A "SUPER FOOD" FOR ALTERNATIVE NUTRIENTS: *Spirulina Platensis*

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Abstract: Nutrition provides the growth and maintains function of organism. In recent years, there has been an increase in importance of alternative foods for feeding and health, especially *Spirulina platensis. S.platensis* is a microalgae called as "Super food" as endorsed by lifestyle personalities, and also has been approved as a health food by the World Health Organization. This study we aimed to evaluate the effects of different doses of *S.platensis* (500-1000 mg/kg bw) on physiological such as growth, haemotogical and biochemical parameters. During trial the rats were weighed weekly and the haemogram parameters (haematocrit, haemoglobin, red-white blood cell counts, leukocyte subtypes, MCV, MCHC, RDW and PLT) were analyzed. Serum total cholesterol, its fractions (LDL, HDL) and atherogenic indices (TC:HDL-C, LDL-C:HDL-C) were observed. Besides that, serum protein, albumin, globulin and albumin/globulin ratio were determined. Although there were no differences occurred among all groups statistically, all parameters were found in their reference values. However, effects of lower dose of *S.platensis* showed the best result for those physiological parameters. As a result that, *S. platensis* with its high concentration of functional nutrients is called as an important alternative therapeutic food and can be said that it can be used safely.
Key words: Alternative food, Spirulina, microalgae, health.

Introduction

Nutritious is important for physiological functions and also growth mechanism of organism. Belong this issue, a quality nutritious is nedded for a healthy life for both animal and human. Recent years, researchers are interested in interesting food supplements for better health. The selection criterias of these products take into account the strengthening of the immune system, maintenance of growth performance and antioxidant properties, especially protein needs. Among these products, a strong antioxidant *Spirulina platensis*, which is a natural protein source, rich in vitamins and minerals, has become a focus of interest. Belong to the increase in interest of *S.platensis*, biotechnological studies about this microalgae have been researched in nowadays.

S. platensis is a microscopic filamentous alga which is rich in polyunsaturated fatty acids, phycocyanin and phenolic compounds (Richmond, 1992). It also does not contain cellulose on its cell wall. In this respect, *S.platensis* stimulates the bowel function and digestion rate by activate the useful microorganism such as *Bifidobacterium* and *Lactobacillus*, and inhibit the harmful bacteries such as *E.coli* and *Candidas*. The improvement of absorption of foods and digestion were reported by some researchers (Pulz and Gross, 2004; Vural and Celen, 2005; Dogan, 2012). Besides that, phycocyanin content of *S.platensis* which has antioxidant and antienflamatuar properties, affects positively on erythropoiesis. Researchers also determined that phycocyanin and also polisaccarides in this microalgae improve values of the erythrocytes, granulocytes, monocytes and fibroblast cells in bone cell marrow (Hayashi et al. 1994; Cheng-Wu et al., 1994). Hayashi et al. (1994) observed the stimulation effects of *S.platensis* on activation of macrophage and leukocyte cells, phagocytosis, interleukin production and immune response in rats. At the same time, more toxicological analysis have been studied for usage this microalga as a natural food additive and reliability (Salazar et al., 1998; Yazıcı and Kaynak, 2001; Belay, 2002). On that point, in terms of preventive medicine or alternative food for health is supported by the macrophage activation and thereby effects on growth and immune system. *S.platensis* has an important role on blood protein and lipid. Researchers indicated that (Nakaya et al., 1988; Kanamaru et al., 2005) cholesterol is decreased by inhibition of the cholesterol absorption from jejunum and bile acid resorption from ileum with phycocyanin in *S. platensis*. Also, it was reported that polyunsaturated fatty acids and phycocyanin in *S.platensis* may help for this purpose. In addition, the plasma proteins (total protein, albumin, globulin) increased by *S.platensis* due to its high contents of essential amino acids and protein with values ranging from 55-65% (Bezerra et al., 2009; Mariey et al., 2012). However, it was only reported that long term of Spirulina intake may caused gout due to the high protein value (Becker et al., 1986; Araújo et al., 2003). In this study, it's aimed to evaluate the effects of different doses of *S. platensis* on hematological and biochemical parameters of rats that fed a long trial period.

Materials and Methods

Animals, Groups And Feeding

In trial, aged 7-8 weeks, 30 male Wistar Albino rat were randomly allocated on a weight basis to three groups: Control, (basal diet), SP-1 (added 500 mg/kgbw *S. platensis*, daily) and SP-2 (added 1000 mg/kgbw *S. platensis*, daily). The rats were housed in purpose-built metal cages. Feed and water were offered ad libitum throughout the 45 day trial. Basal diet was formulated to contain 2000-2500 kj ME/kg metabolize energy, 23% crude protein, 3% crude fat, 7% crude fiber, 8% crude ash and, was projected to take on maintenance requirements according to the NRC (1995). The experimental groups fed by *S.platensis* (Egert, Izmir-Turkey) orally daily, and doses also were provided and modified according to literature (Nagaoka et al., 2005; Moreira et al., 2011).

The experimental protocols were approved by the Animal Care and Use Committee of Namik Kemal University and are in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. The study was carried out with the permission of Namik Kemal University Animal Experimentation Local Ethics Committee (Approval No: 2017/04-4).

Measurement

Body weights and weight gains of each rats were determined for growth performance in each week of the trial. Blood samples were collected for anticoagulant tubes by tail venipuncture on the 45th day from overnight-fasted rats. Haematocrit, haemoglobin, counts of white and red blood cell, platelet (PLT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and red cell distribution widht (RDW) were obtained by Exigo Eox Vet hemogram apparatus that from the laboratory of Namik Kemal University Experimental Research Center. Serum total cholesterol, its fractions (LDL, HDL) and atherogenic indices (TC:HDL-C, LDL-C:HDL-C) were observed by spectrophotometrically. Besides that, serum protein, albumin, globulin and albumin/globulin ratio were determined.

Statistical Analysis

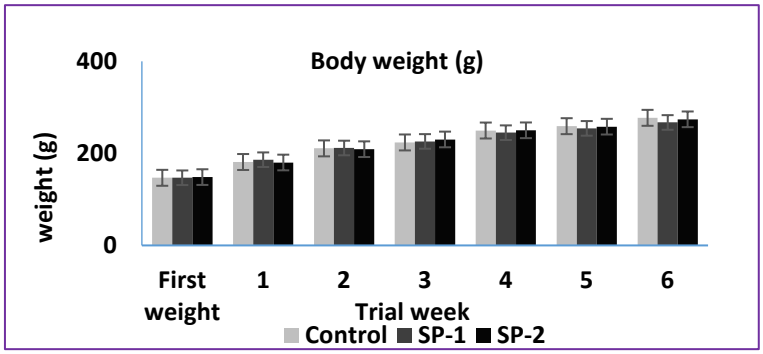
Statistical analyses were performed with SPSS (Version 17,0). Data were tested for normality distribution and variance homogeneity assumptions. All the values were grouped and the means and standard errors were calculated. One-way ANOVA was applied to the all parameters to examine the difference between groups. Differences were considered significant at $P<0.05$. If the difference between groups was provided to be significant ($P<0.05$), differences evaluated by Tukey's test (Dowdy and Wearden, 1981). On the other hand, in non-homogenous groups, differences between means were analyzed by Kruskal Wallis and following Mann Whitney U test between groups one by one (Dawson and Trapp, 2001).

Results and Discussion

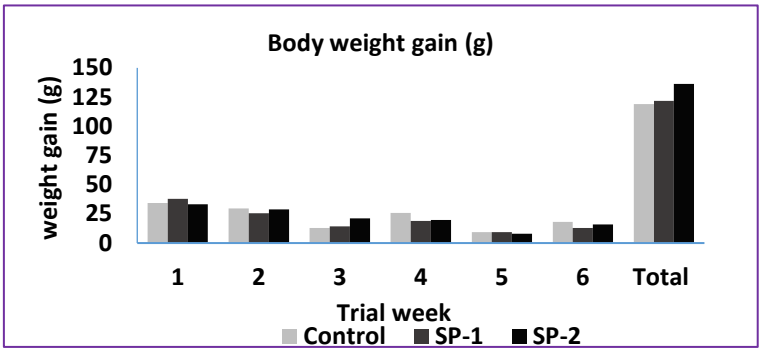
Although there were no statistically differences occurred among all groups, all parameters were found in their reference values. However, effects of lower dose of *S.platensis* showed the best result for those physiological parameters. The body weight and weight gain values obtained in the study groups were shown in Graphic 1 and 2. respectively. There were no significant differences among all groups according to the weekly periods ($p>0.05$). At the end of the study, mean live weights of control and research groups were 277.25±13.22, 267.19±8.13 and 274.033±7.84 g. Also, there were no differences in terms of average weight gains among the groups (Graphic 2, $p>0.05$). The weight gains of rats at the end of the experiment were 118.91 ± 13.60, 121.82 ± 9.93, and 136.23 ± 8.59 g respectively in group control, SP-1 and SP-2. The daha about some physiological and biochemical parameters were given in Table 1 and 2. All parameters were found in normal reference values but interesting in increase of total cholesterol, LDL cholesterol and atherogenic indices.

S.platensis have been used for a natural food additive for animal feeding, recently. Most of researchers reported various results of the effects *of S.platensis* on growth performances. Araújo et al. (2003) studied rats fed by of 5% and 10% *S.platensis.* It was determined that although there were no statistical differences in growth and feed efficiency, there was an increase in live weight in group fed by 10% *S.platensis*. This results were similar to our results. Although there were no significant differences in growth parameters statistically in our study, it was observed that weight and weight gain of rats in experimental groups increased in last weeks (Graphic 1). Nevertheless, Heidarpour et al. (2011) had a study with additives of 0, 2, 6, 25 g *S.platensis* given to ruminants for 15 trial day. They found no differences in weight gain, feed efficiency and daily feed consumption among all groups. Besides that, an other study about growth parameters were detected in fishes which fed by 10%, 20%, 30% and 40% Spirulina, and no statistical differences were observed (Dernekbasi et al., 2010). However, some researchers reported the positive impact of *S.platensis* on growth metabolism in animals (Grinstead et al., 2000; Peiretti and Meineri, 2008; Moreira et al., 2011). The mechanism of *S.platensis* on growth and feed efficiency was explained by its inhibiton effect on harmful microorganism in intestinal mucosa (Bhowmik et al., 2009).

Graphic 1. Body weight of rats in Control and Experimental Groups (g).



Garphic 2. Body weight gain of rats in Control and Experimental Groups (g).



In our study, there were no statistical differences in all hematological parameters among all groups, and also they were in their normal reference values (Table 1; p>0.05). Similary, Simsek et al (2007) determined that *S. platensis* had no differences in hemaotological parameters such as erytrocytes, haemoglobin, but a statistical decrease in haematocrit value in rats. A study about fish nutrition with *S.platensis*, researchers found that an increase in erytrocytes and leucokcytes count statistically (Promya and Chimanat, 2011). They reported that *S.platensis* may stimulate the activities of bone marrow cells and thereby improve the immunity of organism. Also, it was reported that because of the macrophage activity of *S.platensis*, cellular and humoral immunity and survival rate were improved, especially broilers whose immune system was not sufficiently developed (Qureshi et al., 1996; Hamad et al., 2001). There was no mortality and any diseases were excited during this study as well as the results showed that *S.platensis* had no negative effect on hematological characteristics. The Lymphocytes were 69,34±1,96 in control, 70,66±0,98 in SP-1 and 76,05±3,17 in SP-2 (p:0,185). Eosinophils were 10,87±1,64, 14,50±1,68 and 13,70±1,63 in groups control, SP-1 and SP-2 respectively (p:0,116). However, leukocytes counts and ratios of monocytes, neutrophil and basophils were not identified exactly. So, they not rated for the research. This result may be due to an allergic reactions of rats or a mistake of analyzer. Besides that, a recent study, it was reported that using automotical methods (hematology analyzers) for count the leucokcyte and leucokcyte substypes may not be suitable for rats (Messias et al., 2017). So, it was neccessary to analyse these parameters by handled method for future researches.

Table 1. Haematological Indices (mean ± SE, n=30).

Parameters	Groups		
	Control	**Group SP-1 (S. platensis-500mg)**	**Group SP-2 (S. platensis-1000mg)**
Haematocrit (%)	39,91±1,10	36,55±1,34	34,72±2,34
Haemoglobin (gr/100ml)	15,27±0,35	13,93±0,50	13,34±0,90
Erythrocyte (x106/mm3)	7,64±0,19	6,92±0,22	6,71±0,46
Leukocyte (x103/mm3)	8,32±0,90	4,73±1,33	10,18±3,97
MCV (fl)	52,22±0,80	52,74±0,62	51,82±0,74
MCH (pg)	20,02±0,24	20,11±0,18	21,99±2,05
MCHC (g/dl)	38,39±0,29	38,14±0,20	34,73±3,77
RDW (%)	15,11±0,34	14,13±0,29	14,34±0,24
PLT (x106 /mm3)	519,83±143,57	573,67±39,37	556,75±171,69

In addition, *S.platensis* may inhibit the harmful bacteria in intestine (Bhowmik et al., 2009) and thereby inflammatory agents that secreted by enteric bacteria may affect on globulin synthesis of liver. Some researchers reported different results about effects of Spirulina on protein values. (Bezerra et al., 2009; Moreira et al., 2011; Heidarpour et al., 2011). Although, Moreira et al.(2011) indicated no effect of *S. platensis* on serum protein levels, Mariey et al.(2012) stated that SP level at 0.2% had a significant increase in plasma total protein, albumin and globulin in laying hens. On the other hand, Bezeria et al. (2009) determined the high serum protein value in lambs fed 0, 5 and 10 g SP. All these researchers suggested that the protein quality and quantity of *S. platensis* may increase the serum protein level. In recent study, high Spirulina additive showed normal value with control however, in group of lower dose of Spirulina determined insteresting results about cholesterol and its fraction values. All of them are in reference value but interesting in increase of Total cholesterol, LDL cholesterol and atherogenic indices. Total cholesterol (TC) and its fraction, low-density lipoprotein cholesterol (LDL-C), exhibited a rise coupled with a marginal decrease in the level of high-density lipoprotein cholesterol (HDL-C). As a result, increase in the atherogenic indices, TC:HDL-C and LDL-C: HDL-C, was observed. Due to all parameters resulted in their reference value, we can say that different experimental condition or animal may be change the results. However, various studies must be planned for next studies with different aims.

Table 2. Biochemical Parameters (mean ± SE, n=30).

Parameters	Groups		
	Control	**Group SP-1 (S. platensis-500mg)**	**Group SP-2 (S. platensis-1000mg)**
Cholesterol (mg/dl)	41,18±3,25	51,76±3,4	42,78±2,04
Trygliserid (mg/dl)	107,53±10,87	83,08±12,49	107,41±8,97
Total lipid (mg/dl)	252,19±8,37	257,32±16,07	259,44±5,82
HDL-Cholesterol (mg/dl)	30,89±0,86	25,13±3,16	30,91±2,52
LDLD-Cholesterol (mg/dl)	31,80±3,56	43,25±7,81	33,36±2,52
VLDL-Cholesterol (mg/dl)	21,51±2,17	17,46±2,35	21,48±1,79
LDL/HDL ratio	1,04±0,13	1,46±0,43	1,17±0,17
TC/HDL ratio	1,25±0,07	1,52±0,14	1,43±0,11
Total protein (g/dl)	5,63±0,18	6,06±0,20	5,54±0,08
Albumin (g/dl)	2,36±0,07	2,20±0,07	2,28±0,05
Globulin (g/dl)	3,27±0,21	3,86±0,24	3,27±0,10
Albumin/globulin	0,75±0,07	0,62±0,06	0,70±0,03

Conclusion

Good and high quality protein intake from alternative supplements has become important in nowadays. Belong to this issue, researchers attract to attention on the various and natural alternative foods or plants. *S. platensis* which is an interesting plant among these natural additives has a rich biological content. Nevertheless, all studies about plant additives and the results were indicated the controversial nature effects of *S.platensis* on weight, weight gain, blood and biochemical parameters.

It's believed that these differences may due to the sexuality of animals, environmental conditions, trial long and also effective doses of *S.platensis* which have not yet been used. In this study, results showed that long term and high dose of using this microalgae is appropriate for health, but more studies are needed to pointed out the importance of this natural supplement.

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