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Effects of Spirulina on the Weight and Hematological Parameters of Rats

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Abstract **Original Research Article**

The aim of this study was to evaluate the nutritional potential of Spirulina in rats growth and haematological parameters. The study took place along two months. To do this, four batches of six rats were created, with three males and three females in each batch. Batch 1, the control, received conventional pellets and distilled water at 10 ml/kg body weight, while batches 2, 3 and 4 received conventional pellets and Spirulina at 10 (batch 2), 50 (batch 3) and 100 mg/kg body weight (batch 4) respectively. Analysis of the samples taken showed an increase in the weight of the treated animals. Haematological parameters were undisturbed throughout the study. Better still, these haematological parameters improved significantly and the best results were seen in rats given Spirulina at a dose of 100 mg/kg bw.

Keywords: Spirulina, conventional food, weight gain, haematological parameters, rat.

INTRODUCTION

Malnutrition is a scourge that is rife in almost all developing countries and takes a variety forms. The most obvious cases of malnutrition in these regions are undernutrition and protein-energy deficiency. According to the United Nations (UN) report on the state of food security and nutrition in the world in 2022, nearly one person in eight, or a total of 924 million people, said they suffered from severe food insecurity in 2021. This proportion was more than one in four in Africa (322 million people) and around one in ten in Asia (489 million people). In Africa, 40% of people who were severely food insecure in 2021 were from East Africa, while the situation worsened in all sub-regions of the continent, with the most rapid increase occurring in West Africa (an almost 2.5-fold increase in their numbers since 2015) (SOFI, 2022).

To remedy this, international structures such as WHO (World Health Food and Agriculture Organization) and FAO (Food and Agriculture Organization) have proposed solutions based on the nutritional recovery of children suffering from severe malnutrition, supplementary feeding and food fortification. They also asked researchers to re-examine humanity's nutritional potential. It was in response to this call that we turned our attention to Spirulina. Spirulina is a blue-green micro-algae known for its numerous health benefits. It is rich in essential nutrients such as protein (up to 70% highly digestible protein), vitamins, minerals and antioxidants. As a result, it is attracting growing interest in the field of nutrition and biomedical research (Salmean *et al.*, 2015). Spirulina has been proposed for human consumption by a number of scientists and nutritionists thanks to its exceptional nutritional qualities, ease of cultivation, high productivity and low production costs (Kent, 2015).

This study focuses specifically on the effects of Spirulina on the weight and haematological parameters of rats. Weight is a key indicator of an individual's state of health, while haematological parameters reflect the composition of the blood, including levels of red blood cells, white blood cells and haemoglobin.

MATERIAL AND METHODS

Food

Spirulina (*Spirulina platensis*) was used in the feed; it is a cyanobacteria that was supplied by the Spiruline Laboratory in Mé (southern Côte d'Ivoire) in powder form. Spirulina was added as a supplement to the granules. FACI®.

Animal material

The rats used in these experiments were male and female albino white rats of the species *Rattus norvegicus* and the Wistar strain. The rats were approximately 35 days old and had an average body mass of 47.1±0.46 g. They were acclimatised in cages for 11 days before the start of the experiment at the Laboratoire de Physiologie, Pharmacologie et Pharmacopée (LPPP) of the Unité de Formation et de Recherche des Sciences de la Nature (UFR-SN) at Nangui Université Abrogoua (Abidjan, Côte d'Ivoire). During this period, all the animals were fed FACI® brand industrial pellets ad libithum and water ad libitum. The various experimental protocols were followed in accordance with the animal protection protocols of the European Council legislation. 87/609/CEE (UE, 2010).

Table 1: Average weight of rats at the start of experiment

Rats batches	Average initial weight (g)
Batch 1	45,7±1,89
Batch 2	47,2±2,44
Batch 3	47,8±4,62
Batch 4	47,5±2,84

Methods

During the experiment, the treated rats were given different doses of Spirulina, in addition to the pellets, for two months after the acclimatisation phase. The daily ration distributed was the equivalent of 10% of body weight of pelleted food. Feed was distributed once a day between 07:30 and 08:30 in the morning. Water was served in bottles in the morning while the feed was being distributed. It was renewed every two days. Four batches of six rats were created, each containing three males and three females. Lot 1, the control lot, received FACI pellets and distilled water at a rate of 10 ml/kg body weight. The other batches, 2, 3 and 4, received FACI granules and Spirulina at 10 (batch 2), 50 (batch 3) and 100 mg/kg body weight (batch 4) respectively. Feed intake was assessed daily by determining the difference between the amount of feed given and the amount refused using a scale. As for growth monitoring, we weighed the animals individually twice a week. These weights were used to determine the average daily gain (ADG). Blood samples were taken according to the experimental protocol used by Weiss *et al*., (2000), et modified by Descat (2002). Blood was collected by puncture from the retroorbital sinus in rats. Heparinised pasteur pipettes were used, given the quantity to be collected. The operation was carried out under anaesthetic. The volume of blood collected was 0.5 ml, collected in EDTA tubes for blood count.

Statistical Analyses

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. The significance threshold was set at a probability threshold p of less than 0.05 for the expression of results.

RESULTS

Effects of Spirulina on rat growth

After one month of treatment, the control rats reached a weight of 66.45 ± 2.08 g. When this weight was compared with that of the Spirulina-treated rats, a non-significant increase ($P > 0.05$) was noted at doses of 50 and 100 mg/kg. After two months' follow-up, the weights of rats in all groups were significantly increased. Rats in the control group gained 54.48 ± 3.11 g from their initial weight (45.7 ± 1.89 g). Rats given Spirulina pellets at doses of 10 mg/kg, 50 mg/kg and 100 mg/kg bw grew with weight gains of +57.14 ± 2.42 g (10 mg/kg), +60.45 ± 3.52 g (50 mg/kg) and +66.7 ± 1.35 g (100 mg/kg), respectively. The weight gains of rats given 50 mg/kg and 100 mg/kg were significantly greater than those of controls. Similarly, the mean daily gains (MDG) of rats in the 50 mg/kg bw and 100 mg/kg bw dose groups were significantly greater ($P \leq 0.01$) than those of control rats. All these results are reported in Table 2.

Effect of Spirulina on erythrocytes and erythrocyte indices in rats

The influence of Spirulina on red blood cell levels and erythrocyte indices in rats is shown in Figure 1.

During the growth phase of the rats, administration of Spirulina resulted in a significant increase in red blood cell levels in the second month, at all doses, compared with blood sampling in the first month, except at 50 mg/kg bw.

This increase was highly significant ($P < 0.01$) in control rats, significant ($P < 0.05$) at 10 mg/kg and very highly significant ($P < 0.001$) at 100 mg/kg bw (Figure 1-A). In addition, Spirulina administration induced a significant ($P \leq 0.05$) increase in haemoglobin at 100 mg/kg and haematocrit at 10 mg/kg in the second month compared with sampling in the first month. In contrast, VGM decreased in the second month of dosing compared with the first month in all rats. This decrease was highly significant ($P < 0.001$) at all doses and also in the controls, with a greater decrease at 100 mg/kg. Similarly, significant decreases in MCHT were observed at all doses except in control rats. These decreases were significant ($P < 0.05$) at 10 mg/kg, highly significant ($P < 0.01$) at 50 mg/kg and very highly significant ($P < 0.001$) at 100 mg/kg bw from the first month to sampling at the second month (Figure 1-E and 1-F). There was no significant difference in CCMH from month 1 to month 2 sampling (Figure 1-F).

Effect of Spirulina on white blood cells and platelets in rats

Administration of Spirulina to rats at a dose of 100 mg/kg bw induced a highly significant (P < 0.01) increase in the white blood cell count at the second month compared with the first month of sampling. The other doses showed no significant difference at the same time (Figure 2-A) in rats. Lymphocyte levels in rats at month 2 sampling were elevated at all dose levels compared with month 1. This increase was non-significant (P > 0.05) in control rats and at 50 mg/kg, and highly significant (P < 0.01) and very highly significant (P < 0.001) at 10 and 100 mg/kg bw, respectively. Conversely, monocyte levels were very highly significantly (P < 0.001) reduced in the second month compared with the first month at 50 and 100 mg/kg (Figure 2-B and 2-C). Like monocytes, platelet levels were decreased in all rats in the batches. These decreases were insignificant in controls, highly significant (P < 0.01) at 10 mg/kg and very highly significant (P < 0.001) at 50 and 100 mg/kg bw (Figure 2-E). Similarly, granulocytes decreased significantly at all doses, including controls. These decreases were highly significant (P < 0.01) for controls, significant (P < 0.05) at 50 mg/kg and very highly significant (P < 0.001) for doses of 10 and 100 mg/kg bw (figure 2-D).

Table 2: Weight changes during rat growth

Growth factors	Batch 1	Doses of Spirulina (mg/kg body weight)		
		10	50	100
Poids initial (g)	45,7±1,89	47,2±2,44	47,8±4,62	47,5±2,84
Poids au mois 1 (g)	66,45±2,08	63,5±2,41	70,1±1,33	72,4±2,84
Poids au mois 2 (g)	100,18±4,35	104,34±8,21	108,25±3,68*	114,2±3,52**
GP moyen (g)	+54,48±3,11	+57,14±2,42	+60,45±3,52*	+66,7±1,35**
GMQ (g/j)	+0,78±0,13	+0,82±0,2	+0,86±0,28*	+0,95±0,37**

*: p < 0,05; **: p < 0,01; ***: p < 0,001. GMQ: Gain moyen quotidien, GP: Gain pondéral

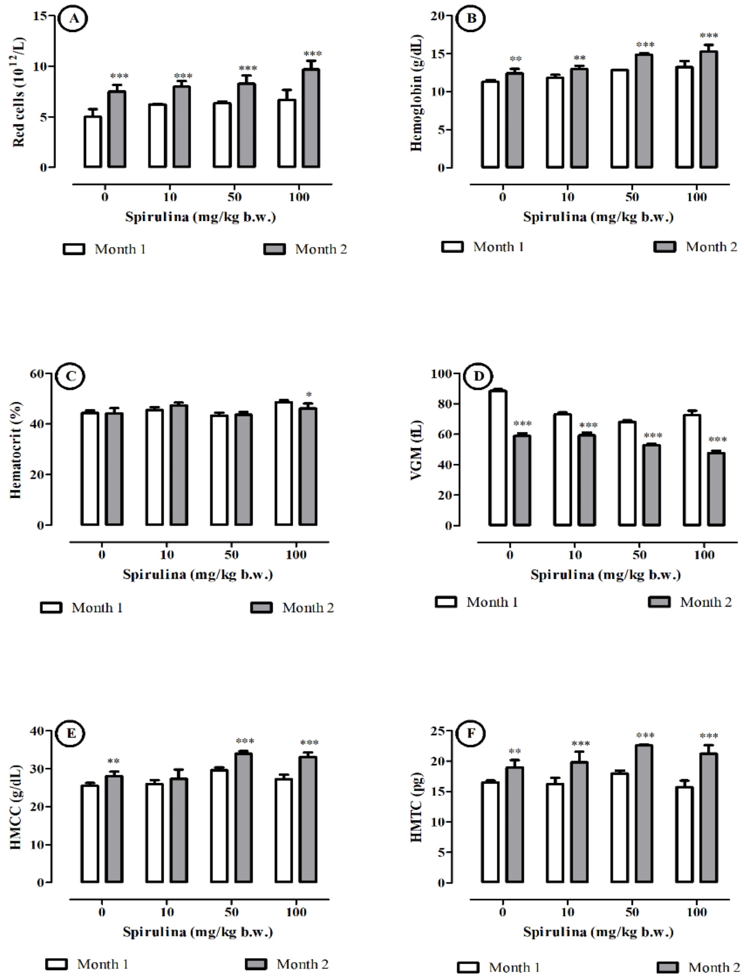

Figure 1: Evolution of red blood cell levels and erythrocyte indices during rat growth
A: Red cells, B: Hemoglobin, C: Hematocrit, D: VGM, E: HMCT, F: HMCC; *: p < 0,05 ; **: p < 0,01; *** p < 0,001. TG: Pellet-fed controls.

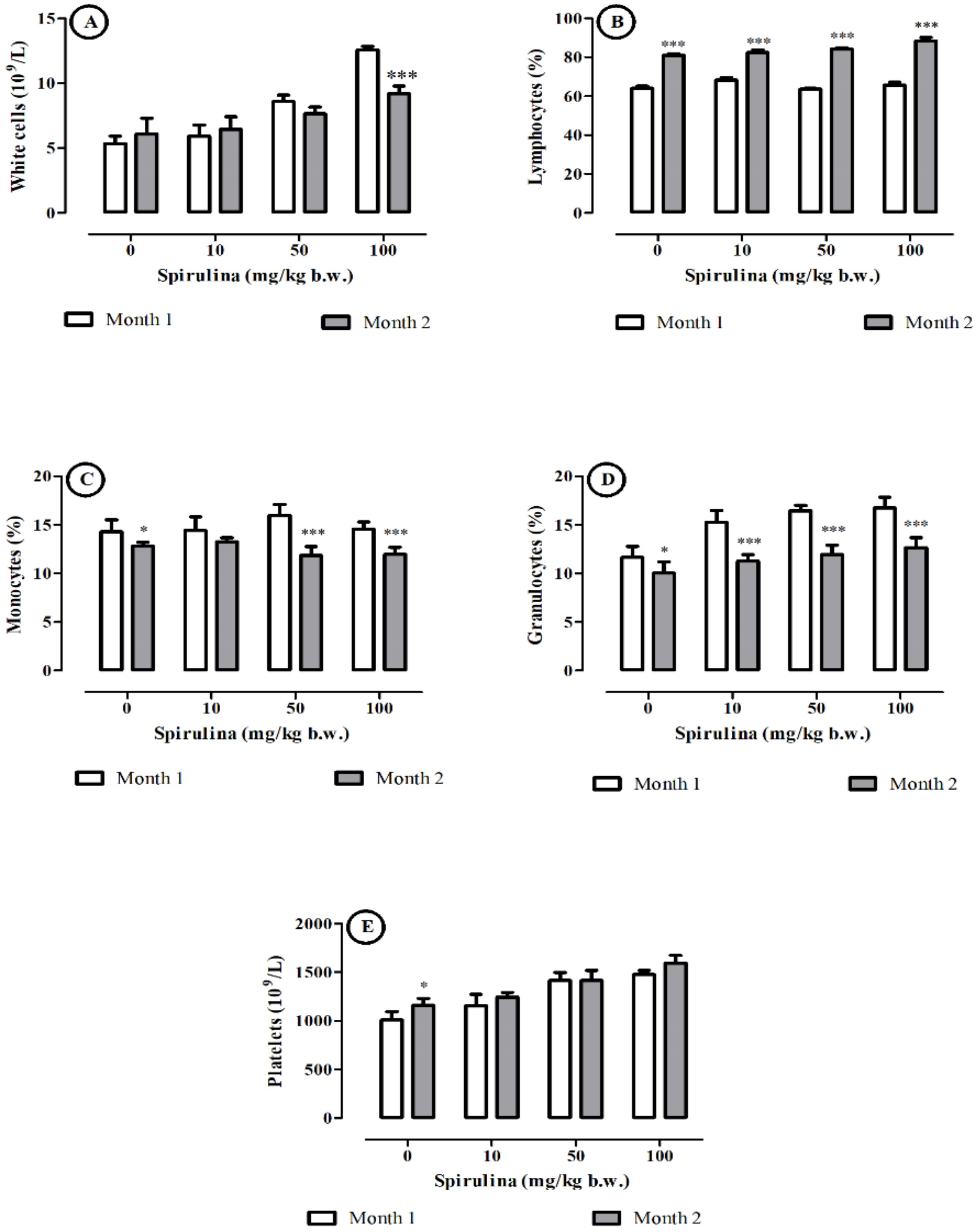

Figure 2: Evolution of leukocyte and platelet during rats growth
A: Whites cells; B: Lymphocytes; C: Monocytes; D: Granulocytes; E: Platelets
*: $p < 0,05$; ** : $p < 0,01$; *** $p < 0,001$. TG: Pellet-fed controls

DISCUSSION

The aim of this study was to determine and evaluate the nutritional performance of Spirulina (Spirulina platensis) produced in Côte d'Ivoire on growth and haematological parameters in wistar rats (Rattus norvegicus).

The administration of Spirulina in addition to granules over a period of two months to the rats resulted in harmonious growth in the treated animals. This growth could be an indication of the good health of the rats, reflecting an absence of nutritional dysfunction that would be corrected by the intake of Spirulina, especially as this algae allows nutritional recovery. Furthermore, the significant observation of weight gain in rats gavaged at the second month with doses of 50 and 100 mg/kg bw suggests that Spirulina does not interfere with weight gain in treated animals. Furthermore, it was at the 100 mg/kg dose and after two months' administration that Spirulina produced the best weight gain. This can be explained by Spirulina's richness in highly bioavailable protein. In fact, Spirulina proteins are complete, containing all the essential amino acids and representing 47% of the total weight of proteins (Manet, 2016). These results are in line with those obtained by Simpore *et al*., (2006). These authors evaluated the nutritional performance of Spirulina on severely malnourished children in Burkina Faso. In all situations, the subjects showed significant weight gain.

As regards the effect of Spirulina intake on haematological parameters, an increase in erythrocyte levels at doses of 50 and 100 mg/kg bw, haemoglobin and haematocrit levels at all doses compared with controls was observed in treated rats. This increase in erythrocyte, haemoglobin and haematocrit levels, which occurred in both the first and second months of sampling, is thought to be due to the presence of Spirulina in the rat diet. For these two parameters, Spirulina showed significant rates of improvement at a dose of 100 mg/kg and after two months' treatment. Spirulina acts directly and indirectly on the proliferation and differentiation of multipotent stem cells in the bone marrow. It stimulates haematopoiesis and especially erythropoiesis by inducing the release of erythropoietin (EPO) (Simsek *et al*., 2009). Spirulina's ability to increase haemoglobin levels promises its use not only in anaemia-inducing diseases such as malaria in southern countries, but also in people taking certain antiretroviral drugs (ARVs) such as zidovudine (AZT) (WHO, 2010). This could be explained by the presence of iron and folic acid (nutrients that promote haematopoiesis) in Spirulina. These results are similar to those of Kambou *et al.*, (2015a) who obtained a significant increase in the number of red blood cells and haemoglobin levels in rabbits treated with Spirulina.

In terms of leukocytes, Spirulina intake led to an increase at all doses in month 1 and at doses of 50 and 100 mg/kg in month 2. For this duration of Spirulina administration, the leucocyte rate was higher at 100 mg/kg bw. According to the standards, the results obtained did not reveal leucopenia or leucocytosis. In fact, the lack of abnormalities in the leucocytes and leucocyte formula of treated rats is attributable to Spirulina's action on the immune system, as reported by Simsek *et al*., (2009) following a study conducted in rats. According to these authors, Spirulina was effective against anaemia and leukopenia caused by lead or cadmium poisoning. The significant increase in leucocyte and lymphocyte levels observed in the first and second months is thought to be due to the body's defence against microbes and other foreign material following tissue damage caused by the samples. These results are in line with those of Deng *et al*., (2010) who showed an increase in white blood cells in their study. There was no significant change in thrombocyte levels in supplemented rats compared with controls. This could be due to the absence of phenomena likely to mobilise or reduce thrombocyte levels in plasma.

In view of these results, Spirulina in this study did not disturb the haematopoietic system by inducing leucopenia, thrombocytopenia and anaemia. Similarly, no polycythemia, leukocytosis, thrombocytosis or hypochromia were observed. The immunostimulant and nutritional effects of Spirulina would be beneficial for laboratory rats, which often suffer from infections and nutritional imbalance. In addition to haematological factors, biochemical parameters may help to better understand the influence of Spirulina on the well-being of treated subjects.

CONCLUSION

The administration of Spirulina as a dietary supplement to rats resulted in good growth and did not profoundly affect the haematopoietic system. This algae has real nutritional effects, demonstrating its importance in the fight against malnutrition.

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