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The Effects of Dietary Supplementation with *Spirulina platensis* in Growing Rats

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ABSTRACT

Spirulina is a nutritional supplement that has gained worldwide popularity due to its high nutritional content. Although the spirulina based products are mainly targeted at adult humans there is an increased use of the supplements by young children and animal owners might feed spiruline to their pets or livestock which could have long term and irreversible effects on them. The effect of *Spirulina platensis* on the viscera, morphology and morphometry of growing rats was investigated in 23 female Sprague Dawley rats. The rats were randomly divided into 3 groups. Group 1 served as a control, group 2 received low dose of 150 mg kg⁻¹ Spirulina daily, and group 3 received a high dose of 1500 mg kg⁻¹ daily to investigate its possible adverse effects. After three and a half weeks of supplementation all groups of rats showed a significant body mass gain. Spirulina significantly increased tibial length (ANOVA; $p = 0.0073$) and visceral fat ($p = 0.0013$) but there was no significant effect on the abdominal viscera morphology and morphometry. Spirulina did not cause any hepato-toxicity as measured by blood alanine amino transaminase and histology.

Key words: Spirulina, abdominal viscera, metabolic substrates, growth, tibia, morphometry

INTRODUCTION

Spirulina, blue green algae, thrives in tropical and subtropical warm lakes with a high pH ranging from pH 9.4 to pH 11.0 (Ciferri, 1983). There are two different species of spirulina, *Spirulina maxima* and *Spirulina platensis*, with varying distribution throughout the world (Oliveira *et al.*, 1999). *S. platensis* is more widely distributed and found mainly in Africa, Asia and South America (Vonshak, 1997). *S. maxima* on the other hand is more confined to areas in Central America.

Spirulina is rich in nutrients such as vitamins, amino acids, gamma linoleic acid, phycocyanins, tocopherols, chlorophyll and beta carotenes (Khan *et al.*, 2005; Abd El-Baky *et al.*, 2003). As a result of its nutritional content spirulina has gained world wide popularity as a nutritional supplement. It has also been reported to have health benefits in conditions such as diabetes mellitus and arthritis (Parikh *et al.*, 2001; Rasool *et al.*, 2006). Spirulina has also been shown to have immuno-stimulatory effects and to have antiviral activity (Khan *et al.*, 2005). Studies on humans have shown beneficial effects of spirulina on the nutritional status of undernourished children as well as HIV positive children (Simpore *et al.*, 2005). In adult oestrogen-insufficient mice, spirulina

has been shown to affect bone mineralization and skeletal muscle protein in rats. However, it does not affect reproductive performance (Ishimi Sugiyama, 2006; Salazar *et al.*, 1996; Voltarelli and de Mello, 2008). Adverse effects of taking high doses of spirulina include rhabdomyolysis (Mazokopakis *et al.*, 2008). Although Spirulina has been shown to have hepatoprotective effects in rats given diethylnitrosamine (Bin-Meferij, 2009), hepatotoxicity has also been reported in cases where the spirulina has been contaminated with microcystins (Iwasa *et al.*, 2002).

The use of Spirulina worldwide, although mainly by adults also extends to children. When dietary supplements are administered *per os*, the first point of contact with the body is the Gastrointestinal Tract (GIT). The GIT is a potent source of regulatory cytokines, growth factors and humoral signals which regulate function of the accessory organs such as the liver and the pancreas as well as the GIT-brain neurohumoral axis (Strader and Woods, 2005). The constituents of spirulina include substances which could potentially affect these neurohumoral signals and consequently alter function especially in the prepubertal animals in the immediate post-weaning period. Dietary manipulations during the suckling period and post weaning can have long lasting and potentially irreversible effects on some of the transport mechanisms in the gut and may even cause precocious maturation of the GIT (Linderoth *et al.*, 2005; Pacha, 2000). It is thus important to investigate some of the potential effects of spirulina in young prepubertal animals as a source of information for the supplementation of spirulina in children and animals.

The major aim of this study was to investigate the effects of dietary supplementation with *S. platensis* on growth performance, abdominal visceral morphology, morphometry and metabolic substrates of growing rats. Most of the studies investigating effects of spirulina have used *Spirulina maxima* and there is a dearth of information on the effects of dietary supplementation with *Spirulina platensis*.

MATERIALS AND METHODS

This study was approved by the Animal Ethics Screening Committee (AESC) of the University of the Witwatersrand, South Africa (AESC approval number: 2009/21/03). The study was conducted in the Central Animal Services animal unit at the University of the Witwatersrand, in 2009.

Animals: Twenty three female *Sprague dawley* rats (108±9 g body mass) were used in the study. The rats were housed in separate solid-bottom cages with wood shavings for bedding. All rats had *ad libitum* access to drinking water and standard rat chow (Epol, South Africa). The lighting was set on a 12 h light-dark cycle with lights from 7:00 and ambient temperature was set at 22±1°C and a relative humidity range of 30 to 40%. The rats were habituated to the housing conditions as well as the experimental interventions (handling and weighing) for three days before starting the experimental protocol.

Feeding and treatments: The rats were randomly divided into three groups. Group 1 (n = 8) served as a control and received plain gelatine cubes once a day. Group 2 (n = 7) received a low dose of spirulina (150 mg kg⁻¹) suspended in gelatine cubes and group 3 (n = 8) received a high dose of spirulina (1500 mg kg⁻¹) suspended in gelatine cubes. All rats received water and rat chow *ad libitem* The *Spirulina platensis* in the form of tablets, (Marcus Rohrer, Gorinchem, The Netherlands) were crushed and suspended in 2 mL gelatine cubes. The rats were weighed twice a week and adjustments to the amount of Spirulina in the cubes were made in order to maintain the

dose rate constant. The rats were on the treatment for a period of three and a half weeks which is adequate to show effects on growth. The gelatine cubes in which the Spirulina was suspended were prepared as described by Kamerman *et al.* (2004).

Blood: After the three and a half week feeding trial, the rats were fasted overnight and two drops of blood were collected from the tail vein via pin prick with a sterile needle after disinfecting the tail with alcohol (Loxham *et al.*, 2007). The blood was used to measure fasting glucose using a glucometer (Glucometer Elite, Kyoto, Japan) and triglycerides using a TG meter (Accutrend Plus Cobas, Roche, Mannheim, Germany).

A further 2 mL of blood was collected into a vacutainer by cardiac puncture, using a 1 mL syringe flushed with heparin and a 21G needle. The Packed Cell Volumes (PCV) were determined by collecting some blood into capillary tubes and centrifuging (Haematokrit 210, Labotech, Germany). The blood was spun for three minutes at $15,000 \times g$ for 20 min. The enzyme activity of alanine aminotransferase (ALT) was determined using some of the collected blood on a calibrated Reflotron machine (Reflotron®, Roche Diagnostics LTD, Burgess Hill West Sussex, United Kingdom) as per manufacturer's instructions. The rats were then killed by anaesthetic overdose of Pentobarbitone (Eutha-naze, Centaur labs, Johannesburg, South Africa).

Visceral measurements and histology: The visceral organs were removed and weighed. The intestinal contents were removed gently before weighing. The length of the large and small intestines was measured by gently stretching them out on a board. Samples of the small intestine, kidney, liver and spleen were collected for histology and preserved in 10% phosphate buffered formalin. The samples were then processed routinely and stained with haematoxylin and eosin. Villus height and crypt depth was measured with a micrometer mounted on the eye piece of a light microscope.

Liver glycogen: Liver glycogen was determined indirectly by hydrolysis to glucose and measurement of glucose (Passonneau *et al.*, 1974). The glucose concentration of the hydrolysate was determined with a glucose (glucose oxidase) assay kit (Sigma Catalogue, GAGO-20) and a spectrophotometer (LKB Ultrospec II, LKB Biochrom Ltd., England).

Liver lipids: Standard procedures were used for lipid extraction (Bligh and Dyer, 1959).

Pancreatic soluble proteins: To determine the concentration of soluble pancreatic proteins using the Lowry *et al.* (1951) method modified to be performed on microplates, Bovine serum albumin (Sigma, St Louis, USA) was used as a standard.

Linear growth: The right femoral head was cut away from the acetabulum at the hip joint and the soft tissues were removed from the bones (tibia and femur). The length of the femur and tibia was then measured. The bones were then dried in an oven (Salvis®) at 40°C for 7 days (until constant mass) and weighed to determine their dry mass. The density of the bones was estimated by the formula: Bone density (mg mm^{-1}) = dry bone mass (mg)/bone length (mm).

Data analysis: All data are expressed as mean \pm SD. A one way analysis of variance (ANOVA) was used to assess the effects of spirulina on the measured parameters. A Tukey's post hoc test was

performed to make comparisons between all groups. Statistical significance was accepted at $p < 0.05$. All statistical analysis were performed using Graphpad Prism 403 for Microsoft Windows (Graphpad Software, USA).

RESULTS

Body mass: No significant difference in the initial mass of rats in the different groups was observed. Although all groups had significant body mass gain after 3.5 weeks (Fig. 1), there was no significant difference between the final mass of the rats in the different treatment groups (ANOVA; $p > 0.05$).

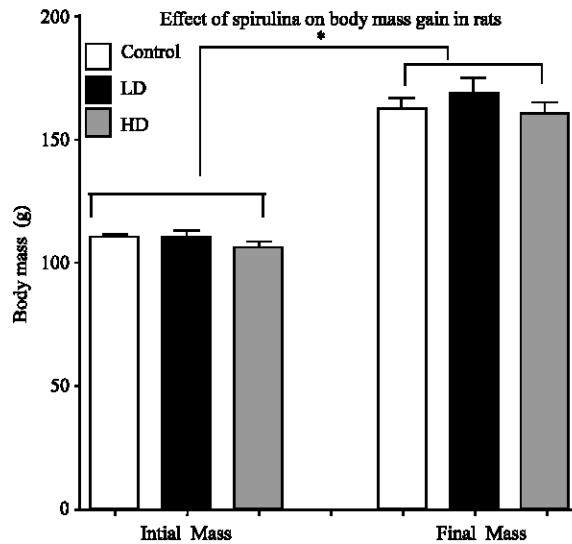


Fig. 1: Body mass of rats ($n = 23$) at the beginning of the study and at the end of the study period (3.5 weeks). LD, low dose; HD, high dose. * Significant increase ($p < 0.05$) in body mass gain for all groups final mass after 3.5 weeks vs initial mass.

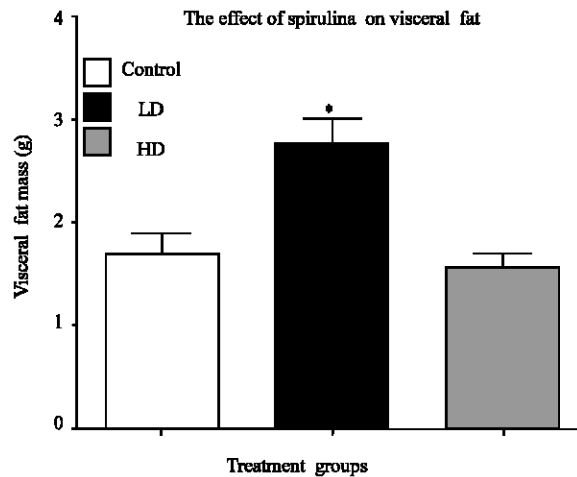


Fig. 2: The effects of dietary supplementation with spirulina at LD (low dose), HD (high dose) on visceral fat mass after the 3.5 week study period. *Significantly increased visceral fat compared to control and high dose ($p < 0.001$)

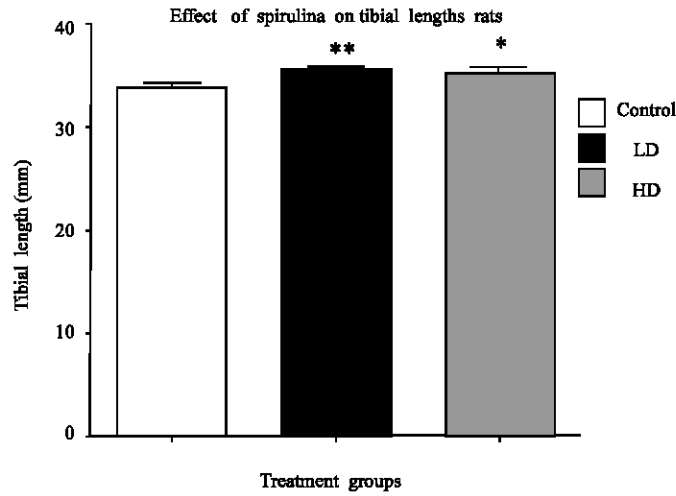


Fig. 3: Effect of Spirulina on linear growth as measured by the tibial length. **Significantly increased tibial lengths ($p < 0.01$) compared to the control; *Significantly increased tibial lengths compared to control ($p < 0.05$)

Table 1: The intestinal segment length (mm), visceral organ absolute (g) and relative (% body mass) mass after 3.5 weeks of dietary supplementation with Spirulina

Organ	Units	Control (n = 8)	LD (n = 7)	HD (n = 8)
Small intestine	g	5.3±0.5	5.6±0.6	5.3±0.6
	% BM	3.3±0.2	3.31±0.21	3.3±0.4
	mm	1051.3±62.2	1047.5±48.3	1081.4±49.5
Large intestine	g	1.3±0.1	1.5±0.4	1.3±0.2
	% BM	0.8±0.1	0.9±0.3	0.8±0.1
	mm	186.9±9.6	183.8±13.0	185.0±13.8
Liver	g	6.1±0.3	6.3±1.6	6.0±0.5
	% BM	3.8±0.3	3.7±0.6	3.8±0.2
Spleen	g	0.5±0.1	0.5±0.1	0.5±0.1
	% BM	0.3±0.1	0.3±0.0	0.3±0.1
Kidneys	g	1.3±0.1	1.3±0.2	1.2±0.1
	% BM	0.8±0.1	0.8±0.1	0.8±0.0
Stomach	g	1.3±0.2	1.3±0.2	1.3±0.1
	% BM	0.1±0.0	0.1±0.0	0.1±0.0
Caecum	g	0.7±0.1	0.8±0.1	0.8±0.2
	% BM	0.4±0.1	0.4±0.0	0.5±0.1
Pancreas	g	0.8±0.2	0.7±0.3	0.8±0.3
	% BM	0.5±0.1	0.4±0.1	0.5±0.1

Data are expressed as mean ±SD. BM: Body mass

Viscera and histological observations: The different doses of Spirulina did not significantly affect the mass, morphometry or morphology of the visceral organs, except for the visceral fat mass which was significantly increased ($p < 0.05$) in the rats that received the low dose of Spirulina

Table 2: Effects of Spirulina on fasting concentrations of metabolic substrates, hepatic glycogen and lipid storage, erythrocyte packed cell volume, Soluble pancreatic proteins and alanine amino transaminase

Parameter	Control	LD	HD
Blood			
Fasting glucose (mmol L ⁻¹)	3.6±0.8	3.6±0.9	3.9±0.3
Fasting triglycerides (mmol L ⁻¹)	1.1±0.2	1.15±0.1	1.2±0.1
PCV (%)	49.8±1.7	47.5±5.7	45.0±1.4
Alanine amino transaminase (U.L ⁻¹)	14.5±2.6	16.05±7.7	16.7±7.4
Liver			
Glycogen (mmol L ⁻¹) as glucose equivalents	7.4±0.7	7.5±0.6	7.4±0.6
Lipids (%)	4.9±0.7	4.7±0.7	4.7±0.6
Pancreas			
Soluble pancreatic protein (mg g ⁻¹)	66.8±4.2	70.1±6.4	68.2±5.7

All data are represented as mean ±SD. LD: Low dose spirulina, HD: High dose spirulina.

(Fig. 2 and Table 1). Supplementation with Spirulina had no significant effect on the small intestinal villus height and crypt depth (ANOVA; $p>0.05$).

Pancreatic soluble proteins: There was no significant difference (ANOVA; $p>0.05$) between groups in the concentration of soluble pancreatic protein (Table 2).

Linear growth: Dietary supplementation with Spirulina at both doses significantly increased the tibial length (ANOVA; $p = 0.0073$) compared to control however there was no difference between Spirulina supplemented groups (Fig. 3). There was no significant difference in the length of the femurs from the rats in the different groups (ANOVA; $p>0.05$).

Blood metabolic substrates and ALT: There was no difference in the blood concentrations of fasting glucose and triglycerides among the groups (ANOVA; $p>0.05$). There was also no difference in the erythrocyte Packed Cell Volume (PCV) amongst the groups (ANOVA; $p>0.05$) (Table 2). The circulating concentrations of ALT were also not significantly affected by the treatments (ANOVA; $p>0.05$).

Liver lipid and glycogen storage: The storage of lipids and glycogen was not significantly affected by the dietary supplementation with Spirulina (ANOVA; $p>0.05$) (Table 2).

DISCUSSION

The present study investigated the effects of dietary supplementation with *Spirulina platensis* on the abdominal viscera, morphology and morphometry of growing rats as a model for the use of supplements by children. After three and a half weeks of supplementation there was no significant difference in all parameters measured except for the tibial length and the visceral fat which were increased in rats supplemented with Spirulina.

The efficacy of a drug or supplement is affected by factors as dose, route of administration as well as the duration and frequency of supplementation (Atassi and Tagnon, 1975). The doses of spirulina used in this study were within the ranges of those previously used by other authors in their investigations on the effects of Spirulina on aspects such as anti-diabetes, anti-inflammatory, muscle structure and immunostimulation and shown to be effective (Parikh *et al.*, 2001; Voltarelli and de Mello, 2008; Hen *et al.*, 2006). In the previous studies, the duration of

administration ranged from one to eight weeks, the present study duration (3.5 weeks) falls within this duration. The route of administration in experiments has been per os either by gavage or as part of the diet as was the case in this study (Simpore *et al.*, 2005; Hen *et al.*, 2006).

The body mass gain for control rats compared to those fed *Spirulina*, suggested that *Spirulina platensis* did not affect the growth of the rats, however body mass alone is not a reliable measure of growth because body is influenced by various factors like obesity (Deng *et al.*, 2001). Tibial lengths have been used in a number of studies as a more accurate indicator of linear growth (Fritton *et al.*, 2005). Interestingly, although the tibial lengths increased significantly following the administration of *Spirulina*, there was no difference in the tibia mass between groups. It would have been more informative to have measured the bone density by densitometry and previous studies have shown *Spirulina* to affect bone mineralization (Ishimi Sugiyama, 2006).

There was a significantly greater amount of visceral fat in the rats that consumed a low dose of *Spirulina* compared to the other groups. However this did not translate into an increased overall body mass, thus the control group and high dose *Spirulina* groups may have had a higher lean body mass than the treatment groups or the fat may have been stored as subcutaneous fat. It would be interesting in the future to investigate the effects of *Spirulina* on the deposition of subcutaneous fat.

There was no difference observed in the absolute and relative organ masses among the three groups showing that spirulina neither promoted or retarded growth of the organs. We did not measure the function of the GIT in terms of digestive, absorptive and secretory capacity. Histological examination did not reveal any hepatic pathology and this was corroborated by the insignificant difference in circulating concentrations of ALT which is an indirect measure of liver function (Srivastava *et al.*, 2007).

In growing diabetic and obese rats spirulina has been found to reduce lipids, cholesterol and glucose (Parikh *et al.*, 2001). These effects were not evident in our study on growing non obese rats.

In rats the composition of pancreatic juices adapts to changes in diet composition (Buddington and Lepine, 1999). In this study we found that the soluble pancreatic proteins were unaffected by the dietary supplementation with *Spirulina*. The soluble proteins are an indirect measure of exocrine pancreatic zymogen concentration. We however did not assay for the specific enzyme activities.

Spirulina has been shown to have beneficial haematological effects where it has been reported to inhibit the development of leucopenia and anaemia induced by lead and cadmium in rats (Simsek *et al.*, 2009). We measured the PCV (haematocrit) and found that spirulina did not have an effect on the PCV. Although PCV can give an indirect measurement of anaemia, it would have been better to measure haemoglobin concentration because haematocrit can be affected by factors such as hydration status and variation in plasma volume.

CONCLUSION

Spirulina platensis which has slight compositional differences to *S. maxima* was not toxic neither did it promote or retard growth of the visceral organs. This is in agreement with a previous study which showed *Spirulina maxima* to be non toxic in mice (Salazar *et al.*, 1996). Supplementation did however promote linear growth and the deposition of visceral fat.

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