

EFFECTS OF *ARTHROSPIRA (SPIRULINA)* ON HEMATOPOIESIS IN RATS

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SUMMARY. The objective of our study was to test the hematopoietic effects of an autohtonous strain of *Arthrospira (Spirulina)* added to the food of white rats. To investigate these effects we compared the reponse of anemiated and healthy rats to *Spirulina* feeding. The animals were divided into 3 groups which were fed bread and milk for the first week. The control group (C) received *Spirulina* powder (3% of the food) for two additional weeks. In the other two groups, during the first week, 25% of the blood was extracted 3 times, in days 1, 2 and 4, in order to induce anemia, while in days 3 and 5, small blood samplings were taken to establish the degree of the induced anemia. One of these two groups, denoted An (anemiated) received only bread and milk for the next two weeks, while the other one, denoted An-Sp, was also anemiated but received *Spirulina* powder. A blood sampling was also performed at the end of the 3rd week. The state of the animals was evaluated by measuring the blood content of 3 parameters: percent reticulocytes, red blood cell (RBC) count and hematocrit value. The percent reticulocytes and RBC count kept relatively constant for the C group, while hematocrit slightly increased. In the An group, the number of reticulocytes was highest at the second sampling (after anemia induction) and slightly decreased afterwards, while in the An-Sp group the number of reticulocytes at the second sampling was moderately lower than in the An group (but significantly higher than in the control) and decreased even more at the final sampling. As expected, the RBC count had a different evolution, increasing in a highly significant way in the An-Sp group, while in the An group it only approached the level of the control, a fact which can be explained by the hematostimulating effect of *Spirulina* in the An-Sp group. Due to the compensating changes between reticulocytes and RBCs, the hematocrit had only slight variations during the experiment. It is concluded that *Spirulina* had a hematostimulating effect not only in group An-Sp but also in group C.

Keywords: antianemic effect, hematocrit, red blood cells, reticulocytes, *Spirulina*.

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Introduction

Arthrospira (Spirulina) is a blue-green cyanobacterium which contains a complex of vitamins (A, B, D, E, K), minerals (calcium, potassium, sodium, phosphorus, magnesium, iron, iodine), amino acids, fatty acids, pigments (phycocyanin, allophycocyanin, chlorophyll and carotenoids), all necessary to an equilibrated diet (see, for example, Belay *et al.*, 1996; Sánchez *et al.*, 2003; Becker, 2004; Spolaore *et al.*, 2006). It represents the richest natural source of easily digestible proteins that contain all the essential amino acids (Guil-Guerrero *et al.*, 2004)

Previous studies have also reported that *Arthrospira (Spirulina)* can reduce the severity of anemia, inducing an increase of hematologic parameters (Kostic *et al.*, 1993; Zikic *et al.*, 1997; Simsek *et al.*, 2009). Apparently, its content of phycocyanin stimulates hematopoiesis and mimics the effect of endogenous erythropoietin (Epo). The role of Epo consists in stimulating the proliferation, growth and differentiation of erythroid precursors, with the consequent increase of the erythrocyte count, the endogenous Epo being normally inversely proportional with the erythrocyte volume.

The objective of the present study was to test the hematopoietic effects of the locally isolated wild strain of *Arthrospira (Spirulina)* added as biomass to the food of white rats. More exactly, we investigated the stimulating effects of this cyanobacterium on both anemiated and normal/healthy rats.

Material and Methods

In our experimental variant, white rats of about 200 g each were monitored for 3 weeks. They were divided into 3 groups: group **C** (control), which was fed for the first week only bread and milk, to which *Spirulina* powder (3% of the food) was added for the last two weeks; group **An** (anemiated), which consumed all the time only bread and milk; group **An-Sp**, also anemiated, but treated with *Spirulina* for the last two weeks, similar to the **C** group. The anemia in groups **An** and **An-Sp** was induced through 3 blood takings in days 1, 2 and 4. In days 3 and 5, small blood samplings were performed to check for the level of the induced anemia. A final sampling (at the end of the 3rd week) was also performed for all groups. For each intervention, the animals were slightly anesthsized with ethyl ether. The blood was taken from the orbital plexus. For inducing anemia, 25% of the blood volume was extracted each time (the blood volume was estimated to 6 ml for each 100 g body weight, according to Chiricuța *et al.*, 1992). For blood samplings, only 0.5 ml were taken each time.

The state of the animals was evaluated by measuring the blood content of 3 parameters: percent of reticulocytes, red blood cell (RBC) count and hematocrit value, using standard clinical procedures.

Results and discussion

At the first/initial blood sampling, the 3 groups displayed very similar/close results, while in the subsequent samplings the results differed statistically significant in a manner suggestive for an antianemic effect of *Spirulina*. The evolution of the 3 parameters monitored, along with the appropriate statistical data for the 3 groups are presented in Table 1 (group C), Table 2 (group An) and Table 3 (group An-Sp). Suggestive bar illustrations of the measured parameters are also presented comparatively in Fig. 1 (reticulocytes), Fig. 2 (RBCs), and Fig.3 (hematocrit).

As can be seen from Table 1, at the final sampling (performed after *Spirulina* administration), the count of both reticulocytes and RBCs increased in a highly significant way, in comparison with either of the previous samplings. The hematocrit also increased significantly.

Table 1.

Evolution of the blood and statistical parameters in group C

| Blood parameter (unit of measure) | Statistical parameter | Sampling before <i>Spirulina</i> administration (S ₂) | Final sampling (S ₃) |
|---|-----------------------|--|--|
| Reticulocytes (/100 erythrocytes) | $\bar{x}\pm ES$ | 0.59±0.02 | 1.08±0.06 |
| | n | 6 | 5 |
| | $\pm D\%(S_2)$ | 0 | +83.05 |
| | p _{3/2} | | < 0.001 *** |
| RBCs (x10 ⁶ /mm ³) | $\bar{x}\pm ES$ | 9.34±1.69 | 12.69±2.29 |
| | n | 6 | 5 |
| | $\pm D\%(S_2)$ | 0 | +35.86 |
| | p _{3/2} | | < 0.001 *** |
| Hematocrit (%) | $\bar{x}\pm ES$ | 43.27±0.98 | 48.26±1.55 |
| | n | 6 | 5 |
| | $\pm D\%(S_2)$ | 0 | +11.53 |
| | p _{3/2} | | < 0.05 * |

Explanations: $\bar{x}\pm ES$ – mean ± standard error of mean; n – number of animals tested; $\pm D\%(S_2)$ – percent difference between final sampling and the sampling before *Spirulina* administration; p_{3/2} – value of p for the above defined difference.

The evolution of the blood parameters in the An group, along with the appropriate statistical parameters are presented in Table 2. As can be seen from this table, at the second sampling (after anemia was produced), a highly significant decrease in RBC count was recorded in the An group, as compared to the initial sampling, while at the final sampling this decrease was smaller, but still statistically

significant. However, as compared to the second sampling, there was a very significant increase in RBC count at the final sampling. This increase is most likely due to the induction of Epo synthesis and its effect on erythropoiesis, as a reaction of the organism to anemia. As regards the reticulocytes, there is a highly significant increase of their number at both final and second sampling, although the increase is somewhat smaller at the final sampling. This can be attributed to the conversion in time of some reticulocytes into adult erythrocytes, as indicated by the increase of RBC count at the last sampling, as mentioned above. Significant increases can also be observed for hematocrit. All these results demonstrate the natural capacity of hematologic recovery for the animals tested.

Table 2.

Evolution of blood and statistical parameters in group An

| Blood parameter | Statistical parameter | Initial sampling (S ₁) | Sampling before <i>Spirulina</i> administration (S ₂) | Final sampling (S ₃) |
|---|-----------------------|------------------------------------|---|----------------------------------|
| Reticulocytes (/100 erythrocytes) | $\bar{x} \pm ES$ | 0.64±0.03 | 3.67±0.46 | 3.03±0.36 |
| | n | 7 | 7 | 7 |
| | $\pm D\%(S_1)$ | 0 | +473.43 | +373.43 |
| | p _{2/1} | | < 0.001 *** | |
| | p _{3/1} | | | < 0.001 *** |
| | $\pm D\%(S_2)$ | -82.56 | 0 | - 17.43 |
| RBCs (x10 ⁶ /mm ³) | p _{3/2} | | | NS |
| | $\bar{x} \pm ES$ | 11.4±0.54 | 8.30±0.15 | 10.07±0.24 |
| | n | 7 | 7 | 7 |
| | $\pm D\%(S_1)$ | 0 | -27.19 | -11.66 |
| | p _{2/1} | | < 0.001 *** | |
| | p _{3/1} | | | < 0.05 * |
| Hematocrit (%) | $\pm D\%(S_2)$ | +37.35 | 0 | +21.32 |
| | p _{3/2} | | | < 0.001 *** |
| | $\bar{x} \pm ES$ | 38.18±3.05 | 40.26±1.32 | 44.54±2.11 |
| | n | 7 | 7 | 7 |
| | $\pm D\%(S_1)$ | 0 | +5.44 | +16.78 |
| | p _{2/1} | | NS | |
| | p _{3/1} | | | < 0.05 * |
| | $\pm D\%(S_2)$ | -5.16 | 0 | 0 |
| | p _{3/2} | | | |

Explanations: $\pm D\%(S_1)$ – corresponding difference between S₂ or S₃ and S₁ p_{2/1} – value of p for the difference between the second sampling (performed before the administration of *Spirulina*) and the initial one; p_{3/1} – value of p for the difference between the final and the initial sampling; p_{3/2} – value of p for the difference between the final and the second sampling. The rest of the notations are identical to those in Table 1.

For the An-Sp group, the results are presented in Table 3, below. As can be seen, all three blood parameters tested show increased values at the final sampling, as compared to the initial one. The same statement is valid if comparison is made with the second sampling, with one notable exception: the reticulocytes. Their count is higher after the induction of anemia (before *Spirulina* addition) than at the final sampling. This can be explained by the fact that phycocyanin has an Epo-like effect, stimulating the hematopoiesis, i.e. the conversion of reticulocytes into RBCs. Indeed, the RBC count is much larger in the An-Sp group than in the An group and even higher than in the control (C) group. Since the control group also received *Spirulina*, the difference should be ascribed to the combined effect of the endogenous Epo (induced by anemia) and the Epo-like effect exerted by *Spirulina*. The value of the other blood parameters is also in line with this conclusion. For example, the hematocrit is significantly increased at the final sampling.

Table 3.

Evolution of blood and statistical parameters in group An-Sp

| Blood parameter | Statistical parameter | Initial sampling (S ₁) | Sampling before <i>Spirulina</i> administration (S ₂) | Final sampling (S ₃) |
|---|-----------------------|------------------------------------|---|----------------------------------|
| Reticulocytes (/100 erythrocytes) | $\bar{x} \pm ES$ | 0.58±0.04 | 3.03±0.20 | 1.53±0.08 |
| | n | 7 | 7 | 6 |
| | $\pm D\%(S_1)$ | 0 | +422.41 | +163.79 |
| | p _{2/1} | | < 0.001 *** | |
| | p _{3/1} | | | < 0.001 *** |
| | $\pm D\%(S_2)$ | -80.85 | 0 | -49.50 |
| RBCs (x10 ⁶ /mm ³) | p _{3/2} | | | < 0.001 *** |
| | $\bar{x} \pm ES$ | 10.85±0.60 | 6.97±1.29 | 13.19±2.39 |
| | n | 7 | 6 | 6 |
| | $\pm D\%(S_1)$ | 0 | -35.76 | +21.56 |
| | p _{2/1} | | < 0.01 ** | |
| | p _{3/1} | | | < 0.001 *** |
| Hematocrit (%) | $\pm D\%(S_2)$ | +55.66 | 0 | +89.23 |
| | p _{3/2} | | | < 0.001 *** |
| | $\bar{x} \pm ES$ | 46.39±1.16 | 41.93±1.14 | 46.60±1.57 |
| | n | 7 | 6 | 6 |
| | $\pm D\%(S_1)$ | | -9.61 | +0.45 |
| | p _{2/1} | | < 0.01 ** | |
| | p _{3/1} | | | NS |
| | $\pm D\%(S_2)$ | +10.63 | | +11.13 |
| | p _{3/2} | | | < 0.05 * |

The same explanations are valid as for Tables 1 and 2, but it should be remembered that this group was both anemiated and given *Spirulina*.

In order to have a general image of our results, a synthetic comparison across the 3 groups at all 3 samplings is also illustrated by bar diagrams in Figs.1-3, separately for each of the 3 blood parameters tested.

Fig. 1 illustrates and compares the reticulocyte count of the 3 groups at the moments of the 3 samplings. As can be seen, there are highly significant increases (***) in both An and An-Sp group as compared to the control, at the second and third (final) sampling, whereas a very significant decrease (+++) can be observed at the final sampling for the An-Sp group as compared to the An group. The significance of these observations has been already discussed.

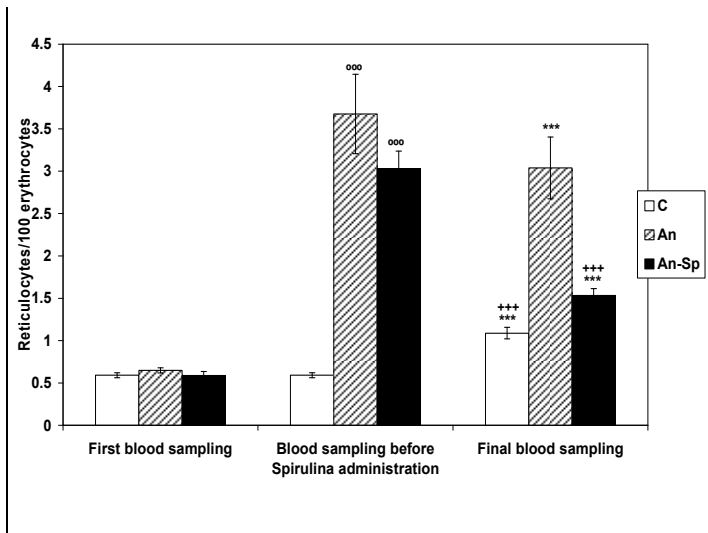


Figure 1. A comparative illustration of the evolution of the reticulocyte count in the 3 rat groups. ° - denotes the significance of the differences between the sampling before *Spirulina* administration and the initial sampling; * - denotes the significance of the differences between the final sampling and the initial sampling while the symbol + is used to show significant differences between the final sampling and the one before *Spirulina* administration.

In Fig. 2 we illustrate the evolution of RBC count in the 3 rat groups. As can be seen, the differences that exist between the groups at each sampling do not appear statistically significant, although there are differences among the 3 moments of sampling as already discussed when we presented Tables 1-3.

Hematocrit values of the 3 groups are presented comparatively in Fig. 3. As already discussed, due to different compensating effects, the hematocrit has a rather slight variation which is either not significant or hardly significant. Occasionally, the hemoglobin concentration was also measured and its values seem to be more relevant than the hematocrit. As expected, the hemoglobin concentration parallels the increase of the RBC count (not shown here).

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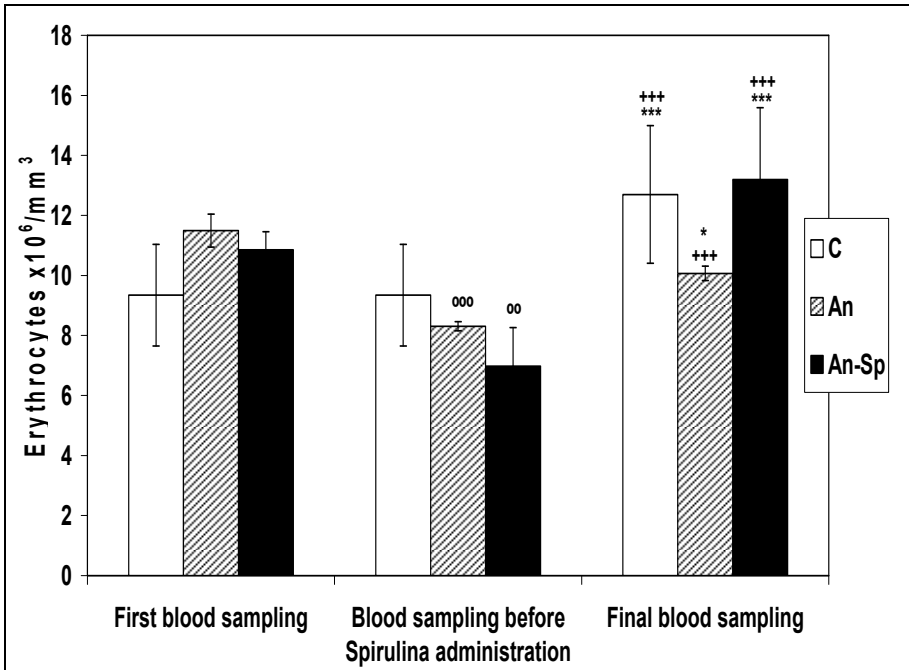


Figure 2. The evolution of the RBC count in the 3 rat groups

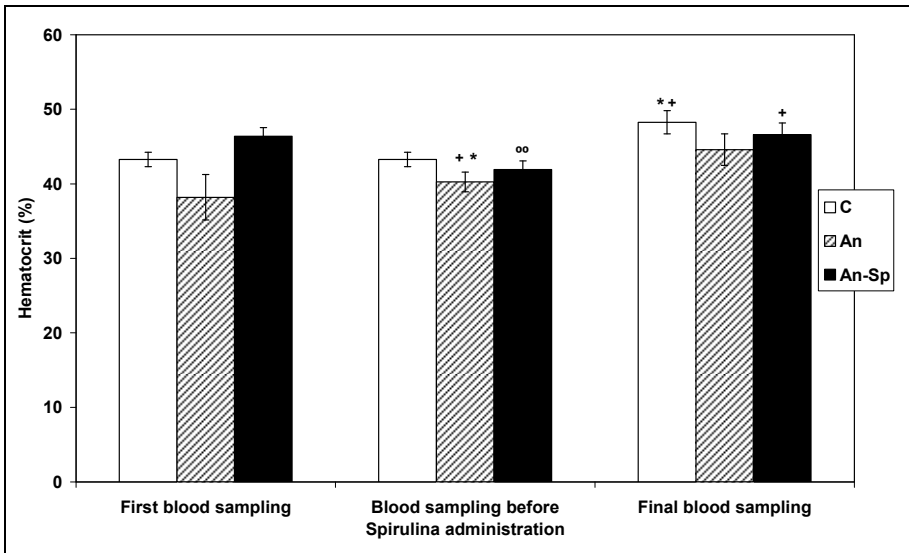


Figure 3. Hematocrit values in the 3 groups of rats at the 3 moments of sampling

Conclusions

The hematostimulating effect of our strain of *Arthrospira (Spirulina)* was observed even in group C (control), in the absence of anemia.

In the rats of the An (anemiated) group, after 14 days since the induction of anemia, the measured parameters (% reticulocytes, RBC count, and hematocrit) partially recovered, as a result of erythropoiesis intensification under the action of the induced endogenous Epo.

The animals in the An-Sp group were more efficient in restoring the affected parameters, as compared to the An group, because the normal reaction of the organism to anemia was supplemented by the effect of phycocyanin contained by the cyanobacterium *Spirulina*.

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