STUDIES ON THE NUTRITIONAL AND TOXICOLOGICAL EFFECTS OF LOCAL MINERAL SALT (KANWA) IN RABBITS

BY

ADEFOLALU FUNMILOLA SHERIFAT (Mrs)
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CERTIFICATION

This project entitled **"Studies on the Nutritional and Toxicological effects of Local Mineral salt (Kanwa) in Rabbits"** was carried out by **ADEFOLALU FUNMILOLA SHERIFAT** under my supervision and has been examined read and found to meet the regulations governing the award of the degree of Masters of Technology in Biochemistry of Federal University of Technology Minna, and is approved for its contribution to knowledge and literary presentation.

.....
Prof T.A. Gbodi (Supervisor)

3rd December 2001
Date

.....
Dr (Mrs) H.O. Akanya
(Head of Department)
Biological Sciences

3rd December, 2001
Date

.....
Prof K.R. Adeboye
(Dean, School of Science and
Science Education)

Date

.....
Prof J. A. Abalaka
Dean, Postgraduate School

Date:

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DEDICATION

Dedicated to my children Tolu, Bidemi and Seyi for every inspiration they gave me in their little and very special ways.

ABSTRACT

The traditional herdsmen in Nigeria are known to routinely administer local rock salts (kanwa) to their ruminant livestocks (cattle, horse, sheep, goats and rabbits) as minerals supplements because of the nutritive and curative properties they belief it possess. Twelve varieties of such local rock salts were obtained from the Minna central market, Niger State in Nigeria, and from constituent analysis, were found to be composed of mineral elements as sodium, potassium, calcium, chloride and carbonates others includes magnesium, iron, manganese, zinc and copper. An estimation of the nutritional and toxicological effects of the local rock salts on rabbits was carried out at 3 weeks interval for 9 weeks on a group of control rabbits by the administration of 200mg/kg, 800mg/kg and 3200mg/kg of the local salt (kanwa) to three groups of rabbits the serum electrolyte level, their weight gain and biochemical parameters were evaluated. There were fluctuations in the level of serum electrolytes and serum biochemical parameters in control animals and treated animals. It was concluded that the salts (Kanwa) contain minerals elements which are required by the animals for growth and enhanced performance, and feeding at lower dosages can infact be beneficial to the animals but feeding at levels above 800 mg/kg may be injurious to the animals. Although no profound observable clinical

toxicological effects were recorded in all the treated animals during the 9 week experimental period it is suggested that, further work needs to be carried out for a longer period, so as to ascertain incontrovertibly the nutritional and toxicological effects of the local rock salts.

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CHAPTER ONE

INTRODUCTION

Man and other livestock require mineral salts for the proper functioning of the body and to maintain good health and growth. These minerals are inorganic materials that constitute about 50% of the animal body weight, and are classified as macro and micro elements. The macro elements are the major elements that are required by plants, animal, and man and they are calcium (Ca) sodium (Na), potassium (K), phosphorus (P), chlorine (Cl), sulphur (S) and magnesium (Mg) and the micro elements (trace minerals) are iodine (I), zinc (Zn), manganese (Mn), cobalt (Co), copper (Cu), iron (Fe), molybdenum, (Mo) selenium (Se) and flourine (F) (Cole, 1966).

Eighty percent (80%) of the total mineral matter of the body is found in bone, and inspite of the fact that bone has all the appearance of being firm and resistant, the bony structure is regarded as active storehouse of mineral matters. When the need arises, the body as a whole can draw upon the bones for constituents such as Ca and P, under certain conditions the bones may give up so much of their mineral matter that they become soft and can no longer function as an effective framework (Dyer, 1969).

The other twenty percent (20%) of the mineral constituents found in the body are distributed throughout the remainder of the soft tissues and fluids of the body. Crampton and Lloyd (1959) stated that 99% of Ca and 80% of P in the body are located in the bones and teeth. Also minerals play a major role in the maintenance of acid – base balance of the body fluids (blood, digestive secretions and saliva) which is measured as pH or hydrogen ion concentration. Blood is maintained at pH 7.4. In the stomach acid conditions of the pH 2.0 to 2.5 facilitate digestion by the enzyme pepsin. The mechanisms involved in acid-base balance are complex but the role of minerals such as Na, K, Ca, and Mg in establishing and maintaining a given acid-base balance is significant. Bicarbonates, sulphates, phosphates, organic acid salts form complexes with proteins to form strong buffering systems that neutralise gastric acids or the acidity or alkalinity of foods and liquid ingested (Cole, 1966).

Some mineral metals are known to play a role in many enzyme reactions as activator components of cell structure. Iron is contained in hemoglobin; (a heme containing enzyme) and cytochromes, (Guyton, 1981). Copper is necessary for oxidation of ascorbic acid (vitamin C) by the enzymes ascorbic oxidase and for elastin formation. (Shaw, 1980 and Corah, 1996).

Mineral element especially Na, K, Ca and Mg in conjunction with organic compounds (acid, protein, colloids) are the chief factors in establishing the osmotic pressure of fluids in biological systems, (Cole, 1966). Cobalt in the form of vitamins B_{12} is necessary to prevent pernicious anemia (Guyton, 1981). Magnesium is essential for several enzymatic steps in the metabolism of carbohydrate. Phosphorus play an important role in the metabolism of many compounds and is a constituent of many cellular moieties such as adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP) which are components of DNA RNA and cAMP. The proper working of the heart depends on a good balance between Ca and K ions. Calcium ions also have functions related to the clotting of blood. Sodium carbonate has a special function of transporting carbonic acid from the tissue to the lungs and to maintain the blood pH within very narrow limits.

Minerals can influence vitamin requirements. Similarly, mineral utilization can be affected by either the availability or lack of vitamins, As can be observed in Ca and P utilization for bone development in young animals is dependent on an adequate amount of vitamin D being present. Selenium functions in a synergistic manner by sparing or replacing vitamin E requirements.

The metabolic regulatory hormone, thyroxine formed in the thyroid glands contains iodine, which is a preventive for goiter. The pathway and rate of excretion of the inorganic elements vary, some are excreted almost entirely in feaces, others almost entirely in urine while others are excreted through both route some are lost through sweat and menstrual cycle (Dyer, 1969). The kidney for the most part regulates in a very selective excretory fashion the output of these various inorganic constituents with great accuracy (Routh, 1973).

In abnormal states however an excess or lack of required minerals may easily arise resulting in specific syndrome (s). Excess may be as a result of too high an oral intake or due to the inability of the kidney to excrete the surplus. Depletion can rapidly occur as a result of excessive sweating, vomiting or its total absence from the diet (Green, 1976). The body requires a constant replenishing of the minerals and electrolytes that are excreted from the body in various forms. Impairment in immune system occurs in trace mineral deficiencies (Corah, 1996). Low level of Na result in agonizing cramps in various muscles groups, this can be cured or prevented by salt tablets.

Agriculturally, the Fulanis under the transhuman system (nomadic) rear most of the cattle and other farm animals (goat and sheep) in Nigeria.

They make use of "kanwa" salt (which is a lake salt, formed from the geological weathering of igneous rocks) as mineral supplements because refined commercial supplements are not readily available and when available they are expensive. The need for mineral supplements depends on the quality of feed and water available to the livestock (Elwood, 1964; McDowell, 1996). This in turn depends upon the geographical area in which the animals are grazing or on the soil where the feed are produced. The mineral content of the plant depends primarily upon the seasonal fluctuations in plant species (Hiornaux *et al,* 1996), abundance of the elements in the soil (Rhodes, 1995), and the pH, moisture, and other conditions affecting the plant growth and consequently, the mineral uptake by the plant, (Underwood, 1981; McDowell, 1996). Other factors affecting mineral requirement as enumerated by Dyer (1969) includes the amount of food ingested per unit of weight, growth rate of the animal and species, ambient temperature, the form in which the element is ingested, dietary balance of other nutrient, level of antagonists in the ration. Younger animals require and absorb a higher percentage of minerals than older animals of the same species (Schlecht *et. al*, 1999). Also health of the animal will affect the dietary requirement for minerals.

Chemical analysis carried out on kanwa and the commercial salt licks revealed that they are both composed mainly of carbonates, bicarbonates, sulphate and chlorides of sodium, potassium, magnesium, calcium, zinc, copper (Peers, 1959; Gbodi *et al.*, 1982; Ako, 1984; McDowell, 1996). Ikwegbu *et al.*, (1983) indicated that supplementation with kanwa or commercial salt lick of animals feed enhance the presence of the necessary and required minerals at the same time for maximal absorption and availability for the animals use for growth and general performance. The commercial salt blocks is refined and of known quantified component elements and compounds but expensive, kanwa on the other hand is cheaper but has the disadvantage of being bulky and with uncertainty of chemical constitution. Even when the commercial salt lick is available, the Fulanis still prefer the administration of kanwa to their cattle because they believe that it has some prophylactic, nutritive and medicinal values in the animals. Kanwa is administered unquantified in its unrefined state; it is largely obtained with carbonaceous organic matters, sand and less soluble salts.

The question of possible clinical or toxicity effects thus arises. From the studies carried out by Ikwuegbu and Gbodi (1983) on the effects of kanwa and commercial salt licks in diets of milking cows, pregnant cows and young bulls respectively, it was observed and suggested that the local

salt preparations (Kanwa) can replace commercial salt lick, since neither milk yield and milk composition were significantly affected nor were the serum levels of Ca, K, Mg, Zn, Fe and Cu affected, the commercial preparations however gave superior results in terms of general performance of the animals. However, it appears that the experimental period of two weeks used was rather short. Also, they were not able to quantitate the amount of kanwa taken by the animals, since it was given ad-libitum, thus, there was that possibility of differential consumption of the supplied kanwa, and therefore the results were inconclusive. They suggested that further experiments was required using local minerals salt lick and similar diet in a longer feeding trial using young bulls. Also, apart from this evaluation no any other clinical parameter had been determined to properly evaluate its safety. Thus the objective of this study is:

i. To evaluate chemical composition of a variety of kanwa samples purchased from different locations at the Minna Central Market in Niger State of Nigeria.

ii. To conduct nutritional and semi chronic toxicological evaluation of kanwa in male and female rabbits.

From the results, we should be able to estimate the average level of mineral elements in each kanwa sample and identify the best kanwa sample

in terms of composition which is closest to the commercial salt lick and which can be recommended to replace the expensive and not readily available commercial salt licks. The presence or absence of toxicological effects will allow us to predict the safety of kanwa in man and livestocks.

CHAPTER TWO

LITERATURE REVIEW

2.1 Mineral Elements in Human and Animal Nutrition

Minerals are naturally occurring inorganic elements, which are required by the body for growth, maintenance, reproduction and lactation. At least 19 minerals are known to be required by man and animals. The mineral elements are classified as macro or micro (trace) elements, depending upon the quantitative requirements for each and the level of their presence in the higher animals.

The macro elements required by man and other farm animals are Ca, Na, K, P, Cl, and Mg, while the micro or trace elements include I, Zn, Mn, Co, Cu, Fe, Mo, Se, Fl (Cole, 1966).

Minerals have important roles as constituents of certain proteins (Iron in hemoglobin) or of vitamins, (Co in Vitamin B_{12}) and acts as coenzymes or cofactors in enzyme reactions, (Fe in cytochromes).

In addition minerals play important roles in maintaining the osmotic pressure of the body tissues and fluids and in the maintenance of acid-base equilibrium, P is involved in intermediary metabolism of carbohydrate and as components of physiologically important substances. Minerals are taken in the food and feed diets of man and animals respectively. The minerals in

water, food and feed diets depends upon the geographical area in which the food and feed are produced because the crops grown on soils are a direct reflection of the mineral content of the area and the water utilized by the plants. Rain water is low in minerals whereas well water from soils rich in lime would be high in Ca (Elwood, 1964). Cole, (1966) outlined factors affecting dietary mineral requirements to include the amount of food ingested per unit weight, growth rate, animals growing rapidly require more minerals than those growing slowly or those fed for maintenance species products of some species are high in certain minerals. For example, milk and whole egg including the shell are high in calcium, ambient temperature kind of feed (natural feeds are not completely digestible because of high percentage of lignin and cellulose contents). Also the form in which the element is ingested can affect it availability in the body, dietary balance of other nutrients, such as the level of fat greatly affects the amount of calcium available for absorption, the level of antagonist in the ration, age (young animals absorb a higher percentage of minerals than older animals of the same species) also metabolic anomalies affects the dietary requirements for minerals (Underwood, 1962; Dyer 1969) .

Although minerals are unchangeable and therefore not subject to degradation they are frequently assimilated as discrete compounds,

(Hammond, 1978). The absorption of minerals takes place in the gut mainly and some throughout the intestinal tract and the re-absorption cycle by the kidney excretory action, excretes the minerals back into the guts. The bioavailability of minerals is dependent upon many factors including the level of elements ingested, age of the animal, pH of the intestinal contents, state of the animal with respect to deficiency or adequacy of the element and the presence of other antagonistic minerals or nutrients by chelation.

All animals are subject to mineral deficiencies, these may be caused by a sub-optimal amount of a given element in the feed, imbalance of another mineral or nutrient which decreases bioavailability or any condition which increase the rate of passage of the element through the gut or body and a metabolic antagonist which causes the animal to require a greater quantity of the element (Dyer 1969). Manston and Allen (1981) explained that the likelihood of a particular element becoming a limiting factor to production and inducing diseases depends upon the relationship between its availability from the diet, the animals requirements for productions and obligatory excretion, the rate of metabolic turnover of the elements and its availability from body stores.

The conditions of mineral deficiencies or imbalance can result in maladies manifested with signs and symptoms of deficiencies or excesses of

minerals. In deficiency conditions the mineral supplementation of diet with salt tablets or other required minerals containing materials like the local salt (kanwa) can reverse or prevent the conditions and enhance balance nutrition for growth, good health and reproduction.

2.2 Chemical Constituents of Kanwa

It has been shown that the chemical constituents of kanwa vary greatly depending on sources and method of treatment and mining. Reaburn and Jones (1934) noted that the lake salt is a mixture of several compounds. In the experimental findings of Makanjuola and Beethlestone (1975), and Ako (1990) it was indicated that the lake salt (kanwa) contains sodium carbonate and sodium hydrogen carbonate in a 1:1 ratio. Gbodi and Ikwuegbu (1982) and Ako (1984) confirmed the presence of sodium chloride, sodium carbonate, sodium bicarbonate, sulphate, phosphates, zinc, copper, calcium and magnesium salts in kanwa samples.

'Kanwa' is the local trade name in Nigeria for the lake salt deposit, primarily from geological weathering of igneous rocks. It is predominantly found in the Lake Chad region. The lake salt which is erroneously referred to as "potash" implying a high concentration of potassium has been found to be a complex mixture of salt compounds composed mainly of sodium

chloride (NaCl), carnalite ($KCl,MgCl_2.6H_2O$), sylvinite (KCl, NaCl) kieserite ($MgSO_4.H_2O$) polyhynite ($K_2SO_4.MgSO_4.2CaSO_4.2H_2O$), kainite ($MgSO_4.KCl.3H_2O$), felspar, potash, potassium, carbonate and potassium bicarbonate (K_2CO_3), KH_2CO_3 (Clarke 1959) and (Reaburn 1934). The potassium in the parent igneous rock is less easily accessible to weathering hence its of lower concentration in Kanwa.

Kanwa is useful industrially as additive in ink making, soap making (washing Soda), baking soda (Sodium bicarbonate) snuff making, cement industries, dying of textiles, tanning and curing of hides (Ako, 1984).

It is domestically used in homes as preservative to prevent growth of microorganisms, which are the main causes of food spoilage. It is also used as appertiser or seasoning, as tenderiser in cooking tough cuts of meat, beans (Vigna Ungulata) (Ankrah and Dovlo 1978) and (Edijala 1980). It is widely used for cooking vegetables such as okra (Hibiscus esculentus), spinach (Amaranthus Spps), ayoyo/ewedu (Corchorus olitorius) to preserve the green vegetable coloration and also in some cases to enhance resilience (Ako, 1984). The Gwaris in Niger Sate make use of Kanwa to prepare their cereal porridge meal for long keep without spoilage. Medicinally, Kanwa is used for curative purposes in the treatment of skin disorders, also as gastric antacid and as antidote to some poisons.

2.3 Chemical Constituents of Commercial Salt Lick

The imported commercial salt licks were found to be composed of cations and anions which are essential and required for proper biochemical functioning of the animal system. Gbodi and Ikwuegbu (1982) revealed that the main chemical composition of the commercial salt lick from imperial chemical industry (ICI) and others are shown in the table below:

Table 2A: Composition/constituents of known commercial salt Licks

(a) Rockies Tithe Barn Limited	Imperial Chemical Industry (ICI)	(b) Kola and Sons Agro-chemical Limited
NaCl - 38.0%	sodium chloride	magnesium – 400mg/kg
Mg - 5%	phosphorous	iron - 144 mg/kg
Cu - 550mg/kg	calcium	calcium - 700 mg/kg
I - 155mg/kg	cobalt	copper - 400 mg/kg
Ash - 93.0%	copper	phosphorous – 1%
Co - 55mg/kg	zinc	sodium chloride – 97%
Zn - 310 mg/kg	magnesium	
	manganese	

a - Winsford, Chesire CWT 3PG, United Kingdom

b - Ilorin (Kwara State) Nigeria

2.4 Biochemical Roles of Minerals found in Kanwa and commercial Saltlick

2.4.1 Calcium

Calcium is the fifth most abundant element in the earth, it constitutes 3.63% of the igneous rocks. It is found in gypsum, limestone, chalk, marble, eggshells, coral, stalagmites and in bone and teeth. Calcium occurs in compounds of chlorides, phosphates, sulphates, and bicarbonate. Calcium is required in large quantity by growing animals mainly for skeletal development. Bone contains approximately 99.5% of the Ca in the body. The reference value for serum Ca in adult man is 2.1 – 2.6 mmol/L (Baron, 1981) and 2.4 – 3.4 mmol/L in rabbits (Okerman 1988), in Pigs is 2.76 mmol/L Saror et. al., (1987), in cattle it ranges between 2.32 and 2.56mmol/L (Gbodi et al., 1990) and 2.1 – 2.8 mmol/L in sheep, as cited by Royal (Dick) School of Veterinary Studies. Veterinary field station, Caster bush Roslin, Midlothian.

Calcium functions in osmoregulation, muscle contraction, blood coagulation, enzyme activation such as succinate dehydrogenase and adenosine triphosphate (ATPase). Calcium ions decrease; neuromuscular excitability, also Ca and cyclic AMP play a role in the transfer of inorganic ions across cell membranes and in the release of neurotransmitters.

Calcium is required in large quantity during lactation, bovine milk contains about 0.12% of calcium (Dyer, 1969). Calcium is absorbed primarily in the gut, with absorption rate decreasing with age of the animal.

Calcium required for metabolic functions depends upon a minimal amount; an excess may result in mineral imbalances having profound physiological effects. Parakeratosis in pigs occur when excess calcium is fed. Calcium increases the chelation of Zn by phytic acid (Byrd and Matrone, 1965). Stott, (1968) indicated that excess Ca may increase the incidence of parturient paresis in mature and aged dairy cattle. High level of calcium in extra cellular fluids can cause the heart to stop in systole and act as a mental depressant (Guyton, 1981). High calcium level may also impair the absorption of phosphorus, copper and manganese (Dyer 1969).

Calcium deficiency effects vary depending upon the age of the animal. In young animals, rickets may be as a result of Ca deficiency characterized by decreased concentration of hydroxyapatite in the organic matrix of the bone and prebone cartilage. In adult, it results in osteomalacia characterized by a decrease content of hydroxyapatite in the bone matrix.

Calcium deficiency may result in parathyroid disease wide spread hemorrhage, lesions in the digestive tract and vision impairment from cataract formation (Follis, 1958).

2.4.2 **Magnesium**

Magnesium is widely distributed in the earth crust as carbonate silicate, sulphate and chloride. It is a constituent of all living cells. It is required for plant growth and functions essentially as catalyst for most enzymatic reactions in carbohydrate metabolism such as kinases, mutases, enolase, atpases, choline esterase, alkaline phosphatase, isocitrate dehydrogenase and arginase (Dyer, 1969).

Guyton, (1981) indicated that increase extracellular concentration of Mg depresses activity in the nervous system, and depression of cardiovascular system reduces renal oxalate deposition and also causes skeletal muscle contraction which can be blocked by administering Ca.

Low level of Mg may occur due to the following conditions absorption and excretion defects, gastro intestinal disorders, protein energy malnutrition and tetany (Wacker, 1980). Other conditions include liver cirrhosis, alcoholism, kidney and endocrine disorder or pancreatitis.

Low level of Mg causes increase irritability of the central nervous system (Dyer, 1969), (Shaw, 1980).

Decline in serum K level and metabolic alkalosis were observed in Mg deficient persons (Prasad, 1978).

Oral ingestion of Mg as therapeutic agent such as magnesium citrate and magnesium containing antacid elevates serum Mg concentration (Wacker, 1980). Normal serum level of Mg in Nigerian cattle is 1.12 mmol/L as published by Gbodi et al., (1990) and that of Pig is 1.02 mmol/L (Saror et.al., 1982). Normal serum level of Mg in rabbits as reported by Okerman (1988) ranges between 0.5 – 0.8 mmol/L.

2.4.3 Sodium

Sodium is widely found in nature as sodium chloride (rock salt), sodium carbonate and sulphate on land and seawater. Sodium chloride is universally used as condiment. Sodium is mostly ingested as sodium chloride, carbonate, phosphate, lactate and proteinate, which then dissociates and is absorbed in the small intestine and stomach.

Sodium is the chief extracellular cation, distributed throughout the body, with the greatest proportion in blood plasma and interstitial fluid. Sodium is also stored in the bone.

Sodium chloride in the blood serves primarily to effect protein dissociation, (Encyclopedia Britannica, 1972).

Sodium bicarbonate helps to transport carbonic acid from tissues to the lungs, the interaction of sodium bicarbonate in the plasma with hemoglobin helps to buffer the blood.

Sodium is connected with regulating acid-base balance (Cole, 1966) and nerve irritability. Oser (1965) expressed the role of Na in nerve irritability with the equation below.

$$\frac{Na^{+} + K^{+} + OH^{-}}{Ca^{++} + Mg^{++} + H^{+}}$$

The greater the numerator, the greater the irritability and vice versa.

Sodium is a plasma electrolyte, it provides 92% of the alkalinity of the plasma fluid. Sodium is also involved in the exchange with K ions during nerve and muscle actions, osmotic pressure regulations require Na. Sodium is necessary for amino acid and glucose transport across the mucosa and cell membrane.

Dietary deficiencies of Na are rare for man, but occur regularly in domestic animals, this may become apparent following excess body fluid loss, precipitated by excess sweating, diarrhea, vomiting, diuresis, and cortical hormone insufficiency (Coleman, 1980).

Sodium deficiency results in depressed appetite (Wilson et al., 1981) decrease growth, agonising cramps in various muscle groups, weakness and

finally vascular collapse (Dyer, 1969). Hair growth may be affected and fertility impaired (Wilson et al., 1981).

High serum level of Na in man and livestock result in hypertension and degenerative disease of arterioles and glomeruli as reported by Dyer, (1969) and Coleman (1980). Excess Na results in antagonism between Na and K (Homer et al., 1980).

The serum reference values for sodium concentration is 136-148 mmol/L in man (Baron, 1981) and 128-148 mmol/L in rabbits. (Okerman, 1988). In catle is 132mmol/L (Gbodi et al., 1990).

2.4.4 Potassium

Potassium is found in combined form as chloride, nitrate, phosphate, sulphate and carbonate. It is widely distributed in all soils and terrestrial water, (Bakken *et al,* 1997). It is important for nutrition of plants and it's widely found in plant and animal tissues. Potassium is the principal intracellular cation. It is absorbed from the gut as an ion.

It has many functions in the body, K influence the osmotic pressure equilibrium, it increase the nerve irritability, by countering the effects of Ca and Mg. Potassium increases the heartbeat and facilitates several enzymes reactions such a pyruvic kinase and myosin ATPase. (Dyer, 1969).

Potassium normal reference value in plasma is 3.8- 5.0 mmol/L and 3.3-4.1 mml/L in man and rabbit respectively.(Baron 1981) and Okerman (1988). Serum K in cattle estimated by Gbodi et.al., (1982) is 4.6mmol/L Potassium influence carbohydrate metabolism through its effect on the adrenal gland. Wilson et al., (1981) noted that K enhanced growth by increasing the rate of transfer of amino acids from aminoacyl-sRNA to polypeptides in unicellular organism.

Dyer (1969) reported that except in rare occasions, K deficiency and excesses are relatively rare. Ruminants consuming high grain diets often do not ingest sufficient K (Telle et al., 1964).

Potassium deficiency can be caused by low K in diet or by diarrhea diuresis or acidosis. A deficiency of K is manifested by muscular weakness, increased irritability, mental disorientation, cardiac irregularities and reduced rate of growth (Dyer, 1969). Sexual maturity may be retarded and fertility impaired (Wilson et al., 1981).

Cardiac arrhythmia occurs when K level in plasma rises from the normal value of 4 – 5 mmol/L to a level of 8mmol/L in man (Guyton, 1981), hypertrophy of zona glomerulosa, hyper-kalemia results from high level of K in the plasma, (Dyer, 1969; Baron 1982). Wilson et al., (1981) stated that too high K intake may upset the availability of other minerals particularly,

Mg and Ca may also have a damaging effect on conditions in the reticule-rumen and hence on the average efficiency of feed utilization.

2.4.5 Iron

Iron is an essential trace element as established by many workers including Lewis (1971), Widdowson, et.al., (1974) and Prasad et. al., (1974). The major proportion of iron in the body is in the form of hemoglobin for oxygen transport and myoglobin. Iron is also found in the liver and bone marrow, in electron carriers cytochromes (Iron sulphur non heme) that are essential for most of the oxidation that occurs in cell, in xanthine oxidase (Dyer, 1969) and in peroxidase and catalase. Iron is stored in combination with apoferitin mainly in the liver, spleen and bone marrow. The normal daily requirement for Fe is about 12mg/L of blood in man (Baron, 1981), normal level of Serum Fe in cattle range between 28 – 32umol/L (Gbodi, et.al., 1990). It is mostly absorbed from diet in the duodenum and jejunum and small amounts from the stomach.

Increase serum or plasma levels of Fe result from conditions characterized by increased red cell destruction such as hemolytic anemia, decreased survival time of red blood cells, decreased utilization of Fe in lead poisoning and pyridoxine deficiency also elevate the serum Fe levels in the

blood. (Dyer, 1969; Underwood, 1977; Lewis 1971). Defective Fe storage and causes of increase rate of absorption increase Fe level. (Smith and Sargent, 1971). Self induced over load due to ingestion of Fe tablets and oral contraceptives for prolonged period may develop into heamodromatosis (Shaw, 1980).

Decrease in serum or plasma Fe levels are generally due to lack of intake or absorption of Fe, increased loss of Fe (nephrosis) or increased demand on the body store (Pregnancy). This is characterized by anemia, depressed growth, anorexia, fatigue, low resistance to infection (Dyer, 1969). An excess of Fe may result in an increased need for Na and K and in a reduced availability and absorption of P and Mg. (Wilson et al., 1981).

2.4.6 Copper

Copper is an essential trace element required by the body. Copper is distributed in muscle tissue, bones, the liver erythrocytes, plasma and other body tissues. Copper is present as a component of a number of metallo-enzymes, as the prosthetic group of hemocyanins, cofactor for tyrosinase, ascorbic acid oxidase, cytochrome C oxidase, uricase, plasma monoamine oxidase, ceruloplasmin and as components of flavoproteins it is necessary for the formation of erythrocytes and increase iron absorption (Guyton,

1981, Dyer, 1969; Corah, 1996). It is also essential for bone formation skin pigmentation, heamoglobin production and hair and wool growth (Wilson et al., 1981).

Most of the Cu that is ingested is lost through the stool, only a small portion is absorbed by the upper small intestine. Copper in plasma is loosely bound to albumin and incorporated into plasma alpha-2-globulin and ceruloplasmin (Cu transport protein).

Elevated serum Cu levels (hyper cupremia) results in hemolysis, jaundice, thyrotoxicosis, and various infections. Serum Cu levels are also high in-patients who are taking contraceptives or estrogens.

Low serum Cu levels have been observed in a number of hypo-proteinemias as a result of malnutrition, malabsorption and nephrotic syndrome, and can have an effect on fertility (Corah, 1996).

The deficiency of Cu results in nomochronic anemia caused by failure of intestine to absorb adequate quantity of Fe, depressed growth, hair or wool depigimentation, bone anomalies, neonatal ataxia, reduced reproductive efficiency in animals. Low levels of Cu also decrease the quantities of certain Cu containing enzymes in the tissue cells such as cytochrome C oxidase and cytochrome oxidase. Excess Cu, in the liver results in haemolytic crisis, causing liver tissue break down, usually ending

in death. (Wilson et al., 1981). Estimated normal level of Serum Cu in cattle was reported by Gbodi *et.al.*, (1990) and McDowell (1996) between 13.65 – 13.95 µmol/L. In pigs it was estimated as 31.97 by Saror et.al., (1982).

2.4.7 Zinc

Zinc is an essentially required trace element by plants, man and animals. (Underwood 1977; Prasad, 1976;). It is widely distributed through out the body. Zinc functions as an integral part of many enzymes (Corah, 1996). One of the most important is carbonic anhydrase present in high concentration in the red blood cells for rapid release of carbon doxide (Guyton, 1981). Zn is an integral part of insulin (Wilson et al., 1981).

Zinc is essential in small quantity for the performance of many reactions relating to carbohydrate metabolism, lactic dehydrogenase, and peptidase.

Zinc is required for ribonucleic acid synthesis and regulation of the activity of ribonucleases, thymidine kinase, RNA polymerase and DNA polymerase are zinc dependent (Prassad and Obserleas, 1974).

Zinc deficiency can occur as a result of mineral antagonism, Ca can induced Zn deficiency (Wilson et al., 1981) and malnutrition due to poor diet high in phytic acid or to intestinal malabsorption, or to effect of

alcoholic cirrhosis (Baron, 1981), pregnancy and contraceptives (Prasad,1974). Deficiency of Zn results in lesions of skin, (Wilson *et al.*, 1981) mucocutaneous junctions and epithelial structures, retarded growth of testes and secondary sex organs in male animals and reduced growth in some species of animals (Dyer, 1969).

Prasad, (1976) related Zn deficiency to growth retardation and gonadal hypofunction. Zinc deficiency can be presented as failure to thrive, hypogonadism, rashes, and infantile diarrhea; it can lead to impaired wound healing (Baron, 1981). Excess Zn may reduce the availability of Cu and Fe, Zinc; toxicity is rare, high level of Zn reduces feed consumption. Reference value for plasma Zn concentration is 12 – 17 µmol/L in normal human.(Baron 1981), for cattle it was estimated to range between 24.02 µmol/L (Gbodi et al., 1990) for Pig it was estimated at 18.5 µmol/L (Saror *et. al.*, 1982)

2.4.8 Chloride

Chlorine is found in animal tissue primarily as the chloride ions. Chloride is the anion that is in the greatest concentration in the extracellular fluid and it occurs in combination with Na in the blood and red blood cell, in

the gastric juice, it appears in combination with hydrogen, rather than Na. It is absorbed in the digestive tract (Dyer, 1969).

Chloride plays a major role in the acid-base regulation of extracellular fluids and the maintenance of proper osmotic concentration. Chloride functions in carbon dioxide transport through the Cl shift (Oser, 1965), activation of enzymes and digestion of proteins.

Deficiency of Cl is not wide spread; it however results in alkalosis deficiency of K, renal lesion achloryhdria, hyper excitability. Decreased level of Cl may be as a result of excess losses due to a digestive tract or metabolic malfunction. Lack of this element can cause unthriftiness, lack of appetite and loss of weight or lowered milk production (Wilson *et al*, 1981).

2.4.9 Carbonate

Carbonate (CO_3) is a carbon compound in combination with oxygen, or with oxygen and hydrogen as bicarbonate. It occurs both as organic and in organic compounds. Metallic carbonates are the salts of carbonic acids (H_2CO_3) such as the carbonates of Na, Ca, Mg, which are particularly important biologically and as industrial chemicals. Many carbonates are found as natural minerals such as cerusite ($PbCO_3$), smithsonite ($ZnCO_3$), calcite ($CaCO_3$), dolomite ($CaCO_3$ Mg CO_3), trona (Na_2

CO_3.NaHCO$_3$.2H$_2$O) (Ako, 1990). The organic CO_3 are the esters of carbonic acid and ortho carbonic acids. Carbonic acid is formed when CO_2 is dissolved in water.

$CO_2 + H_2O \longrightarrow H_2CO_3 \rightleftharpoons H^+ + HCO^{2-}{}_3{}^-$ (Green, 1976). Being a dibasic acid, it forms both the normal carbonates and the bicarbonate or acid carbonate. The mineral salts of carbonate are taken into the body for the proper functioning of the body fluids. The buffering of blood is achieved principally by the interaction of the sodium bicarbonate in the plasma with hemoglobin in the red blood cells (Guyton, 1973). The liberation of oxygen from heamoglobin releases the base (HCO_3) in the carbonic acid to form potassium bicarbonate, the red cells thus acts as factory manufacturing bicarbonate (Green, 1976). The bicarbonate content of the plasma is available to neutralize fixed acids entering the blood.

$HCl + NaHCO_3 \longrightarrow NaCl + H_2CO_3$

Excess intake of carbonate such as sodium bicarbonate may result in alkaleamia (Guyton, 1981), which may lead to tetany when there is arise in blood pH. Deficiency of bicarbonate results in acidaemia.

2.4.10 Sulphate

Sulphate (SO_4) is a mineral salt of Sulphur (S) and is one of the most abundant elements in the earth. It is commonly found as gypsum barite and anhydride. Sulphur is an essential element required for the synthesis of certain amino acids, such as methionine needed to form protein (Wilson 1981). Sulphates have being established to function in the formation of sulphated mucopolysaccharides, the synthesis of taurine and the reactions of detoxification. (Homer et al., 1980). Sulphate is seldom deficient. The presence of excess inorganic sulphates can be detrimental, it causes the destruction of vitamin D, increases the production of feaces, and loss of membrane permeability can result. (Homer et al., 1980).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Sources of Kanwa

Twelve samples of kanwa were purchased from different locations in Minna town central market, Niger State of Nigeria. Some were already in powdered form and others had to be crushed, ground, sieved and were each stored in labelled polythene sample bags from where portions were taken for analysis as required.

3.2 Extraction of Kanwa for analysis

Five grams of the air dried Kanwa samples were transferred into 250ml conical flasks. 125ml of 2.5% acetic acid was added, a polythene screw cap was fitted on the flask and clamped on a wrist action shaker (SGL-700-010V Gallenkamp, Christopher Street London EC2P2ER UK) and was operated for 1 hour. The mixture was filtered through a 440 Whatman filter paper. The filtrate was labelled solution (A) and retained for analysis of Kanwa. (White side, 1981).

3.3 Preparation of Kanwa for dosing of Rabbits

Fifty grams of Kanwa sample was dissolved in 100ml of distilled, deionized water and stored in polythene bottle until required for dosing, a fresh solution was prepared for every dosing.

3.4 Housing and Preparation of animals for Experiment

Thirty two (32) healthy growing rabbits made up of 16 males and 16 females between the ages of 8 – 12 weeks and weighing between 380-779g, were purchased from the Animal Production Farm of the Federal University of Technology Minna in Niger State. Routine treatment for deworming and control of endo and ecto parasites was carried out using Ivomec super (Vermectin and Dorsulon by Merck and Co. Inc, Whitehouse Station N J U.S.A) and 0.2ml Oxytetracycline 20% L.A (Tridox) (Kepro B.V, Compagnieweg 33771 N H Barneveld Holland) was given to each animal as broad spectrum antibiotic before commencing experimentation. The rabbit were housed in the Departmental Animal House in cages made from wood and wire gauze, Wood shavings were used as bedding and no extra light was provided at night. Eight animals made up of 4 male and 4 females each per treatment group and control were randomly allotted to the cages, which were labelled for each of the three treatment groups, and the control. Each cage

housed four rabbits of the same sex, which were identified by their treatment group label using permanent markers to write their identification number on the ears. The cages were cleaned daily and the animals were quarantined for four weeks before experimentation commenced. The animals were weighed using Avery balance weigh (W & T Avery Ltd, Birmingham) weekly for the whole duration of the experiment.

3.5 Feed Preparation and Feeding

Feed (10kg) was compounded with 7kg-ground maize, 2.67kg roasted Soya beans, 0.265kg of bone meal and 0.065 kg of salt. (Aduku and Olukosi, 1990; Villamide 1996), this was supplemented with a variety of succulent leaves and grasses mainly tridax, maize leaves, lemon grass and mango leaves. The feed and water were provided ad-libitum.

3.6 Treatment of Rabbits with Kanwa

The rabbits in the first treatment group received a dosage of 200mg/kg, while the rabbits in the second and third treatment groups received 800mg/kg and 3200mg/kg of kanwa respectively and the fourth group which served as the control received no treatment with kanwa, but was given water. A graduated syringe was used to orally administer the

kanwa solution. Treatment groups I, II, and III received 0.4ml/kg, 1.6ml/kg, and 6.4ml/kg of the kanwa solution respectively. This treatment with Kanwa was carried out twice weekly for a period of 9 weeks to simulate pattern of administration of Kanwa by Fulani herdsmen to their cattle.

3.7 Collection of Blood and Serum Samples

To collect the blood, one male and one female animals from each group were slaughtered at 0, 3, 6 and 9 weeks interval. The rabbit's fur was shaved around the neck area using razor blade sterilized with acetone. The animals were slaughtered using sterile knife, the blood was collected into labelled centrifuge tubes with attached funnel and the collected blood was allowed to stand for about 2 hours, when serum separated out at room temperature. The serum was decanted and then centrifuged at 5,000rpm for 10 minutes, the clear serum using a pasture pipette was transferred into bottles that were washed and soaked in dilute nitric acid and rinsed with deionized water. The serum samples were stored in freezer at -22°C until needed for analysis.

3.8 Determination of Heamatocrit

The microheamatocrit method of Green (1976) was employed in the determination. An uncalibrated capillary tube was filled 2/3 of the volume with blood by capillary action and one end was sealed with a cristal seal (Cat. No. 1503) Hawkley & Sons Limited, Lancing, Sussex and the tubes transferred to the heamatocrit centrifuge and allowed to spin at 12, 000 rpm for 5 min. The packed cell volume was determined using the heamatocrit measuring gauge.

3.9 Quantitation of Electrolytes and other metals in Kanwa

Atomic absorption method as described by Vogel (1964) based on the principle of spectrophotometer (Philips model PU 9100) was used for the quantification of Na, K, Ca, Mg, Fe, Zn, Cu, Mn as described in the Pye Unicam atomic absorption data book (1984) and in the introduction to atomic absorption spectrophotometry scientific analytical equipment manual book by Whiteside (1981).

3.10 Preparation of Stock Solutions for Na, K and Mn

100mg/L Na stock solution was prepared by dissolving 0.2542g of dry sodium chloride in distilled deionized water in 1L volumetric flask mark and made up to mark. 0.1905g of dry potassium chloride was also dissolved in water in 1L volumetric flask and made up to mark with distilled deionized water. 1.80385g of manganese dichloride ($MnCl_2$) Mn4H20 was dissolved in 25ml of hydrochloric acid (S.G 1.81) and made up to mark in 500ml volumetric flask with distilled deionized water.

3.11 Preparation of Calibration Solution for Mn, Na and K

Into three 100ml volumetric flasks 0, 2.5, 5.0ml of each of Mn, Na, and K stock solutions were pipetted, 10ml of acetic acid (25%) was added to each flask and made up to mark with distilled deionized water. All these solutions were stored in polyethylene bottles.

3.12 Preparation of Stock Solution for Ca and Mg

Calcium stock solution (100mg/L) was prepared by dissolving 0.2497g of dry calcium carbonate in 1ml of concentrated hydrochloric acid and made up to mark with distilled deionised water in 1 litre volumetric flask and stored in ethylene bottle.

100mg/L Mg was prepared by dissolving 0.1000g of Mg ribbon in 1ml of 0.1M hydrochloric acid and made up to 1 litre in a volumetric flask and stored in a polythene bottle 5mg/L Mg was prepared from the 100mg stock solution by pipetting 5ml into a 100ml volumetric flask and made up to the mark with distilled deionized water. This was freshly prepared as required.

3.13 Preparation of Calibration Solutions for Ca and Mg

The 0.4% Lanthanum solution was prepared by dissolving 4.7g of Atomic absorption grade Lanthanum oxide in about 300ml of distilled deionized water in a beaker 25ml of hydrochloric acid (SG 1.18) was added, followed by heating and stirring until the salt has dissolved, it was cooled and filtered into a litre volumetric flask and made up to mark with distilled deionized water and stored in polyethylene bottle. Into three 100ml volumetric flasks, 0, 5, and 10ml each of calcium 100mg/ L and 5mg/L magnesium stock solution were pipetted, 20ml of 0.4% lanthanum stock solution and 5ml of 20% sulphuric acid were added to each flask and made up to mark with distilled deionized water.

3.14 Preparation for Stock Solution for Cu, Zn and Fe

1000mg/L of copper stock solution was prepared by dissolving 1.00g of copper metal in 50ml of 5M nitric acid the solution was heated until completely dissolved and then transferred into 1 litre volumetric flask and diluted to mark with distilled deionized water and stored in a polythene bottle.

1.000g of Zn metal was dissolved in 30ml of 5M hydrocloric acid and heated in a beaker, it was transferred into a 1L volumetric flask and diluted to mark with deionized water. Into four 100ml volumetric flasks 0, 0.2, 0.4, 0.8 and 1.6ml each of Zn and Cu stock solutions were pippeted to give 0, 2, 4, 8, 16ppm of Zn and Cu solutions respectively and made up to the mark with deionized water. To prepare Fe stock solution, 0.1000g of iron fillings was dissolved in 20ml of 5m hydrochloric acid and 5ml concentrated nitric acid (S.G 1.42) the solution was heated to allow for dissolving, because the Fe did not dissolve completely, the solution was filtered using a medium speed whatman filter paper and then dried in an oven, the weight of the residue was deducted from the 0.1000g to calculate the concentration. The filtrate was made up to the 1L mark in a volumetric flask and the concentration noted. Calibration solutions of 0, 2, 4, 8 and 10 ppm were prepared from the stock solution.

3.15 Quantification Na, K, Ca, Mg, Zn, Cu, Fe and Mn in Kanwa

The cathode lamp for the element to be quantified was inserted in the lamp holder inside the AAS and switched on. The required wavelength was set. The gas supply was turned on and the air supply turned on and the flame ignited. The air pressure was regulated to the required pressure. The AAS was allowed to warm up for about 10 mins. The AAS was set on the absorbance mode and distilled deionized water was aspirated into the AAS to zero it, this was followed by using the blank solution and the standard solutions to calibrate and the absorbance were read. The absorbance of sample solution were then read, the standard was used to check the calibration after 4 – 5 sample readings. The sample concentration was obtained from the curves plotted for each standard. The absorbance of Na, K, Ca, Mg, Mn, were determined at 589nm, 766.5nm, 422,7nm, 285.2nm and 279.5nm wavelengths respectively. For Cu, Zn, and Fe the AAS was set on the concentration mode and the standard solution of each element was used to calibrate the AAS, after which the sample concentrations were read at wavelength of 324.8nm for Cu and 213.9nm for Zn. The concentration for each element was obtained from the prepared calibration curves of each element (Figures 1.1 – 1.12), the concentration was calculated with the equation below:

Calculation:

For concentration of Mn, Na, K, Zn, Cu and Fe in mg of extractable metal per 100g of kanwa.

$$mg/100g \quad \frac{(Cs - Cb) \times 1.25 \times 10^3}{W_{40} \times 100}$$

For Ca and mg

$$mg/100g \quad \frac{(Cs - Cb) \times 1.25 \times 5 \times F \times 10^3}{W_{40} \times 100}$$

Where Cs = Concentration of element in sample solution (Cppm)

Cb = Concentration of element in extraction blank solution in mg/L

F = Further dilution factor

W_{40} = Weight of soil dried at 40°C = 5g

3.16 Determination of Sulphate in Kanwa Samples

The turbidmetric method as reported by Allen et al, (1974) was employed. The calibration solution was prepared by dissolving 0.3844g of magnesium sulphate, heptahydrate with water in a 1L volumetric flask and made up to mark. Five 100ml volumetric flasks containing 0, 2, 4, 6, 8 and 10ml magnesium sulphate stock solution were treated with 3ml of 25% nitric acid, followed by 50% acetic acid, 1ml phosphoric acid and 1g barium chloride crystals were added. The volumetric flask were inverted twice and

added to the vanadate solution and diluted to mark in a 1L volumetric flask. The spectrophotometer (Spectronic $20D^{+}$) by (Milton Roy 820 Linden Avenue, Rochester NY 14625, USA), was calibrated with phosphorus stock solution. The stock solution was prepared by dissolving 0.4394g of dry anhydrous potassium phosphate in deionized water and made up to 1 litre mark and stored in a dark brown glass bottle. The calibration solution was prepared by pipetting 0,2,4,5,10,15 and 20ml of the stock solution into a series of 100ml volumetric flasks, 45ml of distilled deionised water was added, followed by 20ml of vanado – molybdate reagent and made up to mark. The colour was allowed to develop for 10 minutes and absorbance was measured at 400nm wavelength. The same procedure was repeated for 5ml of sample extract in 100ml volumetric flask. The sample concentration was determined from the calibration curve plotted (Appendix 1.6; Figure 1.6).

3.18 Determination of Chloride in Kanwa Sample

The Mohr Method, as reported by Williams, (1979) was employed.

Kanwa sample extract (25 ml) was pipetted into 100ml conical flask, 1 ml potassium chromate indicator was added and titrated with standardised silver nitrate (0.028N) solution. A blank titration was carried out using

deionized distilled water. The concentration of chloride ion was calculated by the equation;

$$Cl\ (ppm) = \frac{1000 \times (titre - V_{Blank})}{\text{Volume of sample used.}}$$

Where V_{Blank} is blank titre volume.

3.19 Determination of Total Carbonate

The titrimetric method of Vogel (1964) was employed in the determination. Sample (0.2g) was mixed with 25ml of standardized (0.093N) hydrochloric acid and heated to remove carbon dioxide (CO_2), three drops of phenophtalien indicator was added and titrated with standardized sodium chloride (0.073N) solution. The titre value obtained for each titration was used in estimating the total carbonate (Appendix 1.10)

3.20 Monitoring of Animal Weight

The weight of the animals were recorded twice weekly using an Avery balance (W & T Avery Ltd., Birmingham). The record was also used to estimate the dosage for each rabbit in the different treatment groups.

3.21 Kanwa Sample Selection for Treatment

The twelve-kanwa samples purchased from different location were coded from S1 to S12, for ease of identification since it was difficult to identify them by appearance, as most of them had identical colour and texture.

The samples were taken to ten (10) different Fulani cattle rearers in Minna at different locations for identification of "best" kanwa sample. The sample coded (S7) was the choice sample, by many of them based on taste and colour which was powdery, and dark ash in colour.

3.22 Determination of Serum Electrolytes (Na, K, Mg, Fe, Cu, Zn, and Cl)

Determination of serum Mg, Fe, Zn and Cu electrolytes was by Philips atomic absorption spectrophotometer model (PU9100 Pye Unicam Ltd. Yorkstreet Cambridge CB12 PX, England).

The concentrations of the metals in ppm in each of the sample solutions were read off the calibration curves prepared for each metal (Figure 3.1 and 3.4, Appendix 4.1, 4.12) the values obtained were multiplied by the appropriate dilution factors.

3.22.1 Determination of Magnesium and Calcium in serum

The serum samples for Mg and Ca analysis was prepared by the method of Willis (1974). Serum was diluted 1:10 with 0.1% (w/v) lanthanum chloride to suppress any interference from protein and phosphate. The diluted serum solution was aspirated directly into the atomic absorption spectrophotometer as earlier described. The level of Mg and Ca in the serum was obtained from the calibration curve of each element respectively (Figures 3.2 and 3.3, Appendix 4.13 and 4.14).

3.22.2 Determination of Serum iron

Serum Iron was determined by the method of Whiteside et al., (1981). Serum samples were diluted 1:1 with 10% (w/v) trichlorocetic acid to precipitate the proteins. The precipitate and supernatant were separated after centrifugation at 5000 rpm and the supernatant was aspirated directly into the AAS on the concentration mode after calibrating with Fe standard solutions and the serum Fe concentration were directly read off the AAS machine at 562 nm wavelength (Appendix 2.3.4).

3.22.3 Determination of Serum Copper and Zinc

Serum Cu and Zn levels were measured by the method of Parker et al., (1967) and Dawson et al., (1968) as described by Whiteside and Bruce (1981). Serum samples were diluted 1:10 with deionized distilled water in 10ml volumetric flasks. The calibration solution of 0, 5, 10, 20 ppm were prepared by pipetting 0, 0.5, 1, and 2mls each of the 100mg/L stock solutions of Cu and Zn respectively into four 100ml volumetric flasks this was followed by the addition of 10ml each of 140mmol/L sodium stock solution prepared by dissolving 8.2g of dry sodium chloride in distilled deionized water. The calibration solution was made up to mark with deionized water in a 1L volumetric flask. The serum sample was aspirated into the AAS on concentration mode to obtain concentrations for Zn and Cu at their respective wavelengths of 213.9nm for Zn and 324.8nm for Cu (Appendix 2.3.5 and 2.3.6).

3.22.4 Determination of Serum Sodium and Potassium.

The flame photometry method was used as described by the Jenway manual/data book.(Jenway Gransmore Green, Felsted, Dunmow, Essex, CM6, 3LB, England). This is based on the principle that each element when aspirated and sprayed into a flame, very high energy of an electric arc or

spark exhibits characteristic spectra, the wavelength of light which they emit or absorb enters a spectrograph which isolates the wavelength to be measured by means of a filter prism or grating. A photoelectric cell receives the light and converts it into electric energy, the intensity of which is displayed by a spectrometer, this constitutes a convenient qualitative and quantitative analysis of the element (Vogel, 1964). The calibration solution for Na and K were prepared from the stock solutions by dissolving 0.5884g of sodium chloride and 0.7455g of dry potassium in deionized water and made up to 1L mark in a volumetric flask separately, to give 10mmol/L stock solutions and were stored in polyethylene bottles. From the sodium stock solution, 10, 12, 13, 14 and 15ml each was pipetted into five 100ml volumetric flasks and made up to mark with distilled deionized water. 0, 2, 4, 6 and 10ml of potassium stock solution was pipetted into another five 100ml volumetric flasks, 10ml of 5.6mmol/L sodium stock solution (prepared by dissolving 0.3772g of dry sodium chloride with deionized water in 1L volumetric flask and made up to mark), was added to each. The 100ml volumetric flasks were made up to mark with deionized water. Using a micropipette 100µl of each serum sample was pipetted into each 100ml volumetric flask and made up to the mark with deionized water. This was used to determine the absorbance of Na in the serum samples at 589nm

wavelength. To determine the concentration of serum K, 200µl of serum was pipetted into a 50ml volumetric flask and made up to mark with distilled, deionised water and the absorbance was determined at 766.5nm wavelength on the Jenway PFP7 flame photometers. This directly gave the concentration of Na and K from the calibration curve plotted (Fig 3.1 and 3.2, Appendix 2.3.1and 2.3.2).

3.22.5 Determination of Serum Chloride

Serum Chloride was determined by Schales and Schales tritrimetric method (1941). To 0.2ml of serum, 1.8ml of distilled and deionized water was added in a 25ml conical flask, 3 drops of diphenylcarbazone indicator solution was added, standardized mercuric nitrate was added dropwise from a 2ml pipette calibrated at interval of 0.02ml. An intense violet colour was taken as the end point. Mercuric nitrate was standardized with 2ml of sodium chloride standard solution. The concentration of the chloride ion was calculated using the equation:

$$\text{mg \% Chloride} = \frac{585 \times \text{ml mercuric nitrate}}{\text{ml mercuric nitrate for standard}}$$

3.23 Determination of Serum Biochemical Parameters

3.23.1 Determination of Total protein

This was determined by Biuret method as reported by Peter Jr et al., (1982). The Biuret working reagent was prepared by dissolving 9g of sodium potassium tartrate in 500ml of 0.2N sodium hydroxide, 3g of copper sulphate was added and stirred until completely dissolved, 5g of potassium iodide was added and made up to a litre with 0.2N NaOH, in a volumetric flask. 4.9ml of biuret solution was added to 0.1ml each of serum and standard respectively. These were incubated at 37°C for 10 minutes and their absorbance measured at 540nm using water to zero the colorimeter. The concentration was read directly in the colorimeter. Appendix 2.4.1 (Screen master Hospitex diagnosis Transworld medical system groups 27 chemin pre-Bouvier, 1217 Meyrin-Switzerland P.O.Box 433-1215 Geneva).

3.23.2 Determination of Albumin

This was evaluated by the method of Bartholomew and Delaney (1966) which was modified by Doumas et al (1971) as reported by Peters, Jr, et al (1982). 0.1M succinate buffer (pH 4.2) was prepared by dissolving 11.9g succinic acid in 800ml of water and pH adjusted with 0.1M NaOH to pH 4.2 and made to mark with distilled water in 1litre volumetric flask.

0.419g of bromocresol green was dissolved in 10ml of 0.1M NaOH and diluted to 1000ml. A working solution was made by adding 1 volume of bromocresol green solution described above to 3 volumes of succinate buffer and adjusted to pH 4.2 with 0.1M NaOH, using the pH meter. 0.05ml of serum and albumin standard were respectively added to 5ml of working colour solution each and the absorbance measured within 10 secs at 628nm using bromocresol green working solution to zero the colourimeter.The albumin concentration was determined from the absorbance value obtained using the formular below.

$$\text{Albumin level (g/100ml)} = \frac{\text{O.D of Text x Conc. Std (0 g/L)}}{\text{O.D of Std}}$$

O.D = Optical density

The result is shown in Appendix 2.4.4.

3.23.3 Determination of Cholesterol.

The Liebermann-Burchard method described by Warnick and Chien (1982) was used for the determination. Acetic acid/acetic anhydride mixture was made by mixing 67ml of acetic acid with 65ml of acetic anhydride. Standard cholesterol was prepared by dissolving 200mg of pure cholesterol in acetic acid in 100ml volumetric flask and made up to mark. Colour reagent (2.5ml) of the acetic acid/acetic anhydride mixtures was added to

2.5ml of serum, standard and blank respectively, they were mixed and allowed to stand in cold water for 5 minutes. 0.5ml of concentrated sulphuric acid was added, mixed and cooled by immersing in water for 10 minutes. The absorbance was measured at 570nm after zeroing the spectrophotometer with the blank. The concentration was determined from the absorbance value using the formula in Appendix A.

3.23.4 Determination of Urea

This was done by the diacetyl method of Natelson (1951) and March et al., (1965). Acid stock reagent A was prepared by dissolving 5g of ferric chloride in 20ml of water, 100ml of 85% (v/v) phosphoric acid was added and cooled. It was made up to 250ml mark with distilled water.

Acid stock reagent B was prepared by slowly adding 200ml of concentrated sulphuric acid to 800ml of distilled water and cooled under running water. Exactly 0.5ml of stock solution A was added to 1 litre of solution B.

Colour reagent was prepared by dissolving 20g of diacetyl monoxide in one litre of distilled water and was filtered. Stock B solution was made by dissolving 5g/litre of thiosemicarbazide in distilled water. A working colour solution was prepared by adding 67ml of solution A and 67ml of

solution B and made up to 1 litre with distilled water. Preparative diluent was prepared by dissolving 40mg of phenyl mercuric acetate in 250m1 of water and was heated until salt dissolved, cooled and transferred to a litre measuring cylinder, 0.3m1 conc. H_2SO_4 was added and made up to a litre with distilled water. This was used to prepare 50gm/L of stock urea standard, 10ml of urea stock diluted by 250m1.

10ml of distilled water was transferred into each of 3 test tubes with 0.1ml of serum, standard and blank respectively, each tube was thoroughly mixed, 1ml of each was pippeted and 1ml distilled water was added followed by 2m1 mixed colour reagent and 2m1 mixed acid reagent. The tubes were thoroughly mixed and placed in boiling water for 20 minutes, cooled and the optical density was measured at 520nm. The screen master colorimeter directly gave the concentration (Appendix 2.4.3).

3.24 Glucose Determination

Hospitex diagnostic kit was used (Transworld Medical System groups 127 Chemin Pre-Bouvier, 1217 Meyrin-Switzerland, P.O. Box 433-1215, Geneva).

$$\text{Glucose} \xrightarrow{\text{G.O.D}} \text{Glucoronic Acid} + H_2O_2$$

$$2H_2O_2 + \text{Phenol} + 4-\text{Aminopyrine} \xrightarrow{\text{POD}} \text{Red quinoemine} + 4H_2O$$

The reaction principle is based on the oxidation of glucose by glucose oxidase (GOD) to produce hydrogen peroxide, whereby the oxygen from the perioxide is transferred by the peroxidase enzyme (POD) to a suitable acceptor (e.g. amino antipyrine with the production of a coloured end product which can be measured spectrometrically. The intensity of which is proportional to the concentration of glucose in the sample which is determined spectrometrically at 550nm.

3.25 Determination of Serum Enzymes Glutamate Oxaloacetate Transaminase (GOT), Glutamate Pyruvate Transaminase (GPT) and Alkaline Phoshates

Hospitex diagnostic kits were used (Transworld Medical System groups, 27 chemin Pre-Bouvier, 1217 Meyrin-Switzerland, P.O.Box 433-1215, Geneva). GOT at 340nm, GPT at 340nm and alkaline phosphatase was determined at wavelength of 405nm. One centimetre glass cuvettes and spectrophotometer screen master from hospitex diagnostics) were used for all determinations.

GOT is determined by the reaction principle; below:

$$\text{L - Aspartate} + \alpha\text{ -Oxoglutarate} \xrightarrow{\text{AST}} \text{Oxaloacetate} + \text{L} - \text{Glutamate}$$

$$\text{Oxoloacetate} + NADH_2 \xrightarrow{\text{MDH}} \text{L} - \text{malate} + NAD^+$$

Oxaloacetate reacts with aspartate transaminase (AST) which decarboxylates it to pyruvate which is measured by hydrazone formation after the pyruvate has reacted with 2, 4 dinitro-phenylhrazine (2,4 DNPA) spectrometrically at 340nm.

GPT is determined by the reaction principle below:

$$\text{L} - \text{Alaninie} + \alpha - \text{Oxoglutarate} \xrightarrow{\text{ALT}} \text{pyruvate} + \text{L} - \text{Glutamate}$$

$$\text{Pyruvate} + NADH_2 \xrightarrow{\text{LDH}} NAD^+ + \text{L} - \text{lactate}$$

The pyruvate produced by the transamination activity of GPT reacts with 2,4 dinitro-phenyihyrazine which is determined pectrometrically at 340nm.

Alkaline phosphatase (ALP) was determined based on the reaction Principle below:

$$\text{P - Nitrophenylphosphate} \xrightarrow{\text{ALP}} \text{P - Nitro - phenol + phosphate}$$

Phenol released by enzymatic hydrolysis from phenylphosphate and this is estimated spectrometrically at 405nm.

3.26 Statistical Analysis of Results

The data obtained for the estimation of the various biochemical parameters were subjected to regression analysis to obtain the best-fit curves and correlation coefficients of the curves were calculated. Analyses of variance were used to compare and evaluate the degree of differences for different treatment groups using Statographics Computer Software Package and C A Cricket Graph Computer Package was used in plotting the graphs for the serum determinations.

CHAPTER FOUR

RESULTS

4.1 pH

All the kanwa analyzed were found to have pH above 9 and they were thus alkaline in nature, as can be seen in Table 1. Sample eleven (S11) had the highest pH 10.03, while sample eight (S8) had the lowest pH 9.68.

4.2 Mineral composition of Local Salts (Kanwa)

The concentration of eight anions (Na^+, K^+,Mg^{2+}, Ca^{2+}, Mn^{2+},Fe^{2+},Cu^{2+} and Zn^{2+}) and four cations (Cl^-, $Co3^{2-}$, $SO4^{2-}$ and $PO4^{3-}$) were estimated. The value for each ion in each of the twelve samples is presented in Table 1. The correlation coefficient (r) for the regressed standard curves prepared for the estimation of the minerals were 0.998 for Ca, 0.89 for Mg, 0.995 for Mn 0.996 for K, 0.999 for Fe, 0.9998 each for both SO_4 and PO_4 .

Table 1: Concentration of each anion and cation in the Local Salt (kanwa) samples.

Sample Code	Na mg/100g	K mg/100g	Ca mg/100g	Mg mg/100g	Fe mg/100g	Mn (mg/100g)	Cu (mg/100g)	Zn (mg/100g)	Cl mg/100	% (PO4)	% (SO4)	Co_3 mg/100g	pH
S1	25,000	570.00	3.32	2.52	4.58	12.78	0.04	0.50	55.98	1.10	3.89	19.01	9.8
S2	27,000	730.00	14.40	1.82	3.74	0.18	0.04	0.30	61.63	1.35	0.83	19.11	9.72
S3	23,000	6420	58.71	4.86	5.42	0.18	0.03	0.19	143.43	0.99	47.65	16.73	9.70
S4	29,000	2360	109.94	3.40	3.74	12.78	0.02	0.13	120.43	0.39	78.10	14.25	9.96
S5	36,000	5680	105.78	16.57	7.10	0.18	0.02	0.19	110.20	0.02	177.3	16.95	9.72
S6	27,000	1140	3.32	1.24	7.94	1.18	0.02	0.30	64.05	0.19	0.28	20.10	9.94
S7	22,000	3170	75.32	10.48	7.94	0.18	0.05	0.30	126.95	0.14	59.88	14.52	9.99
S8	26,000	1820	216.55	30.96	2.07	0.18	0.04	0.30	61.48	0.29	139.2	13.65	9.68
S9	30,000	5060	67.01	7.56	2.90	0.18	.06	0.38	111.63	0.03	129.1	16.28	9.80
S10	32,000	8690	37.94	0.77	3.74	0.18	0.07	0.69	75.83	0.64	3.89	25.94	9.83
S11	31,000	0630	17.17	2.99	2.90	0.18	0.06	0.56	41.90	0.69	19.51	20.08	10.03
S12	30,000	6350	8.86	0.65	5.42	0.18	0.05	0.38	200.93	0.71	76.72	16.82	9.97

4.3 Selection of Kanwa Sample for Animal Treatment

Sample seven (S7) was chosen by seven of the ten Fulani cattle rearrers as the best Kanwa from the twelve varieties that were obtained for this study. Their choice was based on taste, colour and texture. The chemical composition of the selected sample S7 is given in Table 2.

4.4 Clinical Observations and Weight Changes

Two of the rabbits in the control group died before the commencement of the experiment. No remarkable clinical signs were seen in the control and treated animals during the 9 weeks experimental period.

Figure 1 shows the curve of the mean of three weekly weight changes for 9 weeks. Table 3 shows the group mean and standard deviations for the weight changes in the animals to which varying concentrations of local salt were administered for 9 weeks. The mean weight gain for the control animals is 50.15(g) while the animals in treatment groups 1, 2 and 3 had 55.96, 58.86 and 63.23(g) respectively. The difference in weight gain were however found to be statistically non significant at P=0.05. From Figure 1 the control animals at the start of treatment had the least weight gain of 11.80(g) compared with that of groups 1, 2 and 3 treated animals, which had weight gain of 54.6, 59.7 and 71.3(g) respectively. By the 9^{th} week of

Table 2: Composition of S7 Sample selected for Rabbit Treatment

Constituents	Concentration
Na	22,000mg/100g
K	3170mg/100g
Ca	75.32mg/100g
Mg	10.48mg/100g
Fe	7.94mg/100g
Zn	0.31mg/100g
Mn	0.18mg/100g
Cu	0.05mg/100g
Co_3	14.52mg/100g
So_4	59.88mg/100g
Po_4	0.14mg/100g
Cl	126.95mg/100g

experimentation, the control animals had the highest weight gain of 72.86(g) compared to the treated animals which had 54.07, 59.57 and 64.2(g) for the animals treated with 200mg/Kg, 800mg/Kg and 3200mg of local salt respectively.

4.5 Heamatological Determinations

Table 3 presents the mean and standard deviation values of the packed cell volume (PCV) for the 9week experimental period. The control group had the least mean PCV value of 39.47 % while the treatment groups 1, 2 and 3 had slightly higher but not statistically significant values of 41.16%, 40.35% and 42.31% respectively. Figure 2 shows that at the initial sampling, group 3 animals had the highest PCV of 42.7%, while the control had the least value of 34.67%. By the 9^{th} week the control animals had the highest value of 41.6% and the treatment groups 1, 2 and 3 animals had values of 40.8, 39.0 and 40.75(%) respectively.

4.6 Serum Mineral Determinations

The serum concentration of K, Na, Mg Fe, Zn, Cu, Cl were determined in the control, and the three treatment groups 1, 2 and 3 animals. The "r" (correlation coefficient) values were 0.989 for K, 0.87 for Na and

Table 3: Weight Change and hematological (PCV%) values for four groups of Rabbits treated with varying concentrations of local Salt Lick (Kanwa) after 9 week experimental period

	Control	Group 1	Group 2	Group 3
Weight Change (g)	50.15±26.60	55.96 ± 45.29	58.86±5.38	63.23±9.92
	n = (6)	n = (8)	n = (8)	n = (8)
PCV (%)	39.47 ± 3.22	41.16 ± 1.53	40.35±2.31	42.31± 1.12
	n = (6)	n = (8)	n = (8)	n = (8)

Grp = group
(n) = No. of animals
Control = received no local rock salt (kanwa) treatment)
Group 1 = 200mg/Kg local rock salt (kanwa) treatment
Group 2 = 800mg/Kg local rock salt (kanwa) treatment
Group 3 = 3200mg/Kg local rock salt (kanwa) treatment

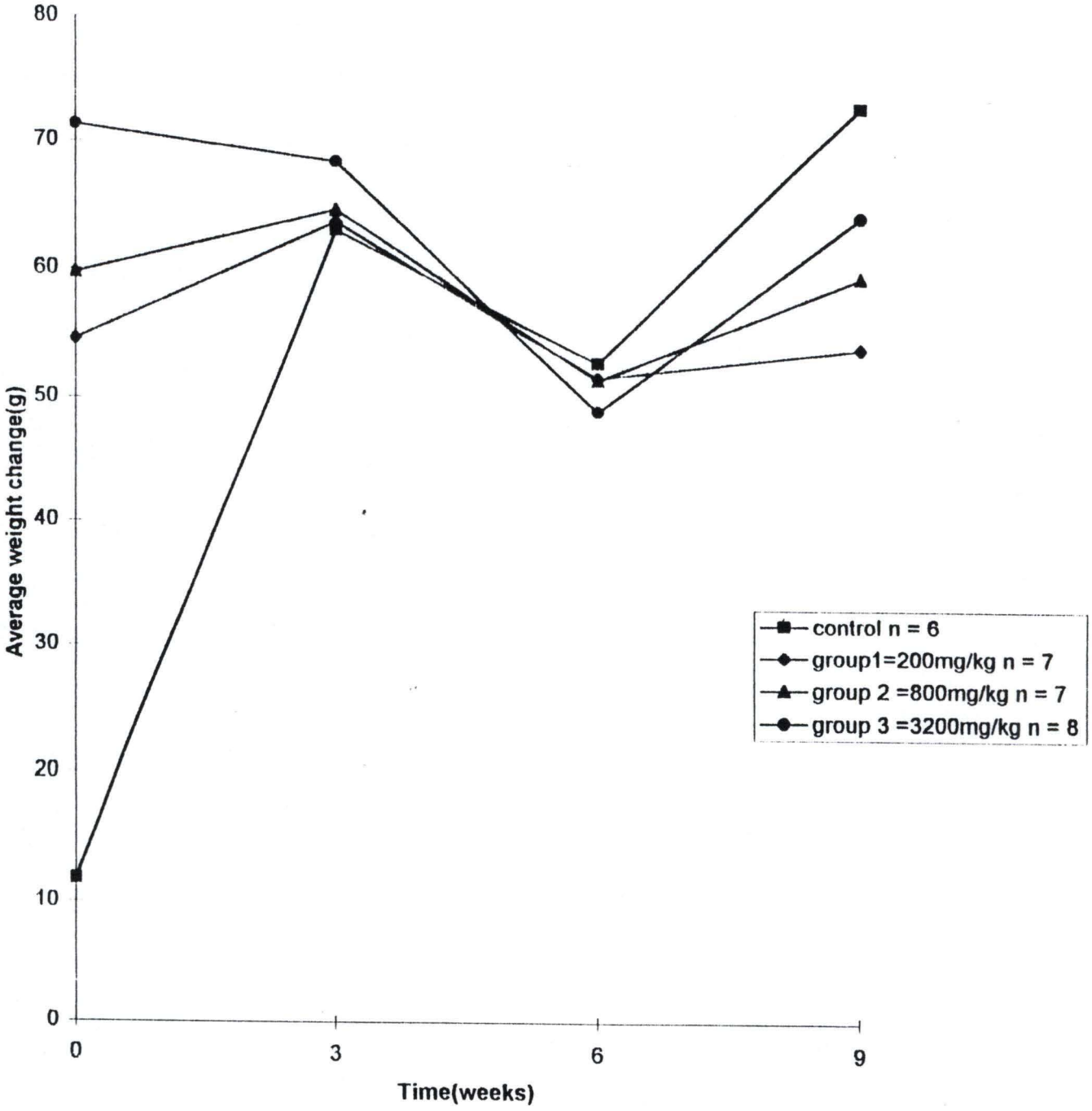

Figure 1: Mean Average Weight change in four groups of rabbits treated with varying concentrations of local salt lick (Kanwa) for 9 weeks

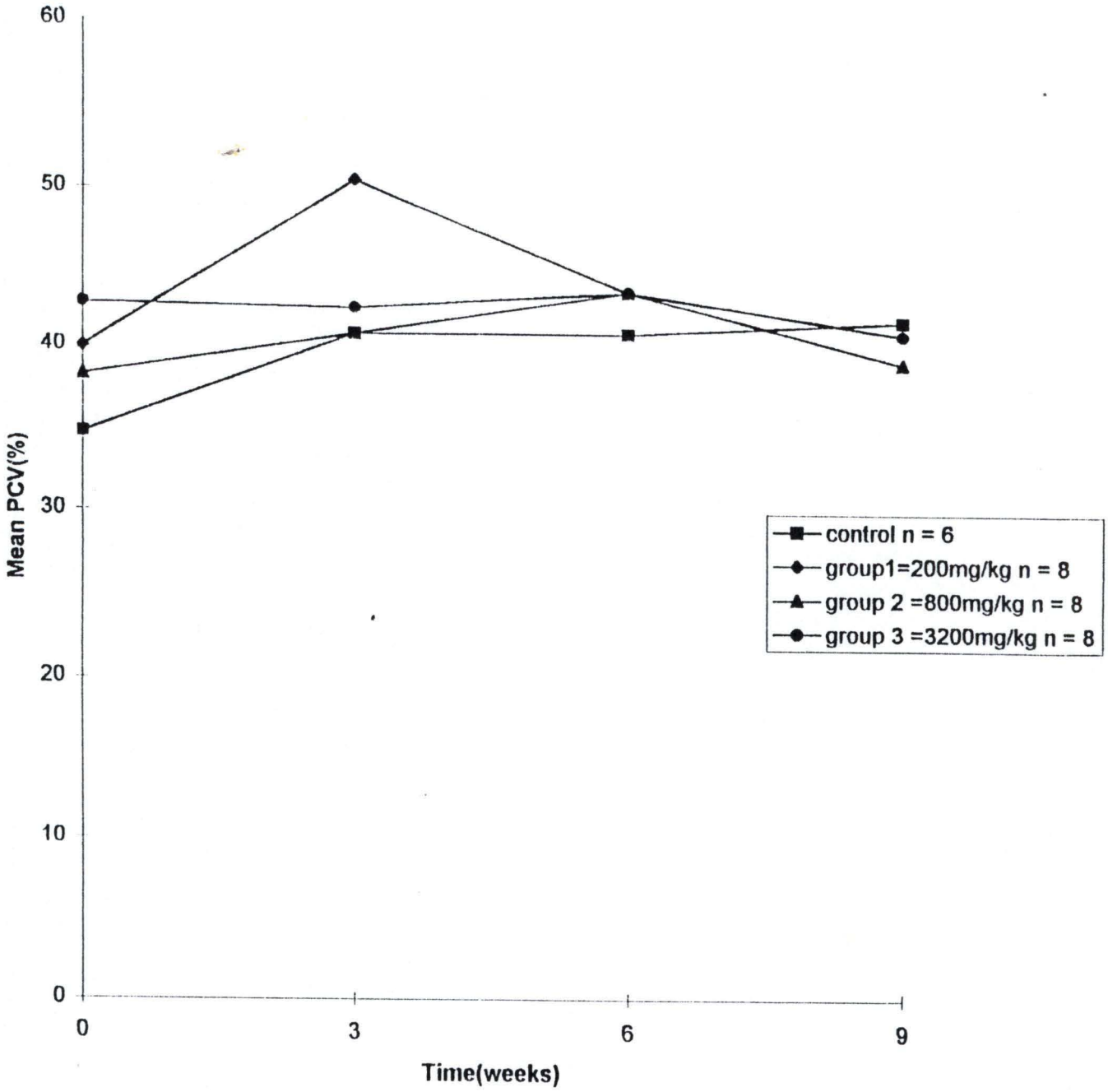

Figure 2: Mean Packed cell volume in four groups of rabbits treated with varying concentrations of local Salt Lick (Kanwa) for 9 weeks

Table 4: Mean and standard deviation of serum mineral in the four groups of rabbits treated with varying concentration of local Salt Lick (Kanwa) for 9 weeks.

Mineral Element	Control	Group 1	Group 2	Group 3
K(mmol/L	3.76±0.50 n = 6	3.73 ± 0.77 n = 7	3.95 ± 0.17 n = 7	3.96 ± 0.3 n = 8
Na (mmol/L)	138.34 ± 4.02 n = 6	140.94 ± 7.4 n = 6	133.62±2.25 n = 8	139.0± 3.69 n = 8
Mg (mmol/L)	0.65±0.11 n = 6	0.74±1.33 n = 7	0.75 ± 0.11 n = 8	0.71 ± 0.11 n = 8
Fe(mmol/L)	0.028±0.004 n = 6	0.034±0.002 n = 8	0.022±0.006 n = 7	0.029±0.007 n = 8
Cl (mmol/L)	103.92 ± 6.41 n = 6	107.49 ± 15.96 n = 8	104.34± 5.33 n = 8	113.12 ± 14.77 n = 8
Cu (mmol/L)	0.014 ±0.003 n = 6	0.013 ±0.003 n = 7	0.015 ± 0.008 n = 8	0.013±0.002 n = 8
Zn (mmol/L)	0.026 ± 0.0019 n = 6	0.034 ± 0.0055 n = 8	0.029 ± 0.004 n = 8	0.027 ± 0.0007 n = 8

Grp = group
(n) = No. of animals
Control = received no local rock salt (kanwa) treatment)
Group 1 = 200mg/Kg local rock salt (kanwa) treatment
Group 2 = 800mg/Kg local rock salt (kanwa) treatment
Group 3 = 3200mg/Kg local rock salt (kanwa) treatment

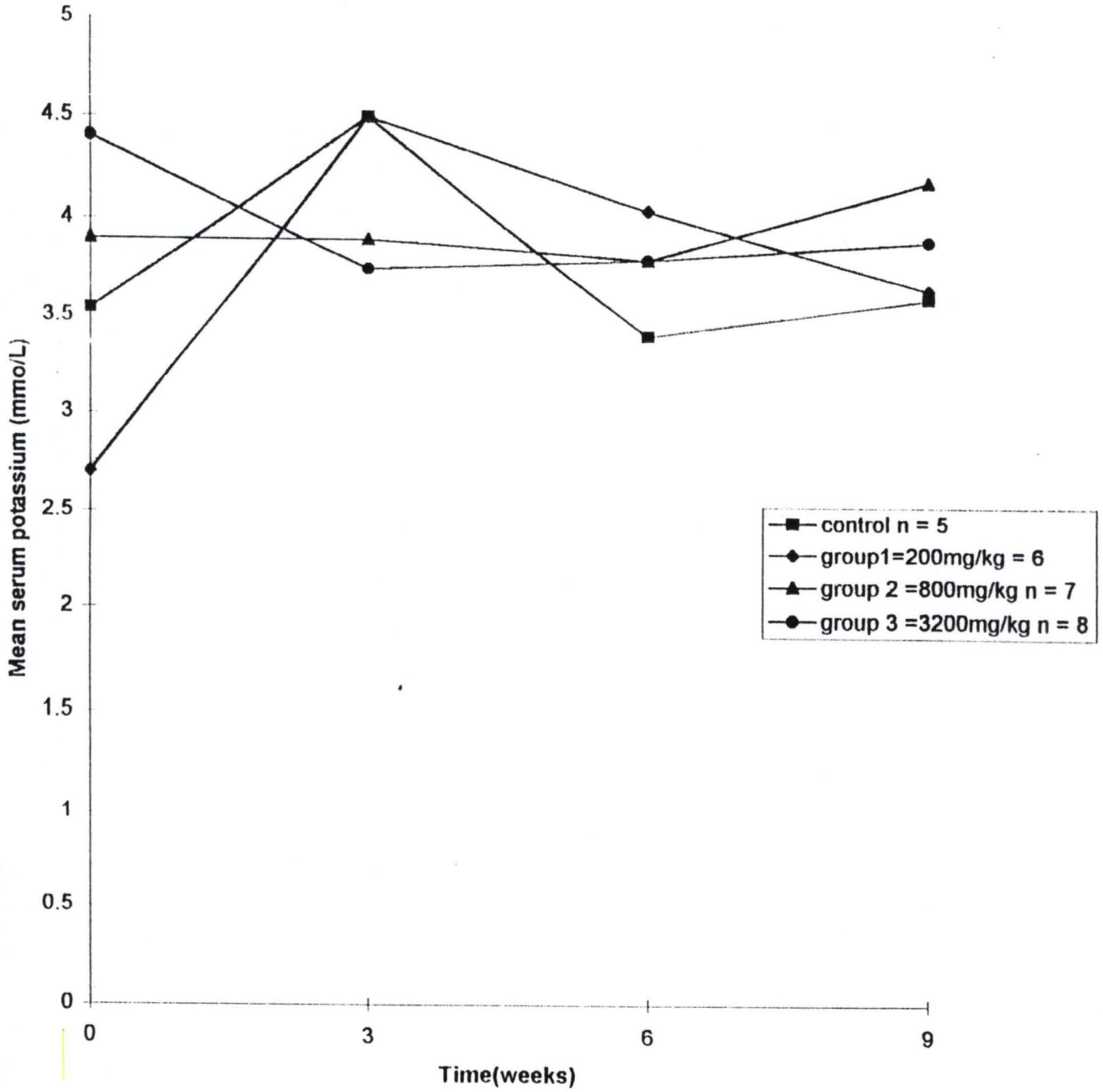

Figure 3: Mean Serum potassium in four groups of rabbits treated with varying concentrations of local Salt Lick (Kanwa) for 9 weeks

0.97 for Mg. Table 4 presents the mean and standard deviation values of the serum electrolytes determined at 3 weekly intervals for 9 weeks.

From Table 4, the mean and standard deviation for serum K shows that group 3 animals has the highest mean value of 3.96 mmol/L, while the other treatment groups 1 and 2 and control had 3.73, 3.95 and 3.76 (mmol/L) respectively. Figure 3 shows the curve of the three weekly mean serum K levels for 9 weeks, 3 weeks after the start of treatment, there was remarkable increase in serum K in animals treated with 200 mg/Kg local salt and the control, the serum K level was 4.5 mmol/L for both. By the 9th week experimental period, animals treated with 800mg/Kg and 3200mg/Kg local salt had higher mean serum K level of 4.2 and 3.9 mmol/L respectively than control (3.60 mmol/L) and 200mg/Kg treated animals had value of 3.65 mmol/L. From Table 4, there was higher level of serum Na in the group 1 animals (140.94 mmol/L) compared with the groups 2, 3 and control animals, which had 133.62, 139.0 and 138.34 (mmol/L) respectively. Figure 4 shows that there were a lot of fluctuations in the level of the mean serum Na in both control and the treatment groups. However by the end of the 9th week experimental period, animals treated with 200 mg/Kg (group 1) of local salt exhibited a significant increase in the mean serum Na level, it had a value of 148.63 mmol/L followed by that treated with 3200 mg/kg (group

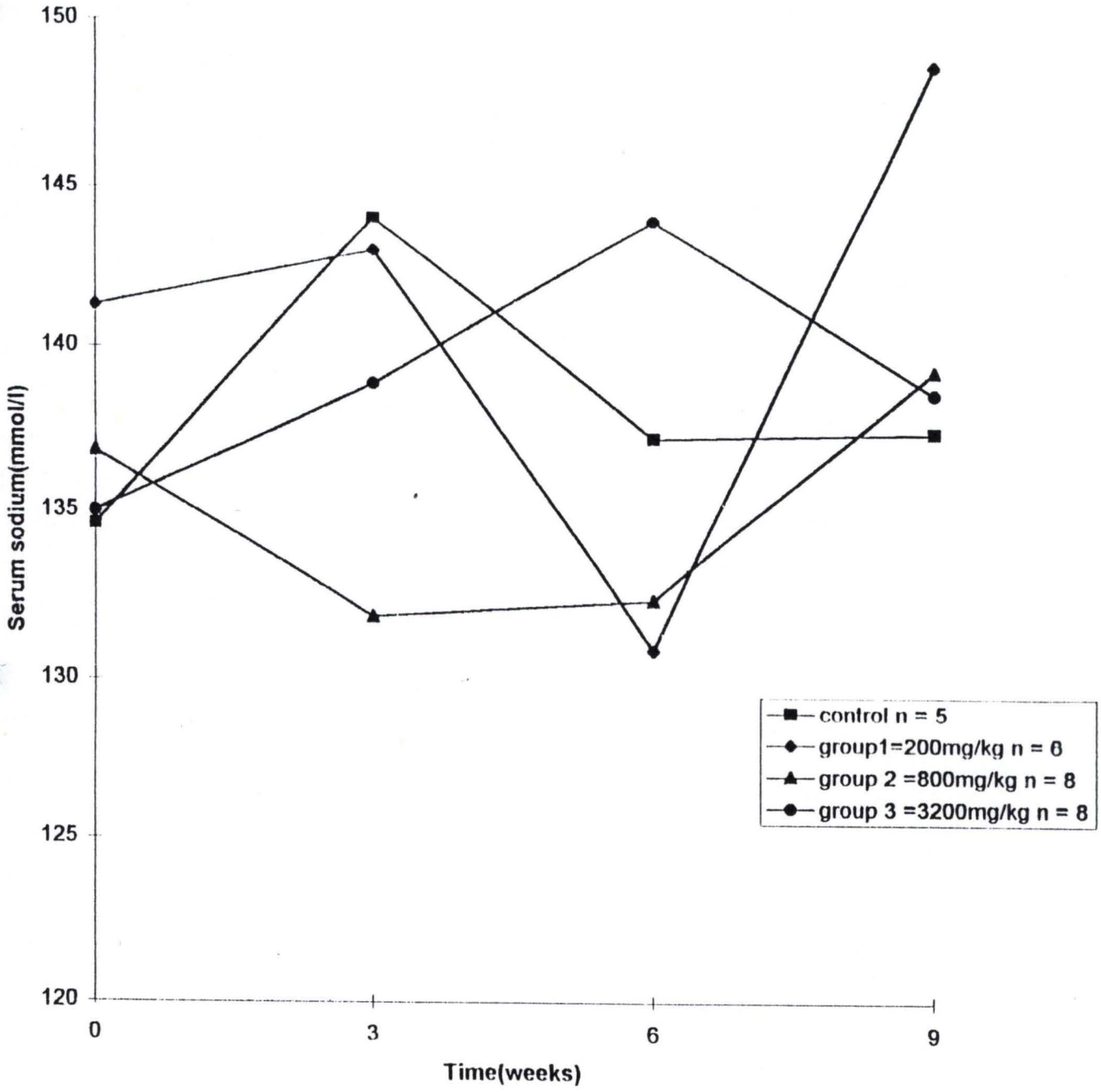

Figure 4: Mean Serum sodium in four groups of rabbits treated with varying concentrations of local Salt Lick (kanwa) for 9 weeks

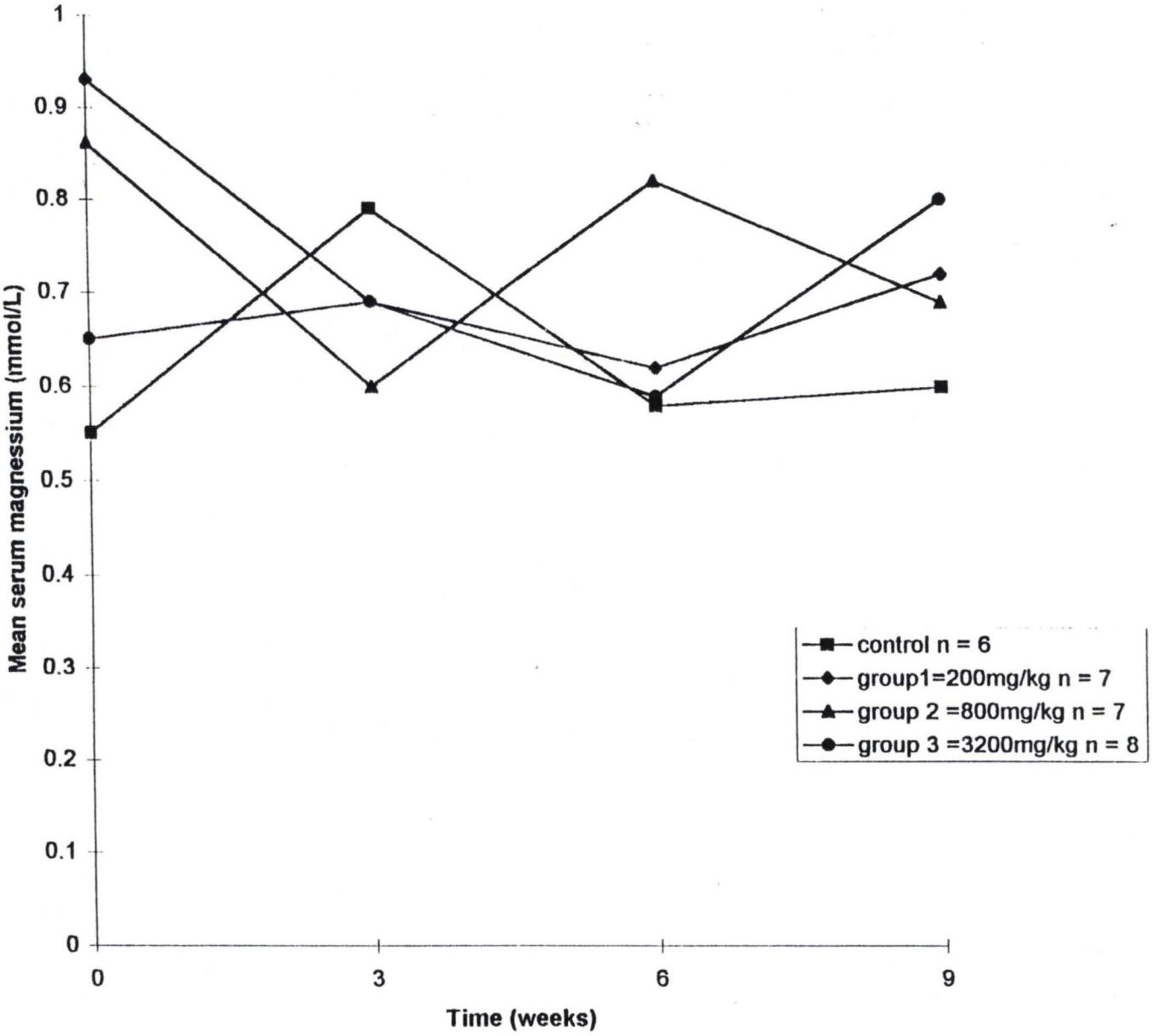

Figure 5: Mean Serum magnesium in four groups of rabbits treated with varying concentrations of local Salt Lick (Kanwa) for 9 weeks

3) and that of 800 mg/Kg (group 2) with values of 138.6 and 133.4 (mmol/L) respectively and the control had 137.45 mmol/L. The mean and standard deviation of serum Mg levels after 9 weeks experimental period are given in Table 4. It shows that the control has the lowest mean value of 0.65 mmol/L while groups 1, 2 and 3 had 0.74, 0.75 and 0.71 (mmol/L) respectively. Figure 5 presents the curve of the three weekly interval serum level of Mg for the 9th week experimental period. A lot of fluctuations was observed in serum Mg, over the duration of 9 weeks experimental period. Both at the commencement and end of the 9 weeks, the control group had the least mean serum Mg level of 0.55 and 0.6 mmol/L respectively. Group 3 had the highest mean serum Mg of 0.80 mmol/L followed by groups 1 and 2 in decreasing order of 0.72 and 0.69 mmol/L respectively. The variations between the control and the rest treatment groups were of no statistical significance.

Table 4 shows the mean and standard deviation values of serum iron at the end of the 9th week experimental period. Group 2 animals have the lowest value of 0.022 mmol/L and group 1 animals have the highest value of 0.034 mmol/L, while the control and group 3 animals had 0.028 and 0.029 mmol/L respectively. Figure 6 shows the mean 3-weekly interval of serum iron in four groups of animals treated with varying amount of kanwa. The control

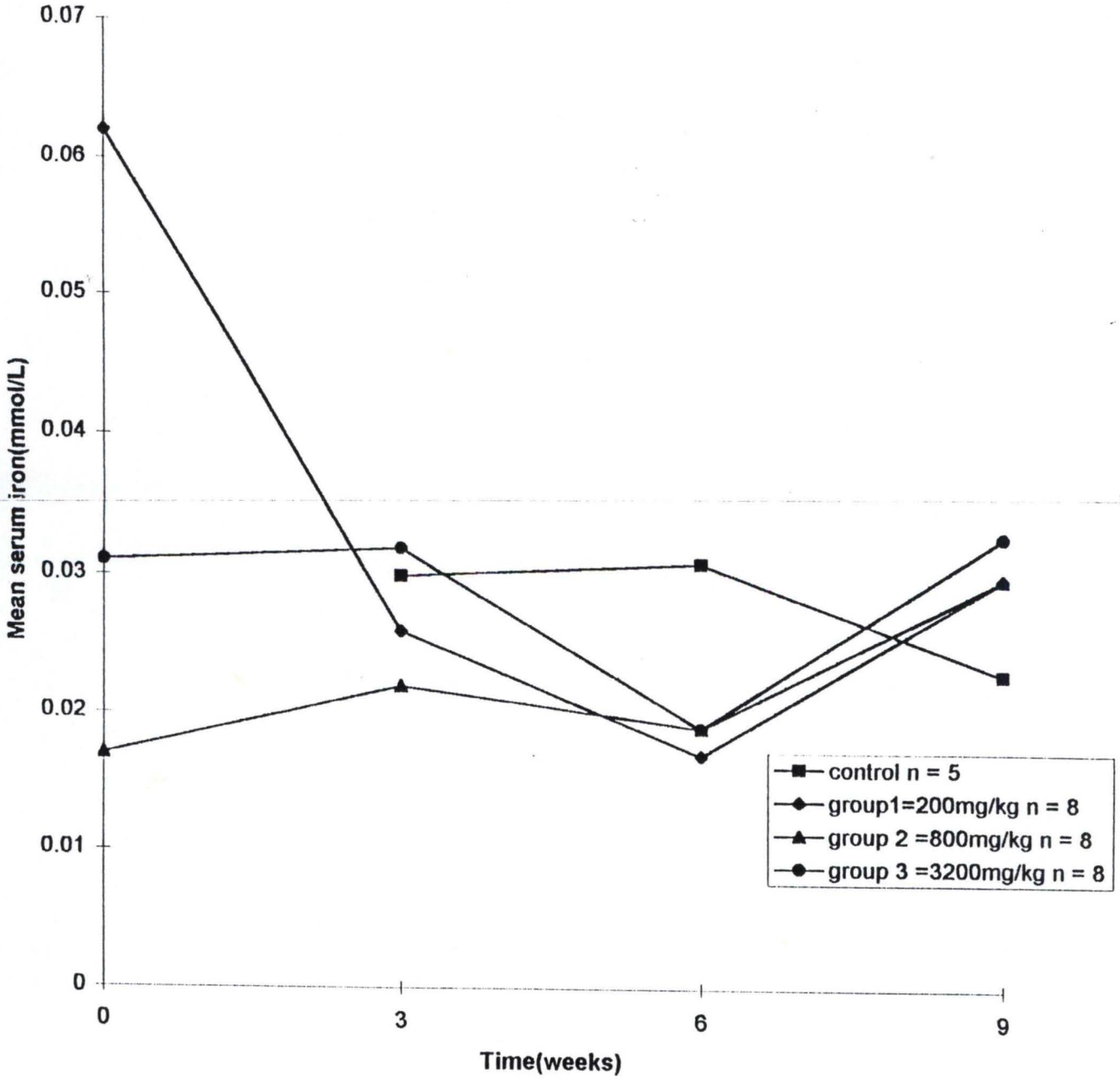

Figure 6: Mean Serum iron in four groups of rabbits treated with varying concentrations of local Salt Lick (Kanwa) for 9 weeks

animal died and there was no sample at the start of sampling (three weeks after the commencement of the experiment). Group 2 animals had the least serum Fe value of 0.016 mmol/L. Groups 1 and 3 animals had 0.062 and 0.031 mmol/L respectively. By the end of the 9th week of experimentation, the control animals had the least value of 0.023 mmol/L and the highest value was observed in-group 3 animals (0.033 mmol/L) followed by groups 1 and 2 with 0.030 mmol/L each.

The mean serum Cu after 9 weeks (Table 4) was slightly higher in group 2 animals (0.015 mmol/L) than in the groups 1, 3 and control, which had 0.013, 0.013 and 0.014 (mmol/L) respectively. Figure 7 presents the mean three weekly interval serum Cu curve for 9 weeks. A very remarkable drop in mean serum Cu was observed between that of commencement point and at 6th week of experimentation in the control and group 2 animals, the control animals had a mean value of 0.018 and 0.012 (mmol/L) at the 6th week while the group 2 animals had 0.019 (mmol/L) at the initial sampling and 0.01 mmol/L at the end of 6th week. A remarkable increase and drop in mean serum Cu was seen in groups 1 and 2 animals respectively by the 3rd week (0.016 mmol/L). The mean and standard deviation of serum Zn level after 9 week experimental period in Table 4 shows the control with a value of 0.026 mmol/L whereas groups 1, 2 and 3 had values of 0.034, 0.029 and

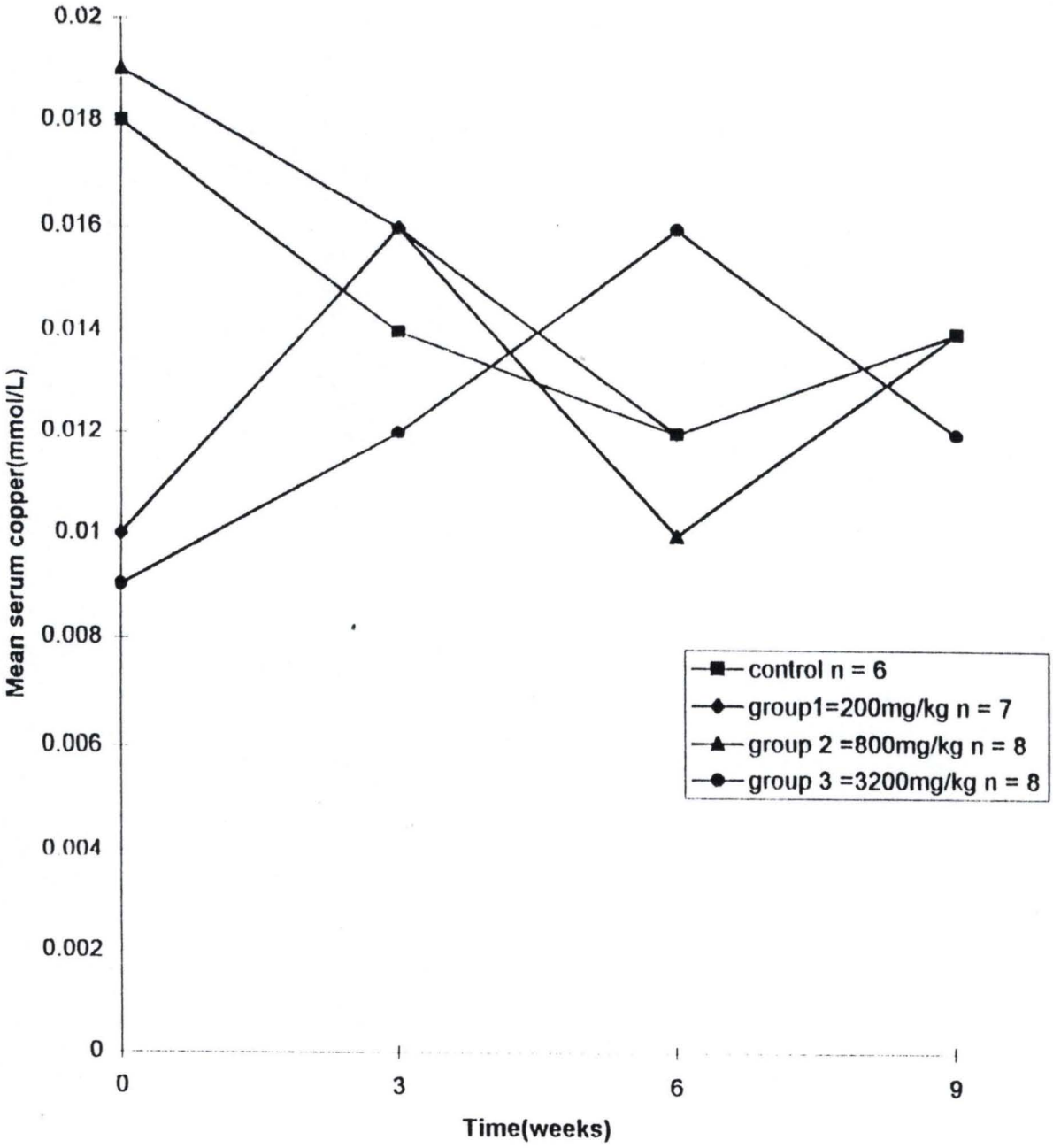

Figure 7: Mean Serum copper in four groups of rabbits treated with varying concentrations of local Salt lick (Kanwa) for 9 weeks

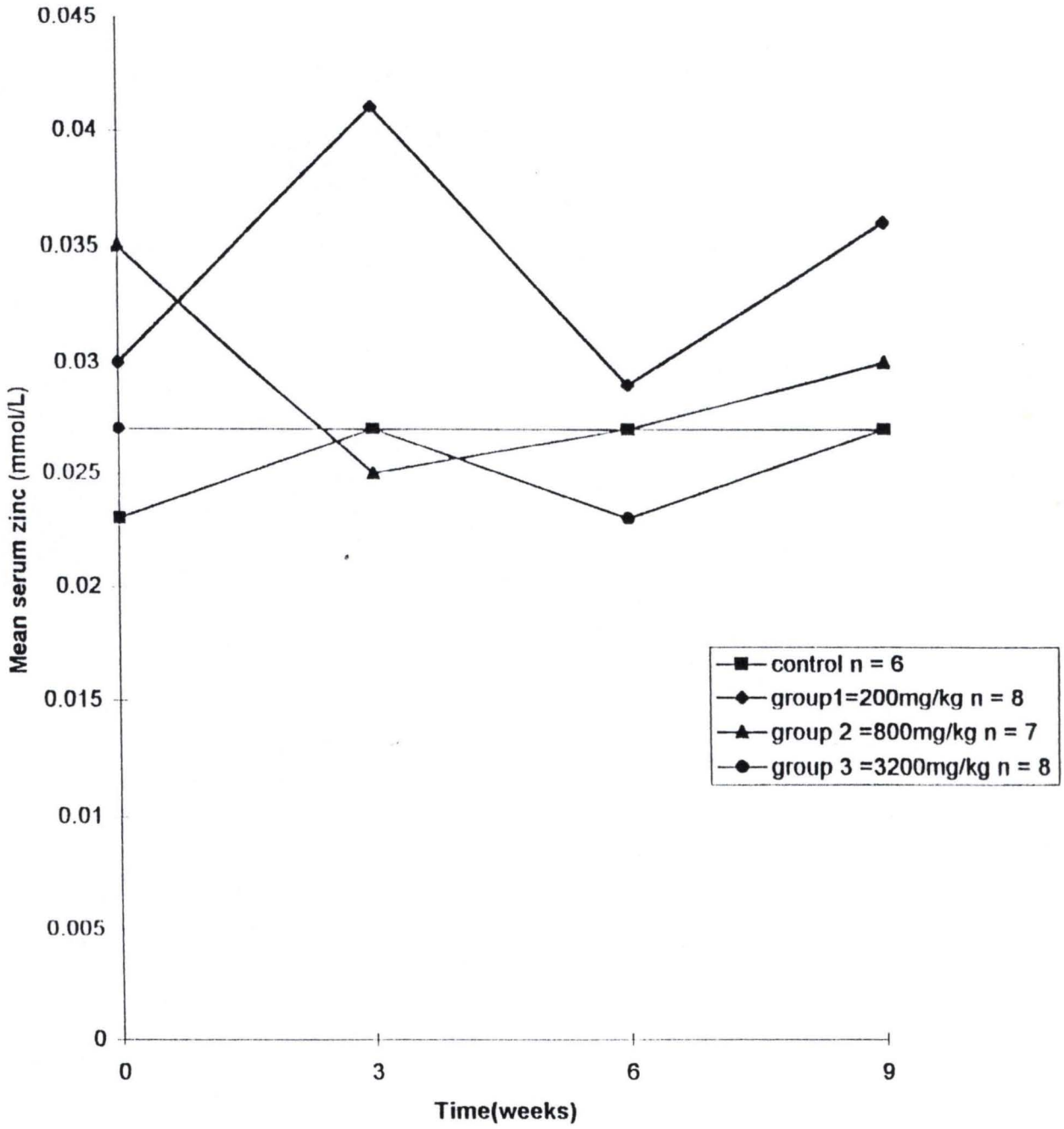

Figure 8: Mean Serum zinc in four groups of rabbits treated with varying concentrations of local Salt Lick (Kanwa) for 9 weeks

0.027 (mmol/L) in decreasing order respectively. Figure 8 shows the mean three weekly mean serum Zn curve for 9 weeks. At the commencement and end of the experiment, the control group had the least mean serum Zn level of 0.023 and 0.027 (mmol/L) respectively.

After the third week group 1 animals had a consistently higher mean serum Zn levels than that of the other groups. At the commencement of experimentation group 1 animals had a mean serum Zn level of 0.03 mmol/L while at 3, 6 and 9 week experimental period, group 1 had values of 0.041, 0.029 and 0.036 mmol/L respectively. The values for groups 2 and 3 were 0.034 and 0.027 (mmol/L) respectively at the initial sampling time and 0.030 and 0.027 respectively at the end of 9th week. The variations observed were however satistically non significant. The mean and standard deviation of serum Cl level after 9 week experimental period is presented in Table 4. The highest value of 113.12 mmol/L was observed for group 3 animals, while the control group had the lowest value of 103.49 mmol/L, groups 1 and 2 had values of 107.49 and 104.21 (mmol/L) respectively. From Figure 9 at the commencement of treatment, group 2 animals had the least serum chloride (103.4 mmol/L), while group 1 had the highest level of serum Cl, (126.4 mmol/L) and group 3 and control had 110.1 and 110.7 mmol/L respectively. By the 3rd week of experimentation period, mean

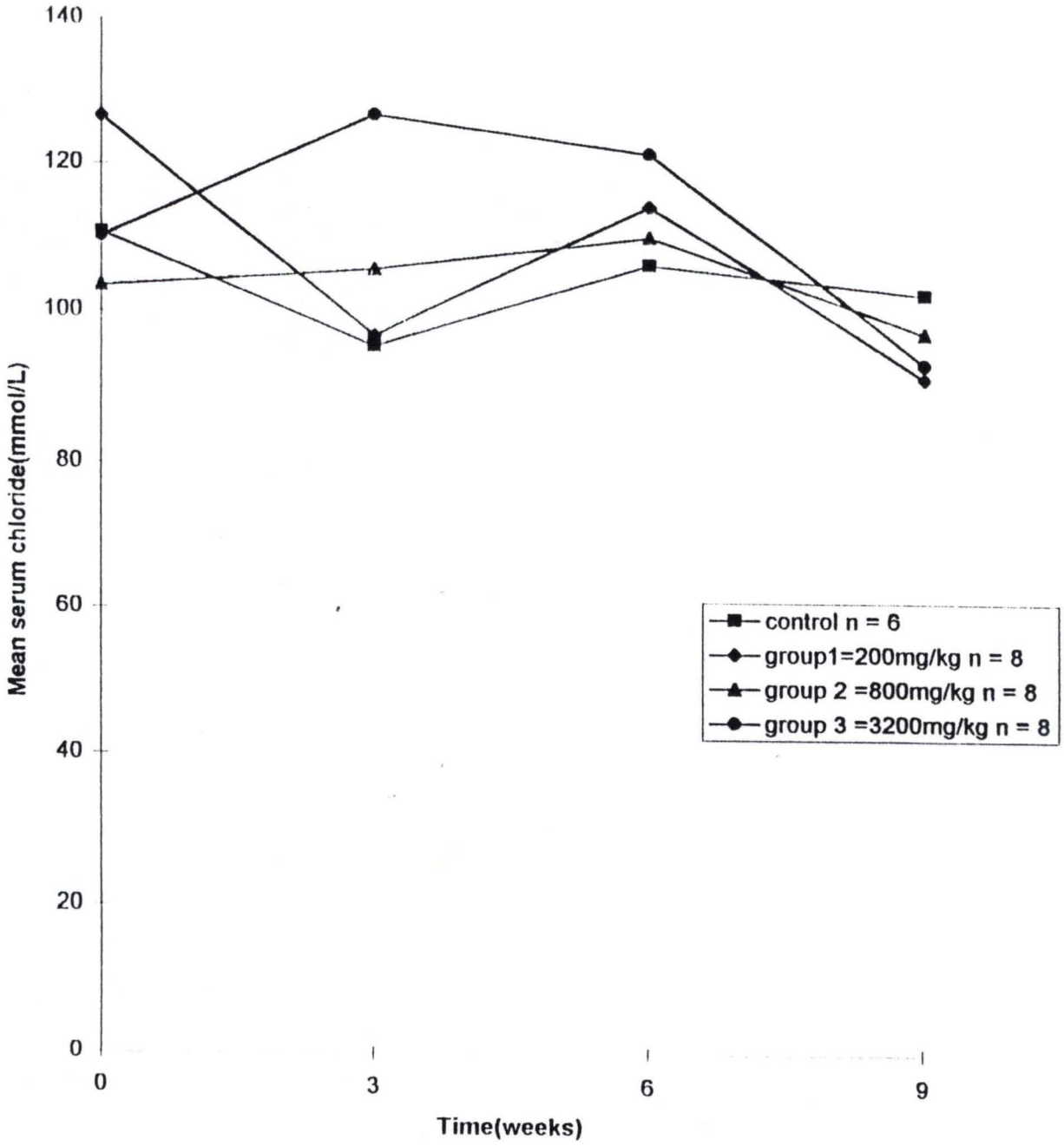

Figure 9: Mean Serum Chloride in four groups of rabbits treated with varying concentrations of local Salt Lick (Kanwa) for 9 weeks

serum Cl of group 1 and control dropped significantly to 97.00 and 95.6 (mmol/L) respectively, whereas that of group 3 rose significantly to 126.9 mmol/L. By the 6th week of the experimental period, the control group had the least mean serum Cl (106.6 mmol/L). But by the end of the 9th week of the experimental period, the control group had the highest mean serum chloride level (102.8 mmol/L) as compared to the values in groups 1, 2 and 3 which had 91.9, 97.7, 9.64 (mmol/L) respectively.

4.7 Serum Biochemical Determinations

Table 5 present the mean and standard deviations of serum total protein, glucose, urea, cholesterol and albumin, at the end of 9 week experimental period. The mean level of serum total protein was lowest in the group 2 animals (6.25 g/100ml) followed by 6.27g/100ml in the control animals while in the groups 1, and 3 animals, the level were 6.67 and 6.62 (g/100ml) respectively. There were fluctuations in the mean serum total protein level after 9 weeks of experimental period (Figure 10). At the commencement of the experiment, the control group had the least mean serum total protein level (4.40g/100ml) as compared with the other 3 treatment groups which had 7.23, 5.94 and 6.85 (g/100ml) for groups 1, 2 and 3 respectively. However, by the end of the 9th week experimental period,

the control group had the higher mean serum total protein (7.5g/100ml) than that of the 3 other treatment groups, which had values of 5.56, 6.68 and 5.29g/100ml for groups 1, 2 and 3 respectively.

The means and standard deviation of serum glucose by the 9th week experimental period is presented in Table 5. It shows that the group 1 animals have the least level of serum glucose (4.55 mmol/L). The highest value was observed in the group 3 animals (6.2 mmol/L) while the control and group 2 had values which were similar (5.70 and 5.76 mmol/L). Figure 11 shows the curve for the three weekly interval of mean serum glucose in the four group experimental animals. At the commencement of experiment, the control group had the least value of 4.66 mmol/L. There was however a progressive increase in the serum glucose level at the 3rd, 6th and 9th weeks with values of 4.92, 6.07 and 7.86 mmol/L respectively. Also at the commencement of experiment, the mean serum glucose level revealed that control group animals had the least mean serum glucose level (4.66 mmol/L) whereas group 3 animals had the highest mean serum glucose level (7.23 mmol/L). By the end of the 9th week of the experimental period, the control group's mean serum glucose was higher (7.86 mmol/L) than that of the treatment groups 1, 2 and 3 which were 5.20, 5.12 and 4.00 mmol/L respectively.

The means and standard deviations of serum urea in the four experimental group of animals after 9th week experimental period is presented in Table 5. It shows that the control animals had the highest level of 9.82 mmol/L, while groups 1, 2 and 3 had values of 7.02, 8.76 and 8.33 (mmol/L) respectively. Figure 12 shows the curve for the mean values of serum urea at three weekly intervals for 9 weeks. At the commencement of experiment, group 1 animals had the least value of 5.03 mmol/L followed by the control (6.96 mmol/L), group 2 (7.06 mmol/L) and group 3 (8.66 mmol/L) in increasing order. By the end of the 9th week of experimental period, the control group had the highest mean serum urea level (11.72 mmol/L) followed by groups 2, 3 and 1 in decreasing order of 9.28, 7.77 and 6.83 mmol/L respectively.

The means and standard deviation of serum cholesterol values after 9 week experimental period is presented in Table 5. It shows that the control animals had value of 5.80 mmol/L while the animals in groups 1, 2 and 3 had values of 4.76, 5.80 and 5.99 (g/100ml) in that increasing order respectively. From Figure 13, at the commencement of the experiment, group 1 animals had the least serum cholesterol level (5.87 g/100ml) followed by the control animals (7.82 g/100ml) and group 3 (8.18 mmol/L) and group 2 (9.95 mmol/L) in that increasing order. By the end of the 9th

week experimental period, the control animals had the highest serum level (7.31 mmol/L) followed by groups 1, 2 and 3 with 6.14, 5.61 and 5.04 (mmol/L) in that decreasing order respectively.

The means and standard deviations value of serum albumin after 9 week experimental period is presented in Table 5. It shows the group 2 animals had the highest level of 6.09 g/100ml while the control had the lowest value of 4.85g/100ml. Groups 1 and 3 animals had values of 6.04 and 5.91 (g/100ml) respectively. Figure 14 shows that at the commencement of the experiment, the control animals had the least mean serum albumin level (2.20g/100ml) as compared to the treated animals in groups 1, 2 and 3 which had values of 7.30, 5.71 and 4.56 (g/100ml) in that decreasing order respectively. This trend was still maintained by the end of the 9th week experimental period, the control group had the least value of 3.56 g/100ml, while groups 1, 2 and 3 had values of 6.03, 5.75 and 5.62 (g/100ml) in that decreasing order respectively.

4.8 Serum Enzyme Determinations

Table 6 presents the means and standard deviation values of SGOT, SGPT and SALP in four groups of experimental animals after 9 week experimental period. The highest SGOT level was observed in the control

Table 5: Biochemical values of four groups of rabbits treated with varying concentrations of local salt (kanwa) for 9 weeks.

	Control n =6	Group 1 n = 8	Group 2 n = 8	Group 3 n = 8
Total Protein(g/100ml)	6.27± 1.35	6.67 ± 0.65	6.25 ± 0.53	6.62 ± 1.30
Glucose (mmol/L)	5.7 ± 1.47	4.55 ± 0.62	5.76± 1.18	6.2± 1.88
Urea (mmol/L)	9.82 ± 2.12	7.02 ± 1.59	8.76 ± 1.88	8.33 ± 0.56
Cholesterol (mmol/L)	5.80 ± 2.11	4.76 ± 1.39	5.80 ± 2.11	5.79 ± 1.96
Albumin (g/100ml)	4.85± 2.34	6.04 ± 1.00	6.09 ± 1.60	5.91 ± 1.68

Grp = group
(n) = No. of animals
Control = received no local rock salt (kanwa) treatment)
Group 1 = 200mg/Kg local rock salt (kanwa) treatment
Group 2 = 800mg/Kg local rock salt (kanwa) treatment
Group 3 = 3200mg/Kg local rock salt (kanwa) treatment

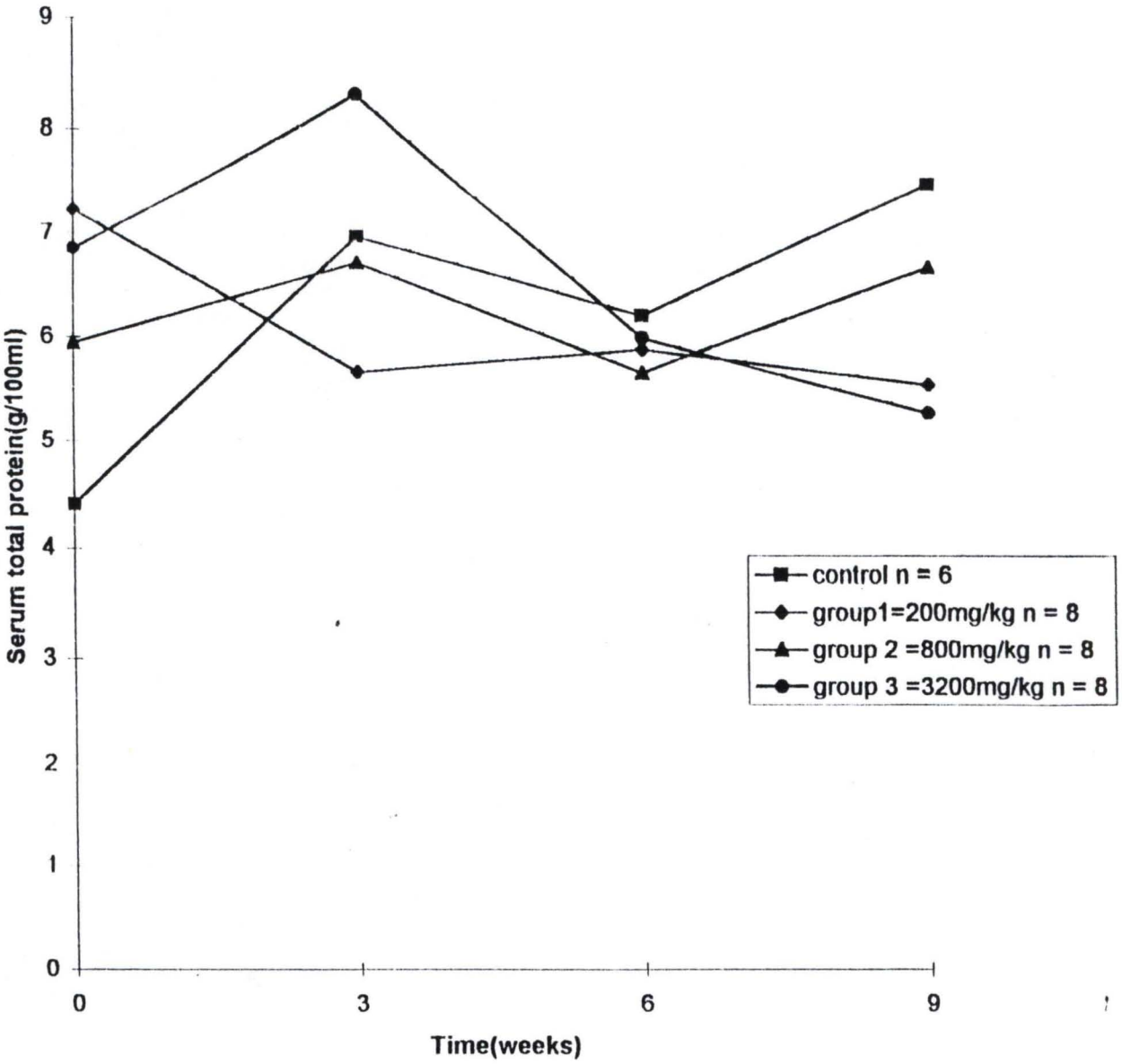

Figure 10: Mean Serum total protein in four groups of rabbits treated with varying concentrations of local Salt Lick (Kanwa) for 9 weeks

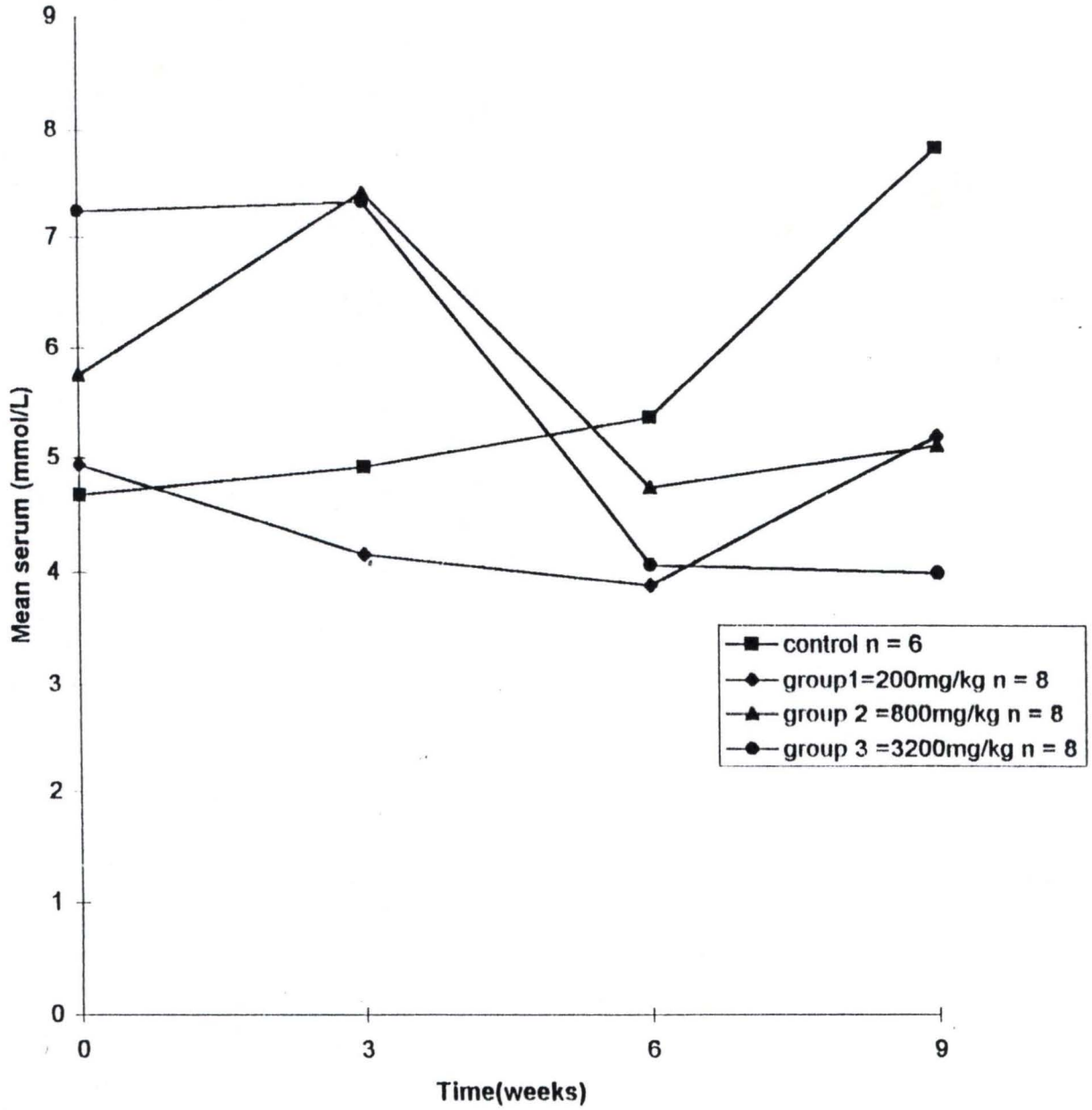

Figure 11: Mean Serum Glucose in four groups of rabbits treated with varying concentrations of local Salt Lick (kanwa) for 9 weeks

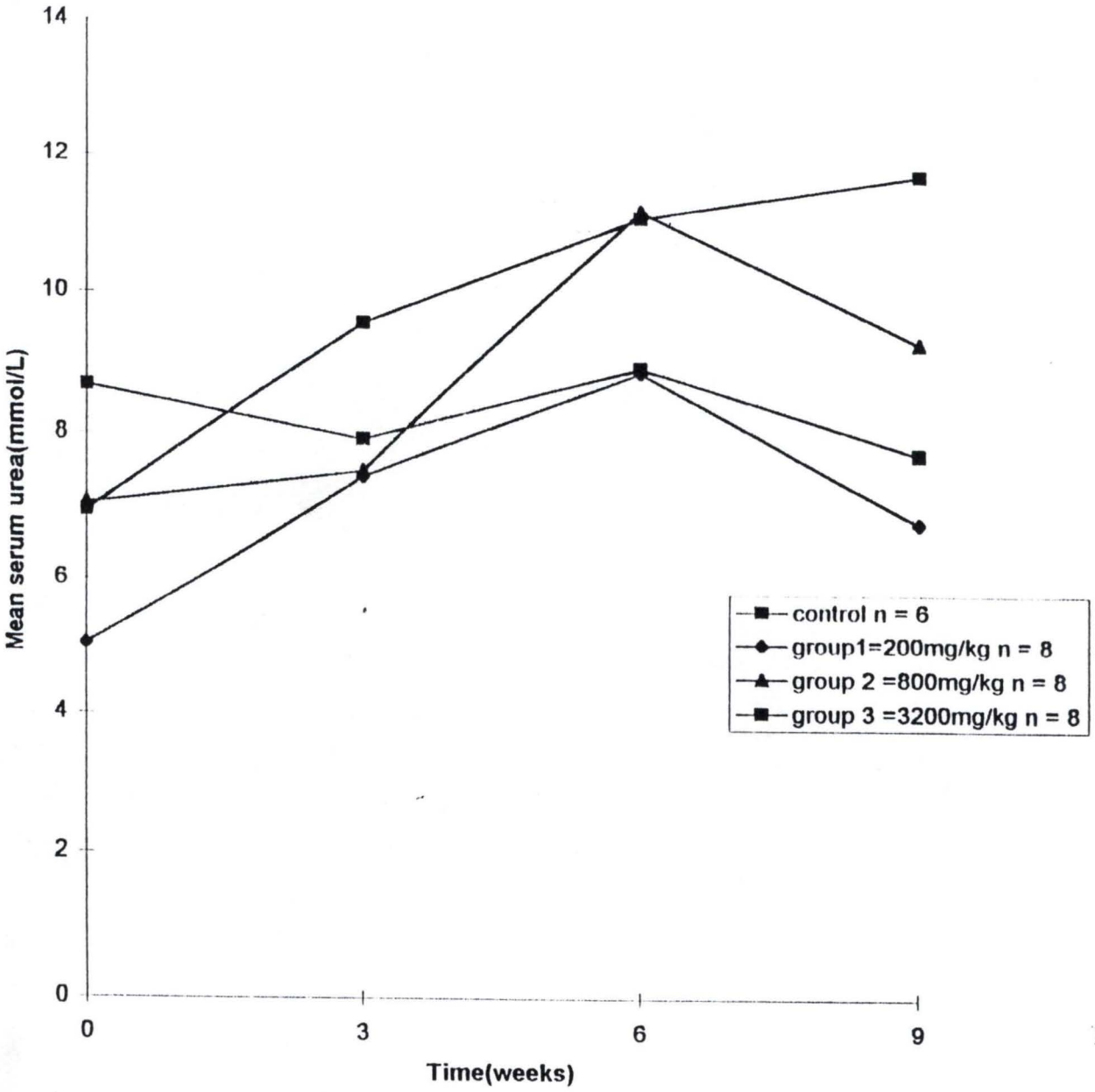

Figure 12: Mean Serum Urea (BUN) in four groups of rabbits treated with varying concentrations of local Salt Lick (Kanwa) for 9 weeks

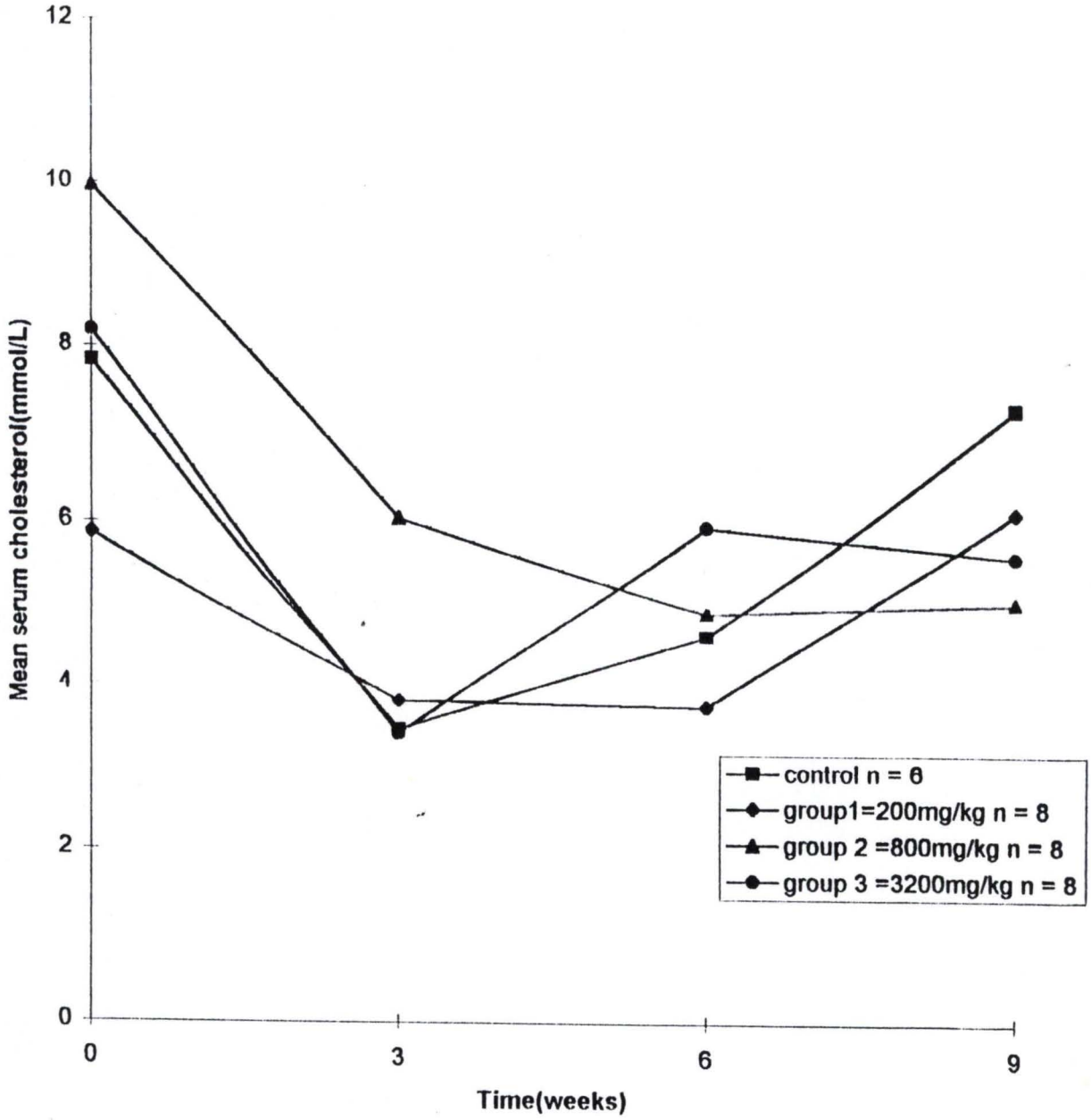

Figure 13: Mean Serum Cholesterol in four groups of rabbits treated with varying concentrations of local Salt Lick (Kanwa) for 9 weeks

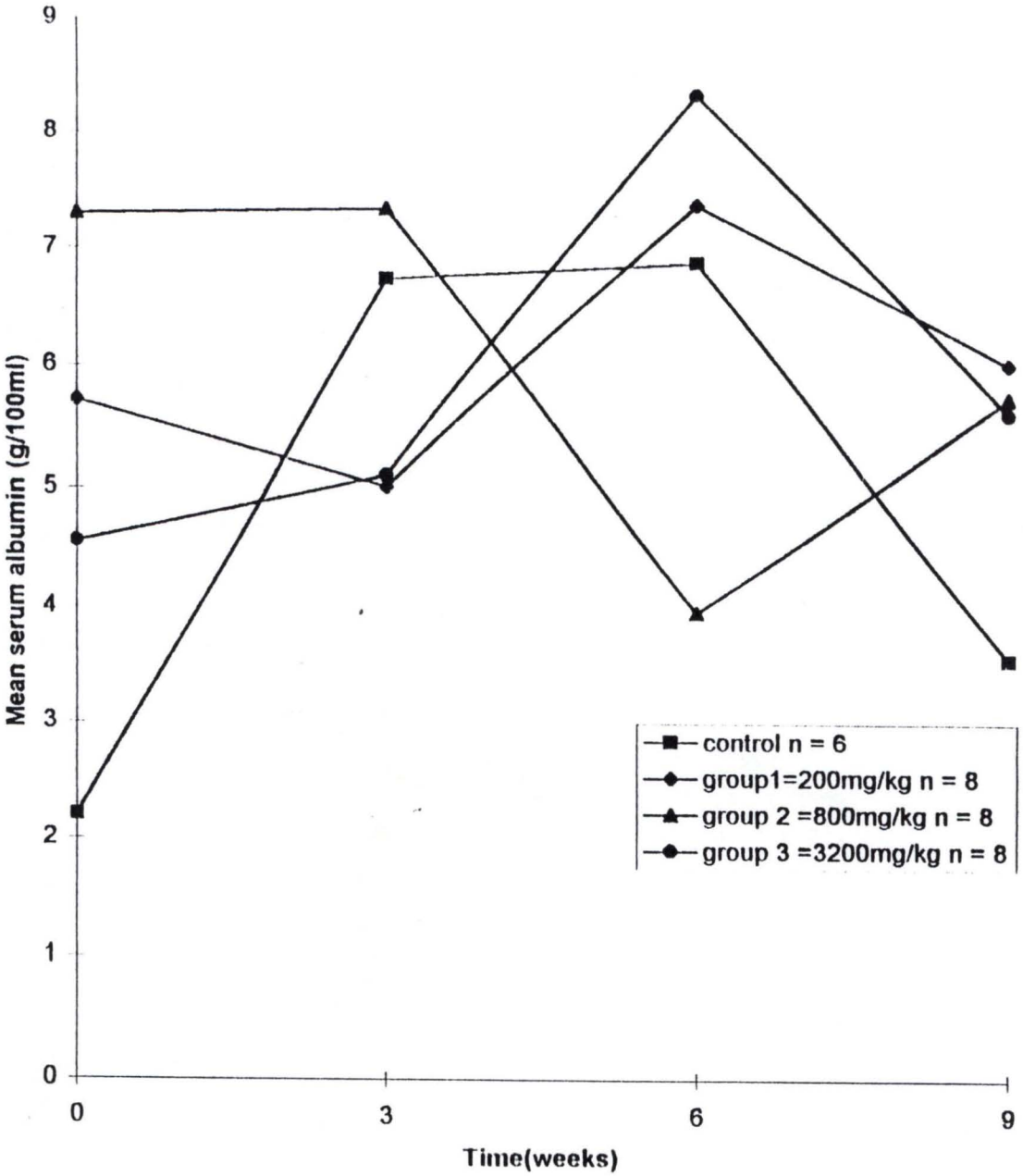

Figure 14: Mean Serum Albumin in four groups of rabbits treated with varying concentrations of local Salt Lick (Kanwa) for 9 weeks

Table 6: Serum enzyme mean standard deviation values of four groups of rabbits treated with varying concentrations of local salt (kanwa) for 9 weeks

	Control	Group 1	Group 2	Group 3
Glutamate Oxoloacetate Transaminase (SGOT) U	19.23± 5.19 n = 6	14.38 ± 3.25 n = 8	18.33 ± 3.81 n = 8	16.72 ± 4.15 n = 8
Glutamate PyruvateTrasaminace (SGPT) U	20.66± 4.27 n = 6	19.17 ± 3.54 n = 8	18.29 ± 5.86 n = 8	20.97 ± 2.49 n = 8
Alkaline Phosphatase U	25.75 ± 15.22 n = 6	22.90 ± 10.80 n = 8	21.71 ± 8.27 n = 8	26.74 ± 12.55 n = 8

Grp = group
(n) = No. of animals
Control = received no local rock salt (kanwa) treatment)
Group 1 = 200mg/Kg local rock salt (kanwa) treatment
Group 2 = 800mg/Kg local rock salt (kanwa) treatment
Group 3 = 3200mg/Kg local rock salt (kanwa) treatment

animals (19.23U) while groups 1, 2 and 3 had values of 14.38, 18.33 and 17.72(U) respectively. From Figure 15, a lot of fluctuations in the mean three weekly SGOT values was observed in all groups during the 9 week experimental period. At the commencement of the experiment, the control group had the highest value of 20.96U, followed by groups 2, 1 and 3 (20.96U, 17.49 and 12.82(U) in decreasing order respectively. **By** the end of the 9th week experimental period, the control group still had the highest level of mean serum GOT (19.38U), then the groups 3, 2 and 1 (17.22, 12.69 and 10.85) in decreasing order respectively. The SGPT mean value (Table 6) was the highest in the group 3 animals (20.97U), while control and groups 1 and 2 had 19.17, 18.29 and 20.66 (U) respectively at the end of the 9 week period. From the 3 weekly SGPT levels depicted in Figure 16, all the treatment groups including the control group, showed decreased SGPT by the 3rd week after commencement of experiment. Also all the groups showed a marked increase except group 1 that showed a further drop. By the end of the 9th week experimental period, the animals with the highest dose (3200 mg/kg) had the highest mean serum SGPT (23.43U) level followed by that of the control (21.76U) while groups 1 and 2 both had 18.80U each. The SALP mean and standard deviation after 9 week experimental period in

Table 6 shows that the control group had a value of 25.75U, while groups 1, 2 and 3 animals had 22.90, 21.71 and 26.74(U) respectively.

Figure 17 shows fluctuations in the mean SALP three weekly values. The fluctuations pattern of SALP levels in all the groups were similar. However, by the end of the experimental period, the control group had the least value of SALP (9.30U) whereas the group 3 animals had the highest serum ALP value of 13.52U. There were no statistically significant difference between the control and the treatment groups at P=0.05.

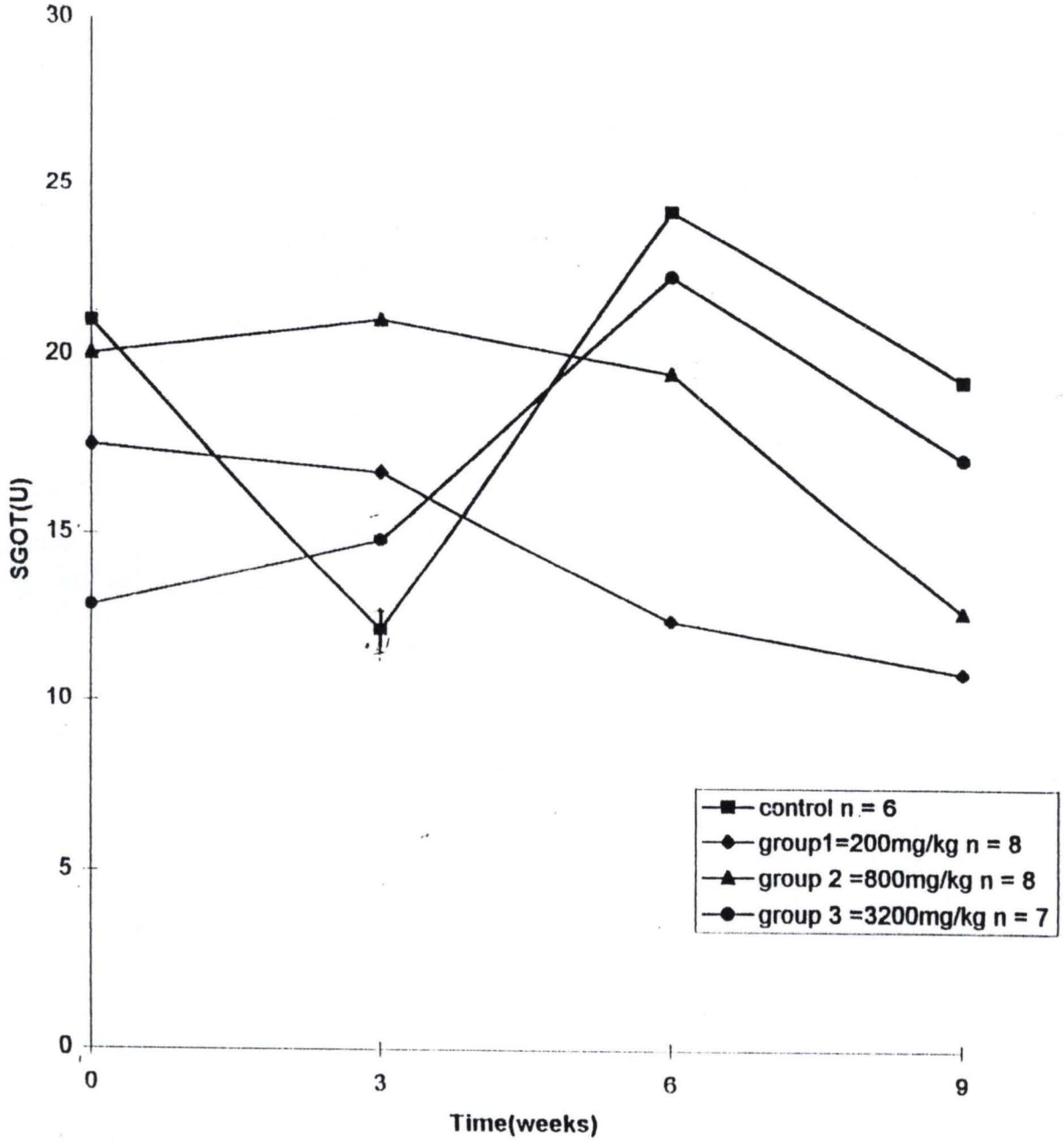

Figure 15: Mean Serum Glutamate oxoloacetate transaminase (GOT) in four groups of rabbits treated with varying concentrations of local Salt Lick (kanwa) for 9 weeks

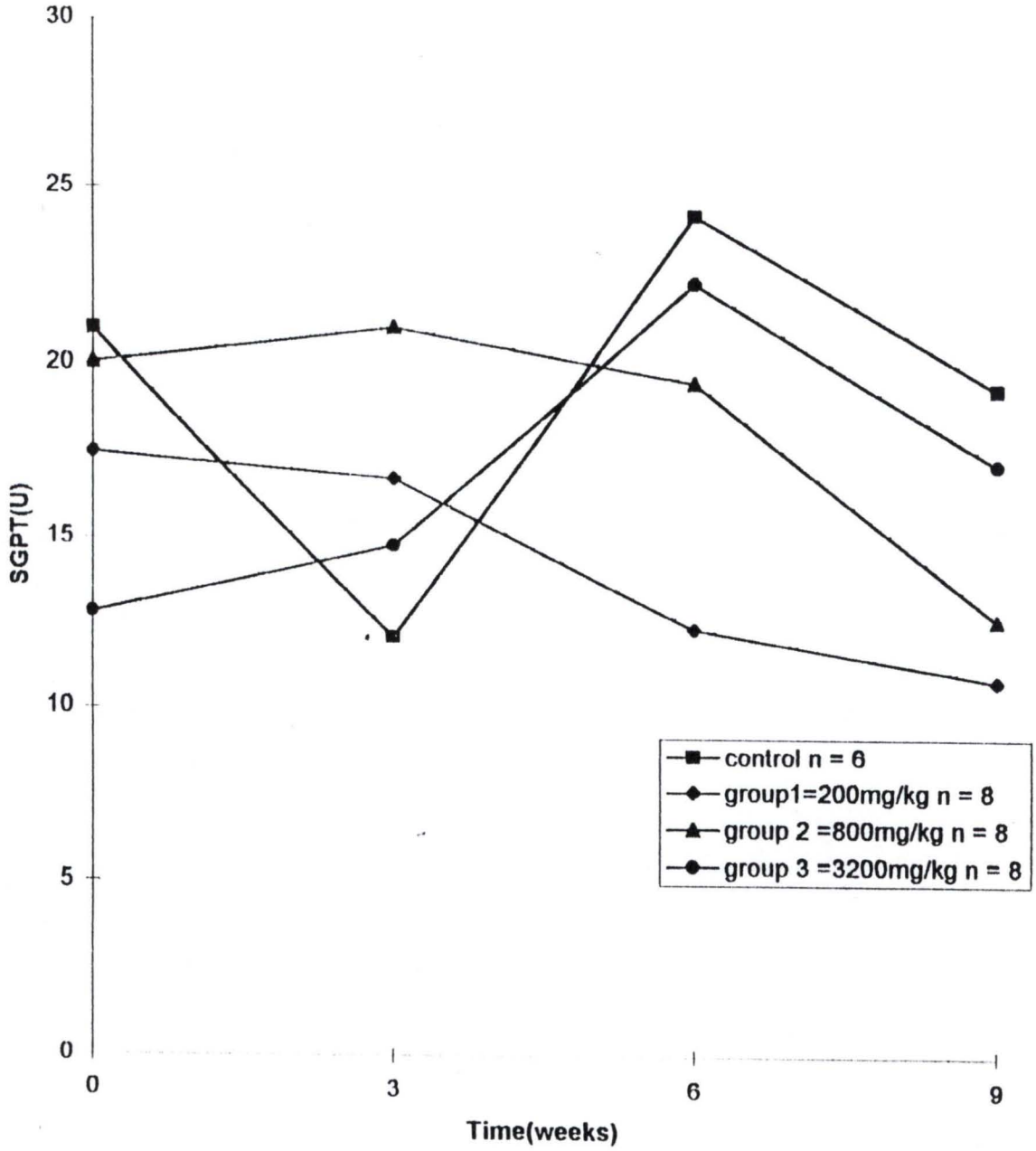

Figure 16: Mean Serum Glumate pyruvate transaminase (GPT) in four groups of rabbits treated with varying concentrations of local Salt Lick (kanwa) for 9 weeks

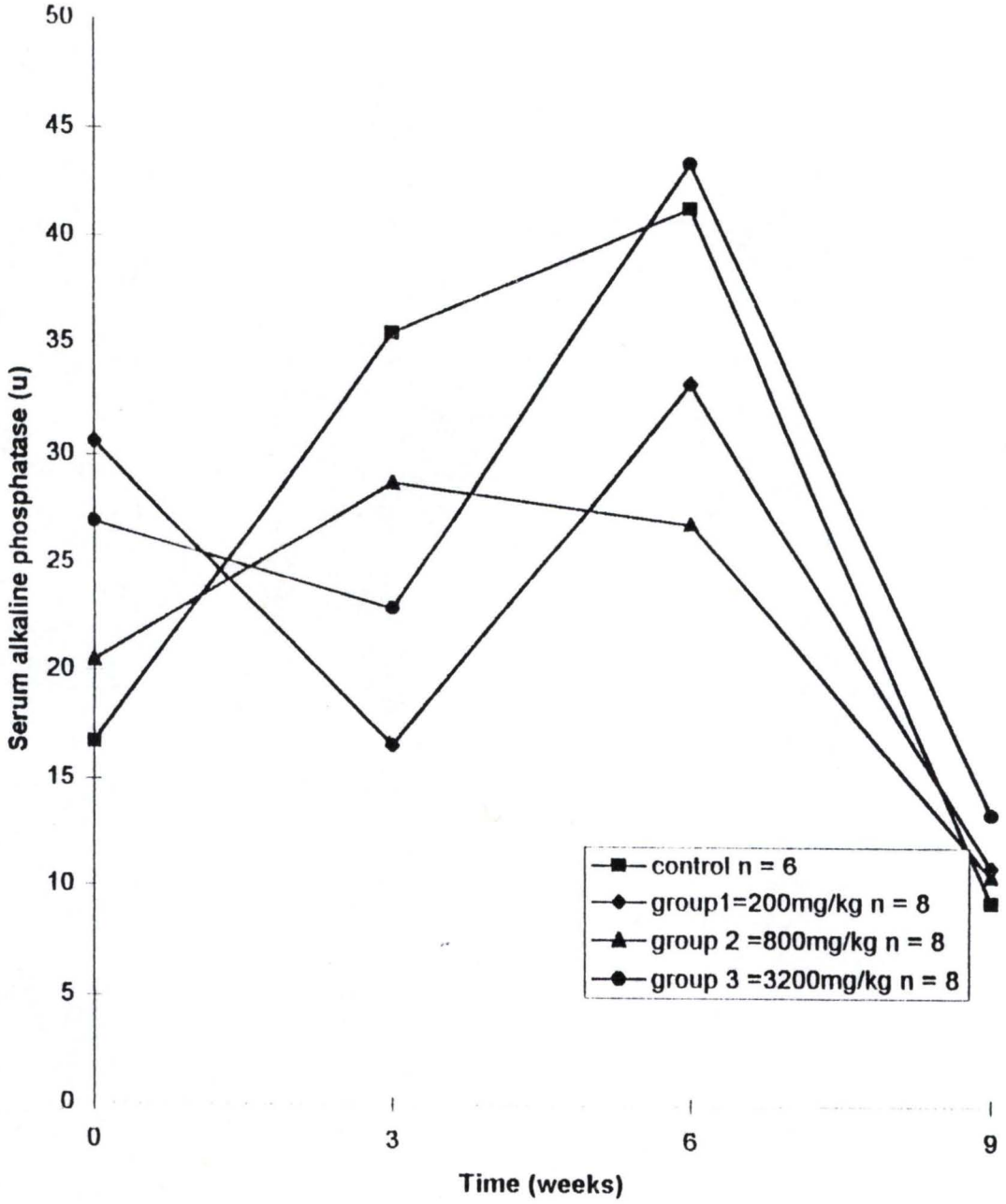

Figure 17: Mean Serum Alkaline phosphatase (ALP) in four groups of rabbits treated with varying concentrations of local Salt Lick (kanwa) for 9 weeks

CHAPTER FIVE

DISCUSSION

5.1 The presence of mineral elements (Na, K, Ca, Mg, Zn, Cu, Fe, CO_3^{2-} SO_4^{2-} and PO_4^{3-}) in the local salts (kanwa) analyzed in this studies agrees with and confirms the reports of other workers (Peers 1959, Makanjuola, 1975; Gbodi et al., 1982 and Ako 1984). The occurrence of these minerals in the local salt (kanwa) also provide evidence for its possible beneficial effects as mineral supplement for animals. From the results, it was observed that the sodium content of most of the kanwa samples were high as noted by Gbodi et al., (1982) and Ako (1984). In comparison with the composition and concentrations of the other kanwa samples that were collected and analyzed, sample 7 (S7) which was fed to the animals was found to have relatively high level of each of the anions and cations analyzed for. Seven other samples (S3,S5,S6,S8,S9,S10 and S12) also had either slightly higher or the same level of the analyzed ions as that of S7 (Sample 7) had the lowest concentration of Na (22g/100g), and had the highest level of Fe (7.94mg/100g).

In toxicologic experiments, change in body weight is an indicator of an adverse effect (Gad and Well, 1982) In this study, weight changes at the end of 9 week experimental period showed a higher mean weight gain in the

treated animals when compared with that of control animals (Table 3). The highest mean weight gain was observed in the group 3 animals (63.5) fed with the highest level of kanwa (3200mg/kg). Animals in groups 1 and 2 fed with 200mg/kg and 800mg/kg respectively also showed a higher weight gain than the control animals. The three weekly interval weight changed in Figure 1 shows that at the start of experimentation, the control animals had a lower weight change (11.8g) than the treated animals. However, by the 9th week of experimentation period the control animals had the highest weight change than the salt treated animals which showed a decreasing trend in their weight change. Thus it would appear that the salt treatment had a low weight gaining effect on the treated animals. This observation appears to suggest the probability that the salt may have induced a decrease of appetite resulting in lower feed intake and resulting in lower weight gain.

Despite the fluctuations observed in the mean PCV values for control and treated animals (Table 3) over the 9 week experimental period, their PCV mean values (range) were within the range obtained by other workers Kozma *et. al* (1974) reported in their work, that their control rabbits had a PCV range value of 35-45(%). A trend was also clearly established despite the fluctuations seen in PCV values for the control and treated animals, the control animals having the least value 39.5% while groups 1, 2 and 3 rabbits

had values of 41.16, 40.35 and 42.31% respectively. The fluctuations were however of no statistical significance. Schermer (1967) reported that rabbits showed an appreciable fluctuations in their hematological parameters as a result of nutrition or environment. From Figure 2, the local salt treatment appears to have a decreasing effect on the PCV, but the range of values in this study (40-42%) are within the range cited in literature (40-45%) by Rozma (1974).

The results on the mean serum electrolytes show no statistical significant variations between the animals that were fed with kanwa and the control. The mean value for serum K in control animals was 3.76 mmol/L, while the literature cited value ranged between 3.3 – 4.1 mmol/L (Okerman, 1988) and 3.6 – 6.9 was reported by Kozma et. al (1974). From the result, (Table 4), no significant difference was observed between the control and treatment group's mean serum K at the end of the 9 weeks experimental period. These findings agreed with that of Ikwuegbu and Gbodi (1983), who found that feeding of kanwa had no significant effect on serum level of Ca, K, Mg, Zn, Fe and Cu in cattle. However Figure 3 suggest that a long time treatment with kanwa may have an elevating effect on serum K level. Incidence of high serum K level is rarely encountered because any K absorbed by the intestinal tracts causes only a slight and temporary increase

in the serum K levels, which is rapidly exreted by the kidney (Tietz, 1973). Shocks however increases serum K level, as is the case in renal failure.

The mean values for serum sodium in control animals was 138.34 mmol/L. This is within the normal range (128-148 mmol/L) as reported by Okerman (1988), and 100-145 mmol/L cited by Kozma *et al* (1974). Result in Figure 4 tend to indicate that high dosage of kanwa may have a depressing effect on serum Na level. The reasons for this might be difficult to explain, since from analysis kanwa had high level of NaCl and thus high dosage of kanwa is expected to increase serum Na level. A possible explanation may be that, with high serum Na, body homeostatic mechanism could have been stimulated resulting in rapid excretion of excess Na or due to the presence of sodium antagonist (Corah, 1996).

The mean serum Mg level in this work was 0.65 mmol/L, which is within the normal range (0.5-0.8 mmol/L) reported by Okerman (1988). From the result in Table 4 and Figure 5, it can be inferred that treating rabbits with high level of kanwa salt may have an elevating effect on the serum Mg. Increased serum Mg levels have been observed in dehydration, severe diabetes, acidosis and in any condition interfering with the glomeruli filtration, this results in the retention and elevation of serum Mg (Tietz,

1973). A reciprocal relationship between serum Mg and Ca levels and also between serum Mg and PO^{3-}_4 have been reported by Cole (1966).

The control rabbits in this work had a mean serum Fe value of 0.028 mmol/L (Table 4) which is within the normal range (0.026-0.033 mmol/L) reported by Gbodi et al, (1990). Group 2 animals had the least serum Fe level of 0.022 mmol/L. Figure 6 suggests that the treatment with the local salt (kanwa) had an elevating effect on the serum Fe level. Elevations in the levels of serum Fe from normal level have been observed by Tietz (1973) in such conditions, as in red cell destruction (hemolytic anemia), decreased formation of blood as in lead poisoning or when there is defective iron storage.

No much difference was observed during the four experimental groups in the serum Cu level after 9 weeks of treatment (Table 4). From Figure 7, it appears that the kanwa have a decreasing effect on the serum Cu level because the value at the commencement were relatively higher than that at the end of the 9th week experimental period. This is contrary to the expected rise in the serum Cu level since Cu was one of the mineral constituent of the fed kanwa. Decreased Cu can be as a result of malnutrition, malabsorption or nephrotic syndromes.

Table 4 shows that treatment of rabbits with kanwa had a lowering effect on the serum Zn level after 9 weeks. But the 3 weekly serum Zn level depicted in Figure 8 tend to indicate that treatment with local salt lick produced a higher serum level. However the elevating effect was not dose dependent, since the group 1 animals treated with the lowest concentration of local salt (200mg/kg) had higher serum Zn level than those treated with higher concentrations. It is difficult to speculate on what could be responsible for this.

From the result (Figure 9), it would appear that the treatment with kanwa containing chloride had an initial elevating effect on the serum chloride. This observation tend to agree with the findings of Tietz (1973) who observed that intake of Cl salts can result in high serum Cl levels.

No much difference was observed in the mean serum total protein between treated and control animals after 9 week experimental period. It appears that the treatment with kanwa may have a lowering effect on the serum total protein level (Figure 10). It has been shown that low level of serum total protein can be caused by salt retention syndrome, resulting in the dilution of protein fractions (Grant, *et al.*, 1973). Ikwuegbu and Gbodi's work on the effect of feeding local salt to young bulls in 1983 showed that there was a non-significant difference in the level of serum total protein.

Lower level of serum glucose was recorded for the group 1 animals as compared to the control group as well as groups 2 and 3 at the end of 9 week experimental period. From Figure 11, treatment with kanwa appears to have a depressing effect on the serum glucose level because, although throughout the experimental period a lot of fluctuations was observed in serum glucose levels in all groups, but by the end of the experimental period, serum glucose level of treated animal groups were far below that of control. A long time low intake or deficient absorption of protein may result in the lowering of the serum level of glucose (Grant *et. al*, 1973) or could be due to technical error.

The level of serum urea in the control animals was slightly higher compared to the values obtained for the treatment groups. The results in Table 5 and Figure 12 tends to indicate that kanwa appears to have a depressing effect on the blood urea nitrogen level. Serum urea concentration is known to vary widely because various factors effect it. Dietary protein levels, the rate of protein catabolism, health status of liver and renal function of kidney are known to be some of the factors affecting it (Whitby *et al*, 1987; Martins *et al*., 1985; Bortolussi *et al*, 1996).

Lower but statistically insignificant mean serum cholesterol was recorded for the animals feed 200 mg/kg salt lick as compared with the

animals in groups 2, 3 and the control animals after the 9 week experimental period (Table 5). Figure 13 suggests that the kanwa appears to have a depressing effect on the serum cholesterol level since by the 9th week experimental period control animals recorded higher serum cholesterol level than all treatment groups. Higher or lower levels of serum cholesterol can be caused by renal disease (Ellefson and Caraway, 1973). Emotional stress has been shown to cause marked fluctuations in serum cholesterol in some individuals, but not in others (Ellefson and Caraway, 1973).

The result on serum albumin level showed variations in each group. After 9 week experimental period, the mean serum albumin levels were in the treatment groups slightly higher in the control animals (Table 5). Figure 14 shows a great fluctuations at 3 weekly intervals in serum albumin levels in all the four groups of animals and this is difficult to interprete. However, the normal albumin value obtained in control animals in this study was slightly higher than that cited in the literature (4.85-6.09 g/100ml). Okerman (1988) reported a range of 3.5-5.5 (g/100ml). The variation were however statistically non significant at P=0.05.

The serum enzyme levels of the rabbits in this study were within the ranges given by Okerman (1988). At the end of 9 week experimental period, treatment with local salt appears to have a slight depressing effect on SGOT

in the treated groups (Table 6), because mean SGOT in the control animals was higher but not statistically significant than in the treatment groups. From Figure 15, it appears as if treatment with kanwa on SGOT was dose dependent by the 9th week experimental period. At the end of the 9 week experiment, treatment with kanwa had no significant or remarkable effect on SGPT (Table 6). However, the 3 weekly mean SGPT levels (Figure 16) tend to suggest that feeding at high dosage (above 3200 mg/kg) may have increasing effect on SGPT. Kachmar et al., (1973) said that serum elevation of SGPT are rarely observed except in parenchyma liver disease. Thus high dosage of kanwa to animals may have effect on the integrity of the liver cells.

The mean serum alkaline phosphate levels in all the studied groups are within the range cited by Okerman (1988). At the end of the 9 week experimental period, slightly higher mean serum SALP level was recorded in the group 3 animals as compared to the control and groups 1 and 2 animals (Table 6). Also, the 3 weekly SALP record as shown in Figure 17 suggest that feeding of local salt licks above 3200 mg/kg can cause an elevation in SALP. Elevation of SALP are usually seen in disorders which involve liver and bone (Kachmar *et al.*, 1973).

Conclusion

From this study the following conclusions can be made:

1. The Kanwa treatment has no statistically significant effect at (P = 0.05) on the levels of packed cell volume, average weight gain, serum electrolytes and microelements. (Na, K, Mg, Fe, Cl, Cu, Zn) in young growing rabbits.
2. Supplementary feeding of local salt lick (kanwa) at lower dosages (i.e 800 mg/kg) may have a beneficial effect on animals but dosages above 3200 mg/kg may likely have injurious effects on the animals.

Suggestion and Recommendations

i. Since there have not been any work done on this topic in this area, it is suggested that the value reported in this study could serve as baseline values for rabbits in the area.

ii. Further work is required to be carried out for longer period to further ascertain the nutritional and toxicological effects of the local rock salt (kanwa) in rabbits.

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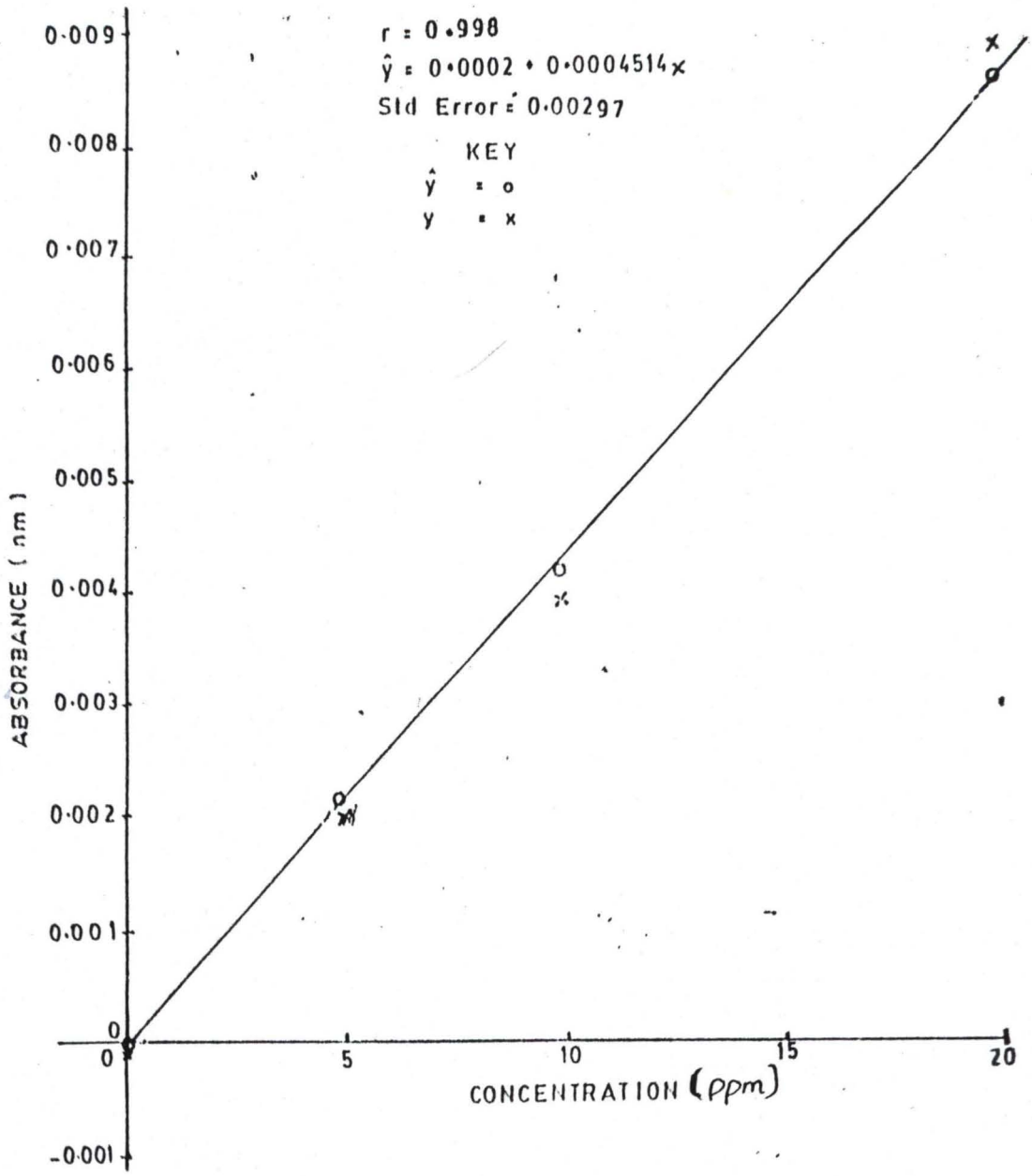


Fig. 1.1: Calibration curve for Calcium in Kanwa.

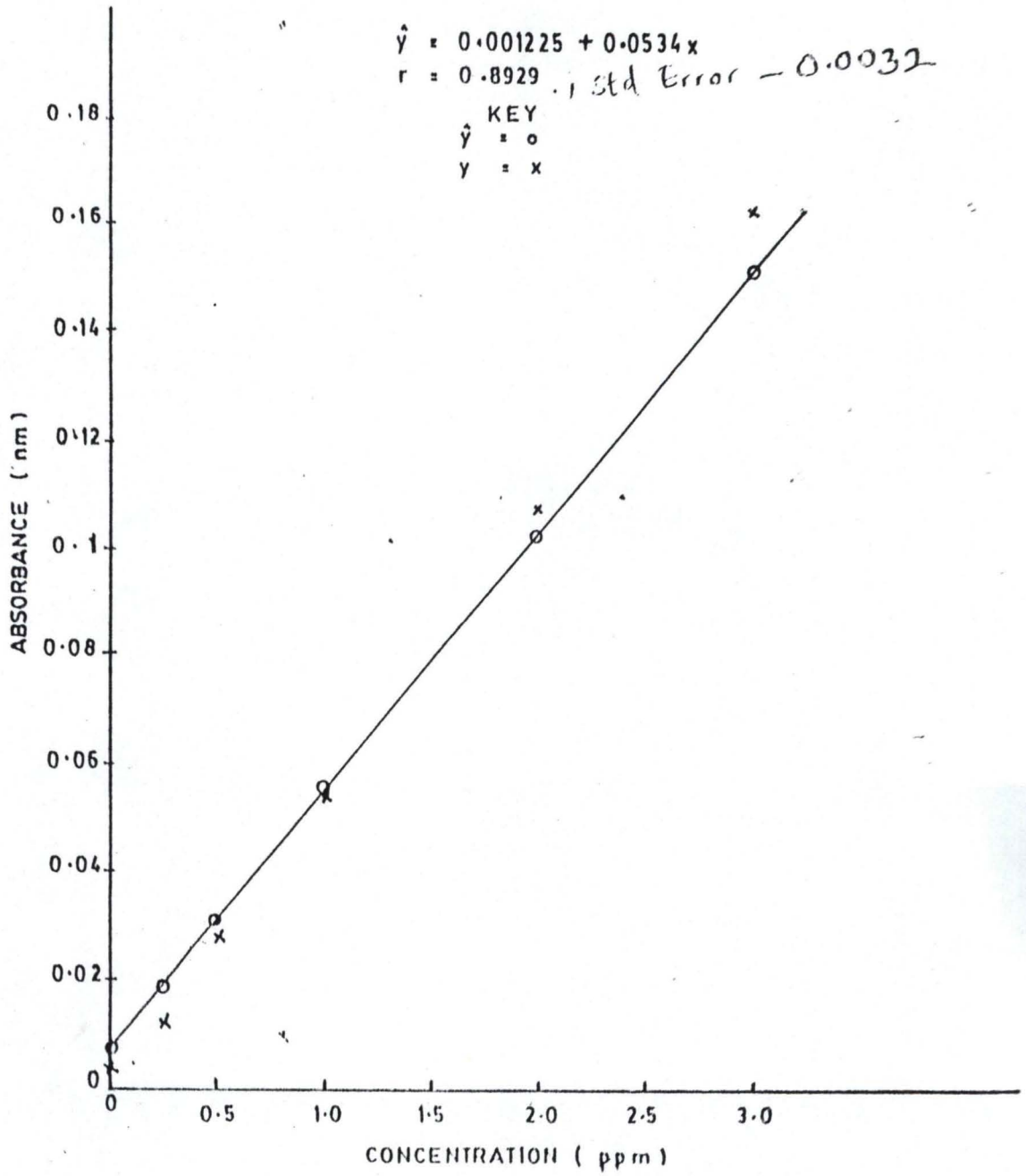


Fig.1.2: Calibration Curve for Magnesium in Kanwa.

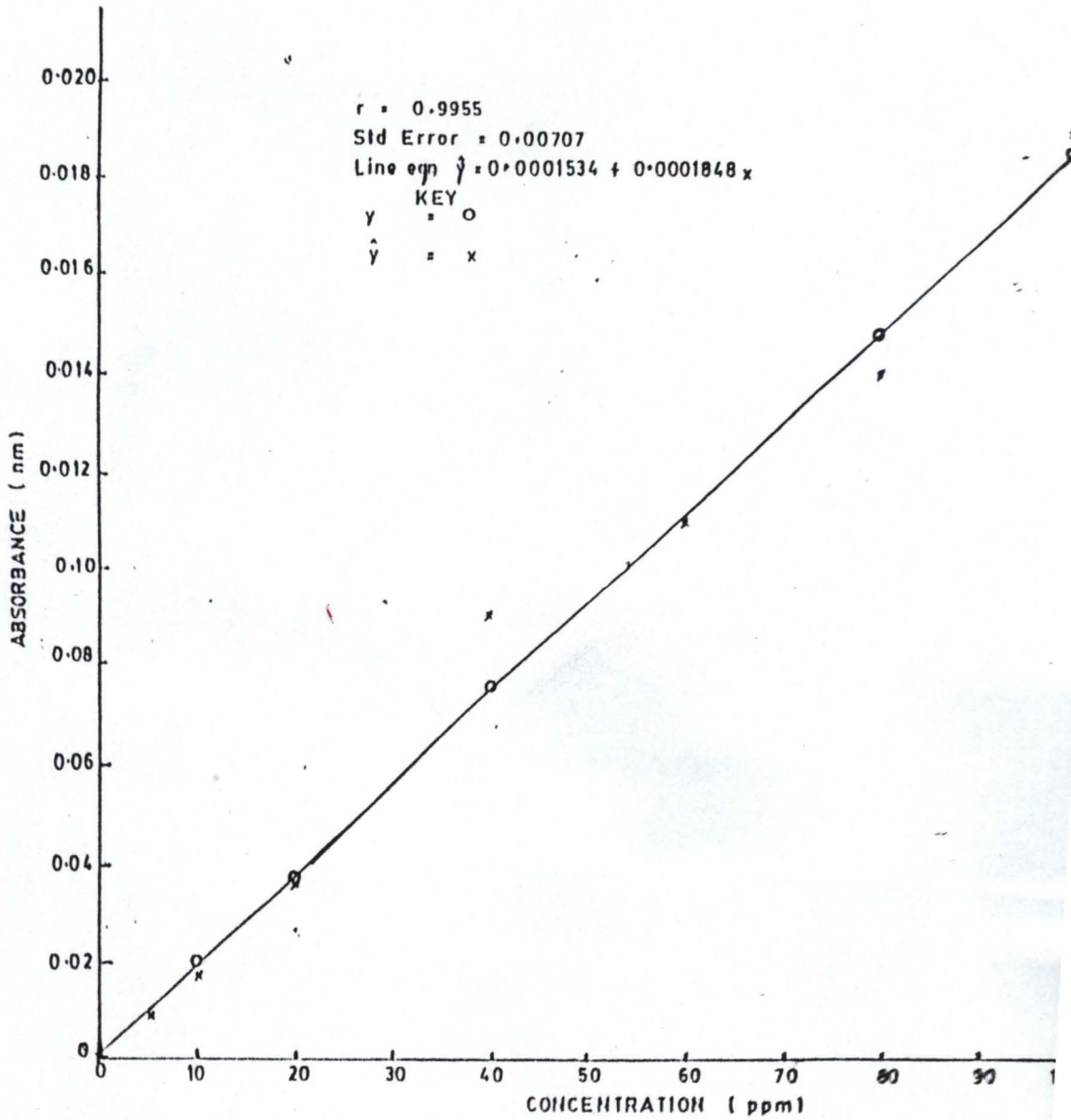


Fig. 1.3 : Calibration Curve for Potassium in Kanwa.

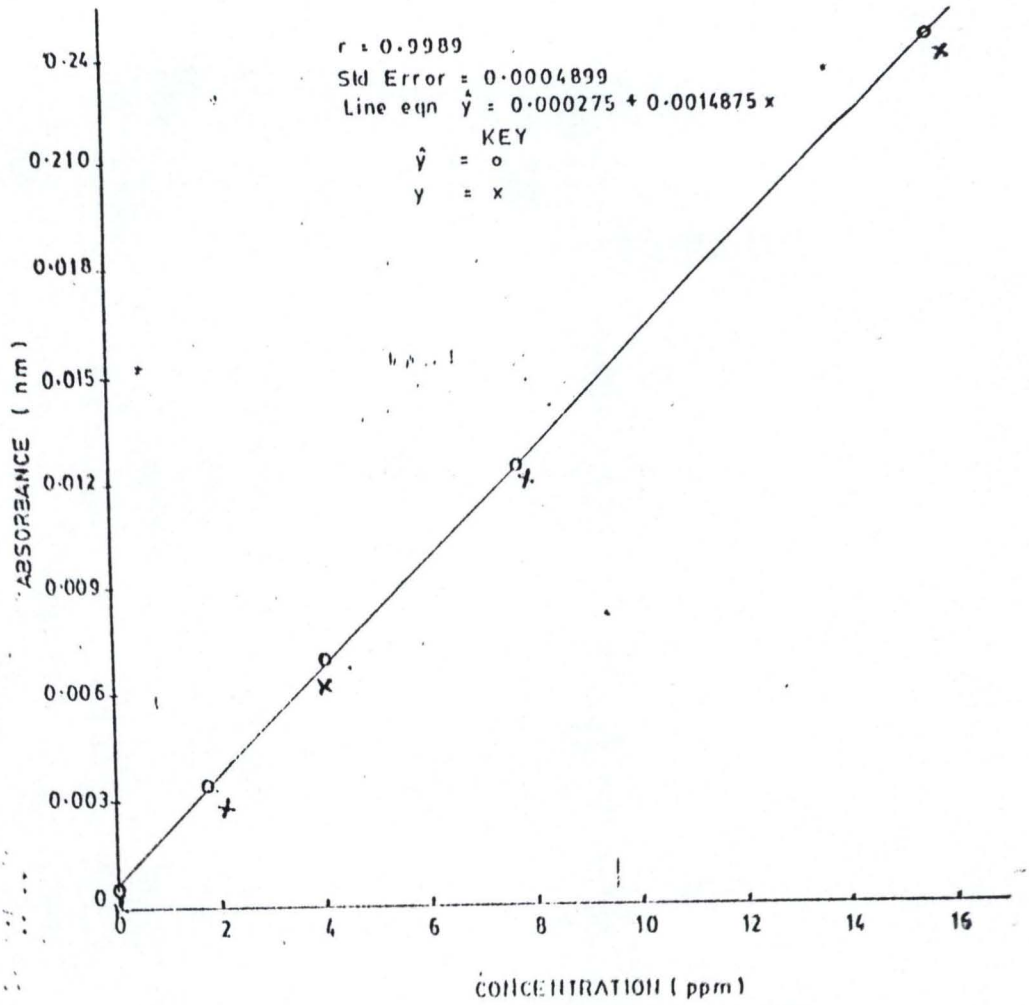


Fig. 1.4: Calibration Curve for Iron in Kanwa

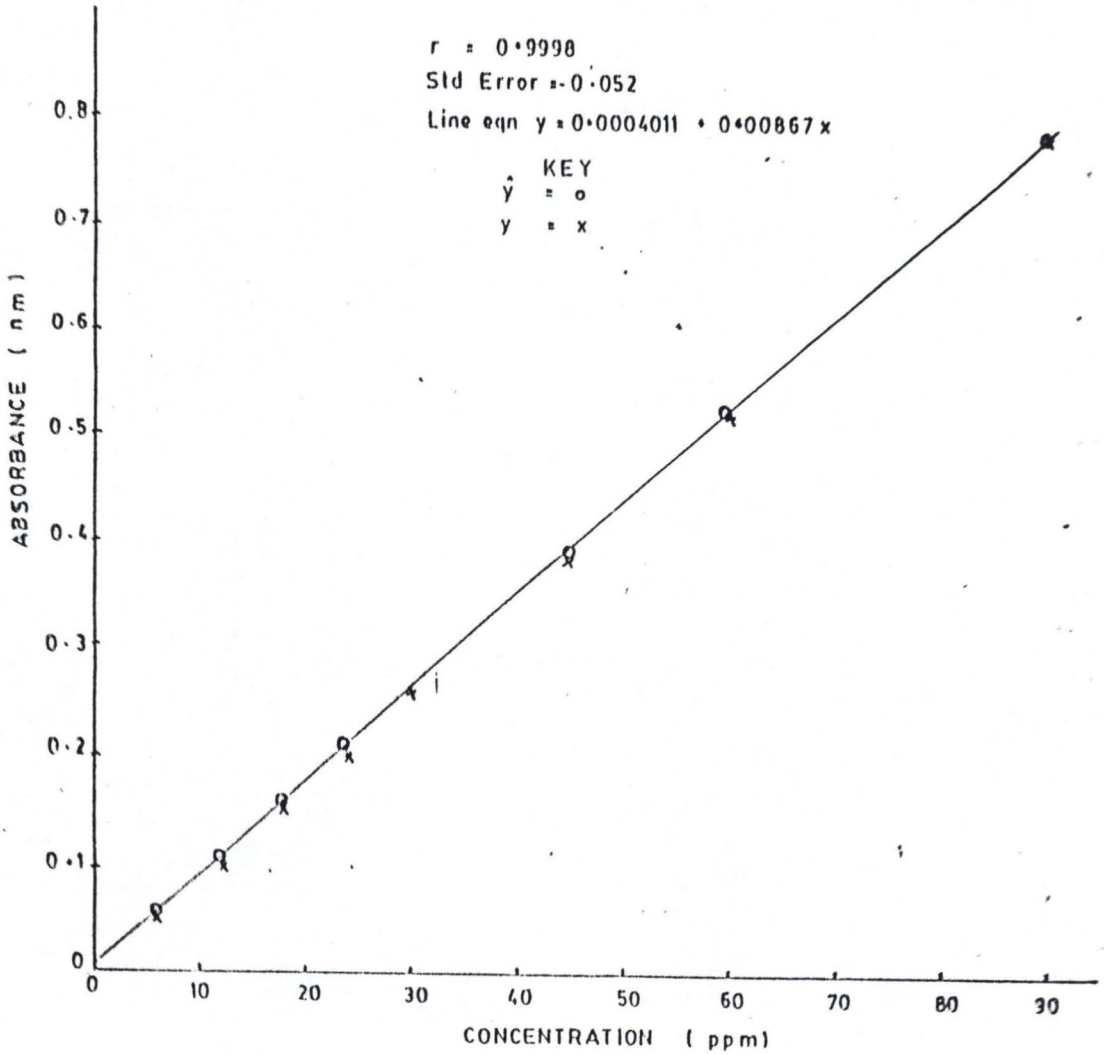
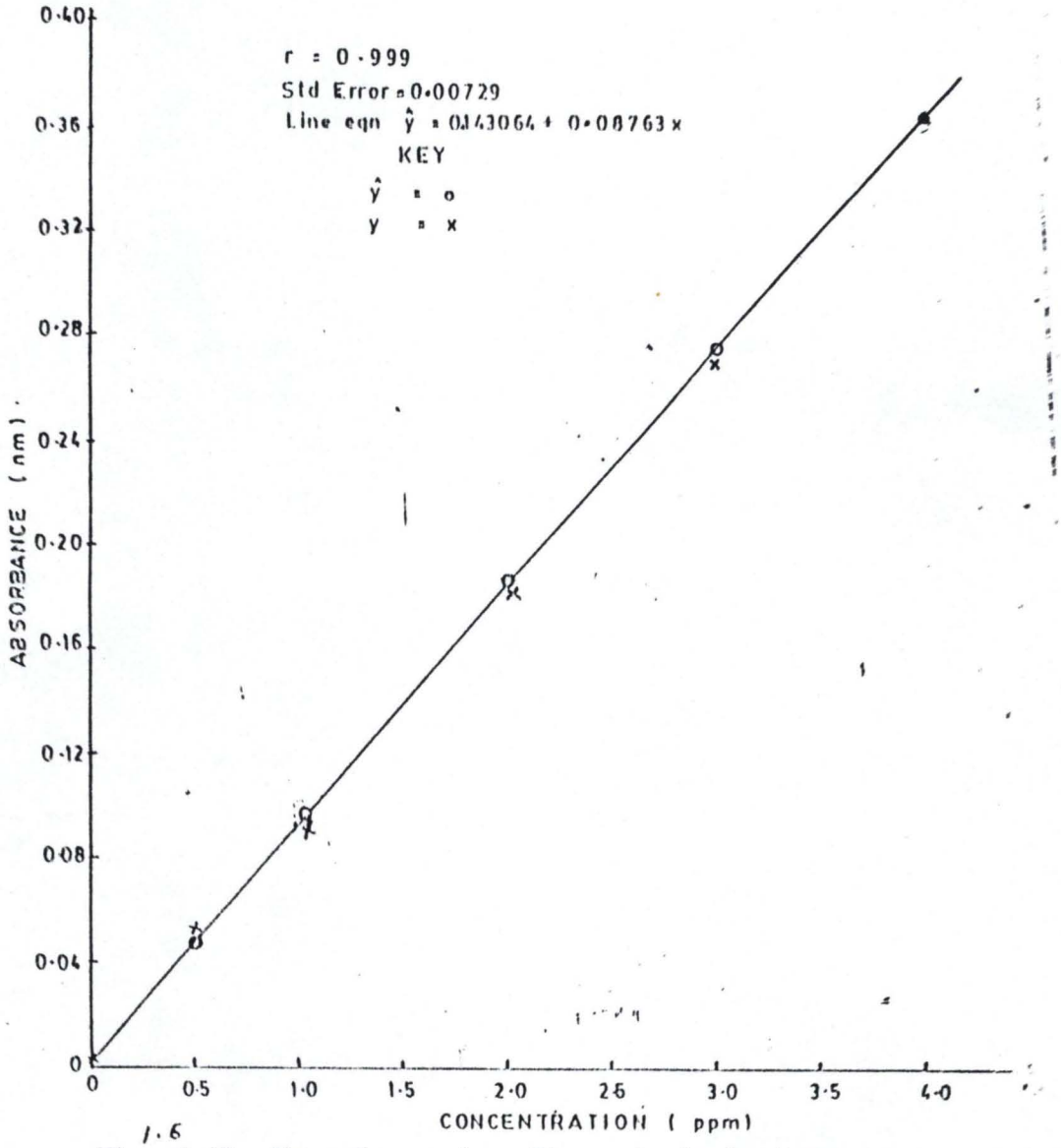


Fig. 1.5 Calibration Curve for Sulphate in Kanwa.



1.6

Fig. 1 Calibration Curve for Phosphate in Kanwa

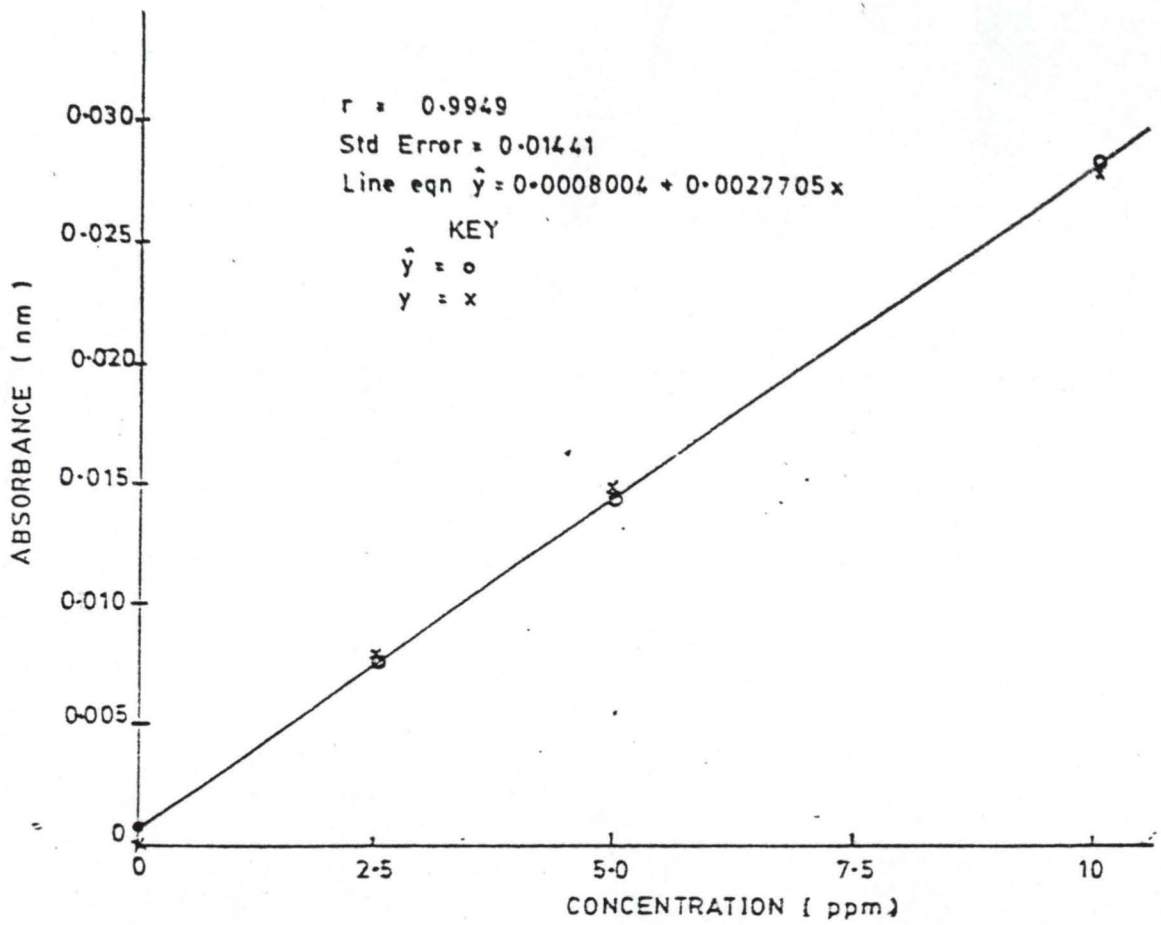


Fig.1.7 Calibration Curve for Manganese in Kanwa.

APPENDIX 1.1

Magnesium In Kanwa Samples

Sample No	Absorbance (Nm)	Off Curve Concentration (Ppm)	Concentration Mg/100g
S1	0.012	0.2018	2.522
S2	0.009	0.1456	1.82
S3	0.022	0.389	4.863
S4	0.020	0.3516	4.395
S5	0.072	1.3254	16.567
S6	0.0065	0.0988	1.235
S7	0.046	0.8385	10.481
S8	0.1335	2.477	30.963
S9	0.0335	0.6044	7.555
S10	0.0045	0.06133	0.7667
S11	0.014	0.2392	2.99
S12	0.004	0.5197	0.6496

APPENDIX 1.2

Potassium in Kanwa

Sample No	Absorbance (Nm)	Off Curve Concentration (ppm)	Concentration g/100g
S1	0.0085	45.1647	0.5645
S2	0.011	58.692	0.7337
S3	0.095	513.2	6.4152
S4	0.035	188.555	2.3569
S5	0.084	453.69	5.6745
S6	0.017	91.1579	1.13958
S7	0.047	253.487	3.1686
S8	0.027	145.268	1.8159
S9	0.075	404.994	5.0624
S10	0.013	69.514	8.6925
S11	0.0095	50.576	0.6322
S12	0.094	507.802	6.3475

APPENDIX 1.3

Iron in Kanwa

Sample No	Absorbance (nm)	Off Curve Concentration (ppm)	Concentration mg/100g
S1	0.003	1.8319	4.57975
S2	0.0025	1.495	3.7395
S3	0.0035	2.168	5.42
S4	0.0025	1.4958	3.7395
S5	0.0045	2.8403	7.1008
S6	0.005	3.1765	7.9412
S7	0.005	3.1765	7.9412
S8	0.0015	0.8285	2.0714
S9	0.002	1.15966	2.8992
S10	0.0025	1.495	3.7395
S11	0.002	1.15966	2.8992
S12	0.0035	2.168	5.42

APPENDIX 1.4

Calcium in Kanwa

Sample No	Absorbance (nm)	Off Curve Concentration (ppm)	Concentration mg/100g
S1	0.001	2.658	3.658
S2	0.005	11.52	14.40
S3	0.021	46.97	58.71
S4	0.0395	87.95	109.94
S5	0.038	84.063	105.78
S6	0.001	2.66	33.25
S7	0.027	60.26	75.32
S8	0.078	173.24	216.55
S9	0.024	53.61	67.01
S10	0.0135	30.35	37.94
S11	0.006	13.735	17.17
S12	0.003	7.09	8.86

APPENDIX 1.5

Sulphate in Kanwa

Sample No	Absorbance (nm)	Off Curve Concentration (ppm)	Concentration mg/100g
S1	0.0173	1.9468	3.8936
S2	0.004	0.4128	0.8256
S3	0.207	23.8269	47.6537
S4	0.339	39.05	78.1036
S5	0.769	88.64	177.2962
S6	0.0013	0.1014	0.2745
S7	0.260	29.9398	59.8798
S8	0.604	69.6169	139.2334
S9	0.560	64.542	129.0839
S10	0.0173	1.9468	3.8936
S11	0.085	9.7554	19.5107
S12	0.333	38.3597	76.7195

APPENDIX 1.6

Phosphorous in Kanwa

Sample No	Absorbance (nm)	Off Curve Concentration (ppm)	Concentration mg/100g
S1	0.206	2.1963	1.09815
S2	0.250	2.6984	1.3492
S3	0.187	1.9795	0.9897
S4	0.081	0.76986	0.3849
S5	0.011	0.0289	0.01448
S6	0.046	0.37045	0.18523
S7	0.038	0.2792	0.13958
S8	0.065	0.5873	0.2936
S9	0.019	0.0623	0.0311
S10	0.126	1.28338	0.6416
S11	0.135	1.38609	0.69305
S12	0.137	1.4089	0.70446

APPENDIX 1.7

Sodium in Kanwa

Sample No	Concentration (ppm)	Concentration g/100g
S1	5.0	25.0
S2	5.5	27.5
S3	4.6	23.0
S4	5.9	29.5
S5	7.2	36.0
S6	5.5	27.0
S7	4.4	22.0
S8	5.3	26.5
S9	6.05	30.25
S10	6.4	32.0
S11	6.25	31.25
S12	6.05	30.25

APPENDIX 1.8

Cupper in Kanwa

Sample No	Off Curve Concentration (ppm)	Concentration mg/100g
S1	0.016	0.0400
S2	0.016	0.0400
S3	0.013	0.0325
S4	0.008	0.0200
S5	0.007	0.0175
S6	0.006	0.0150
S7	0.020	0.0500
S8	0.014	0.0350
S9	0.023	0.0575
S10	0.027	0.0675
S11	0.024	0.0600
S12	0.021	0.0525

APPENDIX 1.9

Zinc in Kanwa

Samples No	Concentration (ppm)	Concentration Mg/100g
S1	0.200	0.500
S2	0.125	0.300
S3	0.075	0.1875
S4	0.050	0.125
S5	0.075	0.1875
S6	0.125	0.300
S7	0.125	0.300
S8	0.125	0.300
S9	0.150	0.375
S10	0.275	0.6875
S11	0.225	0.5625
S12	0.150	0.375

APPENDIX 1.10

Carbonate in Kanwa

Sample No	Volume of HCL (ml)	Volume of NaOH (ml)	% Total Carbonate
S1	25	14.4	19.005
S2	25	14.3	19.11
S3	25	16.45	16.725
S4	25	18.75	14.25
S5	25	16.40	16.95
S6	25	12.6	20.1
S7	25	18.5	14.5
S8	25	19.3	13.65
S9	25	16.9	16.28
S10	25	8.05	25.94
S11	25	12.5	21.075
S12	25	16.4	16.815

APPENDIX 1.11

Chloride in Kanwa

Sample No	Sample Volume (ml)	Volume of 0.0284N $AgNo_3$	Concentration of Cl (ppm)
S1	25	19.90	22.392
S2	25	21.90	24.651
S3	25	50.70	57.365
S4	25	42.60	48.166
S5	25	39.0	44.077
S6	25	22.75	25.617
S7	25	44.90	50.779
S8	25	21.85	24.594
S9	25	39.50	44.645
S10	25	26.90	30.331
S11	25	14.95	16.756
S12	25	70.95	80.372
Blank		0.2	

APPENDIX 1.12

Manganese in Kanwa

Sample No	Absorbance	Off Curve Concentration (ppm)	Concentration mg/100g
S1	0.015	5.1132	12.783
S2	0.001	0.0719	0.17975
S3	0.001	0.0719	0.17975
S4	0.015	5.1132	12.783
S5	0.001	0.0719	0.17975
S6	0.002	0.43197	1.0799
S7	0.001	0.0719	0.17975
S8	0.001	0.0719	0.17975
S9	0.001	0.0719	0.17975
S10	0.001	0.0719	0.17975
S11	0.001	0.0719	0.17975
S12	0.001	0.0719	0.17975

Appendix 3.1: Mean and Standard Deviation Weight Change (g) in Four Groups of Rabbits treated with varying Concentrations of Local Salt (Kanwa) For 9 Weeks

Interval Weeks	Male		Female		Row Mean ± S.D
Control[a]	54.43±41.05	n=3	51.05±20.41	n=3	52.74±2.39
Group 1[b]	62.05±13.52	n=4	49.53±09.32	n=4	56.02±9.17
Group 2[c]	66.09±15.00	n=4	51.10±06.71	n=4	58.60±10.60
Group 3[d]	66.39±11.95	n=4	60.06±15.93	n=4	63.23±4.48
Column Mean ± SD	62.35±05.57	n=4	52.94±04.81	n=4	

a = Control received no local salt (kanwa) treatment
b = Group 1 received 200mg /kg local salt (kanwa)
c = Group 2 received 800mg/kg local salt (kanwa)
d = Group 3 received 3200mg/kg local salt (kanwa)
n = Number of animals

Appendix 3.2: Mean and Standard Deviation Heamatocrit (%) in four Groups of Rabbits treated with varying Concentrations of Local Salt (Kanwa) for 9 Weeks

	Male		Female		Row Mean ± S.D
Control[a]	39.96±4.58	n=3	39.87±1.50	n=3	39.92±0.06
Group 1[b]	41.45±3.44	n=4	40.88±1.72	n=4	41.17±0.40
Group 2[c]	43.47±1.50	n=3	38.35±4.44	n=4	40.91±3.62
Group 3[d]	40.75± 4.39	n=4	43.88±4.67	n=4	42.32±2.21
Column Mean ± SD	41.41±1.50	n=4	40.75±2.33	n=4	

a = Control received no local salt (kanwa) treatment
b = Group 1 received 200mg /kg local salt (kanwa)
c = Group 2 received 800mg/kg local salt (kanwa)
d = Group 3 received 3200mg/kg local salt (kanwa)
n = Number of animals

APPENDIX 3.3a: Mean and Standard Deviation of Serum Potassium (mmol/L) in Four Groups Of Rabbits treated with varying Concentrations of Local Salt (Kanwa) for 9 Weeks

	Male		Female	Row Mean ± S.D
Control[a]	3.69±0.15 n = 2		3.70±0.44 n = 3	3.70±0.01
Group 1[b]	4.14±0.31 n = 4		2.99±0.48 n = 3	3.57±0.81
Group 2[c]	4.19±0.34 n = 3		3.79±0.13 n = 4	3.99±0.28
Group 3[d]	3.48± 0.40 n = 4		4.50±0.13 n = 4	3.99±0.72
Column Mean ± SD	3.88±0.18 n = 4	n=4	3.71±0.20 n = 4	

a = Control received no local salt (kanwa) treatment
b = Group 1 received 200mg /kg local salt (kanwa)
c = Group 2 received 800mg/kg local salt (kanwa)
d = Group 3 received 3200mg/kg local salt (kanwa)
n = Number of animals

APPENDIX 3.3b: Mean and Standard Deviation of Serum Sodium (mmol/L) in Four Groups of Rabbits treated with varying concentrations of Local Salt (Kanwa) for 9 Weeks

	Male	Female	Row Mean ± S.D
Control[a]	138.60±4.02 n = 2	137.77±3.49 n = 3	138.35±4.02
Group 1[b]	138.78±2.69 n = 4	149.27±7.36 n = 2	40.94±7.40
Group 2[c]	134.83±3.57 n = 4	132.39±3.64 n = 4	133.62±2.25
Group 3[d]	132.58± 2.69 n = 4	143.44±3.97 n = 4	139.10±3.67
Column Mean ± SD	136.20±3.02	140.72±7.27 n = 4	

a = Control received no local salt (kanwa) treatment
b = Group 1 received 200mg /kg local salt (kanwa)
c = Group 2 received 800mg/kg local salt (kanwa)
d = Group 3 received 3200mg/kg local salt (kanwa)
n = Number of animals

APPENDIX 3.3c: Mean and Standard Deviation of Serum Magnessium (mmol/L) in Four Groups Of Rabbits treated with varying Concentrations of Local Salt (Kanwa) for 9 Weeks

	Male	Female	Row Mean ± S.D
Control[a]	0.65±0.09 n = 3	0.66±0.05 n = 3	0.66±0.01
Group 1[b]	0.78±0.13 n = 3	0.73±0.04 n = 4	0.76±0.04
Group 2[c]	0.77±0.11 n = 4	0.73±0.06 n = 4	0.75±0.03
Group 3[d]	0.69± 0.05 n = 4	0.73±0.06 n = 4	0.71±0.03
Column Mean ± SD	0.72±0.06 n = 4	0.71±0.03 n = 4	

a = Control received no local salt (kanwa) treatment
b = Group 1 received 200mg /kg local salt (kanwa)
c = Group 2 received 800mg/kg local salt (kanwa)
d = Group 3 received 3200mg/kg local salt (kanwa)
n = Number of animals

APPENDIX 3.3d Mean and Standard Deviation of Serum Iron (mmol/L) in Four Groups Of Rabbits treated with varying concentrations of Local Salt (Kanwa) for 9 Weeks

	Male		Female		Row Mean ± S.D
Control[a]	0.030±0.00	n=2	0.030±0.003	n=3	0.030±0.003
Group 1[b]	0.032±0.007	n=4	0.036±0.013	n=4	0.034±0.003
Group 2[c]	0.024±0.005	n=4	0.019±0.004	n=3	0.022±0.003
Group 3[d]	0.030±0.004	n=4	0.028±0.004	n=4	0.029±0.002
Column Mean ± SD	0.028±0.003	n= 4	0.028±0.004	n=4	

a = Control received no local salt (kanwa) treatment
b = Group 1 received 200mg /kg local salt (kanwa)
c = Group 2 received 800mg/kg local salt (kanwa)
d = Group 3 received 3200mg/kg local salt (kanwa)
n = Number of animals

APPENDIX 3.3e: Mean and Standard Deviation of Serum Copper (mmol/L) In Four Groups of Rabbits treated with varying concentrations of Local Salt (Kanwa) for 9 Weeks

	Male		Female		Row Mean ± S.D
Control[a]	0.014±0.003	n=3	0.014±0.001	n=3	0.017±0.004
Group 1[b]	0.011±0.002	n=4	0.016± 0.001	n=3	0.014±0.003
Group 2[c]	0.011±0.015	n=4	0.018±0.002	n=4	0.015±0.005
Group 3[d]	0.012±0.027	n=4	0.014±0.008	n=4	0.013±0.001
Column Mean ± SD	0.012±0.001	n=4	0.015±0.002	n=4	

a = Control received no local salt (kanwa) treatment
b = Group 1 received 200mg /kg local salt (kanwa)
c = Group 2 received 800mg/kg local salt (kanwa)
d = Group 3 received 3200mg/kg local salt (kanwa)
n = Number of animals

APPENDIX 3.3f: Mean and Standard Deviation of Serum Zinc (mmol/L) in Four Groups of Rabbits treated with varying concentrations of Local Salt (Kanwa) for 9 Weeks

	Male		Female		Row Mean ± S.D
Control[a]	0.027±0.015	n=3	0.027±0.00	n=3	0.027±0.001
Group 1[b]	0.033±0.003	n=4	0.035±0.005	n=4	0.034±0.001
Group 2[c]	0.032±0.035	n=4	0.027±0.001	n=4	0.029±0.036
Group 3[d]	0.027±0.001	n=4	0.025±0.001	n=4	0.026±0.001
Column Mean ± SD	0.029±0.003	n=4	0.029±0.005	n=4	

a = Control received no local salt (kanwa) treatment
b = Group 1 received 200mg /kg local salt (kanwa)
c = Group 2 received 800mg/kg local salt (kanwa)
d = Group 3 received 3200mg/kg local salt (kanwa)
n = Number of animals

APPENDIX 3.3g: Mean and Standard Deviation of Serum Chloride (mmol/L) in Four Groups of Rabbits treated with varying concentrations of Local Salt (Kanwa) for 9 Weeks

	Male		Female		Row Mean ± S.D
Control[a]	100.46±5.69	n=3	103.90±2.68	n=3	103.68±2.88
Group 1[b]	102.67±6.94	n=4	112.34±11.26	n=4	107.51±6.84
Group 2[c]	105.29±5.63	n=4	103.38±1.85	n=4	104.34±1.35
Group 3[d]	113.83±9.94	n=4	112.40±7.60	n=4	113.16±1.01
Column Mean ± SD	105.56±5.86	n=4	105.56±5.05	n=4	

a = Control received no local salt (kanwa) treatment
b = Group 1 received 200mg /kg local salt (kanwa)
c = Group 2 received 800mg/kg local salt (kanwa)
d = Group 3 received 3200mg/kg local salt (kanwa)
n = Number of animals

APPENDIX 3.4a: Mean and Standard Deviation of Serum Total Protein (g/100ml) in Four groups of Rabbits treated with varying concentrations of Local Salt (Kanwa) for 9 Weeks

	Male		Female		Row Mean ± S.D
Control[a]	6.38±1.16	n=3	6.39±0.59	n=3	6.38±0.01
Group 1[b]	5.25±0.33	n=4	6.92±1.05	n=4	6.08±1.18
Group 2[c]	6.76±0.63	n=4	6.73±0.25	n=4	6.25±0.73
Group 3[d]	6.90±0.59	n=4	6.25±0.86	n=4	6.58±0.46
Column Mean ± SD	6.32±0.75	n=4	6.32±0.49	n=4	

a = Control received no local salt (kanwa) treatment
b = Group 1 received 200mg /kg local salt (kanwa)
c = Group 2 received 800mg/kg local salt (kanwa)
d = Group 3 received 3200mg/kg local salt (kanwa)
n = Number of animals

APPENDIX 3.4b: Mean and Standard Deviation of Serum Glucose (mmol/L) in Four Groups of Rabbits treated with varying concentrations of Local Salt (Kanwa) for 9 Weeks

	Male		Female		Row Mean ± S.D
Control[a]	5.50±0.43	n=3	5.53±1.17	n=3	5.52±0.02
Group 1[b]	4.43±0.87	n=4	4.69±0.60	n=4	4.56±0.18
Group 2[c]	5.50±0.73	n=4	6.01±0.75	n=4	5.76±0.36
Group 3[d]	4.83±0.74	n=4	6.24±1.02	n=4	5.54±1.00
Column Mean ± SD	5.07±0.53	n=4	5.62±0.69	n=4	

a = Control received no local salt (kanwa) treatment
b = Group 1 received 200mg /kg local salt (kanwa)
c = Group 2 received 800mg/kg local salt (kanwa)
d = Group 3 received 3200mg/kg local salt (kanwa)
n = Number of animals

APPENDIX 3.4c: Mean and Standard Deviation of Serum Cholesterol (mmol/L) in Four Groups Of Rabbits treated with varying concentrations of Local Salt (Kanwa) for 9 Weeks

	Male		Female		Row Mean ± S.D
Control[a]	5.20±1.33	n=3	5.19±1.15	n=3	5.50±0.01
Group 1[b]	5.56±0.97	n=4	5.82±0.78	n=4	5.69±0.18
Group 2[c]	5.24±2.16	n=4	7.75±0.86	n=4	6.50±1.78
Group 3[d]	5.12±0.83	n=4	8.46±2.95	n=4	6.79±2.36
Column Mean ± SD	5.28±0.19	n=4	6.81±1.55	n=4	

a = Control received no local salt (kanwa) treatment
b = Group 1 received 200mg /kg local salt (kanwa)
c = Group 2 received 800mg/kg local salt (kanwa)
d = Group 3 received 3200mg/kg local salt (kanwa)
n = Number of animals

APPENDIX 3.4d: Mean and Standard Deviation of Serum Urea (mmol/L) in four Groups Of Rabbits treated with varying Concentrations of Local Salt (Kanwa) for 9 Weeks

	Male		Female		Row Mean ± S.D
Control[a]	9.98±1.75	n=3	9.97±0.87	n=3	9.99±0.01
Group 1[b]	7.15±1.18	n=4	6.94±0.51	n=4	7.05±0.15
Group 2[c]	9.26±1.34	n=4	8.26±1.43	n=4	8.76±0.71
Group 3[d]	6.52±0.62	n=4	12.15±0.90	n=4	9.34±3.98
Column Mean ± SD	8.23±1.66	n=4	9.33±2.25	n=4	

a = Control received no local salt (kanwa) treatment
b = Group 1 received 200mg /kg local salt (kanwa)
c = Group 2 received 800mg/kg local salt (kanwa)
d = Group 3 received 3200mg/kg local salt (kanwa)
n = Number of animals

APPENDIX 3.5a: Mean and Standard Deviation of Serum Glutamate Oxaloacetate Transaminase (U) in Four Groups of Rabbits treated with varying Concentrations of Local Salt (Kanwa) for 9 Weeks

	Male		Female		Row Mean ± S.D
Control[a]	19.96±3.43	n=3	17.71±3.70	n=3	18.85±1.59
Group 1[b]	13.93±1.34	n=4	14.81±2.48	n=4	14.37±0.62
Group 2[c]	19.42±4.32	n=4	17.24±2.09	n=4	18.34±1.54
Group 3[d]	17.66±2.45	n=4	21.38±1.71	n=4	19.52±2.63
Column Mean ± SD	17.60±1.50	n=4	16.30±1.12	n=4	

a = Control received no local salt (kanwa) treatment
b = Group 1 received 200mg /kg local salt (kanwa)
c = Group 2 received 800mg/kg local salt (kanwa)
d = Group 3 received 3200mg/kg local salt (kanwa)
n = Number of animals

APPENDIX 3.5b: Mean and Standard Deviation of Serum Glutamate Pyravate Transaminase (U) in Four Groups of Rabbits treated with varying Concentrations of Local Salt (Kanwa) for 9 Weeks

	Male		Female		Row Mean ± S.D
Control[a]	19.99±2.31	n=3	19.79±3.71	n=3	19.89±0.14
Group 1[b]	16.44±2.13	n=4	21.90±1.81	n=4	19.17±3.86
Group 2[c]	17.73±3.60	n=4	18.87±2.47	n=4	18.30±0.81
Group 3[d]	20.99±2.58	n=4	20.98±2.47	n=4	20.99±0.01
Column Mean ± SD	18.79±2.08	n=4	20.42±1.10	n=4	

a = Control received no local salt (kanwa) treatment
b = Group 1 received 200mg /kg local salt (kanwa)
c = Group 2 received 800mg/kg local salt (kanwa)
d = Group 3 received 3200mg/kg local salt (kanwa)
n = Number of animals

APPENDIX 3.5c: Mean and Standard Deviation of Serum Alkaline Phosphatase (U) in four Groups of Rabbits treated with varying concentrations of Local Salt (Kanwa) for 9 Weeks

	Male		Female		Row Mean ± S.D
Control[a]	30.00±7.61	n=3	29.99±12.56	n=3	30.00±0.01
Group 1[b]	32.06±8.79	n=4	13.74±6.18	n=4	22.90±12.95
Group 2[c]	27.35±4.72	n=4	16.05±4.43	n=4	21.70±7.99
Group 3[d]	33.76±6.52	n=4	19.71±6.23	n=4	26.74±9.93
Column Mean ± SD	30.79±2.76	n=4	19.87±7.18	n=4	

a = Control received no local salt (kanwa) treatment
b = Group 1 received 200mg /kg local salt (kanwa)
c = Group 2 received 800mg/kg local salt (kanwa)
d = Group 3 received 3200mg/kg local salt (kanwa)
n = Number of animals

Appendix 5: Weight Gain (g) of Rabbits used for Experimentation

Animals Slaughtered at Start	Control (a)		Group 1 (b)	Group 2(c)		Group 3(d)		
	M3*	Fe(e)	FB3*	M4*	MA2	F7*	M_2*	FC_6*
Initial Weight	550	-	660	520	400	564	777	520
Start (0)	609		1445	714	1248	808	1078	1509
Average Weight Gain	11.8		70.4	38.8	70.6	48.8	60.2	82.4

*	=	Animal Code
a	=	Control received no local salt (kanwa) treatment
b	=	Group 1 received 200mg /kg local salt (kanwa)
c	=	Group 2 received 800mg/kg local salt (kanwa)
d	=	Group 3 received 3200mg/kg local salt (kanwa)
e	=	Animal died

Animals Slaughtered at Week 3

	Control (a)		Group 1 (b)	Group 2(c)		Group 3(d)		
	FC5*	M5*	MA1*	F6*	FB4*	M7*	MA4*	F5*
Initial Weigh	550	545	490	449	410	637	380	649
Start (0)	1707	691	1407	741	1478	920	1328	938
Week 1	1780	718	1452	818	1516	900	1403	990
Week 2	1879	772	1523	826	1595	950	1504	1045
Week 3	1956	804	1593	880	1686	989	1584	1101
Average Weight Gain	93.7	32.4	73.5	53.9	85.07	44	80.27	56.5

* = Animal Code
a = Control received no local salt (kanwa) treatment
b = Group 1 received 200mg /kg local salt (kanwa)
c = Group 2 received 800mg/kg local salt (kanwa)
d = Group 3 received 3200mg/kg local salt (kanwa)
e = Animal died

Animals Slaughtered at Week 6

	Control (a)		Group 1(b)		Group 2(c)		Group 3(d)	
	MB*	F4*	FA2*	M1*	F08*	M6*	MB6*	F1*
Initial Weight	540	460	470	640	420	669	440	649
Start (0)	1415	658	1185	745	1086	800	1161	791
Week 1	1388	653	1204	780	1070	885	1167	865
Week 2	1425	680	1317	829	1140	912	1178	915
Week 3	1448	780	1365	924	1187	910	1240	988
Week 4	1438	810	1420	952	1245	1080	1305	1020
Week 5	1523	939	1491	1069	1296	1296	1345	1095
Week 6	1581	987	1551	1117	1348	1235	1401	1140
Average Weight Gain	57.8	479	60.05	43.4	51.6	51.5	53.39	44.64

* = Animal Code
a = Control received no local salt (kanwa) treatment
b = Group 1 received 200mg/kg local salt (kanwa)
c = Group 2 received 800mg/kg local salt (kanwa)
d = Group 3 received 3200mg/kg local salt (kanwa)
M = Male
F = Female

Animals Slaughtered at Week 9

	Control(a)		Group 1(b)		Group 2(c)		Group 3(d)	
	FA1*	M*	MA3*	F4*	MB7*	F2*	FD7*	M8*
Initial Weight	450		400	473	440	452	520	740
Start (0)	1280	(e)	1219	701	1139	719	1073	979
Week 1	1340		1217	749	1158	775	1085	1100
Week 2	1422		1251	771	1189	839	1154	1100
Week 3	1464		1328	838	1300	913	1200	1200
Week 4	1525		1387	858	1358	968	1265	1307
Week 5	1645		1501	939	1440	1022	1342	1400
Week 6	1620		1440	829	1460	1040	1381	1411
Week 7	1793		1570	882	1540	1141	1470	1579
Week 8	1910		1655	1070	1605	1235	1650	1679
Week 9	1980	(e)	1716	1109	1682	1292	1711	1744
Average Weight Gain	72.86		62.7	45.59	59.14	60.0	56.71	71.7

* = Animal Code
a = Control received no local salt (kanwa) treatment
b = Group 1 received 200mg /kg local salt (kanwa)
c = Group 2 received 800mg/kg local salt (kanwa)
d = Group 3 received 3200mg/kg local salt (kanwa)
M = Male
F = Female
e = Animal died