.; Zangeronimo, M.G.; Da Silva Junior,
 J.W.; Da Silva, L.R.; Dos Santos, L.M. and
 Pereira, L.J. (2011). Energy values and
 chemical composition of spirulina
 (Spirulina platensis) evaluated with
 broilers. Braz. J. Anim. Sci. 40, 992-996.
- Ambrosi, M. A.; Reinhr, C. O.; Bertolin TE, et al. Health properties of Spirulina spp. Rev Cienc Farma Basica Apl 2008; 29: 109-17.
- Andremont, A. (2000). Consequences of antibiotic therapy to the intestinal ecosystem. Ann. Fr. Anesth. Reanim. 19: 395-402. https://doi.org/10.1016/S0750-7658(00)90209-0
- Ariede, M.B.; Candido, T.M.; Jacome, A.L.M.; Velasco, M.V.R.; de Carvalho, J.C.M. and Baby, A.R. (2017). Cosmetic attributes of algae-a review, Algal Res. 25: 483–487, https://doi.org/10.1016/j.algal.2017.05.019
- Ashgan, A. A.; Haiam, M.A; Eman, M. A.; Khaled, A. E. M. and Ola, K. S. (2015).
 Hepato-protective, DNA damage prevention and antioxidant potential of Spirulina platensis on CCl4induced hepatotoxicity in mice. Am. J. Biomed. Res. 2: 29-34.

- Bao, Y.M.; Choct, M.; Iji, P.A. and Bruerton. K. (2007). Effect of organically complexed copper, iron, manganese, and zinc on broiler performance, mineral excretion, and accumulation in tissues. J. Appl. Poult. Res., 16, pp. 448-455.
- Bashandy, S.A.; Alhazza, I.M.; El-Desoky, G.E.; Al-Othman, Z.A. (2011). Hepatoprotective and hypolipidemic effects of Spirulina platensis in rats administered mercuric chloride. Afr J Pharm Pharmacol, 5:175-82.
- Batista, A.P.; Gouveia, L.; Bandarra, N.M.;Franco, J.M. and Raymundo, A. (2013).Comparison of microalgal biomass profiles as novel functional ingredient for food products. Algal Res. 2, 164-173.
- Becker, E.W. (1994). Nutrition. In: Microalgae: Biotechnology and Microbiology. Cambridge University Press, Cambridge. pp. 196-249.
- Becker, E.W. (2007). Micro-algae as a source of protein. Biotechnol. Adv. 25, 207-210.
- Beheshtipour, H.; Mortazavian, A.M.; Haratian, P. and Khosravi- Darani, K. (2012). Effects of Chlorella vulgaris and Arthrospira platensis addition on viability of probiotic bacteria in yogurt and its biochemical properties. Eur Food Res Technol., 235 (4), 719-728.
- Bellof, G. and Alarcon, S.C. (2013). Effect of Spirulina platensis in organic broiler production Archiv. Geflug. 77, 73-80.
- Bonos, E.; Kasapidou, E.; Kargopoulos, A.;
 Karampampas, A.; Christaki, E.; Florou-Paneri, P. and Nikolakakis, I. (2016).
 Spirulina as a functional ingredient in broiler chicken diets. S. Afr. J. Anim. Sci. 46, 94-102.
- Bucolo, G. and David, H. (1973). Quantitative determination of serum triglycerides by the use of enzymes. Clin. Chem.19: 476–482.
- Burgat, V. (1999). Residues of drugs of veterinary use in food. La Revue du Praticien, 41: 985-990.
- Canogullari Dogan, S.; Baylan, M.; Erdogan, Z. (2016). Performance, egg quality and serum parameters of Japanese quails fed diet supplemented with Spirulina platensis. Fresen Environ Bull, 25:5857-62.
- Carrillo, S.; Lopez, E.; Casas, M.M.; Avila, E.; Castillo, R.M.; Carranco, M.E.; Calvo, C. and Perez-Gil, E. (2008). Potential use of seaweeds in the laying hen ration to improve the quality of n-3 fatty acid

enriched eggs. J. Appl. Phycol. 20, 271-278.

- Cavani, C.; Petracci, M.; Trocino, A. and Xiccato, G. (2010). Advances in research on poultry and rabbit meat quality. Ital. J. Anim. Sci. 8, 741-750.
- Christaki, E.; Bonos, E.; Giannenas, I. and Florou-Paneri, P. (2013). Functional properties of carotenoids originating from algae. J. Sci. Food Agric. 93, 5-11.
- Christaki, E.; Karatzia, M.; Bonos, E.; Florou-Paneri, P., and Karatzias, C. (2012). Effect of dietary Spirulina platensis on milk fatty acid profile of dairy cows. Asian J. Anim. Vet. Adv. 7, 597-604.
- Chu, W. L.; Lin, Y. W.; Radhakrishnan A. K., and Lim, P. E. (2010). Protective effect of aqueous extract from Spirulina platensis against cell death induced by free radicals. BMC Complement Altern Med, 10: 53-60.
- Colla, L.M.; Muccillo-Baisch, A.L. and Costa, J.A.V. (2008). Spirulina platensis effects on the levels of total cholesterol, HDL and triacylglycerols in rabbits fed with a hypercholesterolemic diet. Braz. Arch. Biol. Technol. 51, 405-411.
- Danny, S. W. C.; Azhar, K.; Awis, Q. S.; Hishamuddin, O. and Jia, Y. T. (2016). Effect of supplementing Spirulina on live performance, carcass composition and meat quality of Japanese Quail. Walailak J. Sci. Technol. 13: 77-84.
- Deng, R., and Chow, T.J. (2010). Hypolipidemic, antioxidant, and antiinflammatory activities of microalgae Spirulina. Cardiovasc Ther, 28: e33-45.
- Digby, M. R.; Lowenthal, J.W. (1995). Cloning and expression of the chicken interferongamma gene. J Interferon Cytokine Res.Nov;15(11):939–45.
- Dillon, et al. (1995). Nutritional value of Spirulina. World Rev. Nutr. Diet. 77:32-46.
- 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.
- Edis Koru. (2012). Earth Food Spirulina (Arthrospira): Production and Quality Standarts, Food Additive, Prof.Yehia El-Samragy (Ed.), ISBN: 978-953-51-0067-6, InTech.
- Ellefson, R. E. and Caraway, W. T. (1976). Fundamentals of clinical chemistry. Ed Tietz NW, p 506.

- EL-Sabagh, M.R.; Eldaim, M.A.A.; Mahboub, D.H. and Abdel-Daim, M. (2014). Effects of Spirulina platensis algae on growth performance, antioxidative status and blood metabolites in fattening lambs. J. Agric. Sci. 6, 92-98
- Eriksen, N.T. (2008). Production of phycocyanin a pigment with applications in biology, biotechnology, foods and medicine. Appl. Microbiol. Biotechnol. 80, 1-14.
- Evans, A. M.; Smith, D. L. and Moritz, J. S. (2015). Effects of algae incorporation into broiler starter diet formulations on nutrient digestibility and 3 to 21 d bird performance. J. Applied Poult. Res. 24: 206-214.
- Freitas, A.C.; Rodrigues, D.; Rocha-Santos, T.A.P.; Gomes, A.M.P. and Duarte, A.C. (2012). Marine biotechnology advances towards applications in new functional foods. Biotechnol. Adv. 30, 1506-1515.
- Gerencser, Z.; Szendro, Z.; Matics, Z.; Radnai,
 I.; Kovacs, M.; Nagy, I.; Cullere, M.; Dal Bosco, A. and Zotte, D.A. (2014). Effect of dietary supplementation of spirulina (Arthrospira platensis) and thyme (Thymus vulgaris) on apparent digestibility and productive performance of growing rabbits. World Rabbit Sci. 22, 1-9.
- Gershwin, M. E. and Belay, A. (2009). "Spirulina in human nutrition and health," Journal of Applied Phycology, vol. 21, no. 6, pp. 747-748.
- Grinsteal, G.S.; Tokach, M.D.; Dritz, S.S.; Goodband, R.D. and Nelssen, J.L. (2000). Effects of Spirulina platensis on growth performance of weanling pigs. Anim.Feed Sci. Technol. 83, 237-247.
- Gružauskas, R.; R. Lekavicius; A. Raceviciut-Stupelien; V. T. Šašyt; G.J. velis; Švirmickas. (2004). Višiuk broileriu virškinimo procesu optimizavimas simbiotiniais preparatais. Veterinarija ir zootechnika. T. 28(50). pp. 51- 56.
- Guobin, C.; Xiangping, L, and Jin, L. (2011). "Temporal and spatial expression of the Pax-7 gendering chicken embryo and postnatal development," Journal of Animal and Veterinary Advances, 10: 1785-1788. DOI:https://www.doi.org/10.3923/javaa.20 11.1785.1788.
- 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.

- Hasler, C. M. (1996). Functional foods: the Western perspective. Nutr Rev, 54(11): S6-10.
- Holman, B.W.B. and Malau-Aduli, A.E.O. (2013). Spirulina as a livestock supplement and animal feed. J. Anim. Physiol. Anim. Nutr. 97, 615-623.
- Hosseini-Vashan, S.J.; Golian, A.; Yaghob far, A. (2012). Antioxidant status, immune system, blood metabolites and carcass characteristic of broiler chickens fed turmeric rhizome powder under heat stress. Afr J Biotechnol, 11:16118-25.

https://doi.org/10.1051/vetres:2001121

Huntley, M.; Johnson, Z.; Brown, S.; Sills, D.; Gerber, L.; Archibald, I.; Machesky, S.; Granados, J.; Beal, C. and Greene, C. (2015). Demonstrated large-scale production of marine microalgae for fuels and feed, Algal Res. 10: 249–265, https://doi.org/10.

1016/j.algal.2015.04.016.

- Jamil, A.B.M.R.; Akanda, R.; Rahman, M.; Hossain, A. and Islam, S. (2015). Prebiotic competence of spirulina on the production performance of broiler chickens. J. Adv. Vet. Anim. Res. 2, 304-309.
- Jansen, M.; van Schaik, F. M.; Ricker, A.T.; Bullock, B.; Woods, D. E.; Gabbay, K. H.; Nussbaum, A. L.; Sussenbach, J. S.; Van den Brande, J. L. (1983). Sequence of cDNA encoding human insulin-like growth factor I precursor. Nature. Dec 8;306(5943):609–611.
- Jimenez-Colmenero, F.; Carballo, S. and Cofrades, S. (2001). Healthier meat and meat products: Their role as functional foods. Meat Sci. 59, 5-13.
- Kanagaraju, P. and Omprakash, A. V. (2016). Effect of Spirulina platensis algae powder supplementation as a feed additive on the growth performance of Japanese quails. Indian Vet. J. 93: 31-33.
- Kaoud, H. A. (2012). Effect of spirulina platensis as a dietary supplement on broiler performance in comparison with prebiotics. Scientific Journal of Applied Research. 1(2):44-48.
- Khan, M.; Shobha, J. C.; Mohan, I.K, Naidu,M. R, Sundaram, C.; Singh, P. K. andKutala, V.K. (2005). "Protective effect ofSpirulina against doxorubicin-induced

cardiotoxicity" Phytotherapy Research. 19(12): pp.1030-7.

- Kharde, S.D., Shirbhate, R.N., Bahiram, K.B. and Nipane, S.F. (2012). Effect of spirulina supplementation on growth performance of broilers. Indian J. Vet. Res. 21, 66-69.
- Koracevic, D.; Koracevic, G.; Djordjevic, V.;
 Andrejevic, S.; Cosic, V. M. (2001).
 Method for measurement of antioxidant activity in human fluids.J.
 Clin.Pathol.,54:356-361.
- Lokapirnasari, W.P.; Yulianto, A.B.; Legowo, D. and Agustono, (2016). The effect of Spirulina as feed additive to myocardial necrosis and leukocyte of chicken with avian influenza (H5N1) virus infection. Proc. Chem.18, 213- 217.
- Mallery, D. L.; McEwan, W. A.; Bidgood, S. R.; Towers, G. J.; Johnson, C.M., and James, L.C. (2010). Antibodies mediate intracellular immunity through tripartite motif-containing 21 (TRIM21). Proc Natl Acad Sci U S A. 2010 Nov 16;107(46):19985-90.
- Maoka, T. (2011). Carotenoids in marine animals. Mar. Drugs 9, 278-293.
- Maries, Y.A.; Samak, H.R. and Ibrahem, M.A. (2012). Effect of using spirulina platensis algae as a feed additive for poultry diets: 1productive and reproductive performances of local laying hens. Egypt. Poult.Sci. 31, 201-215.
- Mariey, Y. A.; Samak, H. R.; Abou-Khashaba,
 H. A.; Sayed, M. A. M. and Abou-Zeid, A.
 E. (2014). Effect of using Spirulina platensis algae as a feed additive for poultry diets. Egy. Poult. Sci. J. 34: 245-258.
- Mariey, Y.A.; Samak, H.R.; Ibrahem, M.A. (2012). Effect of using Spirulina platensis algae as a feed additive for poultry diets: 1productive and reproductive performances of local laying hens. Egypt Poult Sci., 32:201-15.
- Navacchi, M. F. P.; Monteiro de Carvalho, J.C.; Takeuchi, K. P. and Danesi, E. D. G. (2012). Development of cassava cake enriched with its own bran and Spirulina platensis. Acta Sci. Technol. (Maringa) 34: 465-472.
- 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.

- Ohlsson, C.; Mohan, S.; Sjögren, K.; Tivesten, Å.; Isgaard, J.; Isaksson, O.; Jansson, J. O. and Svensson, J. (2009). The role of liver-derived insulin-like growth factor-I. Endocr Rev., 30:494–535.
- Park, J. H.; Lee, S. I. and Kim, I.H. (2018). Effect of dietary Spirulina (Arthrospira) platensis on the growth performance, antioxidant enzyme activity, nutrient digestibility, cecal microflora, excretanoxious gas emission, and breast meat quality of broiler chickens. Poult. Sci. 97: 2451-2459. https://doi.org/10.3382/ps/psu/093

https://doi.org/10.3382/ps/pey093.

- Peiretti, P.G. and Meineri, G. (2008). Effects of diets with increasing levels of Spirulina platensis on the performance and apparent digestibility in growing rabbits. Livest.Sci. 118, 173-177.
- Petracci, M.; Bianchi, M.; Mudalal, S. and Cavani, C. (2013). Functional ingredients for poultry meat products. Trends Food Sci. Technol. 33, 27-39.
- Pourhossein, Z.; Qotbi, A.A.A.; Seidavi, A.; Laudadio, V.; Centoducati, G. and Tufarelli, V. (2015). Effect of different levels of dietary sweet orange (Citrus sinensis) peel extract on humoral immune system responses in broiler chickens. Anim. Sci. J. 86, 105-110.
- Qureshi, M. A.; Garlich, J. D. and Kidd, M. T. (1996) Dietary Spirulina platensis enhances humoral and cellmediated immune functions in chickens. Immunopharmacol Immunotoxicol. 18, 465-476.
- Qureshi, M.A.; Garlich, D.; Kidd, M.T. and Ali, R.A. (1994). Immune enhancement potential of Spirulina platensis in chickens. Poultry Science. 73: 46.
- 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. Indian Poultry Science Association (Abstr.)
- Raju, M.V.L.N.; Rao, S.V. R.; Radhika, K. and Chawak, M.M. (2004). Effects of Spirulina platensis or furazolidone on the performance and immune response of broiler chickens fed with aflatoxin contaminated diet. Indian J. Anim. Nutr. 21, 40 -44.
- Ravi, M.; Lata De, S.; Azharuddin, S. and Paul, S.F.D., (2010). The beneficial effects of spirulina focusing on its

immunomodulatory and antioxidant properties. Nutr. Diet. Suppl. 2, 73-83.

- Ross, E. & Dominy, W., 1990. The nutritional value of dehydrated, blue-green algae (Spirulina platensis) for poultry. Poult. Sci. 69, 794-800.
- 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., 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.
- Scanes, C. G. (2009). Perspectives on the endocrinology of poultry growth and metabolism. General and Comparative Endocrinology, 163: 24- 32. DOI: https://doi.org/10.1016/j.ygcen.2009.04.01 3.
- Shahidi, F.; Zhong, Y.; Wijeratne, S.S.K. and Ho, C.T. (2009). Almon and Al-mond Products: Nutraceutical Components and Health Effects. Tree nuts: Composition, Phytochemicals, and Health effects. In: Alasalvar, C., and Shahidi, F. (Ed.). CRC press, Boca Raton, FL, pp. 127–138.
- Shanmugapriya, B. and Saravana Babu, S. (2014). Supplementary effect of Spirulina platensis on performance, hematology, and carcass yield of broiler chickens. Indian Streams Res. J. 4, 1-7.
- Shanmugapriya, B.; Babu, S.S.; Hariharan, T.;
 Sivaneswaran, S. and Anusha, M.B. (2015b). Dietary administration of spirulina platensis as probiotics on growth performance and histopathology in broiler chicks. International Journal of Recent Scientific Research 6: 2650-2653.
- Shanmugapriya, B.; Babu, S.S.; Hariharan, T.;
 Sivaneswaran, S.; Anusha, M.B. and Raja,
 P.U., (2015a). Synergistic effect of
 Spirulina platensis on performance and gut
 microbial load of broiler chicks. Indo-Asian
 J. Multidisc. Res. 1, 149- 155.
- Simkus, A., Oberauskas, V., Laugalis, J., Zelvyte, R., Monkeviciene, I., Sederevicius, A., Simkiene, A. and Pauliukas, K. (2007). The effect of weed Spirulina platens is on the milk production in cows. Vet. Ir Zootechn. 38, 74-77.

- Sørum, H and Sunde, M. (2001). Resistance to antibiotics in the normal flora of animals. Vet. Res. 32: 227-241.
- Spolaore, P.; Joannis-Cassan, C.; Duran, E. and Isambert, A. (2006). Commercial applications of microalgae. J. Biosci. Bioeng. 101, 87-96.
- Stengel, A.; Goebel, M.; Jawien, J.; Kobelt, P.; Taché, Y. and Lambrecht, N.W. (2011).
 Lipopolysaccharide increases gastric and circulating NUCB2/nesfatin-1 concentrations in rats. Peptides., 32:1942– 1947. [PMC free article] [PubMed] [Google Scholar].
- Sugiharto, S. (2016). Role of nutraceuticals in gut health and growth performance of poultry. J. Saudi Soc. Agric. Sci. 15, 99-111.
- Sugiharto, S. and Lauridsen, C. (2016). Dietary Chlorella supplementation effect on immune responses and growth performances of broiler chickens exposed to post hatch holding time. Livest. Res. Rur. Dev. 28;

- Świątkiewicz, S.; Arczewska-Wlosek, A. and Józeflak, D. (2015). Application of microalgae biomass in poultry nutrition. World Poult. Sci. J. 71: 663-672
- Tsuchihashi N.; T. Watanabe; Y. Takai. (1987). Effect of Spirulina platensis on caecum content in rats. Bull Chiba Hygiene College. 5:27-30.
- 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. Agric. Sci. Technol. 15: 1353-1360.

http://www.lrrd.org/lrrd28/7/sugi28119.ht ml