

## ***Spirulina platensis* Effects on the Levels of Total Cholesterol, HDL and Triacylglycerols in Rabbits Fed with a Hypercholesterolemic Diet**

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### **ABSTRACT**

*In this work, hypercholesterolemia was induced in rabbits by feeding them a high cholesterol diet (CD, 350 mg/d) and the effects of supplementing this diet with 0.5 g/d *Spirulina platensis* was evaluated by measuring the levels of serum total-cholesterol (TC), triacylglycerols (TAG) and high-density lipoprotein (HDL-cholesterol) at the start of the experiment and after 30 d and 60 d. It was found that the levels of serum cholesterol decreased from 1,054±101 mg.dL<sup>-1</sup> in the rabbits fed a CD without *S. platensis* to 516±163 mg.dL<sup>-1</sup> to those fed with a high cholesterol diet supplemented with *S. platensis* (significant at  $p < 0.0001$ ). The addition of *Spirulina* to the cholesterolemic diet did not cause significant decrease on the levels of triacylglycerols in the animals. The levels of serum high-density lipoprotein (HDL-cholesterol) was 73±31 mg.dL<sup>-1</sup> for rabbits fed a CD without *S. platensis* as compared to 91.0±15.7 mg.dL<sup>-1</sup> in those fed a CD supplemented with *S. platensis* (significant at  $p=0.1533$ )*

**Key words:** *Spirulina platensis*, cholesterol, rabbits

### **INTRODUCTION**

It is known that natural antioxidants can inhibit the hypercholesterolemia, and their effect in many cases has been proved. One of the most known cases is the "French paradox", an apparent compatibility of a diet with high fat ingestion with a low incidence of atherosclerosis, due to the consumption of red wine, which presents considerable amounts of antocyanins, natural pigments with phenolic structure (Renaud and Lorgeril, 1992; Amaral et al., 1995). Considering the high cost of the medicaments, alternative treatments for the control of the

hypercholesterolemia have been used in the popular medicine as the ingestion of capsules of poly-unsaturated fatty acids or fruits and vegetables extracts that present substances with antioxidant potential, as the eggplant and the mate (Gugliucci, 1996; Perugini et al., 2000; Schinella et al., 2000). Among the substances with antioxidant potential, are the ascorbic acid and the phenolic compounds (Gardner et al., 2000). The cyanobacterium *Spirulina* is cultivated and commercialized worldwide due to its nutritional characteristics including high concentrations of protein (~65%), vitamins and mineral salts (Henrikson, 1994; Belay et al., 1993).

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Preparations of *Spirulina* are also used for their therapeutic properties in the treatment of many diseases, including hypercholesterolemia and atherosclerosis (Nakaya et al., 1988; Ramamoorthy and Premakumari, 1996), as well as to reduce body weight in obese humans (Becker et al., 1986). The *Spirulina* components which are responsible for these therapeutic properties are thought to be compounds with antioxidant abilities such as polyunsaturated fatty acids, phycocyanin (Estrada et al., 2001; Bhat and Madyastha, 2001; Reddy et al., 2003, Nagaoka et al., 2005) and phenolics (Miranda et al., 1998). Of the compounds present in *Spirulina*, gamma-linolenic acid (C18:3,  $\omega$ 6, GLA) and phycocyanin are those which have received most attention from researchers.

The aim of this work was to study the opposite effects of cholesterol and *Spirulina platensis* diets on the levels of cholesterol, triacylglycerols and HDL-cholesterol in rabbits.

## MATERIAL AND METHODS

### Animals

Fifteen males New Zealand white rabbits, weighing  $3496 \pm 336$ g, 8 weeks old, were included in this study. Animals were housed individually and water was provided *ad libitum*. After adaptation, rabbits were equally divided in five groups. All animals were fed daily with 100 g of feed, and feed was specific to each group.

### Diets

The first group of animals (SDG-standard diet group) was fed with a standard feed-SD (Nuvital Nutrients, Brazil). The second group (CDG-cholesterol diet group) was fed with a cholesterol added diet (CD-cholesterol diet), containing 3.5 g of cholesterol per 1,000 g of SD (0.35%). The cholesterol was dissolved in 100 g of lard previously heated. The percents of cholesterol used in this kind of studies has been 0.35% (Amaral et al., 1995), 0.5% and 1% (Keaney et al., 1993; Mahfouz et al., 1997, Silva et al., 1997). The third group (CSDG-Cholesterol-*Spirulina* diet group) was fed with a *Spirulina* added diet (CSD-cholesterol-*Spirulina* diet) prepared with the addition of 5.0 g of *S. platensis* biomass (Delaware, Brazil) per 1,000 g of the cholesterol added diet (CD), and the animals were provided

0.5 g of *Spirulina*/d. The fourth group (CSDG30) was fed with the CD in the first month and with the CSD in the second month of treatment. The lard was used to aggregate homogeneously the powder of cholesterol and the *Spirulina* biomass to the feed. However, as the lard is constituted by saturated fat, it was necessary to evaluate the influence of this one on the animals' lipoprotein levels. In this way, a fifth group of animals (FDG-fat diet group) was fed with a fat diet (FD), prepared by the addition of 100 g of lard in 1,000 g of standard feed.

### Determinations of Feed Composition

Determinations of humidity, proteins, lipids and ashes were accomplished following the methodology of A.O.A.C (1995) to determine the final composition of the diets.

### Measurement of Serum Lipids Levels

Approximately 4 mL of blood was drawn from the central ear artery of the rabbits at 0 d, 30 d and 60 d of treatment. Serum total cholesterol, triacylglycerols and high-density-lipoprotein (HDL) were assayed using commercial enzyme kits (Wiener Lab, Argentina; Lab Test, Brazil) on Vitalab Selectra 2 equipment.

### Statistical Analysis

Data are expressed as means  $\pm$  standard deviations of the mean. Statistical differences among the groups were determined using ANOVA and Tukey test with  $p < 0.05$  considered statistically significant.

## RESULTS AND DISCUSSIONS

### Diets and Animals

The composition analysis of the diets, presented in Table 1 demonstrated some variations on the percentiles of humidity, ashes, proteins and lipids. The standard feed (SD) presented the lowest lipid content (5.19%) when compared with the others diets (~13.80%), which could be explained because this diet was not added of lard. The cholesterol added in CD (cholesterol-diet) and CSD (cholesterol-*Spirulina*-diet) ( $350 \text{ mg} \cdot 100 \text{ g}^{-1}$ ) caused no differences on lipids percentiles, even though it could cause significant differences on the serum lipids levels.

Table 2 presents the animals' weights in the beginning and at the end of the treatments. No significant differences were observed ( $p=0.05$ ).

### Serum Lipids Levels

Table 3 presents the values of total-cholesterol (TC), triacylglycerols (TAG) and HDL-cholesterol in the rabbits on 0, 30 and 60 d of treatment. Results of ANOVA demonstrated that the interaction of treatment time (0, 30 and 60 d) and diets groups of rabbits (SDG, CDG, CSDG, CSDG30 and FDG) had significant influence in levels of total cholesterol ( $p<0.0001$ ) and HDL-cholesterol ( $p=0.015$ ) and no significant influence on the levels of TAG ( $p=0.051$ ). Comparing the results of total cholesterol (TC) and

HDL-cholesterol (HDL) at the end of treatment (60 d) on the SDG and CDG groups (Table 3), increasing of the values of serum lipids during the treatment were observed, which presented significant differences at the 95% confidence interval. The rabbits of standard group (SDG) were fed with a standard diet (SD) which was not added of cholesterol and lard, in comparison with the rabbits of cholesterol-diet-group (CDG), which were fed with a cholesterol-diet (CD). The increase of the daily ingesta of cholesterol in CDG caused an increase in lipoproteins in serum since exceeds the necessity required for the organism for synthesis of hormones and biliary salts (Stryer et al., 1996; Champe et al., 1997).

**Table 1** Diets composition

Diet	Humidity (%)	Ashes (%)	Proteins (%)	Lipids (%)
Standard diet (SD)	12.03±1.49	8.04±0.23	17.05±0.17	5.19±0.44
Cholesterol diet (CD)	11.09±0.08	6.56±0.05	15.05±0.67	14.52±1.37
Cholesterol-Spirulina diet (CSD)	11.39±0.29	7.19±0.72	15.98±0.26	11.85±2.04
Fat diet (FD)	10.43±0.06	8.29±0.83	15.02±0.21	15.05±0.04

**Table 2** Animals weights during the treatments

	SDG	CDG	CSDG	CSDG30	FDG
IW (g)	3,413.3±156.7	3,585.8±204.4	3,566.6±264.1	3,087.5±317.5	3,657.5±292.5
FW (g)	3,791.6±183.3	3,845.0±377.4	3,693.3±140.7	3,483.7±227.7	3,765.0±265.0

IW: initial weight; FW: final weight; SDG: standard diet group; CDG: cholesterol diet group; CSDG: cholesterol-spirulina diet group; CSDG30: cholesterol-spirulina diet group-with spirulina after the 30th day; FDG: fat diet group. N=3 in all groups.

**Table 3** Total-cholesterol (TC), triacylglycerols (TAG) and HDL-cholesterol in the rabbits in 0, 30 and 60 days of treatment

	Time (d)	SDG	CDG	CSDG	CSDG30	FDG
TC (mg.dL <sup>-1</sup> )	0	28±9 <sup>a</sup>	59±36 <sup>a</sup>	26±11 <sup>a</sup>	46±15 <sup>a</sup>	21±2 <sup>a</sup>
	30	25±6 <sup>a</sup>	627±77 <sup>cd</sup>	368±51 <sup>bc</sup>	535±305 <sup>c</sup>	68±28 <sup>ab</sup>
	60	24±7 <sup>a</sup>	1054±101 <sup>e</sup>	516±163 <sup>c</sup>	923±101 <sup>de</sup>	43±34 <sup>a</sup>
TAG (mg.dL <sup>-1</sup> )	0	71±18 <sup>ab</sup>	34±14 <sup>ab</sup>	31±13 <sup>a</sup>	59±21 <sup>ab</sup>	47±10 <sup>ab</sup>
	30	64±47 <sup>ab</sup>	59±17 <sup>ab</sup>	62±6 <sup>ab</sup>	71±56 <sup>ab</sup>	41±7 <sup>ab</sup>
	60	66±31 <sup>ab</sup>	167±37 <sup>b</sup>	97±48 <sup>ab</sup>	45±11 <sup>ab</sup>	37±6 <sup>ab</sup>
HDL (mg.dL <sup>-1</sup> )	0	21±2 <sup>ab</sup>	38±22 <sup>abc</sup>	18±6 <sup>a</sup>	29±10 <sup>abc</sup>	15±3 <sup>a</sup>
	30	15±5 <sup>a</sup>	64±18 <sup>abcd</sup>	58±13 <sup>abcd</sup>	37±21 <sup>abc</sup>	23±5 <sup>ab</sup>
	60	15±2 <sup>a</sup>	73±31 <sup>cd</sup>	91±16 <sup>d</sup>	49±14 <sup>abcd</sup>	29±18 <sup>abc</sup>

SDG: standard diet group; CDG: cholesterol diet group; CSDG: cholesterol-Spirulina diet group; CSDG30: cholesterol-Spirulina diet group-with Spirulina after the 30<sup>th</sup> day; FDG: fat diet group.

<sup>a,b,c,d,e</sup> Different superscripts for the same variable mean statistical differences ( $p<0.05$  by Tukey test).

N (number of animals in each group) = 3 in all groups.

Triacylglycerols levels were higher due to the higher ingesta of lipids, since the cholesterol-diet (CD) contained 14.52% of lipids, comparing to 5.19% in the standard diet, but this difference was not significant compared SDG and CDG groups at 60 d of treatment.

Results of total-cholesterol, triacylglycerols and HDL presented no significant differences to all diets groups in the initial time ( $p=0.133$  to TC,  $p=0.051$  to TAG and  $p=0.173$  to HDL). These results showed that rabbits were homogeneous with respect to the serum levels of TC and TAG in the initial of treatment and that modifications in these levels in comparison with the final time had occurred due to the differences in treatments. Similar results of TC, TAG and HDL were reported by others authors in rabbits submitted to control diets. Amaral et al. (1995) found levels of  $40.25 \pm 22.23$  to  $63.75 \pm 10.15$   $\text{mg.dL}^{-1}$  for total-cholesterol and  $26 \pm 13.27$  to  $42.50 \pm 11.12$   $\text{mg.dL}^{-1}$  for HDL-cholesterol. Keaney et al. (1993) found levels of total-cholesterol, HDL and triacylglycerols as  $57 \pm 9$ ,  $30 \pm 5$  and  $115 \pm 15$   $\text{mg.dL}^{-1}$ , respectively. These results demonstrated that the variations of rabbits serum lipids found in this work were in according with the biological variability of these animals. Results of TC to SDG and FDG did not present significant differences in all the treatments time ( $p=0.9234$ ).

The levels of serum cholesterol decreased from  $1054 \pm 101$   $\text{mg.dL}^{-1}$  in the rabbits fed with the cholesterol diet (CD) to  $516 \pm 163$   $\text{mg.dL}^{-1}$  in those fed with the cholesterol-*Spirulina* diet CSD (significant at  $p < 0.0001$ ). The levels of serum high-density lipoprotein (HDL)-cholesterol was  $91 \pm 16$   $\text{mg.dL}^{-1}$  for rabbits fed with the CSD as compared to  $73 \pm 31$   $\text{mg.dL}^{-1}$  those fed a CD. The means were not different at a confidence level of 95%, according to Table 3, but at a confidence level of 85% they were significantly different ( $p=0.1533$ ). This result was in according with Kay (1991) who observed that *Spirulina* decreased total plasma cholesterol levels and increased cholesterol associated to high density lipoproteins, suggesting that *Spirulina* could have a protective effect on the cardiovascular system. Blé-Castillo et al. (2002) observed that when *S. maxima* was added to diet of mice two weeks prior the onset of fatty liver induction with simvastatin, ethanol and a hypercholesterolemic diet, there was a

decrement of liver total lipids (40%), liver triacylglycerols (50%) and serum triacylglycerols (50%) compared to the animals with the same treatment but without the microalgae. In addition, the administration of this alga produced a significant increase (45%) in serum high-density lipoproteins. According to Hill and McQueen (1997), high-density lipoproteins have long been known to be protective against the development of atherosclerosis. There is an inverse relationship between the HDL concentration and the incidence of coronary heart disease. HDL acts as the transport particle and, as such, occupies a central position in the reverse cholesterol transport system whereby cholesterol synthesized or deposited in peripheral cells is returned to the liver for reuse or re-excretion into the bile.

The elevation in the total cholesterol caused by a cholesterol rich diet was suppressed by the supplementation of 16% of *Spirulina* in mice (Kato et al., 1984). In the studies accomplished by Iwata et al. (1987), the addition of 5, 10 and 15% of *Spirulina* in mice diets caused significant inhibition in total cholesterol, triacylglycerols, phospholipids and HDL-cholesterol levels in the hyperlipidemia caused by fructose. Nakaya et al. (1988) demonstrated the hypocholesterolemic effect of *Spirulina* when administrated in humans ( $4.2 \text{ g.day}^{-1}$  for 4 weeks). Torres-Durán et al. (1999) observed that *S. maxima* was able to prevent the changes induced by carbon tetrachloride in liver lipids in rats.

The presence of antioxidant compounds like phycocyanin and phenolic compounds, and poly-unsaturated fatty acids in the microalgae *Spirulina* can be the cause of the properties of *Spirulina* on the decrease of serum lipids levels. According to Nagaoka et al. (2005), both, *S. platensis* concentrates or phycocyanin, a proteic pigment extracted of *Spirulina*, caused hypocholesterolemic activity in rats. The in vivo antioxidant capacity of extracts of *Spirulina* was evaluated by Miranda et al. (1998) in plasma of adult male Wistar rats receiving a daily dose of 5 mg of extract for 2 and 7 weeks. Plasma antioxidant capacity was measured in brain homogenate incubated for 1 h at  $37^\circ\text{C}$ . Upon treatment, the antioxidant capacity of plasma was 71% for the experimental group and 54%

for the control group. The authors considered that the amounts of phenolic acids,  $\alpha$ -tocopherol and  $\beta$ -carotene presents in *Spirulina* extracts were responsible by the antioxidant activity. The microalga *Spirulina* presented poly-unsaturated fatty acids as demonstrated by many authors (Colla et al., 2004; Olguin et al., 2001; Alonso and Maroto, 2000; Quoc et al., 1994; Cohen et al., 1993; Cohen et al., 1987). These compounds have been studied in respect to its therapeutic properties such as its ability to decrease blood cholesterol levels (Ishikawa et al., 1989; Kurushima et al., 1995).

Thus, it could be concluded that supplementing 0.5g. d<sup>-1</sup> of *S. platensis* caused a decrease in the induced hypercholesterolemia in rabbits. The serum levels of cholesterol decreased in the rabbits fed a hypercholesterolemic diet without *S. platensis* in comparison to those fed a hypercholesterolemic diet supplemented with *S. platensis*. In contrast, the serum levels of HDL were higher in the groups fed with *Spirulina*. These results showed the potential of biomass *Spirulina* to decrease the serum levels of total cholesterol and to increase the serum levels of HDL-cholesterol, considered as a protective factor against the development of atherosclerosis.

## RESUMO

A microalga *Spirulina* é cultivada e comercializada no mundo devido a suas características nutricionais (elevada concentração de proteínas, em torno de 65%, vitaminas e sais minerais) e ao seu potencial terapêutico no tratamento de inúmeras doenças, inclusive a hipercolesterolemia. Neste trabalho foi avaliada a inibição da hipercolesterolemia induzida em coelhos por uma dieta adicionada de colesterol (350 mg.d<sup>-1</sup>), pela suplementação de 0,5 g.dia<sup>-1</sup> de biomassa de *Spirulina platensis*, sendo avaliados os níveis de colesterol total, triglicérides e HDL nos tempos de 0 d, 30 d e 60 d de tratamento. Os resultados indicaram que a adição de *Spirulina platensis* na dieta ocasionou decréscimo nos níveis de colesterol total de 1054±101 mg.dL<sup>-1</sup> para 516±163 mg.dL<sup>-1</sup> (p<0,0001), para os coelhos alimentados com a dieta colesterolêmica em comparação com os que receberam a dieta adicionada de *Spirulina*

*platensis*. A adição de *Spirulina* a dieta colesterolêmica não ocasionou decréscimo significativo nos níveis de triglicéridos dos coelhos. Os valores de HDL aumentaram de 73±31 mg.dL<sup>-1</sup> para 91,0±15,7 mg.dL<sup>-1</sup>, comparando-se os coelhos alimentados com a dieta colesterolêmica e os alimentados com a dieta adicionada de *Spirulina*, estatisticamente diferentes a um nível de significância maior que 0,1533.

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