EFFECT OF CASSAVA FIBRE BASED-BISCUIT AS A PREBIOTIC IN RAT ASSAY

WILLIAMS, ADELUSI OLUWAJENYO

FST/04/4218

A THESIS IN THE DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY SUBMITTED TO THE SCHOOL OF AGRICULTURE AND AGRICULTRUAL TECHNOLOGY (SAAT)

IN PARTIAL FULFILLMENT FOR THE REQUIREMENTS OF THE AWARD OF MASTER OF TECHNOLOGY (M. TECH.) DEGREE IN FOOD SCIENCE AND TECHNOLOGY.

THE FEDERAL UNIVERSITY OF TECHNOLOGY, AKURE, ONDO STATE.

MAY, 2009

CERTIFICATION

By Student:- This work to the best of my knowledge has not been presented else where for the award of degree or any other purpose.

WILLIAMS, A.O	.....	3-6-09
CANDIDATE' S NAME	SIGNATURE	DATE

By Supervisor :- This is to certify that research work was carried out by MR. WILLIAMS ADELUSI OLUWAJENYO under our supervision in the Department of Food Science and Technology of the Federal University of Technology, Akure, Ondo State, Nigeria.

DR (MRS) O.F OSUNDAHUNSI	.....	3-06-09
SUPERVISOR	SIGNATURE	DATE
DR I.B. OLUWALANA	.....	3-6-09
CO- SUPERVISOR	SIGNATURE	DATE
PROF. J.B.FASHAKIN	.....	04/06/09.
HEAD OF DEPARTMENT	SIGNATURE	DATE
PROF. J. B ADERIYE	.....	.....
EXTERNAL EXAMINER	SIGNATURE	DATE

DEDICATION

This project is dedicated to God Almighty who is my source of wisdom, knowledge and understanding.

ACKNOWLEDGEMENT

I acknowledge with thanks the mercy and grace of Almighty God that enabled me to start and finish this project with divine wisdom, Peace, good health and in good time.

All gratitude goes to my supervisors, Dr (Mrs.) O.F. Osundahunsi and Dr I. B. Oluwalana, who did a thorough supervision and gave me the necessary guidance in writing this project. The Head of Department, Prof. J .B. Fashakin, Prof. T. N. Fagbemi, Dr (Mrs.) O.S. Fasasi. Technologists and the secretaries were of tremendous help in one way or the other during the period of this work

My loving and caring mother. Mrs. Modupe Williams you are a wonderful and an uncommon mother who has been very supportive indeed. I am grateful to my elder brother Mr. Julius Williams who has been a father and a constant guardian. you are a brother in a million.

I appreciate Dr V. Oyetayo who gave me some relevant and very useful journals. I also appreciate Dr R. O. Osundahunsi and Miss. C. O. Fasakin for their encouragement to me I acknowledge the support of Mrs. T. Olabamerun. Finally, I must also acknowledge the support of Mrs. H. Awolumate, and sweet heart Imisi -Oluwa and my friend sister Bola. Thank you, God bless you richly and abide with you all.

TABLE OF CONTENTS

	Page
Title	i
Certification	ii
Dedication	iii
Acknowledgment	iv
Table of content	v
List of Tables	ix
List of Figures	x
Abstract	xi
CHAPTER ONE	
1.0 INTRODUCTION	1
1.1 Justification	4
1.2 Objectives	4
CHAPTER TWO	
2.0 LITERATURE REVIVEW	5
2.1 Cassava	5
2.2 Processing of Cassava starch	7
2.3 Prebiotics	7
2.4 Probiotics	10
2.4.1 Beneficial effect of prebiotics and probiotics	11

2.4.2 Antimutagenie activities of Prebiotics and Probiotics 12

2.4.3 Immunity of Probiotics 13

2.5 High fibre Food 15

2.6 Resistant starches. The Gut Microflora and

human health 15

2.6.1 Gastrointestinal Bacteria and Human health 17

2.6.2 Resistant Starches and the Microflora 19

2.6.3 Modulation of Gut microflora and the role of starches 21

2.6.4 Starches 22

CHAPTER THREE

3.0 MATERIALS AND METHODS 26

3.1 Sample and sample collection 26

3.2 Processing methods 26

3.2.1 Processing of cassava fibre into Cassava fibre biscuit 26

3.3 Processing of Maize into Ogi 27

3.4 Proximate Analysis 27

3.4.1 Determination of moisture content 27

3.4.2 Determination of crude fat content 31

3.4.3 Determination of ash content 32

3.4.4 Determination of crude fibre content 32

3.4.5 Determination of protein content 33

3.5 Bacteria counts 34

3.6 Experimental diets 35

3.7 Experimental animals 35

3.7.1 Management of experimental animals 37

3.7.2 Slaughtering and collection of blood 37

3.7.3 Haematological studies of blood of rats 37

3.7.4 Liver function tests 39

3.7.5 Determination of glucose and cholesterol levels 40

3.7.6 Determination of Total protein 41

3.7.7 Determination of Albumin concentration 42

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION 43

4.1 Results 43

4.1.1 Proximate composition of cassava fibre biscult, cassava fibre and Ogi 43

4.1.2 Haematological Parameters of the blood of rats 43

4.1.3 Liver function tests 46

4.1.4 Glucose and Cholesterol levels of the rat 48

4.1.5 Protein and albumin values of rats 48

4.1.6 Daily weight gain of the rats 51

4.1.7 Bacteria counts of the rats 53

4.2 Discussion 57

CHAPTER FIVE

5.0. CONCLUSION AND RECOMMENDATION	63
5.1 Conclusion	63
5.2 Recommendation	63
REFERENCES	64
APPENDIX	86

LIST OF TABLES

		Page
Table 3.1	Recipe for production of cassava wheat composite biscuit	28
Table 3.2	Nutritional composition of Ogi diet and cassava fibre biscuit	36
Table 4.1	Proximate composition of Ogi, cassava fibre based biscuit	44
Table 4.2.	Effect of feeds on some vital Haematological factors in rats	45
Table 4.3.	Liver functioning tests	47
Table 4.4.	Cholesterol and Glucose levels of the rats	49
Table 4.5	Effect of feeds on protein and albumin values of the rats	50
Table 4.6	Daily weight gains of the rats	52
Table 4.7	*Lactobacillus* counts	54
Table 4.8.	*Escherichia coli* counts	55

LIST OF FIGURES

		Page
Figure 2.1	Flow chart for processing of cassava starch and how fibre is got	8
Figure 3.1	Flow chart of production of cassava fibre based	29
Figure 3.2	Flow chart of production of fermented maize (Ogi)	30

ABSTRACT

Cassava fibre that used to be discarded as waste product in starch production was incorporated into wheat composite flour. Wet cassava fibre was brought to the laboratory and dried at temperature of 102°C for 15 minutes and later at 60°C for 30 minutes. The dried cassava fibre was mixed with wheat flour at ratio of 50/50, 60/40. 70/30 and 80/20 respectively. The blends were evaluated for Proximate composition. Cracker- like biscuit was produced from the composite Flour with preferred ratios. The cassava fibre- based biscuit groups were milled and mixed with basal diet comprising of vitamin premix, sucrose, salt, bone meal and non-nutritive cellulose. For comparison, growers mash (Top Feeds) and Ogi served as the other groups. During rat bioassay, weight gain was monitored and faeces collected from day 1 and subsequently at interval of 7days up to the 28th day for microbiological analysis. The rats were killed and blood samples were collected into Ethylenediamine tetra acetic acid (EDTA) coated bottles for Haematological analysis, liver function test, protein and glucose determination.

The protein content of the fibre biscuit 50/50 and 60/40 respectively ranged between 10-15%; Crude fibre between 14.1-17.%; fat content 5-9.0%; Ash between 3.0-5.0% and carbohydrate 46.8-51.9% moisture on dry basis 10-13.0%. The group on Ogi diet had protein content 8.8%. crude fibre 1.0%. moisture on dry basis 8.0%, Ash 1.0%, carbohydrate 77.9%. The protein content

of cassava fibre was 0.5%; crude fibre 33.0%; fats 1.0%; moisture on wet basis 53.5%; Ash 2.5%; and carbohydrate 9.5%. The result of the liver functioning test showed that rats fed on 50/50 biscuit and 60/40 fibre biscuit respectively had low cholesterol of 28.75 mg/ul, and 18.75 mg/dl. The result also showed that 50/50 fibre biscuit had low (aspartate amino transferase AST) 44.5 iu/l, (alanine amino transferase ALT) 50/50 fibre biscuit had 27.0 iu/l, and 60/40 fibre biscuit had AST 43.5 iu/l and ALT 19.5 iu/l respectively. These values are significantly lower than values of Top Feeds that had AST 67.75 iu/l ALT 29.5 iu/l and Ogi AST 29.0 iu/l and ALT 29.0 iu/l respectively. The study also revealed rat fed on 50/50 fibre biscuit and 60/40 fibre biscuit to have higher WBC count of 12.0 mm^3 and 11.75mm^3 respectively and higher PCV count of 38.5% and 36.5% for 50/50 fibre biscuit and 60/40 fibre biscuit respectively. This is an index of good health and non anaemics although rat fed on Top feeds had significantly higher PCV value (41.75%) different from 50/50 fibre biscut and 60/40 fibre biscuit because high protein content. The report also revealed that rats fed with Ogi had significantly lower PCV 29.3% because of its low protein content. The report of faecal analysis showed that rat fed with 50/50 fibre biscuit had higher *Lactobacillus* count of 95.0 x 10^3 cfu/g and lower *E coli* count, 62.0 x $I0^3$ cfu/g significantly different from those fed with Top feed. Rats fed with top feed had *Lactobacillus* count of 73.5 x 10^5 cfu/g. The weight observed revealed that rats fed with top feed had highest weight gain 53.7g, 50/50 fibre

biscuit 47.0g, 60/40 fibre biscuit, 43.0g and Ogi 35.3g respectively.This showed clearly that 50/50 fibre biscuit and 60/40 fibre biscuit had good nutraceutic effect, good pre-biotic effect, anticholesterolmic effect and reduction of enterobacteria in the colon than Top feed and Ogi. The top feed and Ogi served as the positive and negative controls respectively.

CHAPTER ONE

1.0. INTRODUCTION

The colonic microflora is important to health. The growth and metabolism of many individual bacteria species inhabiting the large bowel depend primarily on the substrates available to them most of which come from diet (George, 1999). This has led to attempts to modify the structure and metabolic activities of the community through diet using probiotics and prebiotics.

Probiotics are live microbial food supplement or viable organisms that have beneficial affect .They are used to bring balance between the beneficial bacteria in the intestine (Roberfoid, 2000), the best known are lactic acid bacteria and bifidobacteria which are widely used in yoghurts and other diary products. Probiotics have been used as innovative approach to antibiotic in treating gastrointestinal diseases (Fuller, 1992). These organisms are non-toxigenic, non-pathogenic retain viability during storage and survive passage through the stomach and small bowels (Suanne, 2000). Examples of bacteria and yeast used as probiotics are *Bifidobacteria longum, B. Breve, B infantis, B. bifidium, B. adolescents, Lactococcus cremoris, L. lactis, Streptococcus thermophilus, Enterococus faecium, Lactobacillus rhanmnosus, L. acidophilus, L.casei, L. bulgaricus, L. gassen, Saccharomyces boulardii, S. cerevisiae* (George, 1999).

Lactobacilli are important for the maintenance of the Intestinal microbial ecosystem (Sardine. 1979). Colonization of the gut with lactobacillus start within the first week of life. Salmine (1995) reported that the presence of this group of bacteria in the gut is considered to have several potential benefit such as growth enhancement of farm animals and alleviation of lactose intolerance. Casas and Dobrogost (2000) reported that probiotics protect the animal against pathogen. Jiang *et al.,* (1996) also reported that the consumption of foods containing probiotics relieves constipation. Walker and Duffy (1998) reported on anticholesterolemic effect, antibacterial activities or production of antimicrobial compound called bacteriocin (Dodd and Gassin, 1994). Others are reduction of gut p^H, competition for binding receptor sites that pathogens occupy (Kailasapathy and Chin 2000). Stimulation of immunomodulatory cells and competition for available nutrient (Ralfe, 2000).

Bifidobacteria are also of interest. These are anaerobic pleomorphic rods or club shaped organisms which normally have an important role in breaking down dietary carbohydrate and interact directly with the host metabolism (Gibson *et al.,* 1997). Bifidobacteria also synthesis and excrete water soluble vitamins, but there are considerable differences in species and strain. These organisms predominate in the faeces of breastfed babies. They account for up to 95% of all culturable bacteria and protect against infection. Bifidobacteria do not occur in such high numbers in adults.

Commercial prebiotic preparation are usually mixtures of lactobacillus and bifidobacteria, although yeasts auch as Saccharomyees have also been used

(George, 1999). Prebiotics are non-digestible food ingredients which selectively stimulate the growth or activities of lactobacilli or bifidobacteria in the colon, thereby improving health (Aiba, 1998). They are growth factors that promote population of beneficial bacteria in the colon. Prebiotics belong to classes of chemical compound called oligosaccharides. Prebiotics are principally oligosaccharide. Others are disaccharides and polyols. Oligosaccharides are water soluble polymers of a few condensed monosaccharide. The degree of polymerization ranged from two to about ten. More than ten units are usually considered to constitute polysaccharides. The number of possible oligosaccharides is very large, but the most commonly found are maltose, sucrose, lactose, cellobiose, raffinose and stachyose, Invitrio, eight different bifidobacteri species that were grow on fructo-oligosaccharides produced inhibitory substances which were antagonistic against *Campylobacter, Shigella,* and *Vibrio.* Feeding fructo- oligosaccharides (8g/day) to elderly people increase faecal bifidobacteria 10-fold, while ingestion of soybean oligosaccharides (10g/day) resulted in a smaller though still appreciable increase in bifidobacteria.

Fructo- oligosaccharides do more than promote bifidobacteria growth, several other intestinal bacteria are clearly involved in their metabolism. Galacto- oligosaccharides are present in human and cow milk and also produced from lactose by β galactosidase. Feeding 2.5g, 5g of galacto- oligosaccharides to volunteers resulted in a dose related increase in faecal bifidobacteria excretion, although stool weight and frequency did not change noticeably (Ito *et*

al., 1990). One major difference between probiotic and prebiotic is that prebiotic produces short chain fatty acid on fermentation with fibre in especially butyric acid but probiotic produce acetic acid (Suanne, 2000). This butyric acid has been shown to be beneficial to the health of the intestinal cells and to eliminate cancer cells. One possible significant relationship between lipid and prebiotic is the suggested ability of prebiotic to lower cholesterol in human. However information on this subject is still quite small and highly controversial since some studies report slight reductions whatsoever.

1.1 Justification

This research work is carried out because cassava fibre is underutilized in the society. The research is aimed at producing a fibre-wheat cracker biscuit that will serve as functional foods. For diabetics, those with bowel cancer, heart diseases patient. The full potentials of the nutritive value of cassava fibre-based biscuit will be adjudged only after detail evaluation with bioassay on weanling rats for 28 days.

1.2 Objectives

The specific objectives are to:

- determine the chemical composition of cassava fibre and the cassava fibre-based biscuit
- evaluate the nutraceutic effect of cassava fibre biscuit in rat assay
- develop a cassava fibre based biscuit that will serve as prebiotics

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 CASSAVA

Cassava belongs to the family Euphorbiaccae. Both bitter and sweet cassava are classified as *Manihot esculenta or Manihot utilissima or Manihot aipi* Common name for any several related plants native to tropical regions in the Americas. Cassava is the West Indian name, manioc, or mandioc, is the Brazilian name; and juca, or yuccs, is used in other parts of South America. The plant grows in a bushy form, up to .4m (3ft) tall, with greenish-yellow flowers. The roots are up to 8cm (3 in) thick and 91cm (36 in) long. The roots contain from 20 to 32% of starch in 1½ years old plants.

Two varieties of the cassava are of economic value: the bitter, or poisonous; and the sweet, or non-poisonous. Because the volatile poison can be destroyed by heat in the process of preparation, both varieties yield a wholesome food. The root in powder form is used to prepare Farinha, a meal made into thin cakes sometimes called "Cassava" bread. The starch of cassava yield a product called Brazilian arrowroot. In Florida, where sweet cassava is grown, the roots are eaten as food, fed to stock, or used in the manufacture of starch and glucose. In Africa, Garri is a popular food. Tapioca is another popular food product made from cassava. The term "tapioca" is used to designate products made from cassava like starch, dried chips etc. Tapioca is also replacing mung bean starch a prime materials for making clear starch

noodles, however, tapioca starch products made from cassava like starch, dried chips etc. Tapioca

need modifcation to produce a gel with the same strength as mung bean starch, which is very high in amylase (International cassava starch institute Denmark, 2006).Cassava fibre or pomace is the residue obtained after extraction of starch from cassava nuts.

It has been reported that cassava has a crude fibre of about 24% and 55.70% Nitrogen free extract (Devendra, 1977 and Louis, 2002). The inclusion of this by product in broken and pig diets has been as much as 14% (Aritonary and Silalahi, 1992), 30% in cattle diets (Sumagkut *et al.,* 1976) and 20% in sheep diets (Puliengan and Buakti, 1984) .Cassava fibre can be incorporated as functioned foods for diabetes, treating bowel cancer, preventing constipation, coronary heart diseases, reducing cholesterol (Christian, 2004).Functional foods are food that have beneficial effects in a way that is relevant to either improved state of health and well being of disease.

Cassava utilization

- Production of Gari
- Production of Fufu flour
- Production of High quality cassava flour
- Production of Tapioca
- Production of Lafun
- Production of Kpokpogari
- Production of Glucose syrup
- Production of Glue
- Production of Ethanol
- Production of Composite bread

- Used in Livestock feed industry as Livestock feed products
- Starch in paper, etc.
- Starch in food
- Starch production for textile (International cassava starch institute Denmark, 2006)

2.2 Processing of Cassava Starch

The raw cassava is peeled, washed and grinded in hammer mill. After this, it is mixed in mixer for forty five minutes (45 minutes), after it is discharged into a separator.

In the separator the starch and fibre were separated. The starch goes into a sediment tank for six hours, the water suspension is discharged from the starch The starch is now packed into sack and pressed to remove the remaining water. The starch is discharged from the sack into hammer mill for further milling. It is passed into roasting drier and dried. It is finally grinded in hammer mill. The fibre separated is a waste product but can be dried in roasting drier for certain purpose such as poultry feed, fish feed or pig feed (Source: Matna Foods Ltd).

2.3 PREBIOTICS

Prebiotics are non-digestible food ingredients which selectively stimulate the growth or activities of lactobacilli or bifidobacteria in the colon thereby improving health (Aiba, 1998). They are growth factors that promote population of beneficial bacteria in the colon. They belong to classes of chemical compounds called oligosaccharides. Prebiotics are principally oligosaccharide, others are disaccharide and polyols.

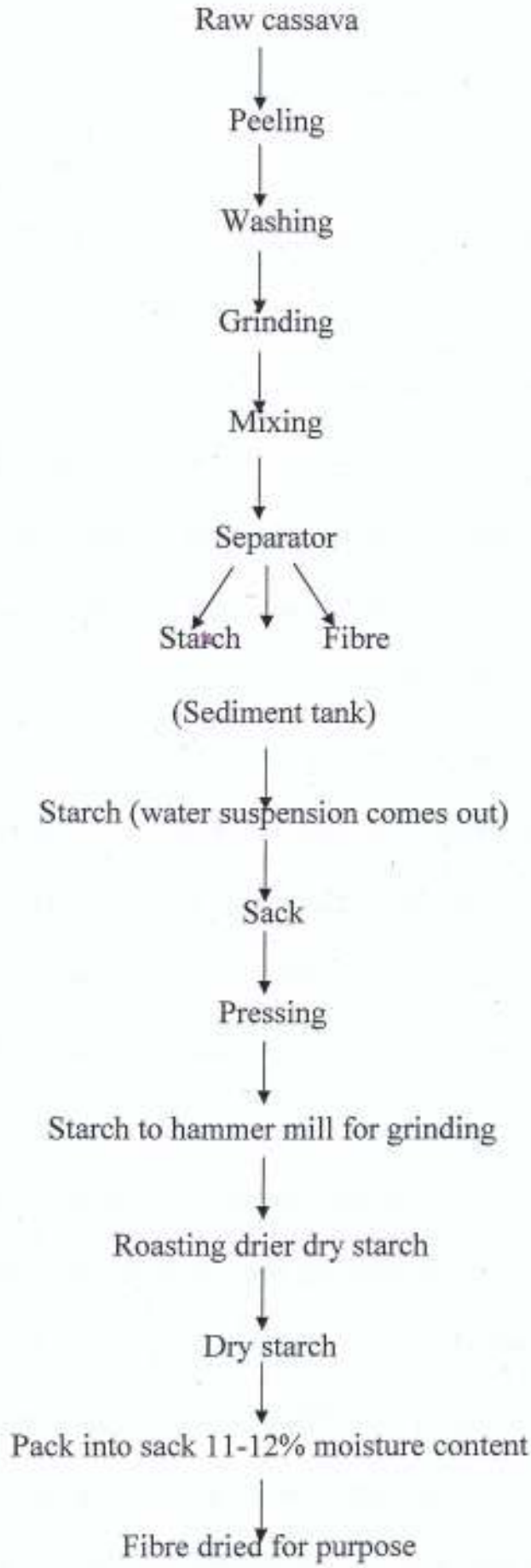


Figure 2.1. Flowchart for processing of cassava starch.

Oligosaccharides are water soluble polymers of a few condensed monosaccharides. Examples of oligosaccharides are: Fructo- oligosaccharide, Inulin Lactose, Lacto sucrose, Lactulose. The degree of polymerization range from two to about ten. The number of possible oligosaccharides is very large, but the most commonly found are maltose, sucrose lactose, cellobiose, raffinose and stachyose. Scientist reported that raffinose and stachyose are found in peas and beans (Suanne, 2000). Raffinose and stachyose are found in peas and bean. Suanne (2000) reported that the most popular is a sucrose molecules linked to two or more sucrose molecule. This can be purchase in syrup and crystalline forms and can also offer some of the physical benefits of sugar serving, as bulking agent, humectant and sweetner (Bruce, 2000). These molecules are naturally found in some foods but when sold in a purified form, they are often made through a chemical synthesis catalyzed by enzymes.

It has been reported by scientists that all non-digestible oligosaccharides have prebiotic properties, and inulin, fructo- oligosaccharide, and (to a lesser degree) galacto- oligosaccharides dominate the group (George, 1999). Fructo-oligosaccharides have energy value of 6KJ/g. They have no genotoxic, carcinogenic, or toxicological effects and are mild laxative, although flatulence is often a complaint when large doses are taken. In controlled dietary studies with human volunteers; fructo- oligosaccharides (15g / day) increase faecal bifidobacteria numbers 10-fold while reducing clostridia and enterobacteria counts, showing that species composition of the microbiota could be selectively manipulated through diets. Suanne, (2000) said most prebiotics are beneficial to

bifidobacteria (bifidogenic) however some particularly the galacto-oligosaccharides and lactulose derived from catalytic treatment of lactose from milk benefit lactobacilli. Some prebiotic are known to result in gastrointestinal discomfort-lactose for example, is a readily available carbohydrate utilized by bacteria. However too much lactose in the colon can result in gaseous by product that is responsible for lactose intolerance. Therefore many companies' selling prebiotics recommend that people who take prebiotics supplement start with a small amount and then ramp as body become accustomed to them. Lipid plays a major role in measuring prebiotics activities and in vivo-studies quantify fatty acid production to guage the effectiveness of a variety of prebiotics. John (2000), reported that lipids are for the most part, extremely well absorbed by humans, so that any fermentative lipids reach the lower bowel to serve as prebiotics.

2.4 Probiotics

Probiotics are live microbial food supplement or viable organisms that have beneficial effect. They are used to bring balance between the beneficial and non-beneficial bacteria in the intestine (Roberfoid, 2001). The best known are lactic acid bacteria and bifidobacteria which are widely used in yogurt and other diary products. Probiotics have being reported by scientists as innovative approach to antibiotic in treating gastrointestinal discomfort or diseases (Fuller, 1992).

These organisms are non-toxigenic, non-pathogenic, retain viability during storage and survive passage through the stomach and small bowel

(Suanne, 2001). Examples of bacteria and yeast used as prebiotics are *Bifidobacterium longum, B. breve, B. infants B. bifidium, B. adolescents, Lactococus cremoris, L. lactis, Streptococcus thermophilis, L. gassen Saccharromyces boulardii, S. cerevisiae* (Fuller, 1989).

2.4.1. BENEFICIAL EFFECTS OF PROBIOTIC AND PREBIOTIC

Prebiotics have been revealed to be beneficial to the health of the intestinal cells by fermentation of fibre to short chain fatty acids (Suanne, 2000). This short chain fatty acids in particular butyric acid have been shown to eliminate cancer cells. Prebiotics have also helped to produce a food for beneficial bacteria (probiotics) (Suanne, 2000).

Furthermore, lactobacilli have being widely used in treating diarrhea disease such as *Pseudomembranous colitis*, but the results have been mixed (Gotz *et al.,* 1979). Feeding freeze dried powder of *L. acidophilus* alc Do1748 had no effect on patients with *Pseudomenbranuous colitis,* but Lactobacillus GG successfully eradicated *Clostridium* sp. in five patients with relapsing colitis. (Arronssum *et al.*, 1987; Gorbach *et al.,* 1987). Viable lactobacilli approximately 10 were fed and concomitant reductions in titre of *C. dificile* toxin in stool. The patients also improved after further administration of antibiotics and prebiotic treatment. *Lactobacillus* GG had been shown to colonise the gut and secrete antimicrobial product that was active against *C. difficile* and range of other micro-organisms (Hidaka *et al.,* 1987).

However, not all lactobacilli are effective in combating enteric pathogens. Twenty health volunteers were given a commercial product

containing *L. acidophilus* and *L. bulgaricus* and then challenged with enteroxigenic *E. coli.* Clement *et al.,* (1981) reported they did not differ in respect of attack rate, incubation period and duration of illness from control subjects given a placebo. The Yeast *Saccharomyces boulardiis* has also been used in studies on prevention and Treatment of diarrhea associated with *C. difficile* infection. Surawicz *et al.,* (1989) reported that of 180 patients in a double blind controlled studies, 9.5% of those receiving the prebiotic had diarrhea compared to 22% of the controls given placebo. The authors concluded that prophylactic use of the prebiotic reduced the incidence of diarrhea associated with *C. difficile* infection, although *Saccharomyces boulardii* did not prevent acquisition of the pathogen.

2.4.2. Antimutagenic activities of prebiotics and probiotics

Probiotics and prebiotics seem to be antimutagenic in several ways. Gram positive and Gram negative bacteria bind mutagenic pyrolysates produced during cooking at a high temperature, and studies with lactic acid bacteria that they can be living or dead, since the process occurs by absorption of mutagen to carbohydrate polymers in the cell wall (Zang *et al.*, 1990). Lactobacilli also degrade carcinogens such as N nitrosamines, which may be important if the process occurs at the mucosa surface. Co-administration of lactulose and *B longum* to rats injected with the carcinogen Azoxymethane reduced intestinal aberrant crypt foci which are preneoplastic markers (Challa *et al.*, 1997; Aderiye, 1999). Purified bifidobacterial cell walls have antitumour activities in

that the cell wall of *B. infantis* induces activation of phagocytes to destroy growing tumour cells. Bifidobacteria probiotics reduced colon carcinogenesis Induced by 1, 2-dimethythydrazine in mice when used with fructo-oligosaccharides and inhibited liver and mammary tumours in rats (Aderiye,1999). When fructo-oligosaccharides at the rate of 4g/day was given to healthy volunteers in the form of chewable tablets, it increased the intestinal bifidobacteria and reduced appreciably the faecal activities of enzymes involved in producing genotoxic metabolites such as β glucuronidase and Glycocholic acid hydroxylase Buddinton *et al.,* (1996) revealing the potential of prebiotics and probiotics to reduce or prevent carcinogenesis.

2.4.3. Immunity of Probiotics

The colonic microbiota affect mucosal and systemic immunity in the host (Famularo *et al.,* 1997). Intestinal epithelial cells, blood leucocytes, B and T lymphocytes, and accessory cells of the immune system are all implicated. Bacterial products with immunomodulatory properties include endotoxic lipopolysaccharide, peptidoglycans, and lipoteichoic acids (Standiford *et al.,* 1997). Lipoteichoic acids of Gram positive bacteria such as bifidobacteria possess high binding affinity for epithelial cell membranes and can also serve as carriers for other antigens, binding them to target tissues, where they provoke an immune reaction. Yoghurt lactobacilli bind in vitro to peripheral blood CD 4 and CD8 T lymphocytes but not to the cells. While lactobacilli which adhere to human intestinal epithelial cells are capable of activating macrophages. There

are yet no experimental data to support the immunostimulatory properties of non- digestible oligosaccharides in humans. However, probiotic organisms interact with the immune system at many levels including cytokine production, mononuclear cell proliferation, macrophage phagocytosis and killing, modulation of autoimmunity, immunity to bacterial and protozoan (Solis *et al.*, 1993). *In vitro*. Bifidobacteria induce formation of large amount of immunoglobin A (lgA) (yasui *et al.*, 1991). Of 120 strains tested belonging to a number of species *(B. animalis. B. longun. B. breve.)* Three *B. breve* strains and one *B. longum* isolate induced appreciable synthesis of lgA. This was confirmed *in vivo* when mice given one of the *B. breve* strains together with cholera toxin had augmented immune responses in lymphoid tissue associated with gut. In mice, *B breve,* fed in fermented milk, include macrophage-like cells in Peyer's patches to realize a factor that stimulated mitosis in B cells and enhanced production antibodies against food allergens and pathogens (Yasui *et al.*, 1992). *L acidophilus* and *B bifidum*, given in capsule form elderly people, effected appreciable changes in inflammatory and immunological responses. They reduced colonic inflammatory infiltration considerably but did not affect the numbers of B Lymphocytes and T lymphocytes. However, study subjects had a greater increase in B cells in peripheral blood than did controls. Lactobacillus GG was used to manage Cow's milk allergy and ectopic eczema in 31 infants aged 2-16 months. It resulted in a considerable improvement in their condition and reduced faecal excretion of a antitrypsin and tumour necrosis factor through α an improvement in antigen elimination by the gut mucosal barrier.

2.5 HIGH FIBRE FOODS

Scientists reported that high fibre food help in treatment of cancer, diabetes reduce cholesterol level, and can also reduce the blood sugar (weight loss) (Christian, 2004). Diets play a major role in the treatment of diabetes. The diet may be used alone or in combination with insulin injections or oral hypoglycemic drugs (Christian, 2004). In case of Insulin dependent diabetes mellitus, the energy intake is based on needs for normal growth and development, physical activity and maintenance of desirable body weight. In case of non-insulin dependent diabetes mellitus, majority of patients are overweight or obese. Therefore, certain restriction is required to achieve a desired weight. Diabetes has already increased by one third during the 1990s, due to the prevalence of obesity and ageing population. There are currently more than 194 million people with diabetes world wide but if nothing is done to slow the epidemic the number will exceed 333 million by 2025 according to the international diabetes federation (Christian, 2004). Examples of high fibre foods are whole cereal like whole wheat, dalia, whole wheat flour, pulses like whole dals and dals with husk, vegetables like peas, beans, fruits like apple, peaches, plums, guava etc. High fibre foods provide nutrients for the bacteria in large intestine

2.6. THE GUT MICROFLORA AND HUMAN HEALTH

Starches are important as energy sources for human and also for their interactions with the gut microflora throughout the digestive tact. Largely,

those interactions promote human health. In the mouth, less gelatinized starches may lower risk of cariogensis. In the large bowel, starches which have escaped small intestinal digestion (resistant starch) together with proteins, other undigested carbohydrates and endogenous secretion are fermented by the resident microflora. The resulting short chain fatty acids contribute substantially to the normal physiological functions of the viscera . Specific type of resistant starch (e.g the chemically modified starches used in the food industry) may be used to manipulate the gut bacteria and their products (including short chain acids) so as to optimize health in the upper gut. These starches may assist in the transport of probiotics organisms thus promoting the immune response and suppressing potential pathogens. However, it appears unlikely that current probiotics organisms can be used to modulate large bowel short chain fatty acids in adults although resistant starch and other prebiotics can do so. Suggestion that starch may exacerbate certain conditions (such as Ulcerative colitis) through stimulating the growth of certain pathogenic organisms appear to be unfounded short chain fatty acids may modulate tissue levels and affects growth factors in the gut and so modify gut development and risk of serious disease, including colon-rectal cancer However, information on the relationship between starches and the microflora is relatively sparse and substantial opportunities exist both for basic research and food product development.

2.6.1. Gastrointestinal Bacteria and Human Health

The microbial population of the digestive tract is largest in the caecum and proximal colon with fewer numbers and species in the mouth, stomach and small intestine. However, these smaller population can be important for health outcomes. Oral colonization (especially by *S. mutans*) varies in individual and is thought to contribute to cariogenesis through leaching of calcium from enamel by organic acids (lactate, acetate) produced by metabolism of dietary carbohydrate (Bibby, 1982). This is a condition of apparently low mortality. Even so, a median estimated cost of dental caries in Australia in 1992 was $478.000.000 (Crowley *et al.,* 1992), which gives some indication of the costs which can be associated with diet-related disease.

Fluoridation of the water supply is an effective mechanism for control and limiting Damage (Morgan *et al.,* 1998) but does not attack the primary problem. An effective preventive strategy appears to be oral hygiene plus limiting the supply of substrate to minimize production of organic acids from fermentable carbohydrates at or near the tooth surface.

The relatively sparse flora of the health stomach is usually less than 10^3 organisms per gram of contents, comprising chiefly facultative anaerobes such as lactobacilli, staphylococci and streptococci. A specific organism (*H. pylori*) appears to infect about 60% of the adult population in more affluent countries Rates of carriage are much higher in some populations which may reflect the possibility that the organism is a zoonosis derived from domestic animals

(Dore *et al.,* 1999). *H. pylori* is found adhering to the gastric epithelium and infections with particularly virulent strains are associated with reflux, gastritis, surface ulceration and cancer (Ernst, 1999). The bacterium secretes an enzyme urease which liberates free NH_3 from urea. NH_3 is a known cytotoxic agent and carcinogen and it is thought that it (or more accurately, NH_4^+) contributes to the local tissue necrosis, ulceration and, ultimately, cancer (Tsuji *et al.,* 1997). In global terms, gastric carcinoma is a major cause of morbidity and mortality (World Cancer Research Fund and American Institute for Cancer Research, 1997).

Eradication of the bacterium through antibiotic therapy is an obvious option for primary prevention but may not be entirely satisfactory for two reasons. Firstly, a direct connection between infection and gastritis which proceeds through ulceration to gastric carcinoma is yet to be shown. Secondly, it is possible that *H. pylori* may be involved to some degree in the maintenance of normal gastrointestinal motility. Affected individuals in whom the organism has been suppressed or eradicated can develop reflux oesophagitis (Richter *et al.,* 1998). This may be associated with a rise in the incidence of Barrett's oesophagus believed to predispose to increased risk of oesophageal carcinoma (Heatly and Guillou, 1997). It appears that control, rather than eradication, of *H. pylori* may be a preferred option. In addition, there is evidence implicating *H. pylori* (and other organisms) in the pathogenesis of human cardiovascular disease.

Treatment of patients with unstable angina with an antibiotic against *Chlamydia pneumoniae* for 30 days lowers the clinical endpoints for up to 6 months (Gurfinkel *et al.,* 1999*). H. pylori has* been shown to be present in atheromatous plaques (Ellis,1997).However, a direct causal role for it and other candidate infectious agents in atherogenesis have yet to be proven (Danesh *et al.,*1998). The gut microflora, however, may influence coronary heart disease (CHD) risk by modulating serum lipid concentrations. Circulating levels of low density lipoproteins (LDL) and high density lipoproteins (HDL) cholesterol, which are established risk factors for CHD appear to be influenced by the population profile and metabolic activity of the colonic microflora (Jenkins *et al.,* 1999).

2.6.2. Starches, Resistant Starch and the Microflora

Starches can influence the gut microflora simply through the provision of extra substrate. This may occur in the mouth as fermentable carbohydrates (particularly sucrose and other disaccharides and monosaccharides) have been implicated in caries formation (Bibby, 1982). The highly gelatinized starches found in processed foods may be even more important. Highly gelatinized starches stick to teeth on chewing (Akerberg *et al.,* 1998) and oral carboxylic acid production is greater with foods which favour this adhesion (Kashket *et al.,* 1996).It seems that factors effecting starch adhesion and/or fermentation could lower caries risk so that processed and unprocessed foods containing resistant starch would affect risk of cariogenesis. Adverse reactions to resistant starch (RS) have been reported under some circumstances. One area of considerable economic

significance is the weaning of piglets where significant mortality accrues from swine dysentery due to a specific organism, *Serpulina hyodysenteriae*. Feeding of RS and fermentable (guar gum) leads to raised large bowel short chain fatty acid colonization with this bacterium and appearance of pathological clinical symptoms (Pluske *et al.,* 1998). These diets increase the number of total culturable bacteria and colonization by synergistic *Fusobacterium* (Durmic *et al.,* 1998). It should be noted that commercial weaning of pigs is effected very abruptly through introduction of the new diet. It may be this rapid transition, and the concomitant destabilization of the enteric microflora, which contribute, to the syndrome. Possibly, the phenomenon may be relevant to human gut disease as starches have been implicated also in human inflammatory bowel disease (IBD). Total carbohydrate (starch and sugar) consumption seems to be higher in IBD patients than in controls (Tragnone *et al.,* 1995). An interaction between the gut microflora and the mucosa is a possible contributor to this condition which could be enhanced by fermentable carbohydrate. Immunoreactive antibodies to specific organisms, specifically Klebsiella, have been found in patients with ankylosing spondyliti, a population affected also by IBD (Tiwana *et al.,* 1997). A diet low in starch has been shown to Lower circulating IgA in these patients leading to the suggestion that the presence of Potentially pathogenic organisms in the faecal microflora may be a trigger for these Conditions (Ebringer and Wilson, 1996). If this were to occur, it could be a serious contraindication for greater RS consumption. However, further study has not supported a primary role for *K. pneumoniae* in spondylitis (Smith *et al.,*

1997) or this Organism, *Escherichia coli* or *Listeria monocytogenes* in IBD (Walmsley *et al.,* 1998). Indeed, it appears that these responses may be a consequence of active disease rather than causative. Removal of RS from the diet induces temporary remission through bowel rest as does a low fibre diet (Klurfeld, 1999). Sulphur-reducing bacteria have been implicated in UC through the production of H_2S and hence blocking of short chain fatty acid (SCFA) metabolism by colonocytes (Roediger *et al.*, 1997). This remains to be established as does any role for RS. Disordered fermentation and, possibly, a disordered large bowel microflora have been implicated in IBS. It has been reported that both faecal SCFA concentration and production rates *in vitro* are lower in patients with diarrhea-predominant inflammatory bowel syndrome (IBS) (Treem *et al.,* 1996). More recently, a study *in vivo* showed that H_2 evolution was more rapid in IBS patients although total gas production was the same as in affected individuals (King *et al.,* 1998). Both of these interesting studies were performed with very limited numbers of volunteers and require replication with larger populations.

2.6.3. Modulation of the Gut Microflora and the Role of Starches

The majority of colonic bacteria are saccharolytic, and several bacterial groups,Including *Eubacterium, Bacteriodes, Bifidobacterium* and *Escherichia* can ferment Starch (Brown *et al.,* 1998; Wang and Gibson, 1993). *Clostridium butyricum* and species of *Bifidobacterium* in particular are effective starch utilisers in vitro (Brown *et al.,* 1998). There are data from studies with "human microflora-associated rats" showing that a retrograded high amylase starch

raised large bowel butyrate and the numbers of lactobacilli and bifidobacteria (Silvi *et al.,* 1999). These data demonstrate the potential for RS to modify the microflora specifically (i.e. to act as prebiotics).

2.6.4. Starches

Prebiotics are defined as "non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and (or) activity of one or a limited number of bacterial species already residy in the colon, and, thus, attempt toimprove host health" (Gibson and Roberfoid, 1995). Perhaps one of the best known of these agents is fructo-oligosaccharide (FOS) but certain other carbohydrates appear to have prebiotic potential. Starch and NSP have been regarded as unlikely prebiotics, largely because many of the colonic bacterial species are capable of metabolizing these carbohydrates. However, several *in vivo* studies in experimental animals and humans suggest otherwise.

Alginates, which are used widely in the food industry, appear to be prebiotics (Terada *et al.,* 1996) and RS may also be useful. During studies with a high amylase RS it was noted that the granules developed a particular etching pattern on passage through the upper gut of either pigs or humans (Topping *et al.,* 1997a). This resulted in surface pitting and it was thought that this could enhance the viability of probiotics by providing a surface for physical adhesion. It was confirmed that specific bifidobacteria adhered to this starch (Brown *et al.,* 1998). Feeding trials in mice (Brown *et al.,* 1998) and pigs (Brown *et al.,* 1997) showed that faecal excretion of bifidobacterial was higher when the

animals were fed live *Bifidobacterium longum* with this high amylase starch (Brown *et al.*, 1997).

Total faecal short chain fatty acid (SCFA) and butyrate excretion were significantly higher when the pigs were fed the high amylase starch. In the absence of *B. longum,* excretion of total SCFA and butyrate were 25.4 and 3.2 mmol/day respectively with low amylase Starch, and 60.7 and 9.1 with the high amylase starch. There was no effect of the probiotic on SCFA levels. Further experiment showed that the effects of FOS and the RS on faecal bifidobacteria numbers were additive. Topping *et al.*, (1997b) opined that the action was by different mechanisms. Fructo-oligosaccharide acted as a substrate while resistant starch served as a physical protector. This concept is supported by the longer survival and maintenance of high numbers of viable organisms in yoghurts containing high amylose starch where the effective shelf life was improved by several weeks (Brown et al., 1998). Prebiotic action does not seem to limited to high amylase starches per se but also to chemically modified variants (i.e. RS4) which are also effective. Studies *in vitro* have shown that bifidobacteria can adhere to starches modified through acylation. Octenylsuccinylation, carboxmethy-lation and succinylation (Brown *et al.*, 1998). This adhesion varied by strain and did not extend to *Lactobacillus casei.* These data show that RS2 and RS4 have the potential to act as prebiotics – an attribute which could be especially useful in delivering organisms to regions where they are needed (e.g. to the distal colon).

Several other studies support the prebiotic action of RS and that the effect varies with type Kleessen *et al.,* (1997b) have shown that retrograded potato starch (RS3), but not. native potato starch (RS2), promotes faecal and caecal Lactobacillus counts in rates. A preliminary report showed that consumption of foods containing high amylose starch (RS2) by volunteers resulted in lower faecal counts of Bifidobacterium, and Higher butyrate levels, compared to a cornflake control diet (Rao *et al.,* 1997).

In young pigs fed rice-based diets formulated to increase the quantity of starch (RS1 and 3) flowing into the caecum, counts of bifidobacteria and lactobacilli in the Proximal colon were similar to those in pigs fed a digestible starch diet (Bird *et al.*,1997). Numbers of coliforms, especially *E. coli,* were several log units lower in pigs offered the RS-rich diet. These limited data suggest that certain starches alter the species composition and metabolic activity of the colonic microbiota. Not only is the total amount of starch reaching the colon important, but the chemical and physical properties of the resistant starch seem to be important in determining the prebiotic properties of resistant starch.

Finally we are entreated to buy the "bio" Yoghurts on sale in supermarkets with promises that they will boost our body's natural resistance, promote health digestion, and improve the balance of our gut microflora. This is to be achieved through their content of probiotics bacteria. Even more remarkable is the suggestion that some dietary carbohydrates can selectively stimulate growth of these organisms when they occur naturally in our gut and

thus produce the same benefits. If true, this is one of the most important stories to emerge in nutrition and gut microbiology since the turn of the century.

Although there are now many published reports on the use of probiotics in Humans, information on prebiotics is limited. Consequently, many of the health claims made in relation to these substances are unsubstantiated. The ability to target specific organisms in the large intestine for defined. Health promoting purposes will clearly be of great value and needs to be developed. However, there are considerable differences in bacterial carbohydrate utilization patterns between strains as well as Species and this is particularly important for the development of prebiotics. A few Strains have been identified as having health promoting potential *in vivo,* but non-specific increases in total bifidobacteria or lactobacillus numbers in the large bowel through the introduction of functional foods will probably be of questionable benefit to health.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Sample and Sample Collection

The wet cassava (*Esculenta sp)* fibre sample was collected from Matna food Ltd Akure, Ondo State. The sample was brought to the Department of Food Science and Technology, Federal University of Technology, Akure for analysis and processing.

3.2. Processing Methods

3.2.1. Processing of Cassava fibre into cassava fibre biscuit.

Cassava fibre biscuit (Cracker biscuit) was processing as described by modifying the method of Duncan Technology of cracker biscuit (Duncan, 2000).The method involves mixing of ingredients and fermentation. The cassava fibre was dried at temperature of 102°C for 30 minutes and the temperature was reduced to 60°C for 30 minutes and allowed to cool. The fibre was mixed with wheat flour in ratio of 50\50, 60\40, 70\30 and 80\20 respectively.

PRODUCTION OF BISCUIT

The wheat flour and cassava fibre were mixed in ratio of 50\50, 60\40, 70\30, were used to make a cracker like biscuit. 60/40 and 50/50 were eventually selected for further study being the best. Other ingredients added are Salt, Fat, Sugar, water as presented in Table 3.1. These were mixed for about 3mins. Yeast was added. It was covered with polythene film and allowed to ferment for 1hr. The dough was cut into sizes and was put in tray at baked at

120°C for 30minutes. The fibre-based biscuit was removed and allowed to cool then milled for experimental analysis. The recipe for the production of cassava fibre composite biscuit is presented in Table 3.1. and method of production cassava fibre biscuit is shown in figure 3.1.

3.3 PRODUCTION OF DEHYDRATED FERMENTED MAIZE (OGI) FLOUR

Fermented maize (ogi) was prepared using the method of Akingbola *et al.*, (1987) that gave higher protein recovery and better quality porridge. Maize was sorted and steeped in water (1:2 w/v) for 72 hours. After decanting the steeping water. The maize was milled in an attrition mill and wet sieved (1:8 w/v) through a sieve (iron) dimension. The through were left to sediment for 12 hours before decanting the water. The fresh Ogi was dried at 60°C for 24 hours and milled (mesh size, 100) the method of processing of maize into Ogi is illustrated in Fig. 3.2

3.4. PROXIMATE ANALYSIS

3.4.1 Determination of moisture content

The moisture content was determined by using an oven drying method, which was based on weight loss of was, due to evaporation. A clean and well labeled dish that has been oven dried was weighed by using a Mettler balance and their respective weights were recorded (WI). Enough samples were added into the dish and weighed (W2). The glass dishes containing samples were transferred from the desiccators into

Table 3.1: Recipe for production of cassava fibre wheat composite biscuit

Component	Weight (g)
Fibre & wheat	**300**
Salt	**10.0g**
Fat	**300**
Sugar	**15.0g**
Water	**100ml**
Yeast	**50**

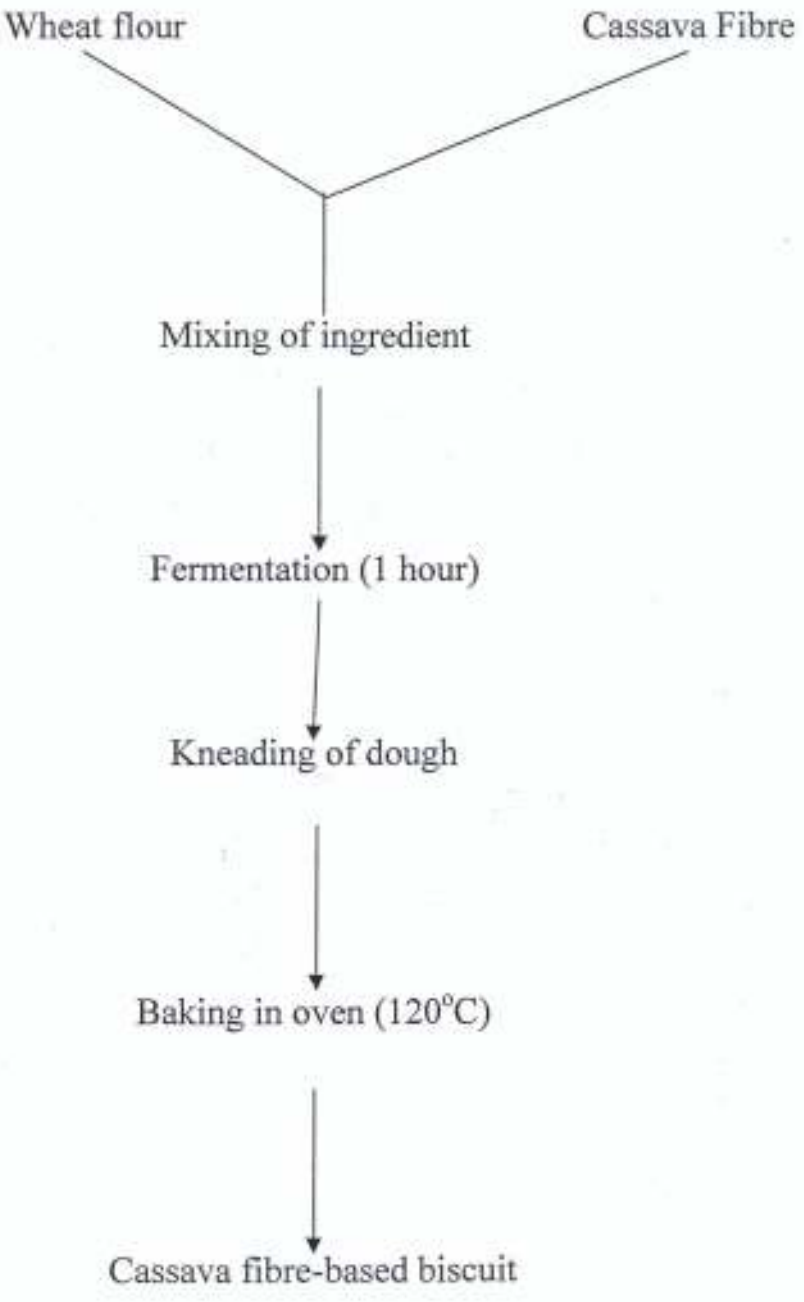


Figure 3.1: Flowchart of production of cassava fibre based biscuit

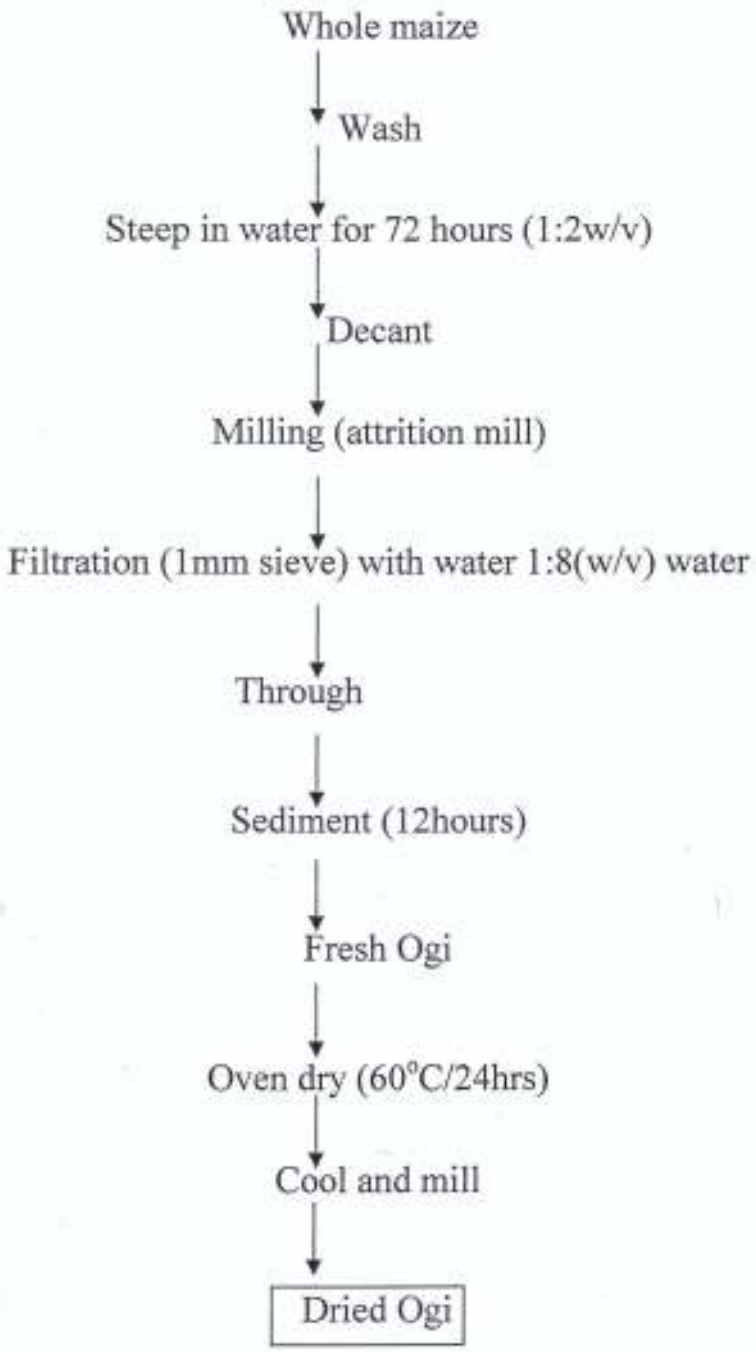


Figure 3.2: Flowchart of production of fermented maize (Ogi).

the thermosetting oven at about 78°C for 3 hours (AOAC 1990.) The dish was transferred from the oven to desiccators and was cooled for about one hour and weighed. The step was repeated three times for constant weigh (W3). The loss in weight during drying in percentage of moisture content was determined (A.O.A.C, 1990)

$$\% \text{ moisture content} = \frac{\text{Loss in weight}}{\text{Weight of sample before drying}} \times \frac{100}{1}$$

$$= \frac{W2-W3}{W2-W1} \times \frac{100}{1}$$

3.4.2 Determination of fat content

The soxhlet extraction method was used. The weight of free filter paper was determined (W1). Sample (3g) was put inside and weighed (W2). The filter paper and sample were wrapped with a twine and packed into a fat free thimble. The thimble was inserted into the soxhlet apparatus and extraction under flux was carried out with petroleum either 2/3 full of extraction. The sample were the 500 ml round bottom flask at temperature ranging between 40-60°C for 8 hours. At the end of the extraction the samples were oven dried at 100°C for 30 minutes to evaporate the solvent and the samples were allowed to cool in a desiccators and later weighed (W3) (Kirk and Sawyer, 1991).

Fat extraction from a given quantity of sample was deduced as:

$$\% \text{ fat} = \frac{\text{Weight of fat extracted}}{\text{Weight of sample before extracted}} \times \frac{100}{1}$$

$$\% \text{ fat} = \frac{W2-W3}{W2-W1} \times \frac{100}{1}$$

3.4.3. Determination of Ash Content

The ash content of the sample was determined by first placing a silica dish or crucible into a muffle furnace for about 15minutes at 350°C. The crucible was removed, cooled in a desiccator weighed (W1) and 0.584g of the sample was added into the crucible and weighed (W2).

The crucible with ash was taken into the muffle furnace. And the temperature was slowly increased from 200°C.to 550°C. Ashing continued until grey or whitish substance was obtained. The crucible was then cooled in a desiccator and weighed thereafter (W3) (A.O.A.C, 1990)

$$\% \text{ Ash} = \frac{W3-W1}{W2-W1} \times \frac{100}{1}$$

3.4.4. Determination of Crude Fibre content

The crude fibre was determined by transferring 5g of sample into 500ml conical flask. Two hundred milliliters of boiling 1.25% H_2SO_4 was added and brought to boiling within one minute and allowed to boil gently for 30 minutes. Then, the sample was filtered through a filter paper with suction using Bucliner funnel well hot distilled water and separated the materials back into the flask by

using spatula. Two hundred milliliters of boiling 1.25% Sodium hydroxide and few drop of antifoaming agent was added brought to boiling within one minute and boiled gently for 30 minutes.

The sample was filtered through a filter paper and washing with hot distilled water. Rinsed four times with hot distilled water, once with 10% hydrochloric acid, four times again with hot distilled water, twice with methylated spirit and three times with petroleum either (or methylated spirit). The residue was savaged into the crucible after drained, dried in the oven at 105°C, cooled in a desiccator and weighed (W2). After that it was placed a muffle furnace at about 300°C for about 30 minute, and sample weighed again (W3) (Kirk and Sawyer,1991).

$$\% \text{ Crude fibre} = \frac{W2-W3}{W1} \times \frac{100}{1}$$

3.4.5 Determination of Protein Content

Nitrogen determination was determined in the cassava and fibre biscuit samples by using a Kjelahl method. The Kjeldahl method was carried out three steps.

The first step involved the digestion of the sample, in which 0.5g of sample was digested with concentrated H_2SO_4 (5ml) together with half tablet of kjeldahl digestion catalyst till a clear solution was obtained.

The second step was the distillation stage. This involved steam distillation of the digested and the dilute sample to which 40% Sodium hydroxide solution was added to make it alkaline. Three drops of methylated

indicator was added to the receiving flask containing 10ml of 2% boric acid solution to produce a pink solution.

The cloudy nature of the sample indicated that Sodium Hydroxide was in excess. The distillation was carried out with a punched cork closed with the end of the condenser touching the surface of the acid solution in the receiver flask. As distillation proceeds, colour changed from pink to deep blue indicating the presence of ammonia. Distillation continued until the distillate was about 50ml after which the delivery end of the condenser was unused with distilled water.

The final step was the titration stage where the received ammonia in the boric acid (H_3BO_3) was titrated with 0.01m HCL solution (Kirk and Sawyer, 1991).

$$\% \text{ Nitrogen} = \frac{\text{Titre value X 0.01 X volume + digest (V1) X 100}}{\text{Weight of the sample of digest used (V2)}}$$

Where T = Titre Value

M = Molarity

V1= Volume of digested sample

V2= Volume of digested sample used for distillation

W= Weight of sample

% Protein= Nitrogen X 6.25

3.5. Enumeration of bacteria

Freshly voided faecal materials were collected and pooled from each rat at day 1, day 7, day 14, day 21and day 28. This was done to confirm the *Lactobacillus* count and enterobacteria, *E coli count.*

The faeces were homogenized in normal saline and serially diluted. Sample (1g) of faeces from the rat was diluted to make 10^{-1} dilution, further dilution was made by pippeting 1m of 10^{-1} to another 9ml distilled water. This continued until 10^{-4} dilution were obtained. It was ensured that for each dilution separate sterile pipette was used. One ml of the dilution 10^{-3} and 10^{-4} was then pippetted into a sterile MRS Agar and EMB Agar for the enumeration of *Lactobacillus* count and *E coli* count. The agar was prepared according to the method of the manufacturer on the label. The plates were incubated at 37°C for 24 hrs and colony forming units on the plate were recorded.

3.6. Experimental Diet/Feed

The diet contained supplement mixture of glucose 50g, sucrose 100g, non-nutritive cellulose 50g, vegetable oil 100g, vitamin premix 20g, oyster shell 10g, bone meal 20g, Sodium Chloride 2.5g. Ogi flour 647.5g to make 1kg of the diet or correspondingly for 50/50 fibre biscuit and 60/40 fibre biscuit respectively. Table 3.2 showed the percentage of each ingredient in the diet

3.7. Experimental animals

Before the arrival of the rats, the rat house and cages were properly cleaned and disinfected using disinfectants (Dettol). The cages were properly arranged and fitted with feeders and the dispensing bottles that can comfortably drop water when nibbled by the rats. The feeders were firmly placed in position to eliminate feeder spillage. A total of 16 weanling albino rats of Wistar strain were purchased from the Department of Animal Production and Health, Federal

Table 3.2. Experimental Diet/Feeds.

Feed formulation (Nutritional composition of Ogi diet and cassava fibre biscuit)

Ingredients	Quantity (g/kg)
Ogi flour and or cassava fibre biscuit	647.5g
Sucrose	100
Non nutritive cellulose	50
Vegetable oil	100
Vitamins premix	20
Oyster shell	10
Bone meal	20
Nacl	2.5

Source: Fanimo, 1991.

University of Technology Akure, Nigeria. The rats 5-6weeks old were divided into four groups of four rats per group the initial weight was taken. The rats were caged with facilities for separate collection of faeces.

3.7.1. Management of experimental animals

Feed and water were provided *ad libitum* for a week to the rats. The rats were fed for 28 days, meanwhile the weight of the rats were taken every day and faeces were collected into polythelene bags.

3.7.2. Slaughtering and collection of Blood.

On the 28th day of experiment, all the rats were starved for about 3 hours and weighed. Each rat was killed by cervical discoloration and the blood collected into bottle coated with Ethylene diamine tetrachloroacetic acid (EDTA) powders as anticoagulant. The bottles were immediately capped and the content mixed gently for about a minute by repeated inversion. Blood collected this way was used for various haematological studies.

3.7.3Haematological studies

From the blood collected (3-9) ml, the packed cell volume (PCV%) was Estimated by spinning of each sample in heparinized capillary tubes in an Haematocit micro-centrifuge for 5minutes. White blood cells (WBC) were determined as described by Lamb (1981) the haemoglobin concentration (HBC) was determined using cyano-methamoglobin Lamb (1981).

Determination of packed cell volume (pcv)

The haematocrit method was used. Capillary tubes were filled with blood

by capillary action up to 2/3 the length of the tube and the vacant end sealed in the flame of a burner. The sealed tubes were centrifuged for 10 minutes at 3000rpm in Hawskley microhaematocrit centrifuge. Each tube was then read using the special graphic reader. The value obtained was expressed as a percentage of the total blood volume and recorded.

Determination of haemoglobin concentration (Hb)

Blood sample (0.02ml) was pipetted into a test tube containing 5ml of Drabkin's solution. The content was then mixed thoroughly by rotation. The optical density at 540 nm of the resulting solution was read using the spectrophotometer. The corresponding haemoglobin concentration was read on a standardized calibration curve and recorded

Determination of white blood cell count.(WBC)

Blood was drawn up to 0.5 mark on the stem of the white blood cell pipette and followed up with diluting fluid sucked up to the 11 mark. The pipette content was shaken for 3 minutes in a mechanical shaker. Some unmixed fluid in the stem of the pipette was expelled and the Neubauer counting chamber was filled as done for red blood cell count. The cells were allowed to settle for 1 minute. The cell in the ruled site centre square of the chamber were counted under high power magnification. The ruler for excluding and including cell touching line as done for erythrocytes was employed.

3.7.4 Liver Function Test

The liver function test-Alanine amino transferase (ALT)

Reitman Frankel method was used. Sample (0.1ml) was pippetted into test tube of sample and 0.5ml of reagent one was pippetted into test tube of reagent blank and another 0.5ml of reagent one was added into test tube of sample. Distilled water (0.1ml) was pippetted into test tube of reagent blank. These was mixed and incubated for exactly 30mins at 37°C. Reagent 2 (0.5ml) was pippetted into test tube reagent blank and another 0.5ml of reagent 2 was also pippetted into test tube of sample. These was mixed and allowed to stand for exactly 20mins at 20 to 25°C. Sodium hydroxide 5.0ml was added into test tube and another 5.0ml of Sodium hydroxide was added into test tube of sample. This was mixed and the absorbance of sample against the reagent blank was read after 5 minutes in Spectrophotometer at 505nm (Cheesbrough, 1991).

ALKALINE PHOSPHATASE (ALP)

Bessey, Lowry and Brock method was used. Water (1.0 ml) was added into test tube that contained sample (plasma) and1.0 ml of water was also pippeted into another test tube that contained standard. One drop of substrate that contained (4-nitrophenyl) phosphate substrate was pippeted into test tube of standard which contains (4-nitrophenol) and another one drop of substrate into tube of sample. The mixture was mixed and incubated at 37°C. In test tube that contained standard (0.1ml) of standard was added and 0.1ml of sample plasma

was added into test with sample these was mixed and incubated at 37°C for 20mins. The reagent used Colour developer was added to the mixture of sample and standard. The absorbance was read in Spectrophotometer at 550nm (Cheesbrough, 1991).

AMINOTRANSFERASE (AST)

Reitman Frankel method was used. Sample (0.1ml) was pippetted into test tube of sample and 0.5ml of reagent one was pippetted into test tube of reagent blank and another 0.5ml of reagent one was added into test tube of sample. Distilled water (0.1ml) was pippetted into test tube of reagent blank. These was mixed and incubated for exactly 30mins at 37°C. Reagent 2 (0.5ml) was pippetted into test tube reagent blank and another 0.5ml of reagent 2 was also pippetted into test tube of sample. These was mixed and allowed to stand for exactly 20min at 20 to 25°C. Sodium hydroxide 5.0ml was added into test tube and another 5.0ml of Sodium hydroxide was added into test tube of sample. This was mixed and the absorbance of sample against the reagent blank was read after 5 minutes in Spectrophotometer at 505nm (Cheesbrough, 1991). The reagents used are p^H 7.5 phosphate buffer substrate containing 200 mmol/l aspartate and 2 mmol/l a-ketoglutarate and pyruvate standard.

3.7.5 The glucose and the cholesterol test

The glucose determination

Glucose reagent (1000 µl) was transferred each into test tube of blank, of

sample and of standard. Sample (10µl) was transferred into test tube of sample and (10 µl) of standard was transferred into test tube of standard. This was mixed and incubated for 10 minutes at 37°C or for 15 minutes at room temperature. The absorbance was read at 500nm.(Cheesbrough, 1991).

Cholesterol test

Cholesterol reagent (1.0ml) was transferred each into test tube of blank, standard, control and test. Standard (1.0ml) was transferred into test tube of standard and 1.0ml of control was transferred into test tube of control and 1.0ml of test sample was added into the test tube of test. The test tube content were thoroughly mixed after the sample preparation and incubated at room temperature for 10 minutes and then the absorbance was read at 505nm. Spectrophotometer was used (Cheesbrough, 1991).

3.7.6 Determination of Total protein

Each of the blood sample collected was centrifuged and the plasma separated into bijon bottle. The plasma was kept at-20°C until analysed. The total protein was determined by the Biuret method using jenway 6100 spectrophotometer.

Sample (0.1ml) of serum was mixed with 2.9ml of distilled water and 3ml of Biuret reagent was added. Another 3ml of Biuret reagent mixed with 3ml of distilled water was used as blank. The sample was read against the blank for determination of optical densities at wavelength 540nm. The instrument was

standardized using sample of known optical density and zeroed with the blank. The corresponding total protein concentration in g/100ml was read off at calibration curve.

3.7.7. Determination of serum albumin concentration.

Sample (0.2ml) of clear serum was pippetted into a centrifuge tube and 5.8ml of Sodium sulphite solution was added. The solution was mixed thoroughly, 2.0ml of the mixture was pippetted into a tube and another 2.0ml of Sodium sulphite was used as blank. To the suspension in the centrifuge tube was added 1.0ml span ether reagent. The tube was firmly capped shaken vigorously for 10 seconds and allowed to stand for 10 minutes. The tube was then centrifuged at 28rpm for 10 minutes. A volumetric pipette was inserted down to the albumin layer of the mixture was sucked up and ran into the albumin tube. To each of the tube was added 3.0ml of Biuret reagent. The mixture was placed in 37°C water bath for 10 min. The optical densities of the total albumin at 540nm was read in the spectrophotometer. The instrument was zeroed using the blank (Cheesbrough, 1991).

CALCULATION

Albumin concentration in the sample was calculated from the following formula:

Albumin concentration

$$g/l. = \frac{A_{sample}}{A_{standard}} \mathbf{X} \text{ concentration of standard (45g/L)}$$

CHAPTER FOUR

4.0. RESULTS AND DISCUSSION

4.1. Results

4.1.1. Proximate composition of cassava fibre biscuit, cassava fibre and ogi

The proximate composition of the cassava fibre-based biscuit, cassava fibre and Ogi are presented in Table 4.1. The moisture content ranged from 8.0 to 53%, ($P< 0.05$). Crude protein content ranged from 8.8 to15%, crude fibre ranged from 1.0 to32.5%, fat content ranged from 3 to9.0%, ash content ranged from 1.0 to5%, carbohydrate content ranged from 46.8 to77.9% there was significant difference among the proximate composition of the feeds. Cassava fibre had 0.5% protein, 33.0% crude fibre, 1.0% fat, 53.5% moisture, ash 2.5%, carbohydrate 9.5%.

4.1.2. Haematological Studies

The result of the haematological studies is presented in Table 4.2.

The packed cell volume (PCV) value of rats fed on Top feed (Positive control) had significantly higher 41.75% value ($P<0.05$) than other groups. There was no significant difference in PCV values of rats fed on 50/50 fibre-based biscuit and 60/40 fibre-based biscuit with 38.5% and 36.5 respectively. Ogi (Negative control) had lowest PCV 29.0%.

Table 4.1. Proximate composition of Ogi, cassava fibre and cassava fibre-based biscuit.

Sample	Protein	Crude fibre	Fat	Moisture	Ash	Carbohydrate
Cassava fibre	0.5±0.3	33.0±0.3	1.0±0.4	53.5±0.4	2.5±0.2	9.5±0.2
50/50 biscuit	15.0±0.3^{c}	14.1±0.5^{b}	9.0±0.2^{a}	10.0±0.4^{b}	5.0±0.3^{b}	46.8±1.0^{a}
60/40 biscuit	10.0±1.0^{b}	17.1±0.4^{a}	5.0±0.3^{b}	11.0±0.5^{c}	3.0±0.3^{b}	53.9±1.0^{b}
Ogi	8.8±0.1^{a}	1.0±0.2^{c}	3.3±0.2^{c}	8.0±0.^{c}	1.0±0.0^{a}	77.9±0.2^{c}

Values with different superscript on the same row are significant ($P<0.05$)

N.B 60 Cassava Fibre, 40 Wheat Flour, moisture on dry basis for Ogi, and the biscuit and moisture on wet basis for Cassava fibre

Table 4.2. Effect of feeds on some vital haematological factors in rats

Feed group	PCV%	HB%	WBCmm^3.
Top feed	41.75±0.63^a	15.00±0.41^a	11.05±0.48^a
50/50 cassava fibre biscuit	38.5±1.32^{ab}	12.5±0.65^b	12.00±0.71^a
60/40 cassava fibre biscuit	36.50±2.06^b	11.75±0.75^b	12.25±0.25^a
Ogi	29.0±1.06^c	12.5±0.87^b	11.69±0.75^a

Values with different superscript on the same row are significant (P<0.05)

Haemoglobin (Hb)

The result showed that rats fed on top feed had significantly higher Hb value (15.0%) than others $P<0.05$. There was no significant difference in the Hb count of the rats fed on 50/50 fibre-based biscuit, 60/40 fibre-based biscuit and Ogi with 12.5%, 11.5% and 12.5% respectively.

White blood cell (WBC)

There is no significant difference in the WBC values among all the groups fed on Top feed, 50/50 fibre biscuit 60/40 fibre biscuit and Ogi with 11.05mm^3, 12.25mm^3, 11.69mm^3 value respectively.

4.1.3. Liver function tests.

The results of the liver function test Alanine amino transferase (ALT), aspartate amino transferase (AST) and Alkaline Phophatase (ALP) are presented in Table 4.3. The liver function test showed that rats fed on 50/50 fibre biscuit and 60/40 fibre biscuit had very low AST, relative to rats fed with Top feed, although higher than for rats feed with Ogi. The result showed that 50/50 fibre biscuit had 44.5 AST IU/l, 27.00 ALT IU/1, while rats fed 60/40 had 43.5 IU/1, AST, 19.5 IU/1 ALP. Rats fed with Ogi diet had 29.0 IU/1 AST and 29.0 IU/1 ALT. Alkaline phosphatase (ALP) of the rats fed on 50/50 cassava fibre and Ogi diet had values that were significantly lower than other groups with 58.25 IU/1 and 46.0 IU/1. The ALP values of rats fed on Top feed and 60/40 fibre biscuit were 99.75 IU/1, 80.75 IU/l respectively.

Table 4.3 Liver function tests

	Metabolic enzymes		
Feed group	**AST (IU/l)**	**ALT (IU/l)**	**ALP (IU/l)**
Top feed	67.75±0.48^{b}	29.50±0.65^{b}	99.75±9.82^{b}
50/50 Cassava fibre Biscuit	44.50±0.65^{a}	27.00±0.91^{b}	58.25±9.25^{a}
60/40 Cassava fibre Biscuit	43.50±0.65^{a}	19.50±3.07^{a}	80.75±2.53^{b}
Ogi	29.00±1.26^{b}	29.00±3.00^{b}	46.00±3.00^{a}

Values with different superscript on the same row are significant (P<0.05)

4.1.4. Cholesterol and Glucose concentration level

Cholesterol concentration level of the rats

The results of the cholesterol concentrations and glucose concentrations are presented in Table 4.4. The result showed that rats fed on 60/40 fibre biscuit had significantly lower cholesterol value 18.75mg/dl ($P<0.05$) than other groups and significantly different from those fed on 50/50 fibre biscuit and Top feed. Although there was no significant difference in cholesterol level of Ogi diet and 60/40 fibre based biscuit. The result further showed that rats fed with 50/50 fibre biscuit and 60/40 had low cholesterol value. Rats fed with Top feed recorded significantly higher value (42.00mg/dl).

Glucose Concentration level of the rats.

There was no significant difference in glucose concentration of rat fed on 50/50 fibre-biscuit and Top feeds with 71.00 mg/dl and 65.75 mg/dl respectively, while the glucose concentration of the rat fed on Ogi was significantly lower, 63.75 mg/dl ($P<0.05$).

4.1.5. Protein and Albumin values of the rats fed with different feeds.

The results of protein content of the rats assessed are presented in Table 4.5. The report showed that rat with Top feed had significantly higher protein and albumin 5.6 g/100ml and 3.05 g/100ml respectively. There was no significant difference in protein content and albumins of rat.

Table 4.4. Glucose and Cholesterol concentration level of the rats.

Feed group	Cholesterol (mg/dl)	Glucose conc (mg/dl)
Top feed	42.00^{c}	65.75^{ab}
50/50 cassava fibre biscuit	28.75^{b}	71.00^{ab}
60/40 cassava fibre biscuit	18.75^{a}	83.00^{ab}
Ogi	21.00^{a}	63.75^{a}

Values with different superscript on the same row are significant ($P<0.05$)

Table 4.5 Effect of feeds on protein and albumin values of the rats

Feed group	Protein (g/100ml)	Albumin (g/100ml)
Top feed	5.6[c]	3.05[c]
50/50 cassava fibre biscuit	4.955[b]	2.83b[c]
60/40 cassava fibre biscuit	4.74[ab]	2.66a[b]
Ogi	4.40[a]	2.43[a]

Values with different superscript on the same row are significant ($P<0.05$) a least significant,

ab slight difference between b and ab have less value than b,

b significantly different from c with less than c or lower value but higher than ab, and c significantly higher

fed on 50/50 fibre biscuit and 60/40 fibre biscuit. Ogi diet had protein content 4.4g/100ml and albumin values 2.43g/100ml respectively.

4.1.6. Weight of the rats

The result of the weight of the rats assessed with feeds and the average daily feed in-take of the rats are presented in Table 4.6. The weight gain of rats fed on Top feed was significantly higher ($P< 0.05$) 53.7g. There was no significantly different in weight gain of rats fed on 50/50 fibre biscuit and 60/40 fibre biscuit with 47.0g and 43.0g respectively. Weight gain of rat fed on Ogi was significantly lower ($P< 0.05$) 35.3g. The rate of food in-take of the rats fed on Top feed was significantly higher ($P<0.50$) with 5.88g of Top feed in-take per day. There was no significant difference in the rate of food in-take of the rats fed on 50/50 fibre biscuit 60/40 fibre biscuit and Ogi with 5.15g, 4.82g and 5.08g of the average daily feed per day respectively.

Table 4.6. Effects of feed on weight gain of rats

Feed group	Initial Weight(g)	Final Weight(g)	Weight Gain	Feed Intake(g)
Top feed	34.0	87.7^{c}	53.7	5.88^{a}
50/50 cassava fibre biscuit	29.0	76.0^{b}	47.0	5.15^{b}
60/40 cassava fibre biscuit	29.0	72.0^{ab}	43.0	4.82^{b}
Ogi	30.0	65.3^{a}	35.3	5.08^{b}

Values with different superscript on the same row are significant ($P<0.05$)

4.1.7 Microbiological Result

The results of enterobacteria count and total viable count of *Lactobacillus* in rat faeces are presented in Table 4.7 and 4.8. The enterobacteria count in rat fed on Top feed in day one ranged from $24x10^3$ cfu/g to $30x10^3$ cfu/g. The result showed clearly there was significant difference in the enterobacteria count in day one. The rats fed on 50/50 fibre biscuit had significantly higher enterobacteria count ($30.0x10^3$) and rats fed on 60/40 fibre biscuit had significantly lower enterobacteria count $24.0x10^3$ cfu/g. On day 7 cfu/g. The enterobacteria count ranged between $15.5x10^3$ to $22.0x10^3$ cfu/g. There was no significant difference in the enterobacteria count in rats fed on 60/40, Top feed and Ogi.

On day 14, rat fed on 50/50 fibre biscuit had significantly lower enterobacteria count $8.0x10^3$ cfu/g. There was no significant difference in rats fed on 60/40 fibre biscuit Top feed and Ogi with $18.5x10^3$ cfu/g, $18.5x10^3$ cfu/g and $20.0x10^3$ cfu/g respectively. On day 21 enterobacteria count ranged between $5.0x10^3$ cfu/g to $18.0x10^3$ cfu/g. The rats fed on 50/50 fibre biscuit had significantly lower enterobacteria count $5.0x10^3$ cfu/g. There was no significant difference in rats fed on 60/40 biscuit, Top feed and Ogi.

On day 28 rats fed on 50/50 fibre biscuit had significantly lower enterobacteria count $3.5x10^3$ cfu/g while rats that fed on Ogi had significantly higher enterobacteria count $16.5x10^3$ cfu/g.

Table 4.7. The *Lactobacillus* count of rats assessed with feeds

	Lactobacillus count (cfu/g)					
Feed group	**Day 1**	**Day 7**	**Day 14**	**Day 21**	**Day 28**	**Total viable count**
50/50 Cassava fibre biscuit	6.0	14.5	11.5	25.5	33.5	$91x10^3$
60/40 Cassava fibre biscuit	6.0	10.0	12.5	12.5	20.5	$61.5x10^3$
Top feed	6.0	11.0	13.0	17.5	14.5	$73.5x10^3$
Ogi	6.5	10.0	11.5	11.5	14.5	$54.0x10^3$

Table 4.8. The *E coli* count of the rats assessed with feeds (enterobacteria)

	E coli count (cfu/g)					
Feed group	**Day 1**	**Day 7**	**Day 14**	**Day 21**	**Day 28**	**Total viable count**
50/50 cassava fibre biscuit	30.0	15.5	8.0	5.0	3.5	$62x10^3$
60/40 cassava fibre biscuit	24.0	21.0	18.5	16.0	12.5	$91.5x10^3$
Top feed	25.5	22.0	18.5	16.0	12.0	$97.0x10^3$
Ogi	28.5	22.0	20.0	18.0	16.5	$105x10^3$

The result in Table 4.7 further showed the *Lactobacillus* count. On day 1 there was no significant difference in the *Lactobacillus* count in faeces of rat fed the control and experimental diet. On day 7 all the rats fed with 50/50 fibre biscuit had significantly higher lactobacillus count $14.5x10^3$ cfu/g ($P<0.05$). The result also showed on day7 that there was no significant difference in *Lactobacillus* count in rats fed with 60/40 fibre biscuit, Top fed and Ogi respetively On day 14 rats fed on 50/50 fibre biscuit had significantly lower lactobacillus count $11.5x10^3$ cfu/g ($P<0.05$). There was no significant difference in rats fed on 60/40 fibre biscuit and Top feed with $12.5x10^3$ cfu/g and $13.0x10^3$ cfu/g respectively. On day 21 rats fed on 50/50 fibre biscuit had $25.5x10^3$ cfu/g significantly higher from other groups fed on other feed ($P<0.05$). There was no significant difference in rat fed on 60/40 fibre biscuit, Top feed and Ogi with $12.5x10^3$ cfu/g, 17.5 cfu/g and 11.5 cfu/g count respectively. On day 28 rats fed on 50/50 fibre biscuit had significantly higher *Lactobacillus* count $33.5x10^3$ cfu/g, Rats fed on Ogi have significantly lower *Lactobacillus* count $14.5x10^3$ cfu/g. Rats fed on Top feed had *Lactobacillus* count next to 50/50 fibre biscuit with $26.0x10^3$ cfu/g while rats that fed on 60/40 fibre biscuit had $20.0x10^3$ cfu/g.

4.2 DISCUSSION

The moisture content of the cassava fibre-biscuit ranged between 10-11.0%. Ogi had moisture content of 8.0% on dry weight basis and protein content of 8.8%, fat content 3.3.% The protein of the cassava fibre biscuit ranged between 10-15% which enhance the growth, repair of tissue and energy formation. The carbohydrate content of the cassava fibre biscuit ranged between 46.0 to 53.9% and that was of Ogi diet is 77.9%.

The carbohydrates contribute to the bulk of the energy of each of the diets and metabolic activities of the animals. Low calories reduce the activities of the animals. The fibre content of the fibre biscuit ranged between 14.1-17.1% the implication is that it improved probiotics activities in the lower digestive system (Suanne, 2000).This is because the fibre enhanced probiotics activities in the lower digestive system as reported by (Suanne, 2000).

The packed cell volume (PCV), Haemoglobin (Hb) of the rat fed on Top feed were significantly higher ($P<0.05$). Packed cell volume is an index of the health of an animal (Schalm *et al.,* 1975; Cheesborough, 1991) A high level of PCV, HB is an indication that the animals (rats) are not anaemic and lower level is a sign of anaemia (Schalm *et al.,* 1975; Cheesborough, 1991). A high count of packed cell volume (PCV) and Haemoglobin (Hb) also indicate high level of protein in the feed (Coward and White head, 1972).

The PCV values of the rats fed on 50/50 cassava fibre biscuit and 60/40

fibre biscuit were 38.5% and 36.5% respectively. This slight difference in PCV values was attributed to the fact that 50/50 fibre biscuit and 60/40 fibre biscult had similar nutritional composition (protein,carbohydrate,fibre,fat). The haemoglobin value of the rats are 12.5% of 50/50 fibre biscuit 11.75% of 60/40 fibre biscuit and 12.5% of Ogi rats group.

The results also show that there was no significant difference in white blood cell count (WBC) for all the four groups. WBC is an indication that there was no infection among the animals. WBC is all important in defending our body against infection. A high count of WBC shows there is infection in the rats (Schalm, 1975; Oyetayo, 2004).

The reports of the liver function test showed that aspartate aminotransferase (AST) and alanine amino transferase (ALT) of rats fed on 50/50 fibre-based biscuit and 60/40 fibre-based biscuit were lower than the negative control. AST is an enzyme that increases in activity in diseases such as severe bacteria infections, malaria, preumonia, pulmonary infant and tumors of organs such as heart and muscle (Cheesborough, 1991).

Lactobacillus can translocate (Bery, 1983) and survive in the spleen, liver and lungs (Bloksma, 1981) in the course of their translocation, they can cause cellular injury that may increase AST level in the serum. A low level of AST was an indication of liver improvement function brought by the effect of the 50/50 cassava fibre biscuit and 60/40 cassava fibre biscuit feed on the rat.

This further revealed that the feed had no toxic substance. ALT is an enzyme that is specific for liver cell damage. This is because ALT is found only in the liver. The lower level of ALT in the rat fed on 50/50 fibre biscuit and 60/40 fibre is also an indication of liver improvement function brought by *Lactobacillus* (Bloksma, 1981). Alkaline Phosphate (ALP) of the rat fed on 50/50 fibre biscuit and ogi fibre biscuit were lower and different from the ALP values of the rats fed on Top feed and 60/40 fibre biscuit. Rats fed with Ogi and 50/50 fibre biscuit had lower ALP values because of the reduced osteoblastic activities in the blood of the rats (Baron *et al.,* 1994). Rats fed with Top feed and 60/40 biscuit had higher ALP due to increased osteoblastic activities and lack of bile flow. Although the rats fed on Ogi had very low AST 44.50 IU/l and 46.00 IU/l ALP, respectively. These values are less than the normal standard which was required for the normal functioning of the liver. We can say that these animals of these group have liver malfunctioning less than the standard according to (Mitruka, 1977). For the normal standard of AST is (46-80), ALT (15-30), ALP (51-128).

The cholesterol concentration of the rat group fed on 60/40 fibre biscuit and Ogi were significantly lower with 18.75mg/dl and 21.00mg/dl respectively. The cholesterol concentration of 50/50 fibre biscuit was lower (28.75mg/dl) than the value recorded for those placed on Top feed. These showed that 50/50 fibre biscuit and 60/40 fibre biscuit can be used to reduce cholesterol concentration. This is because the feeding of the rats with 50/50

fibre biscuit and 60/40 fibre biscuit gave high *Lactobacillus* count in the rats which had direct effect on cholesterol level by assimilation and removal from the growth medium. This has been demonstrated in pigs (Gilliland *et al.,* 1985) and rats (Bertazzoni *et al.*, 2001). Another reason is also that the fat content of the biscuit and Ogi were very low which reduced the cholesterol content. This showed that high fibre food can be used to improve and treat heart diseases (Juliet, 2006). Although Ogi is not among the high fibre food but has very low fat content. High cholesterol content causes heart diseases Artherosclerosis, (Christian, 2004; Aderiye *et al.,* 2005). The 50/50 fibre biscuit and 60/40 fibre biscuit content wheat cereal which is a soluble fibre that also helped in reducing gut cholesterol. The report also revealed that Top feed and 50/50 fibre-based biscuit had with 65.75mg/dl and 71.00mg/dl respectively. These values were within normal level for glucose these show that high fibre foods can be used to maintain or reduce blood glucose. The result of the bacteria counts show that rats fed on 50/50 fibre biscuit have significantly higher *Lactobacillus count* from feacal analysisand significantly lower enterobacteria counts (*E. coli)* with 91.0 $x10^3$ cfu/g and $62x10^3$ cfu/g of lactobacillus count and *E coli* counts respectively. The reasons are that high fibre foods provide enabled environment and acts as substrate for intestinal microorganisms Probiotics which include *Lactobacillus* to grow and multiply and thereby increase the activities of these organisms. These Probiotics produced bacteriocine which is an antibacterial substance which consequently lower the enterobacteria counts (*E coli)* (Juven *et al.,* 1992; Aderiye., 1999). Aderiye (1999) further reported production of the bacteriocine

is affected by P^H and temperature.. Aderiye (1999) revealed both cell growth and bacteriocine activity were much influenced by changes in temperature and pH he said cell grew well at 35°C but no bacteriocine production could be detected at this temperature. Top feed had high *Lactobacillus* counts next to 50/50 fibre biscuit and low enterobacteria count 60/40 fibre biscuit also had higher *Lactobacillus* count next to Top feed but lower enterobacteria counts than rats fed on Top feed. Rats fed on Ogi had significantly lower *Lactobacillus* counts with $54.0x10^3$ cfu/g and significantly higher enterobacteria count *E coli* with $105x10^3$ cfu/g. The factor responsible for this is that Ogi had very low fibre content, low protein. The result of the weight gain showed that rats fed on Top feed have significantly higher weight gain with 53.7g, 50/50 fibre biscuit 47.0g, 60/40 fibre biscuit 43.0g and rats fed on Ogi have significantly lower weight with 35.3g. The factor responsible for these is that Top feed had high fibre content, high protein content vitamins and minerals which enhanced the growth of these animals. Also rats fed 50/50 fibre biscuit and 60/40 fibre biscuit had higher weight because of the high protein content in the biscuit also high fibre content in the biscuit which enhanced the animal growth. The high *Lactobacillus* count is also responsible for the high weight because *Lactobacillus* are growth promoters due to the ability to suppress the growth and activities of growth depressing bacteria and their ability in enhancing absorption of nutrients through the production of digestive enzymes (Fuller and Gibson, 1997). Rats fed on Ogi had significantly lower weight 65.3g. This was

because Ogi had very low protein, low fat and low fibre content although higher carbohydrate content.

Since the rats group fed on 50/50 fibre biscuit and 60/40 fibre biscuit had most of their values between the normal standard values according to Mitruka, (1977) of normal haematological values for rats for Packed Cell Volume PCV (40-50), Haemoglobin HB (11-17) white blood cells WBC (5-13). Alanine amino transferase ALT (15-30), Aspartate amino transferase AST (46-80), alkaline phosphatase ALP 51-128, cholesterol CHOL (10-54), GLUCOSE GLU (50-135), protein PRO (4.7-8.2), albumin (2.7-5.1). We can conclude that 50/50 fibre biscuit and 60/40 fibre biscuit are good prebiotics because they have good anticholesterolmic effect, good liver enzymes values have ability to reduce enterobacteria and to maintain blood glucose.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Cassava fibre-based biscuit would reduce cholesterol in human being cassava fibre-based biscuit would help those with high level of cholesterol in reducing their cholesterol level a great benefit for those with heart problems. Cassava fibre-based biscuit would also improve the liver functioning of the human being, this reduced gastrointestinal cases. Cassava fibre could also reduce enterobacteria bacteria in colon or lower digestive system, this reduce gastrointestinal disease. Cassava fibre-biscuit would also help to reduce glucose level or maintain glucose level.

5.2 Recommendation

1. Cassava fibre should be used in making cassava-fibre biscuit to help those with problems in heart diseases and diabetics.

2. Government should advice those cassava processing factories not to waste their cassava fibre since the importance of cassava fibre have been revealed.

REFERENCES

Abell, L.L., Levey, B.B and Brodie, B.B.(1952). Glucose and Cholesterol determinations and Enzymes *J. of Biol. Chem.* 195:357.

Aderiye, J.B.(1999). Prevention of colonic preneoplastic lesions by the probiotic *Lactobacillus acidophilus. Int J Oncol,* 1999 May, 14(5), 939 - 944.

Aderiye, J.B. (1999). Temperature and p^H conditions that prevail during fermentation of sausages are optical for production of the antilisterial bacteriocin sakacin *J of Appl Environ Microbio.* 1999 Mar, 65 (3), 974-81.

Aderiye, J.B. Odeyemi, A.T., and Olaleye S.A. (2005).Reduction of serum lipid in albino rat using lactic acid bacterial species. *J of Clin MIcrob* 10:8-14

Aguirre, M. M.D. And Willin, L. (1993), Lactic acid bacteria and human clinical infections *Journal of Applied Microbiology* 75:95-107.

Aiba, Y. N. (1998). Lactic acid mediated suppression of *Heliobacter pylori* by the oral administration of *Lactobacillus salvarus* as a probiotic in a gnotobic murine model. *Journal of American Gastroenterology.*93:2097 – 2101

Aiba, Y. N., Suzuki, A.M., Kabir, I., and Takagi, I. (1998). Prebiotics and Probiotics in humans clinical infections

Akerberg, A.K.A., Liljeberg, H.G.E., Granfeldt, Y.E., Drews, A.W.and Bjorck, I.M.E. (1998). An *invitro* method, based on chewing to predict resistant

starch content in foods allows parallel determination of potentially available starch and dietary fibre, *J.* of *Nutrition.* 128:651-660.

Akingbala, J. O., Onochie, E.U., Adeyemi, I.A. and Oguntimehin, G.B. (1987). Steeping of whole and dry milled maize kernels in Ogi preparation, *Journal of Food processing preservation* 11:1-11.

Anderson, A. H Levine, A.S. and levet, M.D. (1981). Incomplete absorption of the Carbohydrate in all purpose wheat flour new *England Journal of Medicine* 304:891-892.

AOAC (1995) Methods of the Association of Official Analytical Chemist AOAC Washington DC.

Arrisson, G. and Roppen, D.L. (1994). Resistant starch chemical structure Versus Physiological function. *Ann Rev. Nutrition* 14:297-320.

Arrisson B. and Barany, P. (1987). *Clostridium difficile* associated diarrhea in uremic patients. *European Journal of Clinical Microbiology.* 6:352-356

ASP, N.G., van Amelsvoort, J.M.M. and Hautvast, J.G.1996. Nutritional implications of resistant starch. *Nutrition Resources Review.* 9:1-31.

Baghurst, P.A., Baghurst, K.I. and Record, S.J. (1996). Dietary fibre, non-starch 35. Polysaccharides and resistant starch-a review in *Food science in Aust.* 48: S3-S35

Baird, M. (1977). Probiotics help boost feed efficiency feed stuffs, 49 11-12.

Baron D.N. A new short text book of chemical pathology, 5th edition Elbs PP 151-156.

Berg, R.D. (1983). Translocation of indigenous bacteria from the intestinal tract in human intestinal microflora in health and diseases hentges D.J. London Academic press PP 333-352.

Bertazzoni, M.E., Benini, A. and Marzotto, M. (2001). Premilinary Screening of Health promotion properties of new Lactobacillus strain: *invitro* and *invivo HEALTH abstract,* Italy

Bibby, B.G (1982). Changing perpectives on dental caries. In : Diet and Dental Caries Changing perspective. E. Story, ed. University of Melbourne, Melbourne.p.1-17.

Bloskma, N., Ettekoven, A., Hothins., E.M., Jansen, I.D. and Revver, M.J. (1981). Effects of Lactobacillus on parameters of non-specific resistance of mice. *Medical Microbiology and Immunology* 170:15-53.

Black, F.T., Andersen, P.L., Orskov, J., Orskol, F., Gaarselu, K. and Lavhead. H. (1989). Prophylactic efficacy of lactobacilli on travelers diarrhea. *Conference. on international travel medicine* I, Zurich Switzerland 33-335.

Brown, I.L., Wang, X., Topping, D.I., Playne. M.J. and Conway, P.L. (1998), High amylose maize starch as a versatile prebiotic for use with probiotic bacteria Food Aust. 50:602-609.

Brown, I., Warhurst, M., Arcot, J., Playne, M., Illman, R.J. and Topping, D.L.(1997). Faecal numbers of bifidobacteria are higher in pigs *Bifidobacterium longum* with a high amylose cornstarch than with a low amylose cornstarch *Journal of Nutrition.* 127: 1822-1827.

Bird, A.R., Illman, R.J. Hayakawa, T., and Topping, D.L. 1997. Brown rice increases faecal and Large bowel short-chain fatty acid levels in pigs. Proc. Nut. Soc. (Aust). 21: 135.

Bruce, N. (2000). German food, Nutrition and health functional food annual Germany

Buddinton, R.K., Williams, C.H. and Chen, S.C. (1996). Dietary supplement of Neosugar alters faecal flora and decreased activities of some reductive enzymes in human subjects. *American J. of Clinical Nutrition* 38:1-10.

Challa, A., Ramkishan, L., Rao, D. and Chawa. C.B. (1997). *Bifidobacteria longum* and Lactulose suppress azoxy methane induced colonic aberrant in rats carcinogenesis 18:517-521

Chang, T., Goldin, B. and Gorbach. S. I. (1987). Successful treatment of relapsing *clostridium difficile* colitis with *Lactobacillus.*

Cheesborough, M. (1991). Medical laboratory manual for tropical countries 2nd Edition Tropical health technology scientific limited Vol 1 PP 494-526.

Christian, R. (2004). Low fat diet study claims to reverse diabetes retrieved 11/8/2006 from http://WWW Nutraingredients.com

Clements, M.L., Leave, M.M., Black, R.E., Ribing, Browne, R.M. and Cisneros, L.A. (1981). Lactobacilli prophylaxis for diarrhea due to enterotoxigenic *Escherichia Coli. Antimicrobial Agents of Chemotheraphy*, 20:104-1080.

Coudrays, Bellanger, J. castigha, D. (1997). Effects of soluble and insoluble fibre *Euro Journal of Clinical Nutrition.*23:1-15

Coward D. G. and Whitehead, R.G. (1972) Experimental protein Energy malnutrition in baby baboons: attempts to reproduce the pathological features of kwashiorkor as seen in Uganda. *British Journal of Nutrition* 28 233-237.

Crowely, S., Antioch, K., Carter, R., Waters, A.-M., Conway, L., and Mathers, C. (1992). The cost of diet-related disease in Austalia. Canberra, Australian Institte of Health and Welfare.

Devendra,B.(1977). Improvement of nutrive quality of crop retrived 17/8/2006 from Http://WWW as edu/proceeding/cassava fibre,

Dodd, H.M. and Gassin, M.J. (1994). Bacteria of lactic acid Bacteria Blackie academic and professional PP 211: 251.

Dore, M.P., Bilotta, M., Vaira, D., Manca, A., Massarelli, G., Leandro, G., Atzei, A., Pisanu, C., Graham, D.Y., and Reali, G. (1999). High prevalence of *Heliobacter pylori* infection in shepherds. Dig. Sci. 44: 1161-1164.

Drasar, B.S. (1988). The bacterial floral and the intestine. In: Rowland I.R., editor. Role of the Gut Flora in Toxicity and Cancer. Academic Press, London.

Duncan, M. (2000) Technology of cracker biscuits, cookies. A professional baking text book from C.H.I.P.S Third edition U.S.A.

Durmic, Z., Pethick, D.W., Pluske, J.R. and Hampson, D.J. (1998) Changes in bacterial populations in the colon of pigs fed different sources of dietary fibre, and the development of swine dysentery after experimental infection. *J. Appl. Microbiol* 85:574-582.

Ebringer, A., and Wilson, C. (1996). The use of a low starch diet in the treatment of patients suffering from ankylosing spondylitis. Clin. Rheumatol. 15:62-6.

Ernst, P.(1999). The role of inflammation in the pathogenesis of gastric cancer. Aliment. Pharmacol. Therapeutics. 13: 13-18.

Famularo, G. Morettis, Marcellini, S. and Desimone, R. (1997). Stimulation of immunity by probiotics in man. *America J. Clinical Nutrition* 66:15-20.

Fanimo, A.O. (1991). Substitution of soyabean and animal by products for fish and in pig rations PhD thesis University of Ibadan.

Fuller and Gibson, G.R (1997). Modification of intestinal flora using probiotics and prebiotics Scandinavian *J. of Gastroenterology* 32:28-31.

Fuller, R.A. (1989). Probiotics in man and animals. *Journal of Applied Bacteriology* 66:365-378.

George, T. Macfarlane, R. (1999). Probiotics and prebiotics can regulate the. activities of intestinal bacterial. *British Medical Journal* 318:999-1003

Gibson, G.R., Saavedra, J.M., Macfarlane S. and Macfarlane, G.T. (1997) Gastrolntestinal microbial disease and probiotics. 125:1401-1412.

Gilliland, S.E., Nelson, C.R. and Maxwell, C. (1985). Assimilation of Cholesterol by *Lactobacillus acidophilus Journal of Applied Environmental Microbiology* 49:377-381.

Golden, B.R. (1998). Health benefits of probiotics *British Journal of Nutrition* 80:S203-207.

Gurfinkel, I.E., Bozovich, G., Beck, E., Testa, E., Livellara, B., and Mauter, B. (1999). Treatment with the antibiotic roxithromycin in patients with acute non-Q-wave coronary syndromes. The final report of the ROXIS Study. *Eur. Heart J.* 20: 121-127.

Gotz, A., Moss, J. and Murray, H. (1979). Prophy laxis against ampicillin associated diarrhea with lactobacillus preparation *American J.of Hosp Pharmacy* 30 754-757.

Heatley,R.V.,and Guillon, P.G. (1997).barrett's oesophagus – a ray of hope.*Eur.J of.Gastroenterol. Hepatol.*9:873-875.

Hidaka, H., Eida, T., Takitawa, T. and Tokunaga, T. (1986). Effects of Fructo-oligosaccharides on intestinal flora and human health review of bifidobacteria and microflora. 5:37-50.

Homa, Deguchi, Y., Muyamin, A., Mat, sumoto, K. and Kikuch, L. (1990). Effects administration of galactooligosacchandes on the human faecal microflora stool weight and abdominal sensation. *Microbial Ecology Health Disease* 3:285-292.

Johnston, D.E. (1999). Special administration in interpreting liver function tests. The American Academy of family physician.Kashket, S., Zhaang,

J., and Van Houte J. (1996). Accumulation of fermentable sugars and metabolic acids in food particles that become entrapped on the dentition. *J. Dent. Res*. 75:1885-1891.

Kirk, R.S. and Sawyer, R. (1991). Pearson's Chemical composition and analysis of food, Scientific and Technical, 8th edition Longmans, Longon, 50-52 Page.

Lamb, G.N. (1981). Manual of Veterinary Laboratory Technique. CIBA-GEIGY. Kenya Pp96-97.

Louis, A. (2002). Improvement of nutritive quality of crop retrieved 17/8/2006 from Http://WWW as edu/proceeding/cassava fibre.

Mitsouka, R. (1992). The human gastrointestinal tract, Elsevier Applied science, London Pp 69-144.

Mitruka, R. (1977). Clinical haematological biochemistry on experimental animals mansson Newyok.

Morgan, M.V., Campain, A.C., Adams, G.G., Crowley, S.J., and Wright F.A. 1998. The efficacy and effectiveness of a primary preventive dental programme in non-fluoridated areas of Victoria, Australia. Community Dent. Health. 15:263- 271.

Oyetayo, V.O. and Osho, O. (2004). Assessment of probiotic properties of a strain of *Lactobacillus plantarum* isolated from fermented corn slurry *Journal of Food Agric and Environment* 2:132-134.Philadephia PP 421-538.

Pluske, J.R., Durmic, Z., Pethick, D.W., Mullan, B.P., and Hampson, D.J. (1998). Confirmation of the role of rapidly fermentable carbohydrates in

the expression of swine dysentery in pigs after experimental infection.*J.Nutr.*128: 1737-1744.

Rao, A.V., Shiwnarain, N., and Jenkins, D.J.A.1979. Effect of high amylose resistant starch on the composition of intestinal microflora. FASEB J.11:3527

Reitman, S. and Frankel. S. (1957). *American Journal of Clinical Pathology,* 28:56-70

Roberfoid, M.B. (2000). Prebiotics and Probiotics are they functional foods *American Journal of Clinical Nutrition 24 1-16.*

Sardine, L. (1979). The role of lactobacillus in the intestinal tract. *J. of Food.*28 1-4

Salminen S. Isolauri E, Onnela T. (1995) Gutflora in animal and disordered states. Chemotheraphy 41 (Suppl) 5-18.

Schalm, O. and. Carrol, E.J. (1975) .Veterinary Haematology 3rd Edition.

Silvi, S., Rumneny, C.J., Cresci, A., and Rowland, I.R. (1999).Resistant starch modifies gut microflora and microbial metabolism in human flora-associated rats inoculated with faeces from Italian and UK donors. *J. Appl Microbiol* 86: 521-530.

Smith, G.W., Blackwell, C.C., and Nuki, G. (1997). Faecal flora in spondyloarthropathy. Br.J. Rheumatol 36: 850-854.

Silva, A.M., Bambirra, E.A., Olivera, A.C. and Nicoli, J.R. (1999). Protective effect of bifidus milk on the experimental infection with *Salmenteridis*

subsptyphilminum in conventional are gnotobic mice *J. Appl. Microbiology* 86:331-336.

Silva, M. Jacobus, N.V., Denukee, I., and Gorback, S.L. (1987). Antimicrobial substance from human lactobacillus strain. Antimicrobial agents. Chemotherapgy 1987: 31-1231 1233.

Solis, Pereyra, B. and Lemonier, D. (1993). Induction of human cytokines to bacteria used in diary. *Food Nutrition Resources* 13:1127-1140.

Soontornchai, S., Sirichakiwa, P. and Puwasten, P. Lacti 101 tolerance in healthy thai adults *European J Nutrition* 2000 38: 218-226.

Standiford, T.K., Arenberg, D.A., Danforth, J.M. and Kankel, S.L. (1994). Lipoteichoic acid induces secretion of interleukin 8 from human blood monocytes. A cellular and molecular analysis infection immunotherapy 119-125.

Suanne, L. (2000). Pro- and prebiotics for health. Functional Food Annual.*American Journal of Nutrition 4* 1-5.

Surawicz C.M., Elmerg, Spelman, P. Lymne, U. and M.C. Farland, L.V, (1989). Prevention of antibiotic associated diarrahea by *Saccharomyces boulardii Journal of Gastroenterology* 9:981-988.

Terada, A.,Hara, H., and Mitsuoka, T.(1996). Effect of dietary alginate on the faecal microbiota and faecal metabolic activity in humans. *Microbial Ecol. Health Dis.* 8:259-266.

Tiwana, H., Wilson, C., Walmsley, R.S ., Wakefield , A.J.,Smith, M.S.,Cox , N.L., Hudson, M.J., and Ebringer, A. (1997).Antibody responses to gut

bacteria in ankylosing spondylitiis, rheumatoid arthritis, Crohn's disease and ulcerative colitis *Journal of Gastrointestinology.* 17:11-16.

Topping, D.L., Warhurst, M.,Illman, R.J., Brown, I.L., Playne, M.J., and Bird, A.R. (1997)b. A high amylase (amylomaize) starch and fructooligosaccharide increase faecal excretion of bifidobacteria pigs fed live *Bifidobacterium longum.* Proc. Nutr. Soc. Aust. 21:134.

Treem, W.R Ahsan, N., Shoup, M., and Hyams, J.S. (1994). Fecal short-chain fatty acid in children with inflammatory bowel disease. *J.Paediatr. Gastroenterol. Nutr.* 18:159-164.

Tsuji, S., Tsuji, M., Sun, W.H., Gunawan, E.S., Murata, H., Kawano, S., and Hori M. (1997). *H. pylori* and gastric carcinogenesis. *J.Clin. Gastroenterol.* 25:S186-197.

Thomas W. (2004) High fibre foods diet for diabetics retrieved 17/8/2006 from Http://WWW .Nutra ingredient Com. Health.

Wang, X., and Gibson, G.R. (1993). Effects of the *in vitro* fermentation of oligofructose and inulin by bacteria growing in the human large intestine.*J. Appl. Bacteriol.* 75:373-380.

Yasui, H. and Ohwaki, L. (1991). Enhancement of immune response in peyer's patch cells cultured with *Bifidobacteria breve. J.of Diary Science* 74: 1187-1195.

Yasui, H., Nagaoka, N. Mike, A. and Hayakawa, K. (1992). Detection of *bifidobacteria* strains that induce large quantity of IgA. *Microbial Ecology of Health Disease.* 155-162.

Zang,X.B. and Olita, Y. (1990). Antimutagenicity and binding of Lactic acid. bacteria from a chinese to mutagenic *pyrolyzates. J. of Diary Science* 73: 2702-2710.

APPENDIX

Nutritional Composition of Top Feeds

Ingredient	Level in diet
Crude protein	20%
Crude Fat	6%
Crude fibre	12.5%
Crude Ash	8%
Calcium	0.9%
Phosphorus	0.9%
Lysine	1%
Methioinine	1.5%
Vit. A	9000,IU
Vit. D_3	2400IU
Vit. E	15mg
Vit. B_2	50mg
Vit. C	50mg
Manganese	30mg
Zinc	30mg
Sodium	0.10%
Metabolisable energy	2300kcal/kg

Source: Top Feeds Nigeria Limited.

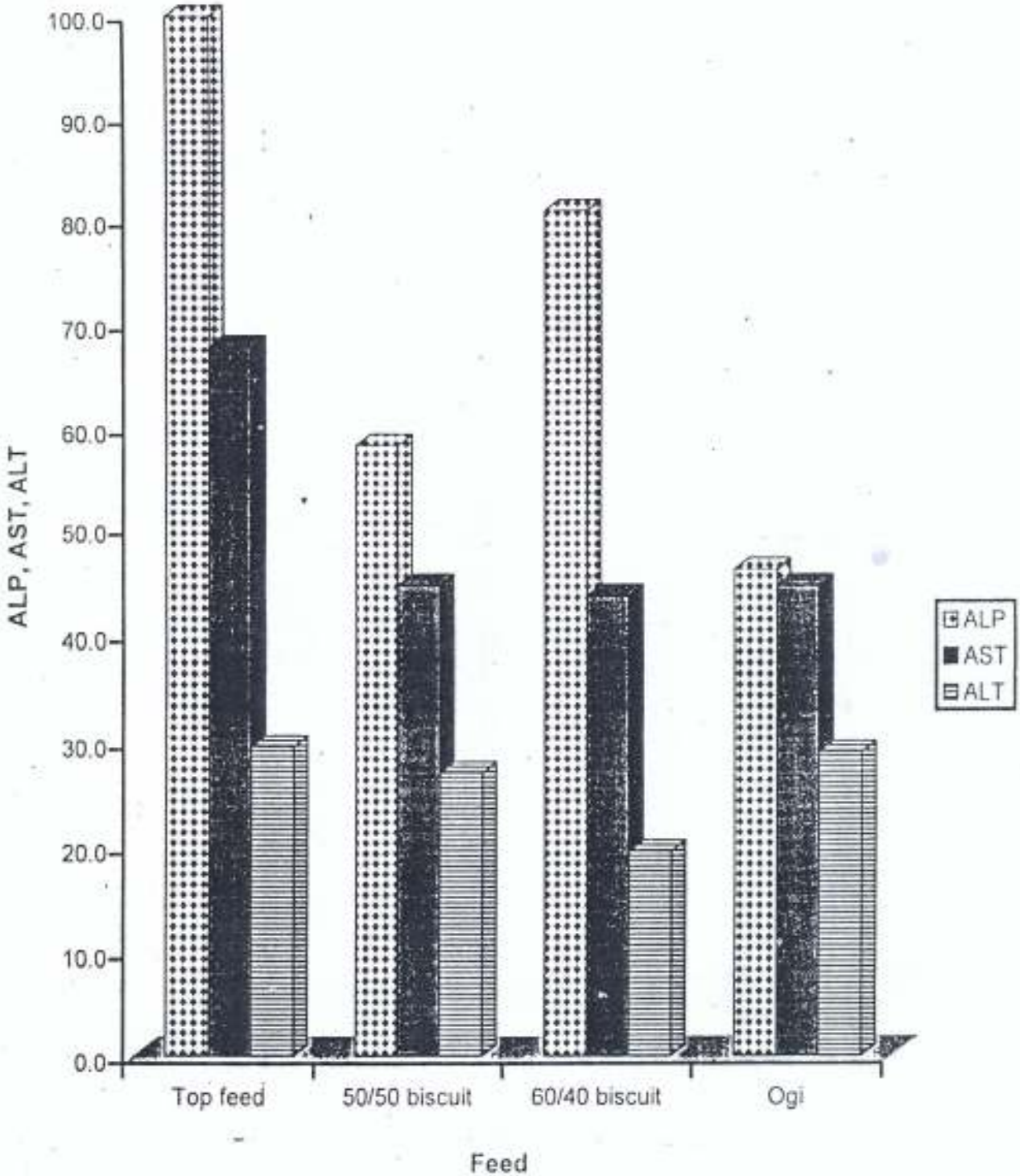
COMPOSITION OF SUPERVITACONC (VITAMIN PREMIX)

Vitamins/Minerals	Quantity
Vitamin A	15, 000,000iu
Vitamin D_3	4,400,000iu
Vitamin E	1,350iu
Vitamin K	4,350mg
Vitamin B_2	4,350mg
Vitamin B_6	2,350mg
Vitamin B_{12}	11,350mg
Vitamin C	1,000mg
Nicotinic acid	16,700mg
Pantothenic acid	5,350mg
Pattasium chloride	87,000mg
Sodium Sulphate	212,000mg
Magnesium Sulphate	12,000mg
Copper Sulphate	12,000mg
Zinc Sulphate	12,000mg
Maganese Sulphate	12,000mg
Lysine	15,000mg
Methioinine	10,000mg

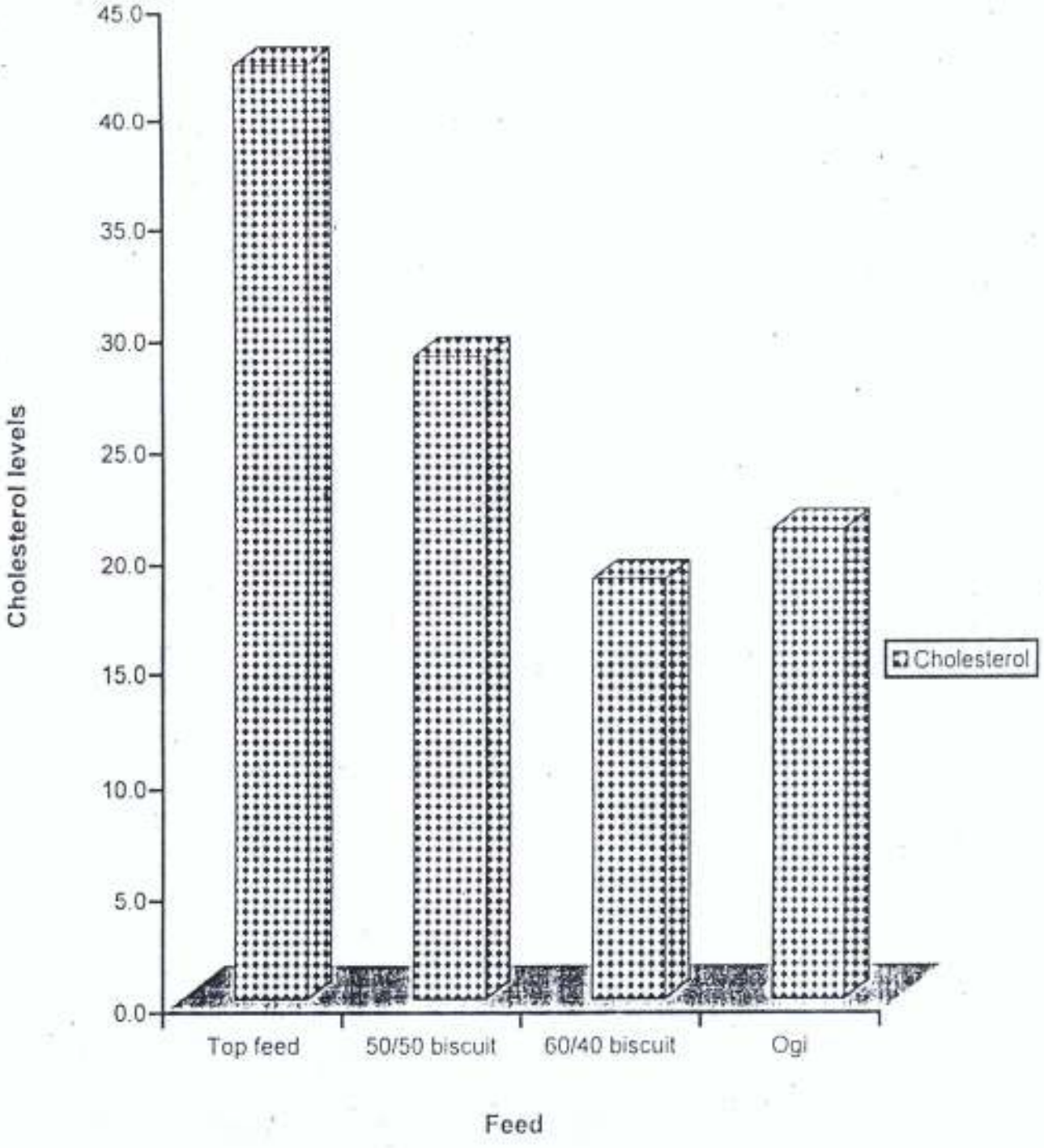
Bioorganic Nigeria Limited

COMPOSITION OF DELTOL DISINFECTANT

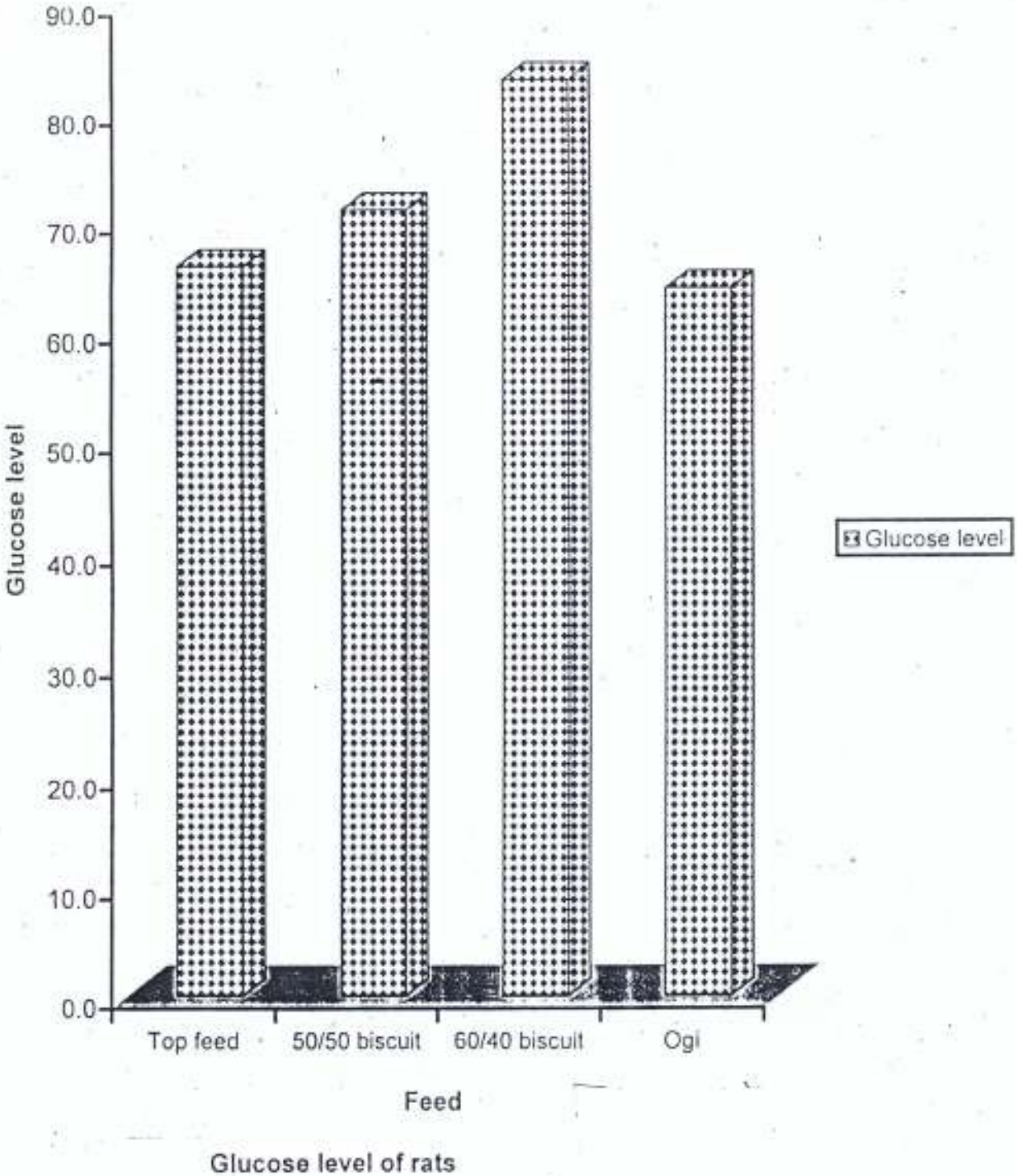
Chloroxylenol B.P.C	4.8% W/V
Oleum pini Aromatium	8.38% W/W
Isopropyl Alcohol	9.43% W/W
Sapo vegetalis	5.6%W/W
Saccharum ustum.q.s	
Aqua	



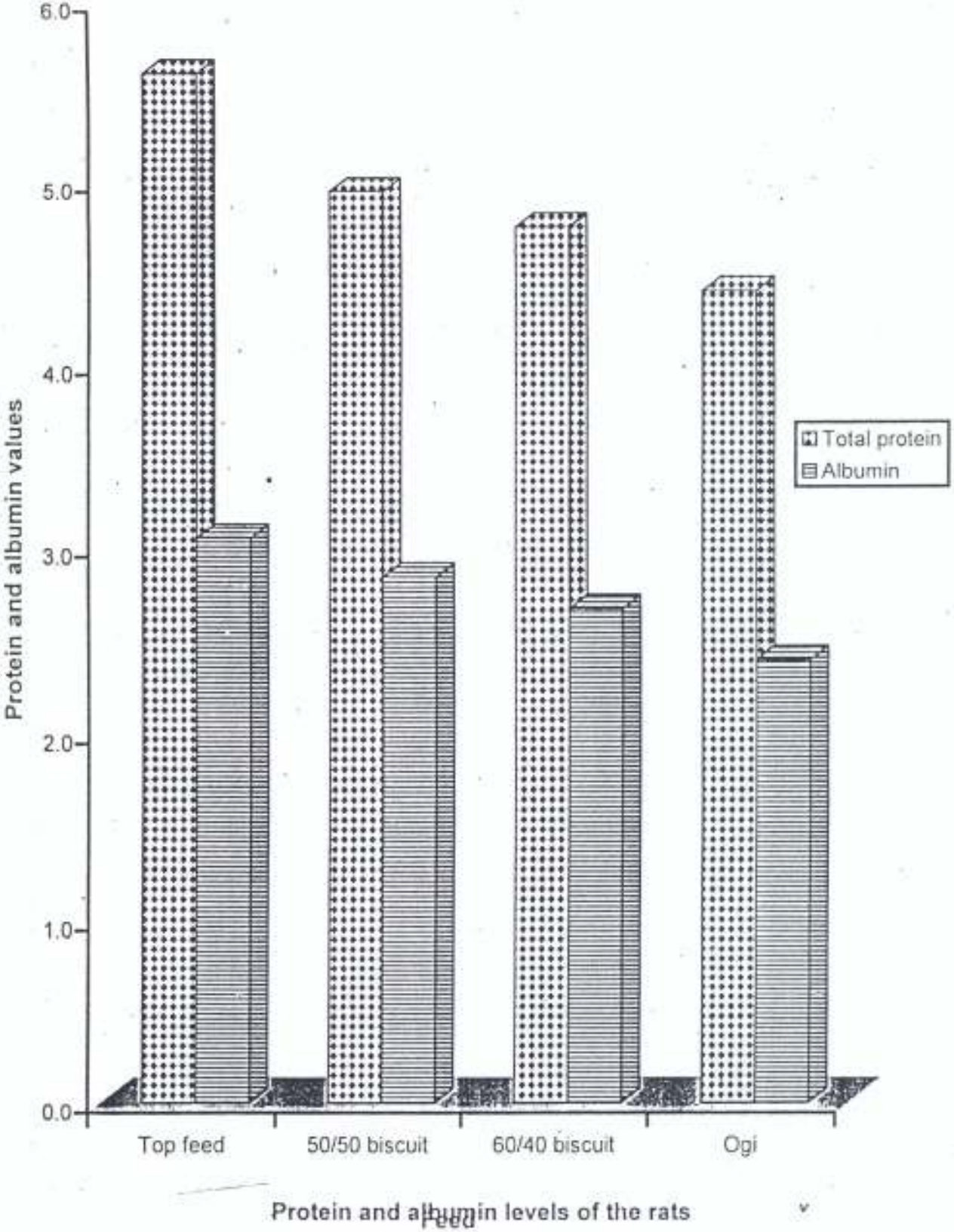
Liver function tests



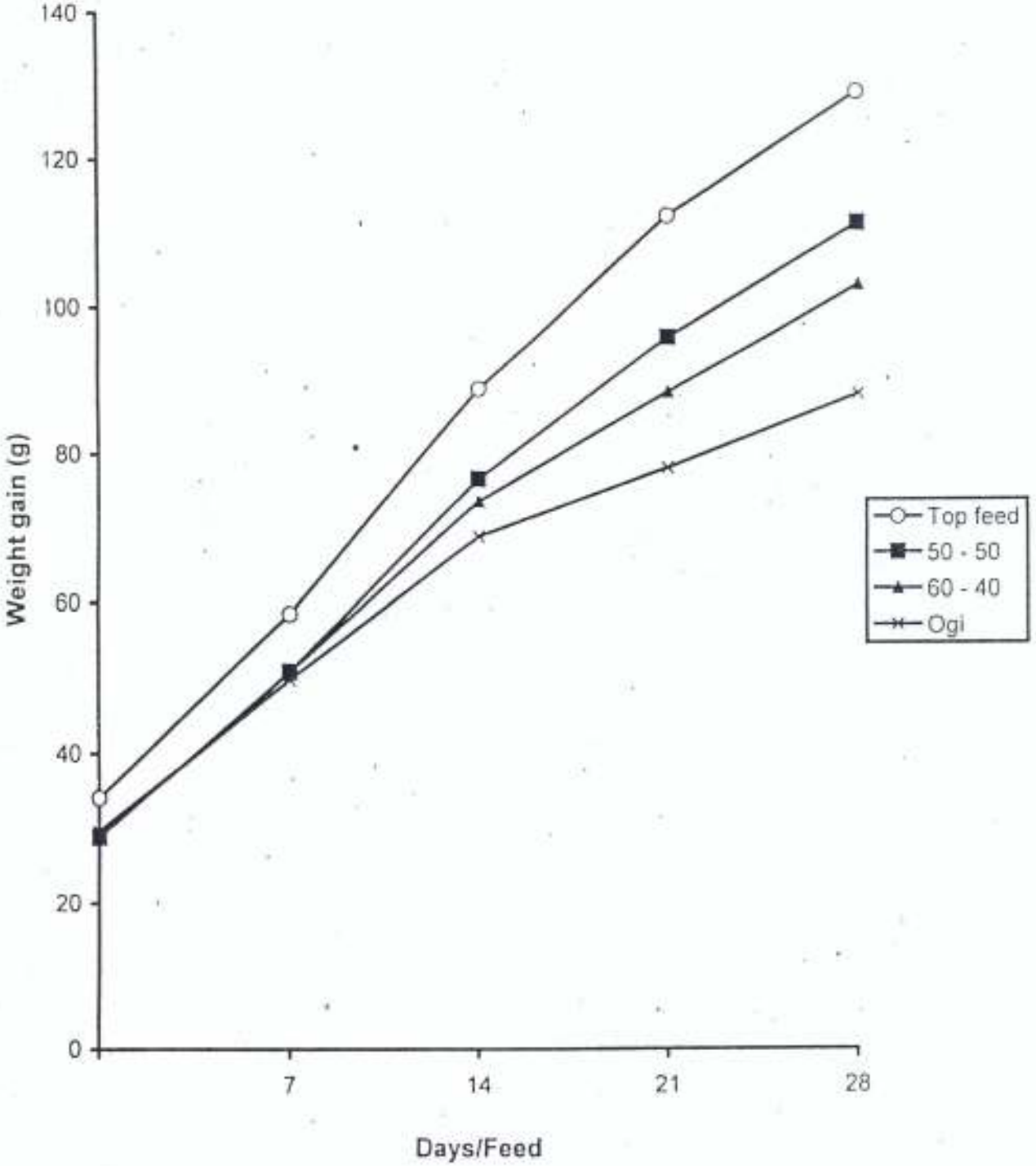
Cholesterol levels of the rats



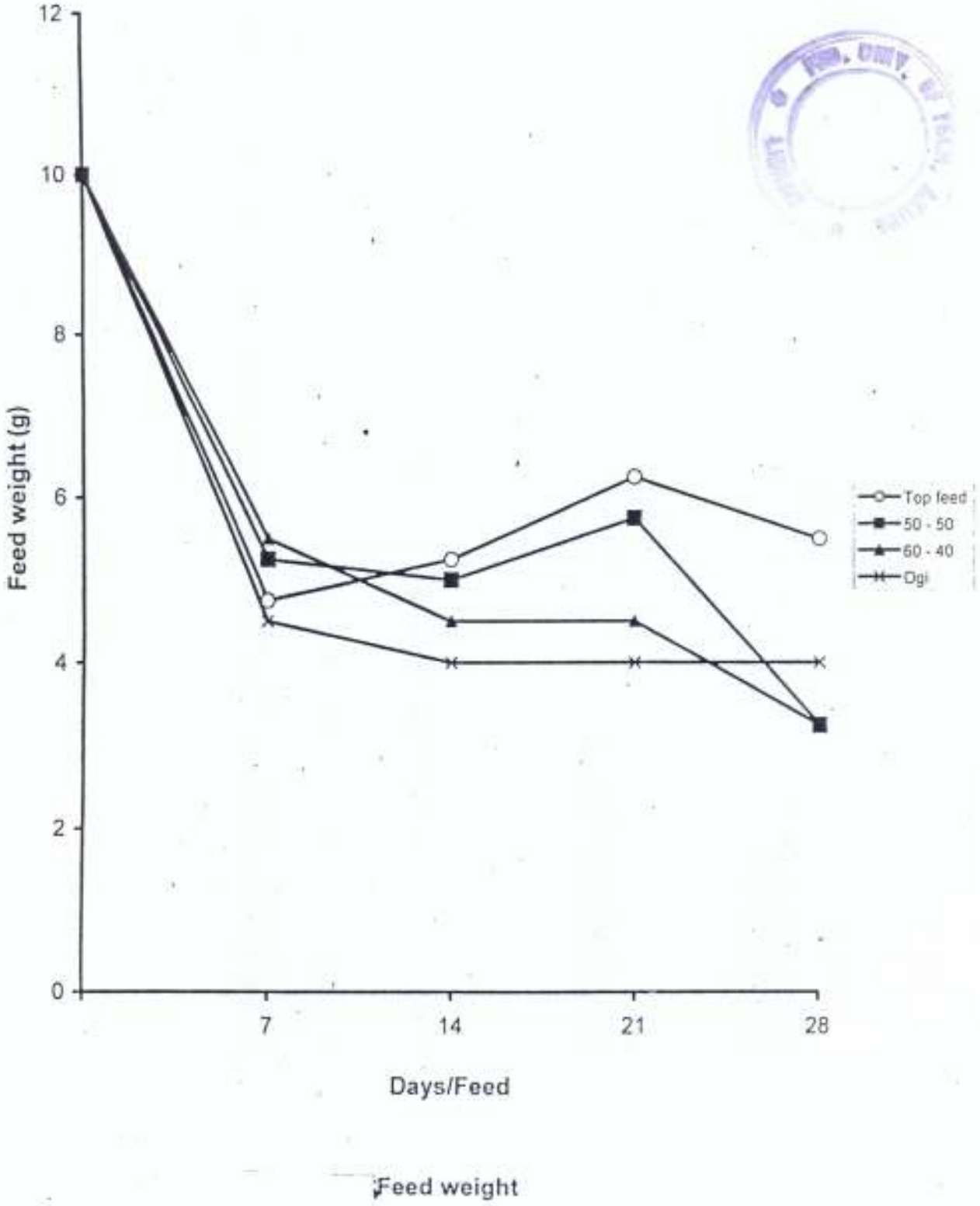
Glucose level of rats



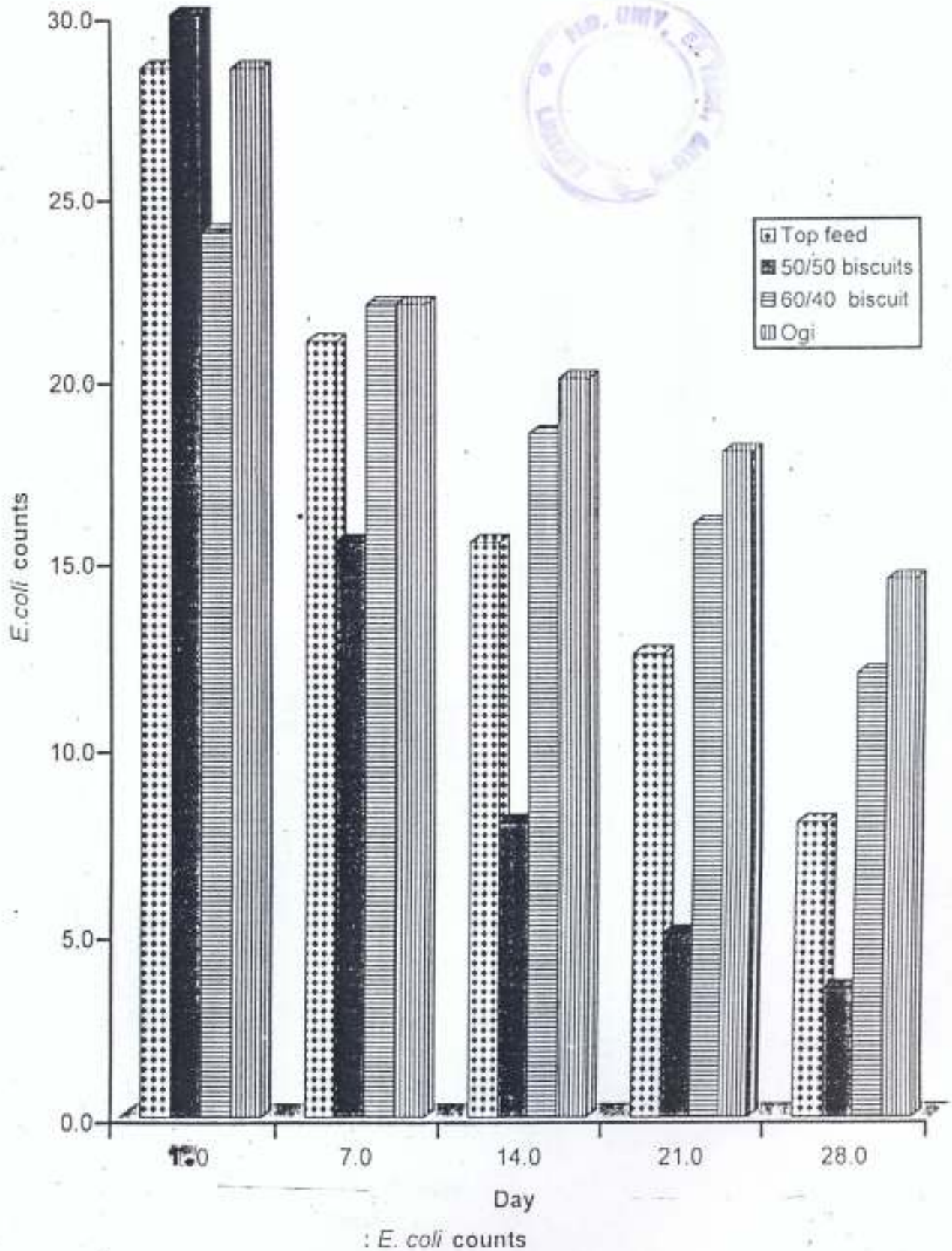
Protein and albumin levels of the rats



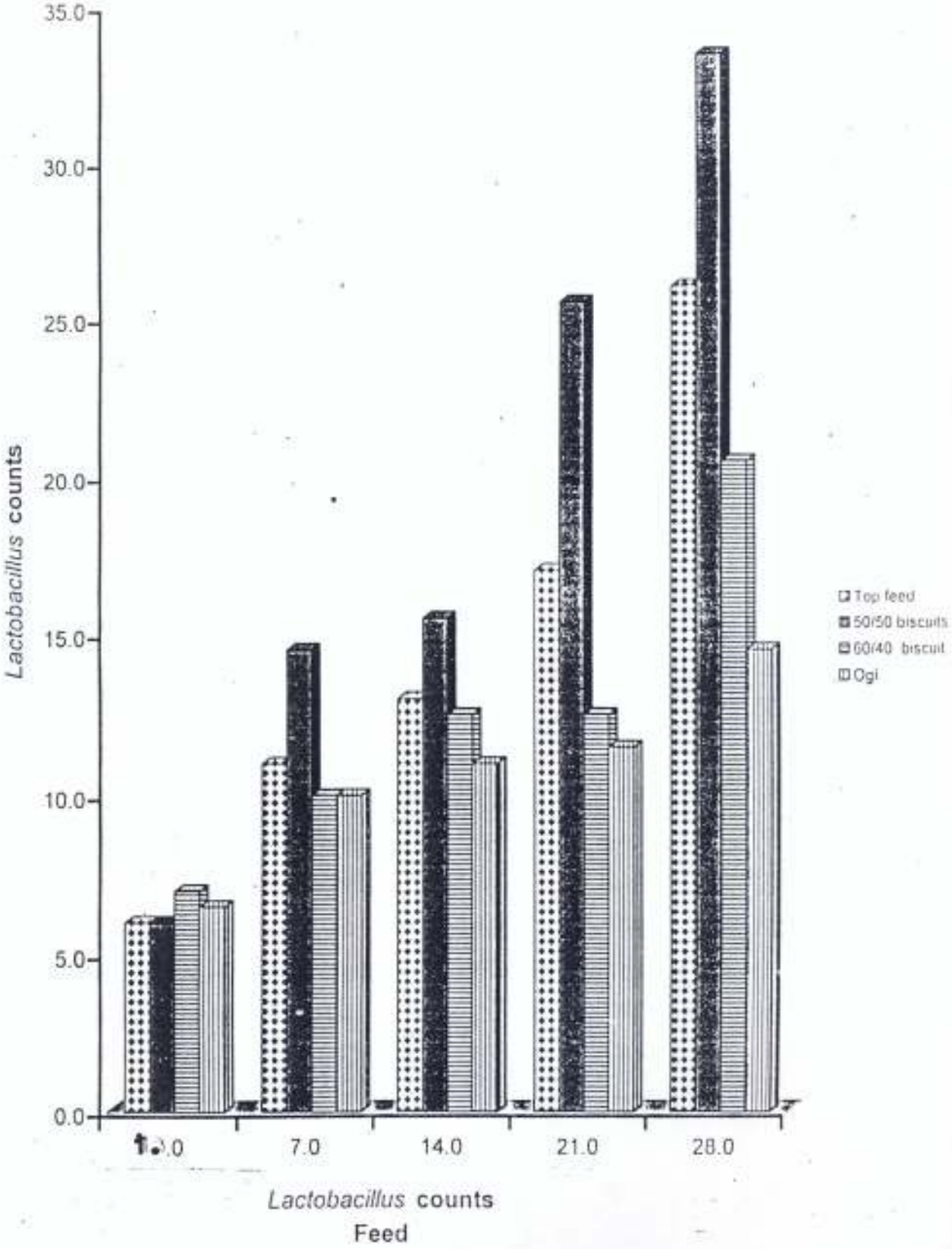
Weight gain of the rats


12
10
8
6
4
2
0
Feed weight (g)
7
14
21
28
Days/Feed
Top feed
50 - 50
60 - 40
Dgi

Feed weight



: *E. coli* counts


35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
Lactobacillus counts
7.0
14.0
21.0
28.0
Top feed
50/50 biscuits
60/40 biscuit
Ogi
Lactobacillus counts
Feed