Spirulina as a Protein Source in the Nutritional Recovery of *Wistar* Rats

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ABSTRACT

This study was aimed at evaluating the effects of Spirulina (Arthrospira) as a protein source in the nutritional recovery of rats subjected to protein malnutrition. In order to understand such effects, responses of the developing body, serum biochemical profile, and blood count of animals were analyzed. A total of, 23 Wistar Rattus norvegicus were given a proteic diet (AIN-93G adapted) in malnutrition phase (phase I) for 10 days. After this period, the rats were separated into four groups according to the protein source (recovery phase): Control (C), casein 12%; Spirulina level I (S1), Spirulina 8.8% + casein 5.0%; Spirulina level II (S2), Spirulina 17.6% + casein 0.15%, and Spirulina level III (S3), Spirulina 26.4%. Recovery phase (phase II) lasted 30 days. In both the phases, the consumption and body weight of the rats were monitored daily and weekly, respectively. The S1 treatment showed the highest values of Feed Efficiency Ratio (FER) and Quotient of Protein Efficiency Ratio (QPE) (p≤0.05). Significant differences among the treatments were found mainly in the levels of serum lipids, and S2 diet caused an increase in such responses (p≤0.05). These results demonstrated that the nutritional recovery of animals was enabled by different concentrations of Spirulina.

Key words: Protein malnutrition, rats, nutritional recovery, *Rattus norvegicus*, *Spirulina*

INTRODUCTION

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). In the last two decades, according to figures provided by the Household Budget Survey, the Northeast region of Brazil decreased more than three times the rates of malnutrition with children aged five to nine, yet this problem remains interfering with the development of these children (Ministry of Health of Brazil 2011).

Whatever the cause of malnutrition is, the body reacts according to the time it is submitted to the lack of nutrients. As Rogatto et al. (2004) have reported, the use of energy substrates decreases in the body as intake is reduced. This automodulation between the intake and use is maintained until a time when energy utilization cannot be offset anymore by insufficient intake. Thus, the body uses up its fat reserves, resulting in the loss of adipose tissue and body weight.

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The metabolism of carbohydrates and proteins also suffer from nutritional deficiency, leading to a reduction of glycogen and protein stores in the muscle and liver. At the same time, higher concentrations of hepatic lipids can be observed due to the difficult transport of fats. The metabolic changes produced by protein insufficiency may be a number of possibilities in the body, from a reduced body weight to a decreased immune system (Quevedo et al. 2002).

According to Alves et al. (2005), authorities within the fields of public health, particularly the WHO, have been concerned with the malnutrition for decades. 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, micro-organisms 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).

Among the microalgae, the genus *Spirulina (Arthrospira)* contains about 60-70% of the proteins, nucleic acids, and amino acids (Pelizer et al. 2003). It also contains beta-carotene and absorbable iron, among other minerals, and high levels of vitamins, phenolic compounds, gamma-linolenic acid, as well as other essential fatty acids (Belay et al. 1993; Von Der Weid et al. 2000; Colla et al. 2008).

The essential amino acids, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine were found in the biomass of LEB-18, strain grown at the Federal University of Rio Grande, south of Brazil. Strain LEB-18 generally presented larger amounts of essential amino acids than the theoretical quantities recommended in dietary protein for children aged 2-5 years (FAO 1990), the exception being lysine which accounted for 2.95% of the dry biomass of LEB-18 as against the 5.8% recommended in the dietary protein of children by FAO (Morais et al. 2009).

Currently in Brazil, *Spirulina* is classified as a new ingredient whose daily intake should not exceed 1.6 g/person (Brazil 2009). The Food and Drug Administration (FDA), after rating this microalga as Generally Recognized as Safe (GRAS), suggested an average daily intake of 3 g/person (FDA 2003). According to Becker (2007), among the factors that limit the use of single cell protein in food there is the presence of cell wall, which can influence the digestibility and absorption, and also the high content of nucleic acids, whose intake in excess can lead to its accumulation in the human body with potential negative consequences. Therefore, this work aimed to evaluate the effects of using *Spirulina* in the nutritional recovery of rats subjected to protein malnutrition on the growth and the biochemical and hematological aspects of the blood.

MATERIAL AND METHODS

Microalgal biomass
Microalga *Spirulina* strain LEB-18 was used, produced by the Federal University of Rio Grande, Brazil (Morais et al. 2008).

Biological models
Twenty three male, recently weaned (21 days) *Wistar* Rattus norvegicus/UFPel weighing 70 g were used, which were kindly supplied by the Central Animal Facility of the Federal University of Pelotas, Brazil. The choice of the number of animals (n) per group and the use of randomization were based on Berndtson (1991) and Kilkenny (2009).

Diets
Five diets (Table 1) based on the formulation for growing the rodents (AIN93G) described by Reeves et al. (1993) were prepared weekly: control diet (C): AIN93G adapted to 12% of protein; aproteic diet (A): no added protein; diet S1: *Spirulina* 8.8% (w/w); diet S2: *Spirulina* 17.6% (w/w); and diet S3: *Spirulina* 26.4% (w/w). The formulation of the C diet, despite the recommendation of eating 20% of protein for growing rodents, was calculated to provide 12% of this nutrient (Miller and Bender 1955; Sgarbieri 1996; Jood and Singh 2001). It is noteworthy that in order to obtain the C diet with 12% of protein, it was supplemented with 120 g casein, since it was not pure (> 85% protein); the rest of the ingredients were as recommended by Reeves et al. (1993) and corn starch to supplement 1000 g of diet. The calculation for formulating the diets S1, S2, and S3 was based on the proximal composition of the biomass (Table 1), the amount of daily intake by rodents (20 g/rat/day) (Souza-Soares et al. 2009), and the limit set by National Agency for Sanitary Surveillance as the maximum daily consumption of *Spirulina* (1.6 g/person/day) (Brazil 2009).

Table 1 - Formulation of diets C (control), S1 (*Spirulina* 8.8%), S2 (*Spirulina* 17.6%), S3 (*Spirulina* 26.4%), and A (aproteic).

Ingredients (g.kg^{-1})	Diets/Treatments				
	C	S1[1]	S2[1]	S3	A
Spirulina LEB-18 (56 % protein; 7.4 % lipids; 10.7 % ashes; 9.5 % fiber)	-	88.0	176.0	264.0	-
Casein (>85 % protein)	120.0	50.5	1.5	-	-
Soybean oil	70.0	63.5*	57.0*	50.5*	70.0
Mineral mix[2]	35.0	25.5*	16.0*	6.5*	35.0
Vitamin mix[2]	10.0	10.0	10.0	10.0	10.0
L- cystine	3.0	3.0	3.0	3.0	3.0
Choline bitartrate	2.5	2.5	2.5	2.5	2.5
Wheat bran	50.0	43.5*	37.0*	30.5*	50.0
Sucrose	100.0	100.0	100.0	100.0	100.0
Corn starch[3]	609.5	613.5	597.0	533.0	729.5

[1] To obtain 12% protein, minimum necessary for the development of rodents, the S1 and S2 diets were supplemented with casein;
[2] Prepared according to AIN-93G (Reeves et al. 1993);   [3] Added to supplement the diet; * Deducting the intrinsic amount present in microalgae.

In vivo experiment

The experiment lasted 45 days, with the top five for adaptation to the environment. At the end of the adaptation period, the rats were weighed and Phase I of the experiment (malnutrition) was begun. In this phase, each rat received daily 20 g of aproteic diet (A) and water *ad libitum* for 10 consecutive days. After this period, the rats were weighed and redistributed at random into four groups (Berndtson 1991; Kilkenny, 2009): C (n = 6), S1 (n = 5), S2 (n = 6), and S3 (n = 6), and Phase II was initiated. In this phase, which comprised 30 days of study, each rat received 20 g of their daily diet with protein for nutritional recovery. Throughout the in vivo experiment, the rats were kept in metabolic stainless steel cages with air exhaust system and 12 h photoperiod at 22 ± 2 °C. The positions of the cages were changed weekly to normalize the impact of the environmental conditions. The implementation of the experiment followed the guidelines of the Brazilian College of Animal Experimentation – COBEA, and was approved by the Ethics Committee of Federal University of Pelotas, Brazil (Case No. 23110. 008077/2009-22).

Weight parameters

During the experiment (phases I and II), daily weighing of the diet remaining at the feeder was performed to determine the food consumption. Likewise, the rats' body weight was registered weekly to assess the weight gain. At the end of the experiment, the rats were evaluated biometrically by measuring the body weight, vertex-coccyx length, forelimb length, waist circumference, weight, body fat (mesenteric, renal, and inguinal) and liver (Águila et al. 1997).

Analyses of diets and feces

The microalgal biomass, diets and feces of the corresponding groups were analyzed following the methods described by the Association of Official Analytical Chemists (AOAC 1995). Total lipids were determined on biomass and diets by the method of Bligh and Dyer (1959). The factor for the conversion of nitrogen was 6.25 for all the samples. The feces collected were analyzed during the last seven days of the experiment.

Nutricional evaluation

The following determinations were performed to evaluate the protein quality of the diets in the study: Feed Efficiency Ratio (FER), given by the ratio between the weight gain (g) and total dietary intake during the experiment (g), and Quotient of Protein Efficiency Ratio (QPE), calculated as the ratio of weight gain (g) and total protein consumed (g) (Sgarbieri 1996).

Biochemical and blood count analyses

At the end of the experiment, after the rats were subjected to a 12 h fasting, the sedation was performed in câmpula with ethyl ether and immediately the blood was collected by cardiac

puncture. Approximately 1.0 mL of the blood was placed in eppendorf with EDTA and a portion was analysed for blood count in automatic system (POCH-100IVDIFF, SYSMEX®). The remaining portion of the blood was centrifuged at 1000 g at 4 ° C for 15 mins in test tubes to obtain the serum that was stored at -18 °C for further analysis in automated equipment (LabMax 240,LABTEST DIAGNOSTIC SA®).

Statistical analyses
The analysis of variance (ANOVA) was performed, followed by the Tukey test for comparison between the means at a significance level of 5% (p ≤ 0.05), using the software Statistica 7.0 (Statsoft, USA).

RESULTS AND DISCUSSION

As seen in Table 2, after the period of malnutrition (Phase I), rats from different groups had similar initial weights, showing an even distribution among the four treatments for subsequent protein recovery (Phase II). There was no significant difference in the weights and weight gains.

Table 2 - Weight and nutritional responses of malnourished *Wistar* rats after recovery for 30 days with diet C (control), S1 (8.8% *Spirulina*), S2 (17.6% *Spirulina*), and S3 (26.4% *Spirulina*)

Determination	Treatments			
	C	S1	S2	S3
Initial weight (g)*	56.00±7.80[a]	54.40±4.95[a]	56.33±3.90[a]	54.65±13.30[a]
Final weight (g)	189.35±11.50[a]	200.00±40.65[a]	165.00±12.25[a]	188.00±15.45[a]
Weight gain (g)	118.35±13.30[a]	130.00±34.35[a]	97.00±11.20[a]	117.00±14.10[a]
Food intake (g)	407.95±46.20[a]	439.20±66.40[a]	560.65±16.60[ab]	626.45±34.30[b]
Vertex-rump (cm)	19.30±0.90[a]	19.90±1.15[a]	19.35±0.60[a]	20.05±1.15[a]
Foreleg (cm)	12.35±0.70[a]	12.30±0.85[a]	11.65±1.05[a]	12.50±0.80[a]
Waist circumference (cm)	13.75±0.70[ab]	14.90±1.15[b]	12.50±1.00[a]	12.60±1.15[a]
Body fat / weight	0.012±0.005[a]	0.022±0.013[a]	0.020±0.007[a]	0.019±0.008[a]
Liver / weight	0.035±0.004[a]	0.035±0.002[a]	0.030±0.006[a]	0.033±0.001[a]
FER	0.18±0.01[a]	0.25±0.05[b]	0.15±0.02[a]	0.17±0.01[a]
QPE	2.10±0.13[a]	2.75±0.40[b]	2.00±0.25[a]	1.60±0.15[c]

Mean ± standard deviation; Different letters in the same row indicate significant difference among groups by Tukey test (p≤0.05); *After 5 days of adaptation; FER: Feed Efficiency Ratio; QPE: Quotient of Protein Efficiency Ratio.

In relation to food intake, S3 showed higher values than C group (p≤0.05); however, differed negatively for QPE. Although the consumption was lower in S1 treatment, this differed from the others positively (p≤0.05) for FER and QPE, indicating improved efficiency. According to Chaud et al. (2008), reduced feed efficiency occurred due to a decreased body metabolism after a specific diet, which caused stagnation or reduction in body weight.
Mitchell et al. (1990) treated male *Wistar* rats for 60 days to evaluate the effect of different concentrations (0, 2.7, 10.7, 18.7 and 26.7%) of *Spirulina maxima* with vitamins A and E and found that the FER was inversely proportional to biomass intake. Rogatto et al. (2004) analyzed a diet with 17% of *Spirulina* as total replacement of the control diet protein (casein) in young male *Wistar* rats for five weeks and FER was found to be 0.21, a value similar to those in the treatments with 17.6 % and 26.4% of *Spirulina* (0.23 and 0.22, respectively) in this study. According to Vieira and Bion (1998), proteins originating from different mixtures and used in different proportions result in variations in the the concentrations of amino acids, which interfere with its efficiency and use by the humans and animals.
S1 treatment differed (p≤0.05) from the others added with *Spirulina* regarding waist circumference, showing higher values; however, it was similar to C treatment. S1 treatment did not differ significantly with regard to the relationship of body fat per body weight, demonstrating that there was no accumulation of abdominal adipose tissue induced by the consumption of seaweed and soybean oil used in the preparation of diets. The groups also showed no significant difference between the other weight parameters (vertex-rump

length, measured from the forelimbs, and liver weight/body weight).

After five weeks of study with male *Wistar* rats fed with *Spirulina*, Rogatto et al. (2004) found average values of vertex-coccyx equal to 24.6 cm. Silva (2009) obtained the measures of the forelimbs around 11.90 cm for the female *Wistar* rats fed with AIN93G. In order to verify some hepatocellular damage from the diet, the ratio of the liver weight (g)/body weight (g) was determined and values ranged from 0.033 ± 0.003 to 0.034 ± 0.003, which showed no significant difference and were in agreement with other studies (Caballero-Cordoba 1997; Shons et al. 2009; Silva 2009). Overall, the results given in Table 2 showed that a diet with less *Spirulina*, despite lower consumption, had higher nutritional responses and consequently higher values in the responses related to the development of animals, even if not significant.

Microalgal biomass has high levels of nucleic acids, which have been reported as about 4-6%. Due to the inability of human body to metabolize uric acid from purine metabolism, an increased consumption of nucleic acids can lead to high levels of uric acid in the serum. This may promote the emergence of diseases such as gout (Becker 1988; Araújo et al. 2003).

Values of uric acid (Table 3) were all within the range of normality, if taken as reference the variation range from 1.2 to 7.5 mg.dL^{-1} described as standard for the rats (Mitruka and Rawnsley 1981), similar to the values obtained (1.77 mg.dL^{-1}) in a study with male *Wistar* rats by Vilela and Sgarbieri (2000). The fraction of non-protein nitrogen in the serum or blood is composed of all the nitrogenous substances other than proteins. Its main component is urea, which is synthesized in the liver from ammonia derived primarily from the protein and amino acids and is the most used resource for the evaluation of renal function in mammals (Devlin 2007).

Table 3 - Responses of blood biochemical levels of malnourished *Wistar* rats after recovery for 30 days with diet C (control), S1 (8.8% *Spirulina*), S2 (17.6% *Spirulina*), and S3 (26.4% of *Spirulina*).

Determination	Treatments			
	C	S1	S2	S3
Uric acid (mg.dL^{-1})	1.55 ± 1.00^a	1.90 ± 0.90^a	1.30 ± 0.90^a	1.40 ± 1.45^a
Urea (mg.dL^{-1})	69.00 ± 10.70^a	66.20 ± 23.70^a	66.65 ± 14.40^a	55.50 ± 8.00^a
Creatinine (mg.dL^{-1})	0.32 ± 0.05^{abc}	0.37 ± 0.04^b	0.27 ± 0.04^c	0.38 ± 0.06^{ab}
Total proteins (g.dL^{-1})	6.10 ± 0.15^{bc}	6.60 ± 0.25^b	5.85 ± 0.50^c	5.85 ± 0.30^c
Albumin (g.dL^{-1})	3.22 ± 0.15^a	3.45 ± 0.15^a	3.20 ± 0.30^a	3.17 ± 0.20^a
AST (u.L^{-1})	33.15 ± 1.35^a	32.20 ± 7.50^a	47.33 ± 3.40^a	32.00 ± 7.95^a
ALT (u.L^{-1})	392.50 ± 27.70^a	424.20 ± 24.25^a	296.15 ± 20.25^a	232.85 ± 11.15^a
Glucose (mg.dL^{-1})	68.00 ± 15.45^a	83.20 ± 18.80^a	65.85 ± 9.80^a	69.00 ± 22.65^a
Total cholesterol (mg.dL^{-1})	80.50 ± 14.65^a	74.00 ± 18.45^a	103.50 ± 10.50^b	86.85 ± 11.95^{ab}
HDL-chol (mg.dL^{-1})	32.85 ± 3.50^a	29.80 ± 6.10^a	35.65 ± 4.05^a	31.35 ± 3.70^a
LDL-chol (mg.dL^{-1})	29.50 ± 10.70^a	30.25 ± 15.60^a	51.95 ± 6.70^b	40.80 ± 8.35^{ab}
LDL/HDL	0.85 ± 0.23^a	0.95 ± 0.35^a	1.45 ± 0.25^b	$1.30 \pm 0,15^{ab}$
VLDL-chol (mg.dL^{-1})	17.85 ± 4.60^a	13.95 ± 2.60^a	16.10 ± 5.30^a	14.70 ± 5.30^a
Triacylglycerols (mg.dL^{-1})	89.35 ± 2.30^a	69.80 ± 12.75^a	80.50 ± 26.50^a	73.50 ± 26.55^a
Iron (µg.dL^{-1})	93.33 ± 13.25^a	116.40 ± 28.35^a	93.15 ± 52.25^a	79.85 ± 17.15^a

Mean ± standard deviation; Different letters in the same row indicate significant difference among groups by Tukey test (p≤0.05); AST: aspartate aminotransferase; ALT: alanine aminotransaminase.

According to Miller (1993), low levels of urea could indicate severe liver failure, nephrosis, malnutrition, and/or hemodilution, which were not observed in this study. Although the rats belonging to the groups that consumed diets with *Spirulina* contain urea serum levels lower than the control group, the difference was not significant. Ori et al. (1990) recommended urea serum levels in the rats around 300mg.dL-1 to be diagnosed with kidney failure, which did not occur with the groups of this study. In order to evaluate the kidney function, it is important to determine creatinine and urea levels,

since they increase as kidney function decreases (Miller 1993). Table 3 showed that all the treatments with *Spirulina* had creatinine levels statistically similar to the control; however, when comparing the treatments with *Spirulina* with each other, S2 showed significantly lower values (p ≤ 0.05). In this study, creatinine levels were between 0.27 and 0.38 mg.dL-1, values considered normal by Mitruka and Rawnsley (1981).

The determination of plasma proteins in serum provides important information about the health conditions and their loss may indicate restriction in the intake of amino acids by eating low-protein diets or food with good protein content, but containing the factors that hinder the digestion and absorption (Campello et al. 2009). The values found for the total plasma proteins were in the range from 6.85 to 6.60 g.dL-1, in which all the treatments with *Spirulina* were similar to the control, but with some differences among themselves (p≤0.05). Regarding albumin, groups did not differ among themselves. These results were close to those obtained by Quevedo et al. (2002) and Rogatto et al. (2004) in the studies with male rats fed with the diets with *Chlorella vulgaris* and *Spirulina*, respectively.

Serum levels of the activities of aspartate aminotransferase (AST) and alanine aminatransaminase (ALT) revealed no values statistically different from the control group. However, all the groups, including the control had AST values higher than reference values from other studies under similar conditions (Caballero-Córdoba 1997; Vilela and Sgarbieri 2000; Anthony et al. 2006; Melo et al. 2008). With respect to the ALT, results were very similar to those of the authors cited previously. According to Devlin (2007), ALT in hepatocytes occurs at concentrations higher than AST; therefore, the determination of its activity in serum is a more effectively diagnosis of liver injury.

The groups did not differ in the levels of blood glucose, with responses between 65.65 and 83.20 mg.dL-1. In a similar study, Alves et al. (2005) evaluated the effect of *Spirulina* on the skeletal muscle of malnourished rats and values were around 105 mg.dL-1 after 30 days of treatment. Rogatto et al. (2004) found in male *Wistar* rats fed with *Spirulina* about 73 mg.dL-1 blood glucose. According to Mitruka and Rawnsley (1981), *Rattus norvegicus* that have glucose levels included in the interval 50 – 135 mg.dL-1 can be considered healthy.

Among the biochemical responses of the blood of the rats analyzed in this study, those related to serum lipids had the most significant difference (p≤0.05) among the treatments. The S2 treatment showed higher values (p≤0.05) than the control for total cholesterol, LDL, and LDL/HDL ratio. The levels of total cholesterol for this treatment were higher than those reported as normal (56 to 76 mg.dL-1) by Souza-Soares et al. (2009) and those obtained by Bertolin et al. (2009). The latter authors, after dealing with male *Wistar* rats for 60 days with hypercholesterolemic diet and *Spirulina*, found 99.1 and 65.1 mg.dL-1 for total cholesterol in the rats with 30 days of hypercholesterolemic diet and 30 days of a diet with *Spirulina*, respectively. As for total cholesterol, the values for LDL were elevated (p≤0.05) in the rats of the S2 treatment. However, this was expected, since this fraction represented about two-thirds of the total cholesterol (Miller 1993). The values of HDL (29.80 to 35.65 mg.dL-1) obtained in this study did not differ among themselves and were similar to those found by Bertolin et al. (2009) for the rats with hypercholesterolemic diet and the diet with *Spirulina* (32.3 mg.dL-1).

The LDL/HDL ratio is commonly calculated to assess the risk of coronary heart disease. Ho et al. (2003) reported that high concentration of LDL showed atherogenic processes, whereas high HDL had a cardioprotective effect. In this study, the LDL/HDL ratio were 0.85, 0.95, 1.45, and 1.30 for the groups C, S1, S2, and S3, respectively. Although the relation between LDL and HDL for the rodents has not been determined and the value set for human cannot be compared directly, the convention is that the LDL/HDL ratio is as small as possible (Smith 2007). Therefore, the ratio obtained for the rats fed with diet S2 was increased in relation to C diet (p≤0.05). The values of VLDL and triglycerides did not differ among the treatments. As shown in Table 3, the values for the VLDL fraction were 17.85, 13.25, 16.10, and 14.703 mg.dL-1 for the treatments C, S1, S2, and S3, respectively. The highest levels of VLDL (C and S2) coincided with the values of total cholesterol and triglycerides, yet no significant difference was found. The levels of VLDL are proportional to total cholesterol and triglycerides, as they comprise a fraction of 60% and 20%, respectively.

The results showed that despite the treatment with 8.8% of *Spirulina* (S1) had the lowest values of triacylglycerol (TAG), there was no significant

difference among the four treatments. The mean TAG among the groups was 78.28 mg.dL-1, which was lower than those obtained by Cerqueira et al. (2008) and Bertolin et al. (2009) for the male *Wistar* rats. The study groups showed no significant difference in the iron content. According to the Vivarium of the Faculty of Medicine, Universidade de São Paulo, adult *Wistar* rats (Rattus norvegicus) must have high iron content in the range of 154 to 279 μg.dL-1 (FMUSP 2011). These data were confirmed by Santos et al. (2004), who found a 254 μg.dL-1 of iron in the rats subjected to protein recovery with casein diet for 28 days.

Hematocrit value reflects the total mass of the cells in unit of blood volume, showing crucial importance in the study of all types of anemia. In addition, their results are less subject to error than the count of red blood cells (Miller 1993). In this study, the treatments did not differ in the levels of hematocrit (Table 4), and the results were similar to other studies (Boaventura et al. 2003; Duarte et al. 2009) and within the range described by Harkness and Wagner (1993) for the rodents, (between 36 and 48%). The groups did not differ with regard to hemoglobin, whose levels were considered normal when compared with other studies. Harkness and Wagner (1993) reported that the mean hemoglobin values for the rats ranged from 11 to 18%. Kappor and Mehta (1993) tested the use of *Spirulina* in a concentration similar to the S3 treatment (24%) in association with the plant and found increased levels of hemoglobin.

Table 4 - Responses of hematological indices of the blood of malnourished *Wistar* rats after recovery for 30 days with diet C (control), S1 (8.8% *Spirulina*), S2 (17.6% *Spirulina*), and S3 (26.4% of *Spirulina*).

Determination	Treatments			
	C	S1	S2	S3
Leukocytes t (10³/mm³)	4.64±1.95[a]	5.50±2.05[a]	5.08±1.08[a]	4.75±2.05[a]
Lymphocytes (%)	80.40±4.45[a]	85.00±4.35[ab]	88.65±2.75[b]	84.00±4.75[ab]
Monocytes (%)	2.40±1.35[a]	1.20±0.85[a]	1.85±1.70[a]	3.50±1.20[a]
Basophils (%)	0	0	0	0
Eosinophils (%)	1.40±1.65[a]	0.20±0.45[a]	0.50±0.55[a]	0[a]
Hematocrit (%)	42.60±0.85[a]	44.00±1.90[a]	45.10±1.80[a]	42.00±6.45[a]
Hemoglobin (%)	13.85±0.15[a]	14.75±0.60[a]	14.20±0.60[a]	13.40±1.85[a]
MCV (%)	59.95±1.75[a]	60.72±0.50[a]	59.10±1.85[a]	61.20±2.05[a]
HCM (%)	32.50±0.75[ab]	32.65±0.20[ab]	31.50±0.55[c]	31.70±0.50[ac]
Erythrocytes (10⁶/mm³)	7.10±0.25[a]	7.25±0.30[a]	7.65±0.25[a]	7.00±1.00[a]
Platelets (10³/mm³)	1087.00±210.7[a]	981.80±92.7[a]	1149.65±162.4[a]	850.35±285.5[a]

Different letters in the same row indicate significant difference among groups by Tukey test (p≤0.05); MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; Normal values of basophils: maximum 1% (Anthony et al. 2006).

The mean corpuscular volume (MCV) and mean corpuscular hemoglobin (HCM) indicate the size of red blood cells and hemoglobin inside red blood cells, respectively. The groups did not differ and the values for MCV were similar to those found for the rats fed with the diets added with S. platensis by Marco (2008). In relation to the levels of HCM, the S2 diet resulted in lower values (p≤0.05) than in groups C and S1. Santos et al. (2004), Marco (2008) and Duarte et al. (2009) found lower values than those of this study. Erythrocytes or red blood cells are formed by the elements present in larger amounts in the blood and its low count indicates iron deficiency and anemic status. For groups C, S1, S2, and S3, there were values included in the range suggested by Souza-Soares et al. (2009), 7 to 10 million/mm³. Marco (2008) added only 2% of S. platensis to the diets offered to female *Wistar* rats, and concluded that it was possible to use the microalgae added with multimixtures for the prevention of iron deficiency anemia.

With respect to the platelets, components responsible for blood clotting, the values (850 - 1087 thousand/mm³) were higher than reported in the literature. Souza-Soares et al. (2009) recommended values between 702 and 796 thousand/mm³ and Duarte et al. (2009) a range of 720 to 746 thousand/mm³ for healthy rats. This increase did not necessarily indicate a problem

with the blood of the rats. Considering that the platelet counts were automated (CAP) this technique was more precise and accurate than counting of the platelets by microscopy (COM), although according to Van der Meer et al. (2003) and Comar et al. (2009), there would be a potential risk to falsely low or high counts. . Erythrocyte fragments, cytoplasmic fragments of leukemic cells, microcytes with volume close to the cutting limit, bacteria and yeasts can lead to falsely increased values.

The colorless formed elements of the blood of the mammals are known as leukocytes, which play an essential role in the mechanism of defense against the infectious actions (Miller 1993). The treatments did not differ for the total leukocytes; however, the figures were slightly below the lower limit recommended by Souza-Soares et al. (2009), i.e. 6000 to 17000 leukocytes/mm³, and also when compared with the study of Santos et al. (2004), who found 6350 leukocytes/mm³. Of the total white blood, lymphocytes are present at higher levels, accounting for about 50 to 95% (Anthony et al. 2006). Thus, the values fit with as recommended, since the groups C, S1, S2, and S3 had 80.4, 85.0, 88.65, and 84.0%, respectively. The results found in this study for the levels of monocytes showed no significant difference among the groups. According to Souza-Soares et al. (2009), monocytes represent 0-3% of total leukocytes. Santos et al. (2004) and Duarte et al. (2009) assessed white levels of young male *Wistar* rats, and found values ranging from 1.2 to 2.7%. Basophils play an important role in the body immune responses, since the slightest contact with an allergenic substance release chemical mediators such as histamine, which attracts other immune cells (Miller 1993). Souza-Soares et al. (2009) and Anthony et al. (2006) mentioned as the normal values the range between 0 and 1% of basophils, which were not detected in the animals under study for all the treatments. According to Feldman et al. (2000), the primary function of eosinophils is combating the toxic substances, and therefore, be present in greater quantities in the places where there are antigen-antibody reactions and at points of penetration of strange substances into the body. According to Souza-Soares et al. (2009) and Anthony et al. (2006), eosinophils must be present around 0-3%. The results obtained were within the range considered normal, with no difference among the treatments.

CONCLUSIONS

The results of this study showed that different concentrations of *Spirulina* strain LEB-18 (8.8, 17.6, and 26.4%) enabled the nutritional recovery of *Wistar* rats/UFPel for physiological, biochemical and hematological characteristics. The diet with 8.8% of *Spirulina* was the most efficient, with equivalent responses to the control and superior to others.

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