THE EFFECT OF SOME PARASITES AND DIETS ON THE RATE OF GROWTH AND BLOOD ASCORBIC ACID CONTENT OF GUINEA FOWLS AND CHICKENS

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CERTIFICATION

I certify that this is the work of Mallam Sani Bagiwa, Idris and that it has not been presented elsewhere for the award of any other Degree.



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A C K N O W L E D G E M E N T S

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S.B. IDRIS

A B S T R A C T

The effects of malarial infection and cecal coccidiosis upon the total ascorbic acid of the whole blood of young chickens and guinea fowls were studied using colorimetric and microfluorimetic techniques. In both cases there appeared to be slight increases in the blood ascorbic acid level. The significance of the findings are discussed in relation to the results of other studies.

The influence of different diets (guinea corn, millet, maize and phizer livestock feed) upon the weight and blood asoorbic acid level of guinea fowls was also investigated. While there appeared to be no definite trend with the ascorbic acid level, the magnitudes of the increase in weight caused by the diets were found to be in the following order:-

Millet > Pfizer commercial feed > Maize > Guinea corn.

OBJECTIVE OF THE PROJECT

The aim of this work is to investigate the effects of some pathological stress and different diets on the growth, and vitamin C content of the blood of some avian species. These avaian species include: chickens and guinea fowls. The pathological stresses are malarial infection induced by the malarial parasites Plasmodium gallinaceum and cecal coccidiosis infection obtained from two coccidia parasites: Eimeria Tenella and Eimeria Acervulnia.

Some investigations on the ascrobic acid contents of the various tissues and organs of avian species have indicated quite a number of interesting things: for example, the vitamin was found to play an important role in the metabolic activities of some organs of avian species, yet these species can do without an external source of the vitamin. For example, the work of Lepkovsky et al revealed that young chickens fed on vitamin C free diets develop no scwivy yet they contained a lot of the vitamin in their liver. However, an investigation carried out by Bell et al[2] it was reported that birds fed with vitamin C free diet developed muscular weakness, loss of action of one leg or both legs, inappetence, loss of weight, definite faecal

changes and even a depression in reproductive system. But when ascorbic acid was injected in such birds, there was a dramatic recovery, including increased egg production. They concluded that under certain nutritional conditions and pathological stress birds may require an external source of the vitamin. Other works that revealed the need for the vitamin in promoting growth include the works of Briggset all[3] and March et al[4].

A fall in the level of the vitamin C of blood and other tissues have been reported in many animals infected with protozoa. Many attempts were made to correlate avian diseases with ascrobic acid level of the blood. An example of this was the work carried out by Moeley et ai[5]. They reported no consistent variation in the vitamin C content of the blood plasma either to the type of disease, nutrition, age or sex from that of similar birds not affected. Other attempts have rather unexpected results. Josephson et al[6] reported no change in the ascorbic acid level of the adrenal glands of chicks infected with Plasmodium gallinaceum but a marked change was observed by Challey[7] when he changed the parasite to E. tenella.

In the present work, the malarial infection was applied to young guinea fowls and the coccidiosis to young chickens. The effects of these on the blood ascrobic acid levels were examined.

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CHAPTER ONE

INTRODUCTION

Ascorbic Acid, also known as vitamic C, is the vitamin whose absence gives rise to scurvy. This disease, so common among sailors at one time, is characterised by a tendency to bleeding, with, among other things, pathological changes in teeth and gums. In guinea pigs, in which experimental scurvy can be induced by a diet lacking in the vitamin, the joints become enlarged and painful. It is for this reason that the vitamin is also called anti-scorbutic factor. Other names include :- cervitamic acid; hexorunic acid; redoxon; L-threo-3-keto hexonic acid-eno-lactone; L-threo-2,3,4,5,6-pentahydroxy-2-hexene-δ-lactone and L-3-ketothreo-hexuronic acid lactone (8)

1.1 Sources of Ascorbic Acid

Vitamin C is distributed rather widely in nature. Important dietary sources include many vegetables and fruits. Brocolli, Kaleparsley and turnip-greens have high content of the vitamin. However, it should be noted that considerable losses of the vitamin are incurred during the processes of

cooking. Fruits such as oranges, straw barries, pear, apples, pineapple, grapefruit and tangerines are also excellent sources of the vitamin and these are consumed largely without processing. However, for economic reasons, they are not important vitamin C source for the overall population.

Certain vegetables constitute an important source by virtue of the large quantity eaten rather than the high percentage content of the vitamin. They include potatoes, beans, peas, etc. (9).

1.2 Dietary Requirements of Ascorbic Acid:-

The optimum human requirement of vitamin C remains a controversial matter. The recommended intakes set by the Food and Nutritional Board of the National Research Council (U. S. A.) in 1963 are:- 70 milligrams per day per adult; 100 milligrams during pregnancy and lactation periods and 30 milligrams for infants, increasing to 80 milligrams in young adults (10).

1.3 Chemistry of Ascorbic Acid

The isolation and characterisation of ascorbic acid involved many workers over along period. It was found to have the molecular formula C6H8O6. The pure compound is a white

crystalline, odourless substance with a sour (acid) taste. It melts at 190°C - 192°C (infact it decomposes).

One gram of the vitamin dissolves in 3 millilitres of water; in 50 millilitres of absolute ethyl alcohol and in 100 millilitres of glycerol. It is insoluble in benzene, ethyl ether, petroleum ether and most organic solvents.

The vitamin has two assymetric carbon atoms and exhibit a specific rotation of +20.5° to 21.5° in water at 20°C and +48° in methanol at 23°C.

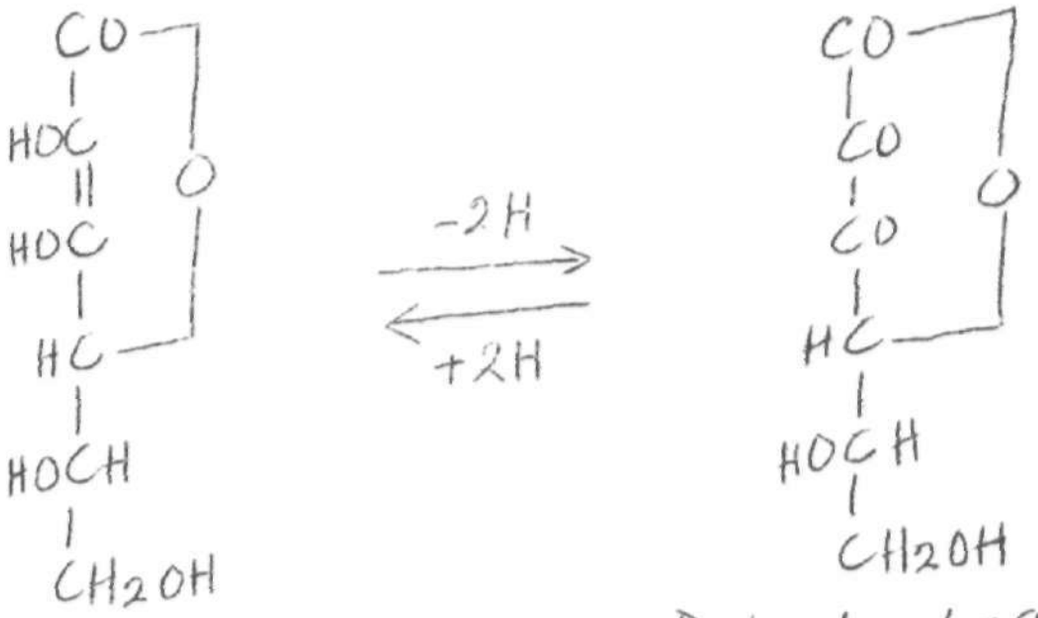
It is comparatively a strong acid as indicated by the acid dissociation constants pK1 and pK2 whose values are 4.17 and 11.5 respectively. The acidic properties are due mainly to the ionisation of the enol group on the third carbon atom.

At neutrality the vitamin has an absorption maximum at around 265mm and this shifts toward the shorter wavelengths with decreasing pH.

Ascorbic acid crystals are stable in air for years. In solution, the vitamin is easily oxidised, the instability being maximum at about pH of 4 and increasing with increasing pH in the alkaline range.

Mild oxidation by air, hydrogen peroxide, ferric chloride,

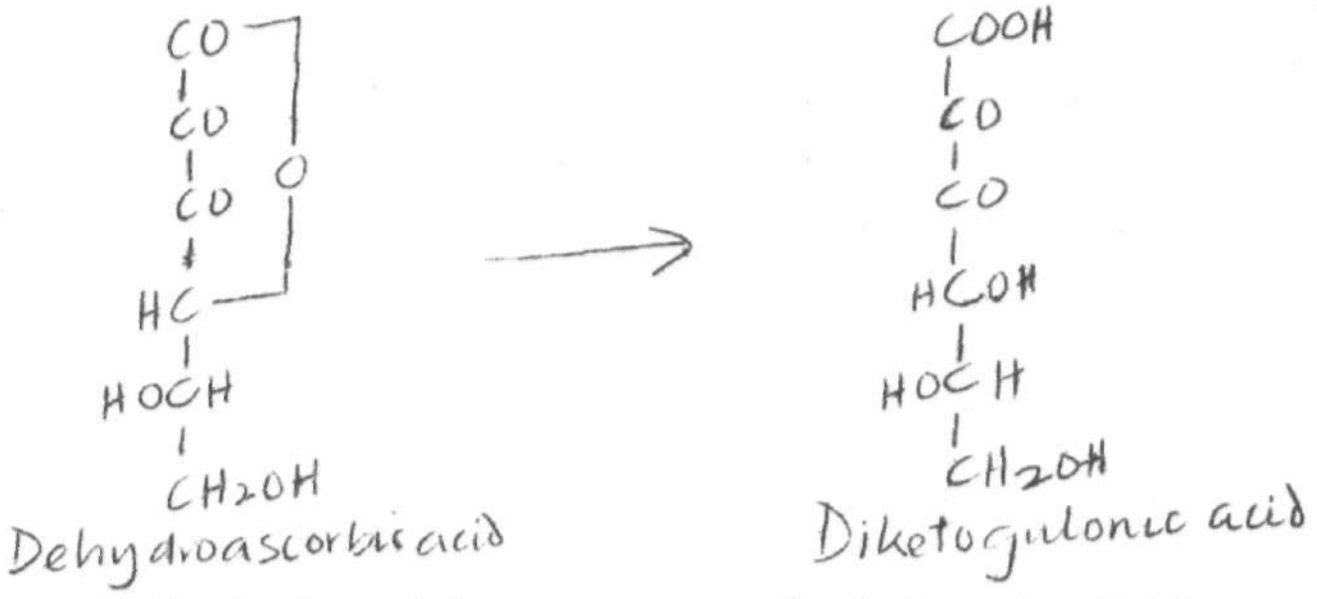
quinione, iodine or 2, 6-dichlorophenolindophenol converts the vitamin to dehydroascorbic acid as shown below:-



L-ascorbic acid

Dehydro-L-ascorbic acid

Dehydro-l-ascorbic acid retains the biological activity of the vitamin. Reduction in vitro, using hydrogen sulphide converts it back to the vitamin. Above pH of 5, dehydroascorbic acid rapidly undergoes rearrangement in which the lactone ring splits to give Diketogul-



Diketogulonic acid does not possess the biological activity of Dehydroascorbic acid. On treatment with hydrogen iodide, it converts back to the dehydroascorbic acid which may subsequently be reduced to ascorbic acid using hydrogen sulphide. Once the lactone ring is opened, the molecule rapidly undergoes further oxidation and may be degraded to oxalic acid.

1.4 Synthesis of Ascorbic Acid:-

Plants and Animals except pig, man and other primates have the ability to synthesize ascorbic acid. The rat for example is resistant to scurvy.

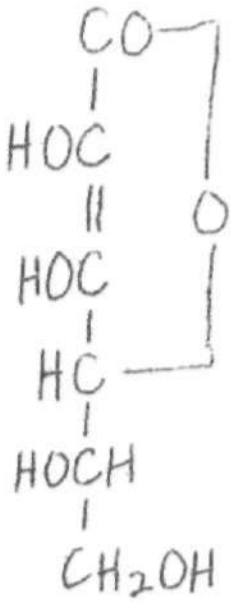
In animals the liver and adrenal (cortex) are probably the main sites for the synthesis of the vitamin. In plants all tissues contain the vitamin except for woody tissues.

Early experiments showed that the administration of D-glucose caused the formation of L-ascorbic acid. In the rat, the administration of hypnotic drugs stimulates the excretion of ascorbic acid as well as of D-glucoronides suggesting that these two compounds may have a common intermediates. By using C-14 labelled sugars, King demonstrated that uniformly labelled glucose is converted to uniformly labelled ascorbic acid (11). This shows that the carbon chain is not broken during the conversion to the vitamin. On the basis of many studies in recent years the following scheme has been presented for the biosynthesis of ascorbic acid. (SEE PAGE 11)

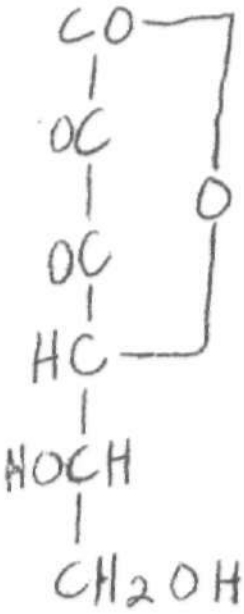
Ascorbic acid can also arise from D-galactose via D-galacturonic acid; L-galactonic acid; L-galactono- -lactone and 2-keto-L-galactono-lactone. What probably prevents the synthesis of ascorbic acid in man is the absence of the enzyme L-gulono-oxidase in the microsomes (12).

1.5. Metabolism of Ascorbic Acid:-

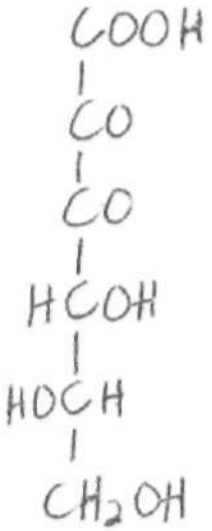
The sequence of reactions below is believed to represent the catabolic breakdown of ascorbic acid in rat kidney (13).



L-ascorbic acid.

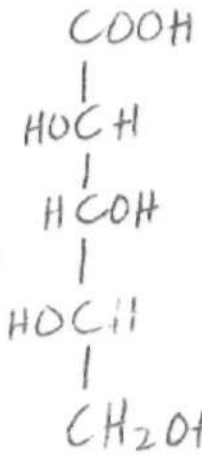


Dehydro-L-ascorbic acid.

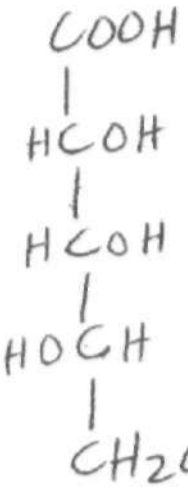


2,3-Diketo-L-gulonic acid

$-CO_2$



L-Xylonic acid



L-Lyxonic acid

Plants contain an enzyme ascorbic acid oxidase which oxidises the vitamin to dehydroascorbic acid. The pure enzyme is blue and contains 0.26% copper. Its molecular weight is 150,000.

1.6. Biochemical functions of Ascorbic Acid:-

Ascorbic acid plays a very important role in the body. It is concerned fundamentally with the formation of intercellular substances, including the collagen of fibrons tissue structures, the matrixes of bones, cartilages and dent-in and all non-epithelial cement substances including that of the vascular endothelium. In the absence of the ascorbic acid, the condition known as scurvy developes (14).

Other uses include its participation in the utilisation of other vitamins. For example: (i) It has the capacity to increase the urinary excretion of citrovorum factor in rats and human subjects; (ii) It influences the storage of folic acid in the liver of chicks; (iii) It relieves the deficiency symptoms of rats put on a ration lacking thiamin, riboflarin or pentothernic acid.

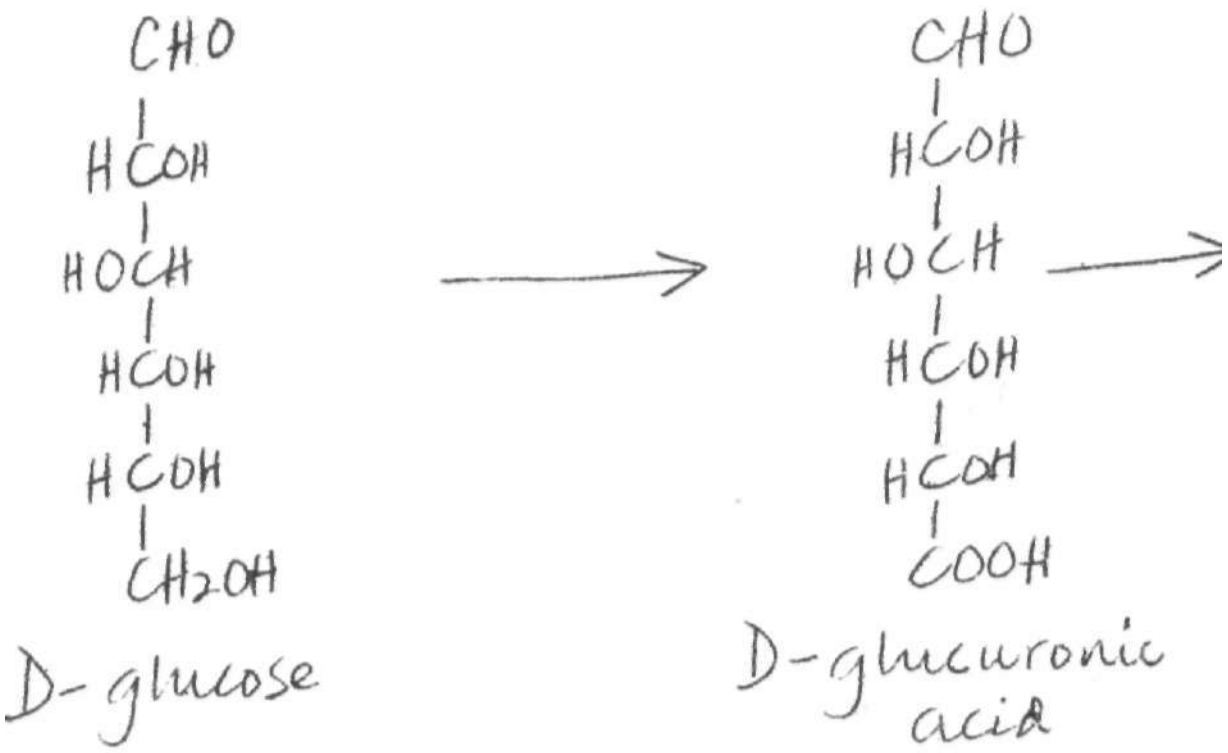
The ease with which the vitamin undergoes redox reaction suggests that it plays a role in cellular respiration.

Ascorbic acid enhances the absorption of iron in human and other animals. This is probably due to the reducing property of the vitamin, since iron in the ferrons state is preferentially absorbed.

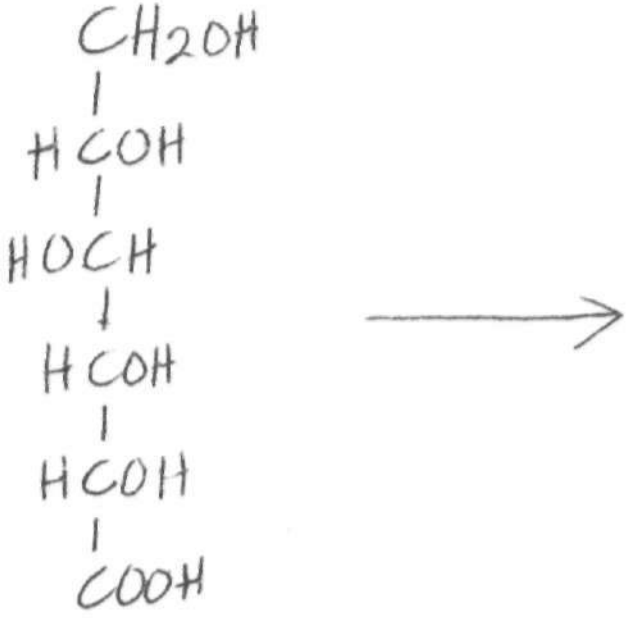
It plays a very important role in the biosynthesis of adrenal steroids. In elderly persons, the vitamin causes a rise in the blood level of 17-hydroxycorticosteroids and increases the urinary out-put of 17-ketosteroids.

SYNTHESIS OF ASCORBIC ACID

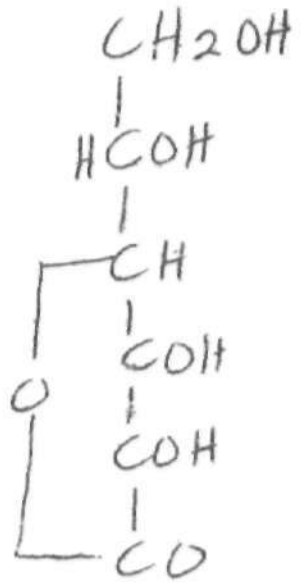
(A SEQUENCE IN RAT)



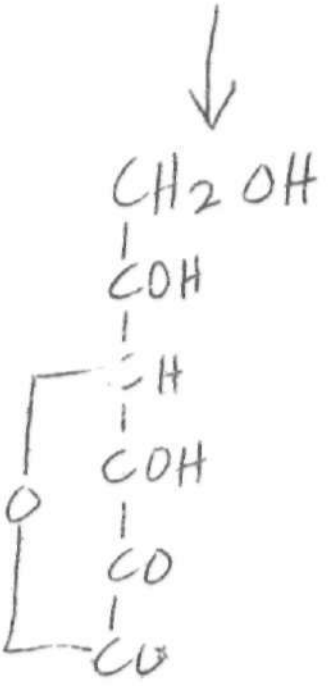
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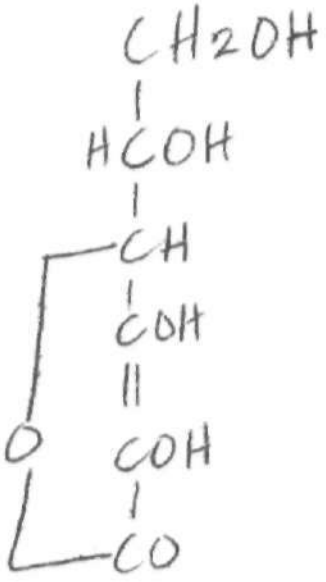
L-Gulonic acid



L-Gulono-γ-lactone



2-Keto-L-gulono-lactone



L-ascorbic acid

C H A P T E R T W O

SOME CHEMICAL METHODS FOR THE ANALYSIS OF ASCORBIC ACID.

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CHAPTER TWO

CHEMICAL METHODS FOR THE ANALYSIS OF ASCORBIC ACID.

A great number of chemical methods have been established for the analysis of ascorbic acid and its derivatives. They are mainly based on the reducing property of the vitamin. These procedures include treatment of the acid extract of the vitamin with oxidising agents such as iodine, methylene blue, 2, 6-dichlorophenolindophenol and many other reagents.

Various acids have been used for the extraction of the vitamin. They include acetic acid, trichloroacetic acid, metaphosphoric acid and oxalic acid. The latter two acids serve not only as extracting agents but as species that reduce the pH of the medium thereby stabilising the vitamin. They also form complexes with metallic ions like cupric ions, thereby preventing the catalytic oxidation of the vitamin.

Most of the methods particularly the titrimetric ones are not very specific. The limitations arise due to the presence of reducing materials such as stannons, ferrons, sulphides and thiosulphate ions. Errors arise particularly in food products because of the presence of reductones and reductinic acid (reductone is a hydroxy pyruvic aldehyde, a compound formed by

the alkaline hydrolysis of sugars; reductinic acid is formed when pentoses are treated with acid. The terms reductone and reductinic acid, however, have been used rather losely to signify other reducing compounds during heat processing and storage of foods).

Inspite of all the problems, numerous attempts have been made to increase specificity as shown in the following methods.

2.1 2, 6-Dichlorondophenol Method [illegible]:

2, 6-dichlorophenolindophenol is one of the most commonly used oxidising agents in the analysis of the ascorbic acid contents of many substances. Ascorbic acid reduces the indicator dye to a colourless solution. At end-point, in a titration involving the two reagents, the dye is rose pink. However, such an analysis cannot be successfully carried out in the presence of ferrons, stannous, cuprous, sulphide and thiosulphate ions. Products containing such species give values in excess of their actual ascorbic acid contents. Several tests have been designed to determine whether these reducing ions are present in such amounts as to invalidate a test. To detect cuprous and ferrous ions, two drops of 0.05% aqueous solution of methylene blue are added to 100 cm of freshly prepared mixture (1 to 1) of the sample

to be analysed and phosphoric acid-acetic acid mixture. Rapid disappearance of the methylene blue colour in 5 to 10 seconds indicates the presence of the interfering substances.

Stannous ions are tested by adding five drops of 0.05% aqueous solution of indigocarmine to a solution containing $10cm^3$ of the sample to be analysed and $10cm^3$ of (1 to 3) hydrochloric acid solution. Disappearance of colour in five to ten seconds indicate the presence of stannous ions.

Other problems encountered with the titrimetric method involving 2, 6-dichlorophenolindophenol include difficulties in judging the endpoint and interference due to turbidity or colour in the unknown extract. These difficulties are largely overcome by using the photometric methods. Here the change in the photometric density of the dye is determined after treatment with the extract containing the ascorbic acid. This change is a direct measure of the amount of ascorbic acid in the extract (15, 16).

2.2 Iodometric Methods (II).

The titrimetric method involving iodine in the estimation of ascorbic acid was first described before the Food and Nutrition Section of the American Public Health Association in 1934 (17).

Since that time, the method has had extensive and satisfactory use.

Initially it had some disadvantages such as the lack of specificity for the vitamin and an indefinite end-point. Now, however, the interference of non-vitamin reducing substances may be reduced and the end-point improved by proper adjustment of the acidity of the titration mixture through addition of strong mineral acid. The end-point may be improved further by the use of double titration. This involves the addition of excess iodine then excess thiosulphate and titrating the excess thiosulphate. If the titration is carried out directly with iodine, the reaction proceeds too slowly near the endpoint giving irreproducible results. By adding an excess of the iodine all substance capable of being oxidised by the reagent under the existing conditions are oxidised quickly and completely. The added thiosulphate then reacts quickly and quantitatively with the excess iodine. Excess thiosulphate is used and then bact titrated with more iodine because in titrating solutions containing large amounts of coloured substances, the endpoint is best detected by the appearance of a colour (blue in this case) than by its disappearance. The addition of few extra drops of iodine solution after the end-point has been exceeded, is more noticeable than in the case of the detection of end-point by the disappearance of colour. Care must always be exercised in applying the method to the estimation of the ascorbic acid

2.4 Diazotised 4-methoxy-2-aniline Method:-

This is another highly specific method for the determination of ascorbic acid. It involves the treatment of an acid extract of the vitamin with a diazotised 4-methoxy-2-nitroaniline followed by the development of a blue coloured product with an absorption maximum at 570nm.

The method was a great success when applied by Wellisched et al (20). They found out that reducing substances such as Cu(II), Fe(II) and SO_2 do not interfere when present in quantities normally enoountered. Vitamins normally present in multivitamin preparations do not interfere even when present in a concentration twice that of the ascorbic acid. Oxidation and decomposition products of the vitamin like dehydroascorbic acid and 2, 3-diketogulonic acid do not interfere when present in twice the quantity of the ascorbic acid. Pantoyl lactone (the possible clearage product from pentothenates) as well as phenols were found to be non interfering when tested in a ratio of 2 parts to one part ascorbic acid. Solutions containing polyhydroxy alcohols and tweens could be successfully assayed. Sugar-containing food in which reductones and reductinic acid may be encountered can be successfully assayed by applying the formaldehyde modification of the method (21).

The method is very rapid, simple and suitable for routine control. It can be carried out directly, requiring extractions only in unusual cases. It is very specific (due to the enediol group of the vitamin) and highly sensitive. It shows good precision and an accuracy which compares favourably with conventional procedures.

2.5 The Gas Chromatography Method:-

Quite often the need may arise for the determination of ascorbic acid and other vitamins simultaneously. Senello and Argoudelis (22) have proposed a gas chromatographic procedure for the simultaneous determination of pyridoxine, ascorbic acid and nicotinamide in Pharmaceutical products.

In the proposed method, BSA [N,O-bis(trimethylsilyl)acetamide] is reacted directly with the above compounds and the reaction mixture chromatographed using phenanthrene as an internal standard.

It was found that varying the concentrations of the vitamins does not affect the result. BSA, unlike hexamethyldisilazane, silanate both vitamins completely and it does not produce any precipitate of ammonium chloride with pyridoxine hydrochloride.

The method is considerably fast and comparatively accurate.

2.6 The Ferrosine 3-(2-pyridyl)-5, 6-bis(4-phenylsulphonic acid)1,2,4-triazine disodium salt Method.

Jaselski and Nelapaty (23) used this method for the determination of ascorbic acid in citrus fruits. It involves the reduction of iron (III) with ascorbic acid followed by the reaction of the iron (II) formed with ferrozine to form iron (II)- ferrozine chelate. The procedure was found to be dependent on many variables, e.g.: (a) The molar ratio of iron (II) to ferrozine, (b) The pH of solution (c) The time necessary to reach maximum intensity and (d) The presence of various interferences. Since iron (III) does not form any stable chelate with ferrozine, the amount of the chelating agent to be added must be at least in 3 to 1 ratio with respect to iron (II) produced in the reaction. Should in case copper (II), cobalt (II) and/or nickel (II) are present, sufficient amount of ferrozine must be present to complex these ions. In actual determinations, it has been suggested that ferrozine in 10 to 1 ratio with respect to iron (II) formed should be used.

The effect of pH is nullified by working in acetic acid-acetate buffer solutions in the pH below 6, the formation of iron (II)-ferrozine chelate is hindered by the hydrogen ion competition, while at pH greater than 7, iron (III) as hydroxo complex is not reduced.

Pure ascorbic acid in an amount as low as 5ng per 25ml and as high as 10ng per 25ml were reported easily determined with relative precision of ± 2% and ± 1% respectively

sugars such as sucrose, mannose, glucose and fructose do not interfere.

The interference of tartaric and citric acids through chelate formation with iron (III) thereby slowing down the reaction, is overcome by the addition of aluminium ion. Oxalic acid in an amount as high as ten times that of iron (III) has no effect. Phosphate in an amount approximately one hundred times that of iron (III) does not interfere. The determination of ascorbic acid in the presence of iron (II) can also be achieved by passing the solution through a cation exchange resin and analysing the elvent for ascorbic acid.

2.7 The 2, 4-dinitrophenyl Hydrazine Method:-

Ascorbic acid and/or its derivatives such as dehydro-ascorbic acid and diketogulonic acid can be osturated using 2, 4-dinctrophenylhydrazine. The method was first developed for the determination of ascorbic acid in blood and urine by Roe and Kuether ( ). It was later adapted by Roe and Osterling (24) for the determination of dehydroascorbic acid in the presence of ascorbic acid in plant materials.

In the estimation of ascorbic acid, the method involves treating the acid extract with norit or bromine to form the dehydroascorbic acid or 2, 3-diketogulonic acid which is later coupled with 2, 4-dinitrophenylhydrazine to form a hydrazone. This hydrazone is insoluble in water and deep red in colour. When it is treated with 85% solution of sulphuric acid, it forms a red coloured solution whose absorption maxima are about 500 - 550nm and 350 - 380nm. In estimating dehydroascorbic acid or diketogulonic acid, the extract need not be treated with norit or bromine.

2.8 The Microfluorimetric Method:-

Trace amounts of ascorbic acid can be estimated by a microfluorimetric method (25) outlined below. The ascorbic acid is oxidised to dehydroascorbic acid in the presence of norit. The dehydroascorbic acid formed is then reacted with O-phenylenediamine to produce a fluorophor having activation maximum at around 350nm and fluorescence maximum at about 430nm. The influence of any extraneous material may be prevented by complexing the derivative of the vitamin with boric acid.

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CHAPTER THREE

ANALYSIS OF THE ASCORBIC ACID CONTENT OF THE BLOOD OF GUINEA FOWLS

3.1 MATERIAL AND METHOD

The effects of different diets and malarial infection upon the ascorbic acid contents of the blood of Guinea Fowls were investigated using a developed Microfluorimetric method (25).

The different diets used include:- Guinea Corn (Dawa); Millet (Gero); Maize (Masara) bought locally from Samaru market and a commercial livestock growers mash from Pfizer manufacturing unit, Kaduna.

The malarial infection was obtained by the intravenous injection of the blood of guinea fowls infected with Plasmodium Gallinaceum, bought locally from Samaru market.

A total of twenty four birds were used. They were grouped into four; each group contained three infected and three uninfected birds, both fed on the same diet.

The microfluorimetric technique applied has been outlined in chapter two. The ascorbic acid in the acid extract was oxidised with norit and the oxidation product reacted with O-phenylenediamine to form the fluorophor whose fluorsence intensity was

determined. Extra care was taken to eliminate the effect of extraneous material by complexing the acid derivative with Boric acid.

The relationship between concentration C and fluorescence intensity F may be derived from the Lambert - Beer light absorption law.

$$I/Io = 10^{-acd} = \text{Fraction of light transmitted}$$

Where

I = Intensity of light transmitted

Io = Intensity of incident light

a = Absorbancy index

b = Concentration

and c = Optical depth

It follows that the fraction of light absorbed is :-

$$1 - I/Io = 1 - 10^{-abc}$$

Therefore the amount of light absorbed is :-

$$Io - I = Io\ (1 - 10^{-abc})$$

But fluorescence intensity F is proportional to the quanta of light absorbed. Hence, where is fluorescence efficient (the ratio of quanta absorbed to quanta emitted)

$$F = k I_o (1 - 10^{-abc})$$

To expand 10^{-abc}, let X be (abc) and since

$$e^x = 1 + X + \frac{x^2}{2} + \frac{x^3}{6} + \frac{x^4}{24} + \cdots \frac{x^n}{n!}$$

and $10 = e^{10}$

$$\therefore 10^{-abc} = 1 + (-2.3abc) + \frac{(-2.3abc)^2}{2} + \frac{(-2.3abc)^3}{6} + \cdots \frac{(-2.3abc)^n}{n!}$$

and

$$\left[1 - 10^{-abc}\right] = \left[1 - (1 + \text{above})\right]$$

For analytical determinations with one reagent in dilute solution where (abc) is less than 0.05 and above 2% of the total extinction energy is absorbed, the latter terms of the expression may be dropped and hence

$$F = \phi k I_o \cdot 2.3abc$$

or

$$F = Kc$$

where K includes the proportionality constant k and the fluorescence efficiency ϕ.

R E A G E N T S

(a) Metaphosphoric Acid - Acetic Acid Solution:

This was made by dissolving 30 grams of metaphosphoric acid sticks in 80cm^3 of acetic acid and 400cm^3 of distilled water. The solution was made up to 1 litre with distilled water and filtered rapidly through fluted filter paper into a glass bottle. It was stored in a refrigerator and a fresh one prepared weekly.

(b) Metaphosphoric Acid - Acetic Acid - Sulphuric Acid Solution:

This was prepared by dissolving 15 grams of metaphosphoric acid sticks in 40cm^3 of acetic acid and 200cm^3 of 0.3N sulphuric acid solution. The solution was diluted to 500cm^3, filtered rapidly through fluted filter paper and kept in a refrigerator.

(c) O - Phenylenediamine Solution:

This was always prepared fresh before use, by dissolving 20mg of O- phenylenediame .2H$_2$O in enough distilled water to make a 100cm^3 solution.

(d) 0.04% Thymol Blue pH Indicator:

This was made by dissolving 0.1gm of the indicator by triturating in agate mortar with 10.75ml 0.02N sodium hydroxide and diluted to 250cm^3 with distilled water.

(j) 10% ($^{W}/_{v}$) Potassium Oxalate Solution:

This was made by dissolving 10 grams of potassium oxalate in enough distilled water to form a 100cm^3 solution.

(k) Stock Standard Ascorbic Acid Solution (1mg/ml):

Accurately weighed 100mg of Analar grade ascorbic acid that has been stored in a dessicator away from direct sunlight were transfered into 100cm^3 volumetric flask and diluted to volume immediately before use with phosphoric acid - acetic acid solution.

(l) Working Standard Ascorbic Acid Solution (100 ng/ml):

25cm^3 of the stock standard ascorbic acid solution were diluted to 250cm^3 using phosphoric acid - acetic acid solution.

A P P A R A T U S

1. Pipettes :- Assorted, ranging from 1 ml to 25 ml.
2. Burettes
3. Glass beakers and Erlenmeyer flasks (assorted)
4. Test-tubes and test-tube racks (assorted)
5. Glass funnels; Buchner funnels and flasks
6. Filter paper (whatman No. 42)
7. Mettler Balance and Top loading balance
8. Water bath ($100^{\circ}C$)
9. Fluorimeter and the fluorescence reading tubes. (The Locarte model).

3.2 CALIBRATION CURVE

Quantitative analysis by fluorimetry are normally based on a calibration curve, plotted using standard solutions of the pure substance in a suitable medium. In preparing the calibration curve for this analysis, 250cm^3 of the working standard ascorbic acid solution (100 microgram ascorbic acid per millilitre of solution) were transfered into an erlenmeyer flask and shaken with about 5 grams of acid - washed Norit. The mixture was filtered through No 42 Whatman filter paper discarding the first few millilitres of the filtrate.

A series of solutions were prepared by pipetting the following volumes (cm^3) of the filtrate:- 2.5, 5.0; 7.5; 10.0; 12.5; 15.0; 17.5; 20.0; 22.5; 25.0 into 100cm^3 volumetric flasks each containing 5cm^3 of Boric acid - sodium acetate solution. The flasks were allowed to stand for fifteen minutes with occasional swirling. They were designated as standard blank solutions.

Another set of solutions was prepared by pipetting the same volumes of the filtrate as above, into 100cm^3 volumetric flasks each containing 5cm^3 of sodium acetate solution and 50cm^3 of distilled water. This set was diluted to volume with distilled water, immediately. Two millilitres of each solution were pipetted into clean test tubes and designated as standard [illegible] tubes.

Five millilitres of O - phenylenediamine solution were added to all the tubes, mixed and allowed to stand for thirty five minutes at room temperature and away from bright light. The fluorescence of each solution was determined using the locarte model fluorimeter.

3.3 D E T E R M I N A T I O N S:

At the initial stage of the analysis, just before the birds were infected and put on the various diets, their weights and whole blood ascorbic acid contents were determined three times in one week. They were then infected and placed on the various diets. All determinations followed immediately for about three weeks.

The birds were bled through prominent jugular reins on their necks using sterilized needles and syringes, constantly flushed with a 10% ($^{W}/v$) solution of potassium oxalate. The flushing prevents clotting of blood. $2cm^3$ of blood were taken from each bird per every analysis.

Each quantity of blood withdrawn from a bird was quickly emptied into a sample bottle containing $10cm^3$ of 6% ($^{W}/v$) trichloroacetic acid solution (an extracting agent and a protein precipitant). The bottle was shaken and kept for about five minutes so that the extraction may come to completion. The content of the bottle was then centrifuged and the supernatant shaken with

BLOOD OF GUINEA FOWLS. A CALIBRATION CURVE USING THE LOCARTE MODEL FLUORIMETER.

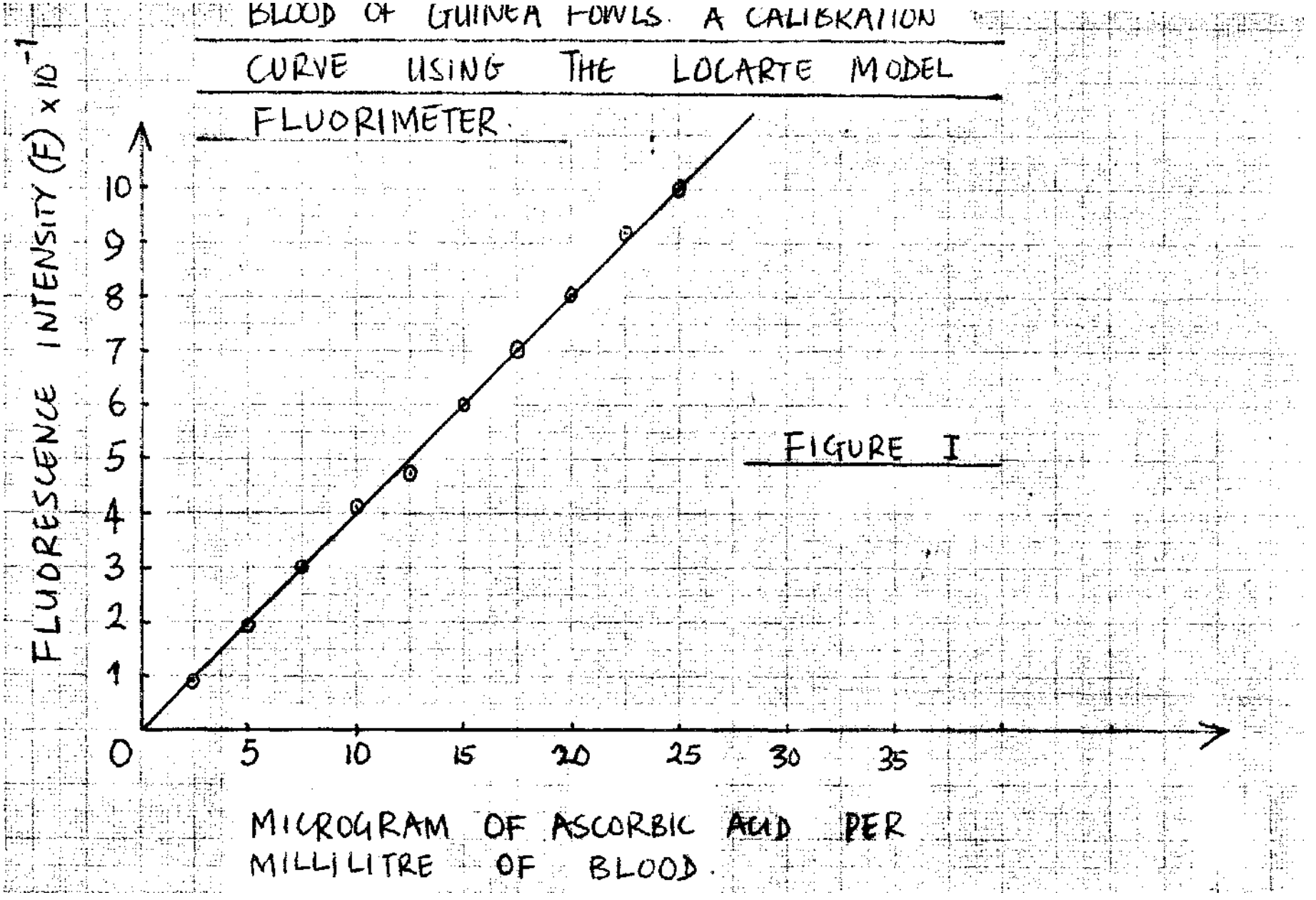


FIGURE I

approximately 0.5 gram of acid-washed Norit which was also previously shaken with 6% ($^{W}/v$) trichloroacetic acid solution. The mixture was filtered through No 42 Whatman filter paper.

$5cm^3$ of the filtrate were transfered into $100cm^3$ volumetric flask containing $5cm^3$ of Boric acid-sodium acetate solution and allowed to stand with occasional swirling for fifteen minutes. The flask was designated as sample blank.

Another $5cm^3$ of the filtrate were transfered into $100cm^3$ volumetric flask containing $5cm^3$ of sodium acetate solution and about $75cm^3$ of distilled water. The content of the flask was diluted to volume with distilled water. Two millilitres were withdrawn and placed in a clean test tube designated as sample tube.

At an appropriate time, the sample blank solution was diluted to volume with distilled water and two millilitres transfered into a clean test tube designated as sample blank tube.

Five millilitres of O- phenylenediamine solution were added to each tube, mixed and kept away from bright light for thirty five minutes. The fluorescence intensity of each solution was determined using the same locarte model fluorimeter.

3.4 RESULTS

Tables I to IV indicate the total ascorbic acid contents of the whole blood of guinea fowls fed with guinea corn (dawa), maize (masara), millet (gero) and Phfizer livestock feed (commercial feed). Looking at these results very closely, it is difficult to say in what way the infection and various diets have influenced the blood ascorbic acid levels. However, taking the mean values of the blood vitamic C level of all the birds in a group and per analysis (Tables V to IX) and their average values (Tables X and XI), there appeared to be some slight variations.

Tables XII to XV show changes in weight for the same guinea fowls feed with the four different diets (guinea corn, maize, millet and phizer feed), both infected and non-infected. A close look at these results indicate that all the birds (infected and nonOinfected) gained weight throughout the period of investigation. This gain in weight becomes more clear when one examines the mean values of the total weights of all the birds in a group and per measurement (Tables XVI to XXI) and their average values (Table XXII). The percentage gain in weight for each group is also shown on Table XXIII.

3.5 DISCUSSION

(a) The effect of plasmodium gallinaceum (a malarial parasite) on the whole blood ascorbic acid level of guinea fowls:-

Birds fed with the same diet (phizer feed) and infected with the malarial parasite: plasmodium gallinaceum shoed a slight increase in ascorbic acid level (Table X). This elevation of blood ascrobic acid level during the period of stress is unexpected in the light of previous observations of depletion as characteristic response to stress in mammals and the general lack of response which has been found in birds. Josephson (6) , found no change in the adrenal ascorbic acid content of chickens infected with plasmodium gallinaceum although adrenal enlargement took place. Full interpretation of the observed slight change in this work must await the elucidation of the role of various organs and tissues in the body, in relation to the blood ascrobic acid level.

(b) The effects of various diets on the whole blood ascrobic acid level of guinea fowls

Table XI indicates that birds fed with guinea corn, maize, and the Pfizer livestock feed showed an increase in the whole blood ascrobic acid level. However, those put on millet as diet suffered a slight blood ascrobic acid depletion. It was also observed that the increase associated

with Pfizer feed exceeds that associated with guinea corn, a diet found to be richer in ascrobic acid compared to the commercial diet. The full interpretation of these results must also await a detailed investigation of the total ascorbic acid contents of the different diets and their utility in the body.

(c) The combined effect of plasmodium gallinaceum and various diets on the whole blood ascrobic acid level of guinea fowls:-

Changing the various diets (from the commercial feed to guinea corn, maize and millet) of guinea fowls and infecting them with a malarial parasite showed a slight increase in the blood ascrobic acid level. This is in line with the observed results in (a) and (b) above except in the case of millet. This would also require a detailed knowledge of the effects of the two factors (diet and parasite) independently before arriving at any reasonable conclusion.

(d) The effects of various diets on the weights of guinea fowls infected and non-infected with plasmodium parasite:-

Both infected and non-infected birds showed some increase in weight when fed with different diets. This is expected since they are growing birds. However, the

observed increase in weight which accompanied the infection is rather surprising. This coupled to the fact that the birds showed no physical sign of the infection other than evidence of multiplication of the parasite in their bodies seem to raise many questions which could not be answered at this moment. This indicates a need for further investigation.

of fission are small spindled-shaped cells known as merozoites. Merozoites enter fresh host cells and usually undergo a second cycle of growth and reproduction by schizogony. After one, two or more cycles of a sexual reproduction, sexual forms develop. Some of the merozoites enter cells, grow and become multinucleate and give rise to many minute flagellated cells. These multinucleate cells are the microgametocytes which produce many motile microgamates. Other merozoites enter the cells and grow without nuclear division. These are macrogamates each of which matures into a macrogamate. A motile microgamate fuses with a macrogamate to form the zygote. The zygote secretes a protective coat and is now called an oocyst. When the host cell disintegrates, the oocysts are released and are passed out in the faeces. The final stage of sporogony or sporulation, takes place outside the host.

Coccidiosis may be suspected when animals are listless and off their feeds - and the faeces are watery or blood stained (27).

4.2 COCCIDIOSIS - A STRESS FACTOR:

The effect of cecal coccidiosis (as a stress factor) upon the weights and whole blood vitamin C (ascorbic acid) contents of some young chickens was investigated using two species of the parasite:- Eimeria Tenella and Eimeria Acervulina. The Eimeria Tenella sporulated oocysts were obtained from an outbreak of coccidiosis in some

poultry farms in Shika, Zaria. The intestinal contents of the most heavily infected birds were collected in a physiological saline solution and centrifuged. The supernatant was separated from the residue and washed with distilled water by sedimentation. It was finally reconstituted in distilled water and counted in a hematocytometer. One millilitre was found to contain 20,000 oocysts. The Eimeria Acervulina isolate was obtained from Utrecht, Holland. It was also washed, reconstituted and counted before use. One millilitre of the isolate was found to contain 800,000 oocysts.

One day old chicks were reared in an electrically heated, sterilised room until they were five weeks old. During the growing period, they were fed with previously boiled water and sterilised, coccidiostat-free diet prepared by the National Animal Production Research Institute (NAPRI), Samaru, Zaria. Fifteen out of the total birds were randomly picked and transfered into three broiler cages labelled A, B and C (each cage contained five birds). The weights and whole blood ascorbic acid contents were determined three times in one week. They were also examined and found free of coccidia parasites.

The chickens in groups A and C were starved for approximately twelve hours. Those in group A were individually innoculated with 20,000 sporulated oocysts of Eimeria tenella placed directly in

their crops using glass pipettes. The ones in group C were also innoculated with 400,000 sporulated oocysts of Eimeria acervulina in a similar way as the Eimeria tenella above. Measurement of weights and the analysis of their whole blood ascorbic acid contents started immediately.

4.3 MATERIAL AND METHOD:

The total ascorbic acid contents of the whole blood of the chickens were determined using the modified Dinitrophenylhydrazine method of Roe el al (28). The acid was oxidised to dehydroascorbic acid with an acid-washed norit. The oxidised form was then treated with 2, 4-dinitrophenylhydrazine to form the hydrazone, which, when treated with 85% sulphuric acid solution, it formed a highly stable, reddish brown product whose absorbance was determined at 515 nm, using Bansch Lomb Spectronic 20.

4.4 A P P A R A T U S:

The following apparatus were used:-

(a) Assorted volumetric flasks, comical flasks, burettes, pipettes, funnels, test tubes, weighing and reagent bottles, syringes and needles.

(b) Mettler and top loading balances

(c) Cotton wool and Plastic trays

(d) No 42 Whatman filter paper

(e) Bansch Lomb Spectronic - 20

R E A G E N T S:

The following reagents were used in the analysis:-

(a) 6% Trichloroacetic Acid Solution:

This was made by dissolving 60 grams of Analar-grade Trichloroacetic in enough distilled water to make a one litre solution.

(b) 85% Sulphuric Acid Solution:

This was prepared by adding 900cm^3 of concentrated sulphuric acid to 100cm^3 of distilled water.

(c) 1% Thiourea Solution:

10 grams of Thiourea were dissolved in 50cm^3 of ethanol and the solution was diluted to 100cm^3 with enough distilled water.

(d) 2, 4 Dinctrophenylhydrazine Solution:

This was made by dissolving 2 grams of 2, 4-dinctrophenylhydrazine in 100cm^3 of approximately 9N sulphuric acid solution.

(e) 5$ _M[e]"kapho.[s]pfopy J-[c] A.cld - 10$ Acetic Acid Solution:

50 grains of reagent grade HPO_3 were dissolved in 800cm' of distilled water. 100cm of glacial acetic acid were added and the solution was made up to a litre with distilled water.

(f) 10% ([w]/v) Potassium Oxalate 3olutj.on:

10 grams of Potassium Oxalate were dissolved in enough distilled water to make a 100cm solution.

(g) 10rt Hydrochloric Aeid Solution:

([h]) Absolute Alcohol:

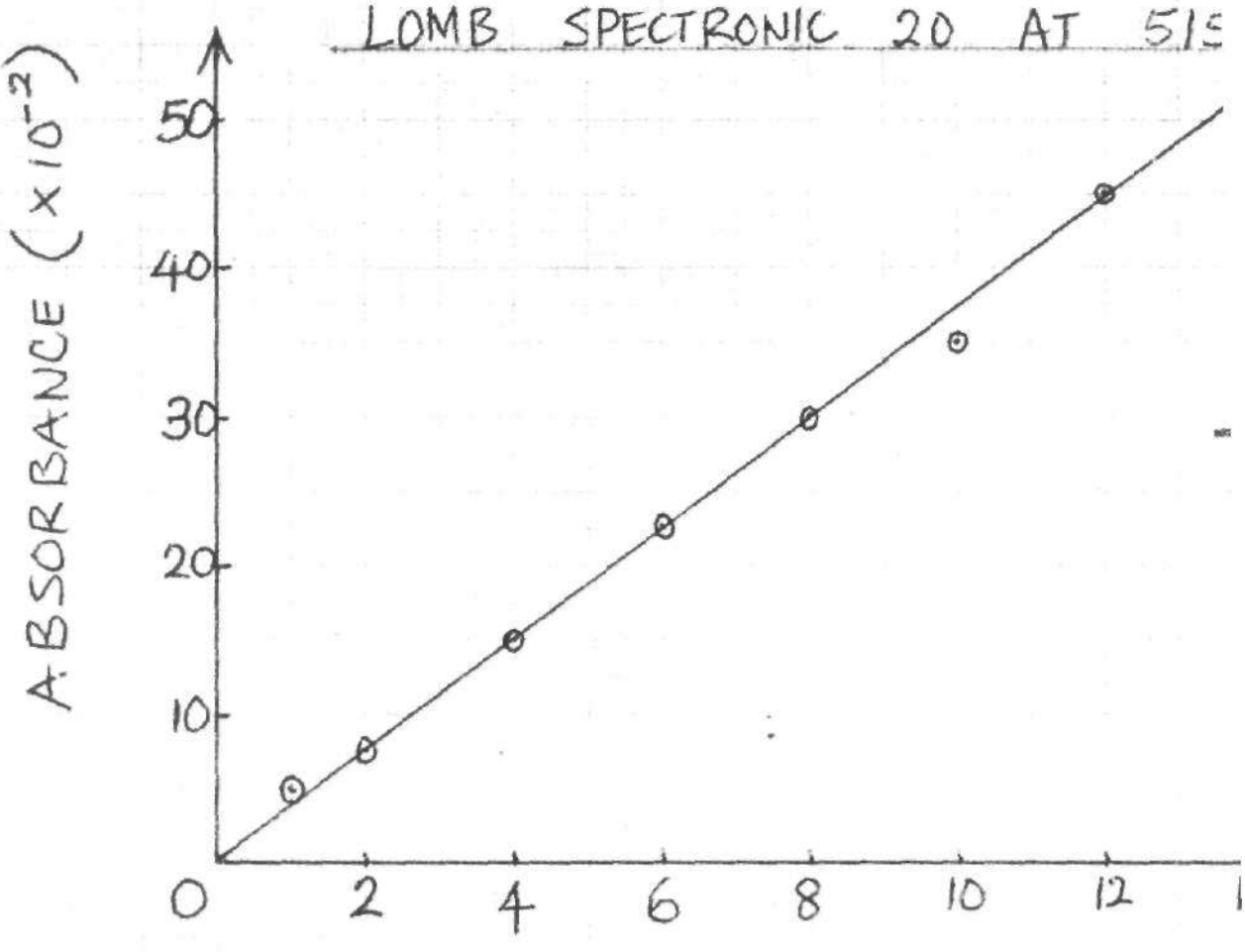
(i) Acid-Washed Nqrit;

This was prepared by heating to boiling 200 grams of Norit and 1 litre of 10^ hydrochloric acid solution. The mixture was filtered by suction. The cake was washed several times with distilled water and. r[q]ried overnight in an oven at 110°C - 120°Cc

(3) Standard Ascorbic Acid Solution XS.1iP.ckJL:

This was prepared by dissolving 100 mg„ of Analar grade ascorbic acid (stored in a dessicator away from direct sunlight) in enough metaphosphoric acid - acetic acid solution to make a 100cm solution.

DETERMINATION OF ASCORBIC
IN THE BLOOD OF YOUNG CHICK
A CALIBRATION CURVE USING BAU
LOMB SPECTRONIC 20 AT 51



(k) Working Standard Ascorbic Acid Solution:

This was prepared by diluting $10cm^3$ of the stock standard ascorbic acid solution to $100cm^3$ using metaphosphoric acid-acetic acid solution as the solvent.

4.4 PREPARATION OF CALIBRATION CURVE:-

A series of standard solution were prepared by pipetting 1, 2, 4, 6, 8, 10 and 12 cm^3 of working standard ascorbic acid solution into $100cm^3$ volumetric flasks and diluting each to mark using metaphosphoric acid - acetic acid solution as the solvent. $4cm^3$ of each standard solution were pipetted into three tubes, two of which were marked standard sample tubes and the other marked standard blank tube. To each of the standard sample tube, $1cm^3$ of 2% 2, 4- dinitrophenylhydrazine solution was added. A drop of thiouren solution was added to all tubes. The tubes were then incubated in a water bath at 100^oC for ten minutes. At the end of the incubation period, the tubes were transfered into a large beaker containing ice water and a generous quantity of ice blocks. $5cm^3$ of 85% sulphuric acid were added slowly to each tube, mixed and kept for ten minutes on a wooden rack. The absorbance of each solution was determined at 515nm using the Bausch Lomb spectronic-20. The results obtained are shown in figure II and appendix II.

4.7 D E T E R M I N A T I O N S:

In analysing the ascorbic acid content of the whole blood of the chickens, two millilitres of blood were withdrawn from each bird through a prominent jugular vein on its neck. The area to be bled was first cleaned with cotton wool soaked in ethyl alcohol. The syringes and needles used were also constantly sterilized and flushed with a 10% solution of potassium oxalate. The flushing prevents blood clotting.

The blood withdrawn was normally emptied into a sample bottle containing $10cm^3$ of a 6% solution of trichloroacetic acid solution (an extracting agent and also a protein precipitant). The bottle was then shaken and kept for five minutes to achieve complete extraction. The mixture was centrifuged and the supernatant shaken with approximately 0.5 gram of acid- washed norit. It was again filtered through No 42 - Whatman filter paper.

Three millilitres of the filtrate were pipetted into two test tubes one of which was marked sample tube and the other blank tube. One millilitre of 2% 2, 4- dinitrophenylhydrozine solution was added to the sample tube. This was followed by the addition of a drop of thiourea solution to all the tubes. They were then incubated in a water bath maintained at $100^{o}C$ for ten minutes. At the end of the incubation period, the tubes were transfered into a large beaker

containing ice water and some ice blocks. 5cm^3 of 85% sulphuric acid solution were added to all the tubes, shakened and kept on a wooden rack for another ten minutes. The absorbance of each solution was determined at 515nm using the same Bousch and Lomb spectronic 20 as in the calibration curve.

The amounts of ascorbic acid in microgram per millilitre of blood were calculated using the simple expression:-

$$\frac{\text{ug/cm}^3 \text{ of Ascorbic Acid from the calibration curve} \times \text{Volume of filtrate}}{\text{Volume of the Sample}}$$

4.6 RESULTS

Tables XXIV, XXV and XXVI indicate the total ascorbic acid content of the whole blood of coccidia-free, Eimeria tenella-infected and Eimeria acervulnia-infected chickens. The trends in these tables shows that the ascrobic acid levels remained rather constant. However, taking the mean values of the total ascrobic acid contents of the birds in the same group per day (i.e. per analysis) and the averages of these values (Tables XXVII to XXIX), there appeated to be a slight increase in the ascrobic acid level. The magnitude of the increase is such that: Eimeria acervulina group showed a more increase than Eimeria tenella group. An increase was also observed with the coccidia-free group.

Tables XXXI to XXXIII also show the change in weight for the three group of chickens. All of them showed an increase in weight. This is expected since they are all young growing birds. The increase in weight becomes more apparent when one examines the mean values of the total weights of all the birds in a group and per measurement, (Tables XXXIV and XXXV). Table XXXVI shows the averages of the mean values indicated in Tables XXXIV and XXXV. The percentage gain in weight appeared in Table XXXVII.

4.7 DISCUSSION

Literature values indicating the expected range of the total ascrobic acid content of the whole blood of chickens were not available for comparisón with the results obtained. The validity of the results and hence deductions made from them depend on many factors. The major one among them is the method of analysis exmployed. The method used in this analysis is of high specificity as evidenced from the results obtained by Roy and Keuther who analysed the blood and urine of guinea pigs with acute scurvy. They obtained whole blood values as low as 0.04 to 0.13 milligram per $100cm^3$ and urine values of 1.0 to 3.0 milligram per litre. Other evidences also obtained from the same workers came through the analysis of interfering substances. For examples alde-hydes and Ketones which were expected to couple with 2, 4-dinitrophenylhydrazine posed no problem. The derivatives of these which are likely to be encountered in such analysis, for example, pyruvic acid and acetoacetic acid, do not react with sculphuric acid under the conditions of the reaction. Moreover, their solutions in acetri acid do not absorb at 540nm, the wavelength at which the vitamic C derviative absorbs. Finally, they performed a series of recovery

experiments which indicate the accuracy and precision of the method. In one of these experiments they added ascorbic acid to blood and urine and obtained good recoveries ranging from 96% to 104%.

In another experiment where ten analyses were carried out on one filtrate, the mean value was found to be 0.17mg per 100cm^3; the standard deviation was $\pm$0.01; the probable error of a single determination was $\pm$0.0007 and the maximum deviation was 0.04 mg per 100cm^3.

Cecal coccidiosis infection appeared to have an effect on the blood ascorbic acid level. It elevates the level of the vitamin. This is unexpected in the light of the previous results of depletion as characteristic response to stress in mammals and the lack of response which has been found in avian species. While ascrobic acid elevation seems to be characteristic of cecal coccidiosis infection, it also seems possible that some ascrobic acid depletion might be present as a transient stage prior to the observed elevation. Such changes might have been missed with the sampling intervals used in this study. The findings of slightly reduced ascrobic acid levels in Tables XXV and XXVI favours this possibility.

The observations also give support to the concept that turnover rate is important in determining the absolute level of blood constituents (29). Thus, the net gain in ascorbic acid which was observed may be one to repletion occuring simultaneously and at a greater rate than the usual stress-induced depletion. Under such a condition ascrobic acid could be playing a fundamentally identical role in situations where either depletion or elevation is the net effect of stress. Studies on the ascrobic acid levels of the various organs on the body in relation to the blood ascrobic acid level might clarify the proble. Furthermore studies on other vitamines may be essential for adequate and conclusive interpretation of results.

The observed increase in weight which accompanied the infection is also surprising. From the results one may be tempted to question the toxic effect of the parasites employed. The finding that the percentage gain in weight associated with E. acervulina exceeds that associated with E. tenella, a more pathogenic parasite, and the fact that the birds did not seem to be affected physically as a result of the infections seem to raise many questions which could not be answered at present. This calls for further investigation.

TABLE I

THE TOTAL ASCORBIC ACID CONTENT (MICROGRAM PER MILLILITRE) OF THE WHOLE BLOOD OF GUINEA FOWLS FED WITH GUINEA CORN FOR THREE WEEKS

CONDITION	IDENTIFICATION MARK	RESULTS (In ug/millilitre of Blood)													
INFECTED	1	19.8	20.0	20.0	21.0	23.0	21.5	21.0	23.0	23.5	23.0	21.5	23.0	23.0	23.5
	2	18.8	21.0	21.0	21.0	21.5	21.0	22.0	21.5	21.0	23.0	21.5	21.0	21.0	21.5
	3	18.6	18.8	19.8	20.5	19.5	20.8	20.0	21.0	21.5	21.5	20.5	22.0	22.0	21.5
NON-INFECTED	4	20.2	20.5	20.0	21.5	21.0	20.5	21.0	21.0	20.8	20.0	21.0	21.0	21.5	20.8
	5	21.4	21.0	21.0	21.8	21.5	20.8	21.5	21.0	22.0	22.5	21.5	21.0	21.5	22.5
	6	22.4	22.0	22.5	21.8	21.0	21.5	22.0	22.8	21.8	22.0	22.0	22.0	22.5	22.0

TABLE II

THE TOTAL ASCORBIC ACID CONTENT OF THE WHOLE BLOOD OF GUINEA FOWLS FED WITH MAIZE (MASARA) FCR THREE WEEKS (MICROGRAMS PER MILLILITRE OF BLOOD)

CONDITION	IDENTIFICATION MARK	RESULTS In ug (Millilitre)											
INFECTED	7	26.0	25.0	25.5	23.0	24.0	24.0	24.5	24.0	24.0	24.5	24.0	22.5	24.5
	8	24.0	24.5	23.5	23.5	224.0	23.8	23.6	23.8	24.0	24.0	24.5	24.0	24.0
	9	19.8	20.0	20.5	20.5	20.0	20.5	21.8	20.5	20.8	20.0	22.8	20.0	20.5
NON-INFECTED	10	23.4	20.4	20.0	20.0	21.8	21.0	21.0	21.8	20.0	19.8	23.0	21.0	21.0
	11	20.2	19.8	19.0	19.8	21.6	21.0	19.5	20.4	20.0	20.0	21.5	20.6	20.0
	12	19.6	19.0	19.5	19.5	19.0	21.8	21.0	20.8	20.6	21.0	21.0	22.5	22.5

TABLE III

THE TOTAL ASCORBIC ACID CONTENT OF THE WHOLE BLOOD OF GUINEA FOWLS FED WITH MILLET (GERO) IN MICROGRAMS PER MILLILITRE OF BLOOD

CONDITION	IDENTIFICATION MARK	RESULTS													
INFECTED	13	20.4	20.0	21.0	20.8	20.5	21.0	20.8	20.0	20.0	21.0	21.0	20.6	21.0	20.0
	14	20.8	23.0	20.5	20.8	20.0	20.0	20.8	20.5	20.0	21.5	23.0	21.8	23.0	20.5
	15	24.0	21.8	21.0	21.0	21.0	22.0	21.5	21.0	20.8	21.0	21.5	21.0	21.0	21.8
NON-INFECTED	16	22.0	22.0	21.8	22.0	21.8	22.4	22.0	22.0	22.8	23.0	21.6	21.0	22.4	24.0
	17	22.5	22.8	22.0	22.5	22.0	22.5	22.4	24.0	25.8	23.4	22.5	22.5	22.6	22.7
	18	26.0	20.4	20.0	20.8	21.0	21.4	20.0	22.0	22.6	22.8	22.0	22.0	22.6	20.8

TABLE IV

TOTAL ASCORBIC ACID CONTENT (MICROGRAM PER MILLILITRE) OF THE WHOLE BLOOD OF GUINEA FOWLS FED WITH PFIZER LIVESTOCK FEEDS FOR THREE WEEKS

CONDITION	IDENTITY	RESULTS													
INFECTED	19	23.0	21.8	22.6	22.0	22.1	22.0	22.6	22.0	21.4	21.0	21.6	22.4	22.0	22.8
	20	17.5	20.0	20.0	20.5	20.0	21.8	20.6	20.8	20.0	21.4	20.5	20.0	20.9	22.0
	21	19.0	19.0	23.4	20.0	20.0	21.3	20.0	21.6	21.0	21.0	20.4	20.0	20.0	20.7
NON-INFECTED	22	19.6	20.8	18.5	18.8	20.4	20.0	20.6	21.4	21.0	21.0	19.5	21.0	21.4	21.8
	23	24.3	22.4	22.2	23.0	23.0	22.8	22.0	22.2	23.0	23.4	22.6	21.4	22.0	21.0
	24	21.4	24.0	21.3	20.8	21.6	21.0	21.0	22.6	21.0	21.9	21.4	20.6	21.0	21.0

T A B L E V

MEAN VALUES (MICROGRAM PER MILLILITRE OF THE TOTAL ASCORBIC ACID CONTENT OF THE WHOLE BLOOD OF MALARIA-FREE GUINEA FOWLS, ALL FED WITH PFIZER LIVESTOCK FEED.

GROUPS	I	II	III
GUINEA CORN	20.33	20.76	21.44
MAIZE	20.73	20.44	21.89
MILLET	20.98	21.34	21.88
PFIZER FEED	21.37	20.64	20.38

T A B L E VI

MEAN VALUES (MICROGRAM PER MILLILITRE) OF THE TOTAL ASCORBIC ACID CONTENT OF THE WHOLE BLOOD OF GUINEA FOWLS FED WITH GUINEA CORN (DAWA).

INFECTED BIRDS	NON-INFECTED BIRDS
19.07	21.33
19.93	21.17
20.27	21.17
20.83	21.70
21.33	21.17
21.10	20.93
21.00	20.93
21.83	21.60
22.50	21.60
21.17	21.43
22.00	21.50
22.00	21.33
22.11	21.83
22.00	21.77

T A B L E VII

MEAN VALUES (MICROGRAM PER MILLILITRE) OF THE TOTAL ASCORBIC ACID CONTENT OF THE WHOLE BLOOD OF GUINEA FOWLS FED WITH MAIZE (MASARA)

INFECTED BIRDS	NON-INFECTED BIRDS
23.27	21.07
21.17	19.73
23.07	19.60
22.33	19.77
22.67	20.80
22.77	21.27
23.30	20.50
22.77	21.00
22.93	20.30
22.83	20.27
23.77	21.83
23.33	21.37
22.83	21.77
23.17	21.77

T A B L E VIII

MEAN VALUES (MICROGRAM PER MILLILITRE) OF THE TOTAL ASCORBIC ACID CONTENT OF THE WHOLE BLOOD OF GUINEA FOWLS FED WITH MILLET (GERO)

INFECTED BIRBS	NON-INFECDED BIRDS
21.73	23.50
21.60	21.67
20.83	21.27
20.87	21.77
20.77	22.50
20.50	21.60
21.00	22.10
21.03	21.47
20.50	22.67
20.27	23.07
21.17	23.07
21.50	22.03
21.13	21.83
21.67	22.53

TABLE IX

MEAN VALUES (MICROGRAM PER MILLILITRE) OF THE TOTAL ASCORBIC ACID CONTENT OF THE WHOLE BLOOD OF GUINEA FOWLS FED WITH PFIZER LIVESTOCK FEED

INFECTED BIRDS	NON-INFECTED BIRDS
19.83	21.77
20.27	22.40
22.00	21.30
20.83	20.87
20.70	21.68
21.70	21.27
21.07	21.30
21.47	22.07
20.80	21.67
20.80	22.10
20.97	22.17
21.83	21.00
21.33	21.47
20.80	23.60

T A B L E X

AVERAGES OF THE MEAN VALUES OF THE WHOLE BLOOD ASCORBIC ACID CONTENTS (IN MICROGRAM PER MILLILITRE) OF GUINEA FOWLS BEFORE AND AFTER INFECTION.

GROUPS	VITAMIN C CONTENT BEFORE INFECTION	VITAMIN C CONTENT AFTER INFECTION	CHANGE IN THE VITAMIN C CONTENT
GUINEA CORN	19.54	21.15	+1.61
MAIZE	20.89	21.04	+0.15
MILLET	22.72	23.03	+0.31
PFIZER FEED	19.57	20.77	+1.20

T A B L E XI

AVERAGES OF THE MEAN VALUES OF THE WHOLE BLOOD ASCORBIC ACID CONTENTS (MICROGRAM PER MILLILITRE) OF GUINEA FOWLS FED WITH DIFFERENT DIETS

GROUPS	VITAMIN C CONTENT BEFORE FEEDING	VITAMIN C CONTENT DURING FEEDING	CHANGE IN THE VITAMIN C CONTENT
GUINEA CORN	20.84	21.43	+0.59
MAIZE	21.40	22.22	+0.82
MILLET	21.0	20.79	-0.21
PFIZER FEED	20.79	21.57	+0.78

TABLE XII

THE WEIGHTS (GRAMS) OF GUINEA FOWLS FED WITH WITH GUINEA CORN (DAWA) RECORDED AT 48-HOUR INTERVAL.

CONDITION	BIRD'S IDENTITY	MEASUREMENTS WEIGHTS (IN GRAMS)						
		I	II	III	IV	V	VI	VII
INFECTED BIRDS	1	550	550	575	575	575	575	600
	2	600	600	600	625	600	625	625
	3	700	700	700	725	725	725	725
NON-INFECTED BIRDS	4	550	550	550	575	575	550	575
	5	600	600	600	625	625	625	625
	6	575	575	575	575	600	600	600

T A B L E XIII

THE WEIGHTS (GRAMS) OF GUINEA FOWLS FED WITH MAIZE (MASARA) RECORDED AT 48-HOURS INTERVAL.

CONDITION	BIRD'S IDENTITY	M E A S U R E M E N T S WEIGHTS (IN GRAMS) I	II	III	IV	V	VI	VII
INFECTED BIRDS	7	625	625	650	625	625	650	650
	8	650	675	675	675	675	675	700
	9	700	675	675	675	700	700	700
NON-INFECTED BIRDS	10	650	650	650	675	675	675	675
	11	675	675	675	700	700	675	700
	12	675	650	650	650	675	675	675

T A B L E XIV

THE WEIGHTS (IN GRAMS) OF GUINEA FOWLS FED WITH MILLET (GERO) RECORDED AT 48-HOURS INTERVAL

CONDITION	BIRD'S IDENTITY	MEASUREMENTS WEIGHT (IN GRAMS) I	II	III	IV	V	VI	VII
INFECTED BIRDS	13	625	625	650	625	625	650	650
	14	650	675	675	675	675	675	700
	15	700	675	675	675	700	700	700
NON-INFECTED BIRDS	16	675	675	675	700	700	675	700
	17	650	650	675	650	675	675	675
	18	675	650	650	675	675	675	675

TABLE XVI

MEAN WALUE (GRAMS) OF THE WEIGHTS OF MALARIA-FREE GUINEA FOWLS BEFORE FEEDING WITH VARIOUS DIETS (ALL BEING FED WITH PFIZER LIVESTOCK FEED).

GROUPS	MEAN VALUES OF WEIGHTS (IN GRAMS)		
	I	II	III
GUINEA CORN	575.00	575.00	583.00
MAIZE	625.00	625.00	641.67
MILLET	633.33	633.33	641.67
PFIZER FEED	575.00	575.00	583.00

T A B L E XVII

MEAN VALUES (GRAMS) OF THE WEIGHTS OF MALARIA INFECTED GUINEA FOWLS BEFORE FEEDING WITH THE VARIOUS DIETS (ALL BEING FED WITH PFIZER LIVE-STOCK FEED).

GROUPS	MEAN VALUES OF WEIGHTS (IN GRAMS)		
	I	II	III
GUINEA CORN	600.00	600.00	616.67
MAIZE	625.00	658.33	658.33
MILLET	641.67	641.67	658.33
PFIZER FEED	583.33	600.00	600.00

T A B L E XIX

MEAN VALUES OF THE WEIGHTS (GRAMS) OF GUINEA FOWLS FED WITH MAIZE (MASARA)

INFECTED BIRDS (WEIGHTS IN GRAMS)	NON-INFECTED BIRDS (WEIGHTS IN GRAMS)
661.67	666.67
658.33	658.33
666.67	641.67
625.00	675.00
666.67	683.83
675.00	675.00
683.33	683.33

T A B L E XX

MEAN VALUES OF THE WEIGHTS (GRAMS) OF GUINEA FOWLS FED WITH MILLET (GERO)

INFECTED BIRDS (WEIGHTS IN GRAMS)	NON-INFECTED BIRDS (WEIGHTS IN GRAMS)
658.33	633.33
658.33	661.67
633.33	666.67
658.33	683.33
666.67	675.00
675.00	683.33
683.33	675.00

T A B L E XVIII

MEAN VALUES OF THE WEIGHTS (GRAMS) OF GUINEA FOWLS FED WITH GUINEA CORN (DAWA)

INFECTED BIRDS (WEIGHTS IN GRAMS	NON-INFECTED BIRDS (WEIGHTS IN GRAMS)
616.67	575.00
616.67	575.00
625.00	575.00
641.67	591.67
633.33	600.00
641.67	591.65
650.00	596.67

T A B L E XXI

MEAN VALUES OF THE WEIGHTS (GRAMS) OF GUINEA FOWLS FED WITH PFIZER LIVESTOCK FEED

INFECTED BIRDS (WEIGHTS IN GRAMS)	NON-INFECTED BIRDS (WEIGHTS IN GRAMS)
616.67	583.33
650.00	583.33
633.33	591.67
650.00	600.00
666.67	616.66
666.67	625.00
675.00	650.00

T A B L E XXII

AVERAGES (GRAMS) OF THE MEAN VALUES OF THE WEIGHTS OF GROUPS OF GUINEA FOWLS FED WITH DIFFERENT DIETS

GROUPS	I N F E C T E D		NON-INFECTED	
	BEFORE FEEDING (WT. IN GRAMS)	DURING FEEDING (WT. IN GRAMS)	BEFORE FEEDING (WT. IN GRAMS)	DURING FEEDING (WT. IN GRAMS)
GUINEA CORN	605.56	632.14	577.78	586.43
MAIZE	647.22	662.38	630.56	669.12
MILLET	647.22	661.90	636.11	668.33
PFIZER FEED	594.44	651.19	577.77	607.14

T A B L E XXIII

PERCENT (%) GAIN IN WEIGHT FOR GROUPS OF GUINEA FOWLS FED WITH DIFFERENT DIETS

GROUPS	INFECTED	NON-INFECTED
GUINEA CORN	4.39	1.50
MAIZE	2.34	6.12
MILLET	2.27	8.21
PFIZER FEED	9.55	5.08

TABLE XXIV

THE TOTAL ASCORBIC ACID CONTENT (MICROGRAM PER MILLILITRE) OF THE WHOLE BLOOD OF COCCIDIA-FREE CHICKENS

NUMBER OF ANALYSIS	BIRDS IDENTITY				
	6	7	8	9	10
I	19.6	19.0	21.4	20.8	18.8
II	19.8	20.1	21.0	20.0	20.6
III	21.4	20.0	21.0	20.0	20.0
IV	21.0	20.0	20.3	20.6	20.4
V	21.6	19.8	20.0	20.0	21.7
VI	20.4	19.0	20.0	21.3	20.0
VII	19.3	20.4	21.3	21.8	21.4
VIII	21.3	20.0	21.0	21.8	21.3
IX	21.6	21.3	21.0	21.0	20.0
X	21.0	20.0	23.3	21.0	20.0
XI	21.3	20.0	20.1	20.4	21.3
XII	20.0	21.3	21.0	20.0	21.4
XIII	20.1	21.0	21.4	20.3	21.0
XIV	20.1	21.0	21.8	20.0	21.0
XV	21.0	20.7	21.0	21.3	20.6

TABLE XXV

THE TOTAL ASCORBIC ACID CONTENT (MICROGRAM PER MILLILITRE) OF THE WHOLE BLOOD OF CHICKENS INFECTED WITH EIMERIA TENELLA

NUMBER OF ANALYSIS	BIRD'S IDENTITY 1	2	3	4	5
I	16.8	20.0	19.4	21.7	18.5
II	20.0	20.2	21.4	20.7	20.4
III	20.8	20.8	20.0	20.5	20.0
IV	20.6	21.5	21.0	21.0	21.5
V	19.9	20.4	20.0	21.8	21.6
VI	20.0	21.5	21.4	20.8	21.0
VII	20.3	20.8	20.5	19.5	19.6
VIII	21.3	20.8	20.6	21.0	21.5
IX	21.0	21.0	20.0	21.9	21.0
X	21.8	21.0	19.8	21.0	21.5
XI	20.9	21.5	20.4	21.0	23.4
XII	19.3	21.0	21.6	20.8	21.0
XIII	20.0	20.8	21.0	20.0	21.8
XIV	19.8	21.0	21.0	19.4	21.0
XV	19.6	20.2	20.8	21.8	20.3

TABLE XXVI

THE TOTAL ASCORBIC ACID CONTENT (MICROGRAM PER MILLILITRE) OF THE WHOLE BLOOD OF CHICKENS INFECTED WITH EIMERIA ACERVULINA

NUMBER OF ANALYSIS	BIRD'S IDENTITY 11	12	13	14	15
I	20.1	19.9	20.0	21.0	20.8
II	19.3	21.3	24.6	21.4	25.0
III	19.0	20.4	21.4	19.0	24.0
IV	20.4	19.5	20.0	21.0	25.0
V	20.4	20.6	19.7	20.8	25.0
VI	20.0	20.4	20.6	20.0	26.0
VII	21.3	19.9	20.0	19.5	26.5
VII	21.0	20.4	21.5	20.3	27.5
IX	21.5	20.0	21.0	20.1	25.8
X	21.0	20.6	21.0	21.4	26.0
XI	22.3	21.4	20.9	21.0	26.0
XII	21.6	21.6	20.0	19.3	26.4
XIII	21.0	20.8	21.7	19.0	27.0
XIV	21.0	21.4	21.1	21.4	27.0
XV	21.4	20.0	21.0	21.0	26.4

TABLE XXVII

MEAN VALUES (MICROGRAM PER MILLILITRE) OF THE TOTAL ASCORBIC ACID CONTENT OF THE WHOLE BLOOD OF THE THREE GROUPS OF CHICKENS ALL BEFORE INFECTION

GROUPS	I	II	III
I (CONTROL; COCCIDIA-FREE	19.59	19.78	20.82
II E. TENELLA	19.56	20.14	20.04
III E.ACERVULINA	20.08	20.98	20.44

TABLE XXVIII

MEAN VALUES OF THE TOTAL ASCORBIC ACID CONTENTS (MICROGRAM PER MILLILITRE) OF THE WHOLE BLOOD OF THREE GROUPS OF CHICKENS

No	COCCIDIA-FREE GROUP	E. TENELLA GROUP	E. ACERVULINA GROUP
1	19.92	19.28	20.36
2	20.30	20.54	22.32
3	20.48	20.42	20.76
4	20.46	21.12	21.90
5	20.62	20.74	21.30
6	20.14	20.94	21.40
7	20.84	20.14	21.44
8	21.08	21.04	22.14
9	20.98	20.98	22.88
10	21.56	21.02	21.88
11	20.62	21.44	22.32
12	20.74	20.74	21.78
13	20.76	20.72	21.90
14	20.78	20.44	22.38
15	20.92	20.54	21.96

TABLE XXIX

AVERAGES (MICROGRAM PER MILLILITRE OF THE MEAN VALUES OF THE WHOLE BLOOD ASCORBIC ACID CONTENTS OF THE THREE GROUPS OF CHICKENS, BEFORE AND AFTER INFECTION

GROUPS	BEFORE INFECTION	AFTER INFECTION
COCCIDIA-FREE	20.06	20.55
E. TENELLA	19.91	20.50
E. ACERVULINA	20.67	21.78

These values were calculated from Tables XXVII and XXVIII.

TABLE XXX

PERCENTAGE GAIN IN THE TOTAL ASCORBIC ACID CONTENT OF THE THREE GROUPS OF CHICKENS

GROUPS	% GAIN
COCCIDIA-FREE	5.05
E. TENELLA	3.82
E. ACERVULINA	6.24

These values were calculated from Table XXIX by finding the difference between the average ascrobic acid level after and before infection and dividing the value with the average ascorbic acid level before infection. Note that with the coccidia-free group the average ascorbic acid level before and after infection correspond to measurements taken together with the other groups, before and after infection.

TABLE XXXI

WEIGHTS (GRAMS) OF COCCIDIA-FREE CHICKENS. MEASUREMENTS TAKEN AT INTERVALS OF THREE DAYS

Bird's Identity	W	E	I	G	H	T	S (in Grams)
6	425	450	425	450	450	450	
7	450	450	475	475	475	500	
8	400	400	425	400	425	425	
9	400	425	425	425	450	450	
10	425	400	425	450	450	450	

TABLE XXXIII

WEIGHTS (GRAMS) OF CHICKENS INFECTED WITH EIMERIA TENELLA MEASUREMENTS TAKEN AT THREE DAYS INTERVAL

Bird's Identity	W	E	I	G	H	T S (in Grams)
1	425	450	450	450	450	475
2	400	425	425	425	450	450
3	375	400	425	425	425	450
4	400	425	425	425	450	425
5	400	375	400	425	425	425

TABLE XXXIII

WEIGHTS (GRAMS) OF CHCIKENS INFECTED WITH EIMERIA ACERVULINA. MEASUREMENTS TAKEN AT THREE DAYS INTERVAL

Bird's Identity	W E I G H T S (In Grams)					
11	400	400	425	400	400	425
12	425	425	425	425	450	450
13	400	400	425	425	400	400
14	400	400	400	425	425	425
15	450	475	475	475	475	500

TABLE XXXIV

MEAN VALUES OF THE WEIGHTS (GRAMS) OF THE THREE GROUPS OF CHICKENS BEFORE INFECTION

GROUPS	I	II	III
COCCIDIA-FREE	400	400	420
E. TENELLA	355	365	380
E. ACERVULINA	370	375	400

TABLE XXXV

MEAN VALUES OF THE WEIGHTS (GRAMS) OF THE THREE GROUPS OF CHICKENS AFTER INFECTION

COCCIDIA-FREE GROUP	E. TENELLA GROUP	E. ACERVULINA GROUP
420	400	415
425	415	420
435	425	420
440	430	420
450	440	420
440	445	450

TABLE XXXVI

AVERAGES (GRAMS) OF THE MEAN VALUES OF THE TOTAL WEIGHTS OF THE THREE GROUPS OF CHICKENS

GROUPS	BEFORE INFECTION	INFECTED
COCCIDIA-FREE GROUP	406.2	425.66
E. TENELLA GROUP	366.67	425.83
E. ACERVULINA GROUP	381.67	424.17

TABLE XXXVII

PERCENTAGE (%) GAIN IN WEIGHT FOR THE THREE GROUPS OF CHICKENS

GROUP	% GAIN IN WEIGHT
COCCIDIA-FREE	6%
E. TENELLA	16%
E. ACERVULINA	11%

The percentage gain in weight was calculated using the simple express

$$\frac{\left\{\begin{array}{l}\text{Average weight after infection} - \text{average weight before infection}\end{array}\right\}}{\text{average weight before infection}} \times 100$$

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APPENDIX II

ABSORBANCE VALUES FOR STANDARD SOLUTIONS OF ASCORBIC ACID USING BAUSCH & LOMB SPECTRONIC 20 AT 515nm

CONCENTRATION (ng/ml)	ABSORBANCE			
	I	II	III	MEAN
1	0.043	0.047	0.057	0.047
2	0.074	0.075	0.076	0.075
4	0.146	0.154	0.159	0.153
6	0.219	0.227	0.229	0.225
8	0.302	0.303	0.296	0.302
10	0.340	0 350	0.357	0.349
12	0.445	0.453	0.455	0.451