

## **EFFECT OF SPIRULINA PLATENSIS SUPPLEMENTATION ON GROWTH, SOME BIOCHEMICAL AND IMMUNOLOGICAL PARAMETERS IN SUCKLING CALVES**

By

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### **ABSTRACT**

The neonatal period of dairy calves may be associated with multiple stressful incidents that negatively impact immune response and animal performance; therefore, there is an increased demand for antioxidants to reduce the deleterious effects of free radicals on the immune system. This work aimed at study the effect of spirulina platensis supplementation on growth, some biochemical and immunological parameters in suckling calves. Sixteen newly born calves were randomly divided into two groups Group (1) served as control. Group (2) treated with spirulina platensis powder, which was added daily at a rate of 6 gm/ calve/ day for 45 days. Body weight recorded for all experimental calves and blood samples collected at days 0, 15, 30 and 45 of spirulina treatments. Erythrocytes and leucocytes count, hemoglobin, total protein, albumin, globulin, immunoglobulin IgG, IgM and total antioxidant capacity and disease incidence were recorded. The results revealed non-significant increase in body weight and a significant increase ( $P<0.05$ ) in Erythrocytes and leucocytes counts, hemoglobin, total protein, globulin and total antioxidant capacity with lower rate of diseases incidence in spirulina platensis treated group compared with control. Albumin and immunoglobulins levels showed non-significant differences. In conclusion, Spirulina platensis supplementation in suckling calf's starter enhanced immunomodulator, antioxidants potential and health status. Therefore, decrease disease incidence rate. Further experiments with different doses and different treatment duration needed to explore the maximum benefit of spirulina.

#### **Key words:**

Spirulina platensis, suckling calves, antioxidants, immunity, the disease incidence rate.

## INTRODUCTION

Calves are the future stock of a dairy enterprise. It is important for every producer to implement best calf management practices to bring up a healthy replacement stock. The identification of new feed resources is therefore crucial for sustainable animal production and future viability. It should have high nutritive value and conversion efficiency (**Panjaitan et al., 2015**). Livestock production systems may be associated with multiple stressful incidents that negatively impact immune response and animal performance (**Lykkesfeldt and Svendsen 2007**). The neonatal period of dairy calves is another time of increased disease susceptibility. High neonatal morbidity and mortality rates are consistent worldwide, (**Mellor and Stafford 2004 and Mee 2013**). Therefore, there is an increased demand for antioxidants to reduce the deleterious effects of free radicals on the immune system (**Carroll and Forsberg, 2007**). The use of natural antioxidants, regardless of the production system, is perhaps one of the safest and most accepted nutritional strategies by consumers in line with the concepts of green economy and food fortification (**Abuelo et al., 2019**). The blue-green algae, *Spirulina platensis*, have been considered as a suitable natural antioxidant and immune-stimulant to humans and animals with fewer side effects and more cost effective than synthetic products (**Abdel-Daim et al., 2013; Belay, 2002; Khan and Zafar 2005**).

*Spirulina* is an edible microalga, and a highly nutritious potential feed resource for many agriculturally important animal species. Research findings have associated *Spirulina* to improvements in animal growth, fertility and nutritional product quality (**Hoseini et al., 2013**). *Spirulina* intake has also been linked to an improvement in animal health and welfare. *Spirulina* algae have high poly-nutrients value and phytopigments in a simple structure with a complex composition (**Abu-Elala et al., 2016**). It contains vital compounds, such as protein (50-70% on DM basis) with all essential amino acids (**Farag et al., 2016**), a source of carotenoids, chlorophyll, pigments and essential fatty acids, alpha-linolenic, gamma-linolenic and linoleic (**Mendes et al., 2003 and Peiretti and Meineri, 2011**), photosynthetic pigments (**Bermejo et al., 2008**), vitamins such as thiamine, nicotinamide, riboflavin, folic acid, pyridoxine, vitamins A, D and E vitamins (**Hoseini et al., 2013**) and minerals like Ca, K, Cr, Cu, Mn, Fe, P, Mg, Na, Zn and Se (**Babadzhanov et al., 2004**), this make spirulina an efficient feed supplementation (**Yousefi et al., 2019**). Dietary spirulina was found to improve crude protein digestibility in rabbits fed diets compared to those receiving

no spirulina (Peiretti and Meineri 2009). Holman *et al.*, (2012) reported that, the average daily weight gain of the pigs fed 2 g of spirulina platensis was higher than control. EL-Sabaghet *et al.*,(2014)reported positive effects of Spirulina platensis powder supplementation to the diets of lambs. Holman and Malau-Aduli (2013) found an improvement in the animal health and productivity. Spirulina supplementation has shown to reduce oxidative stress and enhance humoral and cell-mediated immune functions in human beings. Spirulina is widely available in the market as food supplement for both humans and animals (Farag *et al.*, 2016). Holman *et al.*,(2012) has reported an increase in lamb live weight with dietary spirulina platensis. Bezerra *et al.*, (2010) found that lambs receiving spirulina have higher live weights and average daily gains than control group lambs. Feeding trials with spirulina have been conducted in chickens, pigs, ruminants and rabbits (Furbeyre *et al.*, 2017). However, studies on the use of spirulina platensis as a feed additive in ruminant feeding are still quite limited EL-Sabagh *et al.*, (2014). All these findings encourage us to study the effect of spirulina platensis supplementation on growth, some biochemical and immunological parameters in suckling calves.

## **MATERIAL AND METHODS**

### **1. Animals and housing:**

Sixteen healthy Holstein newly born calves (8 males and 8 females) ranged between (32-37 Kg) with an average (34.34), were selected from a Commercial Dairy Herd, El-Fayoum Governorate. The pregnant cows which show the signs of calving put into a maternity yard, a few days (1-2) before birth, for closer monitoring and to kept clean with fresh water provided. After parturition, once the calf is able to stand, the calf suckles colostrum immediately after birth within the 1st 6 hours, Colostrum feeding continues for about four to five days. Then, calves separated to a special calf's pen.

### **2. Diet:**

The calves were housed in individual pens and fed with whole pooled milk (clean and warm whole milk at body temperature of 37°C) approximately at 10% of birth weight and they had free access to the feed starter and water. Milk was offered in two equal meals daily at 08:00 and 18:00. Spirulina platensis powder was mixed to the upper layer of the starter feed and provided to the animals. The calves weaned at 100 kg body weight or three months of age. The starter diet contains 21% protein, 85% TDN (soya bean, yellow corn, minerals, vitamins and antiacid).

**3. Experiment design:**

Sixteen newly born calves were randomly divided into two groups eight calves of each (4 males and 4 females). Group (1) served as control fed the basal diet only. Group (2) supplemented with spirulina platensis powder, that was added daily in the starter of each calve at a rate of 6 gm/calve/ day for 45 days, according to the manufacture recommendations.

**Spirulina platensis analysis and treatment:**

The Spirulina platensis powder was obtained from a commercial retailer NOUR EL-HOUDA LTD, Egypt. It was analyzed to its nutritional constituents according to AOAC, (2000) as seen in (Table 1).

**Table (1):** Composition quantities in 100 gm of spirulina platensis powder.

Constituents	Ingredient %
Moisture	5.3
Crude protein	60.73
Fat	5.92
Crude fiber	3.34
Ash	10.91
Total carbohydrates	13.8

**Vitamins content:**

Pro-vitamin A	213.00 mg
Thiamin (V.B1)	1.92 mg
Riboflavin (V.B2)	3.44 mg
Vitamin B6	0.49 mg
Vitamin B12	0.12 mg
Vitamin E	10.40 mg

**Mineral content:**

Selenium	5 µg/100gm
Calcium	153.8 mg/100gm
Phosphorous	101.1 mg/100gm
Magnesium	7.9 mg/100gm
Sodium	762.7 mg/100gm

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### Amino acids content:

Amino acid	Content
Threonine	37.66 mg/gm
Valine	56.25 mg/gm
Methionine	71.81 mg/gm
Cystine	17.21 mg/gm
Isoleucine	60.24 mg/gm
Leucine	66.72 mg/gm
Histidine	12.34 mg/gm
Tyrosine	89.67 mg/gm
Phenylalanine	14.74 mg/gm
Lysine	13.59 mg/gm
Aspartic Acid	29.88 mg/gm
Glutamic Acid	14.85 mg/gm
Serine	10.93 mg/gm
Glycine	10.45 mg/gm
Arginine	7.51 mg/gm
Alanine	17.58 mg/gm
Proline	29.78 mg/gm

### Body weight determination:

The body weights of all calves in the two groups were recorded before treatment and every 15 days till the end of the experiment.

### Blood sampling:

Two blood samples were collected from the calves in the experimental groups by jugular vein puncture at days 0, 15, 30 and 45 of spirulina platensis treatment. The 1<sup>st</sup> sample collected on anticoagulant EDTA for hematological parameters. The 2<sup>nd</sup> blood sample was collected in clean, sterilized tubes and centrifuged at 3000 rpm for 15 minutes. Serum was harvested and kept at (-20 °C) until the biochemical analysis.

### Disease incidence recording:

The calves in the 2 groups closely observed and recorded the cases of “calf scour, diarrhea, pneumonia, cough, constipation, joint problems, septicemias, umbilical problems” all-over

the experimental period. The prevention and treatment measures performed according to the farm program.

**Hematology and biochemical analysis:**

**Hematology:**

Total erythrocyte and leucocytic counts were estimated using hemocytometer according to **Schalm (1986)**. Hemoglobin concentration was determined according to **Drabkin, (1932)**.

**Biochemical analysis:**

The serum levels of total protein, albumin, globulin were determined according to **Henery (1968), Drupt, (1974) and Doumas and Biggs (1972)** respectively. Serum immunoglobulin levels IgG and IgM were determined by ELISA according to **Erahard et al. (1992)**.

Total antioxidant capacity was determined calorimetrically according to the method of **Koracevic et al. (2001)**.

**Statistical analysis:**

Data are presented as mean  $\pm$  SE and evaluated by a one-way analysis of variance (ANOVA) using the SPSS 6.1.3 software package (SAS, Cary, NC, USA) and differences between median and range at  $P < 0.05$  were considered significant according to **Snedecor and Cochran, (1989)**.

**RESULTS**

The obtained results showed non-significant increase in the body weight of spirulina treated group (2) compared to control (1), as seen in the (Table 2).

**Table (2):** Calves body weight (Kg) in different groups at different periods of treatment (Mean  $\pm$  SE).

	<b>Pretreatment</b>	<b>15 days after</b>	<b>30 days after</b>	<b>45 days after</b>
<b>Group (1)</b>	<b>34.50 <math>\pm</math> 0.49</b>	<b>40.13 <math>\pm</math> 0.60</b>	<b>46.88 <math>\pm</math> 0.47</b>	<b>53.19 <math>\pm</math> 0.94</b>
<b>Group (2)</b>	<b>34.19 <math>\pm</math> 0.52</b>	<b>40.63 <math>\pm</math> 0.52</b>	<b>48.00 <math>\pm</math> 0.65</b>	<b>56.06 <math>\pm</math> 0.67</b>

**\*: significant at  $P < 0.05$**

Regarding hemogram, there were significant increase ( $P < 0.05$ ) in hemoglobin concentration (gm/dl), erythrocyte count ( $\times 10^6 / \mu\text{l}$ ) and leukocyte count ( $\times 10^3 / \mu\text{l}$ ) in the spirulina treated group ( $11.79 \pm 0.15$ ,  $8.44 \pm 0.13$  and  $9.13 \pm 0.18$ ) compared with control group ( $10.83 \pm 0.24$ ,  $7.83 \pm 0.13$  and  $8.50 \pm 0.09$ ) after 45 days of treatment as seen in (Tables 3, 4 and 5).

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**Table (3):** Hemoglobin concentration (gm/dl) in different groups at different periods of treatment (Mean  $\pm$  SE).

	Pretreatment	15 days after	30 days after	45 days after
<b>Group (1)</b>	<b>11.23 <math>\pm</math> 0.31</b>	<b>11.35 <math>\pm</math> 0.23</b>	<b>10.95 <math>\pm</math> 0.28</b>	<b>10.83 <math>\pm</math> 0.24</b>
<b>Group (2)</b>	<b>11.25 <math>\pm</math> 0.31</b>	<b>11.36 <math>\pm</math> 0.21</b>	<b>11.51 <math>\pm</math> 0.04</b>	<b>11.79 <math>\pm</math> 0.15*</b>

\*: significant at  $P < 0.05$ .

**Table (4):** Erythrocytes count ( $\times 10^6 / \mu\text{l}$ ) in different groups at different periods of treatment (Mean  $\pm$  SE).

	Pretreatment	15 days after	30 days after	45 days after
<b>Group (1)</b>	<b>7.68 <math>\pm</math> 0.18</b>	<b>7.63 <math>\pm</math> 0.12</b>	<b>7.90 <math>\pm</math> 0.13</b>	<b>7.83 <math>\pm</math> 0.13</b>
<b>Group (2)</b>	<b>7.63 <math>\pm</math> 0.20</b>	<b>7.86 <math>\pm</math> 0.21</b>	<b>8.18 <math>\pm</math> 0.14</b>	<b>8.44 <math>\pm</math> 0.13*</b>

\*: significant at  $P < 0.05$ .

**Table (5):** Leucocytes count ( $\times 10^3 / \mu\text{l}$ ) in different groups at different periods of treatment (Mean  $\pm$  SE).

	Pretreatment	15 days after	30 days after	45 days after
<b>Group (1)</b>	<b>8.43 <math>\pm</math> 0.13</b>	<b>8.59 <math>\pm</math> 0.14</b>	<b>8.34 <math>\pm</math> 0.12</b>	<b>8.50 <math>\pm</math> 0.09</b>
<b>Group (2)</b>	<b>8.46 <math>\pm</math> 0.23</b>	<b>8.90 <math>\pm</math> 0.13</b>	<b>8.83 <math>\pm</math> 0.23</b>	<b>9.13 <math>\pm</math> 0.18*</b>

\*: significant at  $P < 0.05$

Regarding protein profile, the results showed a significant increase ( $P < 0.05$ ) in total protein and globulin levels in spirulina treated group ( $8.05 \pm 0.21$  and  $4.31 \pm 0.17$ ) compared to control group ( $7.34 \pm 0.10$  and  $3.51 \pm 0.15$ ), as shown in (Table, 6 and 8). The albumin levels showed non-significant differences between treated and control groups, (Table 7).

**Table (6):** Total protein level (gm/dl) in different groups at different periods of treatment (Mean  $\pm$  SE).

	Pretreatment	15 days after	30 days after	45 days after
<b>Group (1)</b>	<b>7.21 <math>\pm</math> 0.19</b>	<b>7.25 <math>\pm</math> 0.19</b>	<b>7.40 <math>\pm</math> 0.09</b>	<b>7.34 <math>\pm</math> 0.10</b>
<b>Group (2)</b>	<b>7.05 <math>\pm</math> 0.17</b>	<b>7.40 <math>\pm</math> 0.22</b>	<b>7.98 <math>\pm</math> 0.17*</b>	<b>8.05 <math>\pm</math> 0.21*</b>

\*: significant at  $P < 0.05$

**Table (7):** Albumin level (gm/dl) in the groups during different periods of treatment (Mean ± SE).

	Pretreatment	15 days after	30 days after	45 days after
<b>Group (1)</b>	<b>3.96 ± 0.09</b>	<b>3.38 ± 0.10</b>	<b>3.53 ± 0.09</b>	<b>3.83 ± 0.13</b>
<b>Group (2)</b>	<b>3.88 ± 0.17</b>	<b>3.61 ± 0.15</b>	<b>3.63 ± 0.10</b>	<b>3.74 ± 0.15</b>

**\*: significant at P<0.05.**

**Table (8):** Globulin level (gm/dl) in the groups at different periods of treatment (Mean ± SE).

	Pretreatment	15 days after	30 days after	45 days after
<b>Group (1)</b>	<b>3.25 ± 0.15</b>	<b>3.88 ± 0.18</b>	<b>3.88 ± 0.15</b>	<b>3.51 ± 0.15</b>
<b>Group (2)</b>	<b>3.18 ± 0.10</b>	<b>3.79 ± 0.16</b>	<b>4.35 ± 0.19</b>	<b>4.31 ± 0.17*</b>

**\*: significant at P<0.05.**

The results showed non-significant differences in immunoglobulin IgG and IgM in spirulina treated group (2) compared to control group (1), (Tables 9, 10).

**Table (9):** Immunoglobulin IgG (g/L) in different groups at different periods of treatment (Mean ± SE).

	Pretreatment	15 days after	30 days after	45 days after
<b>Group (1)</b>	<b>33.13 ± 2.51</b>	<b>21.00 ± 1.55</b>	<b>24.63 ± 1.63</b>	<b>27.50 ± 2.03</b>
<b>Group (2)</b>	<b>33.38 ± 2.76</b>	<b>20.63 ± 1.91</b>	<b>24.75 ± 1.21</b>	<b>29.88 ± 1.98</b>

**\*: significant at P<0.05.**

**Table (10):** Immunoglobulin IgM (g/L) in different groups at different periods of treatment (Mean ± SE).

	Pretreatment	15 days after	30 days after	45 days after
<b>Group (1)</b>	<b>1.78 ± 0.14</b>	<b>1.49 ± 0.08</b>	<b>1.67 ± 0.10</b>	<b>1.90 ± 0.09</b>
<b>Group (2)</b>	<b>1.71 ± 0.11</b>	<b>1.52 ± 0.08</b>	<b>1.79 ± 0.09</b>	<b>2.13 ± 0.12</b>

**\*: significant at P<0.05.**

The results recorded a significant increase (P<0.05) in total antioxidant capacity (mM/L) in spirulina treated group after 30 and 45 days of treatment (0.54 ± 0.03 and 0.60 ± 0.03) when compared with control group (0.44 ± 0.02 and 0.48 ± 0.03), as seen in (Table 11). The results also showed an increase in the number and (rate) of disease incidence in calves of the control group 35 (9.72%) compared to spirulina treated group 24 (6.67 %), as shown in (Table 12).



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**Table (11):** Total antioxidant capacity (mM/L) in different groups at different periods of treatment.

	Pretreatment	15 days after	30 days after	45 days after
<b>Group (1)</b>	<b>0.45 ± 0.02</b>	<b>0.43 ± 0.02</b>	<b>0.44 ± 0.02</b>	<b>0.48 ± 0.03</b>
<b>Group (2)</b>	<b>0.44 ± 0.01</b>	<b>0.46 ± 0.01</b>	<b>0.54 ± 0.03*</b>	<b>0.60 ± 0.03*</b>

\*: significant at P<0.05

**Table (12):** The health status of experimental groups.

Disease condition		Group (1)	Group (2)
<b>GIT enteritis</b>	<b>Number of cases</b>	<b>15</b>	<b>11</b>
	<b>Rates</b>	<b>4.17 %</b>	<b>3.06 %</b>
<b>Respiratory manifestations</b>	<b>Number of cases</b>	<b>11</b>	<b>7</b>
	<b>Rates</b>	<b>3.06 %</b>	<b>1.94 %</b>
<b>Others</b>	<b>Number of cases</b>	<b>9</b>	<b>6</b>
	<b>Rates</b>	<b>2.5 %</b>	<b>1.67 %</b>
<b>Total incidence of diseases</b>	<b>Number of cases</b>	<b>35</b>	<b>24</b>
	<b>Rates</b>	<b>9.72%</b>	<b>6.67 %</b>

### DISCUSSIONS

Spirulina platensis is a microscopic blue-green alga and it is considered as one of the richest sources of organic nutrients that are making it a good nutritional supplement for human and animal feed worldwide. More recently Spirulina categorized as a genus of photosynthetic bacteria, Arthrospira, (Mirzaie *et al* 2018). Spirulina contains good quality proteins, vitamins and minerals in addition to a wide variety of natural carotene and xanthophyll phytopigments (Farag *et al.*, 2016). These constituents make spirulina unique and impressive nutrient composition to be used as a dietary supplement not only to enhance nutritional qualities, but also for therapeutic purposes (Farag *et al.*, 2016).

The obtained results showed non-significant increase in body weight in spirulina platensis treated group (2) compared to control (1), these results agreed with that mentioned by Heidarpour *et al.*, (2011), Moreira *et al.*, (2011) and Seyidoglu and Galip (2014), where they reported that treatment with spirulina showed a slight increase with non-significant effect on the final body weight compared with the control group. Also, Grinstead *et al.*, (2000)

have established a minimal improvement of the growth of piglets fed with *Spirulina platensis*. On the other side, **Grinstead *et al.* (2000)**, **Peiretti and Meineri (2009)**, **Bezerra *et al.*, (2010)**, **Promya and Chitmanat (2011)**, **Holman *et al.*, (2012)**, **Simkus *et al.*, (2013)**, **Nedeva *et al.*, (2014)**, **Zeweil *et al.*, (2016)** reported a significant increase in live body weight with dietary *spirulina platensis* supplementation compared to control.

Also, **EL-Sabagh *et al.*, (2014)** recorded a significant increase ( $P<0.05$ ) in the final body weights, daily weight gain compared with the control group in *spirulina platensis* powder supplementation to the diets of lambs. This may be due to *spirulina platensis* had pronounced stimulating effect on metabolism during the period of intensive growth of calves organs **Glebova *et al.*, (2018)**. Also, *spirulina* could improve the growth, of the animal through improving feed intake, feed conversion, nutrient absorption and utilization, body weight gain, **El-Desoky *et al.*, (2013)** and **Evans *et al.*, (2015)**.

Regarding hemogram, there were significant increase ( $P<0.05$ ) in hemoglobin concentration (gm/dl), erythrocyte count ( $\times 10^6/\mu\text{l}$ ) and leukocyte count ( $\times 10^3/\mu\text{l}$ ) in the *spirulina* treated group compared with control group after 45 days of treatment. These results were in accordance with that recorded by **EL-Sabagh *et al.*, (2014)** and **Glebova *et al.*, (2018)** who reported that, there was a significant increase effect of the *spirulina* on the hemopoiesis. Similar results were obtained by adding the *spirulina* in experiments with cows (**Simkus *et al.*, 2008a**), fattening pigs (**Simkus *et al.*, 2008b**) and lambs after weaning (**Shimkiene *et al.*, 2010**). It was, probably, due to the antioxidant effect of *spirulina platensis* on hematopoietic cells (**Kong *et al.*, 2004**). Also, the benefits of *spirulina platensis* on hematological parameters may be due to the high content of folic acid and vitamin B12 and their better absorption in *spirulina platensis* (**Nedeva *et al.*, 2014**). While, **Adel *et al.*, (2017)** found that, the lower concentrations of *spirulina* inclusion had no effect on blood parameters of rabbits. Regarding the white blood cells our results agreed with (**EL-Sabagh *et al.*, 2014**) who stated a significant increase ( $P<0.05$ ) in the total leucocytic count in *spirulina* powder supplemented fed group compared to the control in lambs. They added that, the hemoglobin concentration and the total leucocytes count were higher ( $P<0.05$ ) in the *spirulina platensis* fed group compared to the control. Leucocytes play an important role in non-specific or innate immunity and their count can be considered as an indicator of relatively lower disease susceptibility (**Matanović *et al.*, 2007**). The increased leucocytes production may be due to

the presence of phycocyanin and polysaccharides components in spirulina (Zhang, 1994). On the contrary, Nedeva *et al.*, (2014) found non-significant improvement in leucocytes count in spirulina platensis supplemented group compared with control, this also was in accordance with the values established by Ivanova-Peneva (2007) in piglets.

Regarding protein profile, the results showed a significant increase ( $P < 0.05$ ) in total protein and globulin levels in spirulina treated group (2) compared to control groups (1), these results agreed with El-Ratel, (2017) and Glebova *et al.*, (2018) who found a significant increase in protein profile in spirulina platensis supplemented group compared with control in cattle. Also, EL-Sabagh *et al.*, (2014) observed a significant increase in globulin concentration in the spirulina supplemented group compared with control. Increased plasma globulin levels are thought to be associated with a stronger innate response in treated calves and indicate higher resistance (Matanović *et al.*, 2007). This result is supported by increased total leucocytic count in spirulina platensis fed group. The recorded increase in plasma total protein and globulin concentrations may be related to high contents of protein, essential amino acids, vitamins, minerals, phospholipids and antioxidants in spirulina platensis (Gershwin and Belay, 2008; Farag *et al.*, 2016). On the other hand, Heidarpour *et al.* (2011) observed non-significant differences in blood parameters like albumin and globulin in spirulina supplemented and control groups. And EL-Sabagh *et al.*, (2014) recorded non-significant difference in the concentrations of total protein; albumin between spirulina supplemented and control groups.

The results showed non-significant differences in immunoglobulin IgG and IgM in spirulina treated group (2) compared to control group (1), these results were in accordance with Adel *et al.*, (2017) who stated that, the lower concentrations of spirulina inclusion in the diet of growing rabbits had not noticeable effect on blood immunoglobulin levels. On the contrary, Hayashi *et al.*, (1998) recorded that; spirulina may significantly enhance the Ig antibody level to protect against allergic reaction. Also, Hayashi *et al.*, (1994) and Yadav and Kumar (2018) suggested that spirulina enhances the immune response, particularly the primary response, by stimulating macrophage functions, phagocytosis, and IL-1 production.

This may be due to spirulina platensis can produce high protein, amino acids, vitamins, beta-carotene, pigments, and polysaccharides as a bioactive agent. All these components have an enhanced effect on the production of antibodies and cytokines.

The results recorded a significant increase ( $P < 0.05$ ) in total antioxidant capacity (mM/L) in spirulina treated group (2) when compared with control group (1) after 30 and 45 days of treatment, these results agreed with **Abd El-Baky (2003)** and **EL-Sabagh et al., (2014)** who reported that spirulina platensis could be incorporated as an antioxidant, immune-stimulant in fattening lambs diets. The antioxidative effect of spirulina is related to several active ingredients, notably phycocyanin, polysaccharides,  $\alpha$ -tocopherol and  $\beta$ -carotene that have potent antioxidant activities working, individually or in synergy, directly on free radicals (**Riss et al., 2007** and **Kurd and Samavati, 2015**). **Abd El-Baky (2003)** mentioned that, Spirulina can be used for production of some antioxidant compounds. This may be due to its high contents of carotenoids, C-phycocyanin and a potent antioxidant agent as one of its major constituents (**Bhat and Madyastha 2000, Mittler et al., 2004** and **Abd El-Baky et al., 2007**), while **Estrada et al., (2001)** attributed the antioxidant agent to the presence of polyunsaturated fatty acids and phycocyanin in spirulina. Also, **Ismail, (2017)** concluded that, spirulina platensis supplementation protects from disease incidence in rats through its powerful free radical-scavenging, antioxidant, and immuno-stimulant activities. It is a natural product and source of carotenoids, chlorophyll, pigments and essential polyunsaturated fatty acids as gamma linoleic acid (**Peiretti and Meineri, 2011**).

The results also, showed an increase in the number and (rate) of disease incidence in the calves of the control group 35 (9.72%) compared to spirulina treated group 24 (6.67 %) during experiment period, this agreed with **Nedeva et al., (2014)** who observed a tendency of a small number of sick animals (2.40 % and 2.13 %) fed with spirulina platensis compared with those in the control group (5.40 %). **Abuelo et al., (2014)** and **Ranade et al., (2014)** documented that neonatal calves experience oxidative stress during the first few weeks of age that diminishes functional capabilities of immune cell populations and increases the animals' susceptibility to diseases (**Sordillo and Aitken 2009**). This may be due to spirulina has some medicinal properties against inflammation (**Coskun et al., 2011**) beside its antioxidant and immunomodulatory activities (**Jamil et al., 2015**).

In conclusion, the supplementation of spirulina platensis in suckling calf's starter enhanced immunomodulator, antioxidants potential and health status. Therefore, decrease the rate of disease incidence. Further experiments with different doses and different treatment duration needed to explore the maximum benefit of spirulina.

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