

**HEMATOCRIT COUNT OF BLOOD SAMPLES FROM NON ANEMIC
RABBITS *Oryctolagus cuniculus* FED WITH DIFFERENT CONCENTRATIONS
OF SOYBEAN SEEDS *Glycine max* IN A FIVE DAY PERIOD**

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In Partial Fulfillment
Of the Requirements for
SCIENCE RESEARCH 2

by

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Fourth Year Photon

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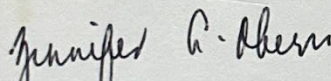
“Hematocrit Count of Blood Samples from Non-anemic Rabbits (*Oryctolagus cuniculus*) Fed with Different Amounts of Soybean Seeds (*Glycine max*) in a Five Day Period.”

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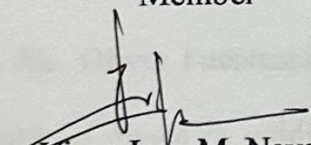
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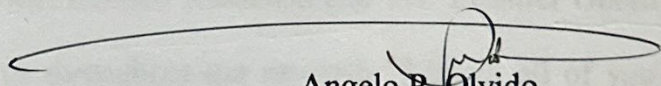
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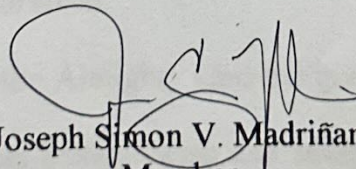
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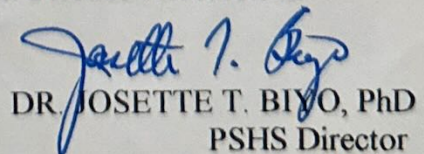


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CHAPTER I

INTRODUCTION

Abstract

There are two types of iron, heme and non-heme iron. One good source of nonheme iron is seeds of plants. Soybean seeds are a rich source of highly bioavailable dietary iron due the presence of stable Fe (II) in its hulls (Laszlo, 1987.) However the relationship between the amount of soybeans ingested and the amount of iron absorbed has not been determined. This study deals with the investigation of the iron content in the blood of non-anemic rabbits fed with different amounts (0, 25, 37.5, 50 g/kg) of soybean seeds in a period of 5 days. Based on the results gathered, it has been observed that Treatment 1 (50g soybean diet) and Treatment 2 (25g soybean diet) shows a positive change in the hematocrit count of the blood after 5 days of feeding. Treatment 2 has the highest change in the hematocrit count of the blood after 5 days of feeding. Treatment 3 (37.5g soybean diet) and Treatment 4 (50g commercial pellet diet) shows a negative change in the hematocrit count of the blood after 5 days of feeding. Treatment 4 has the lowest change in the hematocrit count of the blood after 5 days of feeding.

A. Background of the Study

Iron is a very important mineral for the human body. It is found in every cell of the body and is required for the normal function of each cell.

Approximately 80% of total body iron is ultimately incorporated into red cell hemoglobin. An average adult produces 2×10^{11} red cells daily, for a red cell renewal rate of 0.8 percent per day. Each red cell contains more than a billion atoms of iron, and each ml of red cells contains 1 mg of iron. To meet this daily need for 2×10^{20} atoms (or 20 mg) of elemental iron, the body has developed regulatory mechanisms whereby erythropoiesis profoundly influences iron absorption (sickle.bwh.harvard.edu).

Iron can be acquired by eating iron rich foods but not all the iron taken by the body is absorbed. A healthy adult can only absorb 10% to 15% dietary iron, but individual absorption is influenced by several factors. Iron absorption is influenced by a complex mixture of factors, but by far the major ones are the type of iron, Body iron status, iron absorption inhibitors and iron absorption enhancers. Storage levels of iron have the greatest influence on iron absorption. Iron absorption increases when body stores are low. When iron stores are high, iron absorption decreases to help protect against toxic effects of iron overload (ods.od.nih.gov).

Two types of iron are haem iron and non-haem iron. Haem iron is iron as haemoglobin and myoglobin, found in meat, poultry and fish and non-haem iron is iron in the form of ferrous or ferric iron salts, found in non-animal sources,

including plant foods, supplements and fortificants. The nonheme absorption is greatly influenced by various food components.

One good source of nonheme iron is seeds of plants. Their wide use in the human food industry makes them an ideal candidate for study. Some species of seed producing plants are known to be widely distributed around the world. The seeds of Soybean (*Glycine max*) have been studied in the field of iron bioavailability.

Soybean seeds are a rich source of highly bioavailable dietary iron due the presence of stable Fe (II) in its hulls (Laszlo, 1987.)

A previous study proved that soybean seeds significantly increase the iron content of the blood in non-anemic women (Lonnerdal et al., 2005), but they did not include in their study what amount of soybean diet gives the highest iron content in the blood.

B. Statement of the Problem

This study deals with the investigation of the iron content in the blood of non-anemic rabbits (*Oryctolagus cuniculus*) fed with different amounts (0, 25, 37.5, 50 g/kg) of soybean seeds (*Glycine max*).

C. Objectives of the Study

Main Objective: To correlate the amount of red blood cells to the hematocrit count of non-anemic rabbits (*Oryctolagus cuniculus*) fed with different amounts (0, 25, 37.5, 50 g/kg) of soybean seeds (*Glycine max*).

Specific Objectives:

- To perform hematocrit count of blood samples from non-anemic rabbits (*Oryctolagus cuniculus*) before and after being fed with different amounts (0, 25, 37.5, 50 g/kg) of soybean seeds (*Glycine max*) for a period of five (5) days.
- To compare the hematocrit count of blood samples from non-anemic rabbits (*Oryctolagus cuniculus*) before and after being fed with different amounts (0, 25, 37.5, 50 g/kg) of soybean seeds (*Glycine max*) for a period of five (5) days and correlate the results of the hematocrit count of each blood samples.

D. Hypothesis

- (1) There is no significant increase in the hematocrit count of blood samples from non-anemic rabbits (*Oryctolagus cuniculus*) fed with different amounts (0, 25, 37.5, 50 g/kg) of soybean seeds (*Glycine max*).

E. Research Paradigm

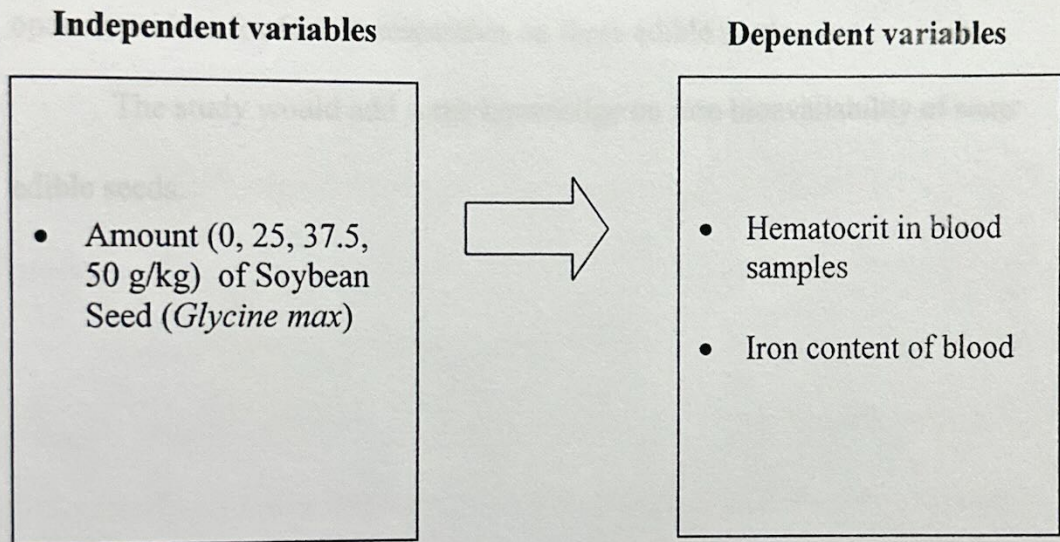


Figure 1. The Research Paradigm

F. Significance of the Study

This study should be able to deal with the investigation of the iron content in the blood of non-anemic rabbits fed with different amounts (0, 25, 37.5, 50 g/kg) of soybean seeds (*Glycine max*) for a period of five (5) days..

In the study, the effect of the amount of iron will be tested whether it will have a great influence on the amount of iron absorbed of a non-anemic organism for a period of five (5) days. If the Soybean (*Glycine max*) would be proven as a useful and practical source for iron even if an organism is non-anemic, it would open new ideas for further researches on these edible seeds.

The study would add some knowledge on iron bioavailability of some edible seeds.

G. Definition of Terms

Amount of Hemoglobin

- The iron-containing respiratory pigment in red blood cells of vertebrates, consisting of about 6 percent heme and 94 percent globin. (Answers.com)

- In this study; it is a parameter that will be related to the amount of red blood cell and to the iron level of a body.

Hematocrit

-measures how much space in the blood is occupied by red blood cells. It is useful when evaluating a person for anemia (Answers.com).

Iron

- A silvery-white, lustrous, malleable, ductile, magnetic or magnetizable, metallic element occurring abundantly in combined forms, notably in hematite, limonite, magnetite, and taconite, and used alloyed in a wide range of important structural materials. (Answers.com)

- In this study; this would refer to the iron converted to hemoglobin.

Iron Absorption

-refers to the amount of dietary iron that the body obtains and uses from food. Healthy adults absorb about 10% to 15% of dietary iron, but individual absorption is influenced by several factors (ods.od.nih.gov)

Rabbit

- Any of various long-eared, short-tailed, burrowing mammals of the family Leporidae, as the commonly domesticated Old World species *Oryctolagus cuniculus* or the cottontail (Answers.com).

Red Blood Cells

- Are the most common type of blood cell and the vertebrate body's principal means of delivering oxygen to the body tissues via the blood. The cells are filled with hemoglobin, a biomolecule that can bind to oxygen. They take up oxygen in the lungs or gills and release it while squeezing through the body's capillaries (ncbi.nlm.nih.gov).
- In this study, it is a parameter that is measured to measure the iron level of a body.

Seed

- A ripened plant ovule containing an embryo. It is also a propagative part of a plant, as a tuber or spore (Answers.com.)

Soybean

- A Southeast Asian annual leguminous plant (*Glycine max*), widely cultivated for forage and soil improvement and for its nutritious seeds.

CHAPTER II

REVIEW OF RELATED LITERATURE

A. Iron

Iron is a trace element which is needed by the body for the formation of blood. The human blood normally contains 3-4 g of iron, more than half of which is in the form of hemoglobin. Hemoglobin transports oxygen from the lungs to the tissues. Iron is a constituent of a number of enzymes. The muscle protein myoglobin contains iron, as does the liver – an important source during the first six months of life. The body's iron balance varies mainly according to dietary intake, as losses from the body are generally small – although women lose iron during menstruation.

B. Soybean (*Glycine max*)

Annual legume soybean (*Glycine max.*) of the pea family has edible seeds. The soybean plant has an erect, branching stem, white to purple flowers, and one to four seeds per pod. It was probably derived from a wild plant of East Asia, where it has been cultivated for some 5,000 years.. Economically the world's most important bean, the soybean provides vegetable protein for millions of people and ingredients for hundreds of chemical products, including paints, adhesives, fertilizers, insect sprays, and fire-extinguisher fluids. Because soybeans contain no starch, they are a good source of protein for diabetics. Processed for food, soybean oil is made into margarine, shortening, and vegetarian cheeses and meats.

Soybean meal serves as a high-protein meat substitute in many food products, including baby foods. Other food products include soybean milk, tofu, salad sprouts, and soy sauce. (Britannica Concise Encyclopedia)

A recent study have results that suggest that IDA in lactation may influence more serious growth and development than those in gestation, soybean hull iron has the higher bioavailability in normal and IDA-induced rats, and thus soybean hull is effective as source of iron supplement. (M. H. Huh. et. al, 1999)

C. Iron Absorption

Numerous factors apparently affect iron absorption. It is known to be dependent upon the iron stores of the test subject, but the results obtained in subjects with apparently similar iron stores and tested under similar conditions vary a great deal indicating that this may not be the primary characteristic affecting iron absorption. The chemical characteristics of the iron source are obviously important but these may react with a variety of other materials in foodstuffs or the gastrointestinal tract which modify iron availability. The relative availability of various iron salts, and of iron in a variety of food stuffs, has been estimated from the rate of hemoglobin synthesis in the body. (E. Amine, D. Hegsted)

The absorption of inorganic iron is obviously dependent upon both the amount and the composition of the salt mixture included in the diet. In some studies the removal of either the phosphate or the calcium from the salt mixture resulted in a greater inhibition of iron absorption than that observed using the

balanced salt mixture. The addition of calcium carbonate itself also inhibited iron absorption. It should be noted that in studies of this kind it is not possible to distinguish clearly between the effects of the material removed from the salt mix and those of the material added. When one compares the effects of the complete salt mixture with those of the calcium- and phosphate-free salt mixtures, one can deduce that the addition of either calcium or phosphate salt may improve iron absorption under certain circumstances whereas excesses of either are detrimental. The production of severe anemia in mice with diets containing 2% calcium carbonate has been reported. The absorption of excessive iron from high iron diets is preventable in rats by the addition of phosphate salts and sodium phytate and phytates also adversely affect the absorption of iron in man. The results can be rationalized as relating to the amount of ionizable phosphate, the formation of insoluble ferric salts, and pH relationships in the gut, but proof is difficult to obtain.

The effects of carbohydrates upon iron absorption have been little studied. The results obtained in the study of E. Hamine and M. Hegsted suggest that a starch diet markedly inhibited iron absorption when compared to the basal diet containing glucose and the disaccharides, especially lactose, which appeared to promote more efficient iron utilization. The effect of lactose on increasing the absorption of calcium salts has usually been attributed to slow rates of absorption and the promotion of acidic conditions in the lower bowel.

Healthy adults absorb about 10% to 15% of dietary iron, but individual absorption is influenced by several factors. Storage levels of iron have the greatest

influence on iron absorption. Iron absorption increases when body stores are low. When iron stores are high, absorption decreases to help protect against toxic effects of iron overload. Iron absorption is also influenced by the type of dietary iron consumed. Absorption of heme iron from meat proteins is efficient. Absorption of heme iron ranges from 15% to 35%, and is not significantly affected by diet. In contrast, 2% to 20% of nonheme iron in plant foods such as rice, maize, black beans, soybeans and wheat is absorbed. Nonheme iron absorption is significantly influenced by various food components

D. Rabbits (*Oryctolagus cuniculus*)

Rabbits are small mammals in the family Leporidae of the order Lagomorpha, found in several parts of the world. Rabbits are ground dwellers that live in environments ranging from desert to tropical forest and wetland. Their natural geographic range encompasses the middle latitudes of the Western Hemisphere. In the Eastern Hemisphere rabbits are found in Europe, portions of Central and Southern Africa, the Indian subcontinent, Sumatra, and Japan. The European rabbit (*Oryctolagus cuniculus*) has been introduced to many locations around the world, and all breeds of domestic rabbit originate from the European (Encyclopedia Britannica.)

Rabbits are herbivores who feed by grazing on grass, forbs, and leafy weeds. In consequence, their diet contains large amounts of cellulose, which is hard to digest. Rabbits solve this problem by passing two distinct types of feces: hard droppings and soft black viscous pellets, the latter of which are immediately

eaten. Rabbits re-ingest their own droppings (rather than chewing the cud as do cows and many other herbivores) to digest their food further and extract sufficient nutrients (oaktreevet.co.uk)

E. Rabbit Pellets

Pellets are most important in the younger stages of rabbit development because they are highly concentrated in nutrients, helping to ensure proper weight gain. A quality pelleted food should be high in fiber (18% minimum) and nutritionally balanced. As a rabbit reaches maturity, however, pellets should make up less of the diet – replaced with higher quantities of hay and vegetables. Overfeeding pellets in mature rabbits can lead to obesity and other medical conditions. (Foster, Smith)

F. Rabbit Blood Collection

This procedure may be used to collect large quantities (10-30 cc) of blood from rabbits, using aseptic technique. In rabbits, a guide to safe blood volume depletion is to collect no more than 5 ml of whole blood per kilogram of body weight every 3 weeks (larger volumes or shorter collection interval need to be discussed with a DLAR veterinarian). DLAR veterinarians are available for training in or consultation regarding any aspect of blood collection in rabbits. Limit a single sampling to 15 % of the total blood volume and allow a 30-day recovery period. Up to 20 % of the blood volume may be feasible in an animal in prime health that is given replacement fluids (4 ml/kg IV isotonic saline). In

general, larger animals have a lower blood volume than smaller animals. If the total blood volume of an animal is unknown, a rough "rule of thumb" is that 6 % of the body weight is blood volume. Rabbit blood volume is normally 56 ml/kg body weight -assuming the animal is mature, healthy, and on an adequate plane of nutrition. Blood loss of 30 % of blood volume, or greater, is life threatening. The common 10 percent-10 percent rule (this estimates a safe volume as 10% of the total blood volume, and that the total blood volume is approximately 10% of the body weight) may result in an excessive blood loss (McGuill, M.W. and Rowan, A.N).

fill a small tube, which is then spun in a small centrifuge. As the tube spins, the red blood cells go to the bottom of the tube, the white blood cells cover the red in a thin layer called the buffy coat, and the liquid plasma rises to the top. The spun tube is examined for the line that divides the red cells from the buffy coat and plasma. The height of the red cell column is measured as a percent of the total blood column. The higher the column of red cells, the higher the hematocrit. (Nardmann, 2006)

G. Hematocrit

The hematocrit measures how much space in the blood is occupied by red blood cells. It is useful when evaluating a person for anemia. The hematocrit is usually done on a person with symptoms of anemia. An anemic person has fewer or smaller than normal red cells. A low hematocrit, combined with other abnormal blood tests, confirms the diagnosis. Some conditions, such as polycythemia, cause an overproduction of red blood cells, resulting in an increased hematocrit.

Blood drawn from a fingerstick is often used for hematocrit testing. The blood fills a small tube, which is then spun in a small centrifuge. As the tube spins, the red blood cells go to the bottom of the tube, the white blood cells cover the red in a thin layer called the buffy coat, and the liquid plasma rises to the top. The spun tube is examined for the line that divides the red cells from the buffy coat and plasma. The height of the red cell column is measured as a percent of the total blood column. The higher the column of red cells, the higher the hematocrit. (Nordenson, 2006)

A. Materials

A1. Pellet Making

- Strainer
- Soybean
- Pigeon pellets
- Measuring cups

A2. Blood Collection

- Hematocrit tubes
- Lancet
- Alcohol
- Sealing clay
- Cotton swab

A3. Equipments

- 2kg weighing scale
- Oven
- Microhematocrit reader
- Centrifuge
- Blender

B. Preparation of Rabbits

B.1 Preparation of Cage

Two cages were prepared with a floor plan of 1m by 1.5m having four occupants each. Each cage will contain two set-ups, having two rabbits for each set-up. Each cage was divided into four parts having one rabbit occupying one part. Each part is provided with two containers, one for water and the other for food.

B.2 Acquisition of Rabbits

Eight male rabbits having approximately the same age have been bought from Panay Rural Development Community Inc. (PRDCI) Matias st. Molo, Iloilo City.

B.3 Assignment of Rabbits

Each rabbit were assigned a number from 1 to 8. The rabbits were then assigned randomly to the four set-ups by drawing lots. After the rabbits are assigned to a set-up, the rabbit will then be assigned a room, but the room assignment will be insignificant to the study.

B.4 Acclimatization of Rabbits

After the rabbits had been transferred to each cage assignment, a two week period will be allotted for the acclimatization of the rabbits by feeding the rabbits daily with commercial pigeon feed along with distilled water supply.

C. Pellet Making and Feeding

C.1 Weighing of Soybeans and Pellets

A two kilograms weighing scale was prepared. A 50 gram diet was prepared for each rabbit. A mixture of 100% ground soybean was weighed and set aside for the first set of rabbits. A mixture of 50% ground soybean and 50% ground commercial pellets was weighed and set aside for the second set of rabbits. A mixture of 75% ground soybean and 25% ground commercial pellets was weighed and set aside for the third set of rabbits. A mixture of 100% commercial pellets was weighed and set aside for the fourth set of rabbits.

C.2 Cooking of Pellets

The pre-weighed mixture of soybean and commercial pellets was mixed with 50g corn starch and 50 ml water. It was then manually formed into long sticks. The solution was then baked in the oven at 200 F for 10 minutes. The cooked pellets were then allowed to cool off. The long sticks was then chopped and prepared for feeding. The pellets are given to the rabbits once a day at the same time.

D. Blood Extraction

D.1 Preparation of Sampling Area

The ears of the rabbits were cleaned using alcohol swabs. The ears were then pierced by using lancets. Clean cotton swabs were used to clean the first drops of blood on the ears.

D.2 Blood Collection

A hematocrit tube was used to collect blood from the wound. The blood was allowed to fill the tubes. It may help if the tube was directed downwards. As the tube gradually fills, it was angled upwards to slow the blood flow into the tube. Ideally no air bubbles should be present in the tube. Air bubbles generally indicate that an inadequate amount of blood has been absorbed by the tube. Squeezing the ears to increase blood flow is not advisable. This can dilute the blood with other body fluids present in the finger and result in an inaccurately low hematocrit value. If the puncture is deep enough there should be adequate blood to fill one capillary tube. When the tube is filled with blood up to the calibration mark (red line), remove the tube from the puncture wound. A clay sealant was then used to seal the tube. The tube was then cleaned using a lint-free tissue to remove the blood outside the tube.

This process can be repeated until three samples are acquired. This process is done everyday for a period of five days.

E. Hematocrit Count

E.1 Operating the Centrifuge

The centrifuge is opened by removing its cover. The capillary tubes are placed in each of the grooves of the centrifuge, and properly numbered for identification purposes. The tubes were oriented in the grooves such that the plugged ends are facing the opening of the centrifuge.

Unless the centrifuge specifies otherwise, the capillary tubes must always balance in the rotor. That is, tubes should always be placed across from each other in the rotor. If there is an odd number of tubes an empty tube should be placed across from the single tube. The top was screwed onto the rotor. The rotor was then firmly attached inside the centrifuge. The lid of the centrifuge was closed and locked. The "Start" button on the centrifuge was pushed. The rotor in the centrifuge should start spinning. The centrifuge will spin for a preset amount of time and then stop. After the rotor had stopped spinning, the lid of the centrifuge was opened and the tubes were removed. The lid of the centrifuge should not be opened while the rotor is still spinning.

E.2 Reading the Hematocrit Tube

On a flat table, the hematocrit tube was laid on the microhematocrit reader. The sealed end of the tube was aligned with the "0" percent mark and the tube was perpendicular to this line. The tube was then moved side to side along the bottom line until the top of the clear fluid (plasma) intersected the "100 percent line." Once the tube was positioned so that the top of the clay is on the "0" line and the top of the plasma is on the "100" line (and the tube should be perpendicular to the "0" line), the line that passes through where the plasma and the red blood cells come together (i.e., the top of the red blood cells) was read. This line gives the value for the hematocrit.

CHAPTER IV

RESULTS AND DISCUSSIONS

This study aims to investigate on the possible increase of the hematocrit count in the blood of rabbits (*Oryctolagus cuniculus*) fed with different concentrations of Soybean Seeds (*Glycine max*).

Specifically, this study aims to determine the hematocrit count of blood samples from rabbits (*Oryctolagus cuniculus*) fed with different concentrations of Soybean Seeds (*Glycine max*) and compare the hematocrit count of each treatment.

Figure 1: The graph shows the day to day change in the hematocrit count of the organisms treated with Treatment 1 in a five day period.

The results show that there is no steady increase in the values. Based on the results shown above there is a 0.00% difference between Day 1 and day 5 in the results given by the rabbit in Treatment 1. There is an increase in the hematocrit count of the blood from the rabbit treated with Treatment 1 (50g soybean diet) between day 1 and day 5.

Results:

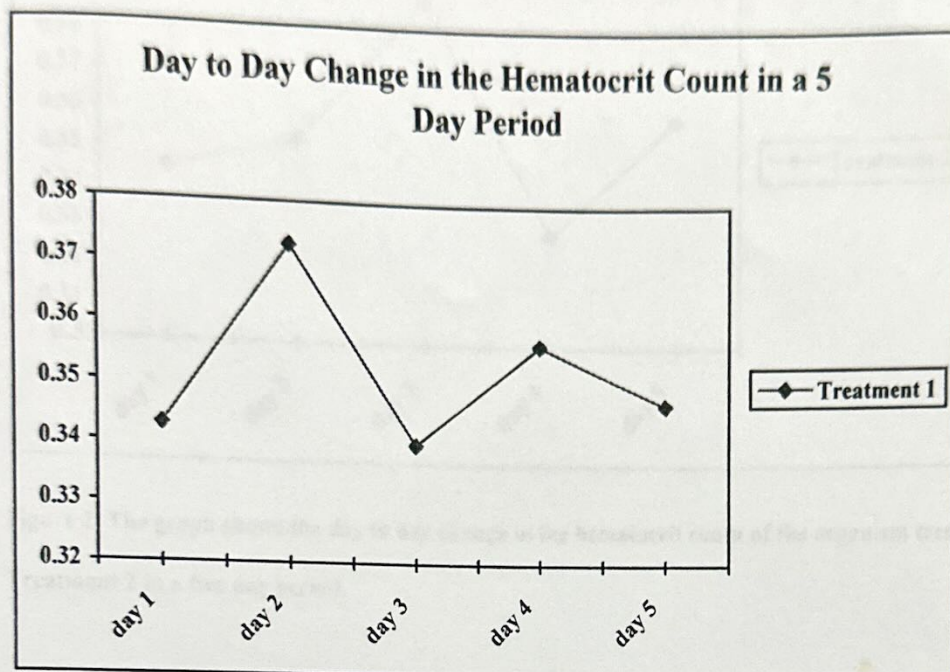


Figure 1. The graph shows the day to day change in the hematocrit count of the organism treated with Treatment 1 in a five day period.

The results show that there is no steady increase in the values. Based on the results shown above there is a 0.004 difference between day 1 and day 5 in the results given by the rabbit in Treatment 1. There is an increase in the hematocrit count of the blood from the rabbit treated with Treatment 1 (50g soybean diet) between day 1 and day 5.

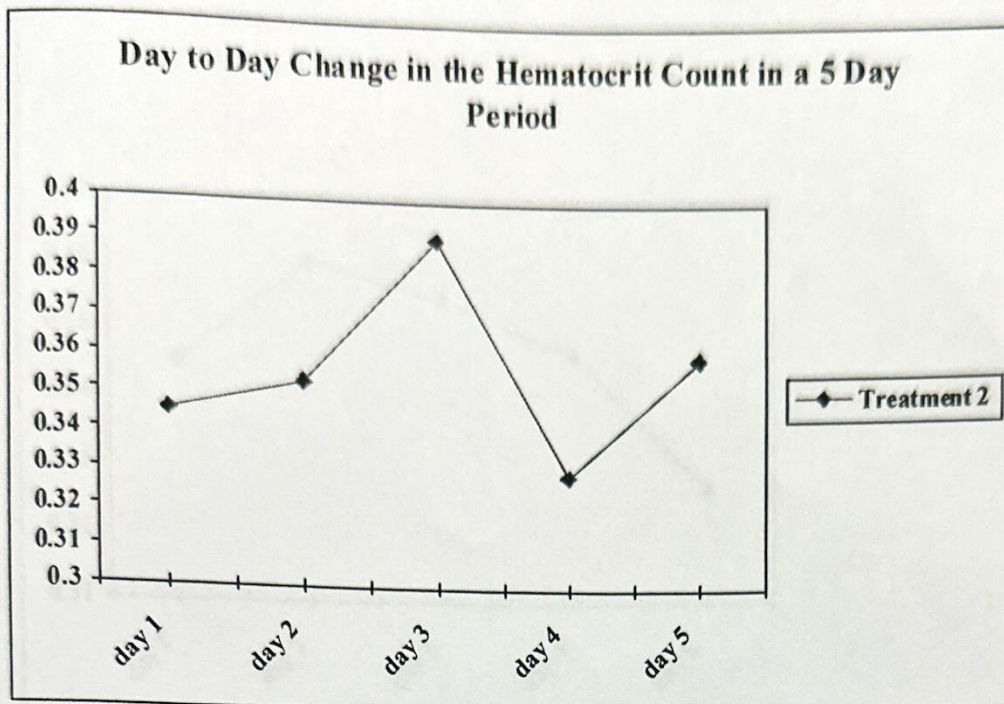


Figure 2. The graph shows the day to day change in the hematocrit count of the organism treated with Treatment 2 in a five day period.

The results show that there is no steady increase in the values. Based on the results shown above there is a 0.015 difference between day 1 and day 5 in the results given by the rabbit in Treatment 2. There is an increase in the hematocrit count of the blood from the rabbit treated with Treatment 2 (25g soybean and 25g commercial pellet diet) between day 1 and day 5.

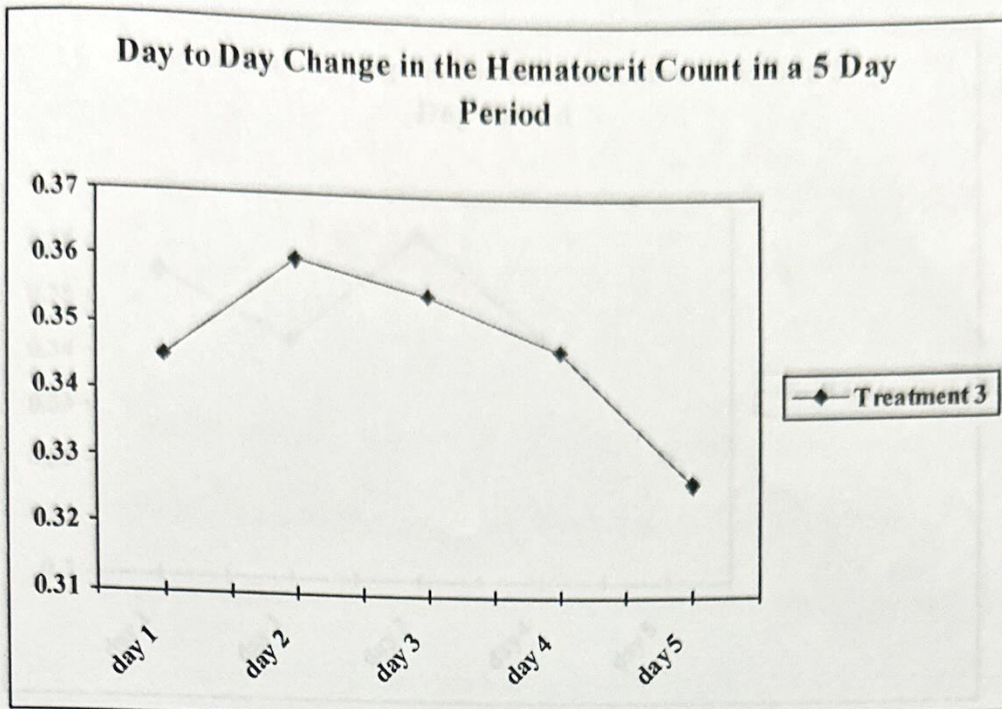


Figure 3. The graph shows the day to day change in the hematocrit count of the organism treated with Treatment 3 in a five day period.

The results show that there is no steady increase in the values. Based on the results shown above there is a - 0.018 difference or increase between day 1 and day 5 in the results given by the rabbit in Treatment 3. There is a decrease in the hematocrit count of the blood from the rabbit treated with Treatment 3 (37.5g soybean and 12.5g commercial pellet diet) between day 1 and day 5.

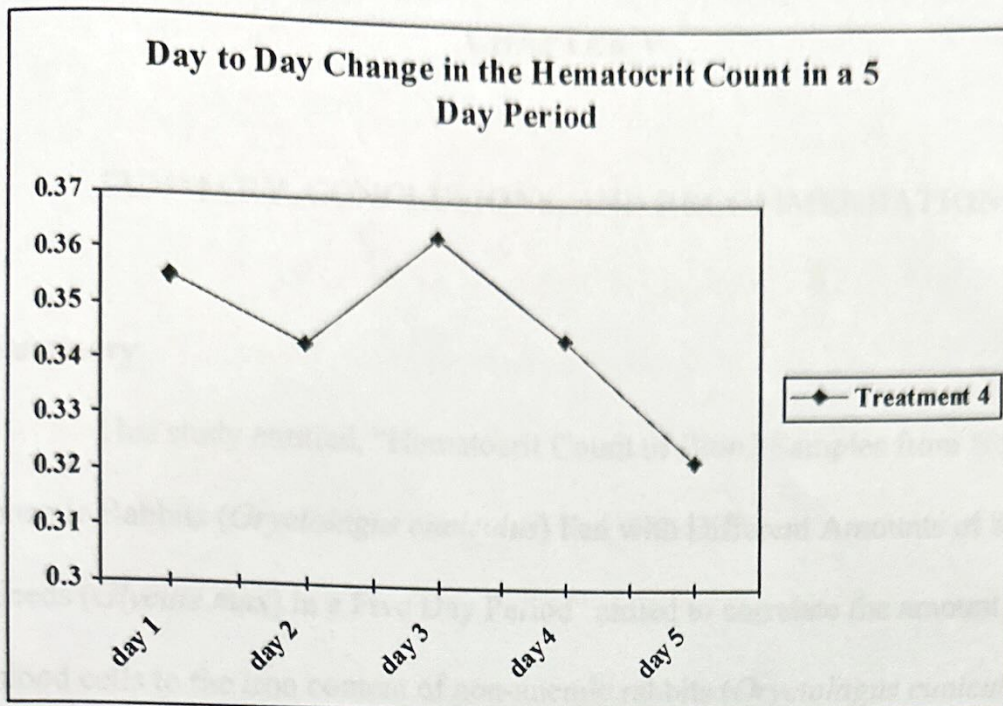


Figure 4. The graph shows the day to day change in the hematocrit count of the organism treated with Treatment 4 in a five day period.

The results show that there is no steady increase in the values. Based on the results shown above there is a - 0.032 difference between day 1 and day 5 in the results given by the rabbit in Treatment 4. There is a decrease in the hematocrit count of the blood from the rabbit treated with Treatment 4 (50g commercial pellet diet) between day 1 and day 5.

CHAPTER V

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

Summary

This study entitled, "Hematocrit Count of Blood Samples from Non-anemic Rabbits (*Oryctolagus cuniculus*) Fed with Different Amounts of Soybean Seeds (*Glycine max*) in a Five Day Period" aimed to correlate the amount of red blood cells to the iron content of non-anemic rabbits (*Oryctolagus cuniculus*) fed with different amounts (0, 25, 37.5, 50 g/kg) of soybean seeds (*Glycine max*).

This study specifically aimed to:

- To determine the Hematocrit count of blood samples from non-anemic rabbits (*Oryctolagus cuniculus*) before and after being fed with different amounts (0, 25, 37.5, 50 g/kg) of soybean seeds (*Glycine max*) for a period of five (5) days.
- To compare the Hematocrit count of blood samples from non-anemic rabbits (*Oryctolagus cuniculus*) before and after being fed with different amounts (0, 25, 37.5, 50 g/kg) of soybean seeds (*Glycine max*) for a period of five (5) days.

Summary of Results

Based on the results gathered it can be observed that Treatments 1 and 2 showed a positive change in the hematocrit count of the blood between day 1 and day 5. However Treatment 2 (25g soybean diet) compared to the other treatments has the highest change in the hematocrit count of the blood between day 1 and day 5. Treatment 3 (37.5g soybean diet) and Treatment 4 (50g commercial pellet diet) does not increase the hematocrit count of the blood and shows a negative change in the hematocrit count of the blood between day 1 and day 5. Treatment 4 (50g commercial pellet diet) compared to the other treatments has the lowest change in the hematocrit count of the blood between day 1 and day 5.

Conclusions

- Treatment 1 and Treatment 2 increased the hematocrit count of the blood from the rabbits.
- After five days of feeding, Treatment 2 (25g soybean diet) gave the highest change in the hematocrit count of the blood from the rabbits.
- After five days of feeding Treatment 4 (50g commercial pellet diet) gave the lowest change in the hematocrit count of the blood from the rabbits.
- Treatment 3 and Treatment 4 did not increase the hematocrit count of the blood from the rabbits.

Recommendations

To further extend the impact and the significance of the study and to provide future view and opinion, we recommend the following:

- Further studies should use a standard of one month or longer for the whole implementation of the diet in order to obtain accurate quantitative values.
- Researchers should use tests organism that have same age and weight in order to obtain accurate quantitative values.
- Future studies should use spectrophotometry to obtain accurate quantitative values to determine which of the different treatments has the highest amount of iron absorbed.

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Appendix

A. Raw Data with their Sample Mean and Variance

Table 4. Raw data of the experiment with their sample mean and variance.

	Day 1	Day 2	Day 3	Day 4	Day 5	Mean
Rabbit 1						
Sample 1	0.34	0.37	0.34	0.36	0.35	0.34
Sample 2	0.34	0.38	0.34	0.36	0.34	0.34
Sample 3	0.35	0.37	0.34	0.35	0.35	0.35
Mean	0.343 ± 0.00577	0.373 ± 0.00577	0.34	0.357 ± 0.00577	0.347 ± 0.00577	0.343 ± 0.00577
Rabbit 2						
Sample 1	0.35	0.36	0.39	-	0.35	0.35
Sample 2	0.34	0.35	0.39	0.33	0.36	0.34
Sample 3	-	0.35	0.39	-	0.37	-
Mean	0.345 ± 0.00577	0.353 ± 0.00577	0.39	0.33	0.36 ± 0.0001	0.345 ± 0.00577
Rabbit 3						
Sample 1	0.35	0.35	0.35	0.35	0.33	0.35
Sample 2	0.34	0.37	0.36	0.35	0.33	0.34
Sample 3	0.34	0.36	0.37	0.34	0.32	0.34
Mean	0.345 ± 0.00577	0.36 ± 0.0001	0.355 ± 0.0001	0.347 ± 0.00577	0.327 ± 0.00577	0.345 ± 0.00577
Rabbit 4						
Sample 1	0.36	0.35	0.37	-	0.32	0.36
Sample 2	0.35	0.34	0.36	0.35	0.32	0.35
Sample 3	-	0.34	0.36	0.34	0.33	-
Mean	0.355 ± 0.0001	0.343 ± 0.00577	0.363 ± 0.00577	0.345 ± 0.00577	0.323 ± 0.00577	0.355 ± 0.0001

1.1 Set-up 1

Rabbit 1 – 100% Soybean Diet

1.2 Set-up 2

Rabbit 2 – 50% Soybean and 50% Commercial Pellet Diet

1.3 Set-up 3

Rabbit 3 – 75% Soybean and 25% Commercial Pellet Diet

1.4 Set-up 4

Rabbit 4– 100% Commercial Pellet Diet

Figure 1: Diagram of the experimental setup.

B.Plates

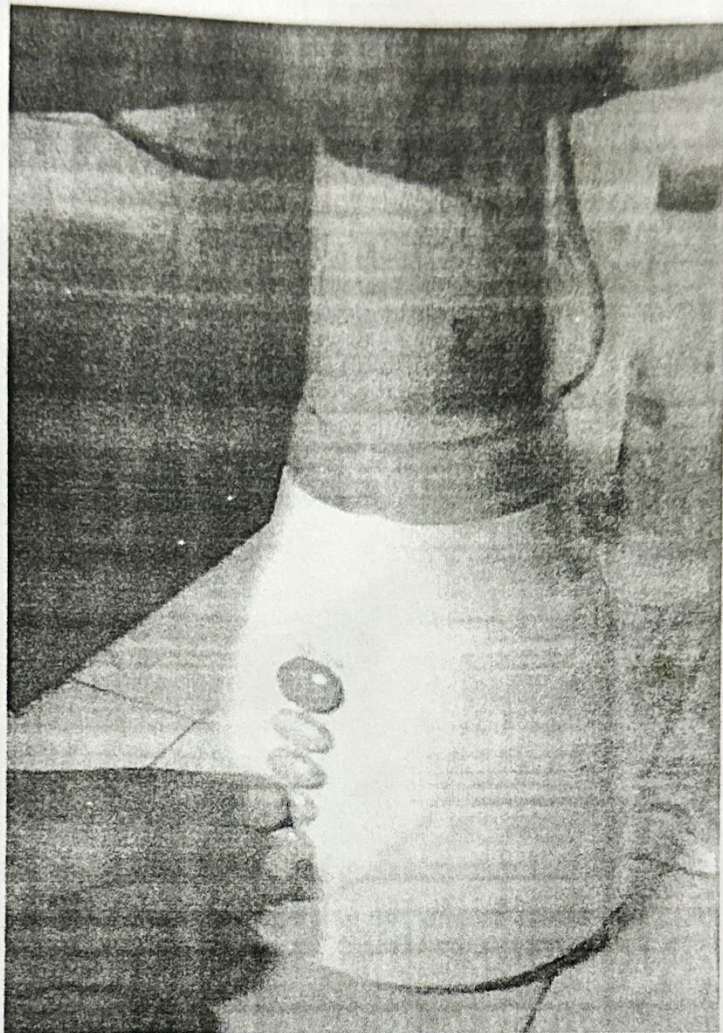


Plate 1: Grinding the soybean seeds.



Plate 2: Screening of ground soybean seeds.



Plate 4: Shaping the ingredients into a specific form.

Plate 3: Weighing the ingredients.



Plate 4: Shaping the dough (mixture of ground soybean seeds and starch).

Plate 5: The formed dough before being cooked in the oven.

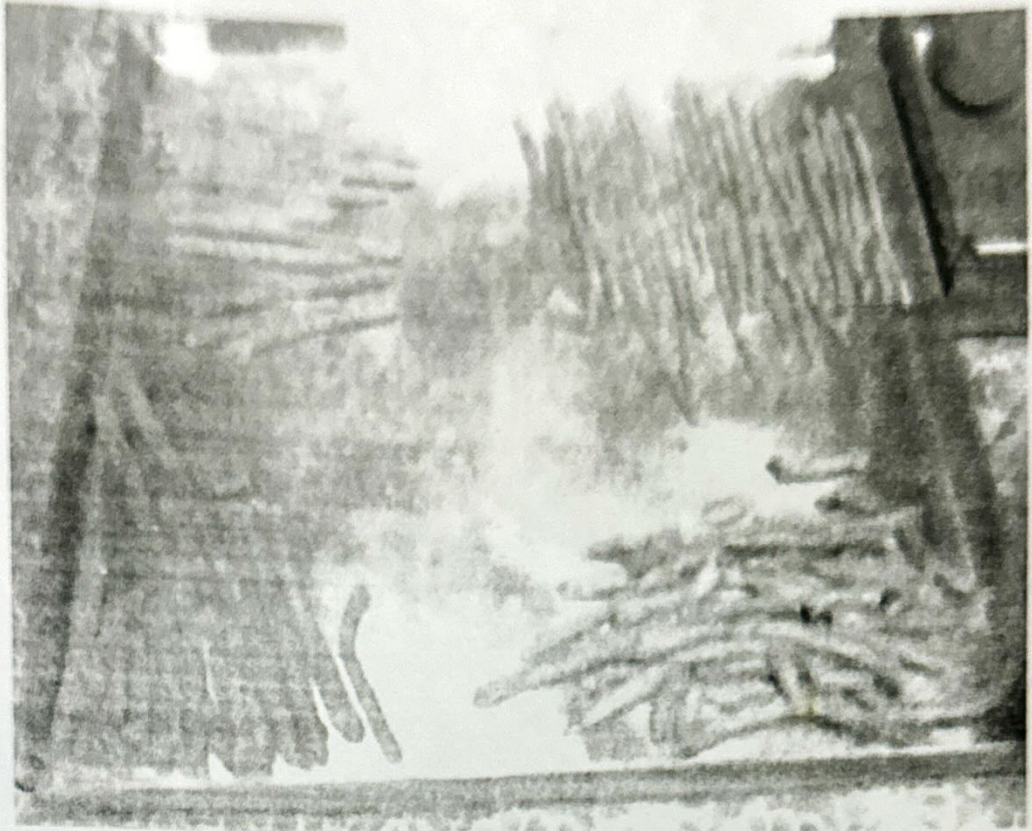


Plate 5: The formed dough before being cooked in the oven.

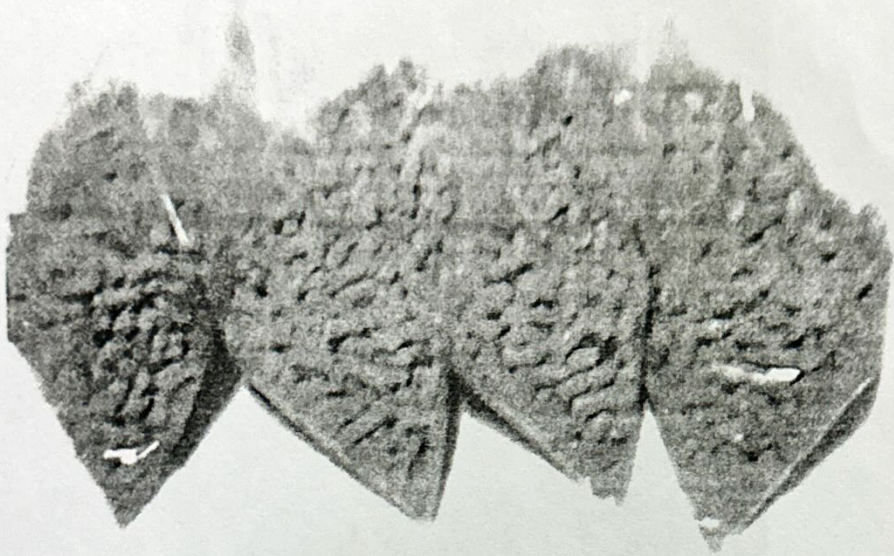


Plate 6: The packed soybean pellets.



Plate 7: The blood collected and labeled.

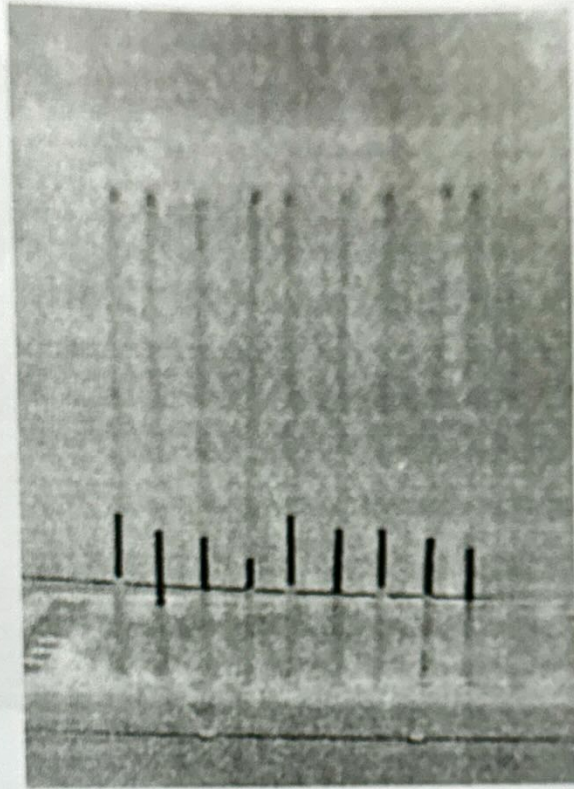


Plate 8: The hematocrit tubes before being centrifuged.

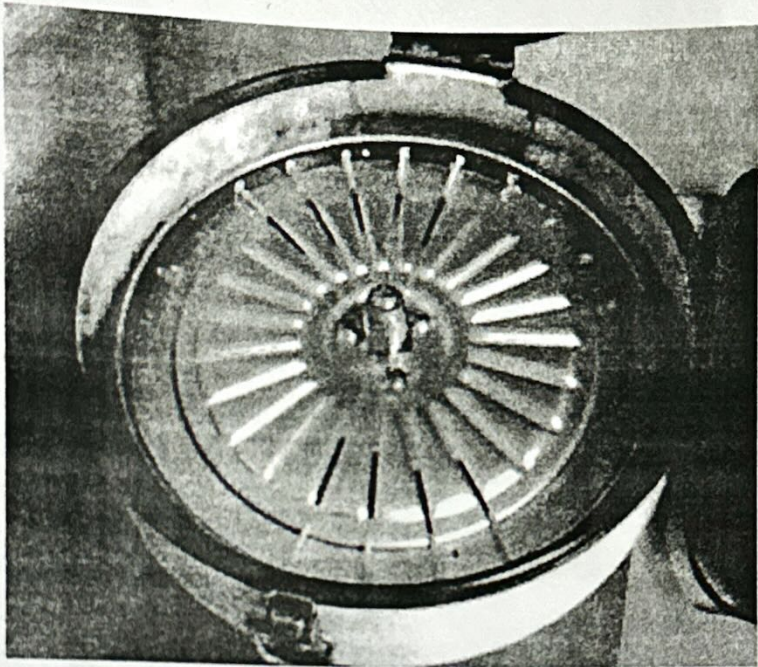


Plate 9: The hematocrit tubes were put in the centrifuge.

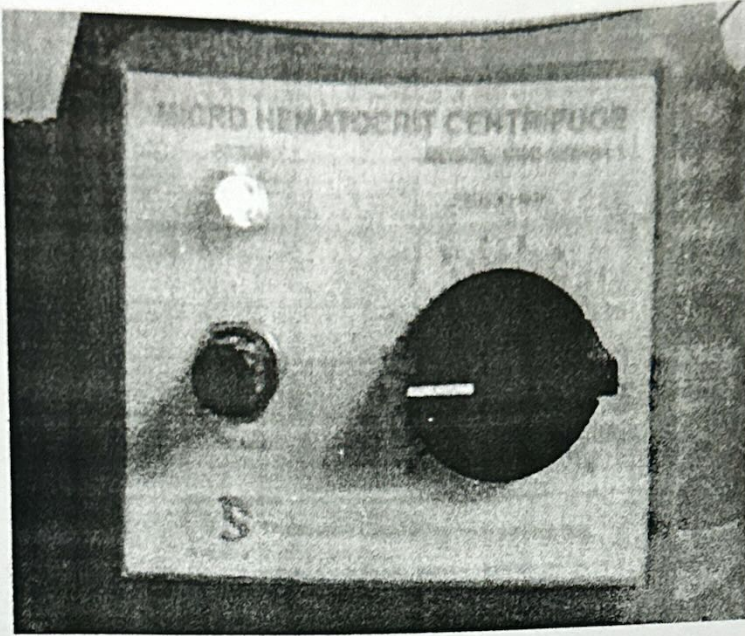


Plate 10: The centrifuge is set for five (5) minutes.

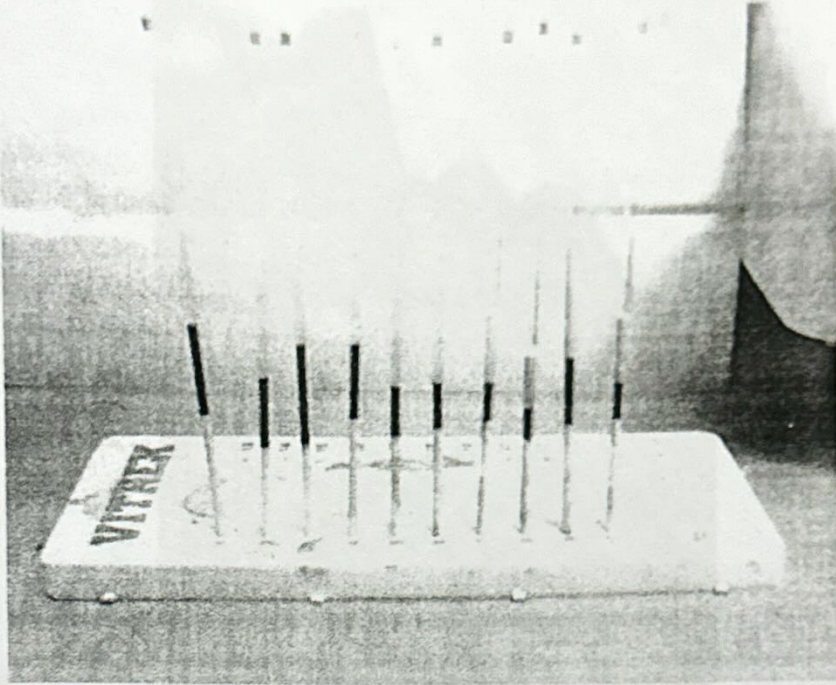


Plate 11: The hematocrit tubes after being centrifuged.

Plate 12: Reading the hematocrit tubes with a Microhematocrit Reader.

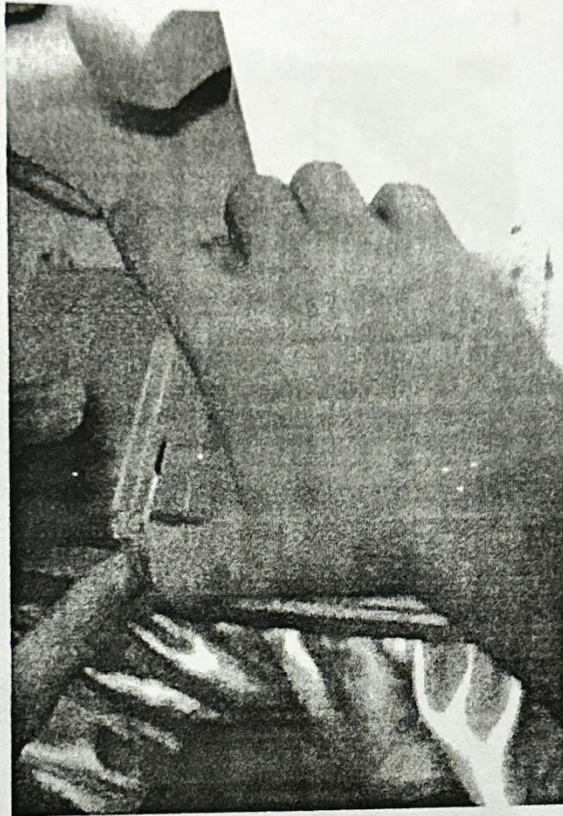


Plate 12: Reading the hematocrit tubes with a Microhematocrit Reader.



Plate 13: Tabulating the results.