Journal of Medical and Biological Science Research
Vol. 6 (4), pp. 51-61, August, 2020
ISSN: 2449-1810
Research Paper
https://doi.org/10.36630/jmbsr_20010
http://pearlresearchjournals.org/journals/jmbsr/index.html

Spirulina and Proportion of Changes in Blood Parameters of Rats (*Rattus Norvegicus*) in Growth

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Accepted 02 August 2020

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ABSTRACT

To demonstrate the safety and active properties of Spirulina, a study was carried out in Wistar rats (*Rattus norvegicus*) which received in addition to the granulated industrial food, Spirulina as a supplement for three months. Four homogeneous groups of six rats each, three males and three females were formed. Group 1 (control) received conventional food (pellets FACI®) ad libitum and distilled water at 10 ml/kg body weight. As for groups 2, 3 and 4, they also received FACI® food, Spirulina at a rate of 10; 50; and 100 mg/kg body weight respectively. Blood samples performed at the orbital sinus were collected in 5 mL EDTA tubes and dry tubes. The collected blood in EDTA tubes was used to assay the blood count (CBC) when dry tubes were centrifuged at 3000 rpm for 10 min. The collected serum made it possible to dose the following biochemical parameters: AST, ALT, triglycerides, total cholesterol, bilirubin, renal markers and glucose. Analysis of obtained results indicated weight gain in treated animals. In terms of hematological parameters, no major disturbance of the hematopoietic system was observed over the entire study period. Results also revealed that transaminases, lipid and carbohydrate markers as well as renal and hepatic markers did not experience any disturbance which could affect the physiological functioning of animals at all doses. Likewise, different proteins presented levels revealing no abnormality. At the end of this study, it was noted that Spirulina supplementation acted favorably on biomarkers and could, therefore, contribute to improving the functioning of vital organs.

Keywords: Blood biomarkers, complementation, rat (*Rattus norvegicus*), Spirulina.
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INTRODUCTION

Many developing countries suffer from malnutrition in various forms, the most obvious cases are still under-nutrition. According to the report on the state of food security and nutrition in the world in 2018, the number of people suffering from hunger has increased to 821 million people in 2017, or one in nine (WHO, 2018). The proportions in sub-Saharan Africa are increasing with an estimated rate of 22.7% in 2016 against 20.8% in 2015 (FAO, 2016a). To remedy this, world organizations such as the WHO (World Health Organization) and the FAO (Food and Agriculture Organization), respectively in charge of health and food policies, have recommended to researchers around the world to re-examine the potentials food of humanity.

It is a response to this call that some researchers have taken interest in Spirulina which is a micro-alga with nutritional and therapeutic properties (Qureshi et al., 1995; Mao et al., 2005; Belay, 2002). In view of its role in the fight against malnutrition, Spirulina is one of the unconventional nutritional and therapeutic sources which is full of essential nutrients. In addition, it was proposed in human food (FAO) by several scientists

Table 1: Content of minerals in *Spirulina platensis.*

	P1	P3	P4	P5	P6	P8
Calcium	1159,83 ±5,64	1064,45 ±3,97	1142,32 ±7,52	1153,2 ± 8,00b	1002,06 ±3,91	1008,08 ±1,88
Magnésium	272,79 ± 4,86	277,36 ± 4,47	228,33 ± 6,91	286,16 ± 9,54	283,96 ± 8,18	267,09 ± 3,86
Phosphore	287 ± 2,00	280,42 ± 1,41	383,73 ± 9,1	235,86 ±1,86	233,38 ± 6,43	392,51 ± 2,8
Potassium	2093,05 ± 5	2090,67 ± 6,02	2104,13 ± 45,8	2083,22 ± 7,32	2074,04 ± 5,94	2077,47 ± 12,71
Sodium	1499,70 ± 9,96	1443,53 ± 6,22	1340,28 ± 15,42	1356,39 ± 5,42	1245,95 ± 6,62	1252,27 ± 8,4
Iron	24,08 ± 1,1	24,32 ± 0,2	23,38 ± 1,1	25,13 ± 1,2	24,18 ± 1,2	24,03 ± 1,5
Manganese	4,03 ± 0,24	4,08 ± 0,38	3,04 ± 0,24	5,05 ± 0,42	4,17 ± 0,62	3,11 ± 0,32
Zinc	2,10 ± 0,13	2,12 ± 0,02	2,14 ± 0,02	2,19 ± 0,04	3,22 ± 0,03	3,25 ± 0,17
Copper	0,017 ± 00	-	-	0,21 ± 0,01	0,14 ± 0,01	0,17 ± 0,02
Fluor	NGV	NGV	NGV	NGV	NGV	NGV
Iode	NGV	NGV	NGV	NGV	NGV	NGV
Selenium	NGV	NGV	NGV	NGV	NGV	NGV

NGV: Non given value ; P ; pond

and nutritionists with its exceptional qualities, ease of cultivation, high productivity and low cost of production (Kent, 2015). If this alga has undeniable nutritional properties, it should be checked for its activity on certain blood parameters. This study aims to assess the changes of hematological and biochemical parameters according to the reference values of rats in growth and subjected to Spirulina supplementation. More specifically, it involved: reporting on the evolution of the weight of the rats in growth; determine the proportions of the main normal blood parameters obtained compared to the reference values for rats; reveal the levels of variations in the main blood parameters that have already been studied previously (Ouédraogo et al., 2018; Ouédraogo et al., 2020); indicate the quantity (administered dose) of Spirulina which would favor a better evolution of the blood biomarkers of rats in growth.

MATERIAL AND METHODS

Spirulina

It consists of *Spirulina platensis*. This cyanobacterium was supplied by the Spirulina Laboratory of Mé (south of Côte d'Ivoire) in powder form; the obtained amount was 400 grams. The minerals and vitamins composition of the alga is listed in the tables 1 and 2 according to Kambou et al (2016).

Rats

Albino rats of *Rattus norvegicus* species and strain Wistar, male and female weighing on mean 26.2 ± 2 g and four weeks old were used. The rats were obtained from the Physiology, Pharmacology and Pharmacopoeia Laboratory (LPPP) of the Natural Sciences Training and Research Unit (UFR-SN) at Nangui Abrogoua University (Abidjan, Côte d'Ivoire). They were acclimated in cages two weeks before the start of the experiment. During these two weeks, they received FACI® food and had unlimited access to water. They were subjected daily to an ambient temperature of 28°C, 12 hours of light and 12 hours of darkness. The different experimental protocols were followed in accordance with the experimental animal protection protocols of the European Council on Legislation 87/609/EEC (EU, 2010).

Administration of Spirulina doses and determination of blood parameters

The Spirulina powder (Spirulina platensis) obtained at SAP la Mé made it possible to prepare the different doses which should be administered to rats orally. So,

Table 2 : Vitamins composition of Spirulina platensis.

water-soluble vitamins	
Vitamin C (mg/100g)	46.80 ± 6,51
Vitamin B1 (mg/100g)	3.10 ± 0,70
Vitamin B2 (mg/100g)	5.60 ± 2,54
Vitamin B3 (mg/100g)	9.50 ± 1,83
Vitamin B5 (mg/100g)	0.87 ± 0,08
Vitamin B6 (mg/100g)	0.58 ± 0,01
Vitamin B8 (µg/100 g)	3.05 ± 1,34
Vitamin B9 (µg/100 g)	17.52 ± 4,72
Vitamin B12 (mg/100g)	0.01 ± 00
Liposoluble vitamins	
Beta-caroten (mg/100g)	55.50 ± 4.72
Vitamin A (µg/100 g)	1.00 ± 0.28
Vitamin E (mg/100g)	36.60 ± 4.99
Vitamin D3 (µg/100 g)	0.60 ± 0.14

we used distilled water as a solvent in which we added Spirulina powder. The preparation method has been described by Ouédraogo et al. (2020). The method of administration used during this study consisted of daily treatment of the animals by giving them all conventional FACI® food at a rate of 10% of their average body mass always at the same time, ie between 7 am 30 and 8:30 am and Spirulina as a food supplement for three months. For this, four homogeneous groups of six rats each, three of which males and three females, were formed. Group 1 (control) received only FACI® food and distilled water at a rate of 10 ml/kg body weight. As for groups 2, 3 and 4, they respectively received FACI® and Spirulina at the rate of 10; 50 and 100 mg/kg body weight. Food intake was assessed daily by determining the difference between the amount of food distributed and refusals using a precision digital scale (SF-400 and S-234 Neo-Tech SA, Belgium). Blood samples were taken on 28th and 70th days from fasted rats the previous evening and anesthetized with ether early in the morning from the orbital sinus using the technique of Jones and Mohr (1990). About 5 mL of blood was collected in EDTA and dry tubes. These latter tubes were centrifuged at 3000 rpm for 10 minutes then transported to the laboratory to measure a few serum parameters including lipids, proteins, ions, renal and hepatic markers by a semi - PLC (RAYTO RT 9200).

Statistical analysis

The results were expressed as means followed by the standard error (M ± SEM) and proportions (%). The evolution of the weight of the rats during the growth was evaluated by one way analyzes of variances (ANOVA1). This statistical test was combined with the Bonferroni test as a *post hoc* test. Statistical analysis of data in this context was performed using GraphPad Prism 5.01 software (San Diego, California, USA). In addition, the obtained proportions according to reference values for each chosen period of growth of the rats on the one hand and on the other hand, the proportions of variation of the main blood parameters during the growth of the rats, were compared by the test G. This test was carried out with the Windows R version 2.0.1 computer program (Ihaka and Gentleman, 1996). The significance threshold was set at a probability threshold p of less than 0.05 for the expression of results.

RESULTS

Weight change

After 28 days of treatment of groups of rats with different doses of Spirulina, control rats treated with FACI® food only experienced growth with a weight gain of 45.7 ± 1.89 g. Comparing this weight with those of Spirulina-treated rats, a non-significant increase ($P > 0.05$) was noted at all doses (10; 50 and 100 mg/kg bw) with values of 47.2 ± 2.44; 47.8 ± 4.62 and 47.5 ± 2.84 g, respectively. Thus, the 70-day extended experiment resulted in increased weights of rats in the constituted groups. The control rats had a weight gain of +54.48 ± 3.11 g from baseline (27.75 ± 1.36 g). Rats that received Spirulina in addition to the granules at 10 mg/kg bw, 50 mg/kg bw and 100 mg/kg bw experienced growth with a weight gain ranging from +57.14 ± 2.42 g (10 mg/kg bw) to +66.70 ± 1.35 g (100 mg/kg bw). In addition, all rats from the three Spirulina-treated lots showed a positive mean daily gain (ADG) compared to controls. The resulting ADGs were +0.78 ± 0.13 g/d (controls), +0.82 ± 0.20 g/d (10 mg/kg bw), +0.86 ± 0.28 g/d (50 mg/kg bw) and +0.95 ± 0.37 g/d (100 mg/kg), respectively (Table 3). In addition, after the first few days, all animals showed harmonious growth. In

Table 3. Weight evolution during rats growth.

Growth factors	Different Spirulina doses (mg/kg bw)				P
	0	10	50	100	
Initial weight (g)	27.75±1.36	25.35±1.07	25.70±1.97	26.10±1.51	> 0.05
Weight on day 28 (g)	45.7±1.89	47.2±2.44	47.8±4.62	47.5±2.84	> 0.05
Weight on 70th day (g)	82.23±12.35	82.49±8.21	86.15±6.68*	92.8±3.52**	< 0.01
GP (g)	+54.48±3.11	+57.14±2.42	+60.45±3.52*	+66.7±1.35**	< 0.01
GMQ (g/j)	+0.78±0.13	+0.82±0.2	+0.86±0.28*	+0.95±0.37**	< 0.01

*: p < 0,05; **: p < 0,01; ***: p < 0,001; DMG: Daily Mean gain, WG: Weight gain.

addition, rats reporting the highest growth were those in the 50 mg/kg bw and 100 mg/kg bw dose groups, while control rats showed the lowest growth. A significant difference (P < 0.05) was noted between the weights of the rats of the different lots at the end of the experiment. The weight gain (W.G.) values recorded ranged from 0.85 ± 0.13 g/d to 1.88 ± 0.18 g/d (Table 3).

Distribution of main haematological parameters proportions

The proportions of normal values presented by all erythrocyte parameters were 100% for all rats with and without Spirulina (Table 4). The study results indicated no anemia, hemodilution, microcytosis and macrocytosis in animals on day 28 of our investigation. No significant difference (P > 0.05) was noted between these proportions across all these red blood cell parameters. Similarly, white blood cells and platelets showed no overall abnormalities. The proportions of normal values were 100% for both treated and control rats (Table 4). After 70 days of treatment, the proportions of hemoglobin, hematocrit and MCV. However, approximately 17% of the rats in the batch supplemented with 10 mg/kg bw Spirulina showed hypochromia (Table 4). In addition, administration of Spirulina at doses ranging from 10 to 100 mg/kg bw resulted in 100% normal lymphocyte counts in the controls and the 10 and 100 mg/kg bw supplemented groups (Table 4). In this vein, no leukopenia, leukocytosis, lymphocytosis, granulocytopenia or thrombocytosis in any animal were recorded. However, 20% of the animals showed thrombocytopenia at 50 mg/kg bw.

Proportions of changes in hematological parameters

With regard to the proportions of variation in haematological parameters, there were three types of changes after the two experimental periods. In the first case of variations, the results showed that supplementation of the rats with Spirulina promoted an increase in the levels of red blood cells, hemoglobin, hematocrit and lymphocyte count.This increase in these parameters was significant at 100 mg/kg bw for red blood cells, hemoglobin and lymphocytes with levels of 57.32%, 18.83% and 34.59% respectively. The lowest levels were 26.48%, 0.39% and 9.52%, respectively (Table 5). In the second category of changes, a decrease over time under the influence of Spirulina was observed for MCV, MCH, monocytes, granulocytes and platelets. Except for platelets which showed the greatest decrease at 50 mg/kg bw, all other parameters showed the greatest rate of decrease at 100 mg/kg bw of Spirulina. The highest rates of decrease were -22.55%, -35.12%, -38.61%, and -86.58% for MCV, MCH, monocytes, and granulocytes, respectively. However, the smallest decreases were scattered; for MCV, MCH, and platelets, the low levels were -16.84%, -20.78%, and -27.37% for controls, respectively. The lowest monocyte level was recorded at 10 mg/kg bw while granulocyte levels were obtained at 50 mg/kg bw. The latter type of variation is represented by the parameters that underwent increases and/or decreases. These were white blood cells and MHC. Thus, for MCH, all groups showed a decrease in their levels during growth with the exception of the lot supplemented at 100 mg/kg bw where an increase of 4.39% was recorded. For white blood cells, the control and 10 mg/kg bw batches showed an increase of 16.1% and 12.16% respectively. At 50 and 100 mg/kg bw, a decrease was noted with values of -17.46% and -30.46%, respectively (Table 5).

Repartition of the main biochemical markers proportions

Blood glucose, protein and lipids

All rats treated or not treated with Spirulina showed results at day 28, fully compliant with blood glucose, A/G ratio, triglycerides and total cholesterol. With regard to total protein, only the control lot showed hypoproteinemia at the proportion of 16.67% (Table 5). Globulin levels were all above normal in treated rats. On the other hand, albumin levels showed normal

Table 4. Proportion of main haematological parameters during rats growth.

Paramètres biologiques	Different Spirulina doses (mg/kg bw)/28th day					Different Spirulina doses (mg/kg bw)/70th day				
	0	10	50	100	P	0	10	50	100	P
Hemoglobin (g/dL)										
Low	0	0	0	0	--	0	0	0	0	--
Normal	100	100	100	100	1	100	100	100	100	1
High	0	0	0	0	--	0	0	0	0	--
Hematocrit (%)										
Low	0	0	0	0	--	0	0	0	0	--
Normal	100	100	100	100	1	100	100	100	100	1
High	0	0	0	0	--	0	0	0	0	--
MCV										
Low	0	0	0	0	--	0	0	0	0	--
Normal	100	100	100	100	1	100	100	100	100	1
High	0	0	0	0	--	0	0	0	0	--
MCH										
Low and High	0	0	0	0	--	0	16.67	20	0	> 0.05
Normal	100	100	100	100	1	100	83.33	80	100	> 0.05
White blood cell (10^3/µL)										
Low	0	0	0	0	> 0.05	0	0	0	0	--
Normal	100	100	100	100	> 0.05	100	100	100	100	1
High	0	0	0	0	--	0	0	0	0	--
Lymphocyes (10^3/µL)										
Low	0	0	0	0	--	0	0	0	0	--
Normal	100	100	100	100	1	100	100	100	100	1
High	0	0	0	0	--	0	0	0	0	--
Monocytes (10^3/µL)										
Low	0	0	0	0	--	0	0	0	0	--
Normal	100	100	100	100	1	100	100	100	100	1
High	0	0	0	0	--	0	0	0	0	--
Granulocytes (10^3/µL)										
Low	0	0	0	0	--	0	0	0	0	--
Normal	100	100	100	100	1	100	100	100	100	1
High	0	0	0	0	--	0	0	0	0	--
Platelets (10^9/L)										
Low	0	0	0	0	--	0	0	20	20	> 0.05
Normal	100	100	100	100	1	100	100	80	80	> 0.05
High	0	0	0	0	--	0	0	0	0	--

values in the range of 83% to 100% at all doses and also in the control rats. In other words, there was no hypoglycemia or hyperglycemia, no hypotriglyceridemia or hypertriglyceridemia. Similarly, no cases of hypocholesterolemia and hypercholesterolemia have been reported. In contrast, hypoproteinemia and hypoalbuminemia occurred in the control rats and at a dose of 10 mg/kg bw supplemented group for 16.67%. Hyperglobulinemia was recorded in virtually all but 16.67% of the control rats. All the rats used in this experiment did not evoke hypoglycemia at the 70th, let alone hyperglycemia. Similarly, there was no hypoglobulinemia or hyperglobulinemia, no hypotriglyceridemia and hypertriglyceridemia and finally, no hypocholesterolemia and hypercholesterolemia were mentioned. In terms of total protein, only the lot supplemented at 50 mg/kg bw showed a hyperproteinemia of 16.67%. With respect to albumin levels and A/G ratio, almost all values obtained were below the norm resulting in hypoalbuminemia

Table 5. Proportion of variations in hematological parameters.

Hematological parameters	Different Spirulina doses (mg/kg bw)				
	0	10	50	100	P
Red blood cell (10^{12}/L)	49.9	34.15	26.48	57.32	< 0.01
Hemoglobin (g/dL)	4.7	0.39	16.59	18.83	< 0.001
Hematocrit (%)	0.89	44.44	1.09	18.83	< 0.001
MCV (fL)	- 16.84	- 20.57	-18.74	-22.55	> 0.05
MCH (pg)	-20.78	- 26.85	- 27.36	- 35.12	> 0.05
MCHC (g/dL)	- 9.46	- 5.02	- 0.71	4.39	< 0.01
White blood cell (10^9/L)	16.10	12.16	- 17.46	- 30.46	< 0.05
Lymphocytes (10^9/L)	9.52	20.78	16.74	34.59	< 0.001
Monocytes (10^9/L)	- 10.14	- 8.10	- 38.23	- 38.61	< 0.001
Granulocytes (10^9/L)	- 48.07	- 75.29	- 22.14	- 86.58	< 0.001
Platelets (10^9/L)	- 27.37	- 35.65	- 63.54	- 49.53	< 0.001

except for 16.67% of the rats in the lot supplemented at 10 mg/kg bw (Table 5).

Ionogram, renal and hepatic blood parameters

When the rats were subjected to the different doses of Spirulina, this did not induce any abnormality at day 28 in the ion levels. All values recorded were in accordance with the reference values. Similarly, the transaminases (ALAT and ASAT) showed values equivalent to the standards at all doses and also in the control lot (Table 5). According to the proportions recorded on day 70, all values obtained for both ions and transaminases (ASAT and ALAT) complied with the reference values (Table 6).

Proportions of variation in blood biochemical parameters

The use of Spirulina at doses ranging from 10 to 100 mg/kg bw from day 28 to day 70 resulted in a decrease in blood glucose, triglycerides, total and LDL cholesterols and the atherogenicity index calculated on the LDL/HDL ratio. These decreases were observed at all doses with very large proportions for total and LDL cholesterols and atherogenicity indices. And the lowest rates of decreases were obtained for triglycerides and blood glucose (Table 7). With respect to the dose-dependent proportions of Spirulina administered, the highest decreases were recorded at 50 mg/kg bw for total and LDL cholesterols. The decrease in triglycerides was largest at the 10 mg/kg dose, while blood glucose was further reduced at the 100 mg/kg dose. As for the various proteins, they were increased over time as a function of the Spirulina doses received. Thus, an increase occurred at all doses for total proteins with the highest rate of 9.36% at 100 mg/kg bw and the lowest at 10 mg/kg bw contrary to the controls who rather observed a decrease. Globulins also showed significant rates of increase at 100 mg/kg bw with 14.37% followed by rats supplemented at 50 mg/kg bw with 5.64%. However, these globulin values were decreased during growth in the controls and the group of rats given 10 mg/kg bw Spirulina. With respect to albumin and A/G ratio, the increase was greatest at 50 mg/kg bw with 19.95% and 13.73% respectively. However, there were decreases at the 100 mg/kg bw dose with the respective rates of -2.04 and -13.64%. The doses received from Spirulina resulted in increases in blood ions with the exception of calcium which was decreased at doses of 50 and 100 mg/kg bw with a higher decrease at 50 mg/kg bw. The increases observed were greatest at 50 mg/kg bw for potassium and 100 mg/kg bw for sodium and chlorine (Table 8). In contrast, renal and hepatic parameters were all decreased at all doses except for conjugated bilirubin and urea, which were not decreased at 10 and 50 mg/kg bw, respectively. AST and total bilirubin expressed a significant decrease at 50 mg/kg bw while creatinine was further reduced at 100 mg/kg bw. The decrease in urea growth and ALT was greatest at 10 mg/kg bw.

DISCUSSION

Evolution of weight and haematological parameters during growth

The administration of Spirulina in addition to the pellets over a period of three months to rats resulted in a harmonious growth in treated animals. This growth could be an indication of the good health of the animals, reflecting an absence of nutritional dysfunction that would be corrected by the intake of Spirulina. Moreover, the significant observation of weight gain in rats treated at day 70 with the doses of 50 and 100 mg/kg bw, suggests that Spirulina does not disturb the weight gain of treated animals. Moreover, Spirulina induced the best weight gain at 100 mg/kg bw. These results are

Table 6. Proportion of glucose, proteins and lipids.

Biochemical blood parameters	Different Spirulina doses (mg/kg bw) /28th day					Different Spirulina doses (mg/kg bw)/70th day				
	0	10	50	100	P	0	10	50	100	P
Glucose (g/L)										
Low	0	0	0	0	--	0	0	0	0	--
Normal	100	100	100	100	1	100	100	100	100	1
High	0	0	0	0	--	0	0	0	0	--
Total proteins (g/L)										
Low	16.67	0	0	0	> 0.05	0	0	0	0	--
Normal	83.33	100	100	100	> 0.05	100	100	83.33	100	> 0.05
High	0	0	0	0	--	0	0	16.67	0	> 0.05
Albumin (g/L)										
Low	16.67	16.67	0	0	> 0.05	0	83.33	100	100	> 0.05
Normal	83.33	83.33	100	100	> 0.05	100	16.67	0	0	> 0.05
High	0	0	0	0	--	0	0	0	0	--
Globulin (g/L)										
Low	0	0	0	0	--	0	0	0	0	--
Normal	16.67	0	0	0	> 0.05	100	100	100	100	1
High	83.33	100	100	100	> 0.05	0	0	0	0	--
A/G (g/L)										
Low	100	100	100	100	1	83.33	100	100	100	> 0.05
Normal	0	0	0	0	--	16.67	0	0	0	> 0.05
High	0	0	0	0	--	0	0	0	0	--
Triglycérides (g/L)										
Low	0	0	0	0	--	0	0	0	0	--
Normal	100	100	100	100	1	100	100	100	100	1
High	0	0	0	0	--	0	0	0	0	--
Total cholesterol (g/L)										
Low	0	0	0	0	--	0	0	0	0	--
Normal	100	100	100	100	1	100	100	100	100	1
High	0	0	0	0	--	0	0	0	0	--

consistent with those obtained by Degbey et al. (2006); Simpore et al. (2006). These authors evaluated the nutritional performance of Spirulina on severely malnourished children in Burkina Faso and Senegal. In all situations, the subjects experienced significant weight gain. However, they do not correspond to those found by Branger et al. (2003); who showed that Spirulina does not bring any benefit compared to traditional nutrition. With regard to the influence of Spirulina on hematological parameters, no abnormalities were identified on either day 28 or day 70. However, 70 days after administration of the various doses, the levels of MCH and blood platelets revealed disturbances at doses of 50 and 100 mg/kg bw in the range of 15-20%. This observed failure could be due to injury at the time of sampling or infection that was facilitated by the injuries. This failure may also be a function of the body's defence against microbes and other foreign materials following tissue damage caused by sampling. These findings are consistent with those of Deng et al. (2010). The normality observed at 100% on the 28th day of treatment and more than 80% on the 70th day was made possible by taking Spirulina. Indeed, Spirulina acts directly and indirectly on the proliferation and differentiation of multipotent bone marrow stem cells (Schwartz et al., 1987). It stimulates hematopoiesis, and especially erythropoiesis by inducing the release of erythropoietin (EPO) (Mathew et al., 1995). Maintaining hemoglobin levels within the normal range due to Spirulina would promise its use not only in pathologies that cause anemia such as malaria in southern countries but also in people on antiretroviral therapy (ART) drugs such as zidovudine (AZT) (WHO, 2010). These results are in agreement with those of Kambou et al. (2015) who obtained a significant increase of red blood cells and hemoglobin levels in

Table 7. Proportion of ionogram, renal and hepatic blood parameters.

Biochemical blood parameters	Different Spirulina doses (mg/kg bw) /28th day				P	Different Spirulina doses (mg/kg bw)/70th day				P
	0	10	50	100		0	10	50	100	
Calcium (mg/dL)										
Low	0	0	0	0	--	0	0	0	0	--
Normal	100	100	100	100	1	100	100	100	100	1
High	1	1	1	1	--	1	1	1	1	--
Sodium (mmol/L)										
Low	0	0	0	0	--	0	0	0	0	--
Normal	100	100	100	100	1	100	100	100	100	1
High	1	1	1	1	--	1	1	1	1	--
Potassium (mmol/L)										
Low	0	0	0	0	--	0	0	0	0	--
Normal	100	100	100	100	1	100	100	100	100	1
High	1	1	1	1	--	1	1	1	1	--
Chlorine (mmol/L)										
Low	0	0	0	0	--	0	0	0	0	--
Normal	100	100	100	100	1	100	100	100	100	1
High	1	1	1	1	--	1	1	1	1	--
ASAT (UI/L)										
Low	0	0	0	0	--	0	0	0	0	--
Normal	100	100	100	100	1	100	100	100	100	1
High	1	1	1	1	--	1	1	1	1	--
ALAT (UI/L)										
Low	0	0	0	0	--	0	0	0	0	--
Normal	100	100	100	100	1	100	100	100	100	1
High	1	1	1	1	--	1	1	1	1	--

rabbits treated with Spirulina while being in line with the reference values. No abnormalities in leukocytes were recorded following the intake of Spirulina at all doses on days 28 and 70. The results obtained did not show any leukopenia or leukocytosis at the reference values. Indeed, the maintenance of leukocyte levels and white blood cell counts in rats treated in accordance with the reference values would be attributable to the immune system activity of Spirulina as reported by Mathew et al. (1995). In view of these results, it is possible to think that Spirulina in this study does not disturb the hematopoietic system by inducing leukopenia, thrombocytopenia and anemia. Likewise, we did not record any polyglobulism, leukocytosis, thrombocytosis or hypochromia. The immunostimulant and nutritional effects of Spirulina would be beneficial for laboratory rats that often experience infections and nutritional imbalance.

However, the abnormalities observed in some rats during the growth period could be due to factors other than nutritional factors including stress, sample-related infections, environmental parameters.

Variation in blood biochemical parameters

The analysis of the blood biochemical parameters of the rats made it possible to estimate the performances related to the consumption of Spirulina (*Spirulina platensis*) and to see if possible abnormalities would have occurred. In general, the results of this work showed that blood glucose levels in the groups of rats treated with Spirulina were in line with the reference values. Indeed, these results indicated a control of Spirulina on the blood glucose level by keeping it within the normal range. Concerning certain compounds with which it abounds, in particular linoleic acid which could participate in the improvement of nervous complications and lipid levels associated with diabetes. These results are consistent with those of Huang (2005). According to him, Spirulina decreases glycemia, total cholesterol and triglycerides in diabetic animals. Similarly, some authors consider Spirulina as a therapeutic source with numerous properties: antioxidant, anti-inflammatory, anti-anemic, anti-diabetic, anti-atherogenic (Thang et al., 2015; Wang et al., 2007). Cholesterol and

Table 8. Proportions of variations in biochemical blood parameters.

Biochemical blood parameters		Different Spirulina doses (mg/kg bw)				P
		0	10	50	100	
Glucose (g/L)		1.94	- 1.94	- 2.17	- 5.49	< 0.05
Total proteins (g/L)		- 2.03	1.29	10.47	9.36	< 0.001
Albumin (g/L)		5.45	8.99	19.95	- 2.04	< 0.001
Globulin (g/L)		- 7.66	- 3.88	5.64	14.37	< 0.05
A/G		14.67	13.43	13.73	- 13.64	> 0.05
Triglycerides (g/L)		0	- 7.5	- 5.8	- 3.17	< 0.05
Total cholesterol (g/L)		- 1.37	- 13.43	- 18.75	- 6.12	< 0.001
HDL cholesterol (g/L)		5.26	5	0	- 5.88	< 0.05
LDL cholesterol (g/L)		- 16.28	- 16.67	- 26.83	- 23.53	> 0.05
Atherogenicity Indices	ChT/HDL	- 6.25	- 17.61	- 17.43	0	< 0,001
	LDL/HDL	- 51.13	- 23.72	- 26.75	- 17.74	< 0,001
Total bilirubin (g/L)		- 9.54	- 12.05	- 15.67	- 10.3	> 0.05
Conjugated bilirubin (g/L)		- 5	5.88	- 14.28	- 9.68	> 0.05
Creatinine (mg/L)		- 8.38	- 9.66	- 5.21	- 9.88	> 0.05
Urea (g/L)		- 6.67	- 13.33	0	- 9.09	< 0.001
Ca^{2+} (mg/dL)		- 0.54	4.94	- 4	- 2.75	> 0.05
K^{+}(mmol/L)		- 3.67	4.05	4.13	2.93	> 0.05
Na^{+} (mmol/L)		3.52	1.7	1.88	2.98	> 0.05
Cl^{-} (mmol/L)		0.32	1.08	2.85	3.67	> 0.05
ASAT (UI/L)		0.99	- 1.08	- 7.85	- 10.1	< 0.01
ALAT (UI/L)		- 6.18	- 3.46	- 3.6	- 3.6	> 0.05

triglycerides are produced in fairly high proportions in the liver to supply the entire body (Thibaut, 2012). Their significant increase could be linked to excess weight. In this study, therefore, these lipid markers presented levels in line with reference values. This could be due to the role of Spirulina in the regulation of lipidemia as confirmed by Chamorro et al. (2002). These results are also in agreement with the studies of Luciane et al. (2008) which showed that *Spirulina platensis* induces a significant decrease in total cholesterol levels in rabbits subjected to a hypercholesterolemic diet. Similarly, Ponce-Canchihuamán et al. (2010) reported that *Spirulina maxima*, another form of Spirulina, prevented the significant lead acetate-induced changes in plasma and liver lipid levels and the antioxidant status of the liver and kidneys in male rats. However, Spirulina maxima succeeded in improving the biochemical parameters of the liver and kidneys towards the normal values of the control group. These beneficial effects could be justified by certain components of Spirulina itself. Indeed, Spirulina is composed of omega-3 and omega-6 fatty acids, beta-carotene, alpha tocopherol, phycocyanin, phenols and certain minerals which are substances reputed to regulate lipidemia. Spirulina would, therefore, be a substance capable of regulating the lipid level and lipid indices according to standards and could have a protective effect on the cardiovascular system. These results are in agreement with those of Nayaka et al. (1988) who demonstrated the capacity of Spirulina to lower serum cholesterol and triglyceride levels in adult men. In addition, the evaluation of transaminases (ALAT and ASAT), which are enzymes with significant metabolic activity within liver cells, has allowed the exploration of liver functions during growth. According to Najafi et al. (2012), the increase in these enzymes is mainly due to leakage through the hepatic cytosol and discharge into the bloodstream (Saba, 2010; Najafi et al., 2012). Also, increases in ASAT are associated with myocardial infarction and liver damage and increases in ALAT are associated with liver damage only (Ndouyang et al., 2018). ALAT and AST levels rise rapidly when the liver is damaged for a variety of reasons including hepatic cell necrosis, cirrhosis, and hepatotoxicity of certain substances (Dufour *et al*., 2000; Pratt and Kaplan, 2000). This study showed a decrease in transaminases while maintaining them in the reference range, thus reflecting the proper functioning of the liver of rats subjected to Spirulina. These results are close to the work of Mazokopakis et al. (2014) when they highlighted the lipid-lowering effect of Spirulina use on hepatic stenosis in adult dyslipidemic patients. These results are also consistent with those given by El-Bialy et al. (2016) when they evaluated the antioxidant effects of Spirulina in male mice where it was found that the alga normalizes the concentrations of transaminases.

In addition to transaminases, bilirubin allows exploration of liver functions. Bilirubin is a bile pigment formed from the degradation of hemoglobin but also other hemoproteins. It is then captured by the liver (conjugated or direct bilirubin) and degraded. It helps to diagnose causes of certain pathologies and

dysfunctions of the organism such as anemia and liver diseases. According to Sutan et al. (1991), the level of conjugated bilirubin is increased in hepatic and biliary diseases, including different types of hepatitis, rare metabolic abnormalities, biliary disorders, biliary lithiasis, pancreatitis, pancreatic or biliary cancer. Its dosage allows the elimination of bile through the bile ducts (Borenstein et al., 2006). Concerning the bilirubin levels, the results obtained did not reveal any abnormal decrease despite the significant decrease in these parameters. It is reported that hemolysis of red blood cells releases heme which is transformed into biliverdin and then bilirubin (Silbernagl and Lang, 2000). It should, therefore, be noted that, following administration of Spirulina, there was no hemolysis which could be the cause of the increase in bilirubin levels. These results show that the daily use of Spirulina does not lead to organ deterioration in relation to the conditions caused. Creatinine and urea are used to assess kidney function. The blood level of creatinine is a better indicator of kidney function. Higher creatinine levels indicate a decrease in glomerular filtration rate and therefore a decrease in the kidneys' ability to excrete waste products (Gbakon et al., 2018). Also, serum calcium, sodium, chlorine and potassium levels showed good renal secretion compared to the nearly stable values obtained following Spirulina administration on day 28 and 70. Sodium, potassium and chlorine ions are very important in the maintenance of osmotic pressure and water movement in the body and the acid-base balance (Dieusaert, 2015). These results confirm the idea that Spirulina is a hepatoprotective substance and reduces nephrotoxicity as suggested by Sami et al. (2016) and Mao et al. (2005). However, our results contradict those of Mazokopakis et al. (2008) who noted adverse hepatic, dermatological, digestive, hematological, renal and electrolyte disorders in subjects in Japan.

Conclusion

The administration of Spirulina as a dietary supplement to rats in addition to the conventional pelleted feed resulted in positive growth with positive mean gains over controls. Also, the hematopoietic system was not disturbed. Moreover, the alga allowed a good improvement of the hematological parameters, in particular, the levels of red blood cells, hemoglobin and also of the white lineage during the whole period of experimentation, from the 28th to the 70th day. The effects of Spirulina were also noted with the erythrocyte and leucocyte indices. It emerged that the action of Spirulina on the hematopoietic system, in general, was direct and indirect on totipotent stem cells particularly at the dose of 100 mg/kg bw. The nutritional effects of Spirulina were also determined through biochemical blood parameters which helped to confirm its intake in vital organs. Thus, blood glucose was well regulated and lipid markers were reasonably reduced. At the vital functions level, the transaminases ASAT and ALAT behaved better, the hepatic markers (total and conjugated bilirubin) were well regulated and finally, the renal markers also decreased like the lipids compared to the controls at 50 and 100 mg/kg with an accentuated effect for the latter dose.

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