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Full Length Research Paper

Nutritional evaluation of single-cell protein produced by *Spirulina platensis*

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The aim of this research was to evaluate the nutritional characteristics and possible toxic effects of microalgae *Spirulina* LEB-18, developed in south of Brazil. The biomass was evaluated for 40 days through nutritional responses, obtained by the development of *Wistar* rats and also by biochemical and hematological study of the blood of these animals. Four isoproteic diets were prepared (12% protein): (1) Control diet, 12% (w/w) of casein; (2) Diet S1, 8.8% (w/w) of *Spirulina*; (3) Diet S2, 17.6% (w/w) of *Spirulina*; (4) Diet S3, 26.4% (w/w) of *Spirulina*, distributed to the animals according to their treatment (n = 6). As to nutritional parameters the S1 treatment was more efficient. The groups did not differ in regard to biochemical determinations in blood, with the exception of S2, which showed creatinine levels lower than all others. In hematologic profile, the S3 treatment showed hematocrit and hemoglobin levels greater than the control diet. These results revealed no toxicity by the use of microalgae and it suggests the use of *Spirulina* as a potential source of single-cell protein.

Key words: Cyanobacteria, growing rats, nutritional evaluation, *Spirulina.*

INTRODUCTION

The use of single-cell protein (SCP) which refers to the dried cells of microorganisms like yeast, bacteria and microalgae (Zepka et al., 2010), has been present for thousands of years in the world population, mainly in the form of drugs. In recent decades biochemical engineering has been devoted to developing new methods for food processing. The cyanobacteria currently cultivated in large scale systems are economically viable sources of protein for the use in food because they often meet the requirements of this nutrient in the diet and moreover, through them you can get other human consumer products (Kuhad et al., 1997). A cyanobacterium as a source of single-cell protein has certain advantages over the use of other microorganisms because of its rapid growth and quantity and quality of protein (Molina et al., 2002). Among the microalgae, the genus *Spirulina* contains about 60 to 70% of proteins, nucleic acids and amino acids recommended by the Food and Agriculture Organization (Pelizer et al., 2003). It also contains beta-carotene and absorbable iron, and other minerals and high levels of vitamins, phenolic compounds, gamma-linolenic acid and other essential fatty acids (Belay et al., 1993; Von Der Weid et al., 2000). 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

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amino acids than the theoretical quantities recommended in dietary protein for children aged 2 to 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 Food and Agriculture Organization FAO (1990) (Morais et al., 2009). Currently in Brazil *Spirulina* is classified as a new ingredient and its daily intake should not exceed 1.6 g/individual (Anvisa, 2009). However the Food and Drug Administration, after classifying this microalga as generally recognized as safe, suggests that the daily intake should be according to the physical input of consumers, ranging from 0.1 to 6 g and the average estimate of consumption 3 g/individual/day (FDA, 2003). Among the factors that limit the use of single-cell protein in food are 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 accumulation in the human body with potential negative consequences (Becker, 2007). The great interest in the use of *Spirulina* as an alternative source of protein led the group to study the action of microalgae in the body of rats, which are constantly used in research animals hormonal, psychological, immunological and nutritional. The research objective was to evaluate the nutritional value of different levels of daily intake of *Spirulina*, using rats as experimental model.

MATERIALS AND METHODS

Biomass

We used the *Spirulina* strain LEB-18 produced by the Federal University of Rio Grande, Brazil (Morais, 2008).

Test animals

For *In vivo* experiments 24 *Rattus norvegicus* strain *Wistar*, males, recently weaned (21 days) from Federal University of Pelotas, Brazil, were randomly divided into 4 groups (n = 6), being housed in cages of galvanized steel.

Composition of diets

Four diets were prepared following the determinations of the AIN-93G (Reeves et al., 1993). The protein content was adjusted to 12% and protein sources were: (1) Control diet (C): commercial casein, (2) Diet S1: 8.8% (w/w) *Spirulina*, (3) Diet S2: 17.6% (w/w) *Spirulina*, (4) Diet S3: 26.4% (w/w) *Spirulina* (Table 1). The formulation of the control diet (C), despite the recommendation of eating 20% protein for growing rodents, was calculated to provide 10% of this nutrient (Miller and Bender, 1955; Sgarbieri, 1996; Jood and Singh, 2001). It is noteworthy that to obtain the C diet the 12% protein were added: 120 g casein since it is not pure (> 85% protein), the rest of the ingredients were recommended (Reeves et al., 1993) and corn starch added to diet supplement 1000 g. Formulation of diets for S1, S2 and S3 the calculation was based on the proximal composition of biomass (Table 1), the amount of daily intake by rodents (20 g/rat/day) (Souza-Soares, 2009); and the limit set by the maximum daily consumption ANVISA as *Spirulina* (1.6 g/person/day) (Brazil, 2009).

Analytical methods

The microalgal biomass, diets and feces of the corresponding groups were analyzed following the methods described by the Association of Official Analytical Chemists (AOAC, 2000). Total lipids were determined on biomass and diets by the method of Bligh and Dyer (1959).

Growth experiment

The experiment was performed over 45 days; the 5 first days were for adaptation of the animal to the environment. During the experiment, the laboratory remained under light conditions (12 h photoperiod) and temperature (22±2°C) controlled, as also under renovation air by exhaust system. The diets were offered daily (20 g/rat) and weighing the remainder of the same to determine the daily intake. The animals' body weight was recorded every 7 days for evaluation of weight gain from them. The implementation of the experiment followed the standards of the Ethics Committee of UFPEL-RS, Brazil (Case No. 23110. 008077/2009-22) and the Brazilian College of Animal Experimentation- COBEA.

Nutritional evaluation

The following determinations were made to evaluate the protein quality of diets in the study: Food Efficiency Ratio (FER), given by the ratio between the weight gain (g) and total dietary intake during the experiment (g) Protein Efficiency Ratio (PER), were calculated as the ratio of weight gain (g) and total protein consumed (g) and apparent digestibility (AD), obtained by calculating: [nitrogen ingested - nitrogen excreted / N ingested] 100 (Sgarbieri, 1996; Souza-Soares, 2009).

Analysis of serum

At the end of the experiment, after the animals have been subjected to a 12 h fasting, the sedation was performed in câmpula with ethyl ether and immediately blood was collected by cardiac puncture. Approximately 1 mL of blood was placed in eppendorf with ethylene diamine tetraacetic acid (EDTA) anticoagulant and it was intended for the analysis of blood count in automatic (POCH-100IVDIFF, SYSMEX®). The remainder centrifuged at 1000 g x 15 min at 4°C in test tubes to obtain serum that was stored at -18°C for further analysis in automated equipment LabMax 240 (LABTEST DIAGNOSTIC SA®).

Statistical analysis

The values obtained from the experiments were analyzed by analysis of variance (ANOVA) and difference between the means of the Tukey test using the software Statistica 7.0 (Statistica, 2004).

RESULTS AND DISCUSSION

By comparing the nutritional responses (Table 2) of

Table 1. Composition of diets control (C) and experimental with different concentrations of *Spirulina* (S1, S2 and S3) (Mean values with their standard errors).

Ingredients (g.kg^{-1})	Diets			
	C	S1*	S2*	S3
Spirulina LEB18 (56% protein; 7.4% lipids; 10.7% ash; 9.5% fiber - dry weight)	-	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
Mineral mix **	35.0	25.5	16.0	6.5
Vitamin mix**	10.0	10.0	10.0	10.0
L-cystine	3.0	3.0	3.0	3.0
Choline bitartrate	2.5	2.5	2.5	2.5
Wheat bran	50.0	43.5	37.0	30.5
Sucrose	100.0	100.0	100.0	100.0
Corn starch***	609.5	613.5	597.0	533.0

*To obtain 10% protein, minimum necessary for the development of rodents, the S1 and S2 diets were supplemented with casein. ** Prepared according to AIN93G. *** Added to supplement the diet.

Table 2. Nutritional responses of diets control (C) and experimental with different concentrations of *Spirulina* (S1, S2 and S3) (Mean values with their standard errors).

	Treatments							
	C		S1		S2		S3	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Initial weight (g/rat)*	70.65^{a}	11.15	70.33^{a}	7.10	68.33^{a}	8.80	73.65^{a}	7.10
Final weight (g/rat)	253.33^{a}	27.35	263.65^{a}	20.0	200.35^{b}	19.25	232.65^{ab}	14.50
Weight gain (g/rat)	182.65^{a}	35.00	175.33^{a}	20.90	134.33^{b}	14.05	156.15^{ab}	15.75
Total consump. (g/rat)	622.10^{a}	22.30	573.45^{b}	26.6	575.85^{b}	30.25	654.25^{a}	24.00
FER	0.28^{ab}	0.05	0.32^{b}	0.04	0.23^{a}	0.04	0.22^{a}	0.03
PER	2.55^{a}	0.50	2.90^{b}	0.30	2.00^{c}	0.25	2.05^{ac}	0.20
AD	75.45^{a}	2.85	61.50^{ab}	11.70	43.50^{c}	11.70	60.30^{b}	7.45

Within the same line, means having different superscripts are significantly different by ($p \leq 0.05$; n=6) Tukey test. *After 5 days of adaptation. FER: food efficiency ratio, PER: protein efficiency ratio, AD: apparent digestibility.

control (C) with the other we observed that the total food consumption was lower ($P \leq 0.05$) in S1 treatment. Analyzing the treatments with different levels of *Spirulina* is observed that food consumption increased with the concentration of biomass, demonstrating an acceptance of microalgae by the animals. Although the consumption was lower in S1 treatment, this differed from the others positively for FER ($P \leq 0.05$) and PER ($P \leq 0.05$), indicating improved efficiency. The reduced FER occurs due to decreased metabolism a specific diet, causing stagnation or reduction in body weight (Chaud et al., 2008). In a study with male *Wistar* rats under different concentrations (0, 2.7, 10.7, 18.7 and 26.7%) of *Spirulina maxima,* for 60 days, the FER was inversely proportional intake of biomass (Mitchell et al., 1990). A diet with 17% (Rogatto et al., 2004) in total replacement of *Spirulina* protein control diet in young male *Wistar* rats for five weeks had FER (0.21) (Rogatto et al., 2004), a value similar to that found treatments with 17.6 and 26.4% de *Spirulina* (0.23 and 0.22, respectively) in this work. In relation to microalgal digestibility we observed that the only treatment that was similar to C was S1. In study with algae as a source of protein found higher values for AD, but smaller values for PER (Becker, 2007). Protein originating from different mixtures and used in different proportions can result in variations in the concentrations of amino acids, which interfere in its efficiency and use by humans and animals (Vieira and Bion, 1998).

The results of biochemical levels of blood of rats are

Table 3. Biochemical levels of blood of rats (mean values with their standard errors).

	Treatments							
	C		S1		S2		S3	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Uric acid (mg.dL^{-1})	1.63^{a}	1.01	1.36^{a}	0.54	1.20^{a}	0.89	1.20^{a}	0.89
Urea (mg.dL^{-1})	74.83^{a}	15.21	62.50^{a}	11.74	57.59^{a}	7.06	59.16^{a}	7.80
Creatinine (mg.dL^{-1})	0.44^{a}	0.06	0.44^{a}	0.05	0.34^{b}	0.04	0.42^{a}	0.07
Albumine (g.dL^{-1})	3.21^{a}	0,18	3.32^{a}	0.11	3.21^{a}	0.25	3.34^{a}	0.10
TPP (g.dL^{-1})	6.20^{a}	0.26	6.30^{a}	0.21	6.00^{a}	0.16	6.11^{a}	0.22
AST (U.L^{-1})	185.5^{a}	115.0	208.5^{a}	55.0	319.0^{a}	254.0	294.5^{a}	228.0
ALT (U.L^{-1})	32.65^{a}	18.55	28.15^{a}	3.90	41.65^{a}	20.32	38.00^{a}	18.62
AST/ALT	5.60^{a}	0.35	7.45^{a}	1.90	6.85^{a}	2.85	7.05^{a}	3.00

Within the same line, means having different superscripts are significantly different by ($p \leq 0.05$; n=6) Tukey test.TPP: serum proteins, AST: aspartate aminotransferase, ALT: alanine. Aminotransaminase.

shown in Table 3. Microalgal biomass has high levels of nucleic acids, which have been reported as about 4 to 6%. Due to the inability of the human body to metabolize uric acid from purine metabolism, the increase in consumption of nucleic acids can lead to high levels of uric acid in serum. Thus, it may promote the appearance of diseases such as gout (Becker, 1998; Araújo et al., 2003). Values for uric acid, if taken as a reference, the variation range from 1.2 to 7.5 mg.dL^{-1} described as standard for rats (Mitruka and Rawnsley, 1981), are all within the normal range. Likewise, when compared with values obtained from other studies with male *Wistar* rats used as control (Vilela et al., 2000; Rodrigues et al., 2006; Duarte et al., 2009; Denardin et al., 2009). The fraction of non-protein nitrogen in 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 protein and amino acids. The ammonia released in the deamination of amino acids is almost completely removed from the blood by conversion to urea, then, if essentially all the urea is formed in the liver, even in the absence of or presence of any severe liver disease, ammonia accumulates in the blood (Devlin, 2007).

It is recommended that serum urea levels in rats around 300 mg.dL^{-1}, indicate possible kidney failure (Guyton, 1984). Low levels of urea may be indicative of severe liver failure, nephrosis, malnutrition and/or hemodilution (Ori et al., 1990). The animals belonging to groups that consumed diets with *Spirulina* contain serum urea levels lower than the control group; the difference was not significant ($P \leq 0.05$), and it represents about 45% of the total and it is the most used resource for evaluation of renal function in mammals (Duarte et al., 2009). For the purposes of evaluation of kidney function, it is very important to determine creatinine levels together with urea, because its levels increase when there is a decrease of kidney function (Miller, 1993). This study showed that creatinine levels were within a range of 0.34 to 0.44 mg.dL^{-1}, no significant difference between treatments, except for S2 ($P \leq 0.05$). Several studies with male *Wistar* rats reported for values creatinine were in the range mentioned above (Anthony et al., 2006). Assessment of the serum proteins provides important information about clinical conditions such as hydration status, existence of inflammatory diseases and protein metabolism, and its decrease in healthy animals may be indicative of a restriction on intake of amino acids, either by consumption of diets low in protein, or by food intake with satisfying protein content, but containing factors that hinder the digestion and absorption (Campello et al., 2009). According to the literature healthy rats have serum proteins between 5.6 to 7.5 g.dL^{-1} and albumin content in the range of 3.4 to 4.3g.dL^{-1} (Morton et al., 1993; Souza-Soares et al., 2009).

The values found for total serum protein and albumin is in the ranges of 6.00 to 6.30g.dL^{-1} and 3.21 to 3.34 g.dL^{-1}, respectively, suggesting a similarity with the control diet. But the content of albumin for groups C, S1 and S2 are located slightly below the minimum established as normal (3.4 g.dL^{-1}). The serum levels of aspartate aminotransferase (AST) and alanine aminatransaminase (ALT) values revealed no statistically significant difference to the control group, implying that there was no lesion in the liver tissue of animals. The ALT in hepatocytes occurs at concentrations higher than AST, therefore, the determination of its activity in serum blood is a more effective diagnosis of liver injury (Lima et al., 1985). The relationship AST/ALT can give an idea of the degree of hepatocyte injury. Increased ALT/AST ratio may be indicative of the extent of cellular damage (Adeyemi et al., 2010). A ratio below 1.0 suggests cytoplasm of hepatocyte injury, however, when the ratio is above 1.0 reveal cytoplasmic mitochondrial hepatocyte injury (Caballero-Córdoba and Sgarbieri, 2000). Such

Table 4. Hematological parameters of rats fed different diets (mean values with their standard errors).

	Treatments							
	C		S1		S2		S3	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
T leukocytes (10^3/mm^3)	6.60^a	1.30	6.75^a	2.75	5.60^a	2.90	5.65^a	1.90
Lymphocytes (%)	72.65^a	5.80	83.00^a	6.45	65.50^a	32.35	75.35^a	6.05
Band n (%)	0	0	0	0	0	0	0	0
Segmented n (%)	21.35^a	7.10	15.15^a	5.55	14.65^a	8.40	20.50^a	6.15
Monocytes (%)	2.15^a	1.50	1.35^a	1.20	2.50^a	2.35	3.85^a	1.15
Basophils (%)	0	0	0	0	0	0	0	0
Eosinophils (%)	3.85^a	0.75	0.50^b	0.85	0.65^b	0.80	0.35^b	0.50
Hematocrit (%)	43.80^a	1.45	45.00^{ab}	1.15	45.95^{ab}	1.15	46.75^b	2.10
Hemoglobin (%)	14.10^a	0.40	14.65^{ab}	0.40	14.60^{ab}	0.20	14.90^b	0.55
MCV (%)	57.70^a	2.30	59.00^a	0.95	58.25^a	2.10	57.50^a	1.45
MCH (%)	32.20^{ab}	0.45	32.50^{ab}	0.40	31.60^a	0.30	31.90^{ab}	0.45
Erythrocytes (10^6/mm^3)	7.60^a	0.45	7.60^a	0.20	7.90^{ab}	0.15	8.10^b	0.20

Within the same line, means having different superscripts are significantly different by ($p \leq 0.05$; n=6) Tukey test.

information may suggest the possibility that the animals had lesions cytoplasmic and/or mitochondria of hepatocytes, since the AST/ALT ranged from 5.60 to 7.45. In order to confirm or not these hepatocellular damage, we determined the liver weight ratio (g)/body weight (g), obtaining values ranging from 0.033 ± 0.003 to 0.034 ± 0.003, which showed no significant difference and they are in agreement with other studies (Caballero-Córdoba and Sgarbieri, 2000; Shons et al., 2009). The fact that a single parameter studied to indicate hepatological lesions can be explained by the high concentration of AST in erythrocytes, where hemolysis occurrence can affect the test giving falsely elevated results (Miller, 1993). A series of investigations have shown that components of the microalga *Spirulina* such as phyocyanin (Richmond, 1990; Henrikson, 1994), vitamin A, absorbable iron, phenolic compounds, essential fatty acids (Belay, 1993; Colla, 2008), exert very important physiological actions in the body. A large number of peptides derived from food proteins indicate the presence of functional properties such as immunostimulation (Mills, 1992). The results found for total leukocytes (Table 4)can be considered normal when compared with the track recommended, 6000 to 17000/mm^3 (Sanchis and Silbiger, 1986; Souza-Soares et al., 2009). Of the leukocytes total, the lymphocytes are present at higher levels, accounting for about 50 to 95% (Anthony et al., 2006). Band and segments constitute together 10 to 42% of the leukogram (Sanchis and Silbiger, 1986), and the segmented represent virtually the entire sum, therefore the values achieved in the current study are consistent with the literature. The results found in this study for levels of monocytes (Table 4) did not differ significantly between groups, but also are consistent with other studies (Santos et al., 2004; Duarte et al., 2009).

The monocytes represent 0 to 3% of total leukocytes (Sanchis and Silbiger, 1986; Souza-Soares et al., 2009). Basophils play an important role in body immune responses, because the slightest contact with an allergenic substance release chemical mediators such as histamine, which attracts other immune cells (Miller, 1993). In this study, we found 0% of basophils for all treatments, as reported in the literature (Sanchis and Silbiger, 1986; Anthony et al., 2006; Souza-Soares, 2009). Eosinophils must be present at around 0 to 3% (Sanchis and Silbiger, 1986; Anthony et al., 2006; Souza-Soares, 2009), so the results for treatments with *Spirulina* are within the range considered normal. Group C was slightly higher, indicating food allergy, possibly due to casein (Harkness and Wagner, 1993; Bernard et al., 2000). In this study the treatment S3 added to the diet showed higher levels than the control diet ($P \leq 0.05$), showing its efficiency. Hematocrit values for rodents vary between 36 and 48% (Harkness and Wagner, 1993). The mean hemoglobin in the group S3 was significantly higher than the control group ($P \leq 0.05$), agreeing with the results of hematocrit. Hemoglobin levels in all the treatments can be considered normal when compared with other studies (Harkness and Wagner, 1993; Kapoor and Mehta, 1993). The values for Mean corpuscular volume (MCV) did not differ among themselves. In relation to levels of MCM, only S1 and S2 diets were different ($P \leq 0.05$). Erythrocytes are the figurative elements and are most present in the blood and a low count indicates iron deficiency and anemic status. For groups C, S1, S2 and S3 values were found that are included in the normal range (Sanchis and Silbiger, 1986; Souza-Soares et al., 2009). We also observed that the percentage of

Spirulina in the diet is proportional to the levels of erythrocytes and that treatment with higher amounts of microalgae (S3) had higher values ($P \leq 0.05$) than those of control.

Conclusion

The current study demonstrates that diets with 8.8, 17.6 and 26.4% of *Spirulina* strain LEB-18 resulted in the development adequate of rats without significantly changing the majority of indices (biochemical, hematological, nutritional and physiological) studied. 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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