HAEMATOLOGICAL AND SEROLOGICAL CHARACTERISTICS OF HETEROTIS NILOTICUS, (CUVIER AND VALENCIENNES 1829): OSTEOGLOSSIDAE

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CERTIFICATION

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DEDICATION

This research is dedicated to

my loving parents,

Evangelist (Late) J.K. Ayotunde

and

Mrs. L.A. Ayotunde

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ABSTRACT

Heterotis niltiocus, is a freshwater fish, endermic to Africa. It has become popular in fish farming in Nigeria, largely due to its ability to breed in captivity, fast growth rate and large attainable size.

Studies on the biology of Heterotis niltiocus has been previously focused on the food and feeding habits, as well as age and growth. No information exists on its haematological and serological profile.

Haematological characteristics of 25 adults Heterotis niloticus, specimen (standard length, 30.4cm - 39.5cm) (weight, 340g - 610g) collected from, Osun State Ministry of Agriculture and Natural Resources Fish Farm, Osogbo, Osun State, were established.

The blood parameters determined were, red blood cells, white blood cells, haemoglobin, haemotocrit, mean cell haemoglobin concentration, mean cell volume, mean cell haemoglobin, protein, glucose, albumin, sodium, chloride, potassium, magnesium and phosphorus.

The red blood cells and white blood cells counts did not positively correlate with physical parameters of length and weight. There were siginificant and positive correlation between the blood cells, haemoglobin and pack cell volume, at $P < 0.01$ and $P < 0.001$).

The serological (blood group, genotype and agglutination test) result were (92% 0+ve and 8% 0-ve), (56% AA and 44% AS) and 100% -ve respectively, and the result obtained were found to be similar to the pattern in humans.

Factors which affected the haematology of the fish were discussed, and these include, stress due to capturing, transportation and sampling, age, sex and vessels in which the blood is taken.

The knowledge of haematological and serological characteristics of Heterotis niloticus is important in

(1) determining health status of fish.

(2) comparative haematological studies, for disease, toxicological and parasitological investigations.

(3) selecting brooders for breeding purposes.

(4) Seprating thhe sexes of the fish.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 TAXONOMY/IDENTIFICATION OF HETEROTIS NILOTICUS

The family Osteoglossidae is a primitive fish family. It is represented by one species in Africa, Heterotis nitoloticus (African bony tongue). It is easily recognised by its very large bony scales spineless long dorsal and anal fin and small rounded tail seemingly out of proportion to the size of the body. The head is short, thick and smooth except for prominent sensory pit (Wellcome 1988).

Heterotis grows to about one meter in length and exceed 6kg in weight. The large bony scales are patterned in a mosaic fashion which make the age rings difficult to distinguished (Reed et al 1967). The lateral line extend in almost a straight line from the gill cover to the middle of the caudal penduncle and the canals which are tubular in the young and deeply grooved in adults are very conspicuous.

The colour varies from brownish to greenish - olive and is fairly uniform. The young specimens have the dorsal and anal fins marked with indistinct longitudinal streaks and a round dark sport on each of the scale in the caudal region of the body.

Heterotis niloticus is a tropical species inhabiting swamps lower reaches of rivers, flood plains, and creeks where the current is not strong, (Reed et al 1967). It

auxiliary branchial air breathing organ enables it to survive in deoxygenated water. It is microphagus and feeding mostly on plankton which include benthic materials, algae, invertebrates, seeds, and plant debris.

The flesh is fairly tough and dries with low oil contents and a strong flavour. Heterotis niloticus is a substrate breeder, and spawns during the wet season (July - November) with the breeding pair building a characteristic grass nest about a meter in diameter and nearly a meter deep. They lay their eggs in the centre of the nest. Their gonads are very large, and the eggs are about 1-2mm in diameter (Reed et al 1967). Heterotis niloticus has a habit of leaping high out of the water and landing on their side with a resounding slap, this make it difficult to catch them in seine net. This is why this fish is commonly called slapwater.

The hardiness of this fish coupled with its fast growth rate make it suitable for aquaculture in Africa, (Reed et al 1967). In order to enhance aquacultural development, and ultimate production of sufficient protein, knowledge of haematological propertiies of fish should be given high priority towards solving fish diseases, and monitoring the good health of fish.

However, little work has been done on the haematological studies of fish in Nigeria, while much work have been done in other aspects of fish biology.

1.2 HAEMATOLOGY AND CIRCULATORY SYSTEM IN FISH

Haematology is the study of blood. The blood, has two components, one is fluid and other is solid. The fluid part is the plasma in which the solid part, the blood cells are transported (Karl et al 1977). The cells are red blood cell (erythrocyte) white blood cells (leucocytes) and blood platelets. Haemoglobin in red cells enhance the ability of blood to transport oxygen.

The blood vessels of the body of a fish are classified as the major arteries and veins which follow a long axis of the body. The branches of the major vessel (the capillaries) supply the skin, skeleton, musculature, brain, spinal cord, and the intestinal organ. The dorsal aorta is the main route of blood transport from the gill in fishes and it lies directly beneath the vertebral column. The size of dorsal aorta reduces as it supplies series of dorsal vein and lateral segmental vessel and it is referred to as dorsal artery which returns blood towards the heart. The numerous aorta branched vessels and the corresponding vein arrange to match the body segment, the arteries possess valves to prevent back flow of blood towards the aorta. The venous system in teleosts have elaborate sinus, blood returning to the heart flows through sinus like area of the hepatic and renal portal system. The renal portal vein that arise from the caudal vein break-up into a network of increasing smaller capillaries, which then collect blood into the posterior cardinal or post cardinal veins.

The post cardinal of each side terminates in the cardinal sinus or duct of curvier,

which in turn passes into the sinus venosus. The vein from the stomach and the intestine of jawed fishes collects into the hepatic portal vein giving rise to a myriad of sinusoids which transfer dissolved food substances to the liver tissue.

The blood is then collected into the hepartic vein which enters into the hepartic vein which enters into sinus venosus. The hepartic vein in teleost varies between 1 and 3, but it is two in Heterotis niloticus (Kart et al 1977).

1.3 OBJECTIVES OF THE STUDY

The objective of this study is to establish a normal haematological profile of Heterotis niloticus, specifically;

(1) To provide a basis for comparative survey for all the blood parameters such as Red blood cell, White blood cell, Mean cell corpscular haemoglobin concentration, Haemoglobin, Pack cell volume, Erythrocyte sedimentation rate, Mean haemoglobin concentration and Mean cell volume.

(2) To establish a normal serological profile such as blood group, genotype and agglutination test.

(3) To determine a normal biochemical analysis such as plasma glucose protein and albumin contents.

(4) To establish a normal plasma electrolytes such as sodium, potassium, magnesium, phosphorus and chloride.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 HAEMATOLOGICAL CHARACTERISTICS

The use of haematological values as indices of diagnosing diseases and stress induced conditions in fish has received a lot of research in Nigeria, (Kori-siakpere 1985, Rambhaskah and Rao 1990, Erondu et al. 1993, Fagbenro et al. 1993). Changes in cellular composition of blood have been associated with environmental stress, (Casillas and Smith 1977; Hilmy et al, 1980), dietary deficiency and starvation (Smith 1968, Wenberg et al 1976) and disease (Amond and Smith 1975; Lester and Budd 1976).

The blood volume of teleost is small compared with other classes of vertebrates, being in the region of 5% of the body weight (Ronald 1989). There are variations within the blood parameters of a teleost fish. The oxygen transport system has been shown to be sensitive to day length as well as to temperature and oxygen tension (Denton and Yousef 1975).

Considerable range exists in the amount of blood haemoglobin found in blood air breathing fishes, the range being from 4.8 - 14.6g/100mL^{-1} (Jeffrey 1985). Oxygen transport in blood depends on the iron compound haemoglobin, the haemoglobin content of whole fish blood varies with the number of erythrocytes present, express as the percentage of dry weight of erythrocyte representing 14-19% in dog fish and 37-79% in marine and fresh water teleost (Karl et al 1977).

Karl et al 1977 reported that the red blood cells of teleosts range from 20,000 to 3,000,000 per cubic millimeter of blood, while blood cells vary between 20,000 and 150,000/mm^3.

The variations in blood parameters has been attributed to different factors. Karl et al (1977), reported that the effect of diseases as a valuable cause of variation and that there are changes in the haemoglobin content of the blood as well as in the number of cell count of common carp (Cyprinus capio), with a common sore-disease due to the bacterium (Psedomonas punctata), which show the following changes, lymphocyte fall in number from 92.0 - 49.4% of all the white blood cells, monocytes rise from 5.7 - 38% and polymorphonuclear lencocytes increase from the normal of 2.3 - 12.6%, the number of erythrocyte fall from more than 2 million/mm^3 of blood to <1 million/mm^3 and the haemoglobin content of the blood is reduced accordingly.

The trend in the relationship of blood characteristics of fishes with the environmental variables are species specific (Cameron 1970, Soivo et al 1974, Smith et al 1981). The effect of environmental variables on haematological characteristics of fish has been reported by many workers, the most effective are temperature related changes, which were observed in fish blood (Allanson et al 1971, Houston and Smeda 1979, Joshl et al 1980 and Dwyer et al 1983), other parameters include salimity, and dissolved oxygen concentration. The effects of environmental variable are profound on the survival and oxidative metabolism of

fish, and increase in plasma volume resulting in decrease in haematocrit (Joshl et al 1980)

Increase in water temperature also increases plasma sodium. Cameron (1970) reported that a negative relationship of haemoglobin and haemotocrit with dissolved oxygen deficiency.

Raila et al (1985), reported that sampling stress caused an increase in haematocrit value and decrease in mean cellular haemoglobin concentration of rainbow trout, Salmo gairdnari, indicating red cell swelling. He went further that blood ionic balance is affected in that potassium concentration increases and plasma chloride concentration decreases.

Blood sampling stress causes notable changes in the blood status of fish, (Railo et al (1985), Handling stress cause haemoconcentration in the pike Esox Lucius, and in rainbow trout. One minute handling is adequate to cause an increase in the heamatocrit values, lifting fish out of water for 20 seconds change blood parameters (Soivio et al 1977).

Intraspecises variation in haematological parameters can be attributed to many factors, Larson et al (1979) disregarding minor variation. (due to in accuracy of analytical method,) classified these factors into two broad groups.

The first group consists of those factors which course haematological difference

in fish in their natural environment, including age and size of the fish. Das (1965) stated that both the red blood cell number and haemoglobin observed in the plaice, Pleuronectes platesca by Preston, (1960) correlated with the result obtaine for Clarias isheriensis between the physical parameters of length and weight and certain blood parameters. Other factor include season, spawning migration, sex, and genetical variations.

The major cause of variation in plasma glucose in plaice, Pleuronectes platesca L. is environmental factor. Hyperglycemia is a useful indication of the environmental stress, White and Fletcher (1985), did not report any significant changes in plasma glucose as a result of environmental changes.

Seasonal fluctuations in haemotological status have also been reported by Preton (1960) and Mahagan Dheer, (1979). Smith (1977) noted a correlation between mean cell volume (MCV) and weight ranging from highly negative to highly positive depending on season. Khanna and Singh (1971), found seasonal changes in the blood glucose of Channa punctation. Plasma osmotic concentration appear to change seasonally, reflecting a modification in the distribution of water and salt in both plasma and tissue (Roberson et al 1961, Hoagen and Perez 1975). Shell, (1959) reported an apparent seasonal changes in the electrolytes of the fresh water teleost, Micropterms dolomine which was indicative of the presence of some cyclic metabolic pattern. He related these cycles to a presumed seasonal variation in electrolytes level.

The second group which can cause intraspecific haematological variation is stress, due to capture, handling and sampling procedure of which capture is most significant (Brouck and Ball 1966). Stress of handling has been shown to produce a haemoconcentration, elevated blood lactate, increased glucose concentrations and alteration in the plasma electrolyte in the plasma electrolyte balance in two groups - blackish and freshwater of northern pike Esox lucius L. (Soivo and Oikari 1976).

Similarly, haemolobin concentration and haematocrit of fish blood decrease after the stress of capture and transportation, (Hattingh 1976). Erondu et al (1993) reported that significant variations were obtained in the results between juveniles and adults of catfish except Clarias gariepinus in which no variation were observed between spent female and post-juveniles/other adults. There was low inter-species variations between the overall result between C. gariepinus, H. longifilis, F_1 hybrid (C.g. x H.l) and C. nigrodigitatus, the reason could probably be explained by the fact that all the fish specimens used were collected from the same environment semi-intensively managed pond with similar nutritional condition) and during similar season (rainy season). It was, however, not known whether the inter-specific variation in haematocrit and haemoglobin levels were intrinsic or genetic, this was because the result showed some similaries among species from different families,while different values were also obtained among species of the same family. However, the value obtained for H. longifilis were consistently higher than those of the other species, this was probably as a result

of the higher activity rate of H. longifilis which was supposed to elicit a higher ratio of erythrocyte to plasma (haemotocrit) in blood (Erondu et al 1993).

Siakpere (1985) reported that high value of the haemoglobin concentration and enythrocyte count reflect a high oxygen carrying efficiency of the blood. This is consistent with the correlation of haemoglobin concentration with fish activity that is the higher the fish activity the more the haemoglobin, thus varying physiological requirements are compensated by regulating the level of the haemoglobin (Hall and Gray, 1929; Eusler, 1965). Furthermore, Lenfant and Johansen (1972) have shown that there is a tendency for haemoglobin concentration to be higher in these fishes which get their oxygen from the air. Thus the high haemoglobin value in C. isheriensis reflect it air-breathing character and high activities. (Kori-siakpere 1985).

Fagbenro et al (1993) reported high erythrocyte count >1 x $10^6/mm^3$ in Heterobranchus bidorsalis which is considered high and is indicative of high oxygen carrying capacity of the blood and characterize fishes capable of aerial respiration and with high activity. This is particularly true for H. bidorsalis, an active swimmer and predator (Fagbenro et al 1993, Akegbejo-Sampson (IN press) which like all clariid catfishes is capable of aerial respiration.

The plasma protein in fish blood can be affected by the stress due to capture handling and sampling procedure (Bouck & Ball 1966), such stress situation and

probably associated with an increase secretion of catecholamine, increas. concentration of adrenaline and noradrenaline in the blood of rainbow trout Salmo gairdneri has been observed in response to physical disturbance (Nakaro and Tomlinson 1967).

Karl et al (1977) reported the difference in sedimentation coefficient of principal plasma protein, and he went further that the fish have low level of plasma protein compared with higher vertebrate, the basic blood plasma protein of fish are albumin (control osmostic pressure), lipoprotein (transport lipids), globulin (blind heem), ceruboplasmin (bind copper), Fibrinogen (blood clooting), Karl et al 1977).

Wedemeyer, (1981) reported that hyperglyceamia can be a useful indication of environmental stress, but there are other factors which cause increases in serum glucose level. Many authors have reported an inverse relationship between temperature and glucose (Joshi, 1980, Tondon and Joshi 1980), but the pattern is inconsistent (Uniminger 1969). After capture, transportation and handling the mean serum glucose for 10 plaice was 73mg glucose/100ml serum, dropped to 29mg/glucose/100ml after 48 hour in aquarium. Plaice kept for 15 days post capture showed no significant changes in serum glucose coinceded with an increase in plasma cortisol in both carp, Cyprinus carpio and Tilapia mossambcus, serum cortisol in the plaice is higher during and just after spawning (White and Fletcher 1984).

Johansen et al (1978) reported that considerable range exist in amount of blood haemoglobin found in blood, of difference species of air breathing fish, in a number a air-breathing teleost blood haematocrit are as high or higher than those of many mammals and birds (Johansen et al 1978). The possible reason for this have been frequently discussed and factors such as habitat, water, gas tension (PO_2, PCO_2) or pH, fish activity level, extent or reliance upon air breathing, relative size of the air breathing organ, and pathway of blood circulation to and from air breathing organ, have been involved to explain both the haemoglobin value of some species as well as interspecific difference in this property.

Erondu et al (1993) stated that the negative correlation of fish size to erythrocyte leucocyte counts, haemotocrit and haemoglobin concentration was probably due to the relative reduction or decrease in metabolic rate of the individuals as the grew. There was therefore a concomitant reduction in oxygen demand and hence the low blood value. They also reported positive correlation of condition factor to the blood value, as indication that this variable could be used to assess the health status of a fish population (Erondu et al 1993).

Erondu et al (1993) reported low haemotocrit and leucocrit value among infected fish groups. They concluded that parasitic infections could depress the value of total plasma protein and haematocrit in fish.

Faturoti and Obasa (1992) reported increase in Serum protein of Heterotis as the

protein level increased the increase was an indication that the dietary protein have effect on the serum protein component of the fish, Fagbenro and Akegbejo (INpress) observe similar trend, the observed a significant increase in the total protein of fish fed with high protein and high calorie diets over those fed with high protein and high calorie diet over those fed with low protein-low calorie diet. He also observe a 75% increase in the serum albumin and a 73% increase in their albumin (A/G) ratio, (Faturoti and Obasa 1992).

Sandstrom (1989) stated that the main consistent in plasma are chloride, sodium, potassium, magnesium and calcium, he said that plasma ion concentration may be sensitive to temperature change, and that some of these changes could not have been generated by a temperature oscillation, the main effect of temperature on biological rhythms should be on amplitude, while day length is the most forceful synchronizer to the seasonal changes (Sandstrom 1989).

Karl et al (1977) reported that the electrolyte composition in cod (Gadus collorias) in mMole as follows sodium (180), potassium (4.9), magnesium (3.8), calcium (5.0), chloride (158), phosphate (3.1) and that the concentration of Na^{+} and ca++ were generally lower in fresh water teleost. Love et al (1970), stated that the Channa punctatus can tolerate $1.2gl^{-1}$ level of sodium chloride stress in ambient water without any obvious change in feeding behaviour. They further stated that NaCl concentration at 25% and 50% of LC50 q6h value in the ambient water produced statistically significant effect in haematological parameters accompainy

by both loss of growth and mortality.

Love (1970) stated that the calcium content in female plasma has been reported to vary seasonally and to respond to gonad activity.

Erondu et al (1993) reported that the result of the serological studies showed similar antigen reaction as observed in human blood, he went further that the fish genotype and blood group (A, B, O systems) seemed to be similar to those of human standard, individual differences were observed in the antigenic constitution if the various species examined, also some genetic information might be acquired by comparing erythrocyte antigen of female and their offspring. Blood group frequencies could be used to determine the level of reproductive isolation of fish population in pond and in the wild (Erondu et al 1993).

2.1.1 THE CIRCULATORY SYSTEM

The blood circulation in fish is describe as single circulation, with a simple heart (Figure 1) with four chambers, sinus venosus, strium, ventricle and either a bulbus or a conus (Karl et al (1977), Bultler and Matcalfe 1983). The blood is pumped by the heart firstly to the gill where gaseous and osmoregulatory exchange occur, and the flow on to the system circulation before returning to the heart (Butler and Marcalfe 1983).

The conus in teleost is equivalent to bulbus arteriosus which are simple elastic

reservoir, which are positively enlarge by blood driven forward from the ventricle, the blood in fish circulate by mean of a more or less continuous tabular system of heart and vessel.

2.1.2 THE HEART

The heart in teleost is situated inside the pericardium anterior to the main body cavity and usually ventral to the pharynx (Ronald 1989), it has four chambers through which blood flows in simple succession. The atrium has a thin wall and the ventricle has a much thicker wall than the atrium, the whole heart is enclosed in the visceral pericardial sac which is associated with the cardiac surface.

The heart is a valved pump that forces blood forward toward the gills for aeration, arteries carry blood away from the heart and veins toward the gill (Karl et al 1977). The rate of blood flows depend on the rate on force of contraction, the heart of fish possesses the ability to contract rhythmically in the absence of any external stimuli (Butler and Matcalfe 1983). This is as a result of the presence of pacemaker cells within the cardiac tissue which generate a rhythm of action potential by virtue of their unstable membrance potential which propagates rapidly over the myocardium and initiates its contraction (Butler and Matcalfe 1983).

2.1.3 FISH PLASMA

Butler and Matcalfe (1983), stated that

blood consists of fluid plasma and plasma is a clear liquid in which are found, the

blood cells, dissolved minerals, absorbed products of digestion, waste products of tissue, special secretions, enzymes, antibodies and dissolved gases. A number of enzymes have been isolated from fish plasma, among them are, lipase and carbonic anhydrase which occur more abundantly in marine than fresh water fishes.

2.1.4 RED BLOOD CELLS

The red blood cells are yellow, red and nucleated and are found in the blood of all fishes. Mature red blood cell of fishes are oval, tiny and range in long diameter from 7 - 36μm.(Karl et al (1977)

Oxygen transport in blood depends on the iron compound, haemoglobin, the respiratory pigment of the blood, fish responds to shock with a lowering in number of red blood cells, they can rapidly increase the number of young erythrocytes in the blood.(Butler and Matcafe1983)

2.1.5 WHITE BLOOD CELLS

Karl et al (1977) reported that fish blood contains several types of colourless of white cells (leucocytes) of which are ovoid to spheroid in shape. Among the white cells, there are granulocytes, that may make up between 4 and 40% of all the white cells.

The diameter of the leucocytes is about 10μm. There are different types of white blood cells which include the granulocytes, lymphocytes, and monocytes, the

agranular leucocytes are the most numerous white cell component of fish blood, the monocytes serve as macrophase function, lymphocyte serve dual purpose viz. producing anti-bodies and involvement in cellular immunities. Thrombocytes account for about half of all leucoytes in fish and are involved in blood clotting (Karl et al 1977).

2.2 BIOCHEMICAL PARAMETERS

Chemical and Biological analysis of the blood is of considerable value in confirming the diagnosis, prognosis and respond to treatment in variety of diseases, Kelly (1979), suggested that the interpretation of biochemical analysis require the sound knowledge of the normal range of the most important chemical constituent of the blood. The biochemical analysis of blood is limited to the determination of glucose, protein, ca^{2+}, P^{+}, Mg^{2+}, Na^{+}, Cl^{-} (Kelly 1979).

2.2.1 PLASMA PROTEIN

Proteins are polymers of some twenty two (22) amino acids link linearly by peptide bond. Protein are associated with both structure and function, plasma protein are involved in nutrition, maintenance of osmotic pressure, buffering, transport of small ions and molecules, haemostatis, and the protective effect of the immune protein (Schalm et al 1975).

The plasma protein is a group of heterogeneous molecule which represents a balance between anabolism and catabolism, it also reflect balance between

filtration into the capillaries and return from the tissue via the lymph, this balance according to Schalm et al (1975), depends upon the colloidal osmotic pressure and the circulation dynamics the concentration of protein in the plasma at any given time is a function of the hormonal balance, nutritional status, water balance and other factors affecting the state of health. The plasma protein concentration regulate the metabolic rate that is the concentration of albumin (Schalm et al 1975).

James (1971) reported that plasma contains 8-9% solid material of which about 7% is plasma protein. Plasma protein represents a complex mixture of a number of protein of different structural and functional properties. The major plasma protein includes albumins, globulins, fibrinogen, nucleoprotein, conjugated protein, such as lipoprotein and seromucoid protein, while the bulk of plasma protein consists of albumin and globulins (James 1971).

The albumin molecules are significant in the maintenance of a stable plasma pH. James (1971), stated that plasma protein functions can be categorized as nutritive, physico-chemical or transportive. The nutritive function of plasma protein has been established by indication that all the nitrogenous requirement of the fasted animal can be adequately supplied by intravenous of administration of plasma protein. Many of the plasma protein are involved in the transport of materials within plasma. The protein permits water insoluble materials to be transported through the vascular system. Coagulation of the blood involves a

series of plasma protein interacting in a specific manner to polymerize plasma fibrinogen into long chain and network of fibrin to form a blood clot (James 1971).

2.2.2 PLASMA ALBUMIN

Schalm (1975) stated that the albumin is the most abundant of plasma proteins. It is a readily available pool of amino acids. Albumin is the first protein to be lost from the blood during tissue injuries, it is important in the regulation and maintenance of colloidal osmotic pressure of the blood. (

PLASMA GLUCOSE

The main sugar in the blood is glucose (dextrose), but fructose, galactose, lactose, and some pentoses may be present in some clinical conditions and experimental procedures (Kronfeld 1974). The blood glucose level reflects the nutritional, emotional and endocrine conditions, it increases after feeding in monogastric animals (alimentary hyperglycemia) excitement, as a metabolic effect of noreprephrine release, it is usually in the range of 70 - 100mg/100cm^3 in monogaster and 30 - 60mg/100ml in the stomach of animal (Kronfeld 1974).

The erythrocyte glucose concentration approximates the plasma glucose concentration in most monogaster, the blood glucose concentration is increased by norepinephrine, epinephrine and glucagon, all of which are glycogenolytic, and by glucocorticoid which inhibits glucose utilization and stimulus to gluconeogenesis, food deprivation tend to diminish the blood glucose concentration. However, severe hypoglycocemia may develop in fasted or spent fish. Prolong exercise tends to lower the blood sugar level, but this is often

counteracted by emotional stress (Kronfeld 1974).

The normal value of sugar in the blood stream is approximately 90mg/100cm^3, the level of glucose in the blood and tissue fluid at any given moment is mainly determined by the relative extent to which these different processes occur in the liver. White and Fletcher (1985) reported that the mean serum glucose for 10 plaice was 73mg/glucose/100ml serum after capture, transport and handling and drop to 29mg/glucose/100ml serum after 48h in the aquarium. There are small amounts of glucose and fats dissolved in the blood plasma. The glucose concentration in plasma varied considerably due to metabolic disorders (Rostagi, 1988).

Ronald and Robert (1978) reported that some fish species, such as salmon, and trout, utilize only small amount of carbohydrate in the diet. The blood sugar reaches very high level which shows that trout are effectively diabetic being able to produce only small amount of insulin to lower the blood sugar.

2.3 PLASMA ELECTROLYTES

Blood is rich in anions and cations which function as electrolytes. The average concentration of electrolytes in the plasma as described by Rastogi (1988) is as follow [sodium (135 - 155), potassium (3.1 - 5.5), calcium (2.1 - 2.7), Magnesium (1.3 - 1.8), Chloride (100 - 107) Phosphate (2) plasma protein (5 - 6)] meq/l

plasma. The plasma anionic charge must be equal to the number of cationic charge to make plasma electrically neutral, or the ionic concentration should be independent of strong electrolytes of sodium chlorides and sodium bicarbonate.

The major types of anions in the blood are the anions of the minerals acid which are chloride and sulphate and the plasma protein, haemoglobin, oxyhaemoglobins, which act as blood buffer (Rastogi 1988).

2.4 SEROLOGICAL PARAMETERS

The rapid diagnosis of fish diseases through serological techniques have developed only slowly in fish clinical medicine (Ronald 1978). Serological tests may determine the presence of antigen in the tissue of fish micro-organism, and the presence of antibodies against a particular antibodies within the blood of the fish (Ronald 1978).

2.4.1 BLOOD GROUP

The ABO antigen of blood group of man was discovered by Landstainer (1900). The real impetus to animal blood grouping was provided by investigation of Fergusan (1955), and Rasmusen (1969). Erythrocyte of animal posses specific antigen belonging to many blood groups. Blood groups are inherited as simple mendelian dominant character (Schalm et al 1975), all blood group genes are located in the cell. Antigene of all blood group system in an individual are present on every one of its erythrocytes and are arranged in a more or less regularly

pattern mosaic structure of the cell surface (Schalm et al 1975). James (1971) reported that the importance of blood group as marker of inheritance of biochemical characteristics in genetic and breeding has been discovered.

The blood group classification is based on the antigenic nature of the erythrocyte of the blood, (James 1971). Blood group in men are classified into four namely, A, B, AB and O. Other blood group observed include M, N, S, Lewis and P blood group. It was also found that a human blood group responded immunologically in a similar fashion to blood group of the rhesus monkey.

James (1971) reported another blood grouping term Rh^- negative if the blood cell were not agglutinated by the isoantibody and rh^+ positive if the blood cell were agglutinate by the antibody.

The blood group refers to the presence in the red cell envelope of certain antigen (isoantigen) known as the blood group substance. Many blood groups have been identified but the ABO, Rh, M,N,S, P and Lewis group are the best known (James 1971).

The organisation of blood groups denotes the presence of certain isoantigene in the red cell. For example group AB contains isoantigen for both type A and type B, groups. A contains only type A isoantigen, and group B contains only type B isoantigen, group 0 does not contain either A or B isoantigen in its red cell (James

1971).

William et al (1969) reported that genetically, each antigen is the product of a specific gene, a single antigen may have more than one blood factor, so that a number of factors may be inherited as a group, the product of any individual allele or gene may therefore be identified by reaction with a number of reagents and is designated as a phenogroup. (William et al 1969).

2.4.2 GENOTYPE

The genetic materials of all cell is contained in the chromosomes which have a complex composition. The chromosomes control all kinds of biochemical and physiological activities through tiny chemical unit cells called gene. Genes are hereditary materials which contain DNA which are arranged on a thread like chromosome (Rastagi 1988). Gene is defined as a unit of recombination which cannot be subdivided by chromosomal breakage or crossing over and can be separated from it neigbouring segment by a crossing over event.

Rastagi (1988) reported that the haemoglobin from sickle-cell trail (Sicklemia), Sickle cell anemia and normal individual show different electrophoretic patterns and that the normal and sickle-cell anemia are different and the heterozygote has both type corresponding to haemoglobin, A,S, and a mixture of A and S respectively. These are due to gene interaction so that the physical as well as the physiological properties of the haemoglobin are altered due to S haemoglobin in

relation to the content in the red blood cells (Rastagi 1988).

Christine (1989) stated that an organism genotype-genetic make up - is composed of a collection of gene stating information which, under certain condition can be translated into phenotype. Gene occurs on a specific loci in linear order along chromosomes and the replicate, when the chromosome replicate, give function by synthesis protein, some of phase complicated molecules makeup the frame work of the cells of living tissues, while the majority function as enzymes which control specific biochemical reaction with the cell (Christine 1989).

CHAPTER THREE

3.0 MATERIALS AND METHODS

The experiment was carried out between May and June 1997. Live Heterotis niloticus, identified using taxonomic key of Reed et al (1967) were obtained from Osun State Ministry of Agriculture and Natural Resources, fish farm Osogbo, Osun State.

The fish were captured from a pond using seine net. No sexual selection was made and fish showed no symptons of stress or diseases.

Measurement of standard lenght was done usiing a measuring board, or and the weighht was taken using a top loading beem balance for individual fish .

Blood sample were taken as soon as the fish were taken out of the water. Blood (5 -10ml per fish was collected from vertebral blood vessel towards caudal penduncle of each fish using seperate heparinized syringes and treated with ethylene - diamine - acetic acid (EDTA), as anticoagulant.le as soon as the fish were taken out of the water. Blood (5-10ml/fish) was collected from vetebral blood vessels, toward the caudal penduncle of each fish using separate heparinized syringes and treated with ethylene-diamine-acetic and (EDTA), used as anticoagulant.

Serum was obtained from clotted or coagulated blood sample by centrifigation,

and this was used for determination of blood genotype. Plasma was obtained from non-clotted or EDTA treated blood by centrifugation. This was used for the determinations of plasma electrocytes and plasma biochemical analyses.

CONDITION FACTOR

The fulton's condition factor 'K' was calculated for each fish using the equation.

$$K = \frac{100W}{L^3}$$

where W = fish weight

L = standard length.

3.1 BLOOD CELL COUNTS

Haemocytometer was used in blood cell counts. The apparatus consists of a counting chamber, a cover slip, white and red blood pipette for diluting the blood, and a rubber tube with a plastic mouth piece for drawing the fluid into a pipette. The blood diluting fluid was prepared as discribed by Svobodova et al (1991).

The cells were counted on the counting chamber of haemocytometer with the aid of a compound microscope.

RBC = (No. of cell counted X 5 X 10 X 200) $10^6/mm^3$

WBC = (No. of cell counted X 0.25 X 10 X 20) $10^4/mm^3$.

3.2 HAEMOGLOBIN ESTIMATION

Haemoglobinometer was used based on acid haematin method (SAHLI). Haemoglobin was converted to acid haematin by action of N/10 HCL. The following apparatus and reagents were used. Sahli graduated tube standard (SAHLINOMETER) N/10 HCL, 0.02ml pipette. The graduated tube was filled to 20ml marked with N/10 HCL and 0.02ml of blood was added and mixed thoroughly. It was allowed to stay for five minute and dustilled water was added drop by drop,and each addition was mixed thoroughly, until the colour matches the standard.

The amount of solution in the graduated tube gives the haemoglobin concentration as a percentage.

$$\text{Haemoglobin} = \frac{\text{Value obtained}}{100} \times 17.2 \text{ gm/100ml}$$

3.3 PACKED CELL VOLUME (PCV)

Blood are drawn by capillary action into microhaemotocrit tubes, one end of the tube is then seal by a synthetic sealant. The sealed tubes are then centrifugated in a microhaemotocrit centrifuge. Centrifugation cost for 5 minutes at 10500rpm.

The packed cell volume was measured by using a microhaematocrit reader, and it was expressed as percentage.

3.4 ERYTHROCYTE SEDIMENTATION RATE (ESR)

Non-clotted blood are drawn by capillary action into microhaematocrit tube, one end of the tube is then sealed, by a synthetic sealant, and placed, onto a special metal holder consiting of two plates slanting at and angle of 45°C.

The rate at which erythrocyte sapontaneousely settled, determined the erythrocyte sedimentation rate. It was allowed to stand for 40 min. The value of erythrocyte sedimentation rate is determined by means of the haematocrit reader. The erythrocytes column is determined as percentage of the total column of the blood. The value of erythrocyte sedimentation rate within the given time interval is the difference between 100% and the percentage part represented by the corpuscle column (Svobodova et al 1991).

3.5 MEAN CELL HAEMOGLOBIN CONCENTRATION

This refers to the percentage of haemoglobin in 100ml of red blood cell.

CALCULATION

Divide the haemoglobin content in g/100ml by the PCV/100ml of blood according to the following formula

$$MCHC = \frac{Hb}{PCV \times 1000} \ T/l^{-1}$$

3.6 MEAN CORPSCULAR VOLUME (MCV)

The value of the mean corpuscular volume was calculated from the haemotocrit

value (PCV) (%) and the erthrocyte count (Er) ($10^6/mm^3$), according to the following formula (Svobodova et al 1991).

$$MCV = \frac{PCV \times 1000}{Er} \ (\mu^3)$$

3.7 MEAN CORPUSCULAR HAEMOGLOBIN (MCH)

Mean corpuscular haemoglobin concentration, expresses the concentration of haemoglobin in unit volume of erythrocyte. It was calculated from the haemoglobin value (Hb) and from the erythrocyte count (Er) according to the following formula.

$$MCH = \frac{Hb}{Er} \text{ (Picogramme) (pg)}$$

3.8.0 BLOOD PLASMA BIOCHEMICAL ANALYSIS

The blood for biochemical analysis was collected as discribed by Svobodova et al (1991), The blood was centrifuged and the plasma stored at 40°C or frozen (-20°C) in microbe with a seal.

3.8.1 TOTAL PLASMA PROTEIN

Photometric method:- The determination used followed the instruction of the Bio-La-Test. Total protein (TP 300) base on the formation of a manure coloured complex, protein standard was used for the determinant. The total protein concentration was calculated according to the formula.

TOTAL PROTEIN = $\frac{\text{Ab test}}{\text{Ab Std.}}$ X Concentration of Std.

where

Conc. of std. = 50g

Absolute std. = 5g

3.8.2 TOTAL SERUM ALBUMIN

BCG solution was prepared, and mixed with 0.02mg of serum and allowed to stand for 10 minutes at room temperature. It was read with the rank of 620nm as described in total plasma protein

TOTAL SERUM = $\frac{\text{Ab test}}{\text{Ab Std.}}$ X Concentration of Std.

where

Conc. of std. = 5S

Absolute std. = 50S.

3.8.3 TOTAL PLASMA GLUCOSE

The Bie-La-Test Oxochrom Glucosa (GLU 250E) was used for the determination of glucose concentration in the blood plasma. The plasma glucosewas oxidized and catalyzed by the glucose oxidase enzyme to hydrogen peroxide and gluconate. The peroxide produced was determined by oxidation coupling with substituted phenol abd 4-amina antipyrin and catalyzed by peroxide. The instrument was set to zero and read with 515nm blank.(Svobodova et al 1991)

TOTAL GLUCOSE = $\frac{T}{Std.}$ X Conc. of Std.

Stand. = 0.19g

Concentration of std. = 100g

3.9.0 SEROLOGICAL ANALYSIS

This is a Serological study for rapid diagnosis of fish diseases. The technique can be used to determine the presence of antigen in the tissue of fish, the presence of micro-organism and the presence of antibodies against particular antigen within the blood of fish.

3.9.1 AGGLUTINATION TEST

This test is usually performed to diagnose suspected pathogenic organism in the body of fish. Slide agglutination method was used. One colony of a young culture of bacteria was mixed with saline on a clean degreased glass slide in two separate drops. A drop of serum was added to one drop, and a further drop of saline to the other. The slide was rocked backward and forward for 30 seconds. A positive reaction was indicated by agglutination in the suspection in which serum was added.(Svobodova et al 1991)

3.9.2 BLOOD GROUP DETERMINATION

In the determination of blood group of an animal, its red cells are tested with specific blood typing reagent prepared from immune antisera.

The rh reagent ABD was used, and a visible reaction between the antigens of the surface of the red blood cells and the antibody (agglutination) will indicate positive result.

Three drops of blood were placed on clean degreased slide and antigen A, B, D was separately drop on each of the blood. Agglutination in A or B or A and B indicate that the blood is in either blood group A or B or AB, while no agglutination in A or B, indicated that the blood is in 0 blood group. While agglutination in D indicated that the blood group is positive (A+, B+, or 0+) and no agglutination indicate negative blood (A-, B-, 0-) group.

3.9.3 GENOTYPE (Hb)

The principle of eletrophoresis Haemaglobin was used. The blood was diluted with water and blood of known genotype "AS" was used as a control. The two were applied on a cellulose acetate paper at different point on a straight line, and was put inside electrophoresia-machine for ten minute. The movement of the blood on the cellulose acetate paper indicated the genotype or the blood. Upward movement is AS and downward movement AA, SS genotype will be stationary after 10 minute in the electrophoresia-machine (Rastagi 1988).

3.10.0 PLASMA ELECTROCYTES ANALYSIS METALS

3.10.1 K^+ AND Na^+

The K^+ and Na^+ were determine using corning 400 flame photometer.

3.10.2 Magnesium was determined using (a back) model 200A flame Atomic absortion spectrophotometer.

3.10.3 NON-METAL CL^- AND P^{3-}

PHOSPHORUS:- The spectrophotometric method employed was that described by Fiskea and Subbarow (1925). The phosphate are conjugated with molybidic acid and the phosphomolybdic acid reduced by an aminonapthol-sulphuric acid to a blue colour which was measured with blank at 660mμ. The graph of standard solution was plotted against the absorbent than the concentration of the sample was read from the graph.

$$\text{PPm Solution} = \frac{R \times Fv \times DF}{\text{Volume of Sample}}$$

where R = Read

Fv = Final Value

DF = Diluting Factor

CHLORIDE (CL^-)

This was done by titrimetric method as discribe by Schales and Schales (1941). The ash was dissolved with little water, 1ml of potassium chromate and it was titrited with 0.1M$AgNo_2$ to redish colour.

1ml $AgNo_3$ ≡ 1Mg chloride.

REGRESSION AND CORRELATION ANALYSIS

Regression analysis and correlation coefficient (F) were used to determine ($P< 0.05$, $P<0.01$) the relationship among the values obtained from findings measured in the blood analyses.

CHAPTER FOUR

4.0 RESULTS

4.1 LENGTH AND WEIGHT

The 25 Heterotis niloticus specimens used for the experiment ranged in weighht and standard length of 300g-610g, and 30.4cm - 39.5cm respectively with meann weight of 429g and mean standard lenght of 34.22cm.

4.2 HAEMATOLOGICAL FINDINGS

The summary of haematological characteristics of Heterotis niloticus is presented in (Table 1) and the data of haematological findings is presented in appendix 2. Erythrocyte and leucocytes counts showed intra-species variation.

There was a wide variation in the value of the parameters determined, the number of red blood cell and white blood cell count did not correlate with the standard length and weight. The number of red blood was higher than the number of white blood cell. There were correlations between blood cells, haemoglobin and pack cell volume.

4.3 BIOCHEMICAL DETERMINATIONS

Some biochemical determination of the blood of Heterotis niloticus, are presented inn table 2 and the data of biochemical analysis in appendix 3.

The mean values for biochemical determination were, plasma protein (mg/dl),

plasma glucose (mg/dl) and plasma albumin (mg/dl) were 5.7, 61.46, 4.74) (P 0.05) respectively.

There were variations in the values of biochemical analysis of Heterotis niloticus (Fig. 3). The value of plasma glucose was higher than the value of plasma protein and albumin. There was no correlation between the weight and length of Heterotis niloticus and the biochemistry of the blood plasma.

4.4 PLASMA ELECTROLYTES

The summary of the plasma electrolytes of Heterotis niloticus is presentedin (Table 3) and the data of the electrolyte analysis is presented in appendix 4.

There were variations within the species (Fig. 4). The plasma contains more phosphorous ($540.6_{mg/l} \pm 59.19_{mg/l}$) than sodium, chloride, potassium and magnesium put together 11.7 ± 0.47, 0.82 ± 0.17, 21.70 ± 1.05 respectively ($P<0.05$).

TABLE I: SUMMARY OF HAEMATOLOGICAL CHARACTERISTICS OF HETEROTIS NILOTICU (P0.05)

	MEAN ± S.E	RANGE

TABLE I: SUMMARY OF HAEMATOLOGICAL CHARACTERISTICS OF [illegible] (P0.05)

	MEAN ± S.E	RANGE
FISH SIZE		
Standard Length (cm)	34.22 ± 0.92	30.4 - 39.5
Weight (g)	429.4 ± 32.17	300 - 610
BLOOD PARAMETERS		
Erythrocyte count ($(10^6/mm^3)$)	1502400 ± 16572.19	[illegible]
Lencocytes count ($10^4/mm^3$)	5724 ± 488.63	3900 - 8700
Mean cell Haemoglobin Conc. (T/l^{-1})	$1.576 \times 10^{-3} \pm 6.429 \times 10^{-5}$	0.0012 - 00.19
Mean cell Haemoglobin (pg)	$2.028 \times 10^{-5} \pm 1.5112 \times 10^{-6}$	3.0×10^{-4} - 3.9 x
Mean cell Volume (μ^3)	$1.912 \times 10^{-2} \pm 1.3735 \times 10^{-3}$	0.013 - 0.031
Haemoglobin (g/mm^3)	44.64 ± 4.26	22 - 62
Haemotocrit (%)	28.12 ± 2.98	13 - 39
Erythrocyte sedimentation rate (%)	40.16 ± 7.84	11 - 80

TABLE II: SUMMARY OF BIOCHEMICAL ANALYSIS OF HETEROTIS NILOTICUS (P<0.05)

Protein (g/dl)	± 0.67	0..
Glucose (mg/dl)	61.46 ± 5.25	
Albumin (mg/dl)	4.76 ± 0.83	0..

TABLE III: SUMMARY OF PLASMA ELECTROLYTES OF HETEROTIS NILOTICUS (P0.05)

	MEAN ± S.E	RANGE
Sodium (Na^+) (mg/l)	11.7 ± 0.47	9.7 - 13.9
Chloride (Cl^-) (mg/l)	0.82 ± 0.17	0.07 - 1.63
Potassium (k^+) (mg/l)	21.70 ± 1.05	18.3 - 29.9
Magnesium (mg^+) (mg/l)	8.32 ± 0.54	6.1 - 10.6
Phosphorus (p^{3-}) (mg/l)	546.6 ± 59.19	202.5 - 752.5

TABLE IV: PERCENTAGE OCCURRENCE OF SEROLOGICAL CHARACTERISTICS OF HETEROTIS NILOTICUS

4.1 BLOOD GROUP

A	B	AB	O+	O-
-	-	-	92	8

4.2 GENOTYPE

AA	AS	SS
56	44	-

4.3 AGGLUTINATION TEST

+ve	-ve
-	100

TABLE V: ***LINEAR REGRESSION ANALYSIS*** OF THE BLOOD PARAME
FUNCTION OF THE PHYSICAL PARAMETERS OF LENGTH AND
AND GAVE THE FOLLOWING RELATION

PCV (%) = 7.20628 + 0.61473 Length (cm) (r 0.197) (P0.05)

RBCC(mm^3) = 162348.0078 + 2573.41572 Length (cm) (r 0.006) (P0.05)

MCHC(%) = 1.892502E-03 - 9.25011E.06 Length (cm) (r 0.132) (P0.05)

Na^+ (mg/l) = 6.56480 + 0.15090 Length (cm) r(0.297) (P0.05)

MCH (%) = 3.108067E-05 - 1.86375E-09 Weight (g) 4(-0.

MCV (%) = 0.40144 - 7.97026E-04 Weight (g) r(-0.328) (P0.

TABLE VI: T FOLLOWING CORRELATIONS EX THE BLOOD PARAMET C ETEROTIS NILOTICUS

PCV (%) AND Hb (mg/mm^3)	= r = 0.9571 (P 0.001)
WBC(mm^3) AND PCV(%)	= r = 0.6409 (P 0.001)
WBC(mm^3) AND Hb (g/mm^3)	= r = 0.5599 (P 0.001)
RBCC(mm^3) AND NacHc (%)	= r = 0.6326 (P 0.001)
WBC(mm^3) AND MCH(%)	= r = 0.5006 (P 0.001)

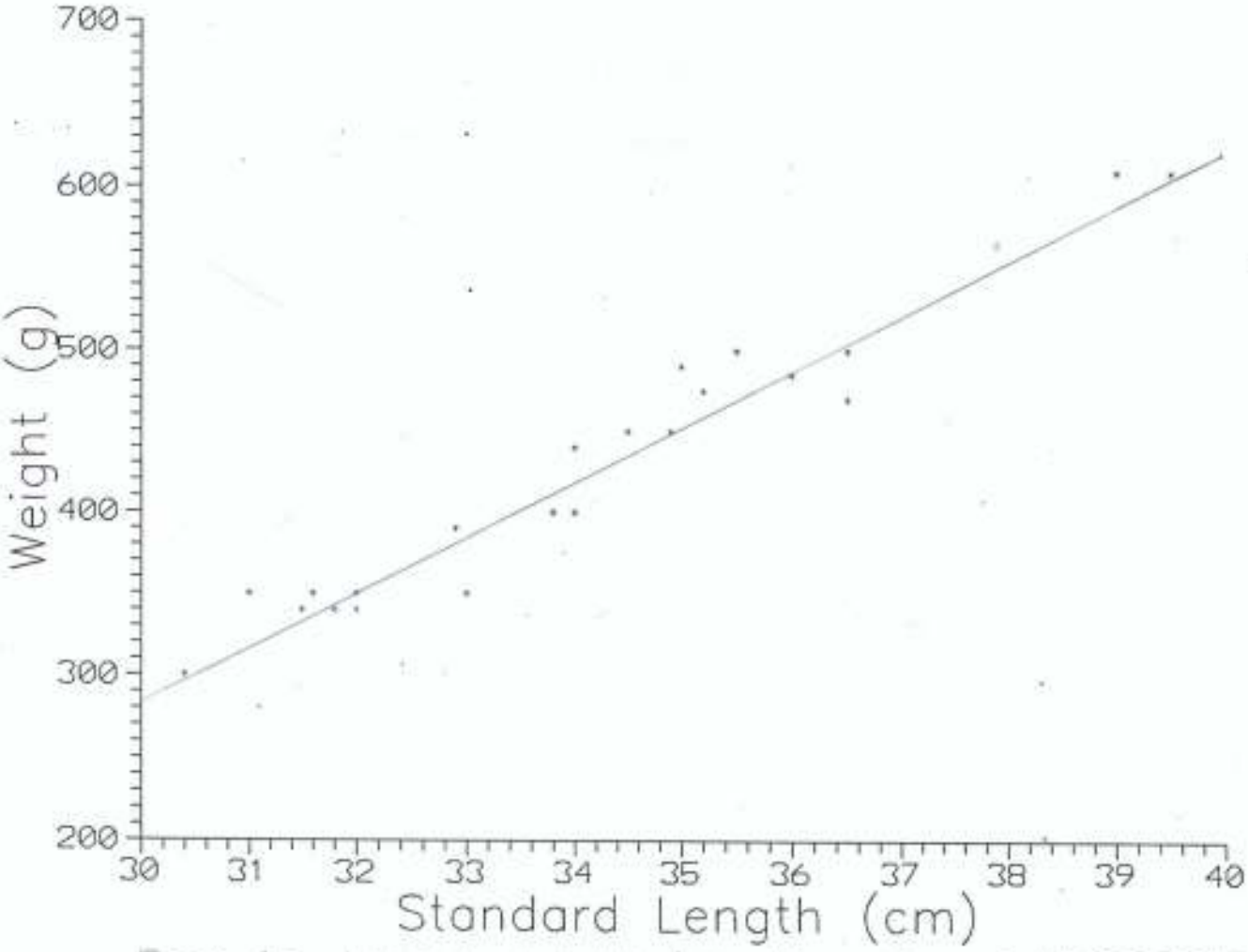
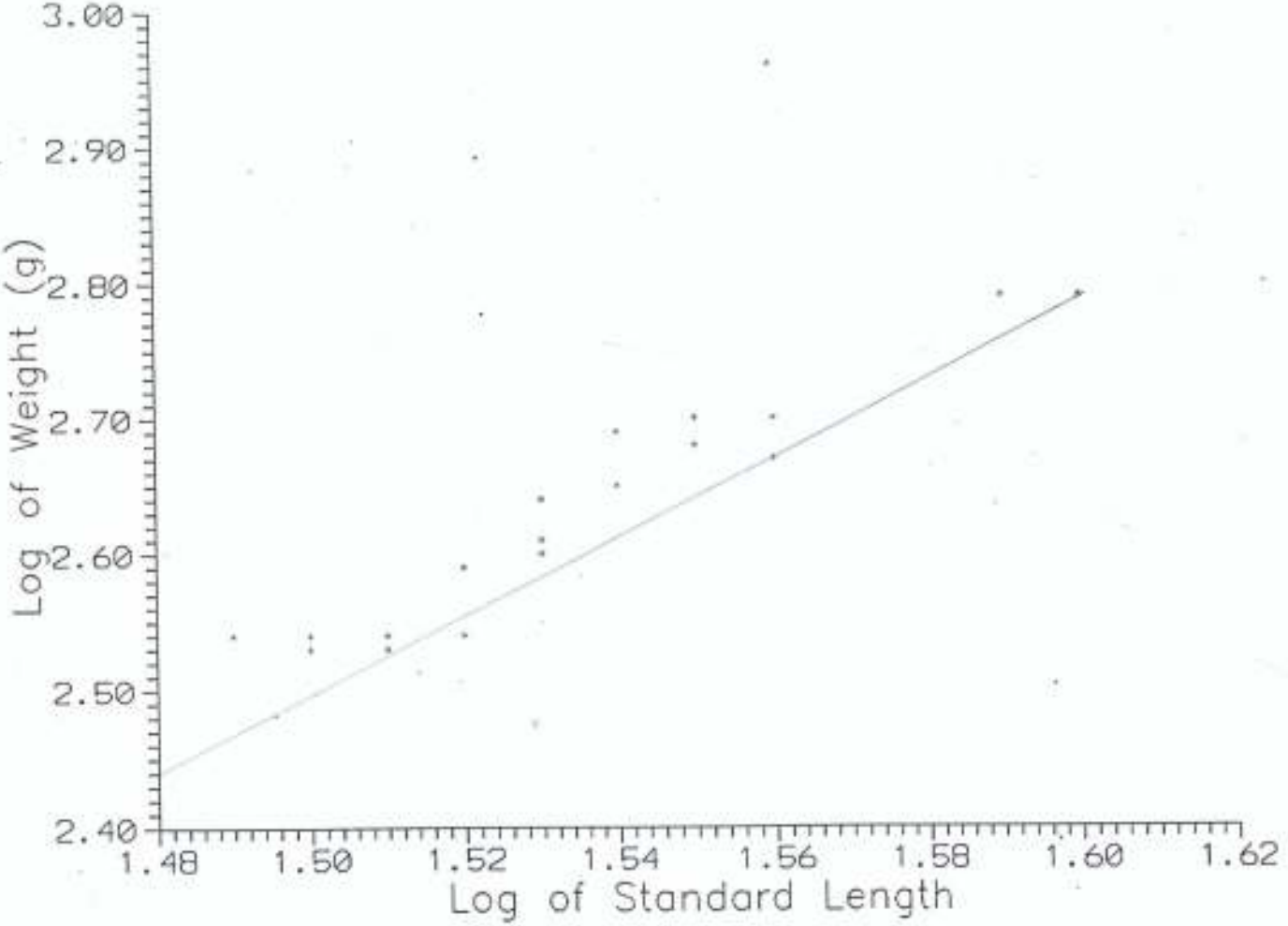


Fig: 1: Length-Weight Relationship of HETEROTIS NILOTICUS



'-nath-Log of Weight Relationship
'TI O ICUS

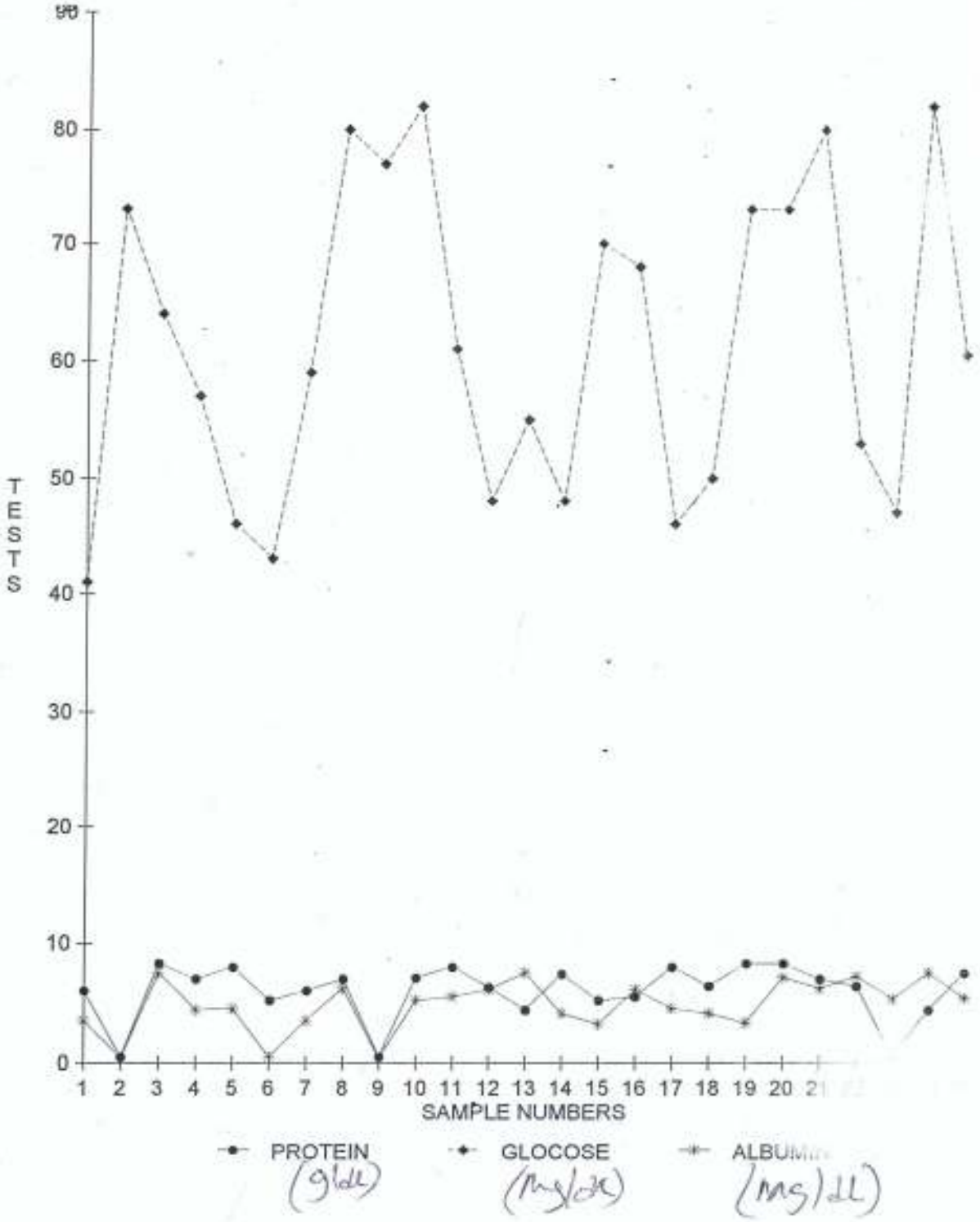


FIG 3: BIOCHEMICAL ANALYSIS OF HETEROTIS NILOTICUS

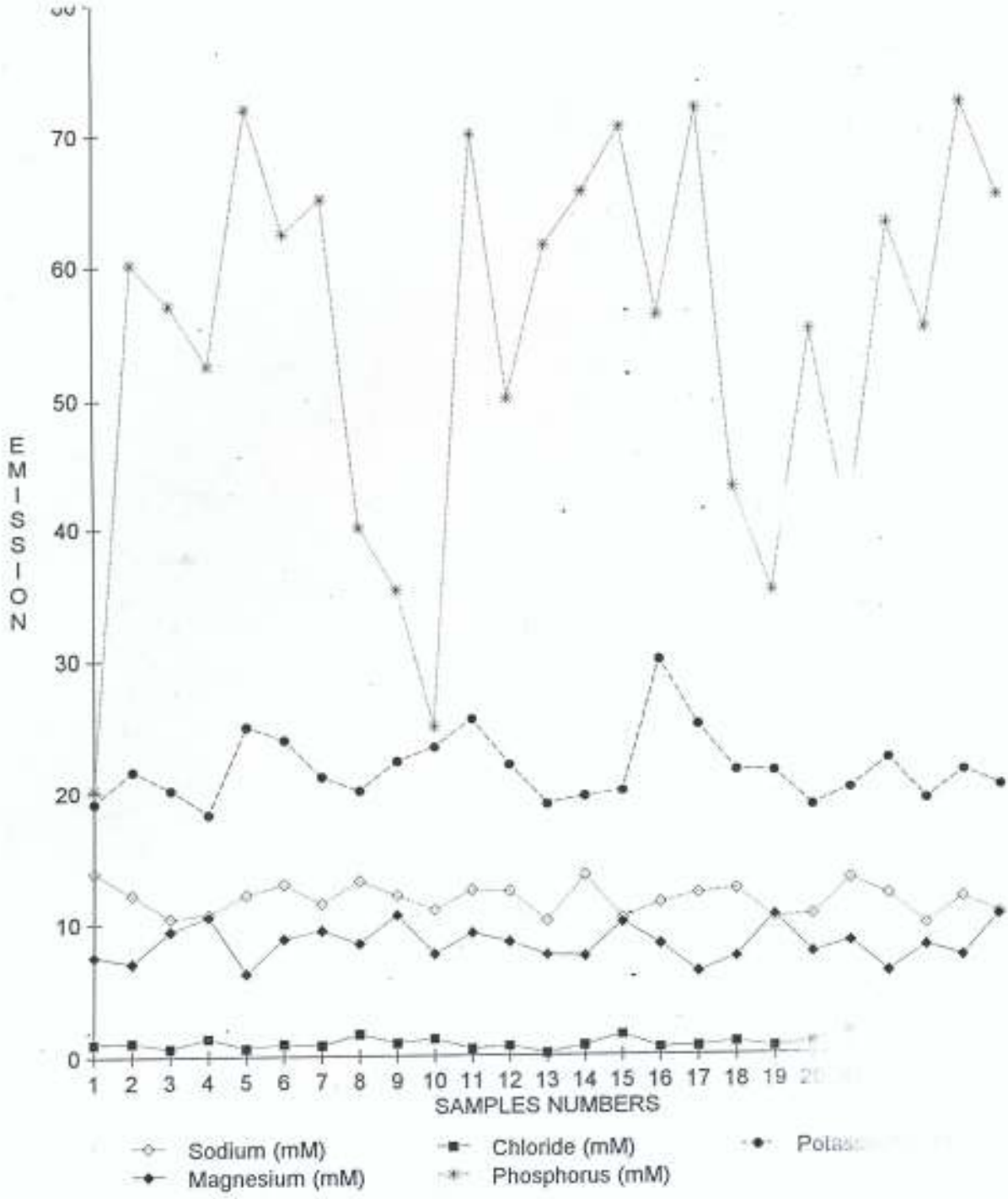


FIG 4: PLASMA ELECTROLYTE ANALYSIS OF HETEROTIS NILOTICUS

4.5 SEROLOGICAL ANALYSIS

The percentage occurrence of serological characteristic of heterotis niloticus is presented (Table 4), and the data of serological characteristic is presented in (appendix 5).

The results show similar antigen ABO. The blood group (table 4.1), shows that out of 25 specimen observed 92% belong to rho+ blood group, while the remaining 8% were rho-blood group.

The genotype A, B and S, observed (Table 4.2), was similar to human, genotype, and 56% out of the 25 specimen observe were in AA normal haemoglobin genotype, while the remaining 44 was in "AS" genotype.

There was 100% negative agglutination (table 4.3) in the blood of heterotis niloticus, which shows that there was no bacterial in the fish during the study period.

CHAPTER FIVE

5.0 DISCUSSION

The blood parameters (Red blood cell, white blood cell, Haemoglobin, Haemotocrits, Erythrocyte sedimentation rate MCHC, MCV, MCH, protein glucose, albumin, sodium, chloride, potassium, magnesium and phosphorus, 1502400 ± 16572.19., 5724 ± 488.63., 44.64 ± 4.26., 28.12 ± 2.98., 40.16 ± 7.84., $1.576 \times 10^{-3} \pm 6.429 \times 10^{-5}$., $2.028 \times 10^{-5} \pm 1.511 \times 10^{-6}$., $1.912 \times 10^{-2} \pm 1.3735 \times 10^{-3}$., 5.7E0.67, 61.46 ± 5.29., 4.76 ± 0.83., 11.7 ± 0.47, 0.82 ± 0.17., 21.70 ± 1.05, 8.32 ± 0.54, 546.6 ± 59.19 respectively of haematological characteristic is presented in (Table 1) of Heterotis niloticus, was similar to those reported by Siakpere (1985), on Clarias isheriensis, Sandless et al (1988), on Atlantic Salmon, Erondu et al (1993), on four catfishes, Fagbenro et al (1993) on Heterobranchus bidorsalis.

There was intraspecies variation in haematological value as indicated by the wide ranges of some parameters in this study, have been attributed to many reasons, for example, Houston et al (1971) reported changes in blood parameters of fresh water fish expose to various handling procedure before experiment.

The effect of stress on fish, such as transport, low oxygen, high ammonia, result in change in haematological picture in fish. (Houston et al 1971)

Ellasser and Clem (1986) reported that the laboratory - acclimated of channel

catfish transported from the field to the laboratory displayed different haematological value from those consider to be normal, and had lost their ability to respond in vitro to both nitogenic and antigenic stimuli.

Railo et al (1985) stated that sampling stress caused an increase in haematocrit value and decrease in mean cellular haemoglobin concentration in rainbow trout (Salmo gairdineri). This is an indication that indicating red blood cell swelling, due to sampling stress, cause haemoconcentration in the pike, Exos lucius. Haemoglobin concentration and haematocrit of fish blood diseases after the stress due to capture, handling and sampling procedure (Bouck and Ball 1966).

Such a stress situation is associated with an increased secretion of catecholine, increase concentration of adrenaline and non-adrenaline in the blood of rainbow trout (Salmo gairdneri), has been observed in response to physical disturbances (Nakano and Tomlison 1967).

There was high erythrocyte count (580000 - 2170000) (Table 1) in Heterotis niloticus. This value was similar to that reported by Korisiakpere (1985), for Clarias isheriensis, Erondu et al (1993) for four catfishes, including Heterobranchus bidosalis (Fagbenro et al 1993) . This high value of red blood cell is an indicative of high oxygen carrying capacity of the blood, which is characteristics of fish capable of aerial respiration and with high activity (Korisiakpere 1985, Fagbenro et al 1993). Sandless et al (1993) reported high

haematological parameter in Salmo gainderi (Atlantic Salmon).

Lenfant and Johansen (1972) stated that there is the tendency for haemoglobin concentration to be higher in those fishes, which utilized atmospheric oxygen.

There was low white blood cell count in Heterotis niloticus (3900 - 8700 mm^3) as compare to 0.80 - 73.60 $10^4/mm^3$) in Heteropbranchus bidorsalis (Fagbenro et al (1993) which could be as a result of the presence of external scales, or other protective measure against pathogenic organisms. Dick and Dixon (1985) reported a decrease leucocyte concentration in the blood of rainbow trout expose to acute water borne copper.

There were variations in the value of haemoglobin (g/mm^3) 44.62 ± 4.26), Haemotocrit, 28.12 ± 2.98% and erythrocyte sedimentation rate, 40.16 ± 7.84%) in Heterotis niloticus. Similar variations was reported by Basia et al (1986), which reported high haemoglobin concentration, and haemotocrit value in the blood sedimentation of Oreochromis mossanbicus and red tilapia (O. niloticus).

The direct proportion between the value of haemotocrit, erythrocytes sedimentation rate and haemoglobin in the blood of Heterotis niloticus, showed that the increases in haemotocrit will increase the amount of haemaglobin concentration in the blood.

Lenfant and Johansen (1972) stated that considerable variations exist in the amount of blood haemoglobin found in blood of different species of air breathing fish. In some air breathing teleost, blood haemoglobin concentration and correspondingly blood oxygen capacity and haematocrit are as high or higher than those of many mammals and bird (Johansen et al 1978).

The possible reason for this could be environment, gas tension, fish activity level, extent of reliance upon our breathing organ and pathway of blood circulation to and from air breathing organ have been used to explain both high haemoglobin value of some species as well as interspecific difference in this property (Johansen 1970, and Johanseen et al 1978).

There was positive correlation between Erythrocyte, leucocyte and PCV, Hb, MCH in Heterotis niloticus.

5.1 BIOCHEMICAL FINDINGS

Table 2 presents the result of biochemical analysis of Heterotis niloticus. A very little work has been done on biochemical studies of fish in Nigeria and in general. The result present is similar to the result of biochemical analysis of Clarias isheriensis (Kori-siakpere 1985).

The mean plasma glucose for Heterotis niloticus was 61.46 ± mg/dl which was in normal range with the work of White and Fletcher (1985), who reported the mean

serum glucose for plaice Kori-siakpere (1985) report high (104.42 ± 4.26 mg/dl) mean glucose value for Claria isheriensis.

Rastagi (1988), stated that there are small amounts of glucose and fat dissolved in the blood plasma. Glucose concentration in plasma varied considerably due to metabolic disorder. White and Fletcher 91985), stated that the normal value of sugar in the blood stream is approximately 90 mg/100cm, they went further to say that the level of glucose in the blood and the tissue fluid at any given moment is mainly determine by the relatively to which these different process occur in the liver.

The mean value for protein in (mg/dl) and Albumin (mg/dl), in Heterotis niloticus were 5.7 ± 0.6, and 4.76 ± 0.83, which does not agrees with Siakpere (1985) who reported the mean value of 7.63 ± 0.11 for plasma protein for Clarias isheriensis.

Raizada et al (1984), reported high value of protein, followed by the value of cholesterol, glucose, urea, calcium, inorganic phosphorus and ceratine in the biochemical constituent of fresh water Cirrhinus mrigala HAM taken under normal condition . Higher value of cholesterol, and creatinines was recorded in female, white globulin where found to be higher in male.

Nakagna (1979) stated that five lipoprotein were fractionated from, a globulin fraction of carp, Cyprinus capio, serum protein, the presence of a small quantity

of the lipoprotein with a large molecular weight and zero mobility in electrophoresis was confirmed.

Craid and Havey (1994) reported that the sex of fishes can be distinguished by a biochemical test. The test depend on the measurement in the blood plasma of alkali libable protein link phosphorus, as specific measure of yolk protein (vitethogenin). In vitellogenic female, values of 70 - 100 μg protein phosphorus ml plasma^{-1} are usually found, while in male non-vitellogenic female and immature fish of both sexes the value is less than 7.5 and usually less than 5μg/ml^{-1}.

5.2 PLASMA ELECTROLYTE

The result of plasma electrolytes is presented in (Table 3) of Heterotis niloticus. showed high value of phosphorus (546.6 ± 59.19 mg/l), which was positively correlated with the work of, Kayame (1983), who reported high inorganic phosphorus in carp Cyprinus carpio.

There were low value of sodium (mg/l), and chloride (mg/l) in Heterotis niloticus. This result did not agree with the work of Kori-siakpere (1985) who recorded high mean values (104.42 ± 4.26, 104.24 ± 3.10) for plasma sodium and plasma chloride respectively.

Fagbenro et al (1993), reported high mean serum value (802.52 ± 36.97 mg/l of sodium in Heterotis niloticus. The generally low mean value in sodium and

chloride plasma electrolytes in Heterotis niloticus may be as a result of seasonal variation in the concentration of sodium and in an aquatic environment.

Sandstrom (1989), reiterated the seasonal variation in sodium chloride concentration in aquatic environment, and that chloride concentration was maximal in spring and summer and decreased in autum down to a winter minimum.

Love (1970) reported that the calcium content in female plasma has been reported to vary seasonally and to respond to gonad activity. The sodium ion concentration, in general, fall greatly in fresh water and increase in higher salinity.

The effect of the ionic environment on the plasma electrolytes has often been demonstrated in salmonids and other eurthaline species, by offering the environmental ion concentration (Sandstrom 1989).

The mean value of potassium and magnesium in (mg/l) is 21.70 ± 1.05, 8.23 ± 0.54 respectively in Heterotis niloticus, this result was higher than the mean value of potassium and magnesium (3.40 ± 0.14, 0.24 ± 0.02 mg/l) in Clarias isheriensis as reported by Siakpere (1985).

Railo et al (1985) reported that the sampling stress affected the ionic balance in fish plasma. The potassium K^+, concentration of plasma increase and plasma

chloride Cl^- decreases during storage.

The plasma, potassium concentration decreased far below resting. Plasma osmotic concentration appeared to change seasonally thus, reflecting a modification in the distribution of water and salt in both plasma and tissue (Roberson et al 1962, Harson and Perez 1957).

Shell (1959) reported an apparent seasonal changes in electrolytes level of the freshwater teleost, Macropterus dolomine, indicative of the presence of some cyclic metabolism pattern and related these cycle to a presumed seasonal variation in electrolytes levels.

Plasma ion concentration may be sensitive to temperature changes. Sandstrom (1989) stated that the seasonal rhythms in perch, Perca fluviatus L. could not have been generated by a temperature oscillation.

The main effect of temperature in biological rhythm should be on amplitude. While day length is the most forceful syncronizer to the changes of season (Sand strom 1989).

5.3 SEROLOGICAL PARAMETERS

The percentage occurrence of serological parameters (Table 4) of Heterotis niloticus, showed that the predominant blood group (Table 4.1) was 92% rho (0^+), while the remaining 8% was 0-rh.

Erondu et al (1993) reported that similar antigen reaction (ABO) as in human blood was observed in four catfish species. This assumption was related to the blood group of Heterotis niloticus.

The predominant genotype (table 4.2) was found to be AA in Heterotis niloticus, which was 56% AA and 44% AS. This result was similar to the result reported by Erondu et al (1993), who reported similar genotype.

There was 100% negative agglutination (table 4.3) test in Heterotis niloticus, which proved that the blood at Heterotis niloticus lack bacterial infection.

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CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

Blood reference value have been established in species of economic value, most primarily in solmonids, Clarias isheriensis, Heterobranchus bidorsalis and four catfishes (C. gariepinus, H. longifilis F_1 hybrid (C.g. x H.l) and C. nigrodigitatus).

Haemalological studies has been widely used as a mean of assessing the state of health of teleost fish, the result of this study will serve as a baseline information for assessing disease condition of this culturable, Heterotis niloticus species under intensive and semi-intensive rearing.

The sexes of Heterotis niloticus can easily be distinguished through the result of biochemical test, through the measurement of yolk protein (vitellogenin) in the blood plasma; The value of vitellogenin in vitellogenic female is higher than in male, non-vitellogenic female and immature fishes of both sexes.

Blood group frequencies could aid in selection of brooder for breeding purposes, the result could aid in genetic selection of brood fish for hatchery management.

Specifically the establishment of the haematological characteristic of Heterotis niloticus, will serve as a standard for physiological, pathological or toxicological investigation.

6.2 RECOMMENDATIONS

The major problem facing aquacultural development in developing world apart from fish seed collection is diseases. In other to solve the above problems, the following require immediates attention:

i) Research into haematological characteristics of cultivable fishes.

ii) Comparative haematological analysis of fishes.

iii) Further research in the serological studies of cultivable fishes.

iv) Further research into the biochemical studies of cultivable species.

v) Finally, special grant should be made available, for fish haemotological studies, since the study is highly technical and expensive.

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APPENDIX 1

RESULT 1: LENGTH-WEIGHT AND CONDITION FACTOR OF HETEROTIS NILOTICUS, (CUVIER 1829)

SAMPLE NO	S.L.(cm)	wt(g)	Log S.L.	Log wt	$K = \frac{100w}{L^3}$
1.	36.5	500	1.56	2.70	1.03
2.	31	350	1.49	2.54	1.17
3.	36	485	1.56	2.96	1.04
4.	34	440	1.53	2.64	1.12
5.	39	610	1.59	2.79	1.03
6.	36.5	470	1.56	2.67	0.97
7.	33	350	1.52	2.54	0.97
8.	34.5	450	1.54	2.65	1.10
9.	35	490	1.54	2.69	1.14
10.	36.5	500	1.56	2.70	1.03
11.	32	350	1.51	2.54	1.07
12.	35.2	475	1.55	2.68	1.12
13.	35.5	500	1.55	2.70	1.06
14.	34.9	450	1.54	2.65	1.11
15.	31.6	350	1.50	2.54	1.11
16.	31.8	340	1.50	2.53	1.06
17.	39.5	610	1.60	2.79	1.04
18.	33.8	400	1.53	2.60	1.09
19.	31.5	340	1.50	2.53	1.09
20.	32.0	340	1.51	2.53	1.04
21.	34.5	450	1.54	2.69	1.10
22.	34.0	400	1.53	2.60	1.02
23.	33.8	400	1.53	2.61	1.06
24.	30.4	300	1.48	2.48	1.07
25.	32.9	390	1.52	2.59	1.10

RESULT 2: HAEMATOLOGICAL CHARACTERISTICS OF HETEROTIS NILOTICUS, (CUVIER 1829)

SAMPLE NO	RBC $(mm)^3$	WBC $(mm)^3$	PCV %	Hb g/mm^3	ESR %	MCHC	MCH	MCV
1.	1410000	4800	19	36	40	0.0019	0.000025	0.013
2.	1730000	5550	32	53	60	0.0017	0.000031	0.018
3.	1800000	7750	37	57	50	0.0015	0.000032	0.021
4.	1850000	6000	33	49	80	0.0013	0.000026	0.018
5.	1400000	4950	32	45	50	0.0014	0.000032	0.023
6.	1350000	5050	25	37	15	0.0015	0.000027	0.019
7.	1710000	7000	35	54	40	0.0015	0.000032	0.021
8.	1920000	5100	37	62	18	0.0017	0.000032	0.019
9.	1810000	7350	33	51	60	0.0015	0.000028	0.018
10.	1600000	6900	30	48	70	0.0016	0.00003	0.017
11.	1670000	5600	29	48	50	0.0016	0.000029	0.017
12.	1590000	6000	32	49	50	0.0015	0.000031	0.020
13.	1160000	4050	22	37	21	0.0017	0.000032	0.019
14.	1970000	5450	39	60	40	0.0015	0.000030	0.020
15.	1750000	5250	33	53	40	0.0016	0.000030	0.019
16.	1570000	6050	28	47	11	0.0017	0.000030	0.018
17.	1430000	5300	30	48	60	0.0016	0.000034	0.021
18.	580000	4200	18	22	41	0.0012	0.000038	0.031
19.	1010000	5200	20	39	18	0.0016	0.000039	0.024
20.	870000	3900	16	29	31	0.0017	0.000031	0.018
21.	1280000	5150	20	36	18	0.0018	0.000028	0.016
22.	1100000	4900	20	36	11	0.0018	0.000033	0.018
23.	2170000	8700	33	46	40	0.0014	0.000021	0.015
24.	700000	4900	13	22	40	0.0017	0.000031	1.019
25.	2130000	8000	36	54	30	0.0014	0.000025	0.014

APPENDIX 3

RESULT 3: BIOCHEMICAL ANALYSIS OF HETEROTIS NILOTICUS

SAMPLE NO	PROTEIN mg/dl	GLUCOSE mg/dl	ALBUMIN mg/dl
1.	6.0	41.0	3.5
2.	0.5	73.0	0.4
3.	8.3	64.0	7.5
4.	7.0	57.0	4.4
5.	8.0	46.0	4.5
6.	5.2	43.0	0.5
7.	6.0	59.0	3.5
8.	7.0	80.0	6.2
9.	0.5	77.0	0.4
10.	7.1	82.0	5.2
11.	8.0	61.0	5.5
12.	6.3	48.0	6.1
13.	4.4	55.0	7.5
14.	7.4	48.0	4.1
15.	5.2	70.0	3.2
16.	5.5	68.0	6.1
[illegible]	8.0	46.0	4.5
18.	6.4	50.0	4.1
19.	8.3	73.0	3.3
20.	8.3	73.0	7.1
[illegible]	7.0	80.0	6.2
22.	6.4	53.0	7.2
23.	0.5	47.0	5.3
24.	4.4	82.0	7.5
25.	7.5	60.5	5.4

APPENDIX 4

RESULT 4: PLASMA ELECTROLYTES OF HETEROTIS NILOTICUS

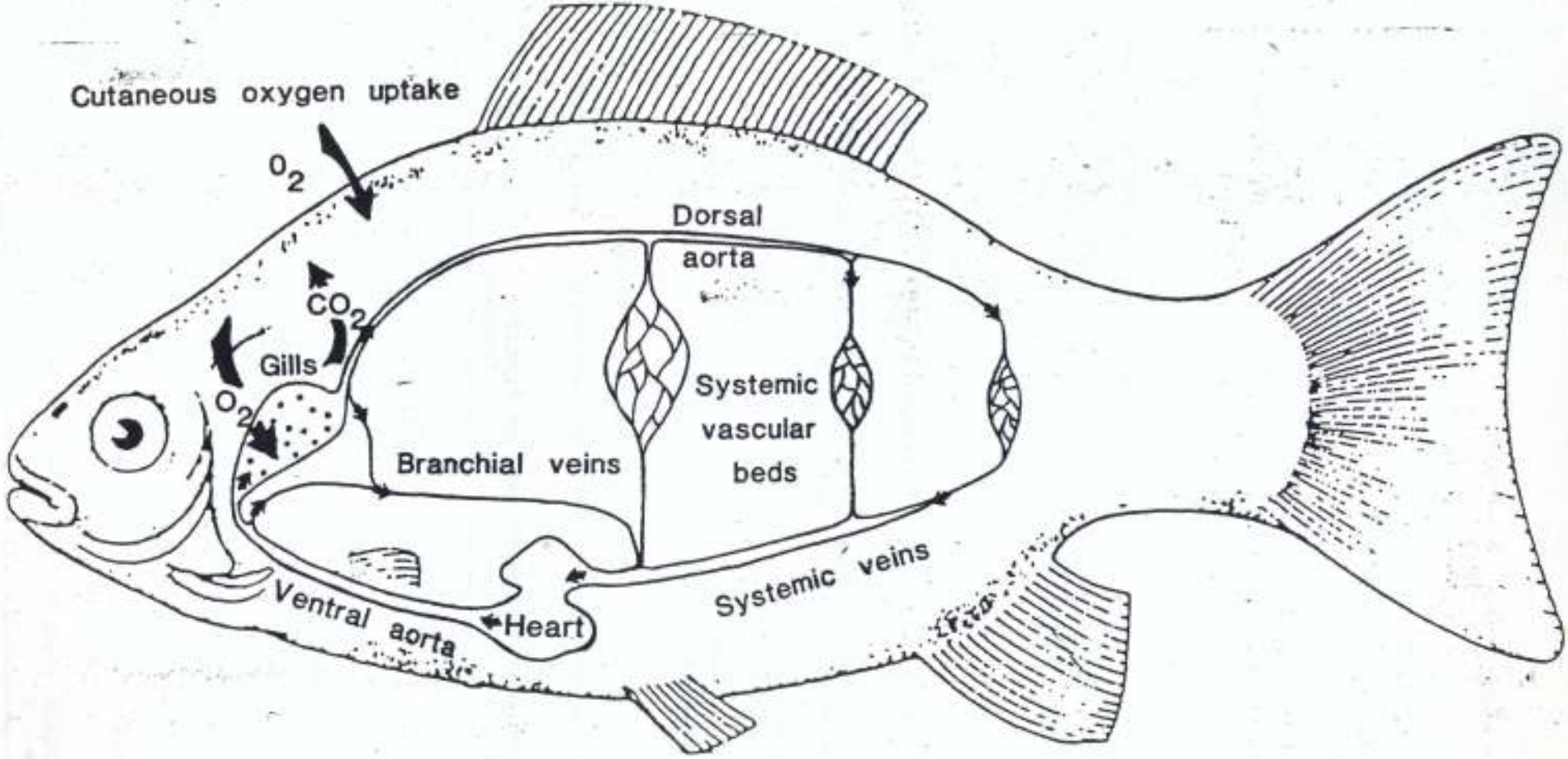
SAMPLE NO	Na^+ (mM)	CL^- (mM)	K^+ (mM)	Mg^+ (mM)	P^3 (mM)
1.	13.9	0.99	19.2	7.5	202.5
2.	12.2	1.05	21.7	7.0	601.5
3.	10.4	0.61	20.2	9.4	570.0
4.	10.7	1.34	18.3	10.5	525.0
5.	12.2	0.61	25.1	6.2	720.0
6.	13.0	0.93	24.1	8.8	624.0
7.	11.5	0.81	21.2	9.4	650.0
8.	13.2	1.63	20.1	8.4	400.0
9.	12.1	0.95	22.4	10.6	352.5
10.	11.0	1.26	23.5	7.6	250.5
11.	12.5	0.49	25.6	9.2	700.0
12.	12.4	0.71	22.1	8.5	500.0
13.	10.1	0.16	19.0	7.5	615.0
14.	13.6	0.72	19.6	7.4	655.0
	10.3	1.53	20.0	10.0	705.0
	11.5	0.55	29.9	8.3	561.5
	12.2	0.61	25.1	6.2	720.0
	12.5	0.93	21.6	7.3	430.0
	10.3	0.56	21.5	10.5	350.5
	10.5	0.88	18.8	7.6	550.0
21.	13.2	1.63	20.1	8.4	400.0
22.	12.0	0.78	22.4	6.1	630.0
23.	9.7	0.59	19.2	8.0	550.0
24.	11.7	0.07	21.4	7.2	752.5
25.	10.5	0.14	20.2	10.3	650.0

RESULT 5: SEROLOGICAL CHARACTERISTICS OF HETEROTIS NILOTICUS

SAMPLE NO	BLOOD GROUP					GENOTYPE			AGGUITINATION TEST	
	A	B	AB	O+	O-	AA	AS	SS	+VE	-VE
1.	-	-	-	O+	-	AA	-	-	-	-VE
	-	-	-	O+	-	AA	-	-	-	-VE
	-	-	-	-	Ove	-	AS	-	-	-VE
	-	-	-	O+	-	AA	-	-	-	-VE
	-	-	-	O+	-	AA	-	-	-	-VE
	-	-	-	O+	-	-	AS	-	-	-VE
7.	-	-	-	O+	-	-	AS	-	-	-VE
8.	-	-	-	O+	-	AA	-	-	-	-VE
9.	-	-	-	O+	-	-	AS	-	-	-VE
10.	-	-	-	O+	-	AA	-	-	-	-VE
11.	-	-	-	O+	-	-	AS	-	-	-VE
	-	-	-	O+	-	AA	-	-	-	-VE
	-	-	-	O+	-	-	AS	-	-	-VE
	-	-	-	O+	-	-	AS	-	-	-VE
	-	-	-	O+	-	-	AS	-	-	-VE
	-	-	-	O+	-	-	AS	-	-	-VE
	-	-	-	O+	-	AA	-	-	-	-VE
18.	-	-	-	O+	-	AA	-	-	-	-VE
19.	-	-	-	O+	-	-	AS	-	-	-VE
	-	-	-	O+	-	AA	-	-	-	-VE
	-	-	-	O+	-	AA	-	-	-	-VE
	-	-	-	O+	-	AA	-	-	-	-VE
	-	-	-	O+	-	-	AS	-	-	-VE
24.	-	-	-	-	Ove	AA	-	-	-	-VE
25.	-	-	-	O+	-	AA	-	-	-	-VE

APPENDIX 6

Figure The basic scheme of 'single circulation' in fish.



```
/COMPRESS=ON/ECHO=OFF/EJECT=OFF/LENGHT=80.
The raw data or transformation pass is proceeding
     25 cases are written to the compressed active file.
```

ANALYSIS 1:REGRESSION (PCV VS LENGTH)

Listwise Deletion of Missing Data

	Mean	Label
PCV	28.240	PCV
SL	34.216	SL

N of Cases = 25

Correlation:

	PCV	SL
PCV	1.000	.197
SL	.197	1.000

ANALYSIS 1:REGRESSION (PCV VS LENGTH)

Equation Number 1 Dependent Variable.. PCV PCV

Beginning Block Number 1. Method: Enter

Variable(s) Entered on Step Number
1.. SL SL

Multiple R	.19655		
R Square	.03863	R Square Change	.03863
Adjusted R Square	-.00317	F Change	.92425
Standard Error	7.34463	Signif F Change	.3464

Analysis of Variance

	DF	Sum of Squares	Mean Square
Regression	1	49.85734	49.85734
Residual	23	1240.70266	53.94359

F = .92425 Signif F = .3464

Condition number bounds: 1.000, 1.000

---------- Variables in the Equation ----------

Variable	B	SE B	95% Confdnce Intrvl B		Beta
SL	.61473	.63943	-.70803	1.93749	.19655
(Constant)	7.20628	21.92795	-38.15515	52.56771	

```
---------------------- Variables in the Equation -----------------------

Variable       SE Beta    Correl Part Cor  Partial  Tolerance        T  Sig T

SL              .20445    .19655   .19655   .19655    1.00000      .961  .3464
(Constant)                                                         .329  .7454

End Block Number   1   All requested variables entered.
------------------------------------------------------------------------------
Page   4                              SPSS/PC+                            7/28/9
ANALYSIS 1:REGRESSION (PCV VS LENGHT)

Equation Number 1     Dependent Variable..   PCV   PCV

                      Summary table
                      -------------

Step   MultR      Rsq     F(Eqn)  SigF       Variable  BetaIn
   1   .1966    .0386       .924  .346  In:  SL          .1966
------------------------------------------------------------------------------
Page   5                              SPSS/PC+                            7/28/9
ANALYSIS 1:REGRESSION (PCV VS LENGHT)

This procedure was completed at 13:26:19
------------------------------------------------------------------------------
Page   6                              SPSS/PC+                            7/28/9
ANALYSIS 2:REGRESSION (RBCC VS LENGHT)

Listwise Deletion of Missing Data

                 Mean  Label

RBCC       1711200.000  RBCC
SL              34.216  SL

N of Cases =     25

Correlation:

                RBCC         SL

RBCC           1.000        .006
SL              .006       1.000

------------------------------------------------------------------------------
Page   7                              SPSS/PC+                            7/28/9
ANALYSIS 2:REGRESSION (RBCC VS LENGHT)

          * * * *   M U L T I P L E   R E G R E S S I O N   * * * *

Equation Number 1     Dependent Variable..   RBCC   RBCC

Beginning Block Number  1.  Method:  Enter


Variable(s) Entered on Step Number
   1..     SL          SL
```

```
Multiple R            .00648
R Square              .00004          R Square Change     .00004
Adjusted R Square    -.04343          F Change            .00097
Standard Error 950596.84395           Signif F Change     .9755

Analysis of Variance
                    DF        Sum of Squares      Mean Square
Regression           1     873726104.64663  873726104.64663
Residual            23 20783590273895.3520 903634359734.581

F =        .00097       Signif F =  .9755

Condition number bounds:      1.000,      1.000

---------------------- Variables in the Equation -----------------------

Variable               B        SE B     95% Confdnce Intrvl B       Beta

SL           2573.41572 82759.65310 -168627.9727 173774.80411 6.4836E-03
(Constant) 1623148.0078 2838079.379 -4247866.574 7494162.5893


------------------------ Variables in the Equation ------------------------

Variable      SE Beta   Correl Part Cor  Partial  Tolerance         T  Sig T

SL             .20851   .00648   .00648   .00648    1.00000      .031  .9755
(Constant)                                                      .572  .5729

End Block Number   1   All requested variables entered.
------------------------------------------------------------------------------
Page   8                          SPSS/PC+                            7/28/97
ANALYSIS 2:REGRESSION (RBCC VS LENGHT)

Equation Number 1    Dependent Variable..   RBCC   RBCC

                     Summary table
                     -------------

Step   MultR      Rsq     F(Eqn)  SigF       Variable  BetaIn
   1   .0065    .0000       .001  .975  In:  SL          .0065
------------------------------------------------------------------------------
Page   9                          SPSS/PC+                            7/28/97
ANALYSIS 2:REGRESSION (RBCC VS LENGHT)

This procedure was completed at 13:26:21
------------------------------------------------------------------------------
Page  10                          SPSS/PC+                            7/28/97
ANALYSIS 3:REGRESSION (MCHC VS LENGHT)

Listwise Deletion of Missing Data

            Mean  Label

MCHC        .002  MCHC
SL        34.216  SL
```

N of Cases = 25

Correlation:

	MCHC	SL
MCHC	1.000	-.132
SL	-.132	1.000

ANALYSIS 3:REGRESSION (MCHC VS LENGHT)

Equation Number 1 Dependent Variable.. MCHC MCHC

Beginning Block Number 1. Method: Enter

Variable(s) Entered on Step Number
1.. SL SL

Multiple R	.13223		
R Square	.01749	R Square Change	.01749
Adjusted R Square	-.02523	F Change	.40933
Standard Error	1.660685E-04	Signif F Change	.5286

Analysis of Variance

	DF	Sum of Squares	Mean Square
Regression	1	.00000	.00000
Residual	23	.00000	.00000

F = .40933 Signif F = .5286

Condition number bounds: 1.000, 1.000

---------- Variables in the Equation ----------

Variable	B	SE B	95% Confdnce Intrvl B		Beta
SL	-9.25011E-06	1.44580E-05	-3.91589E-05	2.065864E-05	-.13223
(Constant)	1.892502E-03	4.95810E-04	8.668402E-04	2.918163E-03	

---------- Variables in the Equation ----------

Variable	SE Beta	Correl	Part Cor	Partial	Tolerance	T	Sig T
SL	.20668	-.13223	-.13223	-.13223	1.00000	-.640	.5286
(Constant)						3.817	.0009

End Block Number 1 All requested variables entered.

ANALYSIS 3:REGRESSION (MCHC VS LENGHT)

Equation Number 1 Dependent Variable.. MCHC MCHC

Summary table

Step	MultR	Rsq	F(Eqn)	SigF		Variable	BetaIn
1	.1322	.0175	.409	.529	In:	SL	-.1322

ANALYSIS 3:REGRESSION (MCHC VS LENGHT)

This procedure was completed at 13:26:22

ANALYSIS 4:REGRESSION (Na VS LENGHT)

Listwise Deletion of Missing Data

	Mean	Label
NA	11.728	Na+
SL	34.216	SL

N of Cases = 25

Correlation:

	NA	SL
NA	1.000	.297
SL	.297	1.000

ANALYSIS 4:REGRESSION (Na VS LENGHT)

Equation Number 1 Dependent Variable.. NA Na+

Beginning Block Number 1. Method: Enter

Variable(s) Entered on Step Number
1.. SL SL

Multiple R	.29721		
R Square	.08833	R Square Change	.08833
Adjusted R Square	.04870	F Change	2.22851
Standard Error	1.16107	Signif F Change	.1491

Analysis of Variance

	DF	Sum of Squares	Mean Square
Regression	1	3.00424	3.00424
Residual	23	31.00616	1.34809

F = 2.22851 Signif F = .1491

Condition number bounds: 1.000, 1.000

---------- Variables in the Equation ----------

Variable	B	SE B	95% Confdnce Intrvl B		Beta
SL	.15090	.10108	-.05821	.36001	.29721
(Constant)	6.56480	3.46648	-.60615	13.73575	

---------- Variables in the Equation ----------

Variable	SE Beta	Correl	Part Cor	Partial	Tolerance	T	Sig T
SL	.19909	.29721	.29721	.29721	1.00000	1.493	.1491
(Constant)						1.894	.0709

End Block Number 1 All requested variables entered.

ANALYSIS 4:REGRESSION (Na VS LENGHT)

Equation Number 1 Dependent Variable.. NA Na+

Summary table

Step	MultR	Rsq	F(Eqn)	SigF		Variable	BetaIn
1	.2972	.0883	2.229	.149	In:	SL	.2972

ANALYSIS 4:REGRESSION (Na VS LENGHT)

This procedure was completed at 13:26:23

ANALYSIS 5:REGRESSION (MCH VS WEIGHT)

Listwise Deletion of Missing Data

	Mean	Label
MCH	.000	MCH
WT	429.600	

N of Cases = 25

Correlation:

	MCH	WT
MCH	1.000	-.040
WT	-.040	1.000

ANALYSIS 5:REGRESSION (MCH VS WEIGHT)

Equation Number 1 Dependent Variable.. MCH MCH

```
Beginning Block Number  1.  Method:  Enter


Variable(s) Entered on Step Number
   1..     WT


Multiple R              .03972
R Square                .00158          R Square Change     .00158
Adjusted R Square      -.04183          F Change            .03634
Standard Error 3.936876E-06             Signif F Change     .8505

Analysis of Variance
                    DF        Sum of Squares        Mean Square
Regression           1                .00000               .00000
Residual            23                .00000               .00000

F =         .03634       Signif F =  .8505

Condition number bounds:       1.000,       1.000

---------------------- Variables in the Equation -----------------------

Variable             B         SE B     95% Confdnce Intrvl B        Beta

WT          -1.86375E-09 9.77684E-09 -2.20887E-08 1.836119E-08     -.03972
(Constant) 3.108067E-05 4.27330E-06 2.224068E-05 3.992066E-05


---------------------- Variables in the Equation -----------------------

Variable      SE Beta   Correl Part Cor  Partial  Tolerance         T  Sig T

WT             .20835  -.03972  -.03972  -.03972    1.00000      -.191  .8505
(Constant)                                                      7.273  .0000

End Block Number   1   All requested variables entered.
------------------------------------------------------------------------------
Page  20                            SPSS/PC+                          7/28/97
ANALYSIS 5:REGRESSION (MCH VS WEIGHT)

Equation Number 1    Dependent Variable..   MCH   MCH


                     Summary table
                     -------------

Step   MultR     Rsq    F(Eqn)  SigF       Variable  BetaIn
   1   .0397   .0016      .036  .850  In:  WT        -.0397
------------------------------------------------------------------------------
Page  21                            SPSS/PC+                          7/28/97
ANALYSIS 5:REGRESSION (MCH VS WEIGHT)

This procedure was completed at 13:26:25
------------------------------------------------------------------------------
Page  22                            SPSS/PC+                          7/28/97
ANALYSIS 6:REGRESSION (MCV VS WEIGHT)
```

Listwise Deletion of Missing Data

	Mean	Label
MCV	.059	MCV
WT	429.600	

N of Cases = 25

Correlation:

	MCV	WT
MCV	1.000	-.328
WT	-.328	1.000

ANALYSIS 6:REGRESSION (MCV VS WEIGHT)

Equation Number 1 Dependent Variable.. MCV MCV

Beginning Block Number 1. Method: Enter

Variable(s) Entered on Step Number
1.. WT

Multiple R	.32752		
R Square	.10727	R Square Change	.10727
Adjusted R Square	.06846	F Change	2.76369
Standard Error	.19306	Signif F Change	.1100

Analysis of Variance

	DF	Sum of Squares	Mean Square
Regression	1	.10300	.10300
Residual	23	.85722	.03727

F = 2.76369 Signif F = .1100

Condition number bounds: 1.000, 1.000

---------- Variables in the Equation ----------

Variable	B	SE B	95% Confdnce Intrvl B		Beta
WT	-7.97026E-04	4.79433E-04	-1.78881E-03	1.947568E-04	-.32752
(Constant)	.40144	.20955	-.03205	.83493	

---------- Variables in the Equation ----------

Variable	SE Beta	Correl	Part Cor	Partial	Tolerance	T	Sig T
WT	.19701	-.32752	-.32752	-.32752	1.00000	-1.662	.1100
(Constant)						1.916	.0679

End Block Number 1 All requested variables entered.

ANALYSIS 6:REGRESSION (MCV VS WEIGHT)

Equation Number 1 Dependent Variable.. MCV MCV

Summary table

Step	MultR	Rsq	F(Eqn)	SigF		Variable	BetaIn
1	.3275	.1073	2.764	.110	In:	WT	-.3275

ANALYSIS 6:REGRESSION (MCV VS WEIGHT)

This procedure was completed at 13:26:26

ANALYSIS 7: PEARSON CORRELATION

Variable	Cases	Mean	Std Dev
PCV	25	28.2400	7.3330
CF	25	1.0696	.0487
RBCC	25	1711200.0000	930601.5975
WBC	25	5724.0000	1246.5051
HB	25	44.7200	10.7375
ESR	25	40.1600	20.0014
MCHC	25	.0016	.0002
MCH	25	.0000	.0000
MCV	25	.0590	.2000
PROTEIN	25	5.9680	2.3636
GLUCOSE	25	61.4600	13.5015
ALBUMIN	25	4.7760	2.1255
NA	25	11.7280	1.1904
CL	25	.8212	.4245
K	25	21.6920	2.6831
MG	25	8.3160	1.3861
P	25	546.6200	150.9890

ANALYSIS 7: PEARSON CORRELATION

Variables		Cases	Cross-Prod Dev	Variance-Covar
PCV	CF	25	1.9924	.0830
PCV	RBCC	25	12682800.0000	528450.0000
PCV	WBC	25	140606.0000	5858.5833
PCV	HB	25	1808.6800	75.3617
PCV	ESR	25	1264.0400	52.6683
PCV	MCHC	25	-.0120	-.0005
PCV	MCH	25	-.0002	-.0000
PCV	MCV	25	-15.2952	-.6373
PCV	PROTEIN	25	-17.3080	-.7212
PCV	GLUCOSE	25	-197.7600	-8.2400
PCV	ALBUMIN	25	-93.2560	-3.8857
PCV	NA	25	-17.7680	-.7403
PCV	CL	25	10.6128	.4422

PCV	K	25	8.3480	.3478
PCV	MG	25	89.9040	3.7460
PCV	P	25	3780.7800	157.5325
CF	RBCC	25	224312.0000	9346.3333
CF	WBC	25	77.7400	3.2392
CF	HB	25	3.1372	.1307
CF	ESR	25	7.4616	.3109
CF	MCHC	25	-.0000	-.0000
CF	MCH	25	-.0000	-.0000
CF	MCV	25	.0003	.0000
CF	PROTEIN	25	-.8733	-.0364
CF	GLUCOSE	25	5.5496	.2312
CF	ALBUMIN	25	-.4372	-.0182
CF	NA	25	.0183	.0008
CF	CL	25	.1006	.0042
CF	K	25	-.7331	-.0305
CF	MG	25	.4292	.0179
CF	P	25	-23.2338	-.9681
RBCC	WBC	25	1327280000.0000	55303333.3333
RBCC	HB	25	-21471600.0000	-894650.0000
RBCC	ESR	25	66185200.0000	2757716.6667
RBCC	MCHC	25	-2317.2800	-96.5533
RBCC	MCH	25	16.8716	.7030
RBCC	MCV	25	-966321.2000	-40263.3833
RBCC	PROTEIN	25	-2813040.0000	-117210.0000
RBCC	GLUCOSE	25	-69213800.0000	-2883908.3333
RBCC	ALBUMIN	25	-8036280.0000	-334845.0000
RBCC	NA	25	2999160.0000	124965.0000
RBCC	CL	25	1279764.0000	53323.5000
RBCC	K	25	-2724760.0000	-113531.6667
RBCC	MG	25	291520.0000	12146.6667
RBCC	P	25	-543398600.0000	-22641608.3333
WBC	HB	25	179868.0000	7494.5000
WBC	ESR	25	206554.0000	8606.4167
WBC	MCHC	25	-1.8306	-.0763
WBC	MCH	25	-.0578	-.0024
WBC	MCV	25	-859.7740	-35.8239
WBC	PROTEIN	25	-20440.8000	-851.7000
WBC	GLUCOSE	25	19974.0000	832.2500
WBC	ALBUMIN	25	-5405.6000	-225.2333
WBC	NA	25	-13106.8000	-546.1167
WBC	CL	25	-1559.7200	-64.9883
WBC	K	25	-260.2000	-10.8417
WBC	MG	25	18930.4000	788.7667
WBC	P	25	-231297.0000	-9637.3750
HB	ESR	25	1443.1200	60.1300
HB	MCHC	25	-.0063	-.0003
HB	MCH	25	-.0002	-.0000
HB	MCV	25	-22.9337	-.9556
HB	PROTEIN	25	-6.9240	-.2885
HB	GLUCOSE	25	137.7200	5.7383
HB	ALBUMIN	25	-109.3680	-4.5570
HB	NA	25	-5.0040	-.2085
HB	CL	25	23.6784	.9866
HB	K	25	14.8440	.6185
HB	MG	25	124.8120	5.2005
HB	P	25	3237.8400	134.9100
ESR	MCHC	25	-.0319	-.0013
ESR	MCH	25	-.0003	-.0000
ESR	MCV	25	-.1452	-.0060

ESR	PROTEIN	25	-227.4720	-9.4780

ANALYSIS 7: PEARSON CORRELATION

Variables		Cases	Cross-Prod Dev	Variance-Covar
ESR	GLUCOSE	25	177.1600	7.3817
ESR	ALBUMIN	25	-337.0040	-14.0418
ESR	NA	25	-34.7120	-1.4463
ESR	CL	25	26.2252	1.0927
ESR	K	25	-74.9680	-3.1237
ESR	MG	25	90.9360	3.7890
ESR	P	25	-6410.9800	-267.1242
MCHC	MCH	25	-.0000	-.0000
MCHC	MCV	25	.0001	.0000
MCHC	PROTEIN	25	-.0002	-.0000
MCHC	GLUCOSE	25	.0172	.0007
MCHC	ALBUMIN	25	.0018	.0001
MCHC	NA	25	.0012	.0000
MCHC	CL	25	.0002	.0000
MCHC	K	25	.0004	.0000
MCHC	MG	25	-.0016	-.0001
MCHC	P	25	-.1059	-.0044
MCH	MCV	25	.0000	.0000
MCH	PROTEIN	25	.0001	.0000
MCH	GLUCOSE	25	.0002	.0000
MCH	ALBUMIN	25	.0000	.0000
MCH	NA	25	.0000	.0000
MCH	CL	25	-.0000	-.0000
MCH	K	25	.0001	.0000
MCH	MG	25	-.0000	-.0000
MCH	P	25	.0005	.0000
MCV	PROTEIN	25	-1.5203	-.0633
MCV	GLUCOSE	25	20.3980	.8499
MCV	ALBUMIN	25	2.7085	.1129
MCV	NA	25	-.0255	-.0011
MCV	CL	25	-.7531	-.0314
MCV	K	25	-.2569	-.0107
MCV	MG	25	-1.1309	-.0471
MCV	P	25	207.1744	8.6323
PROTEIN	GLUCOSE	25	-68.1820	-2.8409
PROTEIN	ALBUMIN	25	52.0408	2.1684
PROTEIN	NA	25	6.1824	.2576
PROTEIN	CL	25	.0370	.0015
PROTEIN	K	25	11.4036	.4751
PROTEIN	MG	25	-1.0272	-.0428
PROTEIN	P	25	362.0460	15.0852
GLUCOSE	ALBUMIN	25	78.7760	3.2823
GLUCOSE	NA	25	-79.5220	-3.3134
GLUCOSE	CL	25	38.7862	1.6161
GLUCOSE	K	25	-28.6580	-1.1941
GLUCOSE	MG	25	128.3160	5.3465
GLUCOSE	P	25	-11155.6300	-464.8179
ALBUMIN	NA	25	-16.4432	-.6851
ALBUMIN	CL	25	-6.2813	-.2617
ALBUMIN	K	25	-15.2648	-.6360
ALBUMIN	MG	25	-18.4104	-.7671
ALBUMIN	P	25	1157.0720	48.2113
NA	CL	25	3.5792	.1491

NA	K	25	15.8556	.6607
NA	MG	25	-13.4612	-.5609
NA	P	25	-762.0340	-31.7514
CL	K	25	-5.8468	-.2436
CL	MG	25	1.9605	.0817
CL	P	25	-703.9036	-29.3293
K	MG	25	-17.7468	-.7395
K	P	25	1906.2740	79.4281
MG	P	25	-937.8480	-39.0770

ANALYSIS 7: PEARSON CORRELATION

Correlations:	PCV	CF	RBCC	WBC	HB
PCV	1.0000	.2325	.0774	.6409**	.9571**
CF	.2325	1.0000	.2063	.0534	.2500
RBCC	.0774	.2063	1.0000	.0477	-.0895
WBC	.6409**	.0534	.0477	1.0000	.5599*
HB	.9571**	.2500	-.0895	.5599*	1.0000
ESR	.3591	.3192	.1482	.3452	.2800
MCHC	-.4142	-.1056	-.6326**	-.3731	-.1483
MCH	-.2249	-.0149	.1959	-.5006*	-.2174
MCV	-.4345	.0015	-.2163	-.1437	-.4449
PROTEIN	-.0416	-.3162	-.0533	-.2891	-.0114
GLUCOSE	-.0832	.3517	-.2295	.0495	.0396
ALBUMIN	-.2493	-.1760	-.1693	-.0850	-.1997
NA	-.0848	.0131	.1128	-.3680	-.0163
CL	.1420	.2028	.1350	-.1228	.2164
K	.0177	-.2338	-.0455	-.0032	.0215
MG	.3685	.2649	.0094	.4565	.3494
P	.1423	-.1317	-.1611	-.0512	.0832

N of cases: 25 1-tailed Signif: * - .01 ** - .001

" . " is printed if a coefficient cannot be computed

ANALYSIS 7: PEARSON CORRELATION

Correlations:	MCHC	MCH	MCV	PROTEIN	GLUCOSE
PCV	-.4142	-.2249	-.4345	-.0416	-.0832
CF	-.1056	-.0149	.0015	-.3162	.3517
RBCC	-.6326**	.1959	-.2163	-.0533	-.2295
WBC	-.3731	-.5006*	-.1437	-.2891	.0495
HB	-.1483	-.2174	-.4449	-.0114	.0396
ESR	-.4052	-.1621	-.0015	-.2005	.0273
MCHC	1.0000	.	.1488	-.0171	.3232
MCH	.	1.0000	.0532	.3822	.1530
MCV	.1488	.0532	1.0000	-.1340	.3147
PROTEIN	-.0171	.3822	-.1340	1.0000	-.0890
GLUCOSE	.3232	.1530	.3147	-.0890	1.0000
ALBUMIN	.2182	.0832	.2654	.4316	.1144
NA	.2511	.0254	-.0045	.0916	-.2062
CL	.1141	-.0738	-.3695	.0015	.2819
K	.0355	.2068	-.0199	.0749	-.0330
MG	-.3006	-.2082	-.1700	-.0131	.2857
P	-.1781	.0331	.2858	.0423	-.2280

N of cases: 25 1-tailed Signif: * - .01 ** - .001

" . " is printed if a coefficient cannot be computed

ANALYSIS 7: PEARSON CORRELATION

Correlations:	NA	CL	K	MG	P
PCV	-.0848	.1420	.0177	.3685	.1423
CF	.0131	.2028	-.2338	.2649	-.1317
RBCC	.1128	.1350	-.0455	.0094	-.1611
WBC	-.3680	-.1228	-.0032	.4565	-.0512
HB	-.0163	.2164	.0215	.3494	.0832
ESR	-.0607	.1287	-.0582	.1367	-.0885
MCHC	.2511	.1141	.0355	-.3006	-.1781
MCH	.0254	-.0738	.2068	-.2082	.0331
MCV	-.0045	-.3695	-.0199	-.1700	.2858
PROTEIN	.0916	.0015	.0749	-.0131	.0423
GLUCOSE	-.2062	.2819	-.0330	.2857	-.2280
ALBUMIN	-.2708	-.2900	-.1115	-.2604	.1502
NA	1.0000	.2951	.2068	-.3399	-.1767
CL	.2951	1.0000	-.2139	.1388	-.4575
K	.2068	-.2139	1.0000	-.1988	.1961
MG	-.3399	.1388	-.1988	1.0000	-.1867
P	-.1767	-.4575	.1961	-.1867	1.0000

N of cases: 25 1-tailed Signif: * - .01 ** - .001

" . " is printed if a coefficient cannot be computed
