

DETERMINATION OF PARAMETERS FROM BLOOD PICTURE OF WHITE LABORATORY RATS FED WITH ENRICHED BREAD

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Abstract

According to figures from the WHO over 30% of the world's population are anemic. If you consider the most important indicators of nutrition, the level of anemia, Macedonia can be considered a medium to high risk of anemia, according to a report by UNICEF. Industrial process of refining of cereal products, leading to loss of important minerals and fiber. Enrichment of flour with micronutrients is outspread procedure that seeks the return of the micronutrients. The aim of this paper is to examine the effect of enriched bread in male rats. Thus is to examine the possibility of future implementation and reduce the number of anemic people in Macedonia.

Material in our study was peripheral blood. The experimental animals were divided as follows: First group (n=8) were fed with white bread. A second group (n= 8) were fed with enriched bread. As controls were used the same animals in which analysis were taken before treatment. Animals were held on the above diet for 21 days. Analysis was performed on hematology counter from the company ABH Micros.

The results of our research showed, statistically significant increase in the RBC $p < 0.0004^*$, the WBC $p < 0.000001^*$, Hb $p < 0.0000001^*$, HCT $p < 0.001^*$ and PLT, $p < 0.000009^*$ comparison between groups that are fed and enriched white bread.

Key words: *Enrichment, iron, folic acid, WBC, RBC, Hb, HCT, PLT.*

1. Introduction

Iron deficiency is the commonest cause of anemia. According to figures from the World Health Organization, 2 billion people - over 30% of the world's

population are anemic (ACC/SCN [1]). If you consider the most important indicators of nutrition, the level of anemia and lack of vitamin A, Macedonia is considered to be country with a medium to high risk of anemia, according to a report by UNICEF.

Iron is an essential element of all human cells. He plays a key role in transporting oxygen and cellular respiration. This feature comes from the knowledge that iron is an integral part of haemoglobin, myoglobin and cytochrome oxidase enzymes, peroxidase and catalase. Proteins containing iron can be divided into:

- proteins that contain heme, in which an iron atom is embedded: haemoglobin, myoglobin, and cytochrome hemoproteids (enzymes catalase, peroxidase, cytochrome oxidase);
- proteins which not contain heme, and bind iron (ferritin, transferrin, haemosiderin, flavoproteins).

In all these proteins, iron, has a central place in the transport of oxygen, and in energetic metabolism (Dzhekova-Stojkova [2] and HEINZ [3]). On the other side the development countries the economic regarding diet is consist mainly of products of plant origin from which iron is not assimilated enough. This is the reason why even in cases when nutrition support is sufficient however, the majority of the population have a chronic iron deficiency. The result of such a chronic lack of effective iron in the human body is the appearing different diseases. One reason for the occurrence of iron deficiency (as well as calcium and zinc) is affected by certain anti-nutritional phytates, tannins and other fibres which substantially reduces the biological absorption of these micronutrients (Harland and Oberleas [4]). Studies show that the industrial process of refining of grain products, leads to a loss of important minerals and fibres, and, on

the other hand, have significant consequences for human health. One of the frequently used procedures, especially in highly developed countries, in order to overcome these shortcomings, is the enrichment of food with micronutrients (Bauernfeind [5]). This method is easily implemented and has a very low price (in case of mineral micronutrients) (Luten *et al.* [6]). The aim of this paper is to examine the effect of enriched bread on the parameters of blood picture in experimental rats of male sex, and thus the possibility of its further application in humans to improve their health and reduce the number of anaemic people in the Republic of Macedonia.

Our results showed that taking of iron and folic acid as supplement in food plays a crucial role in haematopoiesis in animals.

2. Materials and Methods

2.1. Materials

Material in our study was peripheral blood of white laboratory rats weighing 150-270 g total 16 in number. The experimental animals were divided as follows:

The first group of animals ($n = 8$) were fed with white bread. The second group of animals tested ($n = 8$) were fed with enriched bread. As a control were used the same animals on which the analysis were made before the start of treatment with white or enriched bread. Animals from all groups were on a diet 21 days during the experiment had normal access to food and water.

Making bread from enriched flour. In 10 kg white flour type 500 we added 1 g from the product ELCOvit 2049E, product of German company Mühlenchemie, which is pre-mix of iron and folic acid. The iron is 60 mg/kg, and folic acid 1,6 mg/kg. We also added in the flour 5,300 g water and 500 g yeast.

2.2. Methods

The biological material - peripheral blood of laboratory animals was taken twice - at the initial stage at the beginning of the experiment and the end of the experiment. Blood was collected in plastic disposable tubes, which had anticoagulation substance heparin. Analysis of blood parameters was performed on haematology analyser from the company ABX Mikros. The results of the experimental research are represented as mean $X \pm SD$ and Standard deviation. *t* - Test was used to assess the variables between groups, the value of $p < 0.05$ was considered statistically significant. For statistical evaluation of the results was used a computer program EXCEL and statistical package STATISTIKA 7.

3. Results and Discussion

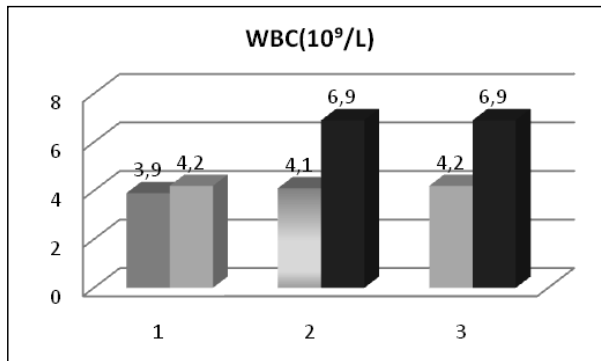
Table 1. Statistical analysis of biochemical parameters compared between the control group and the group fed with white bread, group before the experiment and group fed with enriched bread, and a comparison between the groups fed with white bread and enriched bread at the end of the experiment

	$X \pm SD$ $p < 0,05$		
	1. Control group & Group fed with white bread after the experiment	2. Before the experiment & Group fed with enriched bread after experiment	3. Group fed with white bread after the exp. & Group fed with enriched bread after exp.
WBC($10^9/L$)	3,9 \pm 0,42:4,2 \pm 0,33 $p < 0,04^*$	4,1 \pm 0,26:6,9 \pm 0,71 $p < 0,000001^*$	6,9 \pm 0,71:4,2 \pm 0,33 $p < 0,000001^*$
RBC($10^{12}/L$)	6,7 \pm 0,43:6,7 \pm 0,45 $p < 0,059$	6,8 \pm 0,48:8,2 \pm 0,58 $p < 0,0005^*$	8,2 \pm 0,58:6,7 \pm 0,45 $p < 0,0004^*$
HGB(g/L)	134,1 \pm 1,55:133,9 \pm 2,08 $p < 0,66$	134,5 \pm 1,09:146,7 \pm 1,14 $p < 0,0000001^*$	146,7 \pm 1,14:133,9 \pm 2,08 $p < 0,0000001^*$
HTC(L/L)	0,39 \pm 0,01:0,41 \pm 0,05 $P < 0,29$	0,41 \pm 0,04:0,51 \pm 0,05 $P < 0,0001^*$	0,51 \pm 0,05:0,41 \pm 0,05 $P < 0,001^*$
PLT($10^9/L$)	523 \pm 77,9:573,25 \pm 62,4 $p < 0,04^*$	523 \pm 50,2:695 \pm 63,4 $p < 0,000007^*$	695 \pm 63,4:573,25 \pm 62,4 $p < 0,000009^*$

(Note, significant deviations are indicated by *).

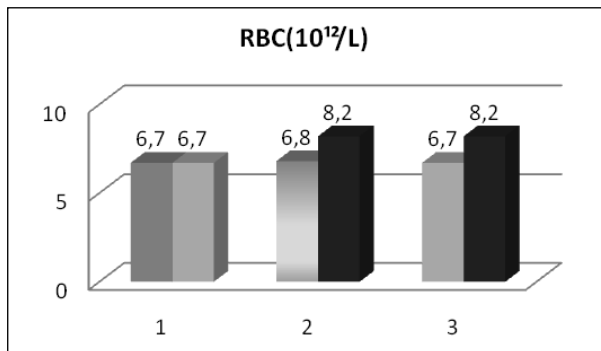
The results are graphically displayed for each biochemical parameter separately.

White blood cells are cells of the immune system and there are involved in protecting the body. There are five different types of leucocytes (Alberts [7]). From the results shown in Table 1 and the graphical review, it is evident that values of WBC in the control group before treatment amount to $3.9(10^9/L)$, and for the group before experiment $4.1(10^9/L)$. At the end of the experiment for group fed with white bread $4.2(10^9/L)$, and for the group fed with enriched bread the rise is significantly increased $6.9(10^9/L)$ (Figure 1). During the statistical processing of the results found no statistically significant difference in the values obtained for the number of leucocytes in all three groups WBC($10^9/L$) of the first $p < 0,04^*$ in the second $p < 0,000001^*$ and third $p < 0,000001^*$. Erythrocytes are known as red blood cells and contain large amounts of haemoglobin. Their functions are to transport oxygen in the blood and are produced through a process called erythropoiesis (Dzhekova-Stojkova [2]). Significant growth factored by the number of red blood cells for the group of rats who are on a diet of enriched bread $8.2(10^{12}/L)$, compared with the values of the group before the experiment $6.8(10^{12}/L)$. The group is fed with white bread remained almost the same values with the control group $6.7(10^{12}/L)$ (Figure 2). When comparing the group fed with the enriched bread with the group fed with white bread got statistical significant difference $p < 0,000001^*$. The results can conclude that addition of iron and folic acid stimulates production of red blood cells and increases their numbers.



1. Control group & Group fed with white bread after the experiment.
2. Before the experiment & Group fed with enriched bread after experiment
3. Group fed with white bread after the experiment & Group fed with enriched bread after experiment

Figure 1. Change of leukocyte number in individual groups before and after investigation

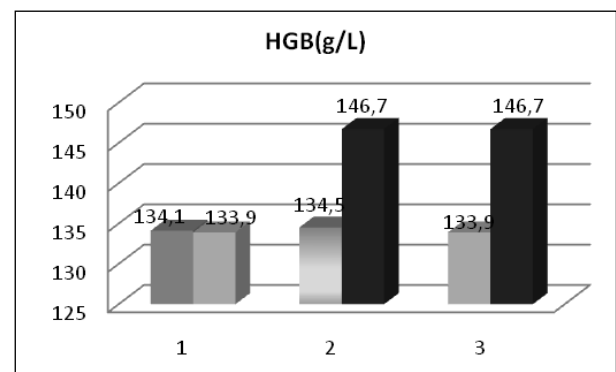


1. Control group & Group fed with white bread after the experiment.
2. Before the experiment & Group fed with enriched bread after experiment
3. Group fed with white bread after the experiment & Group fed with enriched bread after experiment

Figure 2. Change of the erythrocyte number in individual groups before and after investigations

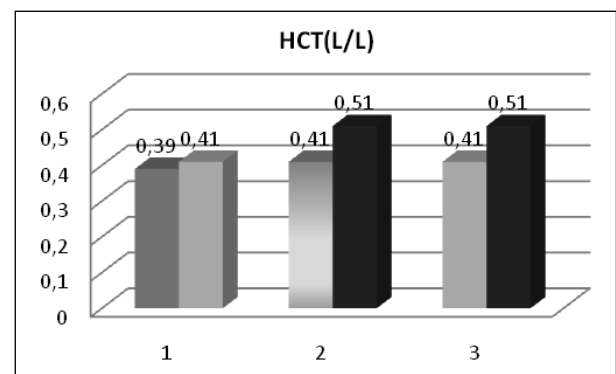
The evaluation of other parameters was determined that the haemoglobin values were statistically significantly changed (Table 1). For the control group, 134,1 (g/L) and the group before the experiment we got almost identical values 134,5 (g/L). In contrast, we found a slightly reduced in the group fed with white bread 133,9 (g/L), and an increase in the group fed the enriched bread 146,7 (g/L) (Figure 3). The increase in haemoglobin in the group fed with the enriched bread was expected, because the number of erythrocyte in that group also increased. Hematocrit is a measure of the total volume of erythrocytes in relation to total blood volume, thus providing, information on the concentration of haemoglobin, which circulates in the blood. For the control group we received 0,39

(L/L), the group fed with white bread and experiment group before 0,41 (L/L) and for the group of animals fed with food enriched with iron, hematocrit increased significantly and reached values of 0,51 (L/L) (Figure 4). Platelet function PLT (10⁹/L) is the maintenance of haemostasis. This is achieved primarily through the creation of blood clots when there is a damage to the blood vessels, endothelium of blood otherwise the creation of blood clots should be blocked (Dzhekova-Stojkova [2]). Significant increase in our study occurs for the number of platelets, where values for the group fed with enriched bread move 695 (10⁹/L) but for the group fed with white bread 573.25 (10⁹/L). The values for the control group and the group before the experiment are the same and is 523 (10⁹/L) (Figure 5).



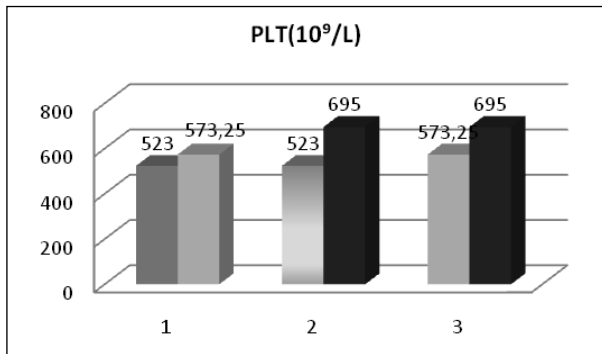
1. Control group & Group fed with white bread after the experiment.
2. Before the experiment & Group fed with enriched bread after experiment
3. Group fed with white bread after the experiment & Group fed with enriched bread after experiment

Figure 3. Change of the haemoglobin in individual groups before and after investigations



1. Control group & Group fed with white bread after the experiment.
2. Before the experiment & Group fed with enriched bread after experiment
3. Group fed with white bread after the experiment & Group fed with enriched bread after experiment

Figure 4. Change the hematocrit in individual groups before and after investigations



1. Control group & Group fed with white bread after the experiment.
2. Before the experiment & Group fed with enriched bread after experiment
3. Group fed with white bread after the experiment & Group fed with enriched bread after experiment

Figure 5. Change of the platelets in individual groups before and after investigations

From our results can be seen that the number of red blood cells for the group fed with enriched bread was significantly increased 8.2 ($10^{12}/L$) of these data we can conclude that the addition of iron and folic acid in bread increases the RBC and their volume and thus gives an opportunity to produce more haemoglobin, which is in fact received with the diet. With increasing the number and size of red blood cells increases the amount of space they occupied and these results in higher levels of hematocrit. In the majority of the consulted literature (Gudumac *et al.* [8] and Sturza *et al.* [9]). We noticed that a particular emphasis on bread production is set to phytates. In our study in the preparation of bread we used the baker's yeast, and we have received satisfactory levels of the tested biochemical parameters, this is probably due to the quality and enhancement not only of iron but of folic acid used premixes. However, in the further future studies will consecrate more attention to the procedures of making bread, because it is very important the added iron to be in adequate quantities, and with it properly and fully used by the body. Because it is not developed a mechanism which helps to be evacuate the accumulated iron from the body and can easily lead to iron overload in tissues and thus to lead to the production of harmful free radicals via the Fenton or Haber-Weiss reaction.

4. Conclusions

The results presented in this study showed that:

- The total number of leukocytes showed a significant difference between groups on a diet of white and enriched bread.
- Total number of erythrocyte were lower in the

blood of rats fed with white bread compared to the group fed with enriched bread

- Haemoglobin is varied in the examined groups, but we found a significant increase in animals who were fed with enriched bread.
- Hematocrit in the differential blood count also showed a significant difference in animals that were fed with enriched bread.
- We found a significant increase and the number of platelets in the comparison group that was on a diet with white bread and with enriched bread.

Studies of experimental animals are numerous, and the results can not be interpreted and applied as such in humans. However, they are precious indicators and guidelines for future research on this field. Since Macedonia is one of the development countries in and also faces the problem of increasing the number of anemic people need to raise the public awareness about the problems that can arise from iron-deficiency anemia and the importance of prevention with the use fortified foods. In this study, it was concluded that the supplement of iron and folic acid play a significant role in the diet of experimental rats and leads an improve of the biochemical indices examined.

5. References

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