SYNERGETIC RESPONSES OF HAEMATOPOIETIC HORMONES ON DIETARY PROTEIN SUPPLEMENTATION IN PHLEBOTOMIZED NEW ZEALAND WHITE RABBITS

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ABSTRACT

Background: Nutrition plays an important role in the management of blood dyscrasia due liver and kidney diseases. These disease conditions are usually accompanied by haematopathology which requires separate managements. There is need to provide information on the role that major components of the diet play in these conditions. This study compared at intervals, the values of erythropoietin and thrombopoietin in phlebotomized New Zealand white rabbits.

Aim: To determine the levels of haematopoietic hormones (erythropoietin and thrombopoietin) in phlebotomised New Zealand white rabbits maintained on protein rich and hypoproteic diets.

Materials and Methods: Twenty (20) New Zealand white rabbits were maintained on a diet containing 30% Protein for a period of four weeks (BASELINE), bled and grouped (TREATMENT) as follows A (n=5)– hypoproteic diet, B (n=5)–30% Protein rich diet, C (n=5)–hypoproteic diet (Phlebotomized), D (n=5)–30% Protein (Phlebotomized). Blood samples were obtained from the marginal earvein of the Rabbits after the period of acclimatization (4 weeks) to obtain baseline data. The animals were maintained on the indicated feed and also bled every other day to reduce the haematocrit by 20% and determine erythropoietin and thrombopoietin levels. The animals were sacrificed and the kidney and liver were harvested for histological studies.

Result: There was a significant decrease ($p \leq 0.05$) in the response of thrombopoietin and erythropoietin in New Zealand white rabbits fed with hypoproteic diet as compared to those fed with protein rich (30% protein) diet.

Conclusion: The outcome of this study suggests that dietary protein supplementation can improve blood dyscrasia resulting from active bleeding, malnutrition and thrombocytopenia caused by increased platelet loss due to bleeding.

Keywords: New Zealand White rabbits, Dietary Protein, Thrombopoietin, Erythropoietin, Phlebotomy.

INTRODUCTION

Proteins are macromolecules, consisting of one or more long chains of amino acid residues which perform a vast array of functions within living organisms, including catalyzing metabolic reactions, deoxyribonucleic acid (DNA) replication, response to stimuli, and transporting molecules from one location to another (Fernandez and Scott, 2003). Proteins differ from one another primarily in their sequence of amino acids, which is dictated by the nucleotide sequence of their genes, and which usually results in protein folding into a specific three-dimensional structure that determines its activity (Dobson, 2000).

Proteins are essential nutrients for the human body and they are one of the building blocks of body tissue. Like other biological macromolecules, proteins are essential parts of organisms and participate virtually in every process within cells (Whitney and Rolfes, 2013). Many proteins are enzymes that catalyze biochemical reactions and are vital to metabolism.

Proteins also play an important role in cell signaling, immune responses, cell adhesion, and the cell cycle (Food and nutrition board, 2005). Proteins are also necessary in animals' diets, since animals cannot synthesize all the amino acids they need and must obtain essential amino acids from food (Whitney and Rolfes 2013).

Once proteins are formed, they only exist for a certain period of time after which they are degraded and recycled by the cell's machinery through the process of protein turnover (Gutteridge, *et al*., 2005). A protein's lifespan is measured in terms of its half-life and covers a wide range. They can exist for minutes or years with an average lifespan of 1–2 days in mammalian cells. Abnormal and or misfolded proteins are degraded more rapidly either due to being targeted for destruction or due to being unstable (Gutteride, *et al*., 2005).

Thrombopoietin, also known as megakaryocyte growth factor is a glycoprotein which is encoded by thrombopoietin (TPO) gene. It is produced by the liver and kidney and it functions to stimulate the production and differentiation of megakaryoctes from which large number of platelets bud off (Kaushansky, 2006).

Thrombopoietin is encoded by the TPO gene which is located on the long arm of chromosome 3 *(q26.3-27).* Abnormalities in this gene occur in some kind of hereditary forms of thrombocytosis (high platelet count) and in some cases of Leukemia (McKusick, 2007).

Thrombopoietin is produced by parenchyma and sinusoidal endothelial cells in the liver and also in the kidney by the proximal convoluted tubule cells. Small amounts are produced by striated muscle and bone marrow stromal cells. Bone marrow stromal cells and the liver are the major sites of thrombopoietin production (Eva-Maria and Wolfgang, 2002).

Normally, the plasma level of thrombopoietin is relatively low in human (about 10-12 mol/l). However, in thrombocytopenic states due to marrow failure or bleeding, the concentration of circulating thrombopoietin may increase greatly. The simple feedback regulation by thrombopoietin and its target cells is efficient in maintaining constant platelet numbers in healthy individuals. Persisting thrombocytopenia develops only in severe liver or marrow failure. On the other hand, an increase in circulating thrombopoietin and interleukin 6 (IL-6) may cause reactive thrombocytosis in inflammatory diseases, including cancer (Jelkmann, 2003).

The rising and dropping of platelet concentrations regulates the Thrombopoietin levels. Reduced platelets lead to a higher degree of Thrombopoietin exposure to the undifferentiated bone marrow cells leaving to differentiation of megakaryocytes and further maturation of the cells. On the other hand, increased platelets leads to reduced availability of thrombopoietin (Kaushasky, 2006).

Erythropoietin (also known as hemopoietin, hematopoietin or EPO) is a glycoprotein hormone that controls the production of red blood cells (erythropoiesis). It is a protein signaling molecule (cytokine) for red blood cell precursors in the bone marrow and it has a molecular weight of 34kDa (Malineux, 2005).

Erythropoietin is produced by intestinal fibroblasts in the kidney in close association with peritubular capillary and proximal convoluted tubule. It is also produced in perisinusoidal cells in the liver. While liver production predominates in the fetal and perinatal period, renal production is predominant during adulthood. In adults, erythropoietin is produced to a lesser extent by the liver (about 10%). The erythropoietin gene has been found on the long arm of chromosome 7(7q21)(Malineux, 2005).

The kidney cells that produce the erythropoietin are sensitive to low oxygen tension in the blood that travels through the kidney. A low oxygen level in the blood may be as result of reduced number of circulating red blood cells (anaemia) or reduced amount of haemoglobin molecules that transport oxygen though the body.

Erythropoietin stimulates the bone marrow to produce more red blood cells which consequently increases the oxygen carrying capability of the blood. As a prime regulator of erythropoiesis, its major functions are to promote the development of red blood cells production and also to initiate the synthesis of hemoglobin (Kaushansky, 2011).

Erythropoietin hormone can be detected and measured in the blood; Normal levels of erythropoietin range from 0-19mU/ml (milliunits per milliliter). An abnormal level of erythropoietin in the blood can indicate bone marrow disorders (such as polycythemia) or kidney disease. Testing erythropoietin blood levels is of value if:

- Too little erythropoietin might be responsible for too few red blood cells (anemia), especially anemia related to kidney disease.
- Too much erythropoietin might be causing too many red blood cells (polycythemia).
- Too much erythropoietin might be evidence for a kidney tumor (Kremyanskaya *et al.,* 2013).

The correct interpretation of an abnormal erythropoietin level depends on the particular clinical situation. Sometimes, the erythropoetin level may be inappropriately normal when it should be elevated (such as when there is an anemia), indicating a problem with the kidneys (Kremyanskaya *et al.,* 2013).

Protein adequacy is a factor in erythropoietin productionas inadequate protein nutrition can cause a reduction in erythropoietin production. The erythroid response to erythropoietin highly depends on the adequacy and quality of dietary protein (El-Nawawy *et al.,* 2002).

According to Akiko *et al.,* 2008 the mouse spleen is an erythropoietic organ, which contains an erythropoietin-responsive cell population that can be easily amplified by administration of the hormone. Researchers determined the effect of a protein-free diet offered freely to mice up to two days after injection of r-Hu EPO (1000mU/200 ul) on the response of the above population. Splenic cell suspensions from control and experimental mice were prepared in microwells containing 400 mU r-Hu erythropoietin and appropriate medium. The response to erythropoietin was evaluated in terms of 3H-thymidine uptake. The results obtained indicate that acutely induced protein restriction suppressed the response of the erythropoietin-responsive splenic cell population to erythropoietin when it was imposed on mice immediately after hormone injection, and suggest the appearance of deficient rates of differentiation of erythropoietic units by protein restriction.

Protein deprivation decreased the number of erythropoietin-responsive cells in spleen. This result indicates the impairment of erythropoiesis during protein deficiency caused by the decrease in serum erythropoietin and the subsequent reduction of the population size of erythroid precursor cells in spleen (Frydlova, 2016).

Two likely causes of the depression of erythropoiesis in protein-deprived rats were suggested to be:

1) Reduced protein synthesis in erythroid precursor cells because of insufficient precursors such as amino acids, and

2) A decrease in erythropoietin in the circulation.

The former possibility was excluded and a decrease in serum erythropoietin upon protein deprivation was found. In those experiments, rats deprived of protein or fed protein were exposed to reduced atmospheric pressure so that the serum erythropoietin would increase to a level detectable by an in vivo assay. The increase in erythropoietin concentration in the rats deprived of protein was lower than that in rats fed protein (Frydlova, 2016).

Without erythropoietin, definitive erythropoiesis will not take place in the bone marrow. Under low oxygen tension (hypoxic conditions), the kidney will produce and secrete erythropoietin to increase the production of red blood cells by targeting CFU-E, proerythroblast and basophilic erythroblast subsets in the differentiation.

It has its primary effect on red blood cell progenitors and precursors found in the bone marrow by promoting their survival through protecting these cells from apoptosis (Malineux, 2005).

Erythropoietin levels in blood are usually low (about 10mU/ml) in the absence of anaemia. However, in hypoxic stress, erythropoietin level may increase upto 1000 folds. Regulation of erythropoietin is believed to rely on feedback mechanism measuring iron availability and blood/tissue oxygenation (Jelkmann, 2007).

Erythropoietin is the primary erythropoietic factor that cooperates with various other growth factors (e.g., IL-3, IL-6, glucocorticoids, and stem cell factor (SCF)) involved in the development of erythroid lineage from multipotent progenitors. The burst-forming unit-erythroid (BFU-E) cells start erythropoietin receptor expression and are sensitive to erythropoietin. Subsequent stage, the colony-forming unit-erythroid (CFU-E), expresses maximal erythropoietin receptor density and is completely dependent on erythropoietin for further differentiation. Precursors of red cells, the proerythroblasts and basophilic erythroblasts also express erythropoietin receptor and therefore affected by it (Rakesh *et al.,* 2016).

The liver plays an important role in the production of hemopoietic hormones, i.e. Thrombopoietin and erythropoietin. It is the primary site of erythropoietin synthesis in the fetal stage, and it is the predominant thrombopoietin-producing organ for life. In contrast to the erythopoietin and other liver proteins, the hepatic synthesis of thrombopoietin is influenced little by external signals. Hepatocytes express the thrombopoietin gene in a constitutive way, i.e. irrespective of the level of platelets in blood, Megakaryocytes and platelets remove the hormone from blood by means of their high-affinity thrombopoietin receptors (Jelkmann, 2001).

Study Area

The study was conducted in the animal house of the College of Medicine and Health Sciences, Afe Babalola University, Ado-Ekiti. Laboratory Analysis was carried out in Department of Medical Laboratory Science, Afe Babalola University, Ado Ekiti.

Animals Housing

The animals were housed in hanging stainless-steel wire cages kept in an isolated room at a controlled temperature (23–25°C) and humidity (40–60%) with 12hours lighting.

Study Population

Twenty (20) New Zealand White Rabbits were used based on calculation by "Resource Equation Method".

In this study, four (4) groups of animals were formed having 5 animals each for different interventions, therefore the total animals were 20 (5 × 4).

Inclusion criteria

1. New Zealand White Rabbit with body weight within the range 1000g ± 50g
2. Apparently healthy animals with normal body temperature and baseline haematological Indicies

Exclusion criteria

1. Rabbits with body weight </>1000g ± 50g.
2. Rabbits showing signs of ill health and/or raised body temperature.

Grouping and Treatments

20 Rabbits was maintained on a diet containing 30% Protein for a period of four weeks (BASELINE), bled and grouped (TREATMENT) as follows:

A (n=5) – hypoproteic diet
B (n=5) – 30% Protein
C (n=5) – hypoproteic diet (Phlebotomized)
D (n=5) – 30% Protein (Phlebotomized)

The animals were maintained on the indicated feed and bled fortnightly for laboratory investigation.

Anaemia was induced in animals in group C and D by phlebotomy. The group specific experimental diet was administered after a reduction in haematocrit by 20%.

Blood Sample Collection

Blood samples were obtained from the marginal ear vein of the Rabbits after the period of acclimatization (4 weeks) which will provide baseline data and at two weeks intervals post-acclimatization.

Samples were taken during the treatment period into 2 ml EDTA tubes and 10ml plain tubes. The Sample obtained into plain tubes were spun in a bucket centrifuge. The serum was aspirated and kept frozen at 4^0C before analysis.

After the experiment, the animals were sacrificed and the kidney and liver were harvested for histological studies.

Measurement of Variables

Measurement of variables were done using the methods described by Lewis *et al.*, 2006.

1. Erythropoietin (ELISA Method)
2. Thrombopoietin (ELISA Method)
3. Organs (kidney and Liver) Histology using Haematoxylin and Eosin staining procedure.

RESULTS

The primary purpose of this study was to examine the differences in the responses of thrombopoietin and erythropoietin to dietary protein supplementation in New Zealand white rabbits.

The results of the analysis showed significant ($p \leq 0.05$) increase in the response of erythropoietin and thrombopoietin in New Zealand white rabbits fed 30% protein diet compared to those maintained on 4% protein diet as seen in tables 1 and 2 respectively.

As expected, the erythropoietin and thrombopoietin levels progressively increased with serial phlebotomy. Values of erythropoietin levels obtained in New Zealand white rabbits fed 30% protein diet and phlebotomized (Group D) was significantly increased ($p \leq 0.05$) at day three of phlebotomy and days two and three post-phlebotomy compared to those obtained from New Zealand white rabbits fed 4% protein diet and phlebotomized (Group C). On the other hand, Values obtained in the New Zealand white rabbits fed 30% protein diet without phlebotomy (Group B) was significantly increased ($p \leq 0.05$) at day two, five and six compared to the values obtained from new Zealand white rabbits fed 4% protein diet (Group A) as seen in Table 1.

The thrombopoietin levels obtained in New Zealand white rabbits fed 30% protein diet and phlebotomized (Group D) was significantly increased ($p \leq 0.05$) at days two and three of phlebotomy compared to those obtained from New Zealand white rabbits fed 4% protein diet and phlebotomized (Group C). On the other hand, Values obtained in the New Zealand white rabbits fed 30% protein diet and unphlebotomized (Group B) was significantly high ($p \leq 0.05$) at days two, four and five compared to the values obtained from new Zealand white rabbits fed 4% protein diet unphlebotomized (Group A) as presented in Table 2.

STATISTICAL ANALYSIS

Data was analysed using the Statsview® statistical program. Significance of differences was determined by One way analysis of variance (ANOVA), with post-hoc tests for variant means (LSD, Duncans). Version 5.0.1 SAS Institute Inc, 1995-1998, Cary, NC). $P<0.05$ was considered significant

Table 1: Serum erythropoietin levels in New Zealand white rabbits fed hypoproteic diet (Group A), 30% protein diet (Group B), hypoproteic diet and phlebotomized (Group C) and 30% protein diet and phlebotomize (Group D).

Groups	A(mU/ml)	B(mU/ml)	C(mU/ml)	D(mU/ml)
Baseline	25±7.5	36±14.4	23±6.9	43±21.3
Phlebotomy	28±5	32±16	27±10.8	88±35.2
	21±7.35	38±11.4*	18±9	62±21.7
	18±7.2	34±13.6	15±9	57±19.95+
Post-phlebotomy	21±6	37±11.1	16±6.4	41±12.3
	23±5	36±21.6*	20±10	38±19+
	17±10.2	32±9.6*	18±7.2	32±11.2+
	23±6.9	31	21±6.3	35±10.5

* -A Vs B- Significant difference ($p \leq .0.05$)
+ -C Vs D- Significant difference ($p \leq .0.05$)

Table 2: Serum thrombopoietin levels in New Zealand white rabbits fed hypoproteic diet (Group A), 30% protein diet (Group B), hypoproteic diet and phlebotomized (Group C) and 30% protein diet and phlebotomize (Group D).

	A(mU/ml)	B(mU/ml)	C(mU/ml)	D(mU/ml)
Baseline	172±43	181±63.35	189±37.8	184±55.2
Phlebotomy	218±32.7	192±57.6	360±72	376±112.8
	254±25.4	187±37.4*	302±75.5	463±115.75+
	226±45.2	176±44	456±91.2	615±184.5+
Post-phlebotomy	228±57	187±37.4*	312±93.6	340±68
	236±47.2	182±36.4*	196±49	185±64.75
	172±43	185±55.5	210±73.5	189±47.25
	172±60.2	182±36.4	193±19.3	181±54.3

* -A Vs B- Significant difference ($p \leq .0.05$)
+ -C Vs D- Significant difference ($p \leq .0.05$)

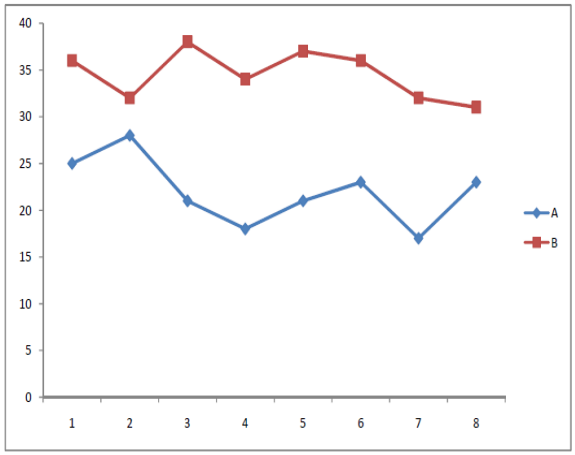

Figure 1: serum erythropoietin levels in new zealand white rabbits fed with 4% protein diet a unphlebotomized (Group A) and those fed 30% protein diet and unphlebotomized (Group B).

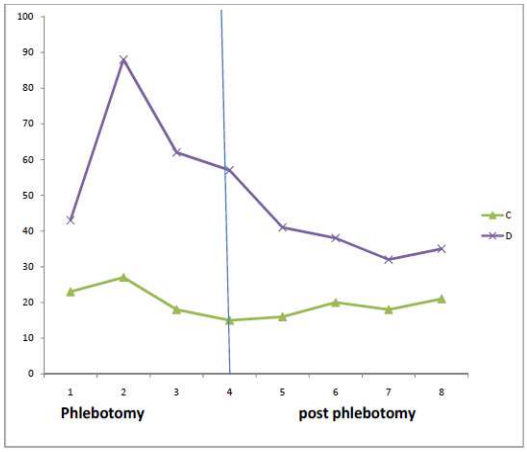

Figure 2: Serum Erythropoietin levels in New Zealand white rabbits fed with 4% protein diet and phlebotomized (Group C) and those fed 30% protein and phlebotomized (Group D).

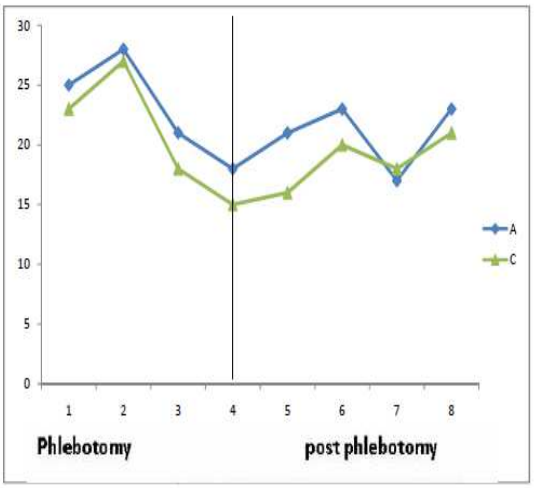

Figure 3: Serum Erythropoietin levels in New Zealand white rabbits fed with 4% protein diet and unphlebotomized (Group A) and those fed 4% protein diet and phlebotomized (Group C).

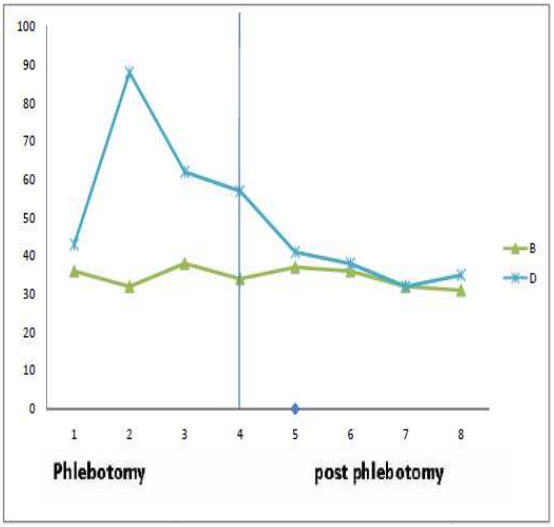

Figure 4: Serum Erythropoietin levels in New Zealand white rabbits fed with 30% protein and unphlebotomized (Group B) and those fed 30% protein diet and phlebotomized (Group D).

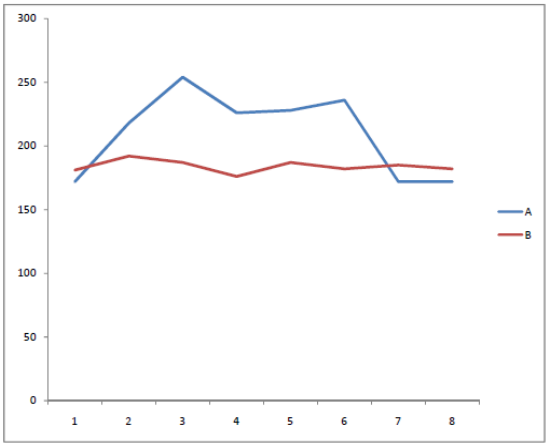

Figure 5: Serum Thrombopoietin levels in New Zealand white rabbits fed with 4% protein diet and unphlebotomized (Group A) and those fed 30% protein and unphlebotomized (Group B).

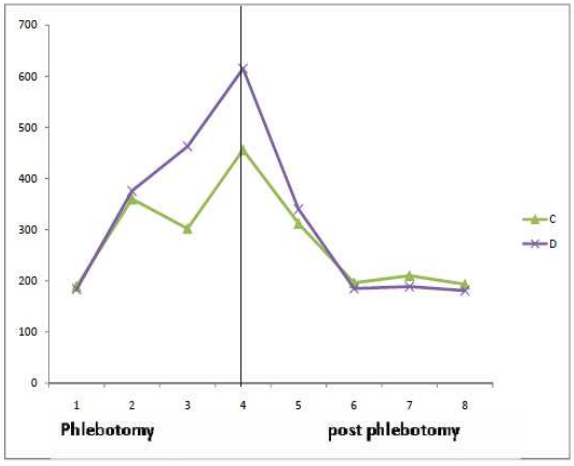

Figure 6: Serum thrombopoietin levels in New Zealand white rabbits fed with 4% protein diet and phlebotomized (Group C) and those fed 30% protein and phlebotomized (Group D).

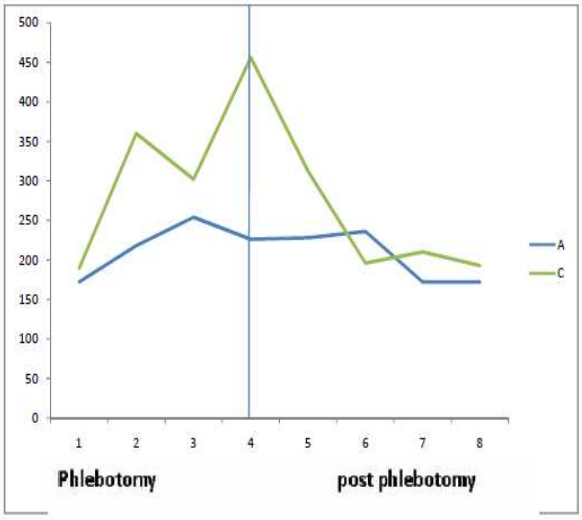

Figure 7: Serum thrombopoietin levels in New Zealand white rabbits fed with 4% protein diet and unphlebotomized (Group A) and those fed with 4% protein diet and phlebotomized (Group C).

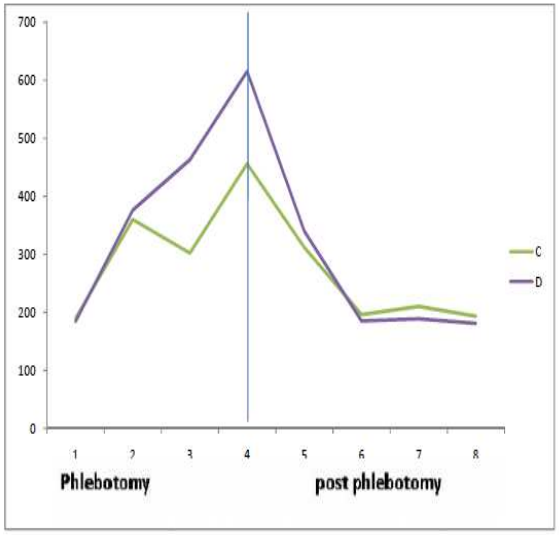

Figure 8: Serum Thrombopoietin levels in New Zealand white rabbits fed with 4% protein diet and phlebotomized (Group C) and those fed 30% protein and phlebotomized (Group D).

DISCUSSION

This study has demonstrated the effect of dietary protein supplementation on the levels of thrombopoietin and erythropoietin in New Zealand white rabbits during and after phlebotomy (reduction of their baseline haematocrit by 20%).

New Zealand white rabbits fed with 4% protein diet (group A), compared with those fed a 30% protein diet (group B) had serum erythropoietin higher than those fed with 4% protein diet (Figure 1). Previous study reported an increase in erythropoietin concentration in the rats fed with protein compared to protein deprived rats and it was suggested that this increase may be as a result of insufficient precursors such as amino acids or a decrease in erythropoietin in the circulation (Kaushanky, 2011).

The serum erythropoietin levels in New Zealand white rabbits fed with 4% protein diet (group C) when compared with those fed with 30% protein diet (group D) after induction of anaemia by phlebotomy. It was observed that the serum erythropoietin level in those fed with 30% protein diet (group D) was drastically elevated compared to the group fed with low protein diet (Figure 2) which is due to the significant reduction in the volume of blood in circulation through phlebotomy which led to blood cell deficiency and required haematopoiesis for correction. This is in accordance with the findings obtained by Primavera *et l.,* 2004 in their study of the mechanism by which erythropoiesis is impaired in rats deficient in dietary protein. They investigated the levels of the immunoreactive EPO (iEPO) in serums and erythroid precursor cells in hemopoietic tissues during protein deprivation. The iEPO level of 32-day-oId rats fed a protein-free diet was lowered to one-third that of rats fed 20% casein at 6 hours after protein deprivation began. They discovered that protein deprivation decreased the number of EPO-responsive cells in the spleen. These results indicate that the impairment of erythropoiesis during protein deficiency is caused by the decrease in serum EPO and the subsequent reduction of the population size of erythroid precursor cells in spleen.

Erythropoietin response to dietary protein examined in nine subjects with a variety of glomerular diseases, they demonstrated that erythropoietin production by the diseased kidney was still responsive to dietary protein manipulation and therefore, conclude that in the diseased kidney, a high-protein diet, perhaps by increasing renal oxygen consumption, directly stimulates erythropoietin production (Biago *et al.*, 2003).

When comparing the serum erythropoietin levels in the group of animals fed with 4% protein diet without phlebotomy (group A) and the group fed with 4% protein diet and phlebotomized (group C), it was observed that erythropoietin levels in those fed 4% protein diet rose up on day two from 23mU/ml to about 27 mU/ml and declined to 15 mU/ml on day four and later rose up to 20 mU/ml on day six and was fairly steady till the end of the experiment while in those fed 30% protein diet, the erythropoietin level rose up from 25 mU/ml on day one to 27 mU/ml on day two and declined to 18 mU/ml on day four after which it rose up to about 23 mU/ml on day six, it fell to 18 mU/ml on day seven and rose up to 23 mU/ml on day eight (Figure 3).

The levels of serum erythropoietin in the group of animals fed with 30% protein diet without phlebotomy (group B) and the group fed with 30% protein diet and phlebotomized (group D), it was observed that the erythropoietin level in erythropoietin level in the animals fed 30% protein diet and phlebotomized was drastically increased and compared to the group not phlebotomized (Figure 4). This is because the significant reduction in the animals' blood volume results in decrease in the number of red cells in the animals' circulation and this consequently leads to reduced oxygen tension in the blood (Hypoxia) which is detected by the animals' kidneys and produce more erythropoietin in response (Yutaka *etal.,* 2015 and Volker, 2010).

Comparing the serum thrombopoietin levels in New Zealand white rabbits fed with 4% protein (4% protein) diet(group A) and those fed with 30% protein (30% protein) diet(group B), both groups at normal conditions without induction of anaemia, it was observed that the thrombopoietin levels in those fed with 30% protein diet was fairly steady at about 184mU/ml throughout the experiment while in those fed with 4% protein diet was, the levels increased for the first two days up to 254 mU/ml, it decreased at day four and was steady till day six after which it fell to about 172 mU/ml at day seven and was same at day eight (figure 5). According to Ampong *et al.,* 2020 in their research on liver diseases on animal subjects, found out that when deficiency of protein is not accompanied by a grossly appreciable calorie deficit, the most salient pathologic feature encountered in the liver is the abnormal accumulation of fat, known as the fatty liver disease which ultimately results in reduced liver functions including thrombopoietin production. It was also suggested that decreased thrombopoietin production accompanying liver dysfunction may be related to thrombocytopenia besides myelosuppression in malnutrition (Yoshiuchi *et al.,* 2010).

The serum thrombopoietin levels in the group of animals fed with 4% protein diet and phlebotomized (group C) and the group fed with 30% protein diet and phlebotomized (group D), it was observed that the thrombopoietin levels in both group rose initially, after the first day, the thrombopoietin level in those fed with 4% protein diet began to fall and rose again after a day fell and remained steady till the end of the experiment. On the other hand, the level of thrombopoietin in animals fed with 30% protein diet rose steadily for the first four days and began to fall till the sixth day after which it remained steady (figure 6). Similar result was obtained by Xavier (2007) in comparing the results obtained from rats given protein diet in controlled amounts, they observed that restriction of the quantity of food protein interferes more seriously with hemopoiesis due to alterations in the histological ultrastructural characteristics of the bone marrow.

The serum thrombopoietin levels in the group of animals fed with 4% protein diet and not phlebotomized (group A) when compared with that of the group fed with 4% protein diet and phlebotomized (group C), it was observed that the serum thrombopoietin levels in those phlebotomized (group C) was a elevated to about 350 MU/ml on day two and fell to about 300MU/ml on day three after which it rose up to about 450MU/ml on day four and subsequently decreased post

phlebotomy to about 200MU/ml on sixth day and remained steady for the remaining days of the experiment while the thrombopoietin levels in the unphlebotomized(group A) was a little raised from 172 MU/ml to about 254 MU/ml on the third day, it reduced a little at fourth day and remained steady till sixth day after which it fell to about 172 MU/ml and remained steady till the end of the experiment (figure 7). A similar study by Edoardo *et al*. (2003), showed that a progressive decline in liver function in malnourished patients with liver disease which is paralleled by a decrease in Thrombopoietin production which was thought to be mainly the result of decrease in hepatic functional mass.

The thrombopoietin levels in the groups of animals fed with 30% protein diet and not phlebotomized(group B) compared to those fed 30% protein diet and phlebotomized (group D), it was observed that in those not phlebotomized, the thrombopoietin levels was relatively steady while in the phlebotomized group (group D), thrombopoietin levels increased steadily from about 184 MU/ml on day one to about 615 MU/ml on day four of phlebotomy after which it reduced steadily post phlebotomy to about 185 MU/ml on day six and remained steady till day eight (figure 2). Similar study by Hobisch-Hagen *et al.* (2000) on multiply bled victims from whom blood was collected within 12hours after trauma as well after every other day during intensive care treatment. Serum thrombopoietin levels were nearly doubled within the first 2 days, reaching its maximum on day six and steadily decreased until day nine. It was suggested that this rise and fall in thrombopoietin levels after blood loss was due to feedback regulation between circulating thrombopoietin and platelet mass (Jelkmann, 2003).

CONCLUSION

The outcome of this study suggests that dietary protein supplementation can improve blood dyscrasia resulting from active bleeding, malnutrition; thrombocytopenia caused by diminished platelet survival or increased platelet loss due to bleeding.

RECOMMENDATION

Further studies should be conducted to detect the effects of reduced dietary protein on other hormones and organs.

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