THE EFFECT OF GERMINATION ON SEED MINERALS ABSORPTION IN ANIMAL ORGANISM

— research paper —

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Abstract: This experiment is an attempt to underline the benefic effect of seed germination on seed minerals absorption in animal organism. For this purpose, we follow the evolution of some minerals of interest content in the blood of two lots of lab rats, W (blind) and T (test), fed with un-germinated respectively germinated integral grain fodder. The necessary determination was carried out with the help of Inductively Coupled Plasma Mass Spectrometry (ICP-MS), a powerful tool for the quantitative and qualitative determination with increased popularity in biologically sample analysis. The experimental results indicate a normalization of the parameter value showing that germinated seeds consumption ameliorates serum calcium, magnesium and iron values.

Keywords*:* seed, germination, ICP-MS, spectrometry.

INTRODUCTION

Inductively coupled plasma mass spectrometry (ICP-MS) is a powerful tool for the quantitative and qualitative determination of the minerals from different sample (De Blas Bravoa et al., 2007). The high sensitivity of ICP-MS has resulted in an increased popularity of this technique for the analysis of the biological sample (Abdelnour and Murphy, 2003). This technique was used to determine the mineral content of biological samples from tested animals (Chambers et al., 1991).

This research aims to emphasize a number of advantages that are obtained as a result of seed germination, in terms of food benefice. We investigate the

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influence of germinated seed consumption on the blood level of three bio minerals, calcium, magnesium and iron, by comparison with two lots of lab rats, W (blind) and T (test) fed with un-germinated respectively germinated integral grain fodder according to a recipe detailed below. In the germination process a series of biochemical transformations increase the mineral ions content in forms that are easier to assimilate from the animal organism. The experiment aims to verify the theoretical hypothesis regarding the superior absorption of bio elements from germinated seeds "in vivo" – respectively on growing organisms.

MATERIALS AND METHODS

We used male Wistar rats. In choosing the animals for the experiment we have looked for homogeneity in age, sex and metabolic activity, thereby the results from different test lots are comparable with minimal error. Considering all this we have bred only healthy animals, the mother and her offsprings were kept under surveillance following their normal development. Following ablactation, baby rats have been separated from their mother and have been kept five days on an adaptation regime consisting of milk and bread, in this period we have observed the way they have adapted at the new condition.
Only healthy animals were grouped in two lots: W (blind) lot of 10 rats and T (test) lot of 10 rats.
For 21 days all animals have been fed „ad libitum" with the fodder described in table 1.

Table 1 Fodder composition

Ingredients – Lot M	**Ingredients – Lot T**
Wheat 50%	Germinated wheat 50%
Barley 20%	Germinated barley 20%
Soya beans 15%	Germinated soya beans 15%
Sunflower 15%	Germinated sunflower 15%

We have used quality wheat, barley, soya, and sunflower seeds with known provenance.
In diet construction four M lot we have kept the nutritional criteria in mind trying to obtain a well balanced fodder containing all the necessary elements in a proportion close to the optimal one. In diet construction for T lot the difference arises due to the fact that we have used germinated seeds instead

of mature seeds, the proportion being the same. In both cases (M and T lots) seeds are turned into flour (10% humidity).
Throughout the experiment animals are held in individual cages with artificial illumination and air-conditioning. After 21 days blood sample ware tacked in order to observe the minerals level.

Germinating the seeds

For germination the seeds have been soaked 30 min in a disinfectant solution (0.7 g/l sodium hypochlorite in water) to prevent microorganisms contamination. After the seeds have been washed with distilled water they have been soaked for another 5h 30 min in clean distilled water. The operation was carried out in a controlled environment at 20^0C, water having the same temperature. After imbibition the seeds are placed on perforated plastic trays in the germination chamber. The process takes place in a controlled temperature, humidity and light environment.
Seeds are sprayed with distilled water every 4h with a programmable electronic device. Seeds germinate for 72h. The prolongation of the germination period leads to significant weight losses due to the accentuation of starch hydrolyzes and respiration process. 3-4 days are sufficient for the macrostructure of the seed components to be affected and the accumulation of nutritional valuable component to reach significant levels.
After germination seeds are dried in a drying chamber at 30°C (high temperatures are avoided in order to protect temperature sensitive compounds).
In both cases (M and T lots) seeds are turned into flour (10% humidity).

Sample testing – ICP-MS presentation

The ICP-MS for simultaneous trace element determination in blood samples has prevailed as the most suitable methodology for clinical aims because of its rapidity, detection limits and minimal sample quantity needed for analysis.
The Emission Spectrometer measures the intensity of light emitted by the elements in a sample. The ISP is a sensitive instrument, capable of detecting a large number of elements down to levels of a few parts per billion or even less. In table 2 we present the wavelength and detection limit, of the ICP model used in this analyze, for calcium, magnesium and iron.

Table 2. Wavelength and detection limit for Ca, Mg and Fe.

Element	Wavelength nm	Limits of detection Mg/l
Calcium	315,887 317,933 393,366	0,1 0,01 0,002
Magnesium	279,079 279,553 285,213	0,03 0,0005 0,001
Iron	259,940 238,20	0,02

The ICP-MS require liquid sample, of a named dilution. Sample introduction system consists of a peristaltic pump, a nebulizer, and a spray chamber and provides the means of getting samples into the instrument. The nebulizer converts the liquid samples into very small droplets. The liquid sample is introduced to a nebulizer where a flow of argon is passed at right angles over the end of the tube that transport the liquid, the flow of argon gas shears the liquid into very small droplets forming an aerosol. The droplets pass through a spray chamber that eliminates all droplets except those that are the right size and velocity for introduction into the plasma.

ICP torch generates the plasma that creates a very hot zone, of approximately 6000°C. The plasma is generated by passing argon through a series of concentric quartz tubes (the ICP torch) that are wrapped at one end by a radio frequency (RF) coil. Energy supplied to the coil by the RF generator couples with the argon to produce the plasma. During their voyage into the plasma the liquid droplets containing the sample matrix and the elements to be determined are dried to a solid, and then heated to a gas. As the atoms travel through the plasma, they absorb more energy from the plasma and eventually release one electron to form a singly charged ion.

ICP require computers and sophisticated software to control the plasma and mass spectrometer as well as perform calculations on the data collected. The CID detector is made up of 262.1444 light-sensitive pixels arranged in a 512 by 512 array. As sample passes through the plasma, the elements present in the sample emit light at different wavelengths. The same element emits light at the same wavelength. Emission at some wavelengths is more intense than at others. CDI detector records an elemental “fingerprint” of the sample. For qualitatively and quantitatively analyses an unknown sample and a full frame

image is acquired (figure 1). Each wavelength falls on an area of the CID detector covering a few pixels. Measuring the amount of light that falls on these pixels allows the spectrometer to determine the concentration of these elements in the sample.
The top of the image appears over-exposed due to the large amount of background resulting mainly from argon continuum emission. A series of other wavelengths appear as bright spots on the lower wavelength region of the image.

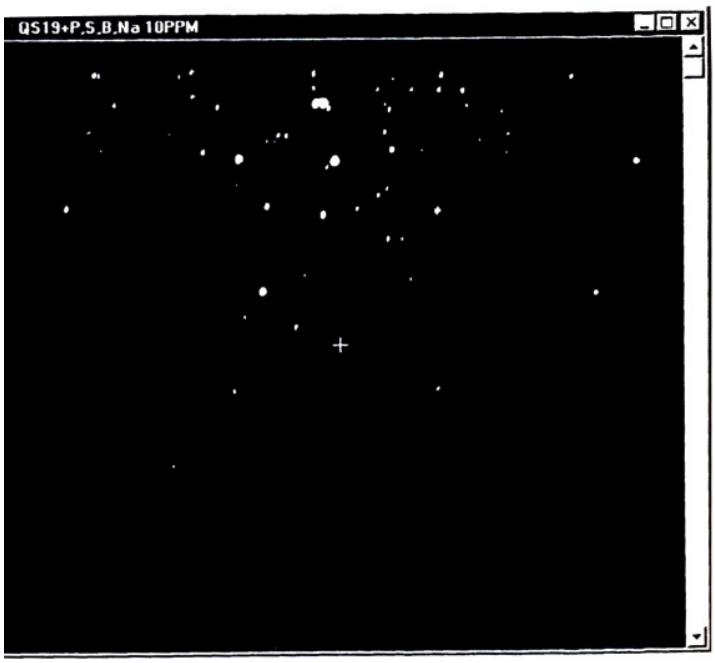

Figure 1. Full frame image

By double clicking on the desired point in the full frame image the point in case is zoomed at the level 4 as we presented in figure 2. Pressing the Enter key the cursor will move to the brightest pixel in the box. For this pixel the computer can display a list of the respective element wavelengths. Based on the knowledge of the sample (witch element is more likely present, considering the sample) and the relative intensities (whose emission at the respective wavelength is much strong) the respective element can be known. To confirm the identity of the element, electronically map the image to find the location of other prominent wavelengths. The base program locates the other prominent lines that will be highlighted by color coded boxes on the

screen. Those lines are investigated by double clicking on each line. If a peak appears in each one, then the element is very probably in the sample. If the map boxes do not center on their wavelengths, they may require mapping (calibration). This procedure is repeated for any number of elements to qualitatively determine the elemental constituents in the sample. As an observation, it is recommended that a maximum of 4 element maps be viewed simultaneously on the screen, as more will make interpretation difficult due to the similarity in box colors.

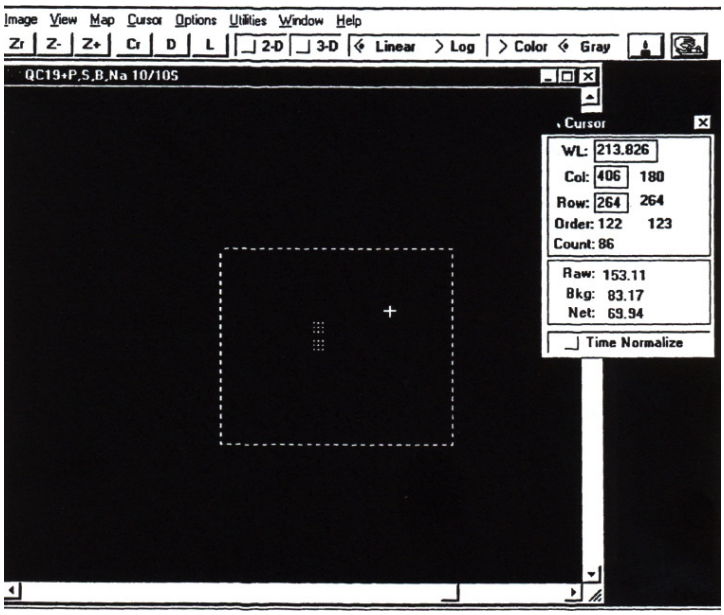

Figure 2. Element identification

Optical emission spectrometry is a comparative technique. Therefore, standard solutions containing known concentrations of all the elements to be determined are used to establish the relationship between raw intensity measurements and reported concentrations. The commonly used process of establishing this relationship is called “standardization”. The intensity is converted to concentration by comparing it with intensities emitted by known standard concentrations of the same elements. To accomplish this task, the relationship between emission intensity and concentrations must be determined. This is done using standard solutions whose concentrations are accurately known. They are used to determine the coefficients of the equations relating the emission intensity concentration.

Sample preparation

Blood samples were diluted 1:10 with a diluent containing the following reagents: 0,07 ammonium hydroxide, 0.01 mM Ammonium EDTA (ethylenediaminetetra-acetic acid, diammonium salt, dihydrate, 97% purity) and 0.07% (v/v) Triton X-100 – only 18 MΩ deionized water was used.
This method ensures a detection limit of the order of μg/l and a reduction of the sample volume.

RESULTS AND DISCUSSION

Figures 3, 4 and 5 presents the obtained data, respectively the calcium, magnesium and iron blood content, for the two case studies, blind lot (W) and test lot (T), after 21 days of regime.

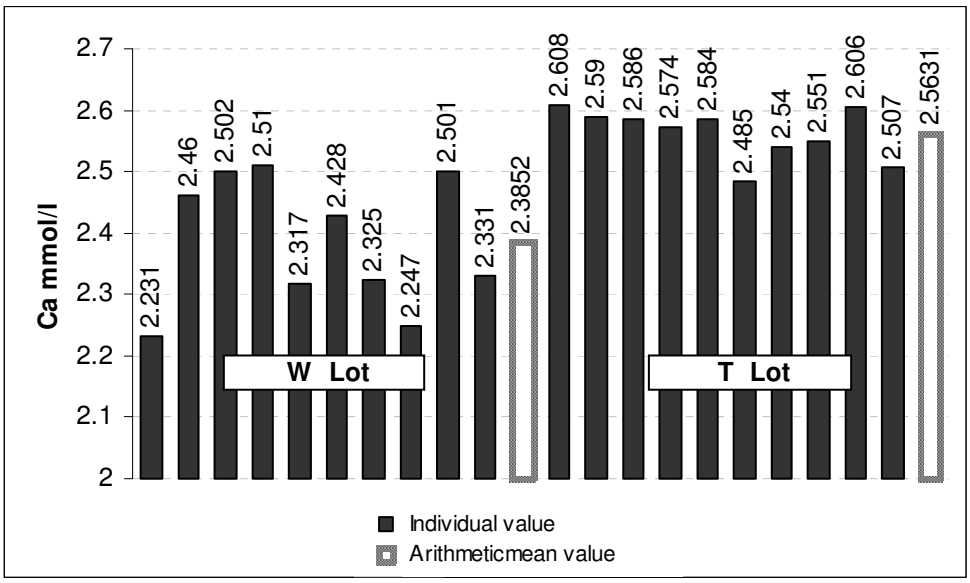

Figure 3. Calcium content of the samples.

Concerning the calcium content, the average of the results in W lot case were inferior to the average of the range of normality: 2,25-2,75 mmol/l (9-11 mg/dl). The variation of the investigated parameter, from one subject to another, were consistent. The dispersion of the T lot results in the range of normality is tighter than in the W lot case, being superior to the average of the interval.

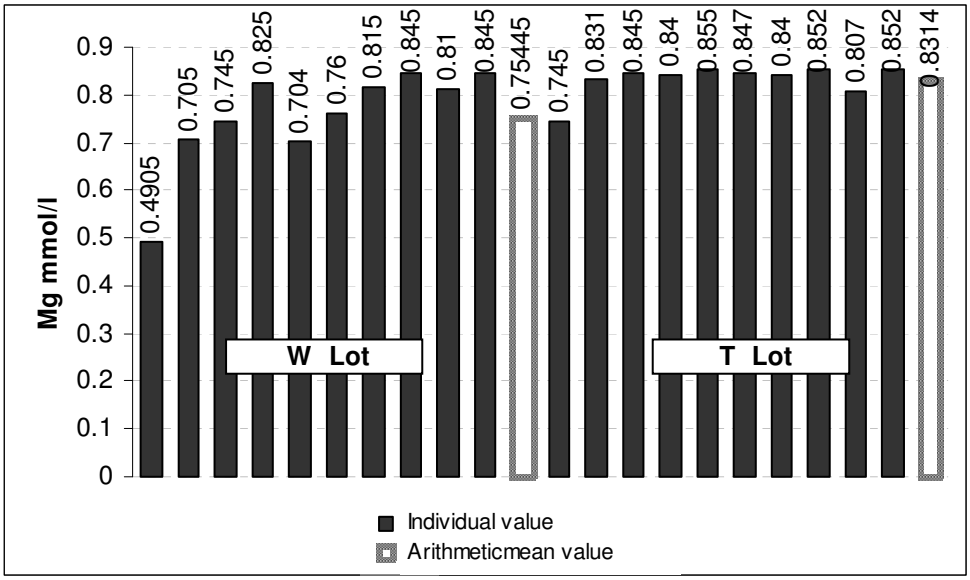

Figure 4. Magnesium content of the samples.

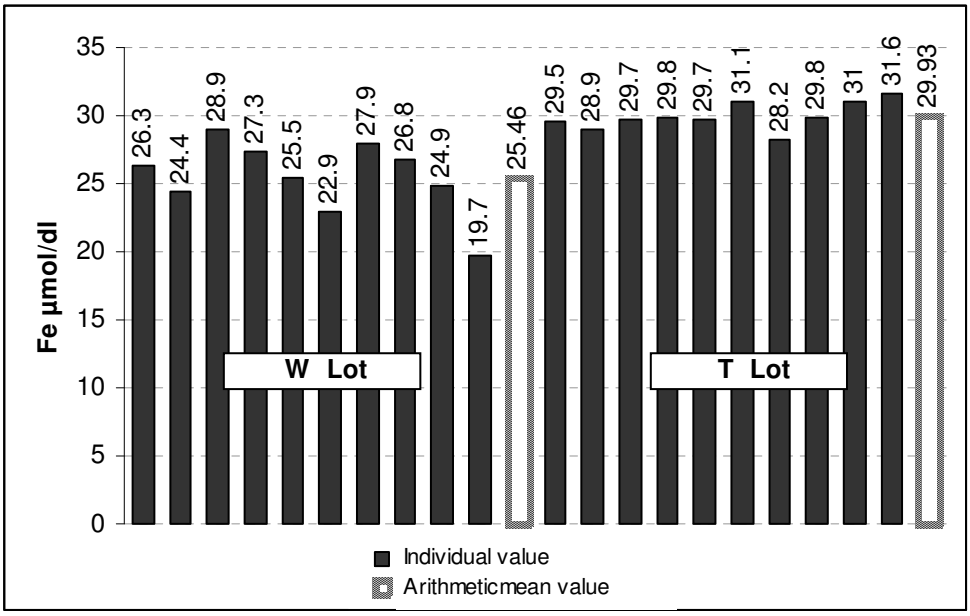

Figure 5. Iron content of the samples.

Regarding the magnesium content (Figure 4), the average of the results in W lot case were inferior to the average of the range of normality: 0,65-1mmol/l (1,6-2,6 mg/dl). The variations of the magnesium content, from one subject to another, were consistent but the dispersion of the results was more tightly than in the case of calcium. The dispersion of the T lot results in the range of normality is tighter than in the W lot case, being closer to the average of the interval but still significantly inferior.
In the case of iron (Figure 5), the average of the results in W lot was superior to the average of the range of normality: 12–32 µmol/dl (65– 175 µg/dl). The variations of the iron content, from one subject to another, were consistent. The dispersion of the T lot results in the range of normality is tighter than in the W lot case, being significantly superior to the average of the normality interval.

In all cases (W and T lots), the analyses results were in the range of normality. The germination process improved the absorption of all the interest minerals that this study analyses due mainly to the biochemical processes that take place in the seed with direct impact upon inositol hexakisphosphate (phytic acid) combination from mature seed and also due to some other complex biosynthesis processes. Tested animals have well accepted and tolerated the flour from germinated seeds, no digestive problems or otherwise have occurred.

CONCLUSIONS

Germinated seeds based products consumption does not generate a “blind” variation of serum calcium, magnesium and iron. In general homeostasis is achieved through complex biochemical processes at the cellular and systemic level by the action of a complex cellular, transport and signaling systems. The biologically active components from germinated seeds interrelate with the ionic homeostasis mechanisms, so the ionic concentration of the respective component in the blood-plasma has a smart variation in the normality interval limits.
The experimental results indicate a normalization of the parameter value showing that germinated seeds consumption ameliorates serum calcium, magnesium and iron values.
This test which has revealed the biogenic and health promoting qualities of germinated seeds recommends them as a natural supplement with applications in the prevention and combat of mineral imbalances, anemia

with deficiency on malabsorption basis, spasmophiliacs, conditions of prolonged stress, etc.

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