EFFECT OF SWEET ORANGE (*Citrus sinensis)* FRUIT WASTE WITH OR WITHOUT ACIDOMIX®AFG ON PERFORMANCE OF WEANER RABBITS

BY

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DECLARATION

I hereby declare that this thesis entitled "EFFECT OF SWEET ORANGE (*Citrus sinensis)* FRUIT WASTE WITH OR WITHOUT ACIDOMIX®AFG ON PERFORMANCE OF WEANER RABBITS" has been written by me from the Department of Animal Science under the supervision of Prof. G.S. Bawa and Dr. T. S. Olugbemi. It is the result of my investigation (except where reference is made to published literature and where any assistance is acknowledged) and has not been part of presentation for any other qualification.

_____ _____

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CERTIFICATION

This thesis entitled "EFFECT OF SWEET ORANGE (*Citrus sinensis)* FRUIT WASTE WITH OR WITHOUT ACIDOMIX® AFG ON PERFORMANCE OF WEANER RABBITS" by Ademu, Lawrence Anebi meets the regulations governing the award of the degree of Masters of Science of Ahmadu Bello University, Zaria and is approved for its contribution to Scientific knowledge and literary presentation.

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DEDICATION

This work is dedicated to my mother; Mrs. E.A Ademu for her love, never-ending prayers and support.

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ABSTRACT

Two studies were carried out to determine the effect of sweet orange (*Citrus sinensis*) fruit waste (SOFW) with or without Acidomix®AFG on performance of weaner rabbits. In the first experiment, a total of thirty weaner rabbits were used to determine the effect of graded levels of SOFW on the performance and carcass characteristics of weaner rabbits. There were five dietary treatments, each having 5 replicates with graded levels of sweet orange fruit waste at 0, 5, 10, 15 and 20 % designated as T_1, T_2, T_3, T_4 and T_5 respectively. The rabbits fed the diet containing 10 % SOFW gave the highest final body weight, weight gain, daily feed intake and feed conversion ratio of 1440.0 g, 16.25 g, 62.32 g and 3.83 respectively, similar to the control. Feed cost per kg gains of 241.07 to 262.52 for the SOFW based diets were similar ($P>0.05$) to the control diet (T_1). No significant ($P>0.05$) difference was observed among the dietary treatment groups for packed cell volume, haemoglobin and white blood cell counts. Total blood protein was highest for rabbits fed diet containing 15% SOFW (T_4). The rabbits on 10 % inclusion of SOFW diet (T_3) had the highest dressed weight, dressing percentage, racks and ribs of 743.23 g, 53.09 % and 27.80 % respectively; while rabbits fed diet containing 20 % SOFW had the highest loin weights. Dietary effect on the organs (liver, kidney, lung, heart) were significant ($P<0.05$). A similar trend was observed for all nutrient digestibility parameters where the values increased ($P< 0.05$) with increased levels of SOFW. It can be concluded that inclusion of SOFW in diet of weaner rabbits up to 10 % is the optimum. The second experiment was conducted with a total of thirty-five weaner rabbits. There were four dietary treatments, each having five replicates consisting of graded levels of SOFW at 0, 10, 15, and 20 % designated as T_1, T_2, T_3 and T_4 respectively treated with Acidomix®AFG at two levels (0.5 % and 0.7 %) for each dietary treatment. Interaction effect results indicated significant ($P<0.05$) linear increases within and across the

treatments for final body weight, weight gain and feed intake with no apparent trend. Also, feed cost per kg gain for the SOFW based diets was lowest for 20 % SOFW+ 0.5 % Acidomix®AFG diet (399.74) and was similar (P>0.05) with the control (365.36). Carcass parameters such as dressed weight, dressing percentage and racks/ribs weight for 20 % SOFW+ 0.7 % (805.0 g, 50.83 % and 31.43 % respectively) gave the highest weight across the treatments. The interaction effect on haematological indices had significant (P<0.05) effect on packed cell volume, haemoglobin, white blood cell and total protein. All haematological indices fell within normal ranges with no observable trend. Treatment of the experimental diets using Acidomix®AFG showed a decrease in coliform bacteria in the intestine of the experimental rabbits. It can be concluded that the inclusion of SOFW in the diet of weaner rabbits up to 10 % is optimal. However, treatment of SOFW with Acidomix®AFG helped achieve inclusion levels of SOFW up to 20 % in diets of weaner rabbits.

TABLE OF CONTENTS

Title page i
Declaration ii
Certification iii
Dedication iv
Acknowledgement v
Abstract vi
Table of contents viii
List of tables xii

CHAPTER ONE

1.0 INTRODUCTION 1
1.1 Objectives of the study 4

CHAPTER TWO

2.0 LITERATURE REVIEW 5
2.1 Origin and description of *citrus sinensis* 5
2.2 Phytochemicals in *Citrus sinensis* 6
2.2.1 Flavonoids 7
2.2.2 Limonoids 8
2.2.3 Limonene 9
2.2.4 Lectins 10
2.3 Utilization of *Citrus sinensis* 10
2.3.1 Medicinal properties 10
2.3.2 Use of sweet orange peel in pest control 12
2.3.3 Antimicrobial activity of *Citrus* peel 12
2.4 Utilization of C*itrus sinensis* in animal nutrition 13
2.5 Use of post-harvest wastes in animal nutrition 14
2.6 Animal feed contamination 17
2.7 The rabbit 18
2.7.1 Origin and distribution 18

2.7.2 Digestive system of the rabbit 19
2.8 Intestinal health of the rabbit 23
2.9 Acidifiers in animal nutrition 26
2.9.1 Effect of acidifiers in feed 29
2.9.2 Effect of acidifiers in the intestinal tract 30
2.9.3 Effect of acidifiers on metabolism 33
CHAPTER THREE
3.0 MATERIALS AND METHODS 35
3.1 Experimental site 35
3.2 Source and processing of the sweet orange fruit waste 35
3.3 Proximate analysis 36
3.4 Microbiological enumeration 36
3.5 Experiment 1: Effect of graded levels of sweet orange fruit waste on the performance and carcass characteristics of weaner rabbits 37
3.5.1Experimental diets 37
3.5.2 Management of experimental animals and data collection 37
3.5.3 Nutrient digestibility study 38
3.5.4 Carcass evaluation 38
3.5.5 Haematological study 40
3.6 Experiment 2: Effect of graded levels of sweet orange fruit waste (SOFW) treated with Acidomix® AFG on the performance and carcass characteristics of weaner rabbits 40
3.6.1 Experimental diets 40
3.6.2 Management of experimental animals and data collection 40
3.6.3 Carcass and haematological study 40
3.6.4 Coliform count 42
3.7 Data analysis 42
CHAPTER FOUR
4.0 RESULTS 43
4.1 Proximate composition of sweet orange fruit waste 43

4.2 Microbial count of sweet orange fruit waste 43
4.3 Experiment I: Effect of graded levels of SOFW on the performance and carcass characteristics of weaner rabbits 46
4.3.1 Effect of sweet orange fruit waste diets on the growth performance of weaner rabbits 46
4.3.2 Effect of sweet orange fruit waste on the nutrient digestibility of weaner rabbits 48
4.3.3 Effect of sweet orange fruit waste on carcass characteristics and organ weights of weaner rabbits 48
4.3.4 Effect of sweet orange fruit waste on the haematological parameters of weaner rabbits ... 50
4.4 Experiment II: Effect of graded levels of sweet orange fruit waste (SOFW) treated with varying levels of Acidomix®AFG 53
4.4.1 Effect of sweet orange fruit waste diets on the growth performance of weaner rabbits 53
4.4.2 Effect of levels of Acidomix®AFG on the growth performance of weaner rabbits 53
4.4.3 Interaction effect of sweet orange fruit waste diets treated with Acidomix®AFG on the growth performance of weaner rabbits 56
4.4.4 Effect of sweet orange fruit waste diets with or without Acidomix®AFG treatment on the carcass characteristics of weaner rabbits 58
4.4.5 Interaction effect of sweet orange fruit waste diets treated with Acidomix®AFG on the carcass characteristics of weaner rabbits 61
4.4.6 Effect of sweet orange fruit waste diets on the haematological parameters of weaner rabbits 61
4.4.7 Effect of levels of Acidomix®AFG on the haematological parameters of weaner rabbits 64
4.4.8 Interaction effect of sweet orange fruit waste diets treated with Acidomix®AFG on haematological parameters of weaner rabbits 64
4.4.9 Effect of sweet orange fruit waste diets on the coliform count in the small intestine of weaner rabbits 67
4.4.10 Effect of levels of Acidomix®AFG on the coliform count in the small intestine of weaner rabbits 67
4.4.11 Interaction effect of sweet orange fruit waste diets treated with Acidomix®AFG on the coliform count in the small intestine of weaner rabbits 70
CHAPTER FIVE
5.0 DISCUSSION 72
5.1 Proximate composition of sweet orange fruit waste 72

5.2 Microbial count of sweet orange fruit waste 72
5.3 Experiment I: Effect of graded levels of SOFW on the performance and carcass characteristics of weaner rabbits 73
5.3.1 Effect of sweet orange fruit waste on the nutrient digestibility of weaner rabbits 76
5.3.2 Effect of sweet orange fruit waste on the haematological parameters of weaner rabbits ... 76
5.4 Experiment II: Effect of graded levels of sweet orange fruit waste (SOFW) treated with varying levels of Acidomix® AFG 77
5.4.1 Effect of sweet orange fruit waste diets treated with or without Acidomix® AFG on the haematological parameters of weaner rabbits 80
5.4.10 Effect of Acidomix® AFG treatment on coliform count 81
CHAPTER SIX
6.0 SUMMARY, CONCLUSION AND RECOMMENDATION 83
6.1 SUMMARY 83
6.2 CONCLUSION 83
6.3 RECOMMENDATION 84
REFERENCES 85

LIST OF TABLES

Tables

3.1: Composition and calculated analysis of sweet orange fruit waste based diets fed to weaner rabbits 39

3.2: Composition and calculated analysis of Acidomix® AFG treated sweet orange fruit waste based diets fed to weaner rabbits 41

4.1: Proximate composition of sweet orange fruit waste 44

4.2: Microbial count of Sweet orange fruit waste..... 45

4.3: Effect of sweet orange fruit waste on the growth performance of weaner rabbits 47

4.4: Effect of sweet orange fruit waste on the nutrient digestibility of weaner rabbits 49

4.5: Effect of sweet orange fruit waste on the carcass characteristics and organ weights of weaner rabbits..... 51

4.6: Effect of sweet orange fruit waste on the haematological parameters of weaner rabbits 52

4.7: Effect of sweet orange fruit waste diets on the growth performance of weaner rabbits 54

4.8: Effect of levels of Acidomix® AFG on the growth performance of weaner rabbits fed sweet orange fruit waste 55

4.9: Interaction effect sweet orange fruit waste diets treated with Acidomix® AFG on the growth performance of weaner rabbits 57

4.10: Effect of sweet orange fruit waste diets on the carcass characteristics of weaner rabbits 59

4.11: Effect of levels of Acidomix® AFG on the carcass characteristics of weaner rabbits fed sweet orange fruit waste 60

4.12: Interaction effect of sweet orange fruit waste based diets treated with Acidomix® AFG on the carcass characteristics of weaner rabbits..... 62

4.13: Effect of sweet orange fruit waste diets on the haematological parameters of weaner rabbits 63

4.14: Effect of levels of Acidomix® AFG on the haematological parameters of weaner rabbits.. 65

4.15: Interaction effect of sweet orange fruit waste based diets treated with Acidomix® AFG on the haematological parameters of weaner rabbits 66

4.16: Effect of sweet orange fruit waste diets on the coliform count at the small intestine of weaner rabbits 68

4.17: Effect of levels of acidomix® AFG on the coliform count at the small intestine of weaner rabbits 69

4.18: Interaction effect of sweet orange fruit waste based diets treated with Acidomix® AFG on the coliform count at the small intestine of weaner rabbits 71

CHAPTER ONE

1.0 INTRODUCTION

The continuous rise in the demand for cereal grains and oil seeds by both man and animal has led to a steady rise in the cost of these feed sources with a consequent rise in the cost of animal feeds. In order to increase farm incomes from livestock in developing countries, an adequate low-cost feeding system must be developed (Chedly *et al*., 1999). This has led to efforts by researchers to discover non-conventional feed resources to cope with the ever increasing demand for these conventional feed resources. Large quantities of agricultural by-products which are regarded as non-conventional feed sources are produced in Nigeria (Hamzat *et al.*, 2005). The use of these agricultural by-products for livestock has been reported to reduce dependence of livestock on grains that can be consumed by humans and eliminates the need for costly waste management programmes which have become very important in recent years. This has become more important as the world human population and the amount of crop and food by-products have increased, particularly in developed countries (Grasser *et al.*, 1995).

Rabbits have been recognized to have a very important role to play in the supply of animal protein to humans, especially in tropical and subtropical areas. Rabbits occupy a vital midway between ruminants and monogastric animals and can Rabbits can utilize low-grain and high roughage diets (McNitt *et al*., 1996). Simple biological characteristics, short breeding cycle, high prolificacy and better feed conversion efficiency logically place rabbit just below poultry (Hasanat *et al*., 2006).

An agro-industrial waste available in the country is sweet orange fruit waste (SOFW). Nigeria produces 3% of fresh citrus in the world, and Africa produces 3,741, 000 tonnes of different

varieties of citrus fruits of which Nigeria contributes 3,240,000 tonnes (FAO, 2004). Citrus of all species are widely grown in tropical and subtropical regions. The fruits are used mainly as dessert or breakfast fruits and for making juices and other citrus products. Citrus production in the producing countries is increasing (Arribas, 2001). Citrus fruits are principally consumed by humans as fresh fruit or processed juice, fresh, chilled or concentrated.

Sweet orange (*Citrus sinensis*) production is significant and it is available all year round with higher production from the month of October through to March. Oranges accounted for 61 % of the world citrus production (82 million tonnes) (USDA-FAS, 2010). Sweet orange fruit is palatable, rich in nutrients, easily mixed with other feed ingredients and exerts a mildly laxative effect. It can be stored for all-year feeding and deteriorates less in storage than many other feeds. *Limonin* is a tri-terpenoid present in the seeds and skins that imparts a bitter taste to citrus pulp. If large quantities of seeds are present their *limonin* content may render the pulp toxic to non-ruminants even at 2.5 % in their diets (Rihani, 1991). *Limonene* is another terpenoid present in large quantities in citrus peels and that is responsible for the characteristic odour of citrus. *Limonene* is practically non toxic to birds and mammals (EPA, 1994). Compounds such as tannins, saponin, phytate, oxalate and flavonoids have been identified in citrus peels but they are below the levels reported to be toxic to livestock species (Oluremi *et al*., 2007).

Waste is an inevitable consequence of the food industry. Appropriate handling of waste has become an essential part of modern processing management. As concerns over the environment have increased, processes which generated lucrative products, but have attendant unmanageable waste problems, can become unaffordable because of prohibitive disposal costs (Rodríguez *et al*, 2003). World citrus production generates many residues (citrus pulp, unmarketable fruit) that constitute a great environmental problem for the production areas (Piquer *et al.,* 2009). Due to

the perishable property of surplus fruit during seasons of over production in tropical countries, it would be convenient to develop methods of preservation that would enable this plant material to be utilized as animal feeds for longer periods of time (Aguilera *et al*., 1997).

Organic acids, marketed as acidifiers have a long history in the food and feed industry. Acidomix®AFG which is a blend of organic acids (formic acid, propionic acid, ammonium formate and ammonium propionate) improves feed hygiene due to strong antimicrobial effects. The inclusion of Acidomix®AFG in feed for rearing animals results in the stabilization of the gastro-intestinal micro flora. Furthermore, the digestion of protein (activation of enzymes) is stimulated by lowering pH and acid binding capacity of the feed. The use of Acidomix®AFG is a preventive measure to control salmonella (Novus, 2007).

Although feeding trials concerning citrus by-products (peel, pulp, seeds and molasses) abound, studies carried out on the entire fruit is lacking. Citrus pulp has been used extensively in the feeding of cattle and rabbits with favourable results (Leon *et al*., 1999; Tokarnia *et al*., 2001; Volanis *et al.,* 2004; Hon *et al*., 2009). It is the abundance of SOFW, non-competitiveness of use, non-requirement of complex processing methods and its potential as an energy source that motivated this study.

1.1 Objectives of the study

The objectives of the study were:

i. To determine the proximate composition and microbial count of sweet orange fruit waste.
ii. To determine the performance, carcass characteristics and haematological indices of weaner rabbits fed varying levels of sweet orange fruit waste based diets treated with or without Acidomix®AFG.

iii. To determine the economic benefits of replacing maize with sweet orange fruit waste.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Origin and description of *Citrus sinensis*

The orange is a hybrid of ancient cultivated origin, possibly between *pomelo* (*Citrus maxima*) and *mandarin* (*Citrus reticulata*) (Nicolosi *et al.,* 2000). The orange fruit is a *hesperidium*, a type of berry (Webster's New World College Dictionary, 2010). Taxonomy of the orange and citrus in general presents difficulties; the ability of citrus to interbreed has resulted in numerous hybrids, bud unions, and cultivars; taxonomy is often controversial, confusing, or inconsistent (Nicolosi *et al.,* 2000). Oranges probably originated in Southeast Asia and were cultivated in China by 2500 BC. The fruit of *Citrus sinensis* is called sweet orange to distinguish it from *Citrus aurantium*, the bitter orange. The name is thought to be ultimately derived from the Sanskrit for the orange tree, with its final form developing after passing through numerous intermediate languages. In a number of languages, it is known as a "Chinese apple" (e.g. Dutch *Sinaasappel*, "China's apple", or northern German *Apfelsine*). In English, however, "Chinese apple" generally refers to the pomegranate.

The orange tree is a small to medium sized evergreen tree with smooth bark and branches. It is an evergreen flowering tree generally growing to 9–10 m in height (although very old specimens have reached 15 m) (Reuther and Lawton, 1989). The leaves are arranged alternately, are ovate in shape with crenulated margins and are 4–10 cm long (EOL, 2011). The oval to round shaped orange fruits (or simply oranges) has a leathery skin that contains orange oil. The skin of orange is green or yellow. The white part of the orange is called *albedo* and is rich in pectin. Inside the orange are segments which contain fragile vesicles containing the juice. The orange seeds are manly found in the part adjacent to the core. Oranges are grown in enormous quantities and are

readily available as an inexpensive fruit at anytime of the year (Arthey and Ashurst, 1996). Orange fruit are the most popular ones for consumer throughout the world due to their pleasant flavours and nutritional value (Dhuique-Mayer *et al.,* 2005). The fruits are both consumed fresh and industrially processed. Orange peels contain abundant fragment substances which are extensively applied for processing into essential oils.

Orange trees are widely cultivated in tropical and subtropical climates for the delicious sweet fruit, which is peeled or cut (to avoid the bitter rind) and eaten whole, or processed to extract orange juice, and also for the fragrant peel. In 2008, 68.5 million tons of oranges were grown worldwide, primarily in Brazil and the State of Florida in the US (FAO, 2008).

Citrus (*Citrus spp*) was introduced to Nigeria by the Federal Department of Agriculture and missionaries in the 1930s (Adigun, 1993). Subsequently, it spread throughout the country and is currently rated as the most widely planted fruit tree in Nigeria. It currently features in diverse cropping systems which include the multi-storied home gardens, cocoa plantations, food crop plots and a few pure stand citrus orchards (Oladokun, 1990). The sweet orange *Citrus sinensis* L. Osbeck is very tasty and nutritious, containing 5-10 % sugar, 1-2 % citric acid. They are fat free, sodium free and cholesterol free. In addition they contain potassium, calcium, foliate, thiamin, niacin, vitamin B_6, phosphorus, magnesium and copper.

2.2 Phytochemicals in *Citrus sinensis*

In recent research studies, the healing properties of oranges have been associated with a wide variety of phytonutrient compounds (Davis, 2004). These phytonutrients include citrus flavanones (flavonoids that include the molecules hesperedin and naringenin), anthocyanins, hydroxycinnamic acids, and a variety of polyphenols. When these phytonutrients are studied in

combination with oranges' vitamin C, the significant antioxidant properties of this fruit are understandable. Orange peel contains high concentrations of phenols (Manthey, 2004). Its extract contains significant amount of beta-carotene (Ghazi, 1999) and is a good source of vitamin C (Rinzler, 1990).

2.2.1 Flavonoids

Flavonoids are plant pigments that are capable of inhibiting or killing many bacterial strains, some pathogenic protozoa and scavenge free radicals (Zhang *et al.,* 2006). However, their toxicity to animal cells is low. But it is yet another flavanone in oranges, the hesperidin molecule, which has been singled out in phytonutrient research on oranges. Arguably, the most important flavanone in oranges, hesperidin has been shown to lower high blood pressure as well as cholesterol in animal studies (Cushnie and Lamb, 2005) and to have strong anti-inflammatory properties (Kim *et al.,* 2004). Importantly, most of this phytonutrient is found in the peel and inner white pulp of the orange, rather than in its liquid orange centre, so this beneficial compound is too often removed by the processing of oranges into juice. This phytonutrient contributes to reduced blood pressure and cholesterol levels and also provides and anti-inflammatory effect. Hesperidin can improve capillary health and connective tissue and works with vitamin C to maintain the health of collagen (Benavente-García *et al.,* 1997). Because of this, hesperidin has been suggested to help with bruising, varicose veins, fragile capillaries and sagging, wrinkled skin. Flavonoids are polyphenolic compounds, widely distributed in the plant kingdom, many of which have antioxidant and antithrombotic properties (Folts *et al*., 1994; Wang *et al.,* 1996).

Epidemiological studies showed an inverse correlation between the level of intake of dietary flavonoids and death from coronary artery disease (CAD) (Hertog *et al*., 1993 and 1995; Knekt

et al., 1996; Rimm *et al*., 1996). Hertog *et al.* (1993) found lower rates of myocardial infarction and cardiac death in the upper trials of flavonoid intake among 805 elderly men in Zutphen, The Netherlands. In a larger group of 16 cohorts in seven countries, flavonoid intake was inversely related to CAD mortality (Hertog *et al*., 1995). In Finland, a study of over 5,000 men and women suggested increased incidence of CAD in the population with the lowest flavonoid intake (Knekt *et al*., 1996).

2.2.2 Limonoids

Limonoids are secondary metabolites in all citrus fruit tissues whose role in human nutrition has not been established. These tri-terpenoid compounds occur in citrus as either limonoid aglycones or limonoid glycosides. Limonoid glucosides occur in large amounts in citrus fruit and juice with levels in juice reported to be as high (500 μg/ mL) (Fong *et al.,* 1990). Limonoid glycosides, like *limonin* glucoside are water soluble, tasteless, non-mutagenic (Lam *et al.,* 2000), and have shown no significant toxic effects when fed to hamsters up to 0.5 % of the animal diet (Mill*er et al.,* 1999). The limonoid aglycones, like *limonin*, occur at low levels in citrus juices. Several of the water insoluble aglycones are responsible for the development of delayed bitterness in citrus juices in concentrations as low as 6 ppm (Hasegaw*a et al.,* 1996). Several studies have established citrus limonoids as having significant biological activity in mammalian systems. In vivo animal tests with mice have shown that citrus limonoids induce glutathione S-transferase activity (Hasegawa *et al.,* 1996) and inhibit fore stomach (Lam *et al.*, 1989), oral (Lam *et al.,* 1992), lung (Lam *et al.,* 1994), skin and colon (Tanaka *et al.,*2000; Tanaka *et al.,* 2001) tumors in animals. In vitro studies with human breast cancer cells have shown limonoids to be potent inhibitors of the proliferation of estrogen receptor negative and estrogen receptor positive human breast cancer cells in culture (Guthrie *et al.,* 1997). In vitro studies have shown selected citrus

limonoids, including limonin to effectively reduce the production of medium *apo* B in cultured human liver cells suggesting that the limonoids could contribute to the cholesterol lowering effect of citrus juices (Kurowska *et al.,* 2000).

Limonin is a tri-terpenoid present in the seeds and skins that imparts a bitter taste to citrus pulp. If large quantities of seeds are present their *limonin* content may render the pulp toxic to non-ruminants even at 2.5 % in their diets (Devendra, 1988; Rihani, 1991). It has been suggested that *limonin* caused intestinal irritation and poor absorption of the nutrients in broilers (El Boushy *et al.*, 2000). *Limonin* should not be confounded with *limonene,* another terpenoid present in large quantities in citrus peels and that is responsible for the characteristic odour of citrus. *Limonin* glucoside is the most prominent limonoid glucoside found in citrus juices and is readily available from orange juice or citrus juice processing by-products (Ifuku *et al*., 1998; Schoch *et al*., 2002).

2.2.3 Limonene

Limonene takes its name from the lemon, as the rind of the lemon, like other citrus fruits, contains considerable amounts of this compound, which contributes to their odour. *Limonene* is a chiral molecule. D-*Limonene* is obtained commercially from citrus fruits through two primary methods: centrifugal separation or steam distillation. *Limonene* is considered by some researchers to be a potential chemo preventive agent (Crowell, 1999) with value as a dietary anti-cancer tool in humans (Tsuda *et al.,* 2004). There is no evidence for carcinogenicity or genotoxicity in humans. *Limonene* is practically non-toxic to birds and mammals (EPA, 1994).

2.2.4 Lectins

There have been reports of a Type IV hypersensibility reaction that caused the death from haemorrhagia and heart failure of several cows fed citrus pulp. This inflammatory reaction may

be the result of a lectin-like hemagglutinating activity thought to be present in citrus pulp. This toxicosis could not be reproduced in sheep and rabbits (Saunders *et al.*, 2000; Tokarnia *et al.*, 2001).

2.3 Utilization of *Citrus sinensis*

The increased interest in their consumption is not only due to their sweet refreshing properties but also as a result of increased knowledge of their nutritional and medicinal values. Orange fruit and its juice have several beneficial, nutritive and health properties (Okwu and Emenike, 2006). They are rich in vitamins especially ascorbic and folic acids. Over the last decades, many other virtues and medicinal benefits of orange fruits have been discovered besides their anti-scurvy property (Rapisararda *et al.*, 1999).

2.3.1 Medicinal properties of *Citrus*

The protection that orange fruits provide against these diseases has been attributed to the various antioxidant phytonutrients contained in citrus species (Rapisararda *et al.*, 1999; Okwu and Emenike, 2006). Yusuf and Salau (2007) reported that citrus fruits are rich in Vitamin C or ascorbic acid and folic acid, as well as a good source of fibre. Citrus fruit is an important source of vitamin C, because of the large quantities consumed (Biolatto *et al.,* 2005). They may help to reduce the risk of heart diseases and some types of cancer. They are also helpful to reduce the risk of pregnant women to have children with birth diseases. As a citrus fruit, the importance of sweet orange in nutrition and human health can therefore hardly be over emphasized. Bakhru (2000) noted that regular intake of orange prevents frequent attacks of common cold, influenza, bleeding as well as contributes to one's healthy living and longevity. In addition to that, the report observed that orange juice compared with all of the fruits juices is more suitable for all ages and can be given with advantages in all kinds of diseases. Apart from human consumption, the use to which sweet orange has been put includes the production of feeds for livestock

especially ruminant animal (Oluremi *et al.,* 2006). The most important part is the orange fruit, which is mostly squeezed to obtain orange juice.

The orange oil is used to treat chronic bronchitis. Tea made from dried orange flowers stimulates the nervous system. Orange peel is traditionally used to treat sleeping problems. The phyto-chemicals *limonene* and flavonoids appear to have anti-carcinogenic properties. They can block the carcinogenesis by acting as a blocking agent (Moon *et al., 2006).* Studies have shown that *limonin* and *limonene* can induce the enzyme activity of glutathione S-transferase, which is an important detoxifying enzyme. Leaf decoction with salt is taken orally for digestive tract ailments, nerve disorders, fever, asthma, blood pressure, general fatigue and vomiting. Crushed leaves or fruit juice is massaged into the skin to relieve itching. Macerated root, leaf or fruit mesoderm is taken orally for urethritis; macerated fruit mesoderm or bark decoction is taken orally for liver ailments. Fruit juice or leaf decoction with sugar is taken orally for cold and loss of appetite, while crushed leaf decoction as a bath relieves headache and rheumatism. Broken bones are massaged with roasted fruit. Leaf oil exhibits carminative properties and light antispasmodic and sedative properties (Orwa *et al*., 2009). In Samoa, a leaf infusion made from sweet orange is used against mouth sores in infants (Goethesson, 1997).

2.3.2 Use of sweet orange peel in pest control

The toxic effect of sweet orange (*Citrus sinensis*) peel has been applied in studies on pest control. In a study by Abolusoro *et al*. (2010) sweet orange peel was compared with nematicide, for the suppression of soil and root population of root-knot nematode *Meloidogyne incognita* on tomato. The significance of the study thus underscores the potency of citrus peel extracts as a

viable alternative to harmful synthetic nematicide for the control of nematode pest on susceptible crops. The exhibited nematicidal properties may be due to the presence of bioactive chemical components including Saponin, flavonoid and tannin in the citrus peel extracts (Olabiyi *et al.,* 2008). Such effects of citrus extracts on plant parasitic nematode have been reported (Oyedunmade *et al.,* 2001; Oyedunmade, 2004).

2.3.3 Antimicrobial activity of *Citrus* peel

The peel of *Citrus* fruit is a rich source of flavanones and many polymethoxylated flavones, which are very rare in other plants (Ahmad *et al*., 2006).The antimicrobial abilities of essential oils, among which citrus oils, are also shown to be a particularly interesting field for applications within the food and cosmetic industries (Caccioni *et al*., 1998). It has also been used as a hypotensive agent (Kumamoto *et al.* (1986), antifungal (Stanger *et al.,* 1993), antimicrobial (Caccioni *et al.,* 1998), carminative, insect repellent, antibacterial, larvicidal, antiviral, uricosuric, anti-yeast, anti-hepatotoxic and anti-mutagenic agent (Han, 1998), anti-diabetic (Hamendra and Anand, 2007), antioxidant (Kanaze *et al.* (2008).

2.4.0 Utilization of *Citrus sinensis* in animal nutrition

The use of *citrus* pulp for animal feeding was found to be an effective way to decrease waste output, and an exhaustive analysis should include an assessment of the environmental burdens associated to substitute feeds and of the associated costs of other methods of citrus pulp disposal (Beccali *et al*., 2010). The palatability of dried citrus pulp is variable, as it may have a bitter taste due to *limonin* and other compounds present in the seeds and peels. It can result in a

decrease in intake if introduced too quickly in the diet, and a more progressive introduction is recommended. It is well accepted by animals that are accustomed to it and can then increase intake (Rihani, 1991; Wing, 2003). In fattening lambs, palatability decreased when diets contained more than 40 % dried citrus pulp (Bhattacharya *et al*., 1973).

Dried citrus pulp is a good ingredient for rabbit feeding with a nutritive value about 2700 kcal/kg (as fed) and can be introduced in diets at 20-30 % levels (de Blas *et al*., 1991; Papadomichelakis *et al*., 2002; Papadomichelakis *et al*., 2004; Pereira *et al*., 2005; Hon *et al*., 2009). In one experiment, a 25% inclusion of dried citrus pulp was found to be economically beneficial compared to the conventional diet for rabbits (Leon *et al*., 1999). No mortality or distress was recorded at 25 % inclusion (Hon *et al*., 2009). Also a study by Ojabo *et al.* (2012) showed that sun dried sweet orange peel can be used as a replacement feedstuff for maize in the ration of grower rabbits at a level of 40 %.

Studies on poultry have shown that sweet orange peel meal can be included in the diet of broilers, substituting maize to levels of up to 20 % (Agu *et al.*, 2010). Oluremi *et al*. (2006) reported a 15 % replacement of dietary maize with sweet orange rind (SOR) was possible in the diet of broilers with increasing dietary SOR content beyond 15 % reducing growth rate, which cumulatively caused a decrease in final live weight. It has been reported that weight gain was negatively affected as the level of citrus in poultry diet increased (Yang and Chung, 1985). Thus far, nutritional trials with monogastric animals have shown that the meal of sun dried peels of

Citrus sinensis can replace dietary maize in broiler diet up to 20 % (Agu, 2008), and 40 % in rabbit (Oluremi *et al.,* 2005), without any adverse effect on their performance.

2.5 Use of post-harvest wastes in animal nutrition

The use of food wastes as an alternative animal feed is of high interest because it produces an environmental and public benefit besides reducing the cost of animal production (Myer *et al*., 1999; Westendorf, 2000). Long ago, farmers reused food waste directly for animal feed without any treatment. In the last century, the risk of spreading disease was a concern for producers, and regulations were enacted to avoid this risk (McChesney, 2000). At present, the legislation of the European Union (EU) forbids the use of meat wastes to feed farm animals and only allows the use of wastes with a minimum microbial and toxic content (Garcia *et al*., 2005). This fact reduces the wastes to be used as animal feed to fruit and vegetable wastes. One of the main drawbacks of using fruit–vegetable in the formulation of animal diets is that their composition may be extremely variable depending on the area of production and the period of the year when they are produced (Westendorf, 2000). Another drawback is the high moisture content that may make the handling difficult and favour microbial contamination (Garcia *et al*., 2005). Heat treatment of these types of wastes is necessary not only to ensure microbial quality (Sancho *et al*., 2004) but also to reduce the moisture content in order to facilitate their inclusion in animal diets. However, a heat treatment can reduce the digestibility of the wastes, thereby decreasing the nutritional value of a diet including fruit and vegetable wastes (Esteban *et al.*, 2007) and can increase the price of the feed formulated with the wastes as a substitute for the cereal typically used.

Recent regional economic growth and changes in dietary patterns have made both the production and consumption of fruit and vegetables increasingly important. The fruit and vegetable sector

has a vital role in farm income enhancement, poverty alleviation, food security, and sustainable agriculture, especially in developing countries. This sector, however, suffers greatly from post-harvest losses. Some estimates suggest that about 30–40 % of fruit and vegetables are lost or abandoned after leaving the farm gate. Heavy crop losses usually occur while the citrus fruit is being transported along its marketing chain. Loss in transit is great for citrus fruit because the fruits are fragile and juicy as well (Adeyemi and Ogazi, 1998). Of the 36.0 metric tonnes world production, Nigeria produces 0.3 million tonnes and has the potential to produce more orange wastes in high proportion. Though Nigeria is not well noted for the exportation of citrus fruits, she has the potential to produce more for both local and international markets.

Presently, the local processing of citrus fruits is on the increase to meet increasing local demands for fruit juice that was previously met by large-scale importation. Of all the citrus fruits, sweet orange is the commonest and the most widely cultivated and consumed in the major 16 citrus growing states in Nigeria, namely: Abia, Anambra, Bauchi, Benue, Cross River, Enugu, Imo, Kaduna, Kogi, Kwara, Lagos, Ogun, Ondo, Osun, Oyo and Plateau states (Odbanjo and Sangodoyin, 2002). This also means quantum increases in the volume of waste derived from its fruit juice production. Huge postharvest losses result in diminished returns for producers. International markets reject fruits and vegetables containing unauthorized pesticides, with pesticide residues exceeding permissible limits, and with inadequate labelling and packaging. At the market place, the seller is also faced with the problem of spoilage as the fruits have to be sold within the shortest time possible before they get spoilt.

The sweet orange fruit perishability problem is also compounded by the poor storage/shelf life of the fruit (Babatola, 2004). In Nigeria, oranges (*Citrus sinensis* L Osbeck) are produced and transported to other parts of the country where the consumption is highest and where the orange

command better prices. The oranges in Nigeria are packed into an open truck and transported during which they are exposed to high ambient temperature which will result into shrivelling due to weight loss and the appearance will be poor. Another problem is that of pests and diseases attacks on the crop. Fruits such as the sweet orange are attacked by pests and diseases which reduce their market quality and quantity (Apata, 2002).

Similarly there have been increasing concerns over food-borne diseases and poisoning such as *Escherichia coli* or *Salmonella* outbreaks. One of the limiting factors that influence the fruit's economic value is the relatively short shelf-life period caused by pathogens attacked. It is estimated that about 20-25 % of the harvested fruits are decayed by pathogens during post-harvest handling even in developed countries (Droby, 2006; Zhu, 2006). Obviously, post-harvest management determines food quality and safety, competitiveness in the market, and the profits earned by producers. In developing countries, postharvest losses are often more severe due to inadequate storage and transportation facilities. Fungal fruits infection may occur during the growing season, harvesting, handling, transport and post-harvest storage and marketing conditions, or after purchasing by the consumer. Fruits contain high levels of sugars and nutrients element and their low pH values make them particularly desirable to fungal decay (Singh and Sharma, 2007).

Generally, spoiling fungi are considered toxigenic or pathogenic. Toxigenic fungi have been isolated from spoiling fruits (Stinson *et al*., 1981). Pathogenic fungi, on the other hand, could cause infections or allergies (Monso, 2004). *Aspergillus spp*. are known to produce several toxic metabolites, such as malformins, naphthopyrones (Frisvad and Samson, 1991) and they can produce ochratoxins (OTA), a mycotoxin which is a very important toxin worldwide because of the hazard it poses to human and animal health (Peraica *et al.*, 1999; Petzinger and Weidenbach,

2002) thus extra care should be taken during personnel handling of these fruits; such as harvesting, cleaning, sorting, packaging, transport and storage. Recycling of fruit and vegetable waste is one of the most important means of utilizing it in a number of innovative ways yielding new products and meeting the requirements of essential products required in human, animal and plant nutrition as well as in the pharmaceutical industry (Gautam and Guleria, 2007).

2.6 Animal feed contamination

Animal feed, due to its composition, provides a favourable environment for the micro organism's growth. Micro organisms found in the feedstuffs can be saprophytic, pathogenic, conditionally pathogenic and toxic. Their growth and proliferation in the feed depends on numerous factors, such as moisture, temperature, type of feed, aerobic and anaerobic conditions, chemical and physical properties of raw material feed pH value, presence of feed supplements, storage periods and conditions as well as feed decomposition products (Radanov-Pelagic, 1999; Đorđević and Dinic, 2007). Feed may be contaminated during processing, storage or transport. Contaminated feed frequently causes zoonozes and for that reason, it is necessary to establish surveillance programmes for microbiological feed hazards (Radanov- Pelagic, 2000).

Feed ingredients must be safe and suitable for storage. Only one unsafe component may jeopardize quality of compound feed. Good quality raw materials produce good quality feed. Seasonal raw materials, like grains, must be stored for a certain period of time. Some micro organisms introduced during storage, primarily moulds, can negatively affect feed quality including reducing dry matter and nutrients, causing musty or sour odours, and producing toxins. Mouldy raw materials are not appetizing and can considerably reduce feed consumption (Meeusen, 1997). Any piece of equipment, process, or production line or entire feed manufacturing plant pose a source of contamination threatening quality of final product

(Sredanović *et al.,* 2005). Though it is not possible to produce microbial-free feed without adversely affecting its nutritional values, microbial feed safety hazards can be minimized through appropriate agricultural and storage practices.

2.7 The rabbit

2.7.1 Origin and distribution

The domestic rabbit (*Oryctolagus cuniculus)* is a descendent of wild rabbits of southern Europe and North Africa. The rabbit is thought to have been discovered by Phoenecians when they reached the shores of Spain about 1000 BC. During the times of the Romans, the rabbit was still emblematic of Spain. It appears that the Romans spread the rabbit throughout the Roman Empire as a game animal. The Romans, like Spaniards of that time ate foetuses or newly born rabbits, which they called laurices. In their natural environment, rabbits are gregarious and prolific. They are completely herbivorous (eat only plants) and most actively forage in the twilight or in the dark. The average lifespan of a rabbit is 5-10 years (potential life span of 15 years is possible) (Bartash-Dawley, 2006). Rabbits have been recognized to have a very important role to play in the supply of animal protein to humans, especially in tropical and subtropical areas. Rabbits occupy a vital midway between ruminants and monogastric animals and can effectively utilize cellulose rich feed with ration containing less than 20 % grain. Simple biological characteristics, short breeding cycle, high prolificacy and better feed conversion efficiency logically place rabbit just below poultry (Hasanat *et al*., 2006).

In 1994, world's production of rabbit meat was estimated to be 1.5 million tons per annum. This would mean *per caput* annual consumption of 280 g per person per year. In Africa, the leading rabbit producing countries are Morocco and Nigeria and these are reported to produce 20000 to 99000 tonnes meat per year (Moreki, 2007).

2.7.2 Digestive system of the rabbit

The digestive system of the rabbit is characterized by the relative importance of the caecum and colon when compared with other species (Portsmouth, 1977). As a consequence, the microbial activity of the caecum is of great importance for the processes of digestion and nutrient utilization, but also in the control of digestive pathologies. Furthermore, caecotrophy, the behaviour of ingestion of soft faeces of caecal origin, makes microbial digestion in the caecum more important for the overall utilization of nutrients by the rabbit. Additionally, the rabbit has developed a strategy of high feed intake (65–80 g kg−1 body weight) and a rapid transit of feed through the digestive system to meet nutritional requirements. To reach its full functional capacity, the digestive system of the growing rabbit must go through a period of adaptation from milk based feeding to the sole dependence on solid feed. This adaptation process not only affects the digestion processes, but also microbiota colonization and the development of gut barrier mechanisms that protect the animal against digestive pathologies.

The first important compartment of the digestive system of the rabbit is the stomach. This has a very weak muscular layer and is always partially filled. After caecotrophy, the fundic region of the stomach acts as a storage cavity for caecotrophs. Thus, the stomach is continuously secreting and the pH is acidic. The stomach pH ranges from 1 to 5, depending on site of determination (fundus versus cardiac-pyloric region) (Gutierrez *et al*., 2003; Chamorro *et al*., 2007; Orengo and Gidenne, 2007; Gomez-Conde *et al*., 2009), the presence or absence of soft faeces (Griffiths and Davies, 1963), the time from feed intake (Alexander and Chowdhury, 1958) and the age of the rabbit (Grobner, 1982). The lowest figures (from 1 to 2.5) are determined in the cardiac region, in the absence of soft faeces, after 4 h of diet ingestion and in rabbits older than 3 weeks with

low presence of milk (Orengo and Gidenne, 2007). The capacity of the stomach is about 0.34 of the total capacity of the digestive system (Portsmouth, 1977).

The stomach is linked with a coiled caecum by a small intestine approximately 3 m long, where the secretion of bile, digestive enzymes and buffers occurs. The pH of the small intestine is close to 7 (Nicodemus *et al*., 2002). The small intestine is the site where the greater part of digestion and absorption take place by passive or active transportation throughout the mucosa.

Digestibility at the end of the ileum accounts for 0.8–1 of the total dietary amino acid and starch digestibility (Gutierrez *et al*., 2002; Garcia *et al*., 2005; Carabano *et al*., 2009).The caecum is characterized by a weak muscular layer and contents with a dry matter (DM) of 200 g kg−1. The caecal contents are slightly acid (pH 5.4–6.8) (Garcia *et al*., 2002). The rabbit's cecum is proportionally the largest of any mammal. It is twice the length of the abdominal cavity and 40–60 % of the total volume of the gastrointestinal tract (Jenkins, 2000). The proximal colon can be further divided into three segments: the first segment possesses three *taeniae* with *haustra* between them; the second segment has a single *taenia* covering half of the circumference of the digestive tube; and the third segment or *fusus coli* has no *taeniae* or *haustra*, but is densely enervated. Thus, it acts as a pacemaker for the colon during the phase of hard faeces formation (Snipes *et al*., 1982).

The different segments of the digestive system of the rabbit grow at different rates until reaching maturity. The development of the digestive tract begins in the foetal stage; at birth, the stomach and small intestine are the main components of digestive tract. According to Sabatakou *et al*. (1999), the stomach glands are evident in late fetuses (26 days' gestation) and true villi and intestinal glands (Crypts of Lieberkuhn) are observed at 29 days' gestation. At birth however, the intestine of the newborn does not possess all of the mucosal constituents that are present in the

adult. These appear in the first week of age (Brunner's glands in the duodenum) and the adult morphology is not completed until 20 days of age. The developmental pattern follows a cranio-caudal gradient. The early development of these two segments is important to ensure the survival of the newborn. From birth to 18–20 days of age, kits suckle large amounts of milk during a once-daily nursing, an amount that can reach 0.12 of their body weight. This explains the importance of the relative weight of the stomach when its contents are also recorded. At around 18 days of age the suckling rabbit begins to eat solid food and decrease its milk intake and the caecum and colon develop faster than the rest of the digestive tract.

Rabbits are herbivores, are concentrate selectors, and are classified as hindgut (Caecum and colon) fermentors (McNitt *et al*., 1996). Because there are no mammalian enzymes to break down the cellulose components of their plant-based diets, rabbits as well as other herbivores have a symbiotic microbe population (Primarily *Bacteroides).* In rabbits, the microbial population is found in the caecum. The rabbit caecum is very large, compared with the rest of the gut (Stevens and Hume, 1995) and forms a spiral that fills the abdominal cavity. The caecum has a capacity 10 times that of the rabbit's stomach, about 40 % of the gastrointestinal tract (Jenkins, 1999). Instead of completely fermenting fibre, rabbits utilize a mechanism to sort out indigestible fibre and expel it from the body, a process that is a specialized feeding strategy that overcomes poor-quality protein (Carabano and Piquer, 1998; Jenkins, 1999). This sorting mechanism occurs as digesta enters the rabbit's large intestine and muscular contractions facilitate the separation of fibre and non-fibre fractions. A series of peristaltic and anti-peristaltic waves separate out non-fibre fractions for further fermentation in the caecum (Cheeke, 1987; Carabano and Piquer, 1998); particle size and density aid separation (Cheeke, 1994). The fibre components are voided from the body about 4 hours after consumption of the diet (Cheeke, 1994). After fermentation of

the non-fibre components in the caecum, a pellet is formed (called a cecotrope or soft faeces) that is voided from the body approximately 8 h after consumption of the diet (Cheeke, 1994). A neural response (Jenkins, 1999) or the strong odour of VFA (Stevens and Hume, 1995) in the cecotrope seems to stimulate its consumption directly from the anus. This practice of consuming cecotropes is called coprophagy, or cecotrophy (Cheeke, 1987). In natural settings, coprophagy usually occurs during the day, opposite of feed intake and the voiding of hard faeces, in a circadian rhythmic pattern (Carabano and Piquer, 1998; Jenkins, 1999), and is an integral part of the rabbit's digestion process (Cheeke, 1994). If a rabbit is equipped with a collar preventing coprophagy, the digestion of the diet is significantly reduced, even when a highly digestible diet is fed.

Gut micro flora of rabbits are sensitive to most antibiotics (McNitt *et al*., 1996). If antibiotics are fed, the microbe population is altered, favouring *E. coli* and *Clostridia* organisms that produce toxins harming the gut lining, causing diarrhoea and enterotoxemia (Cheeke, 1994; Stein and Walshaw, 1996; Brooks, 1997). Antibiotics that may cause this effect include lincomycin, ampicillin, amoxicillin, procaine penicillin, cephalexin, erythromycin, clindamycin, tylosin, and metronidazole. The actual effect from each of the drugs will differ between animals (Stein and Walshaw, 1996). Oxytetracycline, virginiamycin (Cheeke, 1994), or tetracycline (Brooks, 1997) are exceptions and are used as growth promoters, and sulfaquinoxaline is to control coccidia (Brooks, 1997). Under no circumstances should the inophore monensin be fed to rabbits; it is toxic even at low concentrations (McNitt *et al.,* 1996; Martin, 2000).

2.8 Intestinal health of the rabbit

In rabbits, dietary fibre has a critical role in maintaining gut health, stimulating gut motility (Insoluble fibre only), reducing fur chewing, and preventing enteritis (McNitt, *et al*., 1996;

Brooks, 1997). Rabbits need a minimum dietary fibre level of 20 to 25 % to maintain gut health. Diets with less than 20 to 25 % fibre result in gut hypo motility, reduced cecotrope formation, prolonged retention time in the hindgut and often enteritis (Cheeke, 1994; Jenkins, 1999). Composition of the hard faeces and the cecotrope is influenced by the diet. If dietary fibre concentration increases, the fibre composition of the fecal pellets also increases. Thus, high-quality fibre is essential for gut health in rabbits (McNitt *et al*., 1996; Stein and Walshaw, 1996). Fiber fermentation in rabbits does not seem to be enhanced by coprophagy (Cheeke, 1994).

Infectious diseases of the digestive system currently account for 70 % of all rabbit diseases. That percentage has always been quite high, but in recent years it has increased further due to epizootic rabbit enteropathy (ERE), which can cause up to 60 % mortality, increase morbidity and delay the end of the fattening period by one to two weeks (Licois, 2004). Antibiotics have been widely used to control mortality but the corresponding veterinary treatments have increased by 2.5 fold, reducing the benefit margin for many farms. Apart from ERE, several other digestive diseases remain, mostly caused by different strains of *Escherichia coli*. The European ban on the use of antimicrobials to prevent digestive diseases and the narrow range of molecules allowed as treatment in rabbit farming have stimulated the search for alternative solutions. Among those, nutritional management has become a priority, especially given the importance of digestive diseases (Carabano *et al.,* 2008).

Nonetheless, the mortality by ERE in rabbits consuming the same feed, can range from 0 to 70 %, depending on factors such as season, farm, and type of medical treatment. These results suggest that non-dietary factors are also required to begin the process. The main cause of enteric disease is the presence of the pathogen. However, factors such as intestinal saprophyte micro biota and the animal's own defence mechanisms will tend to impede or reduce the growth of

pathogens and the development of the disease. This explains why some animals survive controlled infections caused by pathogenic strains, even though they present symptoms (morbidity) (Carabano *et al.,* 2008). The mechanisms for survival to infection are quite complex. This is especially important in the case of the ERE, where the pathogenic flora involved has not been completely identified up to now. Nutritional strategies have been directed towards avoiding structural damage of the mucosa and favouring repair mechanisms by supplying the necessary nutrients. Adequate characteristics of the mucosal barrier not only improve the immune system, but also the digestive and absorptive efficiency, decreasing the flow of nutrients to the caecum that can favour growth of pathogens. Those strategies are not easy to develop since there are many interactions among the factors involved, but, as our knowledge increases, it appears that they are a useful way to control digestive diseases. The interaction of mucosa with intestinal micro biota seems to play a key role both in the development of defence mechanisms as well as pathogenicity (Colonization and translocation of bacteria and toxins). The main pathogens of the gastrointestinal tract need to be in contact with the mucosa to develop properly. Regarding nutrition, the existing information is scarce and contradictory (De Blas *et al.*, 1999a). Design of starter feeds should take into account both minimizing the weaning stress (By providing starter diets containing highly digestible nutrients, richer in fat and protein, and lower in fibre content than traditional commercial fattening feeds) and the limited capacity for hydrolysis of several components at early ages (Especially for fat and starch). Dietary factors most related with the appearance of digestive diseases were the level and type of fibre (De Blas *et al.*, 1999b; Gidenne, 2000) and the level and type of protein (Carabaño *et al.*, 2006). The beneficial role of fibre in preventing digestive diseases is mostly based on the control of intestinal microbiota through its effects on the digestive transit, and its availability as substrate for bacterial growth. Using

common sources of fibre (Alfalfa, straw and wheat bran) in weaning rabbits diets, Gutiérrez *et al.* (2002) and Feugier *et al*. (2006) showed that a reduction in fibre content from 36-38 to 30-32 % NDF reduced mortality and enhanced performance and feed efficiency, in association with an improvement of mucosal structure (Álvarez *et al*., 2007). Otherwise, a minimal content of insoluble fibre is also required to reduce diarrhoea incidence.

Many studies have shown that virulent strains of *E. coli* need to adhere to the mucosa to be pathogenic. Other pathogenic bacteria in the gastrointestinal tract of the rabbit include the genera *Clostridium*, which tend not to adhere but produce toxins instead (*C. spiroforme, C. perfringens, C. difficile, C. sporogenes*). The death of the animal due to epizootic rabbit enteropathy is produced by a toxin (Still unclassified) or a strain of *C. perfringens* that damages the liver and kidney (Pérez de Rozas *et al.*, 2005). Nonetheless, for these toxins to arrive to the host organs they have to pass through the mucosal layer. The adhesion of *C. perfringens* toxins to the membrane, as well as the initial damage that it causes, seem to be necessary for translocation to occur (McClane, 2001).

2.9 Acidifiers in animal nutrition

Rabbits are sensitive to multi-factorial digestive disorders, which can cause high mortality and morbidity rate, and seem to be related to microbial dysbiosis in the caecum (Cesari *et al.,* 2008). During the first growing period, in fact, changes in feeding behaviour and in housing conditions together with an incomplete maturation of the digestive immune system could promote the development of potentially pathogenic micro flora, which could cause digestive troubles and lower zoo-technical performance. In intensive rabbit farms, antibiotics are often added to feed or water from weaning till 8 weeks of age, in order to prevent enteric diseases and reduce high economic losses. Routine use of antibiotics as growth promoters is a matter of debate in the

animal farming industry. The EU has banned all antibiotic growth promoters (AGP) from livestock production with effect of January 2006. Alternatives to AGP are sought in a variety of forms. The ban on antibiotic growth promoters creates significant opportunity for feed acidification (Frost and Sullivan, 2004). The term acidification includes the reduction of pH in feeds and in the animal digestive tract, mainly in the stomach. Acidification of young animal diets is not a new concept in the feed industry and its ultimate aims are to help to maintain the optimal pH in the stomach, allowing a correct activation and function of the proteolytic enzymes, thus permitting total protein digestion in the stomach and stimulate feed consumption. Young animals have limited ability to produce hydrochloric acid in the stomach. At birth, hydrochloric acid production is negligible, but it increases with advancing age. The greater the production of acid in the stomach, the lower the gastric pH (Peris and Calafat, 2001)

In the stomach, pH can regulate the movement of viable bacteria and molds from the animal's environment to the small intestine and is involved in the activation of pepsin, a proteolytic enzyme. Pepsin is secreted as an inactive zymogen, pepsinogen, and its conversion to the active pepsin is catalyzed by the action of acid and any existing pepsin in the stomach. Additionally, the optimal pH for pepsin activity is 2.0. At higher pH, activity is severely reduced. The initial proteolytic activity carried out by pepsin is necessary for the subsequent activity of trypsin in the small intestine. An additional important role for stomach acid is the protection of the lower part of the digestive tract from bacterial invasion (Easter, 1988). The low pH (2.0 or less) commonly found in normal, growing and adult animals have been shown to have a pronounced bacteriostatic effect. Thus, viable microorganisms entering the digestive system via the mouth are unable to pass through the stomach and successfully colonize in the small intestine.

Organic acids and salts have a long-history in the food and the feed industries, which commonly use them as preservatives. Some authors (Hebeler *et al*., 2000; Hellweg *et al.*, 2006; Øverland *et al*., 2000; Walsh *et al*., 2003) also consider them to be a viable alternative to antibiotics in pig feeds where they already have had considerable success. According to Partanen and Mroz (1999), formic, acetic, propionic, butyric, lactic, sorbic, fumaric, tartaric and citric are the most promising of organic acids in this regard. Organic acids are weak acids that are commonly found in fruit juices and fermented foods and that are added to foods as preservative agents (Lück and Jager, 1997). Organic acids, such as propionic acid, have been used for more than 30 years to reduce bacterial growth and mould in feedstuffs and thus preserve hygienic quality. In feed legislation they are registered as preservatives, but their positive effects on animal health and performance, if they are added to feed in sufficient amounts, are also well documented. Acids used as feed additives are predominantly compounds that naturally occur in cell metabolism, thus they are natural products with low toxicity (Kirchgessner and Roth, 1988).

Health and performance promoting effects have been demonstrated for a number of organic acids, including formic, fumaric, citric and lactic acid and their salts. Besides improvement in hygiene and a corresponding reduction of pathogen intake, effects on feed digestion and absorption and on stabilization of gut flora eubiosis have been demonstrated in a number of investigations. Organic acids also improve the digestibility of proteins and amino acids and the absorption of minerals (Mroz *et al*., 2000; Omogbenigun *et al*., 2003), modulate endocrine and exocrine secretions and influence the mucosal morphology (Partanen and Mroz, 1999).

In animal husbandry, higher feed conversion rates and improved daily gain, as well as reduced incidence of diarrhoea, enhance economic return by lower feed costs and shorter time to market. The greatest response to supplementation with acids has been recorded in piglets, especially

during the weaning period. In the first three to four weeks of life gastric hydrochloric formation and pancreatic enzyme secretion is poor in the digestive tract. Moreover, piglets can be subjected to stress due to separation from the sow, and feed intake may be low for some days post-weaning. After recovery, high amounts of feed are consumed in compensation, and these volumes cannot always be acidified and digested properly, leading to diarrhoea and oedema. These problems are reduced in older pigs but the growth promoting effects of organic acids can still be achieved, though to a lower extent (Baustadt, 1993; Meyer *et al.*, 2006). However, a literature survey revealed considerable variation in effects between trials (Freitag *et al.,* 1998) that can be caused by differences in feeding, housing or hygienic conditions. In animal nutrition, acidifiers and salts exert their performance promotion effects via three different ways: feed, intestinal tract and metabolism.

2.9.1 Effect of acidifiers in feed

Even in a hygienic environment, feed can be infected with a certain amount of fungi, bacteria or yeast. Under favourable conditions, microbes can multiply rapidly during storage, especially at higher moisture level and in a warm environment. Conserving agents reduce microbial growth and, depending on the agent, even microbial numbers, and can lower the uptake of pathogenic organisms by the animal. For conserving purposes, acid concentrations are in general lower than for performance promotion. However, for each acid, its specific inhibiting effect on bacteria, yeast or mould has to be considered when recommendations for feed supplementation are made. The antimicrobial effects of organic acids in feed and their minimum inhibition concentrations are reviewed in detail elsewhere (Strauss and Hayler, 2001).

Besides its hygienic effect, reduction in the acid binding capacity of feed ingredients can promote animal health, especially in piglets. At weaning the withdrawal of milk reduces bacterial

fermentation and results in production of lactic acid in the stomach while the secretion of hydrochloric acid is still low (Kamphues, 1991). Moreover, the high crude protein and mineral content of feed ensures rapid animal growth, but generates high dietary buffering capacity at the same time, thus reducing levels of free hydrochloric acid. Pepsin activation and pancreatic enzyme secretion are reduced, impairing nutrient digestion. Lowering the dietary buffering capacity has been related to beneficial effects on feed digestion (Eidelsburger, 1997).

2.9.2 Effect of acidifiers in the intestinal tract

The effects of organic acids in the intestinal tract are two-fold. They reduce pH in the stomach and small intestine. Moreover, acid dissociation in the bacterial cell and the accumulation of salt anions inhibit microbial growth. Dose response studies reveal a substantial pH reduction in feed with the most effective acid concentrations, while their salts have low effects on the pH value (Roth and Kirchgessner, 1989). Besides acid concentration, pH reduction in feed also depends on acid strength (pH value) and the buffering capacity of feed ingredients. Animal protein, extensively used in piglet feed, has a 15 fold higher buffering capacity compared to cereals such as wheat (Kirchgessner and Roth, 1988). There is also a great variation in buffering capacity of mineral sources; MgO and CaCO3 buffer acids to a greater extent than mineral complexes with organic salts. These effects are especially important in view of the low hydrochloric acid output in the young piglet. Inadequate pH reduction in the stomach inhibits pepsin activity and impairs protein digestion. Effective proteolytic activity requires a pH below 4, and is increased at lower pH values. Positive effects of organic acids on protein hydrolysis have been demonstrated in many published trials (Eckel *et al.*, 1992; Eidelsburger *et al.*, 1992; Mroz *et al.*, 2000). Likewise

duodenal secretion of pancreatic enzymes is reduced at high pH values thus impairing overall digestion.

Feed supplementation with organic acids leads to lower duodenal pH, improved N retention and increased nutrient digestibility (Øverland *et al.*, 2000; Kluge *et al.,* 2004). Experimental evidence demonstrates an increase in apparent ileal lysine digestibility of up to 8 % in weaned piglets and 5 % in fattening pigs compared to controls. Response seems to be higher with feed that has lower digestibility (Partanen and Mroz, 1999). In growing pigs, formic acid supplementation resulted in improved fat digestibility (Partanen *et al.*, 1998) and improved nutrient retention leads to higher feed conversion rate and daily gain (Paulicks *et al.*, 1999; Hasselmann *et al.*, 2003; Ettle *et al.*, 2004). These effects are most pronounced in piglets and young pigs (Roth *et al*., 1998a; Eidelsburger *et al.*, 2000; Øverland *et al.*, 2000). The effects of various acids, salts and acid-salt combinations on feed intake, daily gain, feed conversion ratio and incidence of diarrhoea have been extensively reviewed (Freitag *et al.*, 1998). Improved apparent digestibility and nutrient retention applies also to minerals, either due to homoeostatic effects in faster growing animals or to the formation of mineral and acid complexes, e.g. with Cu (Roth *et al.*, 1998b; Partanen, 2001) leading to reduced mineral excretion and beneficial environmental effects.

Organic acids and salts exert their growth inhibiting effects on stomach and gut microbes through pH reduction and anion and proton effects in the microbial cell. Growth rates of many microbes like *Clostridium perfringens*, *Escherichia coli* or *Salmonella* ssp. are reduced below pH 5, while acid tolerant microbes are unharmed. Low pH also provides a barrier against microbes ascending from the ileum and large intestine. Moreover, small acids are lipophilic and can diffuse across the cell membrane, where, in the more alkaline cytoplasm, they dissociate and the released protons will subsequently lower the internal pH. This reduction in pH alters cell

metabolism and enzyme activity, thus inhibiting growth of intra-luminal microbes, especially pathogens.

Several investigations have demonstrated a reduction in bacterial count in the stomach (Kluge *et al*., 2004) and the duodenum (Kirchgessner and Roth, 1991; Hebeler *et al*., 2000; Hellweg *et al*., 2006). However *Lactobacillus* spp. numbers seem to be unaffected, or may even be enhanced (Hellweg *et al.*, 2006). This will help promote eubiosis in the piglet's intestinal microflora during the post-weaning period. However, the rate at which organic acids kill bacteria depends on the time of exposure, ambient temperature and specific properties of the acid used. Minimum inhibiting concentrations of organic acids are specific for each acid (Strauss and Hayler, 2001), for example, gram negative bacteria are only sensitive to acids with less than eight carbon atoms, whereas gram positive bacteria are sensitive to longer chain acids (Partanen, 2001). Formic acid seems to be more effective than propionic or lactic acids against the majority of bacteria (Strauss and Hayler, 2001). Since salts cannot reduce environmental pH, their effects can only be asserted via acid anions.

The importance of anions in overall benefits for performance can be demonstrated by comparison of acids and salts in feeding trials, as demonstrated by Kirchgessner and Roth (1987) with formic acid and Na-formate, where equal amounts of anions were added to both groups. Growth promoting effects, such as daily gain and feed conversion ratio, were around 50 % in the salt group compared with those receiving formic acid. This appeared to be due to the effects of anions, whereas the higher effects observed for the formic acid group were due to pH reduction. Conversely, reduction of gut microbe counts seems to be due to the anionic effect. Experimental evidence suggests that numbers of *E. coli* and *Enterococcus* ssp. can be reduced to similar levels with either Ca-formate or formic acid (Kirchgessner *et al.*, 1992). Data from feed trials

demonstrated the effectiveness of anions in the regulation of gut microbe composition and the performance promoting effects of organic salts. Changes in the gut micro flora caused changes in the intestinal environment, resulting in reduced ammonia and lactic acid concentrations in the stomach and small intestine (Eckel, 1990; Hebeler *et al*., 2000). An overall reduction in gut bacteria reduced metabolic needs of microbes and enhanced absorption rates of nutrients, especially energy and amino acids (Hebeler *et al*., 2000; Øverland *et al*., 2000), leading to better feed efficiency and improved daily gain (Sebastian *et al.,* 1996; Eidelsburger *et al*., 2000; Øverland *et al*., 2000 and Hasselmann *et al*., 2003). This effect is most distinctive in younger animals, but recent studies demonstrated similar benefits in finishing pigs (Meyer *et al*., 2006). However, sufficient amounts of anions are essential, as acids with low molecular weight, e.g. formic acid, seem to have more pronounced activities than those with higher molecular weights, e.g. fumaric or citric acid (Eidelsburger, 1997).

Eubiosis of intestinal flora and the reduction in the activity of microbes relieved the metabolism of the host animal due to reduced intestinal ammonia, amine and toxin concentrations (Hebeler *et al*., 2000), leading to less digestive disorders, e.g. diarrhoea. In a recent study, feed supplementation with potassium diformate significantly enhanced dry matter content in the caecum (Hellweg *et al*., 2006). In this respect, salts in sufficient concentrations seem to be as effective as acids (Eidelsburger *et al*., 1992). Diarrhoea can also be prevented by inhibition of pathogens like *E. coli* to adhere to the gut wall (Gedek, 1993). Most microbes can only proliferate, alter epithelial function or produce toxins after binding to specific receptors on the gut wall. Again, these effects seem to be more closely related to anions rather than to undissolved acids (Diebold and Eidelsburger, 2006).

2.9.3 Effect of acidifiers on metabolism

Most organic acids contribute a considerable amount of energy. Organic acids are absorbed through the intestinal epithelia by passive diffusion. Short chain acids can be used for ATP generation in the citric cycle, for example, 1M of fumaric acid generates 18M ATP. If ATP formation is compared to the energy content of 1340 kJ/M fumaric acid, it becomes obvious that 74.3 kJ are required per M ATP, approximately the same amount of energy that is involved in ATP generation from glucose. Thus fumaric acid can be compared with glucose in energetic terms. The same is true for citric acid, while acetic and propionic acid need 18 % and 15 % more energy for 1M ATP synthesis (Kirchgessner and Roth, 1988). Sorbic acid is metabolised via ß-oxidation, as are long chain fatty acids (Sofos and Busta, 1993). As the energy content of organic acids is made completely available during metabolism, it should be considered in the energy calculation of feed rations. For example, propionic acid contains up to five times more energy than wheat (Diebold and Eidelsburger, 2006).

Organic acids and their salts can be used to maintain animal health and performance, and as such can be used to replace sub-therapeutic antibiotic growth promoters. Their mode of action includes stimulating gastric enzyme secretion, nutrient digestibility and retention, leading to improved feed conversion rate and daily gain. Economic benefits result from a reduction in feed costs and a shorter time to market. Acids and salts also reduce microbial growth in feed as well as in the intestinal tract, and the combination of improved hygienic standards in feed and eubiosis of intestinal flora reduces the incidence of diarrhoea. An overall reduction in gut microflora reduces the nutrient and metabolic requirement generated by bacteria, leading to better feed efficiency and improved daily gain. Effects are most pronounced in younger piglets, especially during the weaning period. However, several studies have also demonstrated growth

promoting effects in fattening pigs (Freitag, 2007). Reports by Ocak *et al.* (2007) and Ogunwole *et al.* (2011), who included lactic acid and Biotronic®SE in the diet of broilers, respectively both observed that higher levels of organic acids in broiler diets increased feed intake. Studies by Cave (1984) indicate the addition of propionate in the diet increased feed consumption of broiler chicks. Sweet *et al.* (1990) and Mathew *et al.* (1991) reported improved growth rates when weaner piglet diets were supplemented with propionic acid (from 0 .1 to 1 %).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental site

The experiment was conducted at the Teaching and Research farm, Animal Science Department, Ahmadu Bello University, Samaru Zaria in the Northern Guinea savannah zone of Nigeria. The farm is located at latitude 11° 9′ 45′′ N and longitude 7° 38′ 8′′ E, at an altitude of 610 m above sea level (Ovimaps, 2009). The wet season occurs between early May and early October. The dry season occurs between middle of October and early May. Zaria is located in a region with a mean annual rainfall of about 1000 mm (mean of 1976 - 2005). August has the highest mean value (267 mm) while October has the least (19 mm). Daily maximum temperatures may rise to between 32 and 34 °C. Relative humidity is fairly high (60 – 65 per cent but could rise as high as 85 % during the rainy season) (Sawa and Adebayo, 2011). The first and second experiments were carried out between the months of August to October and November to January respectively.

3.2 Source and processing of the Sweet orange fruit waste (SOFW)

The sweet orange fruit waste used in the study consisted of discarded sweet oranges gathered from sweet orange traders at the Railway station market in Kaduna, Kaduna State. The oranges consisted of mixed varieties of sweet orange. The oranges collected were washed with water, split open, then sun dried until they became brittle. The oranges were however used unpeeled. After sun drying, they were then stored in polythene bags to prevent moisture absorption until they were milled using a hammer mill and incorporated into the experimental diets.

3.3 Proximate analysis

Sweet orange fruit waste and feed samples of each of the five treatments were collected and analysed at the Department of Animal Science Biochemical Laboratory for their proximate composition according to AOAC (1995).

3.4 Microbiological enumeration

This was carried out at the Department of Microbiology, Ahmadu Bello University Zaria, to enumerate the bacteria and fungi from the sweet orange fruit waste using the Spread Plate technique as described by Seeley *et al.* (1990). Twenty-five grams of ground sweet orange fruit waste was weighed and dissolved in 225 ml of 0.1 % peptone water. The stock solution was obtained by taking out 10 ml in a test tube. Ten fold dilution of the sweet orange fruit waste was prepared by withdrawing 1 ml from the stock solution into 9 ml of the sterile peptone water to obtain 10^1 dilution with the aid of a sterile pipette from this dilution.1ml was also taken into another 9 ml of sterile peptone water; this procedure was repeated until 10^5 dilution was obtained.

0.1 ml of 10^3, 10^4, and 10^5 dilutions were aseptically transferred into sterile plates of plate count agar for bacteria and sabouraud dextrose agar for fungi in duplicate plates. With the aid of sterile bead glass rod, the innoculum was spread on the culture media. The inoculated plates were then incubated at 37 °C for 24 hours (bacteria growth) while the plates of sabouraud dextrose were incubated at room temperature for 5 days (fungi growth). The number of bacterial and fungal colonies was counted by using a colony counter and the result was recorded as colony forming units per gram (cfu/gm). Total colony forming unit per gram was expressed as total number of colonies counted multiplied by the dilution factor and then divided by the volume of the inoculums.

3.5 Experiment 1: Effect of graded levels of sweet orange fruit waste on the performance and carcass characteristics of weaner rabbits

3.5.1 Experimental diets

The diets consisted of the sweet orange fruit waste replacing maize at graded levels of 0, 5, 10, 15, and 20 % respectively (Table 3.1). The dietary treatments were designated as T_1, T_2, T_3, T_4 and T_5.

The diets were formulated to meet the nutritional requirements for weaner rabbits.

3.5.2 Management of experimental animals and data collection

A total of 30 weaner rabbits were used for the study. The experimental design was a completely randomized design, consisting of five treatments with six replicates each. The animals were kept individually in cages equipped with feeders and drinkers. The cages had wire screen bottoms; this allowed faeces and urine to pass into a collection grid. The rabbits were allowed a two-week adjustment period before the feeding trial commenced. The initial weights of the animals were taken before the experimental diets were introduced to the animals. Feed was supplied daily and water administered *ad lib*. Collection of data included a weekly feed intake, weekly measurement of weights of each individual rabbit, from which daily feed intake, daily weight gain, feed conversion ratio were calculated. At the end of the experiment, the rabbits were weighed to obtain their final live weights. The experiment lasted for a period of 56 days.

3.5.3 Nutrient digestibility study

A total of 15 rabbits were used in a completely randomized design to determine the nutrient digestibility of rabbits fed sweet orange fruit waste based diet. There were 3 rabbits per

treatment, each rabbit serving as a replicate. The study lasted for 14 days including a 7-day adjustment period. Faecal collection for individual rabbits was done 24 hourly before fresh feed was given in the morning. Daily feed intake per replicate was recorded during this period. Collected faeces from each animal were oven dried at 70 ºC for 72 hours and bulked and used for proximate analysis according to AOAC (1995). The nutrient composition of the faeces for each rabbit was used in determining the nutrient digestibility of the experimental diets fed to the rabbits.

3.5.4 Carcass evaluation

At the end of the experiment, three rabbits per treatment representing the average weights of their group were selected for carcass evaluation at the Animal Product Laboratory of the Department of Animal Science. The rabbits were fasted overnight prior to slaughter. They were bled skinned and eviscerated. Retail cuts of fore limbs, hind limbs, loin, rack and ribs were weighed. The weights of the retail cuts and the internal organs were expressed as a percentage of the live weight for each rabbit.

Table 3.1: Composition and calculated analysis of sweet orange fruit waste based diets fed to weaner rabbits

	Experimental diets				
Ingredients	T_1(Control)	T_2	T_3	T_4	T_5
Maize	45.95	40.61	35.26	29.90	24.55
Soyabean meal	25.20	25.54	25.89	26.25	26.60
Sweet orange fruit waste	-	5.00	10.00	15.00	20.00
Palm kernel cake	10.00	10.00	10.00	10.00	10.00
Rice offal	15.00	15.00	15.00	15.00	15.00
Bone meal	3.00	3.00	3.00	3.00	3.00
Salt	0.30	0.30	0.30	0.30	0.30
Vitamin premix*	0.25	0.25	0.25	0.25	0.25
Methionine	0.20	0.20	0.20	0.20	0.20
Lysine	0.10	0.10	0.10	0.10	0.10
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Calculated analysis					
Metabolizable energy, Kcal/Kg	2604	2628	2651	2675	2699
Crude protein, %	18.00	18.00	18.00	18.00	18.00
Ether extract,%	4.52	5.19	5.86	6.53	7.21
Crude fibre, %	10.15	10.31	10.46	10.62	10.78
Feed cost/kg (N)	68.31	65.71	63.12	60.53	57.94

*0.25 kg of premix will supply the following: Vitamin A 1500 IU, Vitamin D 300 IU, Vitamin E 3.00, Vitamin K 0.25 g, Thiamine 0.2 mg, Riboflavin 0.6 mg, Pantothenic acid 1.00 mg, Pyridoxine 0.4999 mg, Niacin 4.00 mg, Vitamin B12 0.002 mg, Folic acid 0.10 mg, Biotin 0.008 mg, Choline chloride 0.05 g, Antioxidant 0.012 g, Manganese 0.0096 g, Zinc 0.0060 g, Copper 0.0006g, Iodine 0.006 g, Iodine 0.00014 g, Selenium 0.024, Cobalt 0.004 mg

3.5.5 Haematological study

Five millilitres (5ml) of blood per animal was collected via the jugular vein of each of the three rabbits per replicate used for carcass evaluation into sample bottles containing ethylenediaminetetraacetic acid. The samples were analysed for total protein (TP), haemoglobin (HB), red blood cell (RBC), white blood cell (WBC) and pack cell volume (PCV). The analysis was carried out at the Clinical Pathology Laboratory of the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria.

3.6 Experiment 2: Effect of graded levels of sweet orange fruit waste (SOFW) treated with Acidomix®AFG on the performance and carcass characteristics of weaner rabbits

3.6.1 Experimental diets

The diets consisted of sweet orange fruit waste replacing maize at graded levels of 0, 10, 15 and 20 % with the dietary treatments designated as T_1, T_2, T_3 and T_4 respectively. The diets containing sweet orange fruit waste were treated with Acidomix®AFG at 0.5 % and 0.7 % levels, respectively (Table 3.2). The acidifier used in the study was Acidomix®AFG and the diets were formulated to meet the nutritional requirements for weaner rabbits.

3.6.2 Management of experimental animals and data collection

A total of 35 weaner rabbits were used for the study. The experimental design was a completely randomized design in a 3 × 2 factorial arrangement (SOFW × acidifier) with a control. The management procedure was the same as described in Experiment I.

3.6.3 Carcass and haematological study

Three animals from each level of the four treatments were selected for carcass and haematological study. The same procedures as described in Experiment I were applied.

Table 3.2: Composition and calculated analysis of Acidomix®AFG treated sweet orange fruit waste based diets fed to weaner rabbits

	Experimental diets						
Ingredients	0% (Control)	10%+0.5 acidomix	10%+0.7% acidomix	15%+0.5 acidomix	15%+0.7% acidomix	20%+0.5 acidomix	20%+0.7 acidomix
Maize	45.95	35.26	35.26	29.90	29.90	24.55	24.55
Soyabean meal	25.20	25.39	25.19	25.75	25.55	26.10	25.90
Sweet orange fruit waste	-	10.00	10.00	15.00	15.00	20.00	20.00
Palm kernel cake	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Rice offal	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Bone meal	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Acidomix®AFG	-	0.50	0.70	0.50	0.70	0.50	0.70
Vitamin premix*	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Methionine	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Lysine	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Calculated analysis							
Metabolizable energy, Kcal/Kg	2604	2651	2651	2675	2675	2699	2699
Crude protein, %	18.00	18.00	18.00	18.00	18.00	18.00	18.00
Ether extract, %	4.52	5.86	5.86	6.53	6.53	7.21	7.21
Crude fibre, %	10.15	10.46	10.46	10.62	10.62	10.78	10.78
Feed cost/kg (N)	68.31	70.07	72.85	67.48	70.26	64.89	67.67

*0.25 kg of premix will supply the following: Vitamin A 1500 IU, Vitamin D 300 IU, Vitamin E 3.00, Vitamin K 0.25 g, Thiamine 0.2 mg, Riboflavin 0.6 mg, Pantothenic acid 1.00 mg, Pyridoxine 0.4999 mg, Niacin 4.00 mg, Vitamin B12 0.002 mg, Folic acid 0.10 mg, Biotin 0.008 mg, Choline chloride 0.05 g, Antioxidant 0.012 g, Manganese 0.0096 g, Zinc 0.0060 g, Copper 0.0006g, Iodine 0.006 g, Iodine 0.00014 g, Selenium 0.024, Cobalt 0.004 mg

3.6.4 Coliform count

To evaluate the effect of dietary treatment on coliform bacteria population in the small intestine, 3 rabbits were randomly selected from each of the seven dietary treatments. Immediately after slaughter, swabs were taken from the duodenum for total coliform count, lactose fermenting and non-lactose fermenting bacteria using the Spread Plate technique according to Seeley *et al.* (1990). Mac-Conkey agar was used for the culture of the swabs.

3.7 Data analysis

Parameters collected were subjected to analysis of variance (ANOVA) using SAS (2002). Test of significance and difference between means among the dietary treatments were separated using Duncan multiple range test (Duncan, 1955). The data obtained in Experiment 1 was analyzed using the model:

$Y_{ij} = \mu + O_i + e_{ij}$ where,

Y_{ij} = Individual observation

μ = overall mean

O_i = effect of the ith sweet orange fruit waste level

e_{ij} = random error

The data obtained in Experiment 2 was analyzed using the model:

$Y_{ij} = \mu + O_i + A_j + OA_{ij} + e_{ij}$ where,

Y_{ij} = Individual observation

μ = overall mean

O_i = effect of the ith SOFW level

A_j = effect of the jth acidifier level

OA_{ij} = interaction effect of the ith and jth levels of factors O and A

e_{ijk} = random error

CHAPTER FOUR

4.0 RESULTS

4.1 Proximate composition of sweet orange fruit waste

The results of the proximate composition of sweet orange fruit waste is presented in Table 4.1. The results showed a dry matter content of 93.46 %; crude protein, 6.44 %; crude fibre, 4.89 %; ether extract, 17.0 %; ash, 4.01 % and Nitrogen free extract (NFE), 67.66 % for sweet orange fruit waste. Mineral analysis results showed calcium content of sodium (Na), 0.68 %; potassium (K), 0.92 %; total phosphorus (P), 0.11 %; calcium (Ca), 0.62 % and Magnesium (Mg), 0.15 %.

4.2 Microbial count of sweet orange fruit waste

The result of the microbial count of sweet orange fruit waste is presented in Table 4.2. The total count of bacteria and coliform count at 10^4 dilution factors was 6.8×10^6 and 2.8×10^5 cfu/gram respectively. Of the coliform counted, all were identified as lactose fermenting. Total fungal count was 1.6×10^6 cfu/gram. None of the bacteria counted were identified as non-lactose fermenting bacteria.

Table 4.1: Proximate composition of sweet orange fruit waste

Nutrient	Sweet orange fruit waste (SOFW)
Dry matter (%)	93.46
Crude protein (%)	6.44
Crude fibre (%)	4.89
Ether extract (%)	17.00
Ash (%)	4.01
Nitrogen free extract (%)	67.66
Neutral detergent fibre (%)	19.11
Acid detergent fibre (%)	17.75
Minerals	
Na (%)	0.68
K (%)	0.92
Total Phosphorus (%)	0.11
Ca (%)	0.62
Mg (%)	0.15
[1]Metabolizable energy (Kcal/kg)	4030

[1](37×%CP) + (81.8×%EE) + (35.5×%NFE); Pauzenga (1985)

Table 4.2: Microbial count of sweet orange fruit waste

Total aerobic count	
Fungi, cfu/gram	1.6×10^6
Bacteria, cfu/gram	6.8×10^6
Coliform bacteria count	
Lactose fermenting bacteria, cfu/gram	2.8×10^5
Non-lactose fermenting bacteria, cfu/gram	-

cfu – colony forming unit; ($x10^4$) - dilution factor

4.3 Experiment I: Effect of graded levels of SOFW on the performance and carcass characteristics of weaner rabbits

4.3.1 Effect of sweet orange fruit waste diets on the growth performance of weaner rabbits

Growth performances of rabbits fed diets containing 0, 5, 10, 15, and 20 % of sweet orange fruit waste are presented in Table 4.3. Differences among the dietary treatments for average final weight ranged from 1160.0 to 1440 g and was significant ($P<0.05$), with rabbits fed diet containing 10 % sweet orange fruit waste (T_3) having the highest final weight compared to other dietary treatments. Animals in T_5 had the least final live weight.

The average daily feed intake varied from 51.43 to 62.32 g/rabbit/day across the dietary treatments. Animals fed diet containing 20 % sweet orange fruit waste (T_5) had significantly ($P<0.05$) lower feed intake compared to those in (T_3). No significant ($P>0.05$) difference was observed between T_1, T_2, T_3 and T_4.

The effect of the experimental diets on the average weight gain ranging from 11.79 to 16.25 g was also significant ($P<0.05$). Rabbits fed diet containing 10 % sweet orange fruit waste (T_3) had the highest daily weight gain while animals fed diet containing 20 % sweet orange fruit waste (T_5) had the least weight gain.

Feed conversion ratio differed significantly ($P<0.05$) among the treatment groups ranging from 3.67 to 4.53 with animals in T_5 performing the least. Animals in T_1, T_2, T_3 and T_4 were similar ($P>0.05$) but differed from those in T_5.

It was observed that differences in feed cost per kg gain across the treatment groups were not significant ($P>0.05$). A decrease in feed cost per gain was observed from the control (T_1) to T_4. Animals in T_5 had the highest feed cost per kg gain.

Table 4.3: Effect of sweet orange fruit waste on the growth performance of weaner rabbits

	SOFW Inclusion levels, %					SEM
Performance indices	T_1(0)	T_2(5)	T_3(10)	T_4(15)	T_5(20)	
Initial weight (g)	490.00	510.00	510.00	540.00	500.00	35.2
Final weight (g)	1280^{bc}	1270^{bc}	1440^{a}	1330^{ab}	1160^{c}	48.5
Feed intake (g/rabbit/day)	54.38^{ab}	56.43^{ab}	62.32^{a}	54.11^{ab}	51.43^{b}	3.06
Weight gain (g/rabbit/day)	14.46^{ab}	15.17^{ab}	16.25^{a}	13.93^{bc}	11.79^{c}	0.73
Feed conversion ratio	3.82^{a}	3.67^{a}	3.83^{a}	4.07^{ab}	4.53^{b}	0.19
Feed cost/kg gain	261.22	241.07	241.56	246.07	262.52	11.5

^{a,b,c} Means within rows with different superscripts are significantly different ($p<0.05$)
SEM = Standard error of means

4.3.2 Effect of sweet orange fruit waste on the nutrient digestibility of weaner rabbits

Results showing the effect of sweet orange fruit waste on the nutrient digestibility of weaner rabbits are presented in Table 4.4. It showed that the digestibility of dry matter significantly increased ($P<0.05$) as the level of sweet orange fruit waste in the treatment groups increased. Similar increases ($P<0.05$) in the digestibility of crude protein, ash, fibre, ether extract and nitrogen free extract was observed. Crude protein digestibility of the treatment groups ranged from 85.50 to 90.08 %. Crude protein digestibility of rabbits fed diet containing 15 % sweet orange fruit waste (T_4) had the highest value while rabbits in T_2 had the lowest crude protein digestibility. Crude protein digestibility of the treatment groups ranged from 68.30 to 79.15 %. Rabbits in T_3 had the highest crude fibre digestibility while rabbits fed diet containing 5 % sweet orange fruit waste (T_2) had the least crude fibre digestibility.

4.3.3 Effect of sweet orange fruit waste on carcass characteristics and organ weights of weaner rabbits

The effect of sweet orange fruit waste on carcass characteristics and organ weights of weaner rabbits is presented in Table 4.5. The inclusion of sweet orange fruit waste in rabbits diet had significant effects ($P<0.05$) on live weight, dressed weight, dressing percentage, forelimbs, hind limbs, rack/rib, loin and carcass length among the dietary treatments. The live weight of rabbits slaughtered ranged from 1175 to 1425 g and rabbits in T_2 were significantly heavier than the live weights of each of the other dietary groups, which were statistically different ($P<0.05$) from each other with rabbits in T_5 being the least.

Dressed weights and dressing percentages ranged from 611.17 to 743.23 g and 43.66 to 53.09 % respectively. Dressed weights and dressing percentages of rabbits in T_2 and T_3 were higher and significantly different ($P<0.05$) from the other treatments. The dressing percentage for

Table 4.4: Effect of sweet orange fruit waste on the nutrient digestibility of weaner rabbits

	SOFW Inclusion levels, %					SEM
Nutrients	T_1(0)	T_2(5)	T_3(10)	T_4(15)	T_5(20)	
Dry matter, %	69.11[b]	68.50[b]	69.42[b]	78.36[a]	77.04[a]	1.41
Crude protein, %	85.89[b]	85.50[b]	88.80[a]	90.08[a]	88.67[a]	0.92
Fibre %	76.62[b]	68.30[b]	79.15[a]	78·49[a]	77.68[a]	1.44
Ash, %	62.81[b]	60.10[b]	77.18[a]	76.81[a]	73.81[a]	1.85
Ether extract, %	73.93[b]	73.30[b]	72.44[b]	82.71[a]	75.02[b]	1.47
Nitrogen free extract, %	67.44[b]	64.53[b]	74.99[a]	74.60[a]	74.97[a]	1.63

[a, b] Means within rows with different superscripts are significantly different (P<0.05)
SEM = Standard error of mean

rabbits in T_5 was similar (P>0.05) to those in the control (T_1). Rabbits in T_4 had the lowest dressed weight and dressing percentage.

The rack and rib portion of the carcass differed significantly across the treatments with T_1, T_2, T_3 and T_5 being similar (P>0.05) and different (P<0.05) from T_4. The loin portion were similar (P>0.05) across treatments with the exception of those in T_5 which differed significantly (P<0.05) from the other treatments.

There were significant differences (P<0.05) in the weights of liver, paired kidneys, lungs and heart expressed as percentages of live weight among the sweet orange fruit waste based treatments and the control group. Rabbits in T_3 had the highest weights of liver, paired kidneys, lungs and heart.

4.3.4 Effect of sweet orange fruit waste on the haematological parameters of weaner rabbits

The effect of diets containing sweet orange fruit waste on the haematological parameters of weaner rabbits is presented in Table 4.6. Pack cell volume ranged from 37.67 % to 39.67 %, haemoglobin, 12.47 to 13.10 dm/dl; white blood cell, 4.70 to 5.40 x 10^5; red blood cell, 6.20-6.57 x 10^6 and total protein, 5.70 to 6.30 gm/dl. Pack cell volume, haemoglobin and white blood cell were statistically similar (P>0.05) across dietary treatments. Red blood cell count for animals in T_1, T_2, T_3 and T_5 being similar and differed significantly from animals in T_4.

Table 4.5: Effect of sweet orange fruit waste on the carcass characteristics and organ weights of weaner rabbits

	SOFW Inclusion levels, %					SEM
Carcass indices	T_1(0)	T_2(5)	T_3(10)	T_4(15)	T_5(20)	
Live weight (LW), g	1275^{b}	1425^{a}	1375^{a}	1225^{bc}	1175^{c}	17.57
Dressed weight, g	667.60^{b}	728.53^{a}	743.23^{a}	611.17^{c}	672.47^{b}	16.28
Dressing percentage, %	47.69^{b}	52.04^{a}	53.09^{a}	43.66^{c}	48.03^{b}	1.16
Fore limbs, %	0.98^{a}	0.83^{ab}	0.98^{a}	0.82^{b}	0.84^{ab}	0.05
Hind limbs, %	2.08^{c}	1.97^{c}	2.26^{b}	2.39^{a}	2.41^{a}	0.04
Racks/Ribs, %	28.90^{a}	28.90^{a}	29.70^{a}	27.80^{b}	29.16^{a}	0.33
Loin, %	18.34^{b}	17.60^{b}	17.70^{b}	16.64^{b}	21.98^{a}	0.60
Liver, %	3.10^{c}	3.10^{c}	4.14^{a}	3.41^{b}	3.61^{b}	0.09
Kidneys, %	0.89^{a}	0.71^{b}	0.91^{a}	0.73^{b}	0.92^{a}	0.01
Lungs, %	0.85^{c}	0.65^{d}	1.31^{a}	1.01^{b}	1.12^{b}	0.05
Heart, %	0.22^{b}	0.22^{b}	0.30^{a}	0.29^{a}	0.24^{b}	0.01
Carcass length (cm)	34.27^{a}	29.77^{cd}	29.50^{d}	32.50^{b}	30.37^{c}	0.22

^{a,b,c,d} Means within rows with different superscripts are significantly different (P<0.05)
SEM = Standard error of mean

Table 4.6: Effect of sweet orange fruit waste on the haematological parameters of weaner rabbits

	SOFW Inclusion levels, %					SEM
Haematological indices	T_1(0)	T_2(5)	T_3(10)	T_4(15)	T_5(20)	
Packed cell volume, %	39.00	39.67	38.00	37.67	38.00	0.72

Haemoglobin (gm/dl)	12.97	13.10	12.89	12.47	12.67	0.24
White blood cell (x 10^5)	4.70	4.97	5.27	5.37	5.40	0.30
Red blood cell (x 10^6)	6.50^{ab}	6.57^{a}	6.40^{a}	6.20^{b}	6.30^{a}	0.12
Total protein (gm/dl)	5.70^{c}	5.93^{bc}	6.00^{abc}	6.30^{a}	6.10^{ab}	0.11

[a, b, c] Means within rows with different superscripts are significantly different (P<0.05)
SEM = Standard error of means

4.4 Experiment II: Effect of graded levels of sweet orange fruit waste (SOFW) treated with varying levels of Acidomix® AFG

4.4.1 Effect of sweet orange fruit waste diets on the growth performance of weaner rabbits

Results showing the effect of sweet orange fruit waste diets on the growth performance of weaner rabbits are presented in Table 4.7. Similar trends were observed for final weight, feed intake and feed conversion ratio. Feed intake values ranged from 53.87 g to 68.16 g, with the

lowest and highest values obtained being for T_2 and T_1, respectively. No significant differences ($P>0.05$) were observed between T_3, T_4 and the control (T_1).

Significant differences were however observed for weight gain with the control (T_1) being significantly higher ($P<0.05$) than the sweet orange fruit waste based diets T_2, T_3 and T_4. Feed Conversion Ratio values ranged from 5.56 to 6.95. No significant differences ($P>0.05$) were observed between T_1, T_3 and T_4. Result for feed cost per kg gain showed rabbits on T_2 to have the highest feed cost per kg gain value with the control (T_1) being similar ($P>0.05$) to T_3 and T_4.

4.4.2 Effect of levels of Acidomix®AFG on the growth performance of weaner rabbits

Results showing the effect of levels of Acidomix®AFG on the growth performance of weaner rabbits are presented in Table 4.8. No significant differences ($P>0.05$) were observed for all the performance indices for the two levels of Acidomix®AFG (0.5 % and 0.7 %) used.

Table 4.7: Effect of sweet orange fruit waste diets on the growth performance of weaner rabbits

	SOFW Inclusion levels, %				SEM
	T_1(0)	T_2(10)	T_3(15)	T_4(20)	
Performance indices					
Initial weight (g)	690.00	710.00	695.00	695.00	33.54
Final weight (g)	1400.00^{a}	1191.70^{b}	1321.40^{ab}	1322.2^{ab}	46.28
Feed intake (g/rabbit/day)	68.15^{a}	53.87^{b}	63.78^{a}	64.98^{a}	3.10
Weight gain (g/rabbit/day)	12.80^{a}	8.36^{c}	10.71^{b}	10.71^{b}	0.61
Feed conversion ratio	5.35^{a}	6.95^{b}	6.28^{ab}	6.14^{ab}	0.33
Feed cost/kg gain, N	365.36^{a}	497.76^{b}	431.27^{ab}	407.35^{a}	23.08
Mortality, %	0.41^{c}	0.41^{c}	0.31^{b}	0.10^{a}	0.00

^{a,b,c} Means within rows with different superscripts are significantly different ($P<0.05$)
SEM = Standard error of mean

Table 4.8: Effect of levels of Acidomix® AFG on the growth performance of weaner rabbits fed sweet orange fruit waste

Performance indices	Levels of Acidomix® AFG, %		SEM
	0.5	0.7	
Initial weight (g)	702.50	693.33	33.54
Final weight (g)	1271.43	1336.36	46.28
Feed intake (g/rabbit/day)	60.97	64.12	3.10
Weight gain (g/rabbit/day)	10.41	10.39	0.61
Feed conversion ratio	6.13	6.47	0.33
Feed cost/kg gain	413.25	452.94	23.08
Mortality	0.30[b]	0.27[a]	0.00

[a,b,c] Means within rows with different superscripts are significantly different (P<0.05)
SEM = Standard error of mean

4.4.3 Interaction effect of sweet orange fruit waste diets treated with Acidomix®AFG on the growth performance of weaner rabbits

Results showing the interaction effect of sweet orange fruit waste diets treated with Acidomix® AFG on the growth performance of weaner rabbits are presented in Table 4.9. Significant differences ($P<0.05$) were observed across treatments for all performance indices with observable trend. The feed intake values ranged between 52.38 g and 69.11 g. T_3 and T_4 were observed to be similar ($P>0.05$) at both levels (0.5 % and 0.7 %) of Acidomix®AFG to the control and different from the two levels of Acidomix®AFG of T_2.Weight gain values ranged between 7.14 g and 12.80 g. T_5 (0.7 %) and T_4 (0.7 %) were found to be statistically similar to T_1 (control). Significant differences were also observed for feed conversion ratio with T_2 (0.5%), T_3 (0.7 %) and T_4 (0.7 %) being statistically similar to the control (T_1).

Results on the feed cost per kg gain indicate a rise in costs of sweet orange fruit waste based feeds when replaced at lower levels of 10 % (T_2) compared to 15 % (T_3) and 20 % (T_4) replacement of maize as a result of the inclusion of Acidomix®AFG. The cost of inclusion of sweet orange fruit waste in the diets caused a decrease in the cost of the feed and continued to decline irrespective of the cost of inclusion of Acidomix®AFG in the diets. A higher meat turnover was obtained at T_4 (0.7 %) at a lower feed cost.

Table 4.9: Interaction effect sweet orange fruit waste diets treated with Acidomix®AFG on the growth performance of weaner rabbits

	SOFW Inclusion levels, %							SEM
	T_1(0)	T_2(10)		T_3(15)		T_4(20)		
Levels of Acidomix® AFG, %	0	0.5	0.7	0.5	0.7	0.5	0.7	
Performance indices								
Initial weight (g)	690.00	720.00	700.00	700.00	690.00	700.00	690.00	36.84
Final weight (g)	1400.00^{ab}	1166.70^{d}	1216.70^{cd}	1237.50^{cd}	1433.30^{a}	1287.50^{bcd}	1350.00^{abc}	46.28
Feed intake (g/rabbit/day)	68.15^{a}	52.38^{c}	55.36^{bc}	63.17^{ab}	64.58^{ab}	59.82^{abc}	69.11^{a}	3.10
Weight gain (g/rabbit/day)	12.80^{a}	9.58^{cd}	7.14^{e}	10.27^{bcd}	11.31^{abc}	9.38^{d}	11.79^{ab}	0.63
Feed conversion ratio	5.35^{a}	6.09^{ab}	7.81^{c}	6.45^{b}	6.06^{ab}	6.42^{b}	5.91^{ab}	0.33
Feed cost/kg gain, N	365.36^{a}	426.85^{ab}	568.68^{c}	435.34^{b}	425.85^{ab}	416.87^{ab}	399.74^{ab}	23.09
Mortality, %	0.41^{c}	0.41^{c}	0.41^{c}	0.20^{b}	0.41^{c}	0.20^{b}	0.00^{a}	0.00

^{a,b,c,d} Means within rows with different superscripts are significantly different ($P<0.05$)
SEM = Standard error of mean

4.4.4 Effect of sweet orange fruit waste diets with or without Acidomix®AFG treatment on the carcass characteristics of weaner rabbits

The carcass characteristics of weaner rabbits fed sweet orange fruit waste are presented in Table 4.10. There were significant differences ($P<0.05$) across all carcass indices with the exception of fore limb and loin. Significant differences ($P<0.05$) were observed for live weights across treatments with T_1, T_3 and T_4 being different ($P<0.05$) from T_2. Dressing percentage also showed significant differences ($P<0.05$) among the treatments with values ranging between 46.24 % and 50.10 % and T_1, T_3 and T_4 also being similar. However, no significant difference ($P>0.05$) was observed between the SOFW based diets. The organ weights expressed as percentages of live weight were observed to decrease across treatments as the SOFW levels increased. Similar trends were observed for both lungs and kidneys where T_1 and T_2 were similar and differed from T_3 and T_4 which were similar.

Results showing the effect of levels of Acidomix®AFG on the carcass characteristics of weaner rabbits are presented in Table 4.11. No significant difference ($P>0.05$) was observed for all carcass indices except for skin weights and live weight with better live weight obtained at 0.7 % inclusion level of Acidomix®AFG.

Table 4.10: Effect of sweet orange fruit waste diets on the carcass characteristics of weaner rabbits

Carcass indices	SOFW Inclusion levels, %				SEM
	T_1(0)	T_2(10)	T_3(15)	T_4(20)	
Live weight (LW) g	1400.00[a]	1191.70[b]	1314.70[a]	1450.00[a]	42.21
Dressed weight (g)	700.00[a]	558.33[b]	620.83[b]	698.33[a]	22.88
Dressing percentage	50.10[a]	46.24[b]	46.84[ab]	48.16[ab]	1.12
Fore limbs, %	0.63[b]	0.72[a]	0.69[a]	0.63[b]	0.04
Hind limbs, %	2.00[b]	2.16[a]	2.00[b]	1.95[b]	0.05
Rack/Ribs, %	29.09[ab]	25.73[c]	27.96[b]	30.62[a]	0.74
Loin, %	18.66[a]	17.83[a]	18.29[a]	17.90[b]	0.46
Liver, %	2.86[a]	2.88[a]	2.97[a]	2.60[b]	0.07
Kidneys, %	0.85[a]	0.85[a]	0.74[b]	0.69[b]	0.03
Lungs, %	0.94[a]	0.86[a]	0.69[b]	0.70[b]	0.03
Heart, %	0.32[a]	0.29[ab]	0.28[b]	0.22[c]	0.01
Carcass length (cm)	31.67[b]	31.25[b]	32.75[a]	33.67[a]	0.33

[a,b,c] Means within rows with different superscripts are significantly different ($P<0.05$)
SEM = Standard error of means

Table 4.11: Effect of levels of Acidomix®AFG on the carcass characteristics of weaner rabbits fed sweet orange fruit waste

	Levels of Acidomix®AFG, %		SEM
Carcass indices	0.5	0.7	
Live weight (LW) g	1283.33[b]	1411.11[a]	42.21
Dressed weight (g)	608.33	673.89	22.88
Dressing percentage, %	47.54	47.47	1.12
Fore limbs, %	0.69	0.65	0.04
Hind limbs, %	2.05	2.00	0.05
Rack/Ribs weights, %	27.86	28.76	0.74
Loin, %	18.22	17.90	0.46
Liver, %	2.87	2.76	0.07
Kidneys, %	0.80	0.74	0.03
Lungs, %	0.77	0.78	0.03
Heart, %	0.27	0.27	0.01
Carcass length (cm)	32.13	32.83	0.33

[a,b,c] Means within rows with different superscripts are significantly different ($P<0.05$)
SEM = Standard error of means

4.4.5 Interaction effect of sweet orange fruit waste diets treated with Acidomix®AFG on the carcass characteristics of weaner rabbits

The result showing the interaction effect of sweet of sweet orange *(Citrus sinensis)* fruit waste treated with Acidomix®AFG on the carcass characteristics of weaner rabbits are presented in Table 4.12. An increase in live weight was observed with increasing dietary levels of sweet orange fruit waste with the T_4 (0.7 %) being similar ($P>0.05$) with the control (T_1) showing that higher live weights of carcass can be obtained when Acidomix®AFG is used in combination with sweet orange fruit waste up to 20 % for weaner rabbits. A similar trend was observed for carcass indices such as dressed weight, dressing percentage, loin weight, rack and ribs weight where there was an improvement within and across treatments for the sweet orange fruit waste containing diets, with T_4 (0.7 %) being comparable (dressing percentage) or higher (dressed weight and rack and ribs weight) than the control (T_1).

4.4.6 Effect of sweet orange fruit waste diets on the haematological parameters of weaner rabbits

The results showing the effect of sweet orange fruit waste diets on the haematological parameters of weaner rabbits is presented in Table 4.13. The values for the haematological parameters ranged from 40.00 to 37.17, 13.30 to 12.37, 5.97 to 8.72 and 5.83 to 6.67 for pack cell volume, haemoglobin, white blood cell and total protein, respectively. A decrease in values across treatments for the various indices was observed with increase in sweet orange fruit waste levels with the exception of white blood cell count, where no definite trend was observed.

Table 4.12: Interaction effect of sweet orange fruit waste based diets treated with Acidomix®AFG on the carcass characteristics of weaner rabbits

	SOFW Inclusion levels, %							SEM
	T_1(0)	T_2(10)		T_3(15)		T_4(20)		
Levels of Acidomix®AFG, %	0	0.5	0.7	0.5	0.7	0.5	0.7	
Carcass indices								
Live weight (LW) g	1400.00^{b}	1166.00^{d}	1216.70^{c}	1250.00^{cd}	1433.30^{b}	1316.70^{bc}	1583.30^{a}	42.21
Dressed weight (g)	700.00^{b}	550.00^{d}	566.67^{d}	591.67^{cd}	650.00^{bc}	591.67^{cd}	805.00^{a}	22.88
Dressing percentage, %	50.10^{ab}	47.43^{bc}	46.25^{c}	47.15^{bc}	45.33^{c}	45.49^{c}	50.83^{a}	1.12
Fore limbs, %	0.63^{bc}	0.78^{a}	0.66^{abc}	0.74^{ab}	0.64^{bc}	0.61^{c}	0.64^{bc}	0.04
Hind limbs, %	2.00^{bcd}	2.12^{ab}	2.21^{a}	2.04^{bc}	1.95^{c}	2.05^{bc}	1.85^{d}	0.05
Rack/Ribs, %	29.09^{bc}	24.15^{d}	27.30^{c}	28.36^{bc}	27.56^{c}	29.83^{ab}	31.43^{a}	0.74
Loin , %	18.66^{a}	17.17^{b}	18.40^{ab}	18.43^{ab}	18.11^{ab}	18.61^{a}	17.20^{b}	0.46
Liver, %	2.86^{ab}	2.92^{ab}	2.85^{ab}	2.97^{a}	2.97^{a}	2.75^{b}	2.45^{c}	0.07
Kidneys, %	0.85^{ab}	0.94^{a}	0.77^{bc}	0.75^{cd}	0.73^{cd}	0.67^{d}	0.71^{cd}	0.03
Lungs, %	0.94^{a}	0.83^{bc}	0.88^{ab}	0.67^{de}	0.71^{de}	0.65^{e}	0.76^{cd}	0.03
Heart, %	0.32^{a}	0.27^{b}	0.31^{a}	0.32^{a}	0.24^{b}	0.18^{c}	0.26^{b}	0.01
Carcass length (cm)	31.67^{c}	31.00^{c}	31.50^{c}	32.83^{b}	32.67^{b}	33.00^{b}	34.33^{a}	0.33

^{a,b,c,d,e} Means within rows with different superscripts are significantly different (P<0.05
SEM = Standard error of mean

Table 4.13: Effect of sweet orange fruit waste diets on the haematological parameters of weaner rabbits

	SOFW Inclusion levels, %				SEM
Haematological indices	T_1(0)	T_2(10)	T_3(15)	T_4(20)	
Pack cell volume (%)	40.00^{ab}	40.17^{a}	37.33^{b}	37.17^{b}	0.75
Haemoglobin (gm/dl)	13.30^{a}	13.33^{a}	12.42^{b}	12.37^{b}	0.25
White blood cell (x 10^5)	6.70^{b}	8.38^{ab}	8.72^{a}	5.97^{c}	0.56
Total protein (gm/dl)	6.67^{ab}	6.08^{b}	5.85^{b}	5.83^{b}	0.17

^{a,b,c} Means within rows with different superscripts are significantly different ($P<0.05$)
SEM = Standard error of mean

4.4.7 Effect of levels of Acidomix®AFG on the haematological parameters of weaner rabbits

The results showing the effect of levels of Acidomix®AFG on the haematological parameters of weaner rabbits are presented in Table 4.14. Except for the white blood cell count, no significant differences (P>0.05) were observed for haematological indices, with the white blood cell count for the 0.7 % level of Acidomix®AFG inclusion being significantly higher than that of 0.5 %.

4.4.8 Interaction effect of sweet orange fruit waste diets treated with Acidomix®AFG on haematological parameters of weaner rabbits

The results showing the interaction effect of sweet orange fruit waste diets treated with Acidomix®AFG on haematological parameters of weaner rabbits are presented in Table 4.15. Values ranged from 33.67 to 41.00, 11.20 to 13.60, 4.43 to 6.20 and 4.97 to 6.73 for pack cell volume, haemoglobin, white blood cell and total protein respectively. The white blood cell count for all the treatment diet groups containing 0.7 % level of Acidomix®AFG were found to be similar (P>0.05) and statistically different (P<0.05) from the control and the 0.5 % level of Acidomix®AFG treatment groups. For the other haematological parameters, there were however significant differences (P<0.05) across the treatments with no definite trend was observed.

Table 4.14: Effect of levels of Acidomix®AFG on the haematological parameters of weaner rabbits

	Levels of Acidomix®AFG, %		SEM
Haematological indices	0.5	0.7	
Pack cell volume (%)	37.75	39.44	0.75
Haemoglobin (gm/dl)	12.55	13.11	0.25
White blood cell (x 10^5)	6.00^b	9.61^a	0.56
Total protein (gm/dl)	5.89	6.21	0.17

[a,b] Means within rows with different superscripts are significantly different (P<0.05)
SEM = Standard error of mean

Table 4.15: Interaction effect of sweet orange fruit waste based diets treated with Acidomix® AFG on the haematological parameters of weaner rabbits

	SOFW Inclusion levels, %							SEM
	T_1(0)	T_2(10)		T_3(15)		T_4(20)		
Levels of Acidomix®AFG, %	0	0.5	0.7	0.5	0.7	0.5	0.7	
Haematological Indices								
Pack cell volume (%)	40.00^{ab}	39.33^{ab}	41.00^{a}	38.00^{bc}	36.67^{c}	33.67^{d}	40.67^{a}	0.75
Haemoglobin (gm/dl)	13.30^{ab}	13.07^{ab}	13.60^{a}	12.63^{bc}	12.20^{c}	11.20^{d}	13.53^{a}	0.25
White blood cell (x 10^5)	6.70^{b}	6.20^{b}	10.57^{a}	6.67^{b}	10.77^{a}	7.50^{b}	11.50^{a}	0.56
Total protein (gm/dl)	6.67^{ab}	6.00^{c}	6.17^{bc}	4.97^{d}	6.73^{a}	5.93^{c}	5.73^{c}	0.17

[a,b] Means within rows with different superscripts are significantly different (P<0.05)
SEM = Standard error of mean

4.4.9 Effect of sweet orange fruit waste diets on the coliform count at the small intestine of weaner rabbits

The results showing the effect sweet orange fruit waste diets on the coliform count at the small intestine of weaner rabbits are presented in Table 4.16. All coliform indices show a decrease in coliform bacteria with the control diet (T_1) exhibiting higher coliform counts compared to the Acidomix®AFG containing diets. The coliform count for the non-lactose fermenting bacteria of rabbits fed Acidomix®AFG containing diets showed no colony formation irrespective of the dietary inclusion levels of sweet orange fruit waste. Total coliform and lactose fermenting bacteria counts were observed to decrease across the dietary treatments with T_4 having the least total coliform and lactose fermenting bacteria counts while the control (T_1) has the highest counts.

4.4.10 Effect of levels of Acidomix® AFG on the coliform count at the small intestine of weaner rabbits

The effect of levels of Acidomix®AFG on the coliform count at the small intestine of weaner rabbits is presented in Table 4.17. Significant differences were observed between the two levels of Acidomix®AFG with the 0.7 % inclusion level having a lower count than the 0.5 % inclusion level of Acidomix®AFG for total count, lactose fermenting bacteria and non-lactose fermenting bacteria. No colony count was obtained for non-lactose fermenting bacteria at 0.7 % inclusion level of Acidomix®AFG

Table 4.16: Effect of sweet orange fruit waste diets on the coliform count in the small intestine of weaner rabbits

Coliform indices	SOFW Inclusion levels, %				SEM
	$T_1(0)$	$T_2(10)$	$T_3(15)$	$T_4(20)$	
Total coliform	9.00^{d}	3.17^{c}	1.83^{b}	0.17^{a}	0.18
Lactose fermenting bacteria	4.33^{d}	3.17^{c}	1.83^{b}	0.17^{a}	0.17
Non-lactose fermenting bacteria	4.67^{b}	0.00^{a}	0.00^{a}	0.00^{a}	0.13

^{a,b,c,d} Means within rows with different superscripts are significantly different ($P<0.05$)
SEM- Standard error of means
x 10^4- dilution factor

Table 4.17: Effect of levels of Acidomix®AFG on the coliform count in the small intestine of weaner rabbits

	Levels of Acidomix®AFG, %		SEM
Coliform indices	0.5	0.7	
Total coliform	3.58[a]	1.67[b]	0.18
Lactose fermenting bacteria	2.42[a]	1.67[b]	0.17
Non-lactose fermenting bacteria	1.17[a]	0.00[b]	0.13

[a,b,c, d] Means within rows with different superscripts are significantly different (P<0.05)
SEM- Standard error of means
$x10^4$- dilution factor

4.4.11 Interaction effect of sweet orange fruit waste diets treated with Acidomix®AFG on the coliform count in the small intestine of weaner rabbits

The interaction effect of sweet orange fruit waste diets treated with Acidomix®AFG on the coliform count at the small intestine of weaner rabbits is presented in Table 4.18. There was a significant difference ($P<0.05$) across dietary treatments with higher coliform counts obtained for the control (T_1) and a decrease in coliform count with rising levels of sweet orange fruit waste. No significant difference ($P>0.05$) was observed between the two levels of Acidomix®AFG for each dietary treatment groups. No colony count was obtained for non-lactose fermenting bacteria for the sweet orange fruit waste-containing diets.

Table 4.18: Interaction effect of sweet orange fruit waste based diets treated with Acidomix®AFG on the coliform count at the small intestine of weaner rabbits

	SOFW Inclusion levels,%							SEM
	T_1(0)	T_2(10)		T_3(15)		T_4(20)		
Levels of Acidomix®AFG,%	0	0.5	0.7	0.5	0.7	0.5	0.7	
Coliform Indices								
Total coliform	9.00^{d}	3.33^{c}	3.00^{c}	1.67^{b}	2.00^{b}	0.30^{a}	0.00^{a}	0.75
Lactose fermenting bacteria	4.33^{d}	3.33^{c}	3.00^{c}	1.67^{b}	2.00^{b}	0.30^{a}	0.00^{a}	0.25
Non-lactose fermenting bacteria	4.67^{b}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.13

^{a,b,c,d} Means within rows with different superscripts are significantly different ($P<0.05$)
SEM- Standard error of means
$x10^{4}$- dilution factor

CHAPTER FIVE

5.0 DISCUSSION

5.1 Proximate composition of sweet orange fruit waste

Results obtained for the proximate composition of sweet orange fruit waste were within ranges of crude protein (3.30-10.3 %), crude fibre (0.3-6.9 %), Ash (1.99-6.60 %), NDF (4.3-20.6 %) and ADF (2.0-15.6 %) reported by Piquer *et al.* (2009) on whole citrus fruit samples. Its energy content of 4030 kcal ME/kg suggests that sweet orange fruit waste can be classified as energy feed. Its crude protein and crude fibre values are however lower than values recorded by Hon *et al.* (2009) who reported values of 7.71 % and 9.68 % respectively for dried sweet orange fruit pulp meal. The ether extract value obtained was higher than those recorded by Oluremi *et al.* (2007) who reported a range of 3.98-12.6 % for orange pulp. The NDF was lower than that recorded by Hon *et al.* (2009) who reported a NDF of 38.28 % and also the values for ADF (18.32 %) and NFE (75.31 %) for dried citrus fruit peel meal.

5.2 Microbial count of sweet orange fruit waste

The total bacterial and fungal counts of sweet orange fruit waste were found to exceed levels allowed for dried fruit materials (FAM, 1995). Low numbers of coliforms are usually permitted in raw vegetables and raw fruits at numbers ranging from 1 to 200/g or ml (FCD, 1997). No colony count was obtained for non-lactose fermenting bacteria. However, it is not uncommon to find no non-lactose fermenting bacteria such as salmonella in materials of plant origin as their primary habitat is the intestinal tract of animals. Fungal count obtained from the sweet orange fruit waste sample exceeded the limit set for yeast and mould counts (<100 000 cfu/gm) (FCD, 1997). Fruits containing high levels of nutrients with low pH values make them particularly desirable to fungal decay (Singh and Sharma, 2007), which could cause infections or allergies (Monso, 2004).

5.3 Experiment I: Effect of graded levels of SOFW on the performance and carcass characteristics of weaner rabbits

Body weight has been reported to increase in proportion to feed intake (Bogart and Taylor, 1983). The final live body weight sequence that is being associated with the feed intake level seems to be a reflection of the suitability of the diets for rabbits. However, Oluremi *et al*. (2006) reported a 15 % replacement of dietary maize with sweet orange rind (SOR) was possible in the diet of broilers with increasing dietary SOR content beyond 15 % reducing growth rate, which cumulatively caused a decrease in final live weight.

The results obtained on feed intake is contrary to results by Hon *et al*. (2009) and Tokarnia *et al*. (2001) who fed sweet orange fruit pulp meal to rabbits. They observed no difference in feed intake when sweet orange pulp meal replaced maize up to 25 %. The low palatability of sweet orange fruit waste with increasing levels could be responsible for this. Migwi *et al*. (2001) reported depressed feed intake in sheep fed diets containing high levels of dried citrus pulp, presumably due to low palatability. *Limonin* is a tri-terpenoid present in the seeds and skins that imparts a bitter taste to citrus pulp. If large quantities of seeds are present their *limonin* content may render the pulp toxic to non-ruminants even at 2.5 % in their diets (Devendra, 1988 and Rihani, 1991). The palatability of dried citrus pulp is variable, as it may have a bitter taste due to limonin and other compounds present in the seeds and peels. It can result in a decrease in intake if introduced too quickly in the diet. Bhattacharya *et al*. (1973) observed that palatability decreased when fattening lamb diets contained more than 40 % dried citrus pulp.

Results on weight gain shows that sweet orange fruit waste up to 10 % maize replacement in rabbit diet can be used as an alternative dietary energy feed resource. The depressed weight gain observed at inclusion levels above 10 % of sweet orange fruit waste can be attributed to the *limonin* content present in the peels and seeds of sweet orange fruit waste. It has been suggested that *limonin* caused intestinal irritation and poor absorption of nutrients in broilers (El Boushy *et*

al., 2000). It has been reported that weight gain was negatively affected as the level of citrus in poultry diet increased (Yang and Chung, 1985). However, the range in this study is higher than weight gains of 10.76 to 13.05 g reported by Hon *et al.* (2009) for weaned rabbits fed dried sweet orange fruit pulp meal as alternative energy source and within the normal range for most rabbits reared in tropical environment (Cheeke, 1987).

The decreased feed conversion ratio with increasing levels of sweet orange fruit waste can be attributed to the high microbial load contained in the sweet orange fruit waste. An overall rise in gut bacteria increases the metabolic needs of microbes and reduces absorption rates of nutrients, especially energy and amino acids leading to reduced feed efficiency and daily gain as reported by Hebeler *et al*., 2000 and Øverland *et al*., 2000. The competition this creates together with poor absorption caused by the *limonin* contained in the sweet orange fruit waste could be responsible for the poor feed conversion obtained with increasing levels of sweet orange fruit waste inclusion in the diets. The feed conversion ratio obtained showed a negative effect when sweet orange fruit waste was included in the diet up to 20 %. The range obtained in this study is lower than earlier studies conducted by Oluremi *et al.* (2005) in which maize was replaced with sweet orange rind up to 15 % in broiler diet which had a feed conversion ratio of 4.22-4.73 and Ojabo *et al.* (2012) who had a feed conversion ratio of 4.12-7.38 when sweet orange rind replaced maize at the rate of 20-40 % in rabbit diet.

The variation in feed cost per kg observed across the treatments especially in diet containing 20 % sweet orange fruit waste was as a result of the lower cost of sweet orange fruit waste compared to maize in the diets. Feed cost per kg (N/kg) decreased from N68.31 in the control diet to N57.94 in diet with 20 % sweet orange fruit waste. The result on the feed cost per kg gain indicates a possible economic benefit in the partial replacement of maize with sweet orange fruit waste. However, inclusion of sweet orange fruit waste in rabbit diet up to 15 % proves more

economical than the control diet as more meat turnover was obtained at lower cost compared to the control.

The dressing percentage values in this trial were lower than 75.81 to 79.04 % reported when orange peel was used as a dietary substitute for maize in growing rabbit diet (Oluremi *et al.*, 2005) and 60.7 to 65.2 % reported by Hon *et al.,* (2009) who fed dried sweet orange fruit pulp meal to rabbits . The differences may be due to breeds of rabbits used and also that the rabbits were singed rather than skinned and their heads were left as part of the carcass, hence the higher values obtained. From the means of the dressing percentage obtained, it can be concluded that, at levels of up to 10 % replacement with sweet orange fruit waste a higher turnover of rabbit meat for consumption can be obtained than is obtained for the control diet. The liver, kidney, lungs and heart weights of the sweet orange fruit waste containing diets were higher than or similar to the control diet. Green *et al*., (1986) demonstrated that growth of organs can be inhibited when insufficient protein and amino acids are available.

None of the visceral organs in the experimental rabbits appeared to have been damaged by replacing maize with sweet orange fruit waste at up to 20 % level. Since these organs help to determine the heath status of farm animals, replacement of maize with sweet orange fruit waste in the diet of weaner rabbits at levels of up to 20 % did not prove detrimental to their health.

5.3.1 Effect of sweet orange fruit waste on the nutrient digestibility of weaner rabbits

The increase in digestibility of dry matter and crude protein across the treatments is contrary to reports by Gholizadeh and Naserian (2010) who reported that dry matter and crude protein remained unchanged when dried citrus pulp (DCP) was fed to Iranian *saanen* kids.The crude fibre levels in this study were within the acceptable range, thus the digestibility of the nutrients was high. The high digestibility of crude fibre in all the dietary treatment shows that the dietary fibre contains more of digestible fibre than indigestible. Gholizadeh and Naserian (2010)

attributed the increased digestibility of the fibre fractions to the low lignin content of dried citrus pulp (DCP). Dietary inclusion of soluble fibre favours the growth of intestinal villi and the activity of the enterocytes (Chiou *et al.*, 1994; García-Ruiz *et al.*, 1997).

5.3.2 Effect of sweet orange fruit waste on the haematological parameters of weaner rabbits

The fact that no significant difference (P>0.05) was found for pack cell volume, haemoglobin and white blood cell count values agree with the findings of Ojabo *et al*. (2012) who fed sweet orange rind to rabbits at 20-40 % replacement of maize. These values give an indication that the experimental diets are in no way inferior to other conventional rabbit feeds. Pack cell volume values were higher than values reported by Ojabo *et al.* (2012) who reported a range of 27.40-32.8 %. Good adequate nutrition has been recognized as an important factor that enhances erythropoiesis. White blood cell values were below levels of 7.5 - 13.5 x 10^5/dl reported by Harkness and Wagner (1995) but close to values of 4.8-6.3x10^5/dl reported by Mitruka (1981). The superior performance of rabbits fed sweet orange fruit waste diets was corroborated by the numerical increase in total protein which is a good index of the quality of dietary proteins. Results on total protein are however lower than values (7.80-9.50 g/dl) reported by Ojabo *et al*. (2012).

5.4 Experiment II: Effect of graded levels of sweet orange fruit waste (SOFW) treated with varying levels of Acidomix® AFG

Studies on poultry have shown that sweet orange peel meal can be included in the diet of broilers, substituting maize to levels of up to 20 % (Agu *et al.*, 2010). The average daily feed intake of 53.87-65.15 g is similar to that of Ojabo *et al.* (2012) who reported an average daily feed intake of 61.97-67.19g. The feed intake obtained in this study is however higher than that obtained by Oluremi *et al.* (2005), who obtained 48.53-52.13 g/day with sweet orange rind (SOR) in the diets of growing rabbits at 0-15% replacement of maize. The palatability of dried

citrus pulp is variable, as it may have a bitter taste due to *limonin* and other compounds present in the seeds and peels. It can result in a decrease in intake if introduced too quickly in the diet, and a more progressive introduction is recommended. It is well accepted by animals that are accustomed to it and can then increase intake (Rihani, 1991 and Wing, 2003). The average daily weight gain obtained is largely within the range of 10-20 g growth rate for weaner rabbits as reported by Owen (1976) but below the range as reported by Adenkola *et al.* (2009), with an average daily weight gain of 13.35-15.42 g.

While the feed conversion ratio may suggest a nutritive superiority of maize over sweet orange fruit waste, the reduction of the amount of maize by its replacement with sweet orange fruit waste up to 20 % has no negative effect on feed conversion. Hon *et al.* (2009) reported no difference (P>0.05) between the control diet and sweet orange (*Citrus sinensis*) fruit pulp meal based diets with replacement levels of up to 25 %.

Dosage rates of commercially produced acidifiers usually recommend rates between 0.2 % and 1.0 %. Even though the 0.7 % level of Acidomix® AFG proved better than the 0.5 % level in terms of final weight and feed intake, it performed less in terms of weight gain and feed conversion ratio. The recommended rate of Acidomix®AFG places a range of 0.3 % - 0.5 % for standard rabbit diets (Novus, 2007). Based on this result, a higher dose may not be necessary.

The increase in feed intake values observed across the sweet orange fruit waste based diets at both levels of acidifier inclusion agreed with the report by Ocak *et al.* (2007) and Ogunwole *et al.* (2011), who included lactic acid and Biotronic®SE in the diet of broilers, respectively. They both observed that higher levels of organic acids in broiler diets increased feed intake. The increase in feed intake can be explained from the point that the palatability of the feed was improved by the addition of the acidifier. Acidifiers maintain an optimum pH in the stomach, allowing correct activation and function of proteolytic enzymes, optimize protein digestion in stomach, stimulate feed consumption by improving palatability of feed (Awaad *et al.*, 2011).

According to Cave (1984), the addition of propionate in the diet increased feed consumption of broiler chicks.

The improvement in weight gain across the orange based diets can be attributed to the improvement in feed hygiene and utilization due to the inclusion of the acidifier. Inclusion of Acidomix® AFG in the diets helped improve its hygiene as shown in the decrease in coliform bacteria such as *E. coli* and *Salmonella* thereby improving feed absorption and utilization (Sebastian *et al.,* 1996). Sweet *et al.* (1990) and Mathew *et al.* (1991) reported improved growth rates when weaner piglet diets were supplemented with propionic acid (from 0 .1 to 1 %). The variation among the treatments across the performance indices of the orange based diets can be attributed to the organic acids (citric, malic, acetic and formic acids) (Rogers, 1981) contained in citrus and may also be responsible for the better performance observed in T_3 and T_4 compared to T_2 due to the rising levels of SOFW in the diets. Acidifiers increase the acidity of the gastrointestinal tract; thereby improving the secretion of digestive enzymes, feed digestion and hygiene hence utilization was enhanced. Also studies on fattening pigs by Partanen (2001) showed formic acid additions ranging from 5 to 12 g/kg enhanced feed conversion ratio.

Reports on the feed cost per kg gain indicate a rise in costs of sweet orange fruit waste based feeds when replaced at lower levels of 10 % compared to 15 % and 20 % replacement of maize as a result of the inclusion of Acidomix® AFG. The higher cost could pose a barrier to the use of this product at low levels of sweet orange fruit waste.

The carcass characteristics of rabbits fed the sweet orange fruit waste based diets clearly show a rising improvement based on indices such as live weight, dressed weights and dressing percentage as the level of sweet orange fruit waste increased; 20 % being similar to the control. This shows that sweet orange fruit waste could effectively replace maize up to 20 %. Results obtained show improved carcass quality by increasing meat content and carcass length as the level of sweet orange fruit waste inclusion increased. This agrees with the findings of Yang and

Chung (1985) who investigated the effect of different levels of dried citrus pulp on the growth performance and carcass quality of growing and finishing pigs.

Results obtained shows that higher weights of carcass can be obtained when Acidomix®AFG is used in combination with sweet orange fruit waste up to 20 % for weaner rabbits. While literatures on the interaction of feeds with acidifiers are scarce, the growth performance results point to the possible improvement of carcass indices of animals on acidifier treated diets.

It is a common practice in feeding trials to use weights of some internal organs like liver and kidney as indicators of toxicity. Bone (1979) reported that if there is any toxic elements in the feed, abnormalities in weights of liver and kidney would be observed. The abnormalities will arise because of increased metabolic rate of the organs in attempt to reduce these toxic elements or convert anti-nutritional agents to non-toxic metabolites. Our observation with the reported liver and kidney weights in rabbits of different treatment groups suggest that the test diets did not contain any appreciable toxin.

5.4.1 Effect of sweet orange fruit waste diets treated with or without Acidomix®AFG on the haematological parameters of weaner rabbits

Haematological indices are a reflection of the effects of dietary treatments on the animal in terms of the type, quality and amounts of the feed ingested and made available for the animal to meet its physiological, biochemical and metabolic needs (Ewuola *et al*., 2004). The hemoglobin quantity was affected by the high bind ability of the pectin, present in the sweet orange fruit waste, which decreased the availability of many minerals, inclusively iron. The iron, making part of the molecule, is essential to the hemoglobin production that, among other functions, is responsible for the oxygen transport to the cells as reported by Retore *et al.* (2008).

The values for pack cell volume fell within the normal range for rabbit (33.0 %-50.0 %) as reported by Hillyer and Queensberry (1994). Pack cell volume is an index of toxicity; for the

value to fall within the normal range suggests absence of toxins in the diet. The white blood cell count values recorded in this study were comparable to those reported by Schaim *et al*. (1975) who recorded a range of 5.0 – 8.0 x 10^6. Results for total protein fall with the normal range of total protein 5 – 8 g/l (Baner Jee, 1998).

With the exception of the white blood cell count where the 0.7 % level of Acidomix®AFG was higher than that of 0.5 %, no significant differences (P>0.05) were observed between the haematological indices. This high count could be a sign of higher resistance to infection of the animals to that group compared with the lower level of 0.5 %. This established enhancement of immune response associated with dietary acidification could account for their inhibitory effects against the pathogenic microorganisms throughout the gastro-intestinal tract.

No observable trend was observed in the results for the haematological parameters. However, the haemoglobin values obtained fall within the range of 10.0-15.5 gm/dl recommended by Johnson-Delaney (1995). The values for pack cell volume fell within normal range for rabbit (33.0 %-50.0 %) as reported by Hillyer and Quesenberry (1994), suggesting the absence of toxins in the diet as pack cell volume is an index of toxicity. The white blood cell count values recorded in this study were comparable (P>0.05) to those reported by Harkness and Wagner (1995) who recorded a range of 7.5 – 13.5 x 10^3 mm^3. Results for total protein fall with the normal range of total protein (5.0 – 8.0 gm/dl) as cited by Baner Jee, (1998). Total protein is a good index of the quality of dietary protein (Aduku *et al.,* 1988).

5.4.10 Effect of Acidomix®AFG treatment on coliform count

Although coliforms are normally found in the gastro-intestinal tract of animals, it is pertinent to mention that most of the lactose fermenting bacteria and non-lactose fermenting bacteria found in this study are *E. coli* and *salmonella spp.* suspects respectively. *Salmonella* growth requires warmth (35-37 ºC is optimal), a moisture content greater than 12 % and a pH between 4.5 and

9.0 (Joardar *et al.,* 2009). It is no coincidence that the rabbit's gut can provide *salmonella* everything they need to thrive. Cortez *et al.* (1992) reported a low count of 100 ton 10000 cfu/gram for coliform bacteria in the intestinal tract of healthy rabbits. The sweet orange fruit waste diets showed the effectiveness of the acidifier in sanitizing the feed with a gradual decline observed across treatments as the replacement level of SOFW increased up to 20 % irrespective of level of Acidomix®AFG inclusion in the diets.

Organic acid blends based on formic acid and propionic acids are most effective in *salmonella* inhibition (Joardar *et al.,* 2009). In an in *vitro* carried out by Joardar *et al.* (2009), a dose of 0.3 % of Acidomix® AFG could be used for feed sanitation against *Salmonella spp.* The results obtained also showed a clear reduction in lactose fermenting organisms, mainly *E. coli.* The use of organic acid can reduce *E. coli* colonization in the gut (Mathew *et al.*, 1991; Eckel et *al.,* 1992; Isobe *et al*., 1994) as well as the incidence of diarrhoea (Eidelsburger *et al*., 1992). Several investigations demonstrate a reduction in bacterial count in the duodenum (Kirchgessner and Roth 1991; Hebeler *et al*., 2000; Hellweg *et al*. 2006). Reduced bacterial counts have been associated with intestinal tract thinning and a declining mucosal surface area (Gordon and Bruckner-Kardoss, 1961), both of which are correlated with improved performance and basal metabolic rate (Belay and Teeter, 1996).

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 SUMMARY

Two experiments were carried out to determine the effect of sweet orange fruit waste (SOFW) with or without acidifier treatment in the diet of weaner rabbits.

In the first experiment to determine the effect of graded levels (0, 5, 10, 15 and 20 %) of sweet orange fruit waste in the diet of weaner rabbits, rabbits fed on the diet containing 10 % sweet orange fruit waste had the best result in terms of weight gain, feed conversion ratio, feed cost per kg gain and carcass yield. Rabbits fed diet containing 20 % sweet orange fruit waste recorded the lowest values. Nutrient digestibility showed an increasing trend across treatments as sweet orange fruit waste levels increased. The feeding trial revealed that sweet orange fruit waste can be included in the diet of weaner rabbits up to 10 %.

For the second experiment, graded levels of sweet orange fruit waste (0, 10, 15 and 20 %) treated with an acidifier at two levels (0.5 and 0.7 %) for each level of sweet orange fruit waste was used. Results obtained showed that the combination of sweet orange fruit waste and Acidomix® AFG at 20 % and 0.7 % respectively reduced duodenal coliform count to zero as against the high coliform count obtained in the control (9.00). Rabbits in the treatment also had the best result in terms of weight gain, feed conversion ratio and feed cost per kg gain and carcass yield.

6.2 CONCLUSION

To minimize the costs of rabbit production in hot climate countries, low-priced discarded sweet orange fruit wastes can safely partially replace the concentrates in diets of growing rabbits without any deleterious effects, and their inclusion until 10 % in the diet was associated with an increase in carcass performance, clearly being the optimum. However the treatment of sweet orange fruit waste with Acidomix® AFG demonstrated the potential of using acidifiers in

achieving higher inclusion levels of sweet orange fruit waste up to 20 % as obtained in this study. Also, the replacement of maize with sweet orange fruit waste up to 20 % proved beneficial economically and could help improve the profit margin of the farmer.

Salmonella contamination is recognized as one of the most important food safety issue worldwide. Thus the use of acidifiers in the diet of weaner rabbits has shown its effectiveness in maintaining feed hygiene and to checking *salmonella* colonization of the gastro-intestinal tract. Creating and maintaining a healthy intestinal environment is essential to productivity and food safety programmes alike.

6.3 RECOMMENDATION

With rabbits fed sweet orange fruit waste at 10 % level in their diet showing promising response, replacement of maize with sweet orange fruit waste up to 10 % is recommended. However, if higher levels of up to 20 % of sweet orange fruit waste are to be used, it is recommended that treatment using acidifiers be carried out.

However, since the effect of acidification on the caecal characteristics in terms of pH and total volatile fatty acid (VFA) was lacking, there is the need for further studies to explain the mode of action of these products and the relationships between health status, caecal characteristics and micro biota population of the rabbit gastro-intestinal tract. Also, more studies should be carried out to identify other natural sources of organic acids to use as acidifiers to help reduce the costs of feeds to maximize the potential of sweet orange fruit waste for use in animal production.

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