

Nutritional and Biological evaluation of using *Spirulina* algae as a new source of protein

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ABSTRACT

Spirulina platensis (SP) alga has been a common dietary substance around the world from ancient times. This study was aimed to using Spirulina as a novel protein source, especially in growth phase. Thirty six male albino rats aged six weeks were divided into six groups according to the protein source as following: The casein groups (C1, C2, and C3) contain (100, 200, and 300 g casein /kg diet) as (10%, 20% &30% casein), respectively. On the corresponding side, the spirulina clusters (SP1, SP2, and SP3) contained (157, 315 and 472 gm algal protein/kg food) respectively. It lasted 28 days. SP has high total antioxidant activity (39.2 %). SP groups recorded significant increases in feed intake, body weight gain, feed efficiency ratio and protein efficiency ratio compared with casein groups. While casein recorded significant increases in apparent digestibility, Co-efficient compared to groups of SP algae. Eating Spirulina has significantly preserved liver and kidney function. As for complete blood picture, the groups of SP3 recorded highest percentage in Hb and RBCs compared with C3 group, other than WBCs, which was lower in SP3. Significant increase in the antioxidant enzymes in liver tissues were observed in SP groups, while Malondialdehyde recorded a significant reduction in the same groups. SP treatment increased GH levels in all groups. On the other side, the results of alpha tumor necrosis factors recorded a significant decrease in groups of SP compared with the groups of casein. In conclusion, Spirulina can be used as a new protein replacement.

Key words: *Spirulina* algae - protein substitutes - Hematological parameters - antioxidant enzymes.

INTRODUCTION

Protein deficiency, characterized by low protein intake, reduces plasma amino acid concentrations and weakens the immune system (**Fechner et al., 2001**). According to the World Health Organization (WHO), approximately 30% of the world's population is malnourished and 40,000 children die every day due to malnutrition and related diseases (**WHO, 2011**). Protein energy malnutrition (PEM) and micronutrient deficiency leading to early growth traced to poor maternal nutritional and health care before and during pregnancy, resulting in intrauterine growth retardation and children born with low birth weight, while significant progress has been achieved over the past 30 years in reducing the proportion of malnourished children in developing countries (**Udayasree et al., 2013**).

Technicians of the WHO have discussed the discovery and use of new protein sources as well as rules to use them in the food. In that sense, microorganisms have been studied as possible sources of protein. Use of cyanobacteria as a source of protein has certain advantages in relation to other microorganisms due to their rapid growth in addition to the quantity and quality of protein (**Molina et al., 2002**). One of such food, which constitutes the most

remarkable concentration of nutrients, is “*Spirulina*” which combat malnutrition undernourishment, and protein deficiencies. *Spirulina* is a blue-green microalgae has a spiral cellular structure, and has an extraordinary capacity to survive under conditions that are much too harsh for other algae two species of *Spirulina* that are most commonly used in nutritional supplements are *Spirulina platensis*(SP) and *Spirulina maxima* (**Khan et al., 2005**).

Spirulina contains, approximately, 65% to 71% protein by dry weight and is claimed to be non-toxic nutritious food with exceptional properties. It contains 47% of essential amino acids, methionine, which is usually absent in other algae, and cyanobacteria, 15–25% carbohydrates, 8–13% minerals, 3–7% fat, 8–10% fiber. It also contains chlorophyll, phycocyanin, carotenoids, minerals, vitamins, essential fatty acids, and other bioactive components (**Iyer et al., 2008**). *Spirulina* is increasingly incorporated into bread and other bakery products such as cassava cake (**Navacchi et al., 2012**), biscuits (**Sharma and Dunkwal, 2012**) and sweet bread (**Minh, 2014**).

Siva Kiran et al., (2015) mentioned that *Spirulina* is a

simple extract of blue-green algae, which is now used worldwide as a food product and as a dietary supplement. It contains, essential amino acids, lipids, vitamins, minerals and anti-oxidants and can be considered as a wholesome food supplement.

Sinha et al., (2019) evaluated the effects of dietary Spirulina supplementation of protein malnourished mothers during pregnancy and lactation on their offspring's reflex, neurobehavioral and cognitive development. The study has shown improved reproductive performance of Spirulina supplemented protein malnourished dams, accelerated acquisition of neurological reflexes, better physical appearance, enhanced neuromuscular strength, improved spatial learning and memory and partly normalized protein malnutrition induced hyper-activity, anxiolytic and a hedonic behavior in offspring.

Recent studies have associated consumption of Spirulina supplements with significant immunomodulatory, pro-inflammatory, antioxidant, anti-cancer, and antiviral activities, both in animal models and in human subjects. Hypolipidemic, hypoglycemic, and hypotensive effects are also being uncovered, and there is clear evidence of

further beneficial health effects on its consumption; however, research into its hepatic effects is still relatively limited (**Reboleira et al., 2019**). **Cho et al., (2020)** observed that Spirulina enhanced bone growth and bone strength by stimulating parathyroid hormone and growth hormone activities, as well its increased antioxidant activity. Their results have indicated that Spirulina provides a suitable dietary supplement and alternative protein source with antioxidant benefits for growth improvement in early developmental stages. Therefore we hypothesized that spirulina, a plant-origin protein, could be a functional substitution for animal protein, in this case, casein protein. Therefore, this work aimed to evaluate the effects of using Spirulina as a protein substitute and its antioxidant effect on the growth-related hormone levels and the biochemical and hematological aspects of the blood in growing male rats.

MATERIALS and METHODS

Materials:

- *Spirulina platensis* (SP) algae powder: It was obtained from Nour Alhuda for Training and Management of Agriculture Projects, Cairo, Egypt.

- Animals: Thirty-six young male albino rats (*Sprague Dawley* strain) weighed (80 ± 10 g) were obtained from Vaccine and Immunity Organization, Helwan Farm, Cairo, Egypt.

- Kits were purchased from Egyptian American Company for Laboratory Service.

- Corn oil and starch were purchased from the local market. Casein, cellulose, vitamins, minerals, Sucrose and methionine were obtained from the Cairo Company for Chemical Trading, Cairo, Egypt.

- Diet: Composition of experimental diets (g/kg diet) according to NCR, (1995) and some modification.

Methods:

Chemical analysis of *Spirulina* algae:

Carbohydrates, Protein, fat, moisture, fiber and ash of algae were determined according to the methods described by A.O.A.C, (2010). Amino acid analysis is a technique based on ion exchange liquid chromatography, used in a wide range of application areas to provide qualitative and quantitative compositional analysis. In the biochrom systems, this basic principle has been refined to produce fully automatic, high speed, sensitive analyses. This is sometimes referred to as classical

amino acid analysis according to the method described by Pearson, (1981). Antioxidant activity was calculated according to the methods described by Moukette *et al.*, (2015).

Experimental design:

After adaptation period (one week), in well-aerated cages under hygienic condition and fed on basal diet and water supply *ad libitum*. The rats divided into six groups (6 rats each) as following: **Group (1)** fed on basal diet (casein 100g/kg diet). **Group (2)** fed on basal diet (*Spirulina* 157g/kg diet as a casein replacement). **Group (3)** fed on basal diet (casein 200g/kg diet). **Group (4)** fed on basal diet (*Spirulina* 315g/kg diet as a casein replacement). **Group (5)** fed on basal diet (casein 300g/kg diet). **Group (6)** fed on basal diet (*Spirulina* 472g/kg diet as a casein replacement). Total faeces for each rat in each group was collected at the end of the seven days (8 am). Faeces were weighed, mixed thoroughly and sun-dried, reweighed, ground to pass through one mm screen. The ground sample for each rat was stored in paper bags for dry matter and protein analysis. At the end of experiment (28) days, The urine dipstick test was performed along with 24-hour urine samples, the animals were deprived of food

and water overnight before being sacrificed. Blood samples were collected in dry centrifuge tubes from hepatic portal veins. Serum samples were separated by centrifugation at 4000 rpm for 10 minutes and kept in Eppendorf tube at -20 until analysis. The liver was removed, washed with isotonic saline, dried by filter paper and weighted. Liver sample was kept at -20°C for preparation of tissue homogenate for determination of antioxidant parameters. The homogenate was centrifuged at 10,000 rpm for 20 min. The supernatant was used for the assay of some laboratory analyses.

Nutritional and Biological parameters

During the experimental period, the diet consumed was recorded twice weekly, and body weight recorded every week. The body weight gain (BWG) and feed efficiency ratio (FER) were evaluated according to **Chapman et al., (1959)**. Protein efficiency ratio (PER), calculated as the ratio of weight gain (g) and total protein consumed (g) (**Sgarbieri, 1996**).

Apparent digestibility Co-efficient = $\frac{\text{protein intake} - \text{fecal protein}}{\text{protein intake}}$

Hematological investigations

Complete blood count was

performed on blood samples with anticoagulant using hematological analyzer (Exigo Eos Vet, Sweden). The erythrogram consisted of red blood cell count (RBCs), hematocrit (HCT), hemoglobin (Hb) concentration and red cell indices, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and red blood cell distribution width absolute (RDW_a), leukogram (includes white blood cell count (WBCs), lymphocytes (%), monocytes (%) and platelet count (PLT) according to (**Jain, 1986**).

-Biochemical analysis of serum and urine

Serum samples were used for the determination of the concentrations of tumor necrosis factor- α (TNF- α) and growth hormone (GH) measured using automated biochemistry analyzer (SPIN120-Spinreact-benchttop 8 wavelengths: 340 and 670 nm) according to **Gao et al., (2015)**. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined according to **Bergmeyer et al., (1986)**. Alkaline phosphatase (ALP) activity was estimated according to **Kind and King, (1954)**. Albumin (**Drupt, 1974**) and total protein were determined according to (**Sonnenwirth and**

Jaret, 1980). Urea nitrogen, creatinine and uric acid were evaluated in serum according to **Patton *et al.*, (1977); Faulkner and King, (1976) and Fossati *et al.*, (1980)** respectively. Creatinine clearance (Cr Cl) **was calculated by the formula:** $\text{Clcr} = \frac{\text{urine creatinine (mg/dL)} \times \text{urine flow (mL/min)}}{\text{Serum creatinine (mg/dL)}}$

Assessment of Oxidant / Antioxidant Activity in liver tissue

After then liver removed, homogenized and centrifuged at 10,000 rpm at 4°C for 20 min. The supernatant was used for estimation of different antioxidant level by calorimetric method with a spectrophotometer Elisa (micro plate reader Ryt2100 C) at (520 and 535 nm) Malonaldehyde (MDA) by thiobarbuturic acid-reactive substances (TBARS) methods (**Uchiyama and Mihara 1978**), Superoxide dismutase (SOD) by method developed by **Misra and Fridovich, (1972)** and Catalase (CAT) by colorimetric assay (**Sinha, 1972**). Glutathione peroxidase (GPx) was carried out according to **Pablos *et al.*, (1998)**.

Statistical analysis

Results are expressed as mean \pm standard deviation (SD). Differences between means indifferent groups were tested for

significance using a one-way analysis of variance (ANOVA) followed by Duncan's test and P value of 0.05 or less was considered significant according to (**Snedecor, 1969**) using SPSS (version 20).

RESULTS & DISCUSSION

Chemical composition of SP (Protein, carbohydrates, ash, lipids, moisture, and crude fiber) per 100g on the dry weight basis was presented in Table (1). SP contains Protein, carbohydrate and ash (54 ± 1.43 , 12.50 ± 0.40 and 11.05 ± 0.21 (g/ 100g DW), respectively. Also SP has high total antioxidant activity (39.2 %). These data agree with **El-Moataaz *et al.*, (2019)** and **Cho *et al.*, (2020)** who found that SP contains a high amount of protein. The protein content was of high quality as it comprised of essential amino acids and non-essential amino acids.

Data in Table (2) confirm the effect of using SP algae as a casein replacement on feed intake, BWG, FER, and apparent digestibility Co-efficient in young male rats. It could be noticed that the groups of SP (1, 2 and 3) recorded significant increases in feed intake, BWG, FER and PER compared with casein groups. However, on the other side the groups of casein

recorded significant increases in apparent digestibility Co-efficient compared to groups of SP algae. This corresponds to **Gargouri et al., (2020)** who found that treatment of SP improved food, water consumption and body weight gain in rats treated with lead (Pb). Also in another study was performed by **El-Beltagi et al., (2020)** who evaluated hepatoprotective and antioxidant activities of (SP) and pomegranate juice (PJ) in rats against hepatotoxicity induced by carbon tetrachloride (CCl₄). They noticed that (SP) and (PJ) significantly increased in weight gain relative to normal control and CCl₄ treatment groups, It is presumed that treatments used with PJ, SP and their combination may boost appetite and increase weight gain. The improvement in body weight gain also noticed in diabetic Wistar rats treated with SP (**Aissaouia et al., 2017**). **Dernikabsi et al., (2010)** studied the effect of dietary supplementation of different rates of SP on growth and feed conversion in guppy, the groups treated with SP recorded significant increases in feed intake and body weight gain specially the groups of 30 & 40% of spirulina. **Moreira et al., (2013)** evaluated the effects of SP as a protein source in the nutritional recovery

of rats subjected to protein malnutrition. The SP treatment showed the highest values of (FER) and Quotient of Protein Efficiency Ratio (QPE) ($p \leq 0.05$). These results demonstrated that the nutritional recovery of animals was enabled by different concentrations of SP. These results disagree with **Heidarpour et al., (2011)** who investigated the effects of different amounts of SP on the performance and digestibility of dry matter (DM) of Holstein calves. Results showed that treatment effect was not significant on the final weight, daily gain, daily feed intake, feed efficiency and digestibility coefficient ($P > 0.05$), while increase in the SP level up to 25 g, decreased digestibility of DM. Digestibility of DM in the control diet was higher than those of SP diets. It was interesting that increased in the SP level led to decrease in digestibility of all mentioned nutrient materials.

Data in Table 3 show the effect of SP algae as a casein replacement on serum AST, ALT and ALP. The mean values of AST, ALT and ALP in groups of SP (1&2) are similar to groups of casein (1&2). But the mean values of AST, ALT and ALP in groups of SP1 and casein 1 recorded a significant decrease compared to casein 2. SP 2 maintains the liver

biomarker in this experiment. These results are similarly for a study by **Moreira et al., (2013)** who evaluated the effects of SP (Arthrospira) as a protein source in the nutritional recovery of rats subjected to protein malnutrition. They found that serum levels of the activities of AST and ALT revealed no values statistically different from the control group. The results matched with **Cho et al., (2020)** who evaluated the effects of SP on the bone metabolism and antioxidant profiles of three-week-old growing male rats. They found that no significant difference between the spirulina substitution diets and the control diet at 7 weeks in liver functions. SP protects against oxidative stress either by free radical scavenging or by enhancing SOD and CAT activities accordance to **Bin-Jumah et al., (2021)** who evaluated the ameliorative effect of SP against acrylamide (AA) toxicity in rats. They found that SP supplementation at a dose of 500 mg/kg in the AA-intoxicated group markedly reduce ($P \leq 0.05$) the serum levels of ALT, AST, and ALP. **El-Beltagi et al., (2020)** evaluated hepatoprotective and antioxidant activities of SP and pomegranate juice (PJ) in rats against hepatotoxicity induced by carbon tetrachloride (CCl₄). They

found that SP extract and its combination (PJSP) also showed a substantial reduction compared to the liver injury management group of rats. The liver markers were be improved after receiving gradient doses of SP and pomegranate juice. **Xiang et al., (2018)** studied the protective effect and mechanism of selenium-enriched SP on chronic alcohol-induced liver injury. Their results indicated that the protective mechanism of selenium-enriched SP on chronic alcoholic liver injury is associated with the activity enhancement of antioxidant enzymes and immunity, the inhibition of DNA damage and apoptosis, accompanied with autophagy and pyro ptosis. As well as **Aissaouia et al., (2017)** investigated the antioxidant effects of SP in diabetic Wistar rats. They found significant decreased in the groups treated by SP in the oxidative stress parameters AST, ALT ($p < 0.001$) in comparison to those of the controls after 70 days. SP dietary supplementation reduced the serum hepatic biomarkers, had a good protection and maintained the structural integrity of hepatocellular membrane (**Abdel-Daim, 2014**) (**Abdelkhalek et al., 2014**). This may be due to the antioxidant activity of *Spirulina* phycobiliproteins (phycocyanins and allophycocyanins) or phenolic

compounds (Nuhu, 2013; Abdel-Daim *et al.*, 2016). On the other side SP 3 and casein 3 recorded significant increases in these parameters compared to casein2 (control group). This is in agreement with Díaz-Rúa *et al.*,

Data in Table 4 demonstrate the effect of SP alga as a casein replacement on serum total protein, albumin and globulin. Total protein recorded non-significant differences among groups of casein and SP groups (1&2). SP may inhibit the growth of harmful bacteria in intestine, as presented by Bhowmik *et al.*, (2009) who found the lower value of serum globulin of rabbits fed at 5% SP. They mentioned that this might be attributed to the inhibitory effect of SP against harmful intestinal microflora. Because harmful enteric bacteria secrete inflammatory agents and lead to increase in globulin synthesis of liver or of other tissues such as lymphatic tissue or plasma cells. Ramez *et al.*, (2021) investigated the therapeutic effects of SP and *Matcha* green tea (MGT) in *Schistosoma mansoni*-infected mice combined with tracing their possible antioxidant and anti-inflammatory impacts and their protective potency. Treatment with the combination of SP and

(2017) who found that using high protein in diet resulting in a high acid load increased TG deposition and increased signs of health risk (increased inflammation, alterations in the acid-base equilibrium and oxidative stress) in rats.

MGT (SP– MGT-infected group) significantly restored serum total protein and globulin to concentrations similar to those of the uninfected controls ($P < 0.05$).

Khanna *et al.*, (2016) studied the effect of *Emblica officianalis* and *Spirulina platensis* was studied in New Zealand White rabbits. They found there were no significant changes in the serum biochemical indices, only the serum globulin value was significantly ($P < 0.05$) lower and albumin value was found significantly higher in the groups fed *spirulina* supplemented diets as compared to groups fed with non-supplemented diets. On the other side casein 3 recorded a significant increase in these parameters compared to casein2 (normal control). These results agree with Nour El-Deen *et al.*, (2018) who found that a high protein intake in diabetic obese albino rats and normal rats led to changes in serum total protein and urine levels of markers of renal function, which indicated abnormalities in the functions of the kidney.

Data in Table 5 show the effect of SP algae as casein replacement on urea, uric acid, creatinine and creatinine clearance of rats. The mean values of urea, creatinine and creatinine clearance in the groups of SP (1&2) are similar to groups of casein (1&2). Nevertheless, the mean value of uric acid of SP and casein groups 1 recorded a significant increase compared to casein 2. On the other hand, casein 3 and SP 3 recorded a significant increase in these parameters compared to casein 2. SP 3 recorded a significant decrease in these parameters compared to casein 3. **Moreira et al., (2013)** evaluated the effects of SP as a protein source in the nutritional recovery of rats subjected to protein malnutrition. They noticed that all the treatments with SP had creatinine levels statistically similar to the control; however, when comparing the treatments with SP with each other, S2 showed significantly lower values ($p \leq 0.05$). High concentration of casein affect kidney functions. **Aparicio et al., (2013)** found that high-protein diet promoted a worse renal profile, especially on urinary and morphological markers, which could increase the risk for developing renal diseases in the long time. Protein ingestion increases renal acid excretion, and

acid loads, in turn, may be buffered in part by bone, which releases calcium to be excreted by the kidney. This protein-induced hypercalciuria could lead to the formation of calcium kidney stones (**Goldfarb, 1988**). **Gargouri et al., (2020)** noticed that a significant reduction in creatinine clearance, an indicator of glomerular dysfunction, was observed in the Pb-treated female rats. Co administration of SP caused a marked improvement of urea, uric acid and creatinine. **Bin-Jumah et al., (2021)** agreed with the present results, when they evaluated the ameliorative effect of SP against AA toxicity in rats. They found the levels of urea, creatinine were markedly reduced. This improvement was due to the enhanced restoration of the tubular malfunction under the influence of a biliprotein pigment called phycocyanin drug, which is present in SP and had a diuretic activity. Another thing clarify the increase of diuresis is the presence of flavonoids and potassium in SP algae (**Abdel-Daim, 2014**).

Data in Table 6 expression the effect of using SP algae as a casein replacement on Hb, RBCs and WBCs. The results illustration that there were a significant increase in groups of SP compared with the groups of casein. The groups of SP3 and C3

recorded highest percentage in Hb and RBCs compared with C2 group, other than WBCs, which was lower in SP3. The improvement in these parameters for SP group may be due to contain a high percentage of protein. In addition, SP contains high iron, about 28.5 mg /100 gm, of which 58 times more than in spinach, and 18 times higher than that found in meat. Consumption of 100 gm of SP can fulfill 158% of iron needed in a day (**Marlina, 2019**). These results agree with **Gargouri et al., (2020)** who investigated the effect of SP against the hemato-biochemical alterations and nephrotoxicity induced by lead (Pb). They noticed that treated with SP occurs the changes in blood indices shown in Pb-treated group were reverted to near normal levels upon co-treatment with SP. **Mohammadizarm et al.,(2020)** studied the effect of SP meal as a feed additive on growth and physiological response of Oscar fish. They found that Hb was improved markedly in different SP treatments, and develop the cellular immune system and red blood cells. **Marlina, (2019)** stated that SP might ameliorate anemia among women in the second trimester of pregnancy. Also, **Rajachar et al., (2016)** emphasized in their randomized

study of 1000 children that consuming spirulina in doses (1 and 2 grams) led to an increase in hemoglobin level as well as a significant improvement in their mental abilities and their academic level.

The table 7 illustrates the effect of using SP algae as a casein replacement on hematocrit, monocytes, lymphocytes and P.Count. The results of hematocrit, lymphocytes, and monocytes recorded significant increases in groups of SP compared with normal control. In the other side, P.Count recorded a significant decrease in groups of SP compared with the groups of casein. These results agree with **Gargouri et al., (2020)** who investigated the effect of SP against the hemato-biochemical alterations and nephrotoxicity induced by lead (Pb). They noticed that treated with SP occurs the changes in blood indices shown in Pb-treated group were reverted to near normal levels upon co-treatment with SP. The present results were in line with **Moreira et al., (2013)** who evaluated the effects of SP as a protein source in the nutritional recovery of rats subjected to protein malnutrition. Where they found that hematocrit recorded a significant increase in-group 10, 20% of SP compared with control group. However, they

recorded significant decrease in group 30% of SP compared with control group. Other than what was found in this research, SP3 raised the hematocrit level compared with casein group.

As shown in Table 8, SP algae groups recorded significant increases in MCH, MCV and MCHC compared with casein 2. In addition, these results agree with **Mohammadizarm *et al.*, (2020)** who studied the effect of spirulina meal SP as a feed additive on growth and physiological response of Oscar fish. The results of this study showed that fish fed on diets including 13.75–55 g/kg SP had improved, HCT, MCH and MCHC compared with the control. In addition, these results are completely in agreement with **Seyidoglu *et al.*, (2019)** who mentioned that SP could improve hematological and morphological parameters when they studied its effect on morphological and hematological factors resulting from social stress in male rats. **Roberto, (2015) and Balasubramani *et al.*, (2016)** showed that SP contains minerals such as iron, magnesium, calcium, and phosphorus. SP is a splendid source of iron which contains 20 times more iron than wheat germ so spirulina is a good treatment for anemia. These results are

supported by the results published by **Visnegarwala and Mahesh, (2017)** has shown the effects of SP, blue green algae, as an alternative to iron supplements, to not only alleviate the anemia of pregnancy but also have impact on the fetal and maternal outcomes, through its impact on the gut microbiome. Also, **Radha and Chandra, (2018)** has shown that SP is useful for anemic persons because it is a good source of iron, meaning it is excellent for women during pregnancy. In addition, **Pugazhendy *et al.*, (2012)** reported that spirulina could improve Hb, MCH and MCHC in anemic children. The nutritional benefits of the spirulina over the vitamins and mineral supplementations might refer to the functional activities of it.

Table 9 shows the effect of SP algae as a casein replacement on CAT, MDA, GPx and SOD. In all groups of SP (1, 2&3) recorded significant increases in antioxidant enzymes (SOD, CAT& GPx) in liver tissue compared with casein 2. On the other side, the results of MDA recorded significant increase in groups of casein compared with the groups of SP.

In this study, oxidative stress was confirmed by decreased antioxidant enzyme (SOD, CAT& GPx) as well as increased MDA level. Administration of SP

increased antioxidant enzymes, which can be attributed to its antioxidant activities. These findings indicate that SP antioxidant activities due to having several antioxidant compounds such as various vitamins, phycocyanin, selenium and polyunsaturated fatty acids. **Abd el-Baky et al., (2009)** have demonstrated that SP is able to increase its antioxidant activity during oxidative stress elevation and has a self-regulating antioxidant activity against the intensity of oxidative stress. Consistently, many studies have reported similar findings. **Ramez et al., (2021)** have investigated the therapeutic effects of SP and (MGT) in *S. mansoni*-infected mice combined with tracing their possible antioxidant and anti-inflammatory impacts and their protective potency. They reached to the antioxidant effects of SP and/or MGT against *S. mansoni* mediated oxidative stress in serum MDA was significantly elevated, and the CAT, SOD, and GSH-Px activities and TAC levels were significantly reduced in infected untreated mice compared with the control group. Recent studies have shown the ameliorative effect of SP against AA toxicity in rats. They found that rats treated with 500 mg/kg SP only did not show obvious changes in hepatic

oxidative stress and antioxidant biomarkers compared to the normal untreated rats. Similarly, rats treated with 1000 mg/kg SP only did not reveal obvious changes in hepatic oxidative stress and antioxidant biomarkers except GSH, SOD, and CAT, which showed significant increase compared to the normal untreated rats (**Bin-Jumah et al., 2021**). According to **El-Beltagi et al., (2020)** who have evaluated hepato protective and antioxidant activities of SP and pomegranate juice in rats against hepatotoxicity induced by carbon tetrachloride (CCl₄). They have demonstrated that SP and pomegranate juice had hepatoprotective effect on CCl₄-caused hepatotoxicity in rats through its antioxidant activity. As well as **Xiang et al., (2018)** have studied the protective effect and mechanism of selenium-enriched SP on chronic alcohol-induced liver injury. They noticed that Se-SP or SP decreased the MDA level in the livers of alcohol-treated mice. The results have confirmed that pretreatment with Se-SP or SP could improve the activities of SOD and GSH-Px. Furthermore, SP induced a marked elevation in the antioxidant enzyme activities and GSH level in the renal and hepatic tissues (**Wu et al., 2016**). Although an analysis study

revealed that the distributions of the total phenolic compounds varied between commercial products, it was claimed that chlorogenic acid, synapic acid, salicylic acid, trans-cinnamic acid, and caffeic acid were commonly present in spirulina (Miranda et al., 1998). The antioxidant compounds in spirulina, such as phycobilins and phycocyanins, also inhibit the activities of catalytic enzymes, such as lipoxygenase and cyclooxygenase, or enhance the activity of enzymes, such as glutathione peroxidase, catalase, and superoxide dismutase (Bhat and Madyastha, 2001 & Chaiklahan et al., 2013). These polyphenols were reported to have anti-inflammatory, antiviral, antioxidant, antithrombotic, vasodilatory, antidiabetic, neuroprotective, hepato-protective, and anti-carcinogenic properties (Bhat and Madyastha, 2001).

Data in Table 10 show the effect of SP algae as a casein replacement on α TNF and G.H in serum. The mean value of growth hormone recorded a significant increase in SP groups compared with casein 2. On the other side, the results of α TNF recorded a significant decrease in groups of SP compared with the groups of casein. In previous studies, treatment with SP after AA

intoxication reduced TNF- α , IL-1 β and IL-6 serum levels. This is probably because SP has antioxidative and anti-inflammatory properties (Pak et al., 2012 and Salzano et al., 2014). These results are in accordance with Ramez et al., (2021) who have investigated the therapeutic effects of SP and MGT (Magnesium L-threonate) in *S.mansoni*-infected mice combined with tracing their possible antioxidant and anti-inflammatory impacts and their protective potency. They found that treatment with SP and/or MGT attenuated the changes in serum cytokines induced by *S. mansoni* infection; TNF- α , IFN- γ , and IL-13 were reduced, and IL-10 was significantly decreased in SP- and/or MGT-treated groups. As well as the results were agreed with Jin et al., (2020) who have evaluated the effects of SP on the bone metabolism and antioxidant profiles of three-week-old growing male rats. They have observed enhancement of bone growth and bone strength by SP treatment, although the SP-fed groups showed continuous increases in GH level over the 7-week study in a dose-dependent manner. They suggested that SP treatment can increase GH levels over time, potentially allowing bones to grow continuously. Our results are in accordance with Bin-

Jumah et al., (2021) who evaluated the ameliorative effect of SP against AA toxicity in rats. They found that the levels of TNF- α were markedly reduced. The present results also came in accordance with another study, which mentioned that this protection could also be attributed to the abundant content of antioxidant active ingredients, such as C-phycoerythrin, vitamins, β -carotene, and minerals in SP algae (**Avdagic et al., 2008**).

CONCLUSION:

Finally, based on all the results, *Spirulina platensis* can be considered very nutritious. SP has antioxidant and anti-inflammatory properties. In addition, the level of hemoglobin in the blood and the

number of red blood cells showed a significant increase, and the average value of the growth hormone recorded a significant increase in all groups treated with spirulina. Due to the higher content of protein and lower amount of carbohydrates and fats in spirulina powder. SP can be used as a good protein replacement. This makes it an ideal food especially in the growing stage. Therefore, this study recommends the introduction of spirulina in many food products, especially for children, avoiding protein consumption above the current RDA (high protein diet) for healthy children due to its potential disease risks.

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Table1: Chemical composition of *Spirulina platensis* (g / 100g dry weight basis)

Content in <i>Spirulina platensis</i>	Proximate composition (%)
Protein	54.4 ± 1.43
Carbohydrates	12.50 ± 0.40
Ash	11.05 ± 0.21
Lipids	10.23 ± 0.26
Moisture	6.50 ± 0.06
Fibers	5.25 ± 0.25
Total antioxidant activity	39 %
amino acids (g/100g)	
Tryptophan	0.929
Threonine	2.97
Isoleucine	3.209
Leucine	4.947
Lysine	3.025
Methionine	1.149
Cystine	0.662
Phenylalanine	2.777
Tyrosine	2.584
Valine	3.512
Arginine	3.147
Histidine	1.085
Alanine	4.515
Aspartic acid	4.793
Glutamic acid	6.386
Glycine	3.099
Proline	2.382
Serine	2.998

Values are means ± standard deviation of three determinations (n=3)

Table 2: Effect of using Spirulina as a casein replacement on total feed intake, body weight gain, feed efficiency ratio, protein efficiency ratio and apparent digestibility Co-efficient of experimental rats

Parameters / Groups	FI (g./period)	BWG %	FER	PER	A.D. Coefficient
Casein 1	324.33±7.55 ^c	50.35±9.71 ^c	0.18±0.01 ^b	0.22±0.02 ^c	0.93±0.004 ^a
Spirulina1	351.83±12.84 ^a	73.64±8.75 ^a	0.23±0.03 ^a	0.35±0.03 ^c	0.91±0.001 ^c
Casein2*	337.66±15.00 ^{bc}	48.94±10.61 ^c	0.15±0.03 ^b	0.193±0.04 ^c	0.93±0.001 ^a
Spirulina2	355.66±15.83 ^{ab}	67.32±13.47 ^{ab}	0.19±0.03 ^b	0.376±0.04 ^a	0.89±0.001 ^d
Casein3	330.66±14.30 ^c	54.40±15.76 ^{bc}	0.17±0.02 ^b	0.226±0.04 ^b	0.92±0.005 ^b
Spirulina3	343.81±18.4 ^a	59.00±10.50 ^{bc}	0.19±0.04 ^b	0.378±0.06 ^a	0.86±0.007 ^a

*Each value represents the mean ±SD. Means in the same column with different superscript letters were significant at p≤0.05 * Casein2 as a negative control group*

Table 3: Effect of using Spirulina as a casein replacement on aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase activities of experimental rats

Parameters / Groups	AST(IU/L)	ALT(IU/L)	ALP(IU/L)
Casein1	55.60±3.57 ^c	30.00±2.8 ^c	183.50±2.42 ^d
Spirulina1	55.33±2.73 ^c	29.00±2.36 ^c	182.16±5.9 ^d
Casein2*	62.83±5.77 ^d	22.50±1.87 ^d	188.16±4.35 ^c
Spirulina2	61.66±2.42 ^d	21.50±1.87 ^d	187.5±1.8 ^c
Casein3	123.00±4.09 ^a	63.50±1.87 ^a	220.83±10.59 ^a
Spirulina3	87.16±5.26 ^b	45.33±2.10 ^b	190.3±2.90 ^b

*Each value represents the mean ±SD. Means in the same column with different superscript letters were significant at p≤0.05 * Casein2 as a negative control group*

Table 4: Effect of using Spirulina as a casein replacement on serum total protein, albumin and globulin of experimental rats

Parameters / Groups	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)
Casein1	6.10 ± 0.05 ^b	3.23 ± 0.01 ^b	2.85 ± 0.044 ^b
Spirulina1	6.10 ± 0.05 ^b	3.24 ± 0.01 ^b	2.86 ± 0.053 ^b
Casein2*	6.14 ± 0.01 ^b	3.25 ± 0.01 ^b	2.89 ± 0.035 ^b
Spirulina2	6.15 ± 0.02 ^b	3.24 ± 0.01 ^b	2.91 ± 0.034 ^b
Casein3	6.18 ± 0.0 ^a	3.31 ± 0.01 ^a	2.87 ± 0.033 ^b
Spirulina3	6.15 ± 0.14 ^b	3.27 ± 0.012 ^b	2.88 ± 0.024 ^b

Each value represents the mean ±SD. Means in the same column with different superscript letters were significant at $p \leq 0.05$ * Casein2 as a negative control group

Table 5: Effect of using Spirulina as a casein replacement on urea, uric acid, Creatinine and creatinine Clearance of experimental rats

Parameters / Groups	Urea(mg/dl)	Uric acid(mg/dl)	Creatinine (mg/dl)	Creatinine Clearance
Casein1	29.65 ± 1.9 ^c	2.6 ± 0.25 ^c	0.74 ± 0.03 ^c	1.41 ± 0.39 ^b
Spirulina1	28.56 ± 1.8 ^c	2.55 ± 0.15 ^c	0.74 ± 0.02 ^c	1.37 ± 0.02 ^b
Casein2*	28.16 ± 1.4 ^{cd}	2.28 ± 0.06 ^d	0.70 ± 0.04 ^d	1.44 ± 0.63 ^b
Spirulina2	27.80 ± 1.2 ^{cd}	2.24 ± 0.02 ^d	0.71 ± 0.01 ^{cd}	1.43 ± 0.02 ^b
Casein3	37.64 ± 1.8 ^a	3.61 ± 0.30 ^a	0.93 ± 0.02 ^a	1.72 ± 0.02 ^a
Spirulina3	34.22 ± 0.8 ^b	3.24 ± 0.02 ^b	0.83 ± 0.01 ^b	1.69 ± 0.02 ^a

Each value represents the mean ±SD. Means in the same column with different superscript letters were significant at $p \leq 0.05$ * Casein2 as a negative control group

Table 6: Effect of using Spirulina as a casein replacement on hemoglobin, RBCs and WBCs of whole blood of rats

Parameters / Groups	Hemoglobin (g/dl)	RBCs 10 ⁶ μ/L	WBCs Thous and/μm
Casein1	11.33±0.2 ^f	2.49±0.16 ^c	13.10±0.37 ^b
Spirulina1	13.06±0.51 ^d	4.4±0.24 ^c	14.22±1.52 ^b
Casein2*	12.22±0.52 ^e	3.12±0.34 ^d	12.98±0.14 ^{cb}
Spirulina2	14.46±0.55 ^b	4.9±0.47 ^b	13.8±1.20 ^b
Casein3	13.82±0.73 ^c	4.7±0.43 ^{bc}	17.76±0.75 ^a
Spirulina3	15.26±0.52 ^a	5.7±0.26 ^a	12.27±1.10 ^c

*Each value represents the mean ±SD. Means in the same column with different superscript letters were significant at p≤0.05 * Casein2 as a negative control group*

Table 7: Effect of using Spirulina as a casein replacement on P.Count , hematocrit , Lymphocytes and Monocytes of whole blood of rats

Parameters / Groups	Hematocrit (%)	L.Cyte%	M.Cyte%	P.Count(k/ul)
Casein1	33.99±0.31 ^d	89.0.86±0.86 ^d	2.4±0.12 ^d	823.66±11.8 ^c
Spirulina1	39.18±0.46 ^a	90.19±0.56 ^a	3.9±0.49 ^a	725.16±26.9 ^d
Casein2*	36.66±0.36 ^c	90.67±11.5	2.9±0.14 ^{bc}	849.33±6.1 ^c
Spirulina2	43.38±0.58 ^{ab}	96.83±1.3 ^b	2.94±0.29 ^b	822.16±60.95 ^c
Casein3	41.46±0.57 ^{bc}	82.37±0.22 ^d	2.5±0.26 ^{cd}	1182.21±46.5 ^a
Spirulina3	45.78±0.25 ^{ab}	99.01±1.06 ^c	2.64±0.23 ^{cd}	924.66±53.7 ^b

*Each value represents the mean ±SD. Means in the same column with different superscript letters were significant at p≤0.05 * Casein2 as a negative control group*

Table 8: Effect of using Spirulina as a casein replacement on MCV, MCH and MCHC of whole blood of rats

Groups/ parameter	MCH (pg)	MCV (fL)(μm^3)	MCHC (g/dl)
Casein1	66.01 \pm 2.21 ^e	26.94 \pm 0.23 ^e	24.94 \pm 3.50 ^d
Spirulina1	76.60 \pm 4.46 ^d	27.82 \pm 0.51 ^d	34.19 \pm 1.2 ^c
Casein2*	73.09 \pm 3.91 ^d	30.38 \pm 0.78 ^c	25.63 \pm 0.30 ^d
Spirulina2	85.5 \pm 3.49 ^c	31.41 \pm 0.91 ^b	36.72 \pm 2.5 ^b
Casein3	126.6 \pm 0.51 ^b	32.08 \pm 0.65 ^a	38.37 \pm 1.07 ^{ab}
Spirulina3	175.11 \pm 27.3 ^a	33.41 \pm 0.33 ^a	40.55 \pm 1.55 ^a

Each value represents the mean \pm SD. Means in the same column with different superscript letters were significant at $p \leq 0.05$ * Casein2 as a negative control group

Table 9: Effect of using spirulina as a casein replacement on antioxidant enzymes and Malonaldehyde in liver tissue homogenate of experimental rats

Parameters / Groups	MDA(mmol/g)	SOD(u/mg)	CAT(u/mg)	GPx (ng/mg)
Casein1	0.296 \pm .0049 ^a	0.116 \pm .003 ^e	0.089 \pm .0135 ^f	0.125 \pm .003 ^f
Spirulina1	0.277 \pm .0040 ^b	0.179 \pm .002 ^d	0.175 \pm .0041 ^e	0.189 \pm .002 ^e
Casein2*	0.234 \pm .0030 ^d	0.175 \pm .003 ^d	0.201 \pm .0031 ^d	0.199 \pm .003 ^d
Spirulina2	0.200 \pm .0041 ^e	0.229 \pm .003 ^b	0.230 \pm .0032 ^b	0.241 \pm .004 ^c
Casein3	0.242 \pm .0071 ^c	0.195 \pm .004 ^c	0.210 \pm .0023 ^c	0.248 \pm .005 ^b
Spirulina3	0.129 \pm .0032 ^f	0.272 \pm .002 ^a	0.269 \pm .0051 ^a	0.289 \pm .005 ^a

Nutritional and Biological evaluation of using Spirulina algae as a new source of protein

Salha Salem Algohary, Zainab Abdul-Wahhab Salem and Amira M. El-Moslemany

Each value represents the mean \pm SD. Means in the same column with different superscript letters were significant at $p \leq 0.05$. * Casein2 as a negative control group

Table 10: Effect of using Spirulina as a casein replacement on tumor necrosis factor, and growth hormone in serum of experimental rats

Parameters / Groups	G.H (pg/mg)	TNF (pg/ml)
Casein1	0.157 \pm .002 ^f	0.237 \pm 0.0023 ^a
Spirulina1	0.234 \pm .002 ^e	0.218 \pm 0.0070 ^b
Casein2*	0.366 \pm .005 ^d	0.198 \pm 0.0023 ^c
Spirulina2	0.393 \pm .008 ^c	0.179 \pm 0.0036 ^d
Casein3	0.599 \pm .004 ^b	0.241 \pm 0.0228 ^a
Spirulina3	0.716 \pm .006 ^a	0.190 \pm 0.0051 ^c

Each value represents the mean \pm SD. Means in the same column with different superscript letters were significant at $p \leq 0.05$. * Casein2 as a negative control group

التقييم الغذائي والبيولوجي على استخدام طحلب الاسبيرولينا كمصدر جديد للبروتين

صالحة سالم الجوهري* - زينب عبدالوهاب سالم* - أميرة مرسي المسلماني*

***قسم التغذية وعلوم الأطعمة - كلية الاقتصاد المنزلي - جامعة الأزهر**

الملخص العربي

تستخدم طحالب الاسبيرولينا كمادة غذائية شائعة في جميع أنحاء العالم منذ القدم. تهدف هذه الدراسة إلى استخدام الاسبيرولينا كمصدر جديد للبروتين في مرحلة النمو. تم إجراء التجربة بتقسيم عدد ٣٦ من ذكور فئران التجارب البيضاء تتراوح أعمارهم ست أسابيع إلى ست مجموعات وفقاً لمصدر البروتين على النحو التالي: تحتوي مجموعات الكازين (ك١، ك٢، ك٣) على (٣٠٠، ٢٠٠، ١٠٠ جم من الكازين /كجم من النظام الغذائي)، بنسبة (١٠%، ٢٠%، ٣٠% بروتين) على التوالي، على الجانب الآخر مجموعات الاسبيرولينا (س١، س٢، س٣) على (١٥٧، ٣١٥، ٤٧٢ جم بودرة الطحلب /كجم) واستمرت هذه التجربة مدة ٢٨ يوم. تميزت الاسبيرولينا بنشاطها المرتفع المضاد للأكسدة (٣٩،٢%)، وسجلت مجاميع الفئران التي تغذت على الاسبيرولينا زيادة كبيرة في الغذاء المتناول، وزن الجسم المكتسب، ونسبة كفاءة الغذاء، ونسبة كفاءة البروتين مقارنة بمجموعات الكازين. حين أن مجموعات الكازين سجلت زيادة معنوية في معامل الهضم الظاهري بالمقارنة مع مجموعات الطحلب. تناول الاسبيرولينا كمصدر بديل للبروتين حافظ بشكل كبير على وظائف الكبد والكلية. بالنسبة لصورة الدم الكاملة سجلت مجموعات س٣ أعلى نسبة في الهيموجلوبين وعدد كرات الدم الحمراء مقارنة بمجموعة ك٣ بخلاف خلايا الدم البيضاء التي كانت أقل في س٣ وقد يرجع التحسن في مؤشرات مجموعات الاسبيرولينا إلى احتوائها على نسبة عالية من البروتين والحديد. كما لوحظ زيادة كبيرة في الإنزيمات المضادة للأكسدة في أنسجة الكبد لمجموعات الاسبيرولينا مقارنة بمجموعات الكازين بينما المانولدهيد سجل انخفاضاً كبيراً في مجموعات الكازين وسجل استخدام الاسبيرولينا زيادة في مستويات هرمون النمو في جميع المجموعات. كما سجلت النتائج انخفاضاً ملحوظاً في عوامل النخر في مجموعات الاسبيرولينا مقارنة بمجموعات الكازين. وفي الختام: يمكن استخدام الاسبيرولينا كبديل جديد للبروتين.

الكلمات المفتاحية: طحلب الاسبيرولينا - المؤشرات الدموية - معامل الهضم الظاهري - الإنزيمات المضادة للأكسدة