EFFECTS OF AFRICAN SAUSAGE TREE FRUIT PULP ON PRODUCTION AND RESPONSE TO E.COLI ENDOTOXIN IN GROWER PIGS.

BY

MUTANDALIKE S CHOONGA

A dissertation submitted to the school of Agricultural sciences of the University of Zambia in partial fulfillment of the requirements of Master of Science in Animal Nutrition (Animal science).

UNIVERSITY OF ZAMBIA

SCHOOL OF AGRICULTURAL SCIENCES

ANIMAL SCIENCE DEPARTMENT

(2010)

DECLARATION

1, MUTANDALIKE S CHOONGA, DECLARE THAT THIS DISSERTATION REPRESENTS MY OWN WORK AND THAT IT HAS NOT PREVIOUSLY BEEN SUBMITTED FOR A DEGREE AT THIS OR ANY OTHER UNIVERSITY.

Signature……..Date……

APPROVAL

This dissertation of Mutandalike S Choonga is approved as fulfilling part of the requirement of the award of the degree of Master of Science in Animal Nutrition (Animal Science) by the University of Zambia.

Examiner's name and signature. Date

1……. …….

Dr F Haazele

2…….. …….

(Internal examiner)

3……. …….

(Internal examiner)

ABSTRACT

A study was conducted to investigate the potential of ican Sausage Tree Fruit Pulp (ASTFP) in modulating the effects of E.coli endotoxin grower pigs. Twenty weanling pigs from five litters/dams were randomly assigned to two dietary treatments consisting of a basic diet with 0 or 2% ASTFP. The basic diet was a farm made feed formulation fed to weanling pigs. After 14 days on basic diet, the study pigs were fed experimental diets for 42 days. The diets were fed to the study animals in the morning at 8 hours daily. On Day 42, pigs were injected with soy broth or 20 µg/kg body weight endotoxin (Lipopolysaccharide from E coli stereotype 057:B5). Body weights were monitored, rectal temperatures measured and blood samples collected before injection (0 hour) d at 2, 4, 8 and 24 hours post injection (PI). Blood samples were analysed for Urea nitrogen, Total protein, Albumin, Glucose, Cholesterol and white blood cell counts.

Body weights and growth rate were not affected by diet. The pigs fed the diet containing 2% ASTFP had higher urea nitrogen, albumin, glucose and white blood cell counts than pigs fed 0% ASTFP (P = 0.05). The concentration of total protein and cholesterol were the same in both 0 and 2% ASTFP. Endotoxin challenge increased body temperature (P = 0.01) and white blood cells (P = 0.01). The results this study indicate that the feed supplement of ASTFP at 2% in diets of grower pigs was beneficial in protecting pigs against the infection induced by E.coli endotoxin.

DEDICATION

To my daughters Luyando and Lushomo and my wife Megan.

In memory of my late father and mother, Mr. and Mrs. Mutandalike.

To my brother and sisters and the Lord God.

ACKNOWLEDGEMENTS

I wish to thank the Ministry of Defence, Zambia National Service (ZNS) and the Commandant in particular for granting me the study leave that enabled me to undertake these studies. I am greatly indebted to ZNS Chisamba staff and particularly WII Isilimwe I and S/SGT Kanyembo R for their participation and commitment during the field experiment, the supervisors and piggery attendants working at the Piggery Unit, who assisted me in the blood sampling. I am very grateful to them all.

Blood analysis was done in the Biomedical laborattory the school of vet, Biomedical sciences department. I wish to thank the staff of that lab and especially Mr Siyumbi Shebby for his commitment and support.

Funding for part I and II of my studies was provided by the RUFORUM scholaship for part payment. I am very grateful to Dr Judith C.N. Lungu (Dean, School of Agricultural Sciences) for offering me the RUFORUM scholarship.

My supervisors Dr. F Haazele and Dr. M. T. Daura of the University of Zambia, Department of Animal Science, gave me invaluable help, both academic and moral, during my studies which I really appreciate. I am also very grateful to Mr Sinkala for running up and down purchasing analysis reagents from suppliers of the analysis Kits. I thank my coursemate Mr Chongwe Andrew for giving me support when I needed it.

I wish to most sincerely thank my wife and our children Luyando and Lushomo for being there for me.

Lastly and above all I thank our Lord God, without who hing would ever have been possible.

TABLE OF CONTENTS

LIST OF ACRONYMS AND ABBREVIATIONS

AACC	AMERICAN ASSOCIATION OF CEREAL CHEMISTS
AOAC	ASSOCIATION OF ANALYTICAL CHEMISTRY
ASTFP	AFRICAN SAUSAGE TREE FRUIT PULP
FCR	FOOD CONVERSION RATIO
iNOS	IODUCIBLE NITRIC OXIDE SYNTHETASE
LPS	LIPOPOLYSACCHARIDE
MSE	ERROR MEAN SQUARES
NDF	NEUTRAL DETERGENT FIBRE
NS	NATURAL SCIENCES
NO	NITRIC OXIDE
RPM	REVOLUTIONS PER MINUTE
RUFORUM	REGIONAL UNIVERSITIES FORUM
UNZA	UNIVERSITY OF ZAMBIA
WBC	WHITE BLOOD CELLS
ZNS	ZAMBIA NATIONAL SERVICE

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

CHAPTER ONE

1.0 INTRODUCTION

1.1 GENERAL

Pig production is an increasing activity among farmers in Zambia. It is emerging as a major agriculture activity among small scale farmers. re are 1, 250, 000 pigs in Zambia, from both small and large scale farming activities (Sayila, 2006). According to the livestock and veterinary department of the ministry of agriculture, quoted by Sayila (2006) only 605, 800 pigs are grown commercially in Zambia. Therefore, most of the production takes place in the peasant and small scale sectors of the rural areas. Sayila (2006*)* put the number of households in Zambia that rear pigs to be more than 140, 000 with the highest number (43.7%) located in Eastern province, followed by Northern 20.7%, Lusaka 9%, and the rest of the provinces of Zambia 27%. There are however, a lot of difficulties encountered by these peasant and the small scale farmers as they pursue the pig production activity. These difficulties increase with the increase in litter size and the pig unit size. The operational difficulties range from environmental stress to the low levels of management skill and the technical knowhow of the farmer.

Growing piglets post weaning has always been a big challenge among the small scale and peasant farmers in Zambia. They experience a lot of losses through mortality of weanling pigs in the periods after weaning, leading to reduced viability of these Pig farmers. However, a lot of improvements have been done to moderate the high mortalities in weaner pigs. Some of these include late weaning, weaning according to certain weights and the use of subtheraptic antibiotics in the feed. Unfortunately, antibiotics are too costly for small scale and peasant farmers in Zambia to afford and according to Bhandari *et al.* (2008) antibiotics are no longer acceptable to consumers thus alternative to antibiotics are needed. As a result of awareness of society with respect

to quality of production and food safety regarding transmissible pathogens to human from animal, most alternatives to antibiotics are becoming unpopular. Therefore, local medicinal plants may provide a health and less costly alternative to antibiotics especially for small scale and peasant farmers.

Medicinal plants are those plants that are exploited for their healing properties by human beings and wild animals. Peasant farmers, who cannot afford the cost of antibiotics, have also exploited these medicinal plants. Such plants include the African sausage Tree (*Kigelia africana*), which is a semi deciduous plant found in the local forest. It is available in abundance and at no or very low cost especially to the rural communities. African Sausage Tree has been used in Africa, Zambia inclusive, by many herbal medicine practitioners for treating a wide range of hu and animal diseases. It has been used for treatment and prevention of diseases and general improvement of performance of domestic animals.

There is however, very little documented information on the use of African Sausage Tree in domestic animals. There is hardly any documented information on the inclusion of African Sausage Tree in animal feeding or guideline on its use in domestic animals either in water or in feed as a replacer of antibiotics. Therefore, the use of these medicinal palnts (Phyto-medicines) by farmers in domestic animals has been without professional guidance.

Salmonella chloraesus and the *Escherichia coli* (E.coli) are the most common bacterial infections in pigs. The major infection related problem in pigs is the enterotoxigenic E.coli which results in scouring, poor performance and even mortality in the periods immediately post weaning (Bhandar*i et al.,* 2008).

Endotoxin challenge can result in a series of metabolic, behavioural and physiologica changes that include changes in body temperature, depressed feed intake, changes in plasma acute phase protein concentration, activation of the immune stem and activation of the hypothalamic-pituitary-adrenal axis (Bhandari *et al.,* 2008; Cengiz *et al.,* 2008; Liu *et al.,* 2003*)*. These are attributed to the release of pro-inflammatory cytokines, including tumour necrosis factor, interleukin-1 and interleukin-6.

Overproduction of these cytokines can adversely affect feed conversion and growth. Therefore, the modulation of these cytokines may have benefits in alleviating the negative effects induced by an immunological stress (Liu *et al.,* 2003).

African Sausage Tree, which has been traditionally used to cure many diseases conditions contain metabolites such as Naphthoquinones, Iridoids, Sterols and Flavonoids (Houghton, 2008). It is a combination of these metabolites that have shown that the African Sausage Tree can attenuate inflammation in many diseases conditions. The protective mechanism of the African sausage tree as an anti-inflammatory agent is associated with its inhibitory effects on the overproduction of pro-inflammatory cytokines (inhibiting both iNOS *ioducible Nitric Oxide Synthase* expression and NO *Nitric Oxide* release) (Jeyachandran *et al.,* 2007*;* Picerno *et al.,* 2008).

If the African Sausage Tree Fruit preparations have been used to cure many human disease conditions and that they contain metabolites active against inflammation, then the African Sausage Tree Fruit preparations should be to prevent the effects of E.coli endotoxin in pigs. It is the anti-inflammatory properties of African Sausage Tree that this study exploited by supplementing growing pigs with the African Sausage Tree Fruit Pulp (ASTFP), challenging them with the E.coli endotoxin and monitoring parameters defining the biological status of the animal inclusive of Blood Urea Nitrogen, Total Protein, Albumin, Glucose and Cholesterol.

1.2 OBJECTIVES

The overall objective of the study was to investigate potential of the African Sausage Tree Fruit pulp in preventing E.coli endotoxin effects in growing pigs.

The specific objectives of the study were to investigate the effects of dietary;

a. ASTFP on Body weights and weight gains and the efficiency of feed conversion.
b. ASTFP and Endotoxin challenge on blood chemistry focusing on urea nitrogen, albumin, total protein, glucose and cholesterol.
c. ASTFP and Endotoxin challenge on body temperature and white blood cell counts.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 THE AFRICAN SAUSAGE TREE

2.1.1 DESCRIPTION

The African Sausage Tree also called the Sausage Tree, is a small to medium-sized deciduous or semi deciduous tree that grows up to a height of between 15 m and 35 m. The trunk grows up to 60 cm in diameter with grayish bark, it is smooth or flaking and often with a hollow bole and heavy branching forming an open roundish crown, sometimes several crowns. The leaves are opposite or whorled, usually in whorls of 3 and crowded towards the apex. It bears blood-red flowers, in September, which bloom at night and hang on a long ropelike stalk. The flowers after pollination develop into Sausage shaped fruits that hang on long stalks (Storrs, 1995*;* Binutu *et al.,* 2008*;* Houghton 2008; Grace and Davis, 2002).

The African Sausage tree belongs to the genus *Kigelia* in the Family *bognoniacaea*. It is generally accepted that *Kigelia* genus comprises of a single polymorphous species, *Kigelia africana* formerly *Kigelia pinnate* (Storrs, 1995). The word Kigelia is derived from a native tribe of Mozambican Bantu name kigeli-keia. The African Sausage tree is well spread in Africa and occurs throughout tropical Africa, particularly in the drier regions.

The African Sausage Tree is found throughout Zambia and it is most uent in Lake Basin Chipya and in all types of Munga woodland with numerous magical and medicinal uses on record (Storrs, 1995). The common names in Zambia include Muzungulu in Bemba, Munzungulu in Tonga, Mvula in Nyanja and the African sausage tree (also called worsboom) in English (Grace and Davis, 2002; Storrs, 1995; Houghton 2008).

2.1.2 TRADITIONAL USES

The African Sausage Tree is widely used throughout Africa for a variety of purposes, particularly in local medicine. The wide range of diseases which are believed to be cured by the African Sausage Tree include fainting, anemia, sickle-cell anemia, epilepsy, respiratory ailments, hepatic and cardiac disorders, and nutritional illnesses such as kwashiorkor, rickets, wasting and weakness (Grace and Davis, 2002; Lwalewa *et al.*, 2007; Ming, 2001; Owolabi *et al.*, 2008; Omonkhelin *et al.*, 2007). In traditional medicine, all the parts of the African Sausage Tree are used either singular or in combination with other part or parts of the tree. The root preparations are used to treat infections of the genito-urinary tract, particularly venereal diseases (both internally and externally) and worm infections especially tape worms. The leaves are used to prepare a general tonic for improved health and growth. Stem bark is used to treat *Candida albicans* infections (Grace and Davis, 2002).

Fruit preparations that include extracts, poultices and powders which are applied as a wash or rub for many treatment procedures. The unripe fruits are taken as a laxative or emetic. A small amount of unripe fruit is chewed, or an aqueous preparation is taken orally as a sexual stimulant. The fruits in combination with other tree parts (roots, bark, leaves, stems and twigs) are used to treat digestive disorders (Grace and Davis, 2002; Houghton, 2008; Ming, 2001).

Aqueous preparations of the fruits and flowers are administered orally or as a vaginal pessary to treat gynecology problems. The fruits and bark are used to promote breast development in young women, or in contrast to reduce swelling and mastitis of the breasts (Grace and Davis, 2002). Sexual complaints ranging from infertility, poor libido, sexual asthenia and impotence are treated with medicines containing the fruits. However, excessive use in males induces scrotal elephantiasis, while in some regions the fruits are used to remedy this condition (Grace and Davis, 2002).

The decoction of the fruit is used to relieve toothache, headache and treat edema of the legs. Snake bite antidotes are made with an infusion of the fruits, stems, leaves, twigs or bark, taken orally or rubbed onto the bite (Grace and Davis, 2002, Houghton, 2008;

Ming, 2001). Skin complaints and infections, such as whitlows, cysts, acne and boils, are treated with traditional medicines containing the fruits.

The fruit preparations are used in ethno veterinary medicine to treat digestive system disorders, leg edemas, dermal irritations and infections, mastitis and retained placenta (Grace and Davis, 2002). Fallen fruits along with leaves and flowers are browsed or foraged by livestock and game.

During times of famine, the hard seeds are roasted and eaten thereby providing food security. However, the fruit pulp is said to be inedible and toxic and may cause blistering of the tongue and skin. Fruits aid fermentation and enhance the flavor of traditional beers (Ming, 2001).

Other uses of the African Sausage Tree, which are non medicinal include making dugout canoes, planks, fence-posts, drums, stools, yokes, tool handles, mortars and large bowls, weapon bows, mousetraps, dolls, wood fuel, soil erosion prevention, shade and dye prpoduction obtained from the tannin-rich fruit pulp (Grace and Davis, 2002*)*.

The African Sausage Tree is regarded as sacred in many regions; religious meetings are held underneath the tree and the fruits are commonly sold in markets as charms for various uses such as to promote wealth and prosperity, to impart strength and courage on warriors, to increase crop yields, and as a fetish for fecundity, or to avert whirlwinds (Houghton, 2008; Ming, 2001).

2.1.3 COMMERCIAL APPLICATIONS OF THE SAUSAGE TREE.

There are now many commercial applications available of the African Sausage Tree for treating various skin complaints. A commercial product containing the African Sausage Tree stem bark is used to treat *Candida albicans* infections (Grace and Davis, 2002). Commercially manufactured products are used for symptomatic relief or cure of skin conditions including, among others, sunburn, chafing, is, itchy scalp and nappy rash. Other commercial reported uses are for treatment of malignant neoplasm such as skin melanoma, tumors and breast cancer (Grace and Dav 2002).

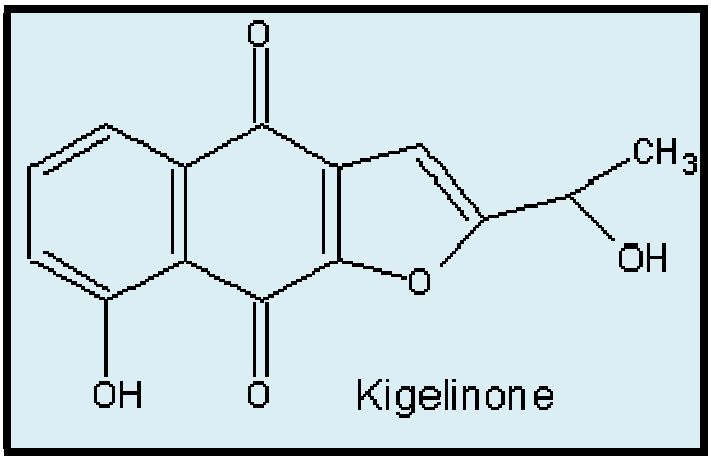
2.1.4 THE AFRICAN SAUSAGE TREE EXTRACTS STUDIES

Studies done with the extracts of the bark, wood, roots and fruits have shown these possess antibacterial and antifungal properties (Jeychandran *et al.,* 2007*;* Omonkhelin *et al.*, 2007). They are also renowned for anti-cancer properties confirmed by laboratory screening of *in-vitro* anti-cancer activity (cytotoxic effects against cancer cells) (Houghton, 2008; Kolodziej, 1997). The fruit extracts exhibit significant effects against inflammation and can be used for anti-inflammatory purposes.

2.1.4.1 NAPHTHOQUINONES

Of the phytochemicals indicated in extracts of the African Sausage Tree, the compound groups to which activity is most frequently attributed are naphthoquinones and iridoids (Houghton, 2008) These extracts exhibit significant inhibitory effects *in vitro* against common Gram-negative and Gram-positive bacteria and yeast (*Candida albicans).* Of the naphthoquinones isolated in fruit and root extracts, kigelinone has shown notable antimicrobial activity. The naphthoquinones have antiprotozoal activity also (Houghton, 2008). The naphthoquinones, lapachol and isopinnatal, exhibit antineoplastic activity against melanoma cell lines. Pinnatal and isopinnatal and kigelinol and isokigelinol are unique to the African Sausage Tree in the bognoniacaea family as isolated from the roots and fruit (Houghton, 2008). Hence overall, Naphthoquinones exhibit antibacterial, antifungal, antiprotozoal and antineoplastic activities (Binutu *et al.,* 2008; Houghton, 2008; Jeyachandran *et al.*, 2007; Omonkhelin, 2007).

Figure 1. **Chemical structure of Kigelinone (Naphthoquinone)**

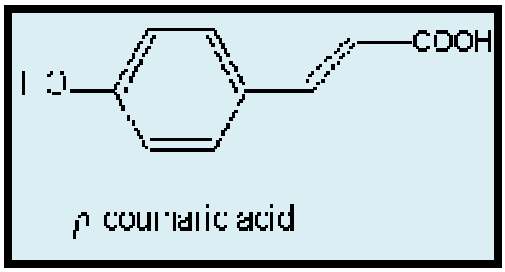


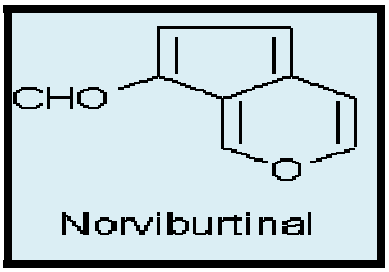
2.1.4.2 IRIDOIDS

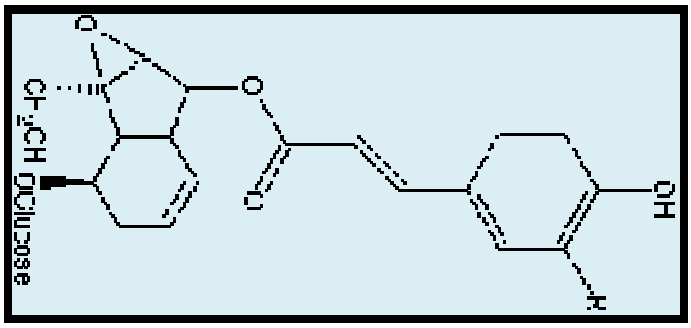
Iridoids and dihydroisocoumarins in extracts of the bark, fruits and roots may enhance the antimicrobial activity of naphthoquinones. Verminoside, an iridoid, and Verbascoside (polyphenols) are responsible for anti-inflammatory activity of the African Sausage Tree extracts (Lwalewa *et al.*, 2007; Lyn *et al.,* 2002). Verminoside has shown (In vitro assays) to have significant anti-inflammatory effects, inhibiting both iNOS expression and NO release in the LPS-induced macrophage cell line. It does not cause release of pro-inflammatory mediators (Kolodzeiej, 1997; Picerno *et al.,* 2008). Verminoside modify the inflammatory responses by eliciting anti-inflammatory activity through the inhibition of prostaglandin synthesis (Kolodzeiej, 1997; Picerno *et al.,* 2008).

The iridoid-related compound norviburtinal is responsible for cytotoxicity activity (Houghton, 2008). Other active antimicrobial compounds present in the fruit extracts are the phenylpropanoids caffeic acid, and *p*-coumaric acid.

Figure 2. **Chemical structures for Iridoids.**







Where R = OH for verminoside,
R = H for minecoside and
R = OCH_3 for specioside

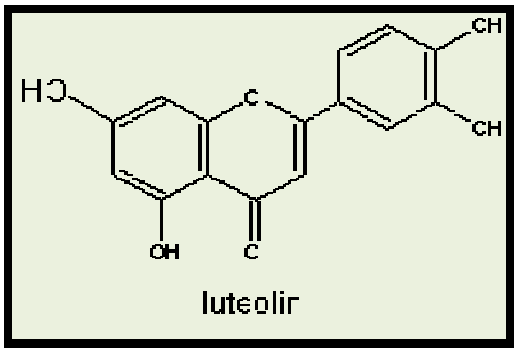
2.1.4.3 STEROLS

Sterols and iridoids are ubiquitous in the plant and may be a factor in the activity against melanoma. Naphthoquinones and sterols isolated in root extracts suggest anti-cancer potential, although *in-vitro* activity is not confirmed (Grace and Davis, 2002). The common sterols isolated from the fruit are b-sitosterol and sitignasterol. Sterols help in a range of skin conditions especially eczema (Houghton, 2008).

2.1.4.4 FLAVONOIDS

The leaves and fruits contain flavonoids. A high concentration of flavonoids may be responsible for antidiarrhoeal properties. In contrast to the use of the African Sausage Tree as a laxative, preliminary studies have shown a preventive effect of leaf extracts against diarrhoea in laboratory rats (Houghton, 2008). Flavonoids have clear hygroscopic and fungicidal properties. According to Houghton and Akunyili (1993) quoted by Houghton (2004), some isolated flavonoids from the fruit include luteol and quercetin and these with steroidal saponins, may be used to develop the bust, and reinforce the strength and stability of the breast col ibers.

Figure 3. Chemical structure of Luteolin (Flavonoids)



2.1.4.5 OTHER COMPOUNDS

The other compounds found in the fruit include Vernolic Fatty acids and Cinnamic acid derivatives which are thought to be responsible for anticonvulsant properties for which the African Sausage Tree is used to prevent epileptic fits. Lignans have not been isolated in the fruit but in the wood of the tree (Houghton, 2004).

Most secondary metabolites of the African Sausage Tree it show activity in some way but do not seem to account for all the activity shown y the extracts. Therefore, the antibacterial activity of the African Sausage Tree plant extracts can be attributed to not only a single bioactive principle but the combined action of the other compounds (Jeyachanran, 2007).

2.1.5 TOXICITY OF THE AFRICAN SAUSAGE TREE

The bark and leaves are bitter tasting, and the bark has been reported to contain a bitter principle. However, acute toxicity tests of the fruits indicate they are non-toxic (Kolodziej, 2007). Both Lorke (1983) and Kolodziej (19) working with mice concluded that the mortality, gross behavior and weight of the mice treated with high doses of African sausage tree mice pellets and African sausage tree extracts was indistinguishable from that of the controls.

2.2 ENDOTOXINS

2.2.1 DEFINITION

The lipopolysaccharides (LPS), sometimes known as lipooligosaccharides, are an endogenous component of the outer envelope of Gram negative bacteria and are commonly called endotoxins. They contain a lipid component, lipid A, which is determined by the number, type and distribution of fatty acids and its activity determines endotoxin activity. LPS trigger a wide variety of adverse and also beneficial reactions and are a potent stimulators of innate immunity (Hauschildt *et al.,* 2000).

Endotoxin is a thermostable polysaccharide toxin which is released from phospholipid and lipopolysaccharide after the destruction or lysis some microorganisms. Lipolysaccharide (LPS) is responsible for many pathophysiological symptoms observed during gram-negative bacterial infections. The symptoms include pyrogenicity (the ability to cause an increase in body temperature), changes in the number of circulating leukocytes (leukocytopenia, leukocytosis), complement activation of macrophages, aggregation of platelets and increase of capillary permeability (Dernfalk *et al.,* 2004).

2.2.2 BASIC LPS STRUCTURE

The LPS core consists of Lipid A in which fatty acid chains are linked to two glucosamine residues, buried in the hydrophobic interior of the envelope. To this is linked a core branched chain oligosaccharide region containing 3-deoxy-d-manno-2-octulosonic acid (KDO). These together constitute a rough LPS which is pharmacologically active and which may be structurally and immunologically diverse due to the wide variety of sugar linkages which are possible. Bacteria with smooth LPS have a variable length polysaccharide side chain of up to 40 sugars, some of which may be extremely uncommon sugars (eg tyvelose in typhoid bacilli). The resultant smooth chains, with its unique components, may enhance the survival of bacteria such as typhoid in the blood stream by preventing the binding preexisting antibody to the immunologically common core oligosaccharide region underneath, making the organism

less susceptible to complement-mediated lysis or to phagocytosis. Despite this common structure, LPS from different bacteria varies enormously in its ability to trigger endotoxic activity in the host (Netea *et al.,* 2003; Dernfalk *et al.,* 2007).

2.2.3 EFFECTS OF ENDOTOXINS

Endotoxins are mitogenic for B cells and activated macrophages, induce the release of pro-inflammatory cytokines and also fever producing agents such as interleukin-1. They may also trigger physiological cascades leading to disseminated intravascular coagulation and or septic shock. However, it has been observed that septic shock may be due to infection related to underproduction of critica proinflammatory cytokines, rather than the more accepted view of endotoxin induced overproduction (Netea *et al., 2003;* Dernfalk *et al.,* 2007).

LPS induces an immune response and the release of a higher dose may produce septic shock. All these biological activities are mediated through the endogenous mediator, tumor necrosis factor-a (TNF-a) (Netea *et al.,* 2003; Dernfalk *et al.,* 2007). TNF-a has an important role as a mediator of the inflammatory response as it induces expression of adhesion molecules on endothelial cells, activates neutrophils and macrophages, and induces production of nitric oxide and complement factors (Jeyachandran and Mehesh, 2007). The same mediators and effector molecules which are involved in microbicidal activity of macrophages, if produced in excess, lead to septic shock. These changes lead to extravasion of activated neutrophils and tissue damage due to release of Reactive Oxygen Intermediates (ROIs). Widespread tissue damage ultimately results in multiorgan dysfunction and septic shock. Therapeutic strategy under such circumstances requires reduction of production of TNF-a by macrophages in response to microbial endotoxin or suppression of TNF-a induced effects on the target cells (*Netea et al.,* 2003; Dernfalk *et al.,* 2007*).*

2.2.4 ENDOTOXIN INFECTION MODEL

Escherichia coli-derived lipopolysaccharide has been used extensively as a model for septic shock. The utility of this model system is because of the ease by which it can be produced and the relative similarity to systemic bacteremia. In pigs, lipopolysaccharide (LPS) challenge causes production of inflammatory cytokines uch as TNF-a, IL-6, and IL-10, as well as other mediators such as Nitric Oxide (Lynn, 2002).

Infections in swine caused by *Salmonella Chloraesus & Escherichia coli c*ause decreased growth performance (Cengiz *et al.,* 200; Lynn, 2002). This is due to the disease stress that causes metabolic changes which result into shifting in nutrient use away from growth process towards support of immune system function.

Bacterial infections induce production of inflammatory cytokines, which are part of, as well as inducers of, the acute phase response. The acute phase response to bacterial infections results in a vast sequel of pathophysiological changes. net effect of these changes results in alterations of drug absorption, distribution, metabolism, and elimination. Because endotoxins such as LPS can elicit inflammatory responses, administration of LPS has been used as a surrogate model of infection (Cengiz *et al.,* 2008; Lynn, 2002; Netea *et al.,* 2003; Dernfalk *et al.,* 2007*).*

Therefore, supplementing weaner pigs with ASTFP would them digest, absorb and accumulate the active metabolites indicated in the African Sausage Tree Fruit. Then the effect of the endotoxin challenge would be assessed to determine the impact of the resulting inflammation on the potential changes in Temperature, white blood cell count, Blood Urea Nitrogen (BUN), Total Protein, Albumin, Glucose and Cholesterol levels in the blood in the presence of ASTFP.

CHAPTER THREE

3.0 MATERIALS AND METHODS.

3.1 ANIMAL MATERIAL

The grower pigs used in the research were provided by Zambia National Service (ZNS) Chisamba camp. These were raised within the camp piggery unit at the normal camp management levels. After the suckling period of 7 to 10 weeks at 18 to 30 kg body weight, the piglets were weaned and some male weaned pigs selected for the experiment. Twenty weanling male pigs of 24 ± 6 kg body weight and of the ages 49 to 70 days old were used in the study. These weanling pigs were uncastrated males of landrace and large white crosses and were picked from litters. There were 4 weanling pigs picked from each litter. These 20 weanling pigs were housed in four and each pen housing 5 pigs. The study pigs were allowed a period of two weeks together before the start of the study for them to adapt to the pens and to each other. All animals were examined at the start of the experiment to ensure that they were clinically health. Allocation of treatments was by random numbers in each block (litter) according to procedure recommended by Gomez and Gomez (1984).

3.2 HOUSING

The piggery pens had a north–south orientation with a rough concrete floor. The walls of the pens were raised to 1.5 m high and the roof was made of corrugated iron sheets. The space between the roof and the walls was open on all the four walls of the pen, with the minimum clearance at 0.5m on the sides and the maximum clearance of 1.5 m at the middle. Each pen was $4m^2$ (2 m X 2 m). Four pens were used in the experiment, two on the eastern side and the other two on the western side separated by a walk way in between of 1.5m.

3.3 PLANT MATERIALS

The fruits of the African Sausage Tree locally known as the sausage tree fruit were collected from Zambia National Service (ZNS) farm in Chisamba between January and April 2009. The fruits were certified by the Resource centre of the department of the biological sciences in the school of natural sciences of the University of Zambia as the fruits of the African Sausage Tree.

3.4 PREPARATION OF AFRICAN SAUSAGE TREE FRUIT PULP

Eighty eight fruits (about 300kg freshly harvested fru were cut into small pieces using a machete and dried in the shade until they were dry enough to be ground (at 11.4% moisture). The particle size was reduced to fine powder at 8000 RPM using a C & N Laboratory Mill Disintergrator, size 8 (Christy and Norris Limited, Process Engineers Chelmsford England). The more fibrous fruit materials were discarded, retaining only the pulp (20,580g) in the pounding and process. The pulp was homogenized and stored for inclusion in test rations.

3.5 FEED FORMULATION

The basal diet used was a formulation used by the farm where the study was located. A pig grower feed (1, 372 kg) having 19.54% Crude Protein (CP) and 14.44 MJ/kg Metabolisable Energy (ME), as-fed basis, was used throughout the study period. The diet was modified by adding 2% ASTFP by weight (13, 720 gms) to half of the feed (686 kg) to suit the experimental diet specification. feed ingredients were acquired locally from the surrounding local farmers and in Lusaka City. The amounts of the various feed ingredients used, and the nutrients supplied are indicated below. (Table 3.5.1).

Table 3.5.1 Composition of basal pig weaner feed formulation

Ingredient	Nutrients							Ingredient
	CP	ME	CF	LYS	MET	Ca	P	
	%	(MJ/kg)	%	%	%	%	%	%
BMM	6.01	8.89	1.47	0.15	0.14	0.02	0.17	61.3
WB	0.85	0.49	0.57	0.01	0.01	0.01	0.07	5
SOYC	9.85	2.96	1.14	0.56	0.16	0.07	0.13	19.7
SFC	3.00	0.90	3.23	0.10	0.08	0.02	0.04	10
DCP						0.18	0.13	0.7
LSM						0.99		2.6
PPM								0.2
FM								0.3
LYS								0
MET								0
ESK								0.2
TOTAL	19.71	13.23	6.41	0.83	0.38	1.29	0.53	100
TARGET	18.00	12.5	=7	0.80	0.25	1.25	0.55	100
EX/DEF	-1.71	-0.73	0.59	-0.03	-0.13	-0.04	0.02	0

MM	Burned Maize meal	WB	Wheat bran	SOYC	Soy cake
SFC	Sunflower cake	DCP	Dicalcium Phosphate	LSM	Limestone
PPM	Pig Premix	FM	Fish meal	LYS	Lysine
MET	Methione	ESK	Eskaline	EX/DEF	Excess/Deficit

The Zambia bureau of Standards (ZS 018:2000) allow min MJ/kg ME and 18% Crude Protein, and Mcdonald *et al.* (1995) indicated 14.0 MJ/kg Digestible energy and 20.5% CP as minimum levels for pig grower feed. The basal diet used in this study had 14.44 MJ/kg ME and 19.54% CP while the modified diet with the inclusion of 2% ASTFP had 14.06 MJ/kg ME and 17.94% CP with the ASTFP having 13.98 MJ/kg ME and 8.35% CP. (Table 3.5.2).

Table 3.5.2 Composition of African sausage tree fruit pulp (ASTFP), 2% Astfp diet and 0% Astfp diet

	COMPOSITION ON AS-FED BASIS		
Food Component	Astfp	Basal with 2% Astfp	Basal without Astfp
Dry Matter %	88.86	91.40	92.10
Crude Protein %	8.35	17.94	19.54
Crude Fibre %	23.66	6.88	11.26
Ash %	3.78	6.02	6.86
Moisture %	11.14	8.60	7.90
Oil %	6.70	16.87	9.36
Calcium %	2.75	1.28	1.45
Phosphorus %	0.17	0.82	0.70
Me Mcal/Kg	3.34	3.36	3.45

Astfp = African Sausage Tree Fruit Pulp

ME Mcal/kg = Metabolisable Energy in Mega calories per kilogram

The maize and the sunflower cake were reduced in size a Saro Engineering Gravity mill using a 3mm sieve. Bags of maize meal, soya cake meal and sunflower meal were weighed on a 300 kg capacity scale supplied Zambia Scale Servises. A Globe Universal scale (100kg X 500g / 220 lbs X 1 lbs) and a Golden lark scale (150 kg X 500g / 330lbs X 24oz) were used to weigh required quantities of Limestone, DCP, Fish meal and pig premix. Mixing of these ingredients done through the ZNS Machine shop feed mixer of a capacity of 500 kg per hour.

3.6 EXPERIMENTAL DESIGN, TREATMENTS AND UNITS.

3.6.1 EXPERIMENTAL DESIGN

The study ran for 42 days after a 14 days adaptation period. The study was carried out in a 2^2 factorial experiment in a Randomized complete block design (RCBD). The two factors being the African Sausage Tree fruit pulp (at 0% and 2% levels) and E.coli endotoxins (at levels of 0µg/kg and 20µg/kg body weight) (Table 3.6.1). There were 5 blocks, representing 5 litters, and 5 replications for each treatment. The number of blocks (replicates) was a balance between the available resources (monetary and the possible number of male piglets per litter) and requirement for appropriate statistical analysis.

The consideration made when making blocks was the possible genetic variability of the pigs from one sow offspring to the other, which could the performance and the reaction of the pigs to the African Sausage Tree fruit pulp and the E coli endotoxin challenge. After these considerations, no physical or ironmental blocking was done. The blocks were given color names according to the color of tags used on the weaned pigs for identification, Red, White Yellow, Orange and Green for Blocks I, II, III, IV and V.

Table 3.6.1. Four Treatment combinations.

Treatment number	Treatment combinations		Treatment code
	African Sausage Tree (%)	Endotoxin (µg/kg body weight)	
1	2	0	K_1E_0
2	2	20	K_1E_1
3	0	0	K_0E_0
4	0	20	K_0E_1

K_0 and K_1 = *Kigelia Africana* levels 1 and 2, E_0 and E_1 = Endotoxin levels 1 and 2

3.6.2 TREATMENTS

The main factor of treatment were the African Sausage Tree fruit pulp at two levels (0 and 2%) and E coli endotoxin challenge at two levels (0 and 20µg/kg body weight). The treatment combinations were 4, replicated 5 times and in each Block (litter). Treatment 1 was made of 2% ASTFP feed supplement and n challenged with endotoxin (0 µg/kg body weight) with treatment code K_1E_0. Treatment 2 was of 2% ASTFP feed supplement and challenged with endotoxin (20 µg/kg body weight) with treatment code K_1E_1. Treatment 3 was of 0% ASTFP feed supplement and not challenged with endotoxin (0 µg/kg body weight) with treatment code K_0E_0. Treatment 4 was of 0% ASTFP feed supplement and challenged with endotoxin (20 µg/kg body weight) with treatment code K_0E_1. The four treatment combinations are presented in table 3.6.1 above. The treatments were assigned to the experimental units using random numbers according to the procedure described by Gomez and Gomez (1984). Treatments 1, 2, 3 and 4 were allocated to pens 1, 2, 3 and 4 respectively. By this arrangement, each pen had one weanling pig (one experimental unit) from each litter.

In the analysis of performance (body weights and weight gains) data, only two treatments were considered; With ASTFP feed supplement treatment code K_1 and Without ASTFP feed supplement (0%), treatment code K_0. These were replicated 10 times and each study pig was considered an experimental unit. (Table 3.6.2)

Table 3.6.2. Treatments for body weights and weight gains.

Treatment number	Treatment	Treatment code
1	Basal with 2% ASTFP	K_1
2	Basal with 0% ASTFP	K_0

ASTFP = African Sausage Tree Fruit Pulp

3.6.3 EXPERIMENTAL UNITS.

Each pig was an experimental unit and each treatment had 5 replicates. The total number of experimental units was therefore, 20 (4 treatment combinations X 5 replications), 5 pigs per Treatment (per pen). The study pigs were fed ccording to average weights per week (Table 3.8.1).

3.7 FEEDING AND WATER SUPPLY

The feed allocation was according to table 3.8.1 below. Two attendants were attached to the project for the purpose of feeding and providing w to the study animals. A day's ration was given at 8 hours daily, clean water was provided throughout the day and the pens were cleaned twice a day. The total amount of feed used was 686 kg basic diet (0% ASTFP) and 699.72 kg of modified basal diet (2% ASTFP) which contained 13.72 kg African sausage tree fruit pulp.

Table 3.8.1. Feeding allocation per week based on expected live wei and ASTFP intake

Week	1	2	3	4	5	6	Total
Body wt (kg)	20-22	22-25	25-30	30-34	34-39	39-45	
Feed allowance (kg/day/pig)	1.0	1.4	1.8	1.8	1.8	2.0	**68.6 kg (9.8X7)**
ASTFP intake at 2.0% (gms)	20.0	28.0	36.0	36.0	36.0	40.0	**13720 gms (196X7X10)**

ASTFP = African Sausage Tree Fruit Pulp

3.8 ENDOTOXIN ADMINISTRATION

On Day 42, Pen 2 (study pigs on 2% ASTFP feed supplement) and pen 4 (study pigs on 0% ASTFP feed supplement) study animals were injected with E coli endotoxins (20µg/kg body weight) while pen 1 (study pigs on 2% ASTFP feed supplement) and pen 3 (study pigs on 0% ASTFP feed supplement) were injected with soy broth (20µg/kg body weight).

The challenged group was administered intraperitoneally with E coli endotoxin (*Escherichia coli* serotype 057:B5 dissolved in soy broth carrier) at 20µ /kg body weight. The pigs were restrained with their abdomen ex sed and their head held downward. The needle was inserted into the abdominal cavity in the animal's lower right quadrant to avoid the cecum and urinary bladder. The needle was directed towards the animal's head at an angle of 15-20 degrees and inserted approximately 1 mm according to recommendation by University of Florida, l Care Department. A 2 ml syringe with a 25G niddle was used to administer the endotoxin.

3.9 HEALTH OBSERVATIONS

Clinical observations were made throughout the entire of the experiment. After endotoxin challenge, the experimental animals were checked for signs of vomiting, dyspneic, labored breathing and changes in feed consumption.

3.10 BLOOD SAMPLING AND TEMPERATURE MONITORING

Blood sampling and temperature monitoring started shortly before endotoxin administration and continued at intervals post administrartion. During blood collection, four men assisted to hold the pigs, all the pigs were standing with the head held with a nose snare and 10 mls of blood sample drawn from the jugular vein. The procedure lasted from 2 to 5 minutes.

The blood samples were collected in sterile 10 ml B-D vacutainer serum tubes (B-D Franklin Lakes NJ USA), immediately chilled on ice and transported to the laboratory. The blood plasma was separated by centrifugation at 3,000 rpm for 10 minutes at $4^{o}C$ and the plasma samples stored at $-20^{o}C$ until the analysis was done.

The body temperature was monitored from the rectum and it was measured using a digital thermometer, model TMO1, at the time the pigs handled for blood collection.

3.11 DATA COLLECTION

The study was conducted for 42 days and the live weights were measured weekly (on days 0, 7, 14, 21, 28, 35 and 42). The feed used was predetermined and recorded every week. The blood samples collection and rectal temperature measurement were done shortly before endotoxin administration (0 hours) and at 2, 4, 8 and 24 hours post endotoxin injections.

The blood samples collected were taken to biomedical laboratory of the School of Veterinary Medicine at the University of Zambia, for analysis on the spectrophotometer. The absorbance for Blood Urea Nitrogen, Total Protein, Albumin, Glucose and

cholesterol before endotoxin challenge (0 hours) and at 2, 4, 8 and 24 hours post injection were recorded for calculation of the respective concentrations in the blood.

Samples of the ingredients, feed formulation and ASTFP were analysed in Animal Science Department Laboratory (University of Zambia) for Dry Matter, Ether Extracts components, Crude Protein, Calcium and Phosphorus.

3.12 CHEMICALS AND CHEMICAL ANALYSIS

The E.coli endotoxin was obtained from the University of Zambia, School of Agricultural Sciences, Department of Food Science and Microbiology laboratory. Proximate analysis was done the Department of Animal Science Laboratory. Analysis kits were obtained from local suppliers in Lusaka city. All reagents were of analytical grade. Five analysis kits were used for the spectrophotometer reading of the absorbance.

Blood Urea nitrogen (BUN) was an enzymatic – colorimetric test with absorbance read at 580 nm wavelength on the spectrophotometer. The color development was through the hydrolysis of urea into ammonia which then reacted with salicylate and hydrochlorite to form a green indophenols whose colour intensity was proportional to the concentration of urea in the blood.

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} CO2 + (NH_4)^{2+}$$

$$NH_4^{+}\text{Salycilate} + \text{NaClO} \xrightarrow{\text{Nitroprusside}} \text{Indophenol}$$

The Total Protein was analyzed through the enzymatic colorimetric test BIURET and reading the absorbance at 546 nm wavelength on the spectrophotometer. The colour development was achieved through the Protein forming a blue colour complex with BIURET in the presence of Basic copper sulphate solution.

The absorbance of Albumin was read at 630 nm on the spectrophotometer after analysis using the enzymatic colorimetric test. The development of colour was through the reaction of Bromocresol Green (BCG) with Albumin to form a coloured complex which is green in pH 3.8.

Glucose concentration was determined through enzymatic colorimetric test. The color development was through the oxidation of Glucose by glucose oxidase to gluconic acid and hydroxide peroxide. The formed hydrogen peroxide (H_2O_2) was detected by a chromogeenic oxygen acceptor, phenol-aminophenazone in the presence of peroxidase.

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{GOD}} H_2O_2 + \text{Gluconic acid}$$

$$H_2O_2 + \text{Phenol} + \text{4-AP} \xrightarrow{\text{POD}} \text{Quinone} + 4H_2O$$

The absorbance was read on the Spectrophotometer measuring at 505 nm wavelength at about 37 ^{o}C. The Cholesterol was an enzymatic – colorimetric test with absorbance read at 505 nm wavelength on the spectrophotometer. The color development was through the release of Cholesterol and its esters from the lipoproteins by detergents. Cholesterol esterase hydrolyses the esters and the hydrogen peroxide was formed in the subsequent enzymatic oxidation of cholesterol by the cholesterol In the last reaction a red dye quinonimine is formed.

$$\text{Cholesterol Esters} + H_2O \xrightarrow{\text{CHE}} \text{Cholesterol} + \text{Fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHOD}} \text{4-Cholestenon} + H_2O_2$$

$$2H_2O_2 + \text{4-AP} + \text{Phenol} \xrightarrow{\text{POD}} \text{Quinonimine} + 4H_2O$$

3.13 DATA ANALYSIS

The data was analyzed as a 2 X 2 factorial array in a complete block design (RCBD) using the analysis of variance procedure. The ASTFP (0% Vs 2%), endotoxin challenge (0 Vs 20µg), Block (litter) and Interaction ASTFP and Endotoxin were analyzed as fixed effects. The values are reported as least Square means and presented with the root mean square error as a measure of variance. Each pig was considered as an experimental unit.

ANOVA for each parameter (immune and blood chemistry) done. The Least Square Difference (LSD) was done on means of the treatment factor for the parameters found to be significant at P = 0.05. Pair wise comparisons were made using the planned pair option of the Least Square Difference (LSD). Then the reatment combination means and the Treatments average means were compared to Treatment mean 3 and effects were considered significant if P = 0.05.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1. GROWTH AND GENERAL PERFORMANCE

The treatments considered under the growth and general performance were the two dietary treatments. Treatment 1 being 2% African Sausage Tree Pulp code K_1 and Treatment 2 being 0% African Sausage Tree Fruit Pulp code K_0. The average body weights per week during the study period are presented in table 4.1.1 while the average weekly gains, growth rate and efficiency of feed conversion over the study period are presented in table 4.1.2. Figure 4 shows a plot of average weekly body weights of the pigs over the study period.

4.1.1 BODY WEIGHTS AND BODY WEIGHT GAINS.

The mean weights of the study pigs at the start of the study was 27.0 ± 0.5 kg while at the end of the study (Day42) it was 44.25 ± 0.75 kg. The total weight gain by Day 42 ranged from 13.0 kg to 20.5 kg in the treatment with 2% ASTFP feed supplement (K_1) and 15.0 kg to 19.5 kg in treatment with 0% ASTFP feed supplement (K_0) (Table 4.1.2). The mean weight gain per treatment for 42 days was 17.5 kg for treatment with 2% ASTFP feed supplement (K_1) and 17.0 kg for treatment with 0% ASTFP feed supplement (K_0) (Table 4.1.2). Eventhough the growth curve of the study pigs in the treatment with 2% ASTFP feed supplement remained above that of the treatment with 0% ASTFP feed supplement (Figure 4), there were no significant differences in weight gain between the two treatments.

The blocks were not significantly different too, therefore, litter/pig source did not affect the performance of experimental pigs in body weight and weight gains.

Table 4.1.1. Mean body weights of study pigs per treatment per week study period (kg)

DAYS	0	7	14	21	28	35	42
Treatment[a]							
K_1	27.5	29.1	32	35.7	39.2	42	45
K_0	26.5	28.6	29.9	33.8	36.6	39.5	43.5

[a] Treatments K_1 and K_0 are dietary treatments of 2% and 0% ASTFP feed supplement respectively.

The rate of body weight gain for treatment with 2% ASTFP feed supplement (K_1) pigs was 0.42 kg per day by Day 42 and 0.40 kg per day for with 0% ASTFP feed supplement (K_0) pigs by Day 42. (Table 4.1.2). The two rates of body weight gains were not significantly different. The feed conversion (68.6 kg feed consumed/17.5 kg weight gain) for treatment with 2% ASTFP feed supplement (K_1) pigs was 3.92 and 4.04 for Treatment with 0% ASTFP feed supplement (K_0) pigs. (Table 4.1.2). Feed conversion of pigs on 2% ASTFP feed supplement was not different to those on with 0% ASTFP feed supplement.

Table 4.1.2. Total weight gains, Growth rate (kg/day) and Effeciency of feed conversion (kg feed/kg gain) of study pigs per dietary treatment per block over a period of 42 days.

	Block/Litter Average Weekly Weight Gain					Treatments		Performance[b]	
Treatments[a]	Red	White	Yellow	Orange	Green	TOTAL	MEAN	GR	EFC
	Rep I	Rep II	Rep III	Rep IV	Rep V				
K_1	18.5	13.0	18.0	20.5	17.5	87.5	17.5	0.42	3.92
K_0	16.5	15.5	19.5	15.0	18.5	85.0	17.0	0.40	4.04
Block total	35.0	28.5	37.5	35.5	36.0	**172.5**			
Block Mean	17.5	14.25	18.75	17.75	18.0		17.25		

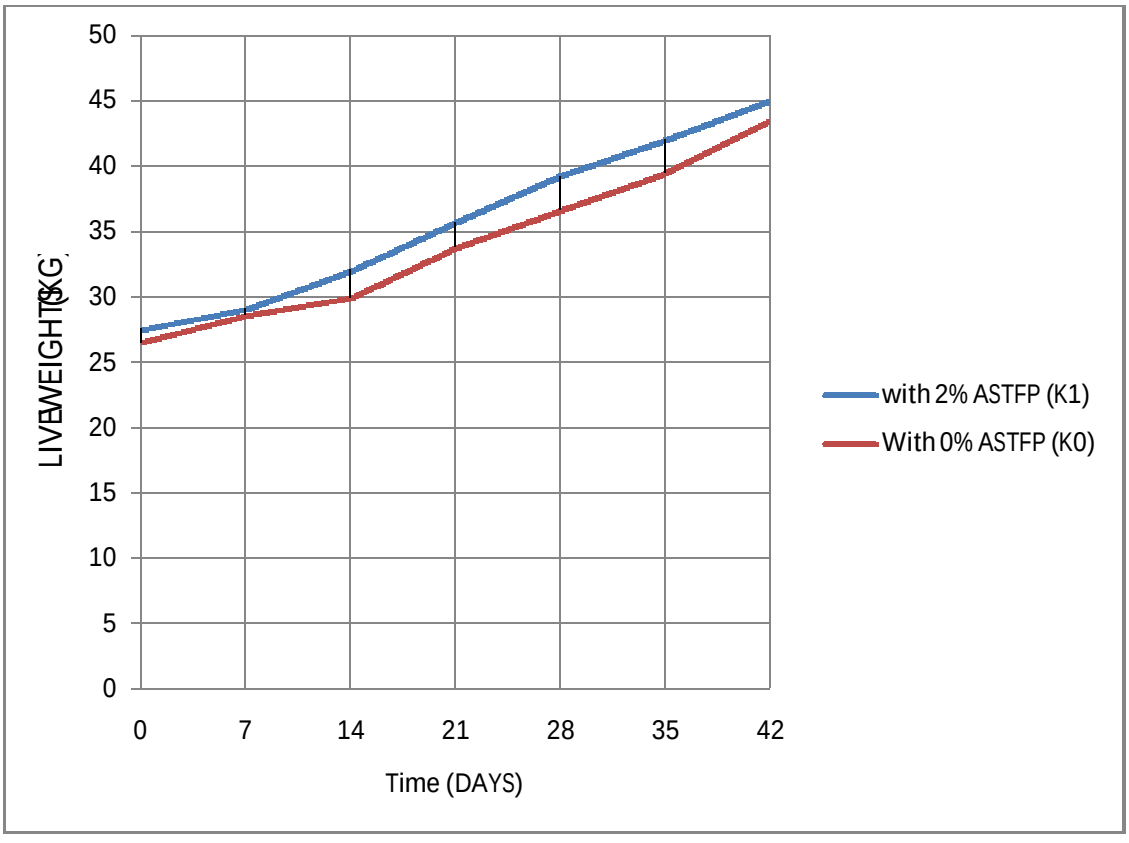
[a] Treatments K_1 and K_0 are dietary treatments of 2% and 0% ASTFP feed supplement respectively.

[b] GR = Growth rate, EFC = Efficient of Food convesion

The growth rate (0.42kg/day) in this study was lower than the average of good management of 0.64 kg per day at 45 kg as recommended by Payne (1990). This could have been due to the quality of the feed ingredients used in the formulation of the farm made feed used in the study (McDonald *et al.*, 1995). There were no significant differences in the growth rates of the two dietary treatment groups by Day 42.

The other observations that were made but not statistically measured include the timings of finishing the days ration, water intake, standing/laying frequency and mounting activities. The day's ration was observed to be completely consumed in treatment with 2% ASTFP feed supplement (K_1) earlier than in treatment with 0% ASTFP feed supplement (K_0). Mounting activities were observed to be more in treatment K_1 than in treatment K_0. The activity of standing and laying down were observed to be the same. Water intake was also observed to be the same in both dietary treatments.

Figure 4 . Average weekly body weights ot the study pigs per dietary treatment during the 42 days period of study.



4.2 IMMUNE FUNCTION

There were four treatments for Immune function studied. Treatment 1 being 2% ASTFP and E.coli endotoxin at 0µg/kg body weight code K_1E_0, treatment 2 being 2% ASTFP and E.coli endotoxin at 20µg/kg body weight code K_1E_1, treatment 3 being 0% ASTFP and E.coli endotoxin at 0µg/kg body weight code K_0E_0 and treatment 4 being 0% ASTFP and E.coli endotoxin at 20µg/kg body weight code K_0E_1. The results of the effect of ASTFP feed supplement and E.coli endotoxin challenge on body temperature and white blood cell count in grower pigs are presented in table 4.2.1. The trends of the body temperature readings and white blood cell counts during the blood sampling period are presented in figures 5 and 7, respectively. Figure 6 elaborates observed interaction of ASTFP feed supplement and E.coli endotoxin challenge on white bloo cell counts at 4 hours post endotoxin injection.

Table 4.2.1. Effect of dietary ASTFP and Endotoxin injection on grower pigs body temperature and white blood cell count.

Effect of dietary ASTFP and E.coli endotoxin injection on immune function in grower pigs.[a]						
		Dietary African Sausage Tree Fruit pulp (%)				
		2		0		
		Injected E coli endotoxin (µg/Kg body weight)				
		0	20	0	20	
Treatment		1	2	3	4	
Parameter	Time (hrs)					**SE**
Temperature (^oC)	0	39.1	39.14	38.96	38.66	**0.13**
	2	39.06	39.52	39.24	39.34	**0.13**
	4[c]	38.36	38.88[#]	38.10	39.08[#]	**0.13**
	8	39.08	39.42	39.22	39.42	**0.08**
	24	39.62	39.44	39.50	39.68	**0.07**
WBC (10^3 cells/mm^3)	0	15.30	14.70	15.90	16.05	**0.65**
	2	14.80	13.60	15.80	14.20	**0.37**
	4[bcd]	12.82	14.84	14.64	22.22[#]	**0.89**
	8[c]	13.64	21.08[#]	14.20	24.55[#]	**1.20**
	24	13.90	14.01	13.50	14.50	**0.22**

[a] Least square means of five pigs per treatment combination
[b] African Sausage Tree Fruit Pulp effect (P = 0.05)
[c] Endotoxin effect (P = 0.05)
[d] ASTFP X Endotoxin interactions (P = 0.05)
[#] Treatment mean significantly different from treatment 3 mean (P = 0.05).
WBC = White blood cells
ASTFP = African Sausage tree Fruit Pulp

4.2.1 BODY TEMPERATURE

There were, no significant differences among the treatment means before (0 hours) and 2 hours Post endotoxin injection (Table 4.2.1).

At 4 hours post injection, there was a drop in body temperature observed, with minimum drop in the endotoxin challenged treatment combinations (Figure 5). This resulted into a significant (P = 0.05) difference among treatment combination means mainly due to endotoxin effect which showed significant effect (Table 4.2.2). The mean body temperature for treatment 4 (0% ASTFP and 20µg endotoxin /kg body weight) was significantly higher (P = 0.01) than the mean body temperature for treatment 3 (0% ASTFP and 0µg endotoxin /kg body weight). Treatment 2 ASTFP and 20µg endotoxin /kg body weight) mean was also observed to be higher (P = 0.05) than treatment 3 mean. When the average of two treatment means, treatments 1 and 2 (2% ASTFP); and treatments 2 and 4 (20µg endotoxin /kg body weight;) were compared to treatment 3 mean, only the average of treatments 2 and 4 was significantly higher (P = 0.01).

Table 4.2.2. Planned pairwise comparison between mean temperature f treatment 3 (K_0E_0) and the other treatments (1, 2 and 4) using the LSD test: 0.66 $t_{0.05}$ (t=2.179, df =12, r= 5 and 0.23 MSE) and 0.93 $t_{0.01}$ (t=3.055, df =12, r= 5 and 0.23 MSE) at 4 hours post injection.

	Treatment	Mean Temperature[b] (^oC)	Difference from treatment 3[d] (^oC)
1	K_1E_0	38.36	0.26^{ns}
4	K_0E_1	39.08	0.98^{**}
2	K_1E_1	38.88	0.78^{*}
1 and 2[a]	(K_1E_0 & K_1E_1)	38.62[c]	0.52^{*}
4 and 2[a]	(K_0E_1 & K_1E_1)	38.98[c]	0.88^{**}
3	K_0E_0	38.10	-

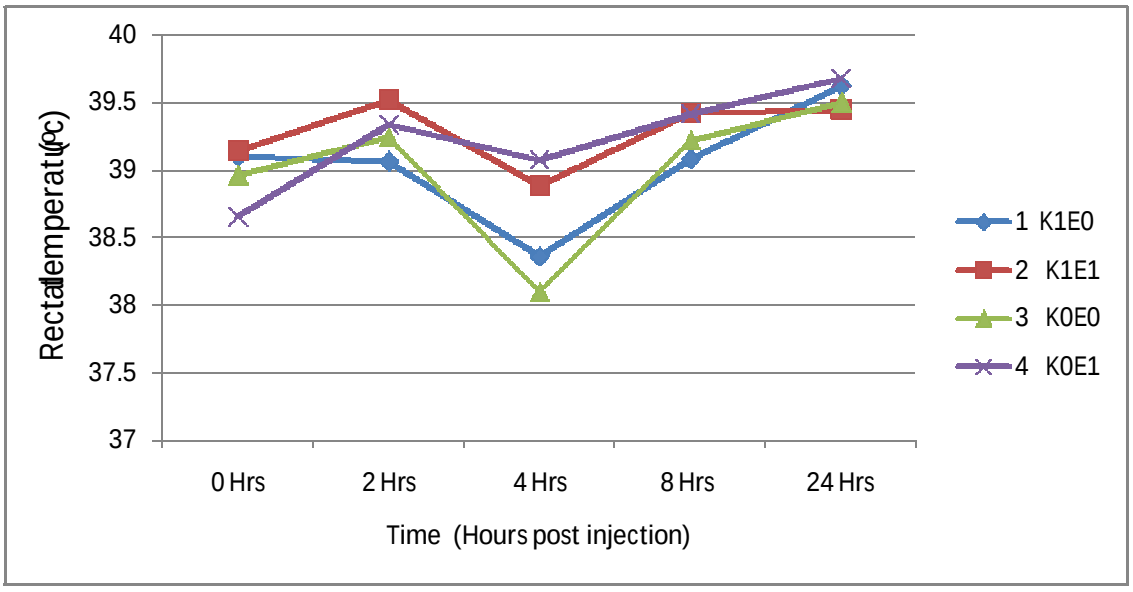
[a]Difference compared with LSD of 0.47 $t_{0.05}$ and 0.66 $t_{0.01}$ calculated by sd = $(s^2/r)^{1/2}$. [b]Average of five replications. [c]Average of two means. [d]** = significant at 1% level, * significant at 5% level, ns = not significant

There were no effects of ASTFP, Endotoxin or Interaction observed at 8 and 24 hours post injection.

The inclusion of ASTFP at 2% of the feed had no signif effect on the body temperature of the study animals throughout the study. The effects of ASTFP at were monitored through treatment 2 mean, whose effects were not significant throughout the blood sampling period. (Table 4.2.1)

It was noticed that in response to endotoxin challenge the study animals increased body temperature and the increase was significant (P = 0.01) by the 4th hour post injection. The increase in temperature in treatment 2, at 4 hours post injection, was significant at P = 0.05 while the increase in body temperature for treatment 4 at 4 hours was significant at P = 0.01. This may indicate the positive effect of ASTFP in modulating, to some extent, the immune function of the study animals by suppressing the synthesis and release of enzymes associated with inflammation in treatment 2 pigs, resulting into the body temperature increase being less than in treatment 4.

Figure 5 Average body temperature readings of weaner pigs per over 24 hours post injection period.



K_1E_0 = treatment 1 (2% ASTFP and 0µg endotoxin /kg body weight)
K_1E_1 = treatment 2 (2% ASTFP and 20µg endotoxin /kg body weight)
K_0E_0 = treatment 3 (0% ASTFP and 0µg endotoxin /kg body weight)
K_0E_1 = treatment 4 (0% ASTFP and 20µg endotoxin /kg body weight)

4.2.2 WHITE BLOOD CELLS COUNT

There were no significant differences among treatment means before (0 hours) and at 2 and 24 hours post injection (Table 4.2.1). Therefore, the ASTFP and endotoxin had no real effects on the white blood cell counts of the study pigs before (0 hours) and at 2 and 24 hours post injection.

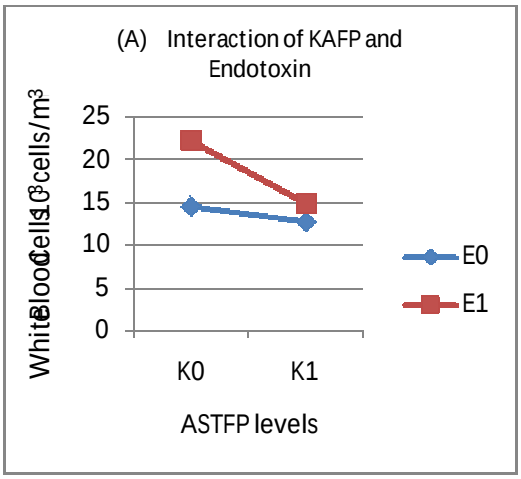
However, there were significant differences (P = 0.01) among the treatment means at 4 hours post injection and these differences were due to both ASTFP and Endotoxin effects (Table 4.2.3). The endotoxin increased the White Blood cell (WBC) significantly (P = 0.01) while the ASTFP decreased the White Blood cells significantly (P = 0.01), both in comparison to treatment 3 (Table 4.2.3). The mean for treatment 4 was significantly (P = 0.01) higher than the mean for treatment 3. The average of treatments 2 and 4 (treatments with endotoxin at 20µg/kg body weight) was also significantly higher than the mean of treatment 3. There was a signi icant (P = 0.01) interaction of ASTFP X Endotoxin observed at 4 hours post endotoxin injection and the nature of this interaction observed was that of change of magnitude (Figure 6). The White Blood cells increase was greater in 0% ASTFP than in 2% ASTFP and ually the decrease in white blood cells was greater in endotoxin at 20 µg/kg body weight than in 0 µg/kg body weight (Figure 6).

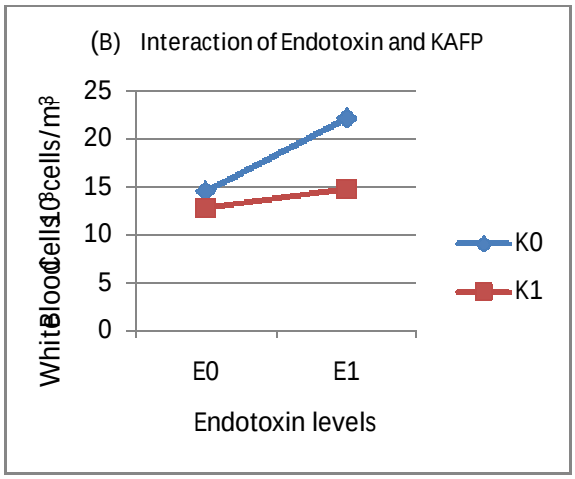
Table 4.2.3 Planned pairwise comparison between mean white blood cell count for treatment 3 (K_0E_0) and the other 3 treatments using the LSD test: 2.17 $t_{0.05}$ (t=2.179, df =12, r= 5 and 2.48 MSE) and 3.04 $t_{0.01}$ (t=3.055, df =12, r= 5 and 2.48 MSE) at 4 hours post injection.

Treatment		Mean WBC concentration[b] 10^3 cells/mm^3	Difference from treatment 3[d] 10^3 cells/mm^3
1	K_1E_0	12.82	1.82^{ns}
4	K_0E_1	22.22	7.58^{**}
2	K_1E_1	14.84	0.20^{ns}
1 and 2[a]	(K_1E_0 & K_1E_1)	13.84[c]	0.81^{ns}
4 and 2[a]	(K_0E_1 & K_1E_1)	18.53[c]	3.89^{**}
3	K_0E_0	14.64	-

[a]Difference compared with LSD of 1.54 $t_{0.05}$ and 2.15 $t_{0.01}$ calculated by sd = $(s^2/r)^{1/2}$. [b]Average of five replications. [c]Average of two means. [d]** = significant at 1% level, [ns] = not significant

Figure 6 **Interaction of dietary ASTFP X Endotoxin injection for white blood cell count in grower pigs at 4 hour post injection.**





K0 = ASTFP at 0%,

K1 = ASTFP at 2%

E0 = Endotoxin at 0 µg/kg body weight and

E1 = 0 µg/kg body weight

At 8 hours post injection, the treatment mean differences continued being significantly different (P = 0.01) mainly as a result of the endotoxin effect (Table 4.2.4). Both means for treatments 2 and 4 were of higher white blood cell count than treatment 3 mean. The ASTFP effect was not significant and no real interaction was observed.

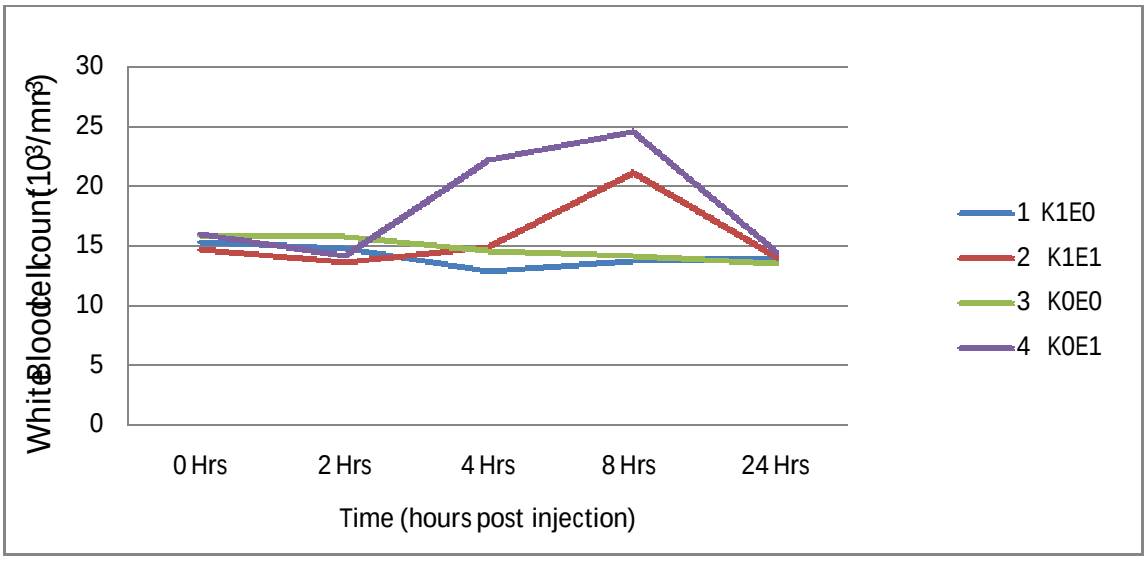
Table 4.2.4 Planned pairwise comparison between mean white blood cell count for treatment 3 (K_0E_0) and the other 3 treatments using the LSD test: 3.46 $t_{0.05}$ (t=2.179, df =12, r= 5 and 6.30 MSE) and 4.85 $t_{0.01}$ (t=3.055, df =12, r= 5 and 6.30 MSE) at 8 hours post injection.

	Treatment	Mean WBC concentration[b]	Difference from treatment 3[d]
		10^3 cells/mm^3	10^3 cells/mm^3
1	K_1E_0	13.64	0.56^{ns}
4	K_0E_1	24.55	10.35^{**}
2	K_1E_1	21.08	6.88^{**}
1 and 2[a]	(K_1E_0 & K_1E_1)	17.36	3.16^{*}
4 and 2[a]	(K_0E_1 & K_1E_1)	22.82	8.62^{**}
3	K_0E_0	14.20	-

[a]Difference compared with LSD of24.45 $t_{0.05}$ and 3.43 $t_{0.01}$ calculated by sd = $(s^2/r)^{1/2}$. [b]Average of five replications. [c]Average of two means. [d]** = significant at 1% level, [ns] = not significant

The white blood cell count was not significantly different among the study pigs at 0 hours and 2 hours post endotoxin injection (Figure 7 and Table 4.2.1). But 4 hours st injection, mean for treatment 4 was significantly higher than mean for treatment 3 (P = 0.01) while mean for treatment 2 was not. However, at hours post injection, both treatment 2 and 4 means were significantly higher than mean for treatment 3 (P = 0.05). The increase in white blood cell count, 8 hours post injection, in treatment 2 compared to increase of white blood cell count, at both 4 and 8 hours post injection, in treatment 4 could be indicative of delayed response to endotoxin induced by the ASTFP. At, 24 hours post injection, the white blood cell counts in treatments 2 and 4 reduced and were not different from treatment 3 mean. Means for treatment 1 were not significantly different from means for treatment 3 throughout the blood sampling period of 24 hours post injection.

Figure 7 Mean white blood cell count for weaner pigs per treatment over 24 hours post injection period.



K_1E_0 = treatment 1 (2% ASTFP and 0µg endotoxin /kg body weight)

K_1E_1 = treatment 2 (2% ASTFP and 20µg endotoxin /kg body weight)

K_0E_0 = treatment 3 (0% ASTFP and 0µg endotoxin /kg body weight)

K_0E_1 = treatment 4 (0% ASTFP and 20µg endotoxin /kg body weight)

4.3 BLOOD CHEMISTRY

There were also four treatments for Blood Chemistry st Treatment 1 being 2% ASTFP and E.coli endotoxin at 0µg/kg body weight code K_1E_0, Treatment 2 being 2% ASTFP and E.coli endotoxin at 20µg/kg body weight code K_1E_1, Treatment 3 being 0% ASTFP and E.coli endotoxin at 0µg/kg body weight code K_0E_0 and Treatment 4 being 0% ASTFP and E.coli endotoxin at 20µg/kg body weight code K_0E_1.

The results of the effect of dietary ASTFP and E.coli endotoxin challenge on Blood Urea Nitrogen (BUN), blood Total Protein, blood Albumin, blood Glucose and cholesterol of study pigs are presented in tables 4.3.1 to 4.3.6, . The trends of the Blood Urea Nitrogen, Total protein, Albumin Glucose and cholesterol over a 24 hour blood sampling period are presented in figures 9, 11, 12, 13 and 14, respectively. Figure 10 elaborates observed interaction of dietary ASTFP and E.coli endotoxin challenge on total protein at 4 hours post endotoxin injection.

4.3.1 BLOOD UREA NITROGEN

Results in table 4.3.1. below show that there were no ificant differences in Blood Urea Nitrogen concentration among the treatment means before (0 hours), 2 and 8 hours post endotoxin injection. Therefore, there were no rea effects of ASTFP, endotoxin and interaction of the two on blood urea nitrogen.

Table 4.3.1. Effect of dietary ASTFP and Endotoxin injection on blood urea nitrogen in grower pigs.

Effect of dietary ASTFP and E.coli endotoxin injection on BUN in growing pigs.[a]						
		Dietary African Sausage Tree Fruit Pulp (%)				
		2		0		
		Injected E coli endotoxin (µg/Kg body weight)				
		0	**20**	**0**	**20**	
Treatment		1	2	3	4	
Parameter	Time (hrs)					**SE**
BUN (mg/dl)	0	53.33	53.81	37.66	50.83	2.70
	2	48.90	42.57	41.76	56.32	3.83
	4[bc]	45.57	35.00	40.20	30.30[#]	1.78
	8	33.30	38.82	37.56	36.36	1.43
	24[d]	36.62	54.50	40.52	35.26	2.88

[a] Least square means of five pigs per treatment combination

[b] African Sausage Tree Fruit Pulp effect (P=0.05)

[c] Endotoxin effect (P = 0.05)

[d] ASTFP X Endotoxin interactions (P = 0.05)

[#] Treatment mean significantly different from treatment 3 mean (P = 0.05).

BUN = Blood Urea Nitrogen

At 4 hours post injection, there was observed decline blood urea nitrogen concentration in all the treatments. (Table 4.3.1). Blood urea nitrogen concentration was less in 2% ASTFP and 0% ASTFP treatment combination challenged with E coli endotoxin (treatments 2 and 4). This resulted into significant differences (P = 0.01) among treatment means (Table 4.3.1.1). The endotoxin effect was significant (P = 0.01) and it was observed in the lowered blood urea nitrogen concentration in treatment 4 in comparison to Treatment 3. The average of Treatments 4 and 2 (0% ASTFP and 20µg endotoxin/kg body weight and 2% ASTFP and 20µg endotoxin/kg body weight respectively) was significantly (P = 0.01) lower than treatment 3 mean. The means of treatments 1 and 2 (2% ASTFP) were not significantly different from Treatment 3 mean, including their average when compared to treatment 3 mean. Therefore, effect of ASTFP on the blood urea nitrogen concentration was not significant. There was also no real interaction of ASTFP X Endotoxin observed for blood urean nitrogen concentration at 4 hours post injection.

Table 4.3.1.1 Planned pairwise comparison between mean blood urea nitrogen for treatment 3 (K_0E_0) and the other 3 treatments using the LSD test: 6.70 $t_{0.05}$ (t=2.179, df =12, r= 5 and 23.60 MSE) and 9.39 $t_{0.01}$ (t=3.055, df =12, r= 5 and 23.60 MSE) at4 hours post injection.

	Treatment	Mean BUN concentration[b]	Difference from treatment 3[d]
		mg/dl	mg/dl
1	K_1E_0	45.57	5.37^{ns}
4	K_0E_1	30.30	9.90^{**}
2	K_1E_1	35.00	5.20^{ns}
1 and 2[a]	(K_1E_0 & K_1E_1)	40.28[c]	0.08^{ns}
4 and 2[a]	(K_0E_1 & K_1E_1)	32.65[c]	7.55^{**}
3	K_0E_0	40.20	-

[a]Difference compared with LSD of 4.73 $t_{0.05}$ and 6.64 $t_{0.01}$ calculated by sd = $(s^2/r)^{1/2}$. [b]Average of five replications.

[c]Average of two means. [d]** = significant at 1% level, ^{ns} = not significant

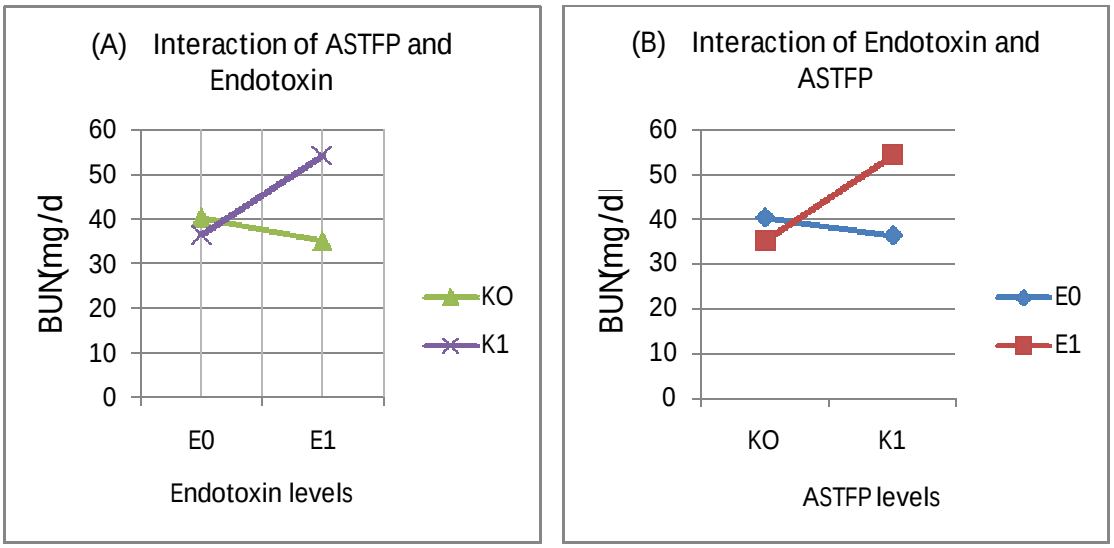
By 24 hours post injection, the blood urea nitrogen concentration of all the treatment combinations increased except the endotoxin challenged treatment combination of 0% ASTFP, (treatment 4) in which blood urea nitrogen reduced. This resulted into significant treatment differences (P = 0.05) observed among four treatment combinations (Table 4.3.1.2). Mean separation using the LSD, however, did not detect any treatment mean different from mean for treatment 3. This implies that when the means of treatments 1, 2 and 4 were compared to mean for treatment 3, no real differences were detected. The interaction (ASTFP X Endotoxin and Endotoxin X ASTFP) observed were of both of change of rank (Figure 8). When the level of endotoxin was increased from 0µg/kg body weight (E_0) to 20µg/kg body weight (E_1) the blood urea nitrogen in 0 % ASTFP (K_0) declined from 40.52 mg/dl to 35.26 mg/dl while increasing from 36.62 mg/dl to 54.50 mg/dl in 2% ASTFP (K_1). (Figure 8A). In figure 8B, when the level of ASTFP feed supplement was increased from 0% ASTFP to 2% ASTFP, the blood urea nitrogen concentration declined from 40.20 mg/dl to 36.62 mg/dl in 0µg endotoxin/kg body (E_0) but it increased from 35.26 mg/dl to 54.50 mg/dl in 0µg endotoxin/kg body weight (E_1).

Table 4.3.1.2 Planned pairwise comparison between mean blood urea nitrogen for treatment 3 (K_0E_0) and the other 3 treatments using the LSD test:14.31 $t_{0.05}$ (t=2.179, df =12, r= 5 and 107.87MSE) and 20.07 $t_{0.01}$ (t=3.055, df =12, r= 5&107.87MSE) at 24 hours post injection.

	Treatment	Mean BUN concentration[b] mg/dl	Difference from treatment 3[d] mg/dl
1	K_1E_0	36.618	3.898[ns]
4	K_0E_1	35.256	5.26[ns]
2	K_1E_1	54.504	13.988[ns]
1 and 2[a]	(K_1E_0 & K_1E_1)	45.561[c]	5.045[ns]
4 and 2[a]	(K_0E_1 & K_1E_1)	44.88[c]	4.364[ns]
3	K_0E_0	40.516	-

[a]Difference compared with LSD of 10.12 $t_{0.05}$ and 14.19 $t_{0.01}$ calculated by sd = $(s^2/r)^{1/2}$. [b]Average of five replications. [c]Average of two means. [d] [ns] = not significant at 5% level

Figure 8 **Interaction of dietary ASTFP X Endotoxin injection for blood urea nitrogen in grower pigs 24 hour post injection**



K0 = ASTFP at 0%,

K1 = ASTFP at 2%

E0 = Endotoxin at 0 µg/kg body weight and

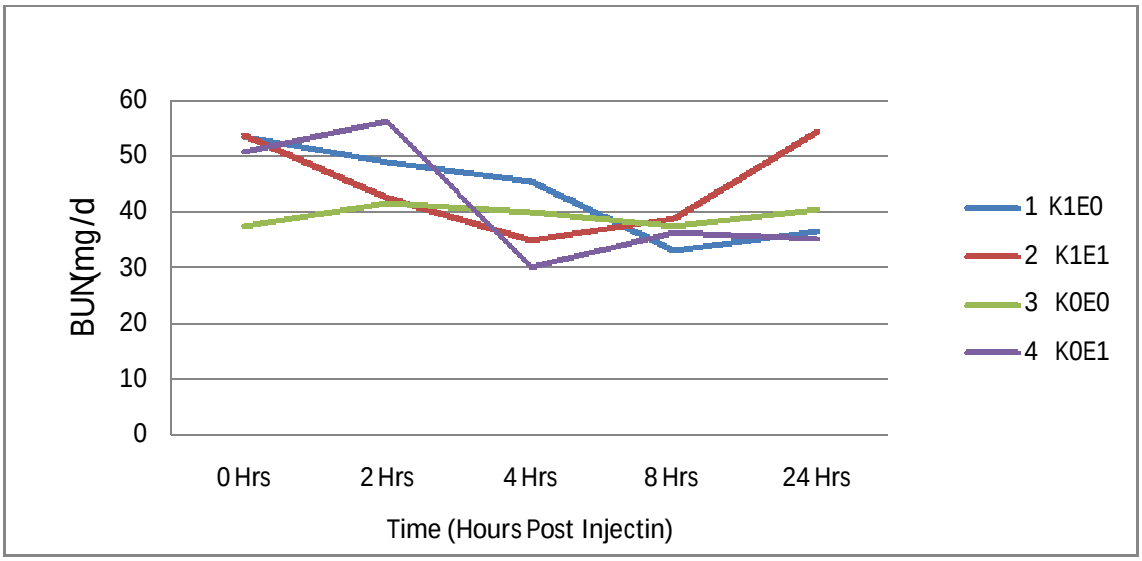
E1 = 0 µg/kg body weight

The trends in blood urea nitrogen concentration levels were similar all treatments declining over the blood sampling period and a slight at the end of the sampling period. (Figure 9). Blood urea nitrogen is a metabolic waste product in the blood generated from the breakdown of protein. Concentrations of blood urea nitrogen in this study were in the upper limit or above the normal range of between 10 and 30 mg/dl (Sunderland *et al.*, 2009), the possible cause of high blood urea nitrogen in t study animals is the stress of handling the pigs to collect blood from the jugular vein during blood sampling time that could have raised the olic state of the pigs hence high blood urea nitrogen.

From table 4.3.1 above, only mean for treatment 4 was icantly different from mean for treatment 3, the other treatment means were not significantly different from mean for treatment 3 at 4 hours post endotoxin injection. The significantly (P = 0.01) lower blood urea nitrogen concentrations for treatment 4 (0% ASTFP and 20µg endotoixn/kg body weight) in comparison to treatment 3 (0% ASTFP and 0µg xin/kg body weight) at 4 hours post injection, may suggest the mobilization of protein (in transamination

process) for pro inflammatory factors and the non significant difference between the treatment means 2 (2% ASTFP and 20µg endotoxin/kg body weight) and 3 may suggest the modulation of synthesis and release of the pro inflammatory factors by the presence of ASTFP active metabolite in the pig bodies. The interaction observed at 24 hours post endotoxin injection was that of change of rank indicating the simple effects of ASTFP and endotoxins. As the level ASTFP increases from 0% to 2%, two things happen; 1) the blood urea nitrogen concentration for treatments challenged with endotoxin increase from 35.26 mg/dl to 54.50 mg/dl and 2) the blood urea nitrogen concentration of treatments not challenged with endotoxin slightly decrease from 40.52 mg/dl to 36.62 mg/dl. (Figure 8B and Table 4.3.1) As the level of endotoxin increase from 0 to 20µg/kg body weight, the blood urea nitrogen concentration, for treatments with 2% ASTFP feed supplement, increases from 36.62 mg/dl to 54.50 mg/dl and a decrease for 0% ASTFP from 40.52 mg/dl to 35.26 mg/dl. (Figure 8A). This interaction my indicate that, in the presence of endotoxin without ASTFP feed supplement, more proteins are used for synthesis of pro-inflammatory factors hence less blood urea nitrogen concentration and in the presence of endotoxin with ASTFP feed supplement, less proteins used for pro-inflammatory factors hence more for metabolic activities resulting in high blood urea nitrogen concentration.

Figure 9 **Mean blood urea nitrogen concentration in weaner pigs over 24 hour blood sampling period.**



K_1E_0 = treatment 1 (2% ASTFP and 0µg endotoxin /kg body weight)
K_1E_1 = treatment 2 (2% ASTFP and 20µg endotoxin /kg body weight)
K_0E_0 = treatment 3 (0% ASTFP and 0µg endotoxin /kg body weight)
K_0E_1 = treatment 4 (0% ASTFP and 20µg endotoxin /kg body weight)

4.3.2 BLOOD TOTAL PROTEIN

Results for blood total protein are presented in table 4.3.2. It can be noted that treatment means for the four treatments were not significantly different from each other before (0 hours), and at 2, 8 and 24 hours post endotoxin injection. Therefore, there was no real endotoxin effect, ASTFP effect or interaction effect before, and at 2, 8 and 24 hours after endotoxin injection.

Table 4.3.2. Effect of dietary ASTFP and Endotoxin injection on blood total protein in grower pigs.

Effect of dietary ASTFP and E.coli Endotoxin injection on blood total protein in growing pigs.[a]						
		Dietary African Sausage Tree Fruit Pulp %				
		2		0		
		Injected E coli endotoxin (µg/Kg body weight)				
		0	**20**	**0**	**20**	
Treatment		1	2	3	4	
Parameter	Time (hrs)					**SE**
Total Protein (g/dl)	0	2.34	2.89	2.90	2.81	0.11
	2	3.52	4.15	3.32	3.11	0.20
	4[d]	2.46[#]	3.61	3.35	2.52[#]	0.15
	8	2.73	3.02	2.87	2.02	0.21
	24	2.90	2.19	2.07	2.25	0.15.

[a] Least square means of five pigs per treatment combination

[d] ASTFP X Endotoxin interactions (P = 0.05)

[#] Treatment mean significantly different from treatment 3 mean (P = 0.05).

However, at 4 hours post endotoxin injection, there was a statistically significant (P = 0.01) effect of ASTFP X Endotoxin interaction on the blood total proteins (Table 4.3.2.1). This resulted into treatments 1 and 4 total protein the blood being lower than the total protein in treatment 3 pigs. The blood total protein for treatment 2 was not different from that of treatment 3. Apart from the effect of interaction, the endotoxin effect and the ASTFP effect on the Total Protein concentration in the blood was not significant.

Table 4.3.2.1 Planned pairwise comparison between mean blood Total Protein for treatment 3 (K_0E_0) and the other treatments (1, 2 and 4) using the LSD test:0.7 $t_{0.05}$ (t=2.179, df =12, r= 5 &0.26 MSE) and 0.98 $t_{0.01}$ (t=3.055, df =12, r= 5 & 0.26 MSE) at 4 hours post injection.

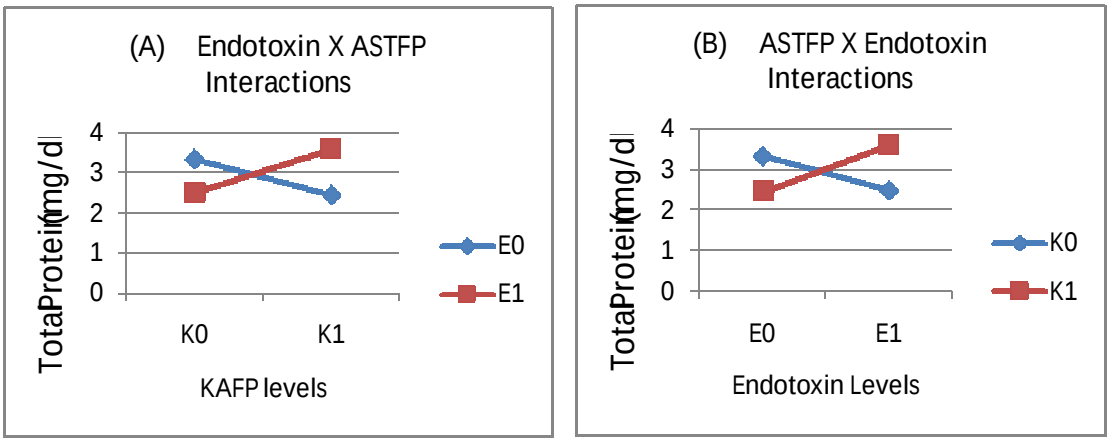
	Treatment	Mean Total Protein concentration[b] g/dl	Difference from treatment 3[d] g/dl
1	K_1E_0	2.458	0.89*
4	K_0E_1	2.516	0.83*
2	K_1E_1	3.616	0.27ns
1 and 2[a]	(K_1E_0 & K_1E_1)	3.037[c]	0.31ns
4 and 2[a]	(K_0E_1 & K_1E_1)	3.066[c]	0.28ns
3	K_0E_0	3.348	-

[a]Difference compared with LSD of 0.49 $t_{0.05}$ and 0.69 $t_{0.01}$ calculated by sd = $(s^2/r)^{1/2}$. [b]Average of five replications. [c]Average of two means. [d] *significant at 5% level ns = not significant at 5% level

When compared to treatment 3 mean, treatments 1 and 4 ASTFP and 0µg endotoxin/kg body weight and 0% ASTFP and 20µ endotoxin/kg body weight) means showed significantly lower total protein concentration. The interaction of ASTFP X endotoxin on blood total protein was that of change of rank in effect. As the ASTFP feed

supplement was increased from 0% to 2%, the total protein concentration in the blood increased (from 2.52g/dl to 3.61g/dl) in study pigs injected with endotoxin while decreasing (from 3.35g/dl to 2.46g/dl) in the study pigs not challenged with (Figure 10A and Table 4.3.2). On the other hand when the endotoxin was increased from 0µg/kg body weight to 20µg/kg body weight, the blood t protein concentration in the study pigs on 2% ASTFP feed supplement increased (from 2.46g/dl to 3.62g/dl) while reducing (from 3.35g/dl to 2.2.52g/dl) in the study pigs on 0% ASTFP feed supplement (Figure 10B). The reduction of total protein in the blood for 0% ASTFP feed supplement when endotoxin was increased from 0µg/kg body weight to 20µg/kg body weight is suggestive of the mobilization of proteins for anti-inflammatory factors hence reduction of blood protein, but the increase total protein in the blood for 2% ASTFP feed supplement when endotoxin was increased from 0µg/kg body weight to 20µg/kg body weight is suggestive of the suppression of the factors that lead to the reduction of blood total protein. It is therefore, indicative of the protective mechanism of ASTFP against the E.coli effect of reducing total protein in the blood of affected pigs.

Figure 10 **Interaction of dietary ASTFP X Endotoxin injection for blood Total protein in grower pigs at 4 hours post injection**

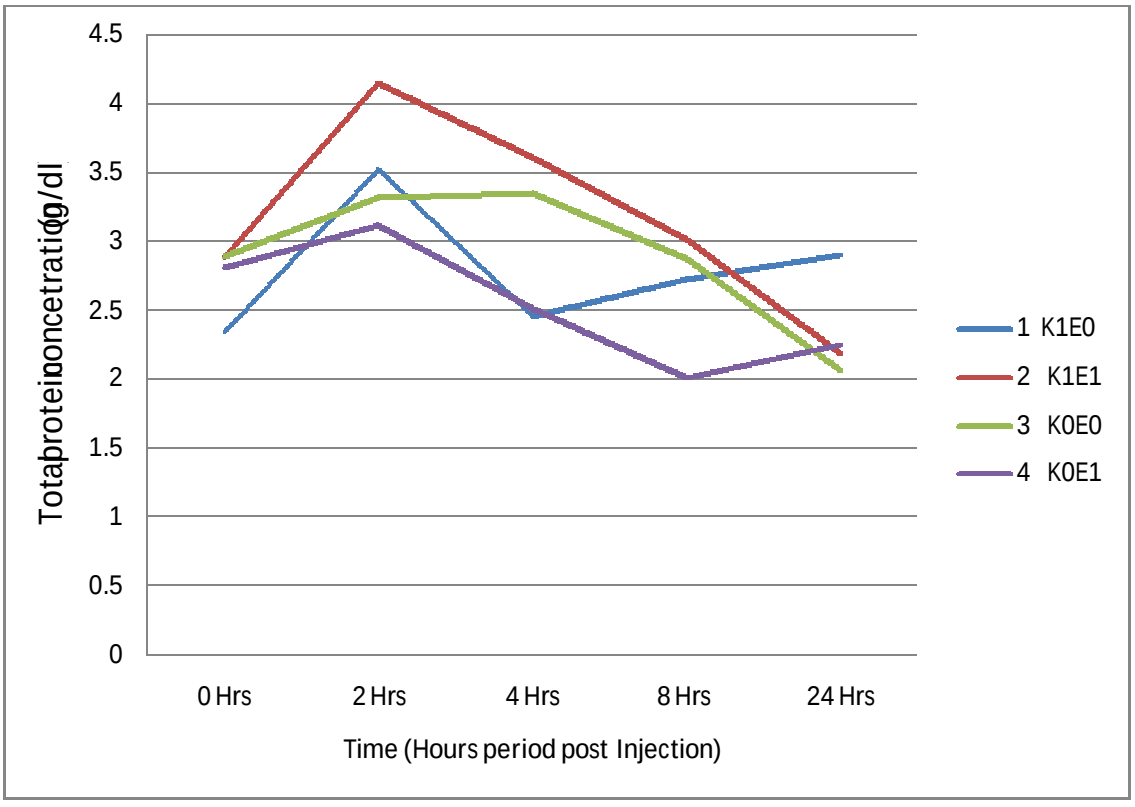


K0 = ASTFP at 0%,
K1 = ASTFP at 2%
E0 = Endotoxin at 0 µg/kg body weight and
E1 = 0 µg/kg body weight

The total protein concentration in the blood increased in t first 2 hours of post endotoxin challenge and there after decreased over the 24 hour sampling period (Figure 11).

Total blood protein concentrations ranged between 2.02 and 4.15 g/dl (Table 4.3.1). This total protein concetration was in the lower range of the normal range for wealing pigs (Sutherland *et al.*, 2009). The Protein concentration in the blood is a marker of protein homeostasis and low total protein concentration was indicative that the study pigs were not dehydrated. The low Protein concentration could also have been due to unchecked lysine levels in the feed used in the study which could have been suboptimal.

Figure 11 Mean Total Protein concentration of weaner pigs per treatment over 24 hour blood sampling period.



K_1E_0 = treatment 1 (2% ASTFP and 0µg endotoxin /kg body weight)
K_1E_1 = treatment 2 (2% ASTFP and 20µg endotoxin /kg body weight)
K_0E_0 = treatment 3 (0% ASTFP and 0µg endotoxin /kg body weight)
K_0E_1 = treatment 4 (0% ASTFP and 20µg endotoxin /kg body weight)

4.3.3 BLOOD ALBUMIN

Results for blood albumin are presented in Table 4.3.3 below. Treatments had no real effects on the Albumin levels in the blood before (0 hours), at 8, and 24 hours after the study pigs were challenged with endotoxin. Therefore, was no real endotoxin, ASTFP or Interaction effect on blood Albumin before (0 hours), at 8 and 24 hours after the study pigs were challenged with endotoxin by injection.

Table 4.3.3. Effect of dietary ASTFP and Endotoxin injection on blood albumin in grower pigs.

Effect of dietary ASTFP and E.coli endotoxin injection on blood albumin in grower pigs.[a]						
		Dietary African Sausage Tree Fruit Pulp (%)				
		2		0		
		Injected E coli endotoxin (µg/Kg body weight)				
		0	**20**	**0**	**20**	
Treatment		1	2	3	4	
Parameter	Time (hrs)					**SE**
Albumin (g/dl)	0	3.87	3.68	3.66	3.03	**0.12**
	2[bc]	3.48[#]	4.27[#]	2.55	3.18	**0.17**
	4[b]	3.58	3.45	3.07	3.05	**0.11.**
	8	3.19	3.49	3.18	2.85	**0.12**
	24	2.77	3.63	2.88	2.56	**0.18**

[a] Least square means of five pigs per treatment combination

[b] African Sausage Tree Fruit Pulp effect (P=0.05)

[c] Endotoxin effect (P = 0.05)

[#] Treatment mean significantly different from treatment 3 mean (P = 0.05).

However, 2 hours post endotoxin challenge, both endotoxin effect (P = 0.05) and ASTFP effect (P = 0.01) were significant and had the effect of increasing the Albumin levels in the blood of the study pigs (Table 4.3.3.1). Therefore, when the treatment means were compared to the mean for treatment 3 (0% ASTFP and 0µg endotoxin/kg body weight), treatment 2 mean was significantly higher (P = 0.01) while treatment 1 mean was also higher (P = 0.05) and treatment 4 mean being not significantly different from treatment 3 mean. Due to the high Albumin levels treatment 2, the average of treatments 1 and 2 (2% ASTFP), and 4 and 2 (20µg endotoxin/kg body weight), were also significantly higher in comparison to treatment 3 mean. No real ASTFP X endotoxin interaction effect was observed at 2 hours post injection for blood albumin.

Table 4.3.3.1 Planned pairwise comparison between mean blood Albumin for treatment 3 (K_0E_0) and the other treatments (1, 2 and 4) using the LSD test: 0.74 $t_{0.05}$ (t=2.179, df=12, r=5 and 0.29 MSE) and 1.04 $t_{0.01}$ (t=3.055, df=12, r=5 and 0.29 MSE) at 2 hours post injection.

	Treatment	Mean Albumin concentration[b] mg/dl	Difference from treatment 3[d] mg/dl
1	K_1E_0	3.48	0.936*
4	K_0E_1	3.18	0.638ns
2	K_1E_1	4.27	1.720**
1 and 2[a]	(K_1E_0 & K_1E_1)	3.87[c]	1.328**
4 and 2[a]	(K_0E_1 & K_1E_1)	3.72[c]	1.179**
3	K_0E_0	2.55	-

[a]Difference compared with LSD of 0.52 $t_{0.05}$ and 0.74 $t_{0.01}$ calculated by sd = $(s^2/r)^{1/2}$. [b]Average of five replications. [c]Average of two means. [d]** = significant at 1% level, ns = not significant

By 4 hours post endotoxin injection, there were no real differences among the treatment means in blood albumin concentration. However, the ave of treatments 1 and 2 was significantly higher (P = 0.05) than the Albumin level in treatment 3 while the average of treatments 2 and 4 (20µg endotoxin/kg body weight) not (Table 4.3.3.2). This

was suggestive of the continued effect of the ASTFP in increasing the blood albumin concentration in the study pigs.

Table 4.3.3.2 Planned pairwise comparison between mean blod albumin treatment 3 (K_0E_0) and the other treatments (1, 2 and 4) using the LSD test: 0.60 $t_{0.05}$ (t=2.179, df=12, r=5 and 0.19 MSE) and 0.84 $t_{0.01}$ (t=3.055, df=12, r=5 and 0.19 MSE) at 4 hours post injection.

Treatment		Mean Albumin concentration[b] g/dl	Difference from treatment 3[d] g/dl
1	K_1E_0	3.58	0.51^{ns}
4	K_0E_1	3.05	0.01^{ns}
2	K_1E_1	3.45	0.38^{ns}
1 and 2[a]	(K_1E_0 & K_1E_1)	3.51[c]	0.45^{*}
4 and 2[a]	(K_0E_1 & K_1E_1)	3.25[c]	0.18^{ns}
3	K_0E_0	3.07	

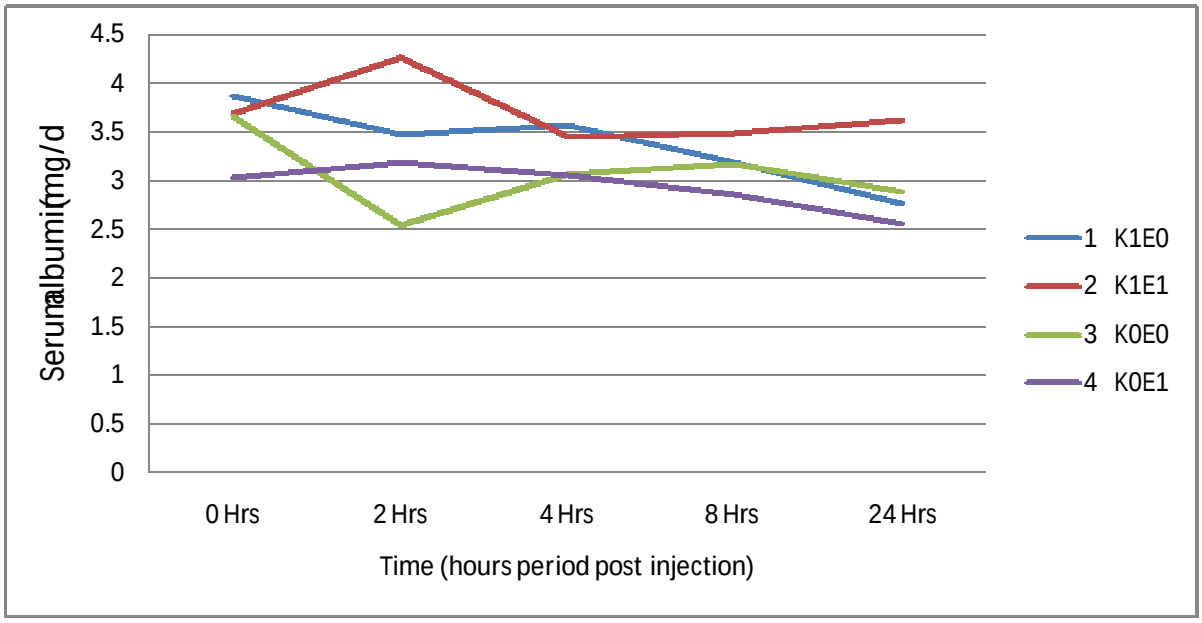
[a]Difference compared with LSD of 0.42 $t_{0.05}$ and 0.60 $t_{0.01}$ calculated by sd = $(s^2/r)^{1/2}$. [b]Average of five replications.

[c]Average of two means. [d]** = significant at 1% level, [ns] = not significant

There was generally a low concentration of blood albumin at the of the blood sampling period which increased by 4 and 8 hours after endotoxin challenge and then reduced to levels lower than at the start of the blood sampling period by 24 hours after endotoxin challenge (Figure 12). The albumin level was a range from 2.55 g/dl to 4.27 g/dl which was within the normal range of the albumin ls in pigs (Sutherland *et al.*, 2009). Albumin is the major plasma protein, and an increase in albumin may indicate an

increase in protein synthesis. Usually Albumin concentrations parallel total protein concentrations. The significantly high levels of Albumin in treatments 1 and 2 at 2 hours after endotoxin challenge and the significantly high a ge of treatments 1 and 2 (2% ASTFP) means at 4 hours after the endotoxin challenge, may indicate increased protein synthesis or the sparing effect of protein use in the 2% ASTFP treatments.

Figure 12 Trends of blood albumin levels of weaner pigs per treatment over 24 hour blood sampling period



K_1E_0 = treatment 1 (2% ASTFP and 0µg endotoxin /kg body weight)

K_1E_1 = treatment 2 (2% ASTFP and 20µg endotoxin /kg body weight)

K_0E_0 = treatment 3 (0% ASTFP and 0µg endotoxin /kg body weight)

K_0E_1 = treatment 4 (0% ASTFP and 20µg endotoxin /kg body weight)

4.3.4 BLOOD GLUCOSE

The results of blood glucose analysis, table 4.3.4, before (0 hours) and 8 hours post endotoxin injection, indicate that treatment effects were not significant.

Table 4.3.4. Effect of dietary ASTFP and Endotoxin injection on blood glucose in growing pigs.

Effect of dietary ASTFP and E.coli endotoxin injection on blood glucose in growing pigs.[a]						
		Dietary African Sausage Tree Fruit Pulp (%)				
		2		0		
		Injected E coli endotoxin (µg/Kg body weight)				
		0	**20**	**0**	**20**	
Treatment		1	2	3	4	
Parameter	Time (hrs)					**SE**
Glucose (mg/dl)	0	20.00	20.91	18.18	17.22	**0.79**
	2[b]	18.18	12.73[#]	19.09	20.91	**1.14**
	4[bc]	70.20[#]	60.00.	60.91	54.54	**2.14**
	8	58.18	57.27	52.724	60.00	**1.03**
	24[c]	51.82	64.55[#]	49.09	56.36	**2.61**

[a] Least square means of five pigs per treatment combination

[b] African Sausage Tree Fruit Pulp effect (P=0.05)

[c] Endotoxin effect (P = 0.05)

[#] Treatment mean significantly different from treatment 3 mean (P = 0.05).

There was ASTFP effect (P = 0.05) on the blood glucose concentration at 2 hours endotoxin injection resulting into significant differences among the treatment means (Table 4.3.4.1). The ASTFP tended to lower the Glucose levels in the blood. When the means were compared to treatment 3 mean, it was mean for treatment 2 that was significantly lower (Table 4.3.4.1). There was no significant endotoxin effect and Interaction effect was also not significant at 2 hour after endotoxin challenge.

Table 4.3.4.1 Planned pairwise comparison between mean blood glucose for treatment 3 (K_0E_0) and the other treatments (1, 2 and 4) using the LSD test: 5.27 $t_{0.05}$ (t=2.179, df=12, r=5 and 14.64 MSE) and 7.39 $t_{0.01}$ (t=3.055, df=12, r=5 and 14.64 MSE) at 2 hours post injection.

	Treatment	Mean Glucose concentration[b] mg/dl	Difference from treatment 3[d] mg/dl
1	K_1E_0	18.18	0.91^{ns}
4	K_0E_1	20.91	1.82^{ns}
2	K_1E_1	12.73	6.37^{*}
1 and 2[a]	(K_1E_0 & K_1E_1)	15.46[c]	3.64^{ns}
4 and 2[a]	(K_0E_1 & K_1E_1)	16.82[c]	2.27^{ns}
3	K_0E_0	19.09	-

[a]Difference compared with LSD of 3.73 $t_{0.05}$ and 5.22 $t_{0.01}$ calculated by $sd = (s^2/r)^{1/2}$. [b]Average of five replications. [c]Average of two means. [d]* = significant at 5% level, [ns] = not significant

At 4 hours after endotoxin injection, the ASTFP effect was ignificant (P = 0.05) and this time it tended to increase the glucose levels significantly, resulting in real treatment differences (Table 4.3.4.2). The mean for treatment 1 was significantly higher when compared to the mean for treatment 3.

Table 4.3.4.2 Planned pairwise comparison between mean blood glucose for treatment 3 (K_0E_0) and the other treatments (1, 2 and 4) using the LSD test: 8.86 $t_{0.05}$ (t=2.179, df=12, r=5 and 41.33 MSE) and 12.42 $t_{0.01}$ (t=3.055, df=12, r=5 and 41.33 MSE) at 4 hours post injection.

	Treatment	Mean Glucose concentration[b]	Difference from treatment 3[d]
		mg/dl	mg/dl
1	K_1E_0	70.198	9.29*
4	K_0E_1	54.544	6.36ns
2	K_1E_1	59.998	0.91ns
1 and 2[a]	(K_1E_0 & K_1E_1)	65.098[c]	4.19ns
4 and 2[a]	(K_0E_1 & K_1E_1)	57.271[c]	3.64ns
3	K_0E_0	60.908	

[a]Difference compared with LSD of 6.26 $t_{0.05}$ and 8.78 $t_{0.01}$ calculated by sd = $(s^2/r)^{1/2}$. [b]Average of five replications. [c]Average of two means. [d]* = significant at 5% level, ns = not significant

There were significant (P = 0.05) differences among treatment means at 24 hours st endotoxin injection (Table 4.3.4.3). This was due to endotoxin effect that increased blood glucose level. The mean that was significantly higher than treatment 3 mean was treatment 2 mean. In addition the average of treatment 2 and 4 means was also significantly higher in comparison with treatment 3 mean (Table 4.3.4.3).

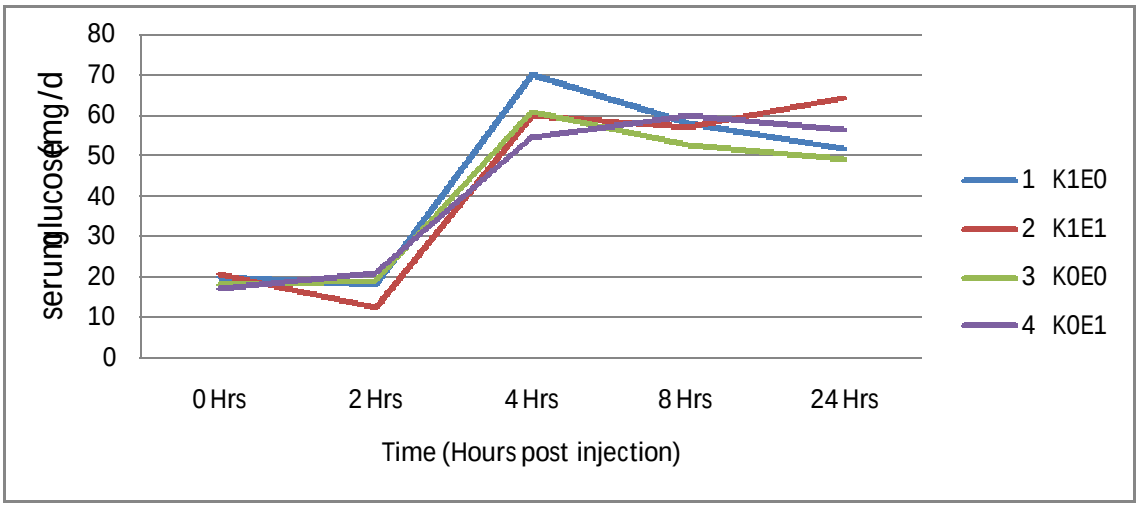
Table 4.3.4.3 Planned pairwise comparison between mean blood glucose for treatment 3 (K_0E_0) and the other treatments (1, 2 and 4) using the LSD test: 13.86 $t_{0.05}$ (t=2.179, df=12, r=5 and 101.22MSE) and 19.44 $t_{0.01}$ (t=3.055, df=12, r=5 and 101.22MSE) at 24 hours post injection.

Treatment		Mean Glucose concentration[b]	Difference from treatment 3[d]
		mg/dl	mg/dl
1	K_1E_0	51.816	2.73ns
4	K_0E_1	56.362	7.27ns
2	K_1E_1	64.546	15.46*
1 and 2[a]	(K_1E_0 & K_1E_1)	58.181[c]	9.09ns
4 and 2[a]	(K_0E_1 & K_1E_1)	60.454[c]	11.36*
3	K_0E_0	49.09	-

[a]Difference compared with LSD of 9.80 $t_{0.05}$ and 13.75 $t_{0.01}$ calculated by sd = $(s^2/r)^{1/2}$. [b]Average of five replications. [c]Average of two means. [d]* = significant at 5% level, ns = not significant

The glucose concentration was low (19.08 mg/dl) at the start of blood sampling decreasing slightly (17.73 mg/dl) at 2 hours post endo xin injection and then increasing (61.41 mg/dl) at 4 hours post endotoxin injection, before decreasing steadily over 8 and 24 hours post endotoxin challenge (57.04 mg/dl and 55.45 mg/dl respectively) (Figure13). The glucose level in the blood of the study pigs was a range of 17.22 mg/dl to 70.20 mg/dl and this was lower than normal range of 80 to 130 mg/dl (Sutherland et al., 2009). The low levels of glucose in the blood could have been due to the low levels of dietary carbohydrates. At 2 hours post endotoxin challenge, the glucose level decreased in treatment 2 and was significantly (P = 0.05) lower than treatment 3 suggesting an early response to the endotoxin challenge. By the 4th hour post endotoxin challenge, the glucose concentration of treatment 2 was no longer different from that of treatment 3 but treatment 1 mean was significantly higher (P=0.05) than treatment 3 mean, indicating the tendency of ASTFP to increase the glucose levels in comparison to 0% ASTFP.

Figure 13 **Blood glucose levels of the study pigs per treatment over blood sampling period.**



K_1E_0 = treatment 1 (2% ASTFP and 0µg endotoxin /kg body weight)

K_1E_1 = treatment 2 (2% ASTFP and 20µg endotoxin /kg body weight)

K_0E_0 = treatment 3 (0% ASTFP and 0µg endotoxin /kg body weight)

K_0E_1 = treatment 4 (0% ASTFP and 20µg endotoxin /kg body weight)

4.3.5 BLOOD CHOLESTEROL LEVEL

The results of blood cholesterol analysis, before (0 hours) and at 2, 4, 8 and 24 hours post endotoxin injection, indicate no real differences among treatment means (Table 4.3.5). Therefore, the treatment effect was not signif the ASTFP effect (factor A), endotoxin effect (factor B) and interaction of ASTFP X endotoxin effect were also not significant through out the blood sampling period.

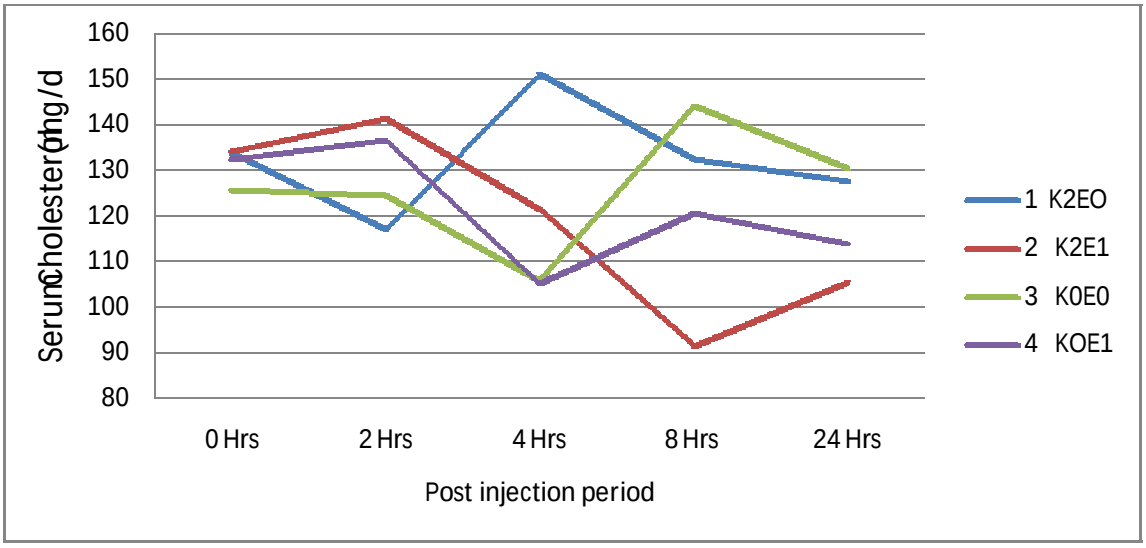
Table 4.3.5. Effect of dietary ASTFP and Endotoxin injection on blood cholesterol in growing pigs.

Effect of dietary ASTFP and E.coli endotoxin injection on blood cholesterol in growing pigs.[a]						
		Dietary African Sausage Tree Fruit Pulp (%)				
		2		0		
		Injected E coli endotoxin (µg/Kg body weight)				
		0	**20**	**0**	**20**	
Treatment		1	2	3	4	
Parameter	Time (hrs)					**SE**
Cholesterol (mg/dl)	0	134.24	134.29	125.72	132.50	**4.93**
	2	117.14	141.43	124.64	136.79	**5.93**
	4	151.43	121.43	105.71	105.36	**7.52**
	8	132.50	91.43	144.29	120.71	**7.93**
	24	127.77	105.66	130.65	113.93	**6.06**

[a] Least square means of five pigs per treatment combination

Figure 14 below shows the trend of Cholesterol levels the blood of study pigs both before (0 hours) and after (2, 4, 8 and 24 hours) endotoxin challenge.

Figure 14 Trends of blood cholesterol concentrations of weaner pigs per treatment during the blood sampling period.



K_1E_0 = treatment 1 (2% ASTFP and 0µg endotoxin /kg body weight)

K_1E_1 = treatment 2 (2% ASTFP and 20µg endotoxin /kg body weight)

K_0E_0 = treatment 3 (0% ASTFP and 0µg endotoxin /kg body weight)

K_0E_1 = treatment 4 (0% ASTFP and 20µg endotoxin /kg body weight)

CHAPTER FIVE

5.0 CONCLUSION

This study investigated the potential of the African sausage tree fruit pulp in preventing the effects of E.coli in growing pigs by focusing on antibacterial activity of the sausage tree fruit pulp. This is related to the traditional uses of the fruit preparations for treating diseases caused by micro organisms.

The inclusion of ASTFP at 2% or less to pig rations for periods of six weeks or less has no significant effect on pig body weights, growth rate and efficiency of feed conversion. At the levels of 2% and below, ASTFP has no observed general symptoms of toxicity effect in pigs. Therefore, ASTFP can be used in pig rations as feed supplement at 2% or less without affecting the performance of the pigs in terms of growth rate and efficiency of feed conversion. However, there are no performance benefits (weight gain and efficiency of feed conversion) at inclusion levels of 2% or less. Further studies are recommended with higher inclusion levels of ASTFP in pig diets.

The ASTFP in this study showed no effects on body temperature and the white blood cell count in the study pigs. However, temperature rise, as a result of endotoxin challenge, was lower in 2% than in 0% ASTFP inclusion level suggesting modulating effect of ASTFP on E.coli endotoxin effects in growing pigs. Also the increase in white blood cell count, due to endotoxin challenge, was lower in 2% than in 0% ASTFP inclusion level further suggesting positive effects of the ASTFP supplement on E.coli endotoxin effects.

The administration of the ASTFP, in this study, showed remarkable protective effects against lowered blood glucose and albumin levels induced by E.coli endotoxins in growing pigs. The interaction of ASTFP X E.coli endotoxin for both blood urea nitrogen and total protein (which was change of rank in nature) was indicative of the sparing

effect of the blood proteins by ASTFP in the presence the E.coli endotoxin effect, making more proteins available for other body activities.

The results in this study suggest that feeding 2% ASTFP was not detrimental and indeed appeared to be beneficial to growing pigs in protecting them against the effects of E.coli endotoxin. This is, therefore, a promising property that could replace the use of antibiotics as preventive measure in grower pig diet.

REFERENCES

Abioye A. and F. D. A. O. Okanlawon. 2003. Aqueous extract of the bark of *Kigelia africana* reverses early testicular damage induced by methanol extract of *carica papaya*. Nigerian Journal of Health and Biomedical Sciences. Volume 2 No2.

Bhandari S.K., B. Xu, C. M. Nyachoti, D. W. Giesting and D. O. Krause. 2008. Evaluation of alternatives to antibiotics using an *Escherichia coli k88*$^{+}$ model of piglet diarrhea: effects on gut microbial ecology. Journal of animal sciences. American Society of animal Science.

Binutu O. A., K. E. Adesogan and J. I. Okogun. 1996. Antibacterial and antifungal compounds from kigelia pinnata. http://www.ncbi,nih.nih.gov/pubmed. PMID: 8792668.

Cengiz A., I. Y Yurtman, T. Savas, M. Gultepe and O. Ozcan. 2008. Effect of weaning on behavior and serum parameters in dairy goat kids. Animal science journal, Japanese Society of Animal Science.

Dernfalk J., K P. Waller and A. Johannisson. 2004. Commercially available antibodies to human tumour necrosis factor-a tested for cross-reactivity with ovine and bovine tumour necrosis factor-a using flow cytometric assays. Acta veterinaria scandinavica (AVS).

Gomez K. A. and A. A. Gomez. 1984. Statistical procedures for agricultural research. 2ND ecition. John wiley and sons. 605 Third Avenue, New York, N.Y.10016.

Grace O.M. and S.D Davis. 2002. *Kigelia africana* (Lam.) Benth. [Internet] Record from Protabase. Oyen, L.P.A. & Lemmens, R.H.M.J. (Editors). PROTA (Plant Resources of Tropical Africa/Ressources végétales de l'Afrique tropicale), Wageningen, Netherlands. <http://database.prota.org/search.htm>. Accessed 27 November 2008.

Graeme T. and G. Roese. 2006. Basic Pig Husbandry - The Weaner. Source: Published by NSW Department of Primary Industries. State of New South Wales 2006.

Hauschildt S., W. Brabetz, A. B. Schromm, L. Hamann, P. Zabel, E. T Rietschel and S. Loennies. 2000. [Internet] Structure and activity of endotoxins in *bacterial protein toxins* (Aktories, K. & Just, I., eds), pp. 619-667. Springer, Heidelberg, Germany.

Houghton P. J. 2008. The sausage tree (Kigelia pinnata): Ethnobotany and recent scientific work. Pharmacognosy research laboratories. of Pharmacy, King's College London

Jeyachandran R. and A. Mahesh. 2007. Antimicrobial evaluation of kigelia africana. Research Journal of Microbilogy 2 (8) 645-649, 2007 ISSN 1816-4935. 2007 Academic journals.

Kolodziej H. 1997. Protective role of kigelia africana fruits against benzo (a) pyrene-induced fore-stomach tumourigenesis in mice and against albumen-induced inflammation in rats. Institut fuer Pharmazie II Freie Universitaet Berlin, Germany. *Pharm Pharmacol Lett 7 (1997) 2/3:67–70*

Liu Y. L., D. F. Li, L. M. Gong, G. F. Yi, A. M. Gaines and J. A. Carroll. 2003. Effects of fish oil supplementation on the performance and the immunological, adrenal, and somatotropic responses of weaned pigs after an *escherichia coli* lipopolysaccharide challenge. Journal of Animal science. 3003. 81:2758 – 2765.

Lorke D. 1983. A new approach to practical acute toxicity testing: Arch. Toxicol. 54: 275-287

Lwalewa E. O., L. J. McGaw, V. Naidoo and J. N. Eloff. 2007. Inflammation: the foundation of diseases and disorders. A review of phytomedicines of south african origin used to treat pain and inflammatory conditions. African Journal of Biotechnology, Vol. 6, No. 25, 28 December, 2007, pp. 2868-2885.

Lynn O. P., D. E. Farrell, C. V. Cope, J. D. Baker and Myers. 2002. The effect of endotoxin and dexamethasone on enrofloxacin pharmacokinetic parametrs in swine. http://jpet.aspetjournals.org. accessed on 25/11/2008.

Mandali S., M.Rincker, S.D. Carter, A.B. Arquitt, E. Droke, B.J.Stoecker and L.J. Spicer. 2000. Effects of dietary zinc and endotoxin challenge on immune function in weanling pigs. Animal research report.OSU . Department of animal science.

McDonald P., R. A Edwards, J. F. D Grenhalgh and C. A Morgan. 1995. Animal nutrition. Riplika press Pvt Ltd. 100% EOU, Delhi 1100 , India.

Ming M. 2001. Kigelia africana for skin conditions. Positive Health Issue 63.april 2001.

Netea M. G., J. W. van der Meer, M. van Deuren and B. Jan Kullberg. 2003. Proinflammatory cytokines and sepsis syndrome: not eno or too much of a good thing? [Internet] *Trends in Immunology* 24, 254 -258.

Ogbeche K.A., Y.O. Ogunbiyi and F.I.O. Duru. 2002. Effect of methanol extract of *kigelia africana* on sperm motility and fertility in rats. Nigerian Journal of Health and Biomedical Sciences. Vol 1 No.2

Omonkhelin J. O., E. K. I. Omogbai and O. Obasuyi. 2007. Antifungal and antibacterial activities of the ethanolic and aqueous of *Kigelia africana* (Bignoniaceae) stem bark. African Journal of Biotechnology Vol. 6 (14), pp. 1677-1680, 18 July 2007. http://www.academicjournals.org/AJB ISSN 1684–5315 © 2007 Academic Journals.

Owolabi O. J., F. C. Amaechina and A. B Eledan. 2008. Central nervous system stimulant effect of the ethanolic extract of *kigelia africana*. Journal of Medicinal Plants Research Vol. 2(2), pp. 020-23, February, 2008. ISSN 1996-0875 © 2008 Academic Journals.

Payne W. J. A. 1990. An introduction to animal husbandry in the tropics. Long man Singapore publishers (Pte) Ltd.

Peter A. A. 1996. Antidiarrheal activity of *kigelia africana* in experimental animals. Journal of herbs, spices and medicinal plants. Volume 4 issue 2; 1SSN 1048-6457.

Picerno P., G. Autore, S. Marzocco, M. Meloni, R. Sanogo and R. P. Aquino. 2008. Anti-inflammatory activity of verminoside from kigelia africana and evaluation of

cutaneous irritation in cell cultures and reconstituted human epidermis. Medline PMID 16309308.

Pluske J. R., J. Le Dividich, W. Martin and A. Verstegen. 2003. Weaning the pig - concepts and consequences. http://www.thebioenergysite.com/

Sayila A. 2006. Pig production in zambia has suffered some severe blows in the last five years or so. Hopes are high, however, that the country's pig production is well on its way again.

Storrs A. E. G. 1995. "Know your trees" Some of the common trees found in zambia. Regional Soil Conservation Unit (RSCU) Nairobi.

Sutherland M. A., P. J. Bryer, B. L. Davis and J. J. McGlone. 2009. Space required of weaned pigs during a sixty-minute transport in summer. Journal of Animal science 2009.87:363-370.dor:10.2527/jas2008-1078. © American society of animal science.

Little T. M. and F. J. Hills. 1977. Agricultural experimentation. Design and analysis. John wiley and sons. 605 Third Avenue, New York, N.Y.10016.

University of florida. Animal care and services. Web page .http://acs.ufl/giudelines.shtml accessed on 15/09/2009.

Wilkinson J. 2006. Anti inflammatory and other effects of *Kigelia africana* extracts. www.phytochemistry.freeserve.co.uk accessed on 26/11/2008.

APPENDICES

APPENDIX A *ASTFP & ENDOTOXIN EFFECT ON METABOLITES IN GROWING PIGS*

The effect of dietary ASTFP and E.coli endotoxin injection on serum metabolites in growing pigs.[a]

		Dietary African Sausage Tree Fruit Pulp (%)				
		2		0		
		E coli endotoxin (0 vs. 20µg/Kg body weight)				
Parameter	Time (hrs)	-	+	-	+	SE
Blood Urea Nitrogen (BUN) (mg/dl)	0	53.33	53.81	37.66	50.83	2.70
	2	48.90	42.57	41.76	56.32	3.83
	4[bc]	45.57	35.00	40.20	30.30	1.78
	8	33.30	38.82	37.56	36.36	1.43
	24[d]	36.62	54.50	40.52	35.26	2.88
Total Protein (g/dl)	0	2.34	2.89	2.90	2.81	0.11
	2	3.52	4.15	3.32	3.11	0.20
	4[d]	2.46	3.61	3.35	2.52	0.15
	8	2.73	3.02	2.87	2.02	0.21
	24	2.90	2.19	2.07	2.25	0.15.
Albumin (g/dl)	0	3.87	3.68	3.66	3.03	0.12
	2[bc]	3.48	4.27	2.55	3.18	0.17
	4[b]	3.58	3.45	3.07	3.05	0.11.
	8	3.19	3.49	3.18	2.85	0.12
	24	2.77	3.63	2.88	2.56	0.18
Glucose (mg/dl)	0	20.00	20.91	18.18	17.22	0.79
	2[b]	18.18	12.73	19.092	20.91	1.14
	4[bc]	70.20	60.00.	60.908	54.544	2.14
	8	58.18	57.27	52.724	60.00	1.03
	24[c]	51.82	64.55	49.09	56.362	2.61
Cholesterol (mg/dl)	0	134.244	134.286	125.716	132.5	4.93
	2	117.144	141.43	124.642	136.786	5.93
	4	151.428	121.428	105.714	105.358	7.52
	8	132.5	91.426	144.286	120.714	7.93
	24	127.772	105.656	130.6534	113.928	6.06

[a] Least square means of five pigs per treatment combination

[b] African Sausage Tree Fruit Pulp effect (P=0.05)

[c] Endotoxin effect (P = 0.05)

[d] ASTFP X Endotoxin interactions (P = 0.05)

[#] Treatment mean significantly different from treatment 3 mean (P = 0.05).

APPENDIX B CHEMICAL ANALYSIS PROCEDURE

I DRY MATER ANALYSIS (According to AOAC, 1998)

Samples weighing 2g were placed in a pre-weighed porcelain dishes with covers. The covers were loosened and the dishes were placed in a moment oven at 110 ^{o}C for 2 hours. The dishes were removed from the oven and the lids tightened. The dishes were left in the dessicator for 30 minutes and then they were weighed. The difference between the weight before and the weight after drying was the evaporated. This was subtracted from the weight of the pre-drying sample to give the weight of DM in the sample. DM percentage in the sample was calculated as:

$$\frac{\text{Weight of dry DM}}{\text{Weight of air dried sample}} \times 100 = \%\text{DM}$$

II CRUDE PROTEIN ANALYSIS (According to AOAC 1998)

Samples weighing 2gms were digested in 24 mls of concentrated sulphuric acid in a Foss Tecator Digestion system at 420 ^{o}C for 1 hour. 8 gms of a mixture of catalyst made by 400g, Potassium sulphate, 16 gms Copper sulphate an 3 gms Selenium powders was added to speed up the digestion. All the organic matter was digested. The digested solution containing Ammonium sulphate (produced from the reaction between the Nitrogen in the sample and the sulphuric acid) was diluted to 250mls. 5 mls of the diluted solution was pippetted into a markhan semi-micro Kjedahl distillation apparatus. 10mls (or excess) of a 40 % Sodium hydroxide was added to release the Ammonia from the ammonium sulphate into the ionized ammonium form. ammonia was distilled into a 1 % Boric acid indicator solution (the indicator was a mixture of 2 parts of 0.2 % Methyl red and 3 parts of 0.2 % Bromocresol green). The solution was green. A 0.1M solution of hydrochloric acid (Hcl) was used to titrate the ammonia, the end point giving a purplish color (due to indicator). The CP % in the solution was calculated as:

$$\frac{0.00014 \times \text{Vol Hcl} \times 250 \times 6.25}{\text{Weight of sample} \times 5} \times 100 = \%CP$$

Where
- 1 ml of 0.1M Hcl = 0.00014 gms Nitrogen
- Vol Hcl = Volume Hcl used in titration
- 250/5 = Dilution rate
- 6.25 = Conversion factor for organic nitrogen to CP

The results of the analysis represents the CP content of the sample because nitrogen also comes from non protein compounds such as free amino acids, small peptides, nucleic acids, phospholipids, amino sugars, porphyrin, some vitamins, uric acid and ammonium ions.

III ETHER EXTRACTS ANALYSIS (According to AOAC 1998)

Samples weighing approximately 5 gms were placed in extraction thimbles. The cotton wool plugged thimbles were placed I reflux condensers d to pre-weighed soxhlet flasks containing petroleum ether with a boiling range of 60-80 ^{o}C. The process involved extraction of the sample using hot condensed petroleum ether in a semi- continuous manner for 8 hours. The ether extract always remained the soxhlet flask. After evaporation of the petroleum ether, the oil and the flask were weighed. The gain in weight was reported as weight of the oil or extract. The EE % of the sample was calculated as:

$$\frac{\text{Weight of oil}}{\text{Weight of air dry sample}} \times 100 = \%EE$$

The EE represents the crude fat content of the sample may be a mixture of simple compound and derived lipids. Protein, amino acids and carbohydrates may also be extracted in the EE (Nielson, 1994). It is possible for these non lipid components to have been extracted in this analysis since the solvent that was used (Petroleum Ether) has a higher boiling point than the usually recommended solvent Ether) which has a much lower boiling point of 34.6 ^{o}C (Nielson, 1994).

IV DETERMINATION OF ASH AND MINERAL EXTRACTION (According to AOAC, 1998)

Samples weighing approximately 2 gms were placed in pre-weighed porcelain crucibles and placed in a Nabertherm muffle furnace to ash at 550 ^{o}C. After 4 hours the crucibles were removed from the furnace and left to cool in a de for 30 minutes. The difference in weight between the empty crucibles and the crucibles with ash was reported as the ash content of the sample. Percentage of ash in the sample was calculated as:

$$\frac{\text{Weight of ash}}{\text{Weight of air dry sample}} \times 100 = \%\text{Ash}$$

The ash was used to determine Calcium and Phosphorus content of the sample. The minerals in the ash were first extracted by boiling ash in 10 mls of 2N Hydrochloric acid. The solution was then filtered out into a 100 mls flask and made up to the mark by washing the residue with hot distilled water.

V DETERMINATION OF CALCIUM (According to AOAC, 1998)

50 mls of the mineral extracts in the ash determination above were pippetted into 400 mls beaker and 100 mls of hot distilled water added. 5 drops of Methyl red indicator were added to each solution which was then brought to a boiling. Approximately 1g powdered Ammonium oxalate was added d to the solution precipitate the calcium in the solution as calcium oxalate. The precipitate was f ltered after being allowed to set for 2 hours. The calcium oxalate residue was dissolved in 20 mls of 2 N Sulphuric acid. After diluting with 100 mls hot distilled water, the solution was titrated to faint pink with N/10 Potassium permanganate. Percent in the sample was calculated as:

$$0.002 \times \frac{\text{Vol Potassium permanganate}}{\text{Weight of air-dry sample}} \times 100 = \%Ca$$

Where 1ml N/10 potassium permanganate =0.002gms Ca

Vol potassium permanganate =volume of potassium permanganate used.

VI DETERMINATION OF PHOSPHORUS (According to AOAC, 1998)

The mineral extracts from the ash determination above were further diluted by a factor of 20. This was done by pippetting 2.5 mls of each of ple's mineral extract into a 50 ml volumetric flask and making up to the mark with lled water. The purpose of the dilutions was to obtain solution with P concentrations whose optical density, when the colors were developed, could be read by a colorimeter at 660 nm wavelength.

Color development was achieved by adding 4 mls acid molybdate and then 3 mls amino naptolsulphonic acid (ANSA) to 1 ml of sample solution in a test tube. A compound which is supposed to have a formula $(MoO_2, {}_4MoO_3)_2.H_3PO_4$ is developed by adding acid molybdate to anorthophosphate. The phosppomolybdate produced is reduced by ANSA to give blue colored compound (Egan et al 1981). test tube was left to stand for 20 minutes to allow the color to develop. The solutions were then put in 15 mm diameter cells and optical density read at 660 nm.

A standard curve was used to determine the concentration of the sample solution. The standard solutions used to produce the standard curve concentrations of 1 mg, 2 mg, 3 mg, 4 mg and 5 mg P per 100 ml. the solution were made by diluting ml, 2 ml, 3 ml, 4 ml and 5 ml of a 1 gm P per ml standard stock solution of potassium dihydrogen phosphate in 100 ml of distilled water. Color was deve for the standard solution as for the sample solution by adding 4 mls acid molybdate and 3 ml ANSA 1 ml of the standard solution in a test tube and leaving for 20 minutes. Optical density of the standard solution was also read at 660 nm. The percent of P in the sample was calculated as:

$$\frac{\text{Mg P/100ml} \quad X \quad 20}{10 \ X \ \text{weight of dry sample}} = \%P$$

Where mg P /100 ml was obtained from the standard curve

20 = Dilution factor for mineral extract solution obtained from 100 ml solution
10 = Dilution factor for ash in 2N Hydrochloric acid.

VII DETERMINATION OF NEUTRAL DETERGENT FIBRE (According to AOAC, 1998)

0.35 gms of sample were boiled for an hour in 35 mls of sodium laural sulphate in 50 ml taylor tubes. The detergent extracts lipids, sugars, organic acids, and other soluble materials, pectin, non protein nitrogenous compounds, soluble proteins and some of the silica and tannin (church and Pond, 1988). The non soluble materials containing cellulose, lignin, some proteins, bound nitrogen, minerals and cuticle was extracted by filtering the taylor tube contents on porous glass crucibles, connected via trap to a vacuum pump. The residue was rinsed with hot water and after removal of most of the free water by suction; the crucibles were placed in a oven to dry at 110 ^{o}C for 2 hours. After cooling in a dessicator for 30 minutes, the crucibles and the dry residue were weighed. The dry residue was then ashed in a nathbertherm muffle furnace at 550 ^{o}C for 4 hours. The crucibles and ash were again cooled in a dessicator for 30 minutes and then weighed. Percent NDF in the sample was calculated as:

$$\frac{\text{Loss in weight from the ash}}{\text{Weight of air dry sample}} \times 100 = \%\text{NDF}$$

VIII DETERMINATION OF GROSS ENERGY (According to AOAC, 1998)

Approximately ½ inch diameter pellets were made from the samples and weighed. The weighed pellets were placed in combustion capsule. The combustion capsules, with sample pellets in them, were placed singly in the comb capsule holder of a parr adiabatic bomb calorimeter bomb. 10 cm of fuse was attached to connect power leads into the bomb. The fuse wire was made into a loop which touched the sample pellet to ignite it when power was passed through the fuse wire. The bomb head was replaced and securely closed. After oxygen at 20 atmospheres was introduced the bomb it was placed in the calorimeter's oval bucket which contained 2000 ml tap water at 24 -27 ^{o}C. Electric fire wires were attached to leads into the bo The lead to the colorimeter was replaced and the temperature in the inner and outer jacket allowed to equilibrate. After firing the sample the temperature was allowed to rise a constant temperature which was within 0.1 ^{o}C for both the inside and the outside jacket.

The bomb was dismantled and inside washed with distilled water. The washings represented heat generated by a chemical reaction in the bomb and were not due to the sample burning. Washings were titrated with sodium carbonate solution after 5 drops of Methyl orange were added to them, up to a straw colored end point. The unburned fuse was also measured. The gross energy of the sample was calculated as:

$$\frac{\text{Rise in temp X Calorimeter heat capacity (Kcal/g)} - \text{washing heat} - \text{burnt fuse wire heat}}{\text{Weight of dry sample}}$$

IX DETERMINATION OF UREA (ENZYMATIC-COLORIMETRIC TEST)

[Burtis A et al. Tietz Textbook of Clinical Chemistry, 3rd ed AACC 1999]

Cypress Diagnostics reagents, code HB022 4 X 250ml wer used. This is a kit containing a buffer (Phosphate pH6.7 at 50 nmol/l, EDTA at 2 nmol/l, Sodium salycilate at 400 nmol/l and Sodium Nitroprusiate at 10 nmol/l), (Sodium Hypochlorite at 140 nmol/l and Sodium Hydroxide at 150 nmol/l), Enzymes (Urease at 30000 U/l) and Standard (Urea aqueous at 50 mg/dl).

The enzyme urease was mixed with the buffer solution to dissolve it gently when make the working reagent. The blank was made of the 1 ml working reagent; the standard was made of 1 ml working reagent mixed with 10 µg of Urea and 10 µg of the sample was mixed with the working reagent. These were incubated at37 ^{o}C for 5 minutes and then 1 ml of NaCIO was added and further incubated at 37 ^{o}C for 5 minutes. The absorbance of the sample and the standard were read against the blank at580 nm on the spectrophotometer.

The color development was through the hydrolysis of urea into ammonia which then reacted with salicylaate and hydrochlorite to form a green indophenol. The colourrintensity is proportionsl to the concentration of urea.

Urea + H_2O $\xrightarrow{\text{Urease}}$ CO2 + $(NH_4)^{2+}$

NH_4^+Salycilate + NaCIO $\xrightarrow{\text{Nitroprusside}}$ Indophenol

The absorbance was read on the Spectrophotometer measuring at 580 nm wavelength using the 1 cm light path cuvettes at about 37 ^{o}C. the Spectrophotometer was switched on an hour before the reading of samples was done and adjusted to zero with distilled water. The calculation of the mg/dl was done as below;

$$\frac{\text{Abs. sample} - \text{Abs blank}}{\text{Abs Standard} - \text{Abs Blank}} \times \text{Standard Concentration} = \text{Urea (mg/dl)}$$

X *DETERMINATION OF TOTAL PROTEIN* (COLORIMETRIC TEST. BIURET)

[Young DS. Effects of disease on clinical lab, Tests, 4rd ed AACC 2001]

Cypress Diagnostics reagents, code HB0190 4 X 125ml were used. This is a kit containing Reagent 1/BIURET (Potassium-Sodium-Tartrate at 15 nmol/l, Sodium Hydroxide at 100 nmol/l, Potassium Iodide at 5 nmol/l and Copper II Sulpahate at 19 nmol/l), and Standard (Bovine albumin at 4.74g/dl).

The Spectrophotometer was switched on an hour before measuring the sample at 546 nm wavelength at temperature of approximately 37 ^{o}C using the 1 cm light path cuvettes. The instrument was then zeroed using distilled water. blank was prepared by getting 1 ml of the BIURET, the standard was prepared by adding 25µg of Bovine albumin (4.74g/dl) into 1 ml of BIURET and the samples were made by pippetting 25 µg into 1 ml of BIURET. These were incubated at 37 ^{o}C in the oven for 5 minutes and then measured the absorbance against the blank at 546 nm wavelength.

The protein together with a basic copper sulphate solution containing tartrate (Biuret reagent) form a violet blue colour complex that is stable for at least one hour at room temperature. The intensity of the colour formed is pro to the total protein in the sample. The Total protein was calculated as;

$$\frac{\text{Abs. sample} - \text{Abs blank}}{\text{Abs Standard} - \text{Abs Blank}} \times \text{Standard Concentration} = \text{Total Protein (g/dl)}$$

XI DETERMINATION OF ALBUMIN (ENZYMATIC COLORIMETRIC TEST)

[Rodkey F. L., Clinical chemistry 10,643 (1964)]

Hospitex Diagnostics s.r.l reagents, code 4001010l pack 4 X 100 ml were used. This is a kit containing a Reagent (Citrate Buffer pH 3.8 at 95 Bromocresol green at 2 nmol/l and detergents and preservatives) and Standard (Human Albumin at 3.5 g/dl).

The Spectrophotometer was switched on an hour before measuring the sample at 630 nm wavelength at temperature f approximately 37 ^{o}C using the 1 cm light path cuvettes. The instrument was then adjusted to zero using distilled water. The blank was prepared by pippetting 2 mls of the working solution and then adding 10 µl distilled water, the standard was made by adding 10 µg of Human albumin (3.5g/dl) to 2 mls working solution and the samples were prepared by pippetting 10 µl of serum into 2 mls of the working solution. The absorbance for the Blank, the standard and the samples was measured at 630 nm wavelength. Before reading the standard and the samples, the instrument was calibrated to zero against the Blank re The calculation was as;

$$\frac{\text{Abs. sample}}{\text{Abs Standard}} \times \text{Standard Concentration} = \text{g/dl of Albumin}$$

The development of colour was through the reaction of Bromocresol Green (BCG) with Albumin to form a coloured complex which is green in pH 3.8. The colour intensity is proportional to the Albumin concentration in the sample and it is stable for 60 minutes at room temperature.

XII DETERMINATION OF GLUCOSE **(**ENZYMATIC-COLORIMETRIC TEST. GOD-POD**)**

[Burtis A et al. Tietz Textbook of Clinical Chemistry, 3rd ed AACC 1999]

Cypress Diagnostics reagents, code HB010 4 X 250 ml were used. This is a kit containing a buffer/Reagent 1 (TRIS Buffer pH 7.4 at 92 nmol/l and Phenol at 0.3 nmol/l), Enzymes/Reagent 2 (Glucose Oxidase at 15000 U/l, Peroxidase at 1000U/l and 4-Aminophenazone at 2.6 nmol/l) and Standard (Glucose aqueous at 100 mg/dl).

The enzymes (Reagent 2) were mixed with the buffer (Reagent 1) solution to dissolve them gently when making the working reagent. The blank l working reagent, the standard was made of 1 ml working reagent mixed with 10 µg of Glucose aqueous and 10 µg of the serum was mixed with 1 ml of the working reagent to make the samples. The samples, the standard and the Blank were incubated at37 ^{o}C for 10 minutes and the absorbance of the standard and of the mples were read against the blank 505 nm on the spectrophotometer.

The color development was through the oxidation of Glucose by glucose oxidase (GOD) to gluconic acid and hydroxide peroxide. The formed hy peroxide (H_2O_2) is detected by a chromogeenic oxygen acceptor, phenol-aminophenazone in the presence of peroxidase (POD). The intensity of the colour formed is proportionsl to the concentration in the sample. The colour developed is stable at room temperature for 30 minutes.

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{GOD}} H_2O_2 + \text{Gluconic acid}$$

$$H_2O_2 + \text{Phenol} + \text{4-AP} \xrightarrow{\text{POD}} \text{Quinone} + 4H_2O$$

The absorbance was read on the Spectrophotometer measuring at 505 nm wavelength using the 1 cm light path cuvettes at about 37 ^{o}C. The Spectrophotometer was switched on an hour before the reading of samples was done and adjusted to zero with distilled water. The calculation of the mg/dl was done as below;

$$\frac{\text{Abs. sample} - \text{Abs Blank}}{\text{Abs Standard} - \text{Abs Blank}} \times \text{Standard Concentration (100)} = \text{Glucose (mg/dl)}$$

XIII DETERMINATION OF CHOLESTEROL **(**ENZYMATIC-COLORIMETRIC TEST. CHOD-POD**) [Burtis A et al. Tietz Textbook of Clinical Chemistry, 3rd ed AACC 1999]**

Cypress Diagnostics reagents, code HB006 2 X 125 ml were used. This is a kit containing Reagent 1/Buffer (Pipes pH 6.9 at 90 nmol/l and Phenol at 26 nmol/l), Reagent 2/Enzymes (Peroxidase at 31250 U/l, Cholesterol esterase at 300 U/l, Cholesterol oxidase at 300 U/l and 4-Aminophenazone at 0.4 nmol/l) and Standard (Cholesterol aqueous at 200 mg/dl).

The enzymes (Reagent 2) were mixed with the buffer (Reagent 1) solution to dissolve them gently to make the working reagent which is stable for 40 days at room temperature in a dark bottle. The Blank was made of the 1 ml working reagent, the standard was made of 1 ml working reagent mixed with 10 µg of Cholesterol aqueous (200 mg/dl) and 10 µg of the serum was mixed with 1 ml of the working reagent to make the samples. The samples, the standard and the Blank were incubated at37 ^{o}C for 10 minutes and the absorbance of the standard and of the samples were read against the blank 505 nm on the spectrophotometer.

The color development was through the release of Cholesterol and its esters from the lipoproteins by detergents. Cholesterol esterase hydrolyses the esters and the hydrogen peroxide is formed in the subsequent enzymatic oxidation of cholesterol by the cholesterol oxidase according to the equation below. In the last reaction a red dye quinonimine is formed. the colour intensity of quinoniminedye is proportional to the Cholesterol concentration in the sample. The colour developed is stable at room temperature for 60 minutes.

Cholesterol Esters + H_2O $\xrightarrow{CHE}$ Cholesterol + Fatty acids

Cholesterol + O_2 $\xrightarrow{CHOD}$ 4-Cholestenon + H_2O_2

$2H_2O_2$ + 4-AP + Phenol $\xrightarrow{POD}$ Quinonimine + $4H_2O$

The absorb ance was read on the Spectrophotometer measuring at 50 nm wavelength using the 1 cm light path cuvettes at about 37 ^{o}C. The Spectrophotometer was switched on an hour before the reading of samples was done and usted to zero with distilled water. The calculation of the mg/dl was done as below;

$$\frac{\text{Abs. sample} - \text{Abs Blank}}{\text{Abs Standard} - \text{Abs Blank}} \times \text{Standard Concentration (200)} = \text{Cholesterol (mg/dl)}$$

APPENDIX C: ANALYSIS OF VARIANCE TABLES

I. ANOVA FOR GROWING PIGS WEIGHT GAINS AT D42.

(a) The weight gains of pigs by ASTFP treatment and block/litter (kg per pig per 42 days)

	Block/Litter					Treatment	
	Red	White	Yellow	Orange	Green	Total	Mean
	Rep I	Rep II	Rep III	Rep IV	Rep V	(T)	( )
Treatment							
1 Level 2 (K_1)	18.5	13	18	20.5	17.5	87.5	17.5
2 Level 1 (K_0)	16.5	15.5	19.5	15	18.5	85	17
Block total	35	28.5	37.5	35.5	36	**172.5**	
Block Mean	17.5	14.25	18.75	17.75	18		17.25

(b) The analysis of variance of total weight gain for D42.[a]

Source of variance	Degree of freedom	Sum of Squares	Mean Squares	Computed F[b]	Tabular F 5%	Tabular F 1%
Total	9	46.125				
Blocks (liter)	4	24.25	6.0625	1.141176[ns]	6.39	15.98
Treatment	1	0.625	0.625	0.117647[ns]	7.71	21.20
Error	4	21.25	5.3125			

[a] cv = 21.68%

[b] [ns] = not significant

II. ANOVA FOR GROWING PIGS BODY TEMPERATURE AT 0 HOURS POS INJECTION (PI).

(a) Rectal temperature reading before endotoxin injection (^{o}C).

	Block/Litter					Treatment	
	Red Rep I	White Rep II	Yellow Rep III	Orange Rep IV	Green Rep V	Total (T)	Mean ( )
Treatment							
1 $K_1 E_0$	39.6	38.7	38.5	38.8	39.9	195.5	39.1
2 K_1E_1	38	38.9	39.6	40.1	39.1	195.7	39.14
3 $K_0 E_0$	38.6	39.8	39.2	39.1	38.1	194.8	38.96
4 $K_0 E_1$	38.9	38.9	38.4	38.4	38.7	193.3	38.66
Block total	155.1	156.3	155.7	156.4	155.8	779.3	
Block Mean	38.775	39.075	38.925	39.1	38.95		38.965

(b) The ASTFP X Endotoxin table of totals for rectal temperature readings (^{o}C).

Endotoxin	Temperature Totals (A X B)		Endotoxin total (B)
	K_0	K_1	
E_0	194.8	195.5	390.3
E_1	193.3	195.7	389
ASTFP Total (A)	388.1	391.2	779.3

(c) Analysis of variance of rectal temperature readings. [a]

Source of variance	Degree of freedom	Sum of Squares	Mean Squares	Computed F[b]	Tabular F 5%	Tabular F 1%
Total	19	6.6055				
Blocks (liter)	4	0.273	0.06825	0.145652^{ns}	3.26	5.41
Treatment	3	0.7095	0.2365	0.504713^{ns}	3.49	5.95
ASTFP (A)	1	0.4805	0.4805	1.025431^{ns}	4.75	9.33
Endotoxin(B)	1	0.0845	0.0845	0.180331^{ns}	4.75	9.33
A X B	1	0.1445	0.1445	0.308376^{ns}	4.75	9.33
Error	12	5.623	0.468583			

[a] cv = 1.51%

[b] ^{ns} = not significant

III. ANOVA FOR GROWING PIGS BODY TEMPERATURE AT 2 HOURS POS INJECTION.

(a) Rectal temperature reading at 2 hours post endotoxin injection (^oC).

	Block/Litter					Treatment	
	Red Rep I	White Rep II	Yellow Rep III	Orange Rep IV	Green Rep V	Total (T)	Mean ( )
Treatment							
1 $K_1 E_0$	39.5	38.3	39.1	39	39.4	195.3	39.06
2 K_1E_1	38.8	39.8	39.3	39.3	40.4	197.6	39.52
3 $K_0 E_0$	39.9	39.6	38.8	39.1	38.8	196.2	39.24
4 $K_0 E_1$	39.5	40.2	38.8	38.4	39.8	196.7	39.34
Block total	157.7	157.9	156	155.8	158.4	785.8	
Block Mean	39.425	39.475	39	38.95	39.6		39.29

(b) The ASTFP X Endotoxin table of totals for temperature readings (^oC).

Endotoxin	Temperature Totals (A X B)		Endotoxin total (B)
	K_0	K_1	
E_0	196.2	195.3	391.5
E_1	196.7	197.6	394.3
ASTFP Total (A)	392.9	392.9	785.8

(c) Analysis of variance for rectal temperature readings.[a]

Source of variance	Degree of freedom	Sum of Squares	Mean Squares	Computed F[b]	Tabular F 5%	Tabular F 1%
Total	19	6.038				
Blocks (liter)	4	1.393	0.34825	1.021511^{ns}	3.26	5.41
Treatment	3	0.554	0.184667	0.541677^{ns}	3.49	5.95
ASTFP (A)	1	0	0	0	4.75	9.33
Endotoxin(B)	1	0.392	0.392	1.149841^{ns}	4.75	9.33
A X B	1	0.162	0.162	0.475189^{ns}	4.75	9.33
Error	12	4.091	0.340917			

[a] cv = 1.44%

[b] ^{ns} = not significant

IV ANOVA FOR GROWING PIGS BODY TEMPERATURE AT 4 HOURS PI.

(a) Rectal temperature reading at 4 hours post endotoxin injection (^oC).

	Block/Litter					Treatment	
	Red Rep I	White Rep II	Yellow Rep III	Orange Rep IV	Green Rep V	Total (T)	Mean ( )
Treatment							
1 $K_1 E_0$	38.3	38.5	38.9	37.9	38.2	191.8	38.36
2 K_1E_1	39	38.6	38.5	39.7	38.6	194.4	38.88
3 $K_0 E_0$	38.2	38.3	37.9	38.7	37.4	190.5	38.10
4 $K_0 E_1$	39	38.6	38.7	39.4	39.7	195.4	39.08
Block total	154.5	154	154	155.7	153.9	772.1	
Block Mean	38.625	38.5	38.5	38.925	38.475		38.605

(b) The ASTFP X Endotoxin table of totals for temperature readings (^oC).

Endotoxin	Temperature Totals (A X B)		Endotoxin total (B)
	K_0	K_1	
E_0	190.5	191.8	382.3
E_1	195.4	194.4	389.8
ASTFP Total (A)	385.9	386.2	772.1

(c) Analysis of variance for temperature readings[a]

Source of variance	Degree of freedom	Sum of Squares	Mean Squares	Computed F[b]	Tabular F 5%	Tabular F 1%
Total	19	6.4295				
Blocks (liter)	4	0.567	0.14175	0.61165^{ns}	3.26	5.41
Treatment	3	3.0815	1.027167	4.432219*	3.49	5.95
ASTFP (A)	1	0.0045	0.0045	0.019417^{ns}	4.75	9.33
Endotoxin(B)	1	2.8125	2.8125	12.13592**	4.75	9.33
A X B	1	0.2645	0.2645	1.141316^{ns}	4.75	9.33
Error	12	2.781	0.23175			

[a] cv = 1.51% [b] ** = significant at 1% level, [ns] = not significant

(d) Comparison between mean temperature for treatment 3 (K_0E_0) and the other treatments (1, 2 and 4) using the LSD test: 0.66 $t_{0.05}$ (t=2.179, df =12, r= 5 and 0.23 MSE) and 0.93 $t_{0.01}$ (t=3.055, df =12, r= 5 and 0.23 MSE).

	Treatment	Mean Temperature[b] ^oC	Difference from treatment 3[d] ^oC
1	K_1E_0	38.36	0.26^{ns}
4	K_0E_1	39.08	0.98**
2	K_1E_1	38.88	0.78*
1 and 2[a]	(K_1E_0 & K_1E_1)	38.62[c]	0.52*
4 and 2[a]	(K_0E_1 & K_1E_1)	38.98[c]	0.88**
3	K_0E_0	38.10	-

[a]Difference compared with LSD of 0.47 $t_{0.05}$ and 0.66 $t_{0.01}$ calculated by sd = $(s^2/r)^{1/2}$. [b]Average of five replications. [c]Average of two means. [d]** = significant at 1% level, * significant at 5% level, [ns] = not significant

V. ANOVA FOR GROWING PIGS BODY TEMPERATURE AT 8 HOURS POS INJECTION.

(a) Rectal temperature reading at 8 hours post endotoxin injection (^oC).

	Block/Litter					Treatment	
	Red Rep I	White Rep II	Yellow Rep III	Orange Rep IV	Green Rep V	Total (T)	Mean ( )
Treatment							
1 $K_1 E_0$	39.1	38.9	39	38.7	39.7	195.4	39.08
2 K_1E_1	39.3	39.5	39.1	39.8	39.4	197.1	39.42
3 $K_0 E_0$	39.3	39.4	38.7	39.1	39.6	196.1	39.22
4 $K_0 E_1$	39.7	39.2	39.7	38.7	39.8	197.1	39.42
Block total	157.4	157	156.5	156.3	158.5	785.7	
Block Mean	39.35	39.25	39.125	39.075	39.625		39.285

(b) The ASTFP X Endotoxin table of totals for temperature readings (^oC).

Endotoxin	Temperature Totals (A X B)		Endotoxin total (B)
	K_0	K_1	
E_0	196.1	195.4	391.5
E_1	197.1	197.1	394.2
ASTFP Total (A)	393.2	392.5	785.7

(c) Analysis of variance for rectal temperature readings.[a]

Source of variance	Degree of freedom	Sum of Squares	Mean Squares	Computed F[b]	Tabular F 5%	Tabular F 1%
Total	19	2.5855				
Blocks (liter)	4	0.763	0.19075	1.624556^{ns}	3.26	5.41
Treatment	3	0.4135	0.137833	1.173882^{ns}	3.49	5.95
ASTFP (A)	1	0.0245	0.0245	0.208659^{ns}	4.75	9.33
Endotoxin(B)	1	0.3645	0.3645	3.104329^{ns}	4.75	9.33
A X B	1	0.0245	0.0245	0.208659^{ns}	4.75	9.33
Error	12	1.409	0.117417			

[a] cv = 0.94%

[b] ^{ns} = not significant

VI. ANOVA FOR GROWING PIGS BODY TEMPERATURE AT 24 HOURS POST INJECTION.

(a) Rectal temperature reading at 24 hours post endotoxin injection (^oC).

	Block/Litter					Treatment	
	Red Rep I	White Rep II	Yellow Rep III	Orange Rep IV	Green Rep V	Total (T)	Mean ( )
Treatment							
1 $K_1 E_0$	39.6	39.8	39.3	39.6	39.8	198.1	39.62
2 K_1E_1	39.5	39.2	39.4	39.7	39.4	197.2	39.44
3 $K_0 E_0$	39	39.9	39.2	39.8	39.6	197.5	39.5
4 $K_0 E_1$	39.3	39.7	39.7	39.4	40.3	198.4	39.68
Block total	157.4	158.6	157.6	158.5	159.1	791.2	
Block Mean	39.35	39.65	39.4	39.625	39.775		39.56

(b) The ASTFP X Endotoxin table of totals for temperature readings (^oC).

Endotoxin	Temperature Totals (A X B)		Endotoxin total (B)
	K_0	K_1	
E_0	197.5	198.1	395.6
E_1	198.4	197.2	395.6
ASTFP Total (A)	395.9	395.3	791.2

(c) Analysis of variance for rectal temperature readings.[a]

Source of variance	Degree of freedom	Sum of Squares	Mean Squares	Computed F[b]	Tabular F 5%	Tabular F 1%
Total	19	1.688				
Blocks (liter)	4	0.513	0.12825	1.546734^{ns}	3.26	5.41
Treatment	3	0.18	0.06	0.723618^{ns}	3.49	5.95
ASTFP (A)	1	0.018	0.018	0.217085^{ns}	4.75	9.33
Endotoxin(B)	1	0	0	0	4.75	9.33
A X B	1	0.162	0.162	1.953769^{ns}	4.75	9.33
Error	12	0.995	0.082917			

[a] cv = 0.75%

[b] ^{ns} = not significant

VII. ANOVA FOR GROWING PIGS WHITE BLOOD CELL COUNT(WBC) AT HOURS POST INJECTION

(a) White blood cell count before endotoxin injection (10^3 Cells/mm^3).

	Block/Litter					Treatment	
	Red Rep I	White Rep II	Yellow Rep III	Orange Rep IV	Green Rep V	Total (T)	Mean ( )
Treatment							
1 $K_1 E_0$	16.05	14.76	17.76	14.10	13.83	76.50	15.30
2 K_1E_1	12.50	14.86	17.93	15.57	12.64	73.50	14.70
3 $K_0 E_0$	12.15	14.55	13.84	16.24	22.72	79.50	15.90
4 $K_0 E_1$	18.88	17.76	18.84	14.76	10.01	80.25	16.05
Block total	59.58	61.93	68.37	60.67	59.20	309.75	
Block Mean	14.895	15.4825	17.0925	15.1675	14.80		15.49

(b) The ASTFP X Endotoxin table of totals for white blood cell count (10^3 Cells/mm^3).

Endotoxin	WBC Totals (A X B)		Endotoxin total (B)
	K_0	K_1	
E_0	79.50	76.50	156.00
E_1	80.25	73.50	153.75
ASTFP Total (A)	159.75	150.00	309.75

(c) Analysis of variance for white blood cell count.[a]

Source of variance	Degree of freedom	Sum of Squares	Mean Squares	Computed F[b]	Tabular F 5%	Tabular F 1%
Total	19	160.1344				
Blocks (liter)	4	14.00865	3.502162	0.299295^{ns}	3.26	5.41
Treatment	3	5.709375	1.903125	0.162641^{ns}	3.49	5.95
ASTFP (A)	1	4.753125	4.753125	0.406203^{ns}	4.75	9.33
Endotoxin(B)	1	0.253125	0.253125	0.021632^{ns}	4.75	9.33
A X B	1	0.703125	0.703125	0.060089^{ns}	4.75	9.33
Error	12	140.4164	11.70136			

[a] cv = 18.74%

[b] ^{ns} = not significant

VIII. ANOVA FOR GROWING PIGS WHITE BLOOD CELL COUNT AT 2 HOURS POST INJECTION.

(a) White blood cell count 2 hours post endotoxin injection (10^3 Cells/mm^3).

	Block/Litter					Treatment	
	Red Rep I	White Rep II	Yellow Rep III	Orange Rep IV	Green Rep V	Total (T)	Mean ( )
Treatment							
1 $K_1 E_0$	15.98	14.24	14.56	16.42	12.8	74	14.80
2 K_1E_1	12.02	15.98	14.88	12.96	12.16	68	13.60
3 $K_0 E_0$	16.04	14.02	16.74	14.34	17.86	79	15.80
4 $K_0 E_1$	15.76	14.56	14.88	12.88	12.92	71	14.20
Block total	59.8	58.8	61.06	56.6	55.74	292	
Block Mean	14.95	14.7	15.265	14.15	13.935		14.60

(b) The ASTFP X Endotoxin table of totals for white blood cell count (10^3 Cells/mm^3).

Endotoxin	WBC Totals (A X B)		Endotoxin total (B)
	K_0	K_1	
E_0	79	74	153
E_1	71	68	139
ASTFP Total (A)	150	142	292

(c) Analysis of variance for white blood cell count.[a]

Source of variance	Degree of freedom	Sum of Squares	Mean Squares	Computed F[b]	Tabular F 5%	Tabular F 1%
Total	19	50.7616				
Blocks (liter)	4	4.8778	1.21945	0.447726^{ns}	3.26	5.41
Treatment	3	13.2	4.4	1.615479^{ns}	3.49	5.95
ASTFP (A)	1	3.2	3.2	1.174894^{ns}	4.75	9.33
Endotoxin(B)	1	9.8	9.8	3.598113^{ns}	4.75	9.33
A X B	1	0.2	0.2	0.073431^{ns}	4.75	9.33
Error	12	32.6838	2.72365			

[a] cv = 11.19%

[b] ns = not significant

IX ANOVA FOR GROWING PIGS WBC COUNT AT 4 HOURS POST INJECTION

(a) White blood cell count 4 hours post endotoxin injection (10^3 Cells/mm^3).

	Block/Litter					Treatment	
	Red Rep I	White Rep II	Yellow Rep III	Orange Rep IV	Green Rep V	Total (T)	Mean ( )
Treatment							
1 $K_1 E_0$	12.96	12.02	13.44	12.82	12.86	64.1	12.82
2 K_1E_1	14.42	16.42	13.45	15.46	14.45	74.2	14.84
3 $K_0 E_0$	16.42	15.57	14.88	13.68	12.65	73.2	14.64
4 $K_0 E_1$	18.98	24.86	24.42	22.08	20.76	111.1	22.22
Block total	62.78	68.87	66.19	64.04	60.72	322.6	
Block Mean	15.695	17.2175	16.5475	16.01	15.18		16.13

(b) The ASTFP X Endotoxin table of totals for white blood cell count (10^3 Cells/mm^3).

Endotoxin	WBC Totals (A X B)		Endotoxin total (B)
	K_0	K_1	
E_0	73.2	64.1	137.3
E_1	111.1	74.2	185.3
ASTFP Total (A)	184.3	138.3	322.6

(c) Analysis of variance for white blood cell count.[a]

Source of variance	Degree of freedom	Sum of Squares	Mean Squares	Computed F[b]	Tabular F 5%	Tabular F 1%
Total	19	299.26				
Blocks (liter)	4	9.85235	2.463088	0.992992^{ns}	3.26	5.41
Treatment	3	259.642	86.54733	34.89149**	3.49	5.95
ASTFP (A)	1	105.8	105.8	42.65319**	4.75	9.33
Endotoxin(B)	1	115.2	115.2	46.4428**	4.75	9.33
A X B	1	38.642	38.642	15.57849**	4.75	9.33
Error	12	29.76565	2.480471			

[a] cv = 24.60% [b]** = significant at 1% level, * = significant at 5% le l, [ns] = not significant

(d) Comparison between mean white blood cell count for treatment 3 (K_0E_0) and the other 3 treatments using the LSD test: 2.17 $t_{0.05}$ (t=2.179, df =12, r= 5 and 2.48 MSE) and 3.04 $t_{0.01}$ (t=3.055, df =12, r= 5 and 2.48 MSE).

	Treatment	Mean WBC concentration[b] 10^3 cells/mm^3	Difference from treatment 3[d] 10^3 cells/mm^3
1	K_1E_0	12.82	1.82^{ns}
4	K_0E_1	22.22	7.58**
2	K_1E_1	14.84	0.20^{ns}
1 and 2[a]	(K_1E_0 & K_1E_1)	13.84[c]	0.81^{ns}
4 and 2[a]	(K_0E_1 & K_1E_1)	18.53[c]	3.89**
3	K_0E_0	14.64	-

[a]Difference compared with LSD of 1.54 $t_{0.05}$ and 2.15 $t_{0.01}$ calculated by sd = $(s^2/r)^{1/2}$. [b]Average of five replications. [c]Average of two means. [d]** = significant at 1% level, [ns] = not significant

X ANOVA FOR GROWING PIGS WBC COUNT AT 8 HOURS POST INJECTION

(a) White blood cell count 8 hours post endotoxin injection (10^3 Cells/mm^3).

	Block/Litter					Treatment	
	Red Rep I	White Rep II	Yellow Rep III	Orange Rep IV	Green Rep V	Total (T)	Mean ( )
Treatment							
1 $K_1 E_0$	12.46	13.88	14.02	12.24	15.6	68.2	13.64
2 K_1E_1	18.64	26.68	23.16	13.86	23.06	105.4	21.08
3 $K_0 E_0$	14.06	13.75	12.46	16.04	14.69	71	14.2
4 $K_0 E_1$	23.55	26.46	24.62	22.08	26.04	122.75	24.55
Block total	68.71	80.77	74.26	64.22	79.39	367.35	
Block Mean	17.1775	20.1925	18.565	16.055	19.8475		18.3675

(b) The ASTFP X Endotoxin table of totals for white blood cell count (10^3 Cells/mm^3).

Endotoxin	WBC Totals (A X B)		Endotoxin total (B)
	K_0	K_1	
E_0	71	68.2	139.2
E_1	122.75	105.4	228.15
ASTFP Total (A)	193.75	173.6	367.35

(c) Analysis of variance for white blood cell count.[a]

Source of variance	Degree of freedom	Sum of Squares	Mean Squares	Computed F[b]	Tabular F 5%	Tabular F 1%
Total	19	551.4256				
Blocks (liter)	4	49.29515	12.32379	1.955147^{ns}	3.26	5.41
Treatment	3	426.4914	142.1638	22.55403**	3.49	5.95
ASTFP (A)	1	20.30113	20.30113	3.220737^{ns}	4.75	9.33
Endotoxin(B)	1	395.6051	395.6051	62.76205**	4.75	9.33
A X B	1	10.58513	10.58513	1.679311^{ns}	4.75	9.33
Error	12	75.63905	6.303254			

[a] cv = 29.33% [b]** = significant at 1% level, * = significant at 5% le l, [ns] = not significant

(d) Comparison between mean white blood cell count for treatment 3 (K_0E_0) and the other 3 treatments using the LSD test: 3.46 $t_{0.05}$ (t=2.179, df =12, r= 5 and 6.30 MSE) and 4.85 $t_{0.01}$ (t=3.055, df =12, r= 5 and 6.30 MSE).

	Treatment	Mean WBC concentration[b] 10^3 cells/mm^3	Difference from treatment 3[d] 10^3 cells/mm^3
1	K_1E_0	13.64	0.56^{ns}
4	K_0E_1	24.55	10.35**
2	K_1E_1	21.08	6.88**
1 and 2[a]	(K_1E_0 & K_1E_1)	17.36	3.16*
4 and 2[a]	(K_0E_1 & K_1E_1)	22.82	8.62**
3	K_0E_0	14.20	-

[a]Difference compared with LSD of24.45 $t_{0.05}$ and 3.43 $t_{0.01}$ calculated by sd = $(s^2/r)^{1/2}$. [b]Average of five replications. [c]Average of two means. [d]** = significant at 1% level, [ns] = not significant

XI. ANOVA FOR GROWING PIGS WHITE BLOOD CELL COUNT AT 24 HOURS POST INJECTION.

(a) White blood cell count 24 hours post endotoxin injection (10^3 Cells/mm^3).

	Block/Litter					Treatment	
	Red	White	Yellow	Orange	Green	Total	Mean
	Rep I	Rep II	Rep III	Rep IV	Rep V	(T)	( )
Treatment							
1 $K_1 E_0$	13.95	12.98	13.77	16.02	12.78	69.5	13.9
2 K_1E_1	14.2	13.55	12.98	13.78	15.54	70.05	14.01
3 $K_0 E_0$	13.99	13.95	13.15	13.01	13.4	67.5	13.5
4 $K_0 E_1$	15.29	14.57	13.95	15.75	12.94	72.5	14.5
Block total	57.43	55.05	53.85	58.56	54.66	279.55	
Block Mean	14.3575	13.7625	13.4625	14.64	13.665		13.9775

(b) The ASTFP X Endotoxin table of totals for white blood cell count (10^3 Cells/mm^3).

Endotoxin	WBC Totals (A X B)		Endotoxin total
	K_0	K_1	(B)
E_0	67.5	69.5	137
E_1	72.5	70.05	142.55
ASTFP Total (A)	140	139.55	279.55

(c) Analysis of variance for white blood cell count.[a]

Source of variance	Degree of freedom	Sum of Squares	Mean Squares	Computed F[b]	Tabular F 5%	Tabular F 1%
Total	19	18.60018				
Blocks (liter)	4	3.96965	0.992413	0.985013^{ns}	3.26	5.41
Treatment	3	2.540375	0.846792	0.840478^{ns}	3.49	5.95
ASTFP (A)	1	0.010125	0.010125	0.01005^{ns}	4.75	9.33
Endotoxin(B)	1	1.540125	1.540125	1.528641^{ns}	4.75	9.33
A X B	1	0.990125	0.990125	0.982742^{ns}	4.75	9.33
Error	12	12.09015	1.007512			

[a] cv = 7.08%
[b] ^{ns} = not significant

XII. ANOVA FOR GROWING PIGS BLOOD UREA NITROGEN AT 0 HOURS POST INJECTION.

(a) Blood urea nitrogen concentration (mg/dl).

	Block/Litter					Treatment	
	Red Rep I	White Rep II	Yellow Rep III	Orange Rep IV	Green Rep V	Total (T)	Mean ( )
Treatment							
1 $K_1 E_0$	54.84	42.65	69.35	50.41	50.41	267.66	53.532
2 K_1E_1	77.42	51.61	51.47	50.41	38.16	269.07	53.814
3 $K_0 E_0$	22.06	51.61	38.16	33.82	42.65	188.3	37.66
4 $K_0 E_1$	54.84	54.84	51.47	54.84	38.16	254.15	50.83
Block total	209.16	200.71	210.45	189.48	169.38	979.18	
Block Mean	52.29	50.1775	52.6125	47.37	42.345		48.959

(b) The ASTFP X Endotoxin table of totals for blood urea nitrogen (mg/dl).

Endotoxin	BUN Totals (A X B)		Endotoxin total (B)
	K_0	K_1	
E_0	188.30	267.66	455.96
E_1	254.15	269.07	523.22
ASTFP Total (A)	442.45	536.73	979.18

(c) Analysis of variance for blood urea nitrogen.[a]

Source of variance	Degree of freedom	Sum of Squares	Mean Squares	Computed F[b]	Tabular F 5%	Tabular F 1%
Total	19	2779.356				
Blocks (liter)	4	288.7931	72.19828	0.537354^{ns}	3.26	5.41
Treatment	3	878.257	292.7523	2.178885^{ns}	3.49	5.95
ASTFP (A)	1	444.4359	444.4359	3.307829^{ns}	4.75	9.33
Endotoxin(B)	1	226.1954	226.1954	1.683517^{ns}	4.75	9.33
A X B	1	207.6257	207.6257	1.545308^{ns}	4.75	9.33
Error	12	1612.306	134.3588			

[a] cv = 24.70%

[b] ^{ns} = not significant

XIII ANOVA FOR GROWING PIGS BLOOD UREA NITROGEN AT 2 HOURS POST INJECTION

(a) Blood urea nitrogen concentrations (mg/dl).

	Block/Litter					Treatment	
	Red	White	Yellow	Orange	Green	Total	Mean
	Rep I	Rep II	Rep III	Rep IV	Rep V	(T)	( )
Treatment							
1 $K_1 E_0$	58.06	30.26	54.41	54.41	47.37	244.51	48.902
2 K_1E_1	48.39	34.21	47.37	52.63	30.26	212.86	42.572
3 $K_0 E_0$	35.53	33.82	28.95	23.68	86.84	208.82	41.764
4 $K_0 E_1$	87.10	54.41	55.63	47.37	37.1	281.61	56.322
Block total	229.08	152.70	186.36	178.09	201.57	947.80	
Block Mean	57.27	38.175	46.59	44.5225	50.3925		47.39

(b) The ASTFP X Endotoxin table of totals for blood urea nitrogen (mg/dl).

Endotoxin	BUN Totals (A X B)		Endotoxin total
	K_0	K_1	(B)
E_0	208.82	244.51	453.33
E_1	281.61	212.86	494.47
ASTFP Total (A)	490.43	457.37	947.80

(c) Analysis of variance for blood urea nitrogen.[a]

Source of variance	Degree of freedom	Sum of Squares	Mean Squares	Computed F[b]	Tabular F 5%	Tabular F 1%
Total	19	5584.706				
Blocks (liter)	4	801.6327	200.4082	0.586788[ns]	3.26	5.41
Treatment	3	684.6588	228.2196	0.668218[ns]	3.49	5.95
ASTFP (A)	1	54.64818	54.64818	0.160008[ns]	4.75	9.33
Endotoxin(B)	1	84.62498	84.62498	0.247779[ns]	4.75	9.33
A X B	1	545.3857	545.3857	1.596868[ns]	4.75	9.33
Error	12	4098.414	341.5345			

[a] cv = 36.18%

[b] [ns] = not significant at level 5%

XIV ANOVA FOR GROWING PIGS BUN AT 4 HOURS POST INJECTION

(a) Blood urea nitrogen concentrations (mg/dl).

	Block/Litter					Treatment	
	Red Rep I	White Rep II	Yellow Rep III	Orange Rep IV	Green Rep V	Total (T)	Mean ( )
Treatment							
1 $K_1 E_0$	44.12	50.41	47.39	40.32	45.59	227.83	45.566
2 K_1E_1	39.70	33.82	33.82	32.35	35.29	174.98	34.996
3 $K_0 E_0$	33.87	40.32	47.39	33.87	45.53	200.98	40.196
4 $K_0 E_1$	33.82	27.94	42.65	19.12	27.94	151.47	30.294
Block total	151.51	152.49	171.25	125.66	154.35	755.26	
Block Mean	37.8775	38.1225	42.8125	31.415	38.5875		37.763

(b) The ASTFP X Endotoxin table of totals for blood urea nitrogen (mg/dl).

	BUN Totals (A X B)		Endotoxin total
Endotoxin	K_0	K_1	(B)
E_0	200.98	227.83	428.81
E_1	151.47	174.98	326.45
ASTFP Total (A)	352.45	402.81	755.26

(c) Analysis of variance for blood urea nitrogen.[a]

Source of variance	Degree of freedom	Sum of Squares	Mean Squares	Computed F[b]	Tabular F 5%	Tabular F 1%
Total	19	1200.929				
Blocks (liter)	4	266.4668	66.6167	2.82255^{ns}	3.26	5.41
Treatment	3	651.2427	217.0809	9.197718^{**}	3.49	5.95
ASTFP (A)	1	126.8065	126.8065	5.372791^{*}	4.75	9.33
Endotoxin(B)	1	523.8785	523.8785	22.19673^{**}	4.75	9.33
A X B	1	0.55778	0.55778	0.023633^{ns}	4.75	9.33
Error	12	283.2193	23.60161			

[a] cv = 21.05% [b]** = significant at 1% level, * = significant at 5% le l, [ns] = not significant

(d) Comparison between mean blood urea nitrogen for treatment 3 (K_0E_0) and the other 3 treatments using the LSD test: 6.70 $t_{0.05}$ (t=2.179, df =12, r= 5 and 23.60 MSE) and 9.39 $t_{0.01}$ (t=3.055, df =12, r= 5 and 23.60 MSE).

	Treatment	Mean BUN concentration[b] mg/dl	Difference from treatment 3[d] mg/dl
1	K_1E_0	45.57	5.37^{ns}
4	K_0E_1	30.30	9.90^{**}
2	K_1E_1	35.00	5.20^{ns}
1 and 2[a]	(K_1E_0 & K_1E_1)	40.28[c]	0.08^{ns}
4 and 2[a]	(K_0E_1 & K_1E_1)	32.65[c]	7.55^{**}
3	K_0E_0	40.20	-

[a]Difference compared with LSD of 4.73 $t_{0.05}$ and 6.64 $t_{0.01}$ calculated by sd = $(s^2/r)^{1/2}$. [b]Average of five replications. [c]Average of two means. [d]** = significant at 1% level, [ns] = not significant

XV ANOVA FOR GROWING PIGS BLOOD UREA NITROGEN AT 8 HOURS POST INJECTION

(a) Blood urea nitrogen concentrations (mg/dl).

	Block/Litter					Treatment	
	Red	White	Yellow	Orange	Green	Total	Mean
	Rep I	Rep II	Rep III	Rep IV	Rep V	(T)	( )
Treatment							
1 $K_1 E_0$	37.1	20.59	32.35	38.24	38.24	166.52	33.304
2 K_1E_1	38.24	36.76	36.76	36.76	45.59	194.11	38.822
3 $K_0 E_0$	38.71	44.12	39.47	39.71	25.81	187.82	37.564
4 $K_0 E_1$	46.05	26.47	32.35	38.24	39.47	182.58	36.516
Block total	160.1	127.94	140.93	152.95	149.11	731.03	
Block Mean	40.025	31.985	35.2325	38.2375	37.2775		36.5515

(b) The ASTFP X Endotoxin table of totals for blood urea nitrogen (mg/dl).

Endotoxin	BUN Totals (A X B)		Endotoxin total
	K_0	K_1	(B)
E_0	187.82	166.52	354.34
E_1	182.58	194.11	376.69
ASTFP Total (A)	370.40	360.63	731.03

(c) Analysis of variance for blood urea nitrogen.[a]

Source of variance	Degree of freedom	Sum of Squares	Mean Squares	Computed F[b]	Tabular F 5%	Tabular F 1%
Total	19	779.7953				
Blocks (liter)	4	152.1102	38.02756	0.838773^{ns}	3.26	5.41
Treatment	3	83.63921	27.87974	0.614942^{ns}	3.49	5.95
ASTFP (A)	1	4.772645	4.772645	0.10527^{ns}	4.75	9.33
Endotoxin(B)	1	24.97613	24.97613	0.550898^{ns}	4.75	9.33
A X B	1	53.89044	53.89044	1.18866^{ns}	4.75	9.33
Error	12	544.0458	45.33715			

[a] cv = 17.53%

[b] ^{ns} = not significant

XVI ANOVA FOR GROWING PIGS BUN AT 24 HOURS POST INJECTION

(a) Blood urea nitrogen concentrations (mg/dl).

	Block/Litter					Treatment	
	Red Rep I	White Rep II	Yellow Rep III	Orange Rep IV	Green Rep V	Total (T)	Mean ( )
Treatment							
1 $K_1 E_0$	33.87	36.76	30.88	33.87	47.71	183.09	36.618
2 K_1E_1	48.39	59.68	46.71	69.35	48.39	272.52	54.504
3 $K_0 E_0$	25	30.88	52.63	32.35	61.72	202.58	40.516
4 $K_0 E_1$	33.87	22.06	30.88	39.47	50	176.28	35.256
Block total	141.13	149.38	161.1	175.04	207.82	834.47	
Block Mean	35.2825	37.345	40.275	43.76	51.955		41.7235

(b) The ASTFP X Endotoxin table of totals for blood urea nitrogen (mg/dl).

Endotoxin	BUN Totals (A X B)		Endotoxin total
	K_0	K_1	(B)
E_0	202.58	183.09	385.67
E_1	176.28	272.52	448.8
ASTFP Total (A)	378.86	455.61	834.47

(c) Analysis of variance for blood urea nitrogen.[a]

Source of variance	Degree of freedom	Sum of Squares	Mean Squares	Computed F[b]	Tabular F 5%	Tabular F 1%
Total	19	3144.242				
Blocks (liter)	4	686.3473	171.5868	1.5907^{ns}	3.26	5.41
Treatment	3	1163.47	387.8232	3.595324^{*}	3.49	5.95
ASTFP (A)	1	294.5281	294.5281	2.73043^{ns}	4.75	9.33
Endotoxin(B)	1	199.2698	199.2698	1.847336^{ns}	4.75	9.33
A X B	1	669.6716	669.6716	6.208207^{*}	4.75	9.33
Error	12	1294.425	107.8688			

[a] cv = 30.83 [b] ** = significant at 1% level, * = significant at 5% le l, [ns] = not significant

(d) Comparison between mean blood urea nitrogen for treatment 3 (K_0E_0) and the other 3 treatments using the LSD test:14.31 $t_{0.05}$ (t=2.179, df =12, r= 5 and 107.87MSE) and 20.07 $t_{0.01}$ (t=3.055, df =12, r= 5&107.87MSE).

	Treatment	Mean BUN concentration[b] mg/dl	Difference from treatment 3[d] mg/dl
1	K_1E_0	36.618	3.898^{ns}
4	K_0E_1	35.256	5.26^{ns}
2	K_1E_1	54.504	13.988^{ns}
1 and 2[a]	(K_1E_0 & K_1E_1)	45.561[c]	5.045^{ns}
4 and 2[a]	(K_0E_1 & K_1E_1)	44.88[c]	4.364^{ns}
3	K_0E_0	40.516	-

[a]Difference compared with LSD of 10.12 $t_{0.05}$ and 14.19 $t_{0.01}$ calculated by sd = $(s^2/r)^{1/2}$. [b]Average of five replications. [c]Average of two means. [d] [ns] = not significant at 5% level

XVII. ANOVA FOR GROWING PIGS TOTAL PROTEIN AT 0 HOURS POST INJECTION.

(a) Blood Total Protein concentrations (g/dl).

	Block/Litter					Treatment	
	Red	White	Yellow	Orange	Green	Total	Mean
	Rep I	Rep II	Rep III	Rep IV	Rep V	(T)	( )
Treatment							
1 $K_1 E_0$	3.41	2.67	2.52	1.93	1.18	11.71	2.342
2 K_1E_1	2.92	2.92	2.81	3.26	2.52	14.43	2.886
3 $K_0 E_0$	2.81	2.67	2.92	3.41	2.67	14.48	2.896
4 $K_0 E_1$	2.81	3.26	2.67	2.52	2.81	14.07	2.814
Block total	11.95	11.52	10.92	11.12	9.18	54.69	
Block Mean	2.9875	2.88	2.73	2.78	2.295		2.7345

(b) The ASTFP X Endotoxin table of totals for blood total protein (g/dl).

Endotoxin	Total Protein Totals (A X B)		Endotoxin total
	K_0	K_1	(B)
E_0	14.48	11.71	26.19
E_1	14.07	14.43	28.50
ASTFP Total (A)	28.55	26.14	54.69

(c) Analysis of variance for blood total protein.[a]

Source of variance	Degree of freedom	Sum of Squares	Mean Squares	Computed F[b]	Tabular F 5%	Tabular F 1%
Total	19	4.809295				
Blocks (liter)	4	1.12172	0.28043	1.27443079^{ns}	3.26	5.41
Treatment	3	1.047055	0.349018	1.58613455^{ns}	3.49	5.95
ASTFP (A)	1	0.290405	0.290405	1.31976277^{ns}	4.75	9.33
Endotoxin(B)	1	0.266805	0.266805	1.21251117^{ns}	4.75	9.33
A X B	1	0.489845	0.489845	2.2261297^{ns}	4.75	9.33
Error	12	2.64052	0.220043			

[a] cv = 18.40%

[b] ^{ns} = not significant

XVIII ANOVA FOR GROWING PIGS TOTAL PROTEIN AT 2 HOURS POST INJECTION

(a) Blood Total Protein concentrations (g/dl).

	Block/Litter					Treatment	
	Red Rep I	White Rep II	Yellow Rep III	Orange Rep IV	Green Rep V	Total (T)	Mean ( )
Treatment							
1 $K_1 E_0$	2.37	3.7	4.3	3.26	4	17.63	3.526
2 K_1E_1	4.89	4.3	4.74	3.56	3.26	20.75	4.15
3 $K_0 E_0$	4.44	1.63	3.86	3.26	3.41	16.6	3.32
4 $K_0 E_1$	1.93	3.26	4	2.52	3.86	15.57	3.114
Block total	13.63	12.89	16.9	12.6	14.53	70.55	
Block Mean	3.4075	3.2225	4.225	3.15	3.6325		3.5275

(b) The ASTFP X Endotoxin table of totals for blood total protein (g/dl).

Endotoxin	Total Protein Totals (A X B)		Endotoxin total (B)
	K_0	K_1	
E_0	16.6	17.63	34.23
E_1	15.57	20.75	36.32
ASTFP Total (A)	32.17	38.38	70.55

(c) Analysis of variance for blood total protein.[a]

Source of variance	Degree of freedom	Sum of Squares	Mean Squares	Computed F[b]	Tabular F 5%	Tabular F 1%
Total	19	14.85858				
Blocks (liter)	4	2.98985	0.747463	1.01225145^{ns}	3.26	5.41
Treatment	3	3.007735	1.002578	1.35774219^{ns}	3.49	5.95
ASTFP (A)	1	1.928205	1.928205	2.61127256^{ns}	4.75	9.33
Endotoxin(B)	1	0.218405	0.218405	0.29577508^{ns}	4.75	9.33
A X B	1	0.861125	0.861125	1.16617895^{ns}	4.75	9.33
Error	12	8.86099	0.738416			

[a] cv = 25.07%

[b] ^{ns} = not significant

XIX ANOVA FOR GROWING PIGS TOTAL PROTEIN AT 4 HOURS POST INJECTION

(a) Blood Total Protein concentrations (g/dl).

	Block/Litter					Treatment	
	Red Rep I	White Rep II	Yellow Rep III	Orange Rep IV	Green Rep V	Total (T)	Mean ( )
Treatment							
1 $K_1 E_0$	2.22	2.22	2.52	2.81	2.52	12.29	2.458
2 K_1E_1	3.56	2.81	3.41	4.3	4	18.08	3.616
3 $K_0 E_0$	4	2.67	4.15	2.81	3.11	16.74	3.348
4 $K_0 E_1$	2.22	2.81	2.96	2.22	2.37	12.58	2.516
Block total	12	10.51	13.04	12.14	12	59.69	
Block Mean	3	2.6275	3.26	3.035	3		2.9845

(b) The ASTFP X Endotoxin table of totals for blood total protein (g/dl).

	Total Protein Totals (A X B)		Endotoxin total (B)
Endotoxin	K_0	K_1	
E_0	16.74	12.29	29.03
E_1	12.58	18.08	30.66
ASTFP Total (A)	29.32	30.37	59.69

(c) Analysis of variance for blood total protein.[a]

Source of variance	Degree of freedom	Sum of Squares	Mean Squares	Computed F[b]	Tabular F 5%	Tabular F 1%
Total	19	9.047695				
Blocks (liter)	4	0.82552	0.20638	0.80301419[ns]	3.26	5.41
Treatment	3	5.138095	1.712698	6.66402298[**]	3.49	5.95
ASTFP (A)	1	0.055125	0.055125	0.2144886[ns]	4.75	9.33
Endotoxin(B)	1	0.132845	0.132845	0.51689321[ns]	4.75	9.33
A X B	1	4.950125	4.950125	19.2606871[**]	4.75	9.33
Error	12	3.08408	0.257007			

[a] cv = 23.12% [b] ** = significant at 1% level, [ns] = not significant

(d) Comparison between mean blood Total Protein for treatment 3 (K_0E_0) and the other treatments (1, 2 and 4) using the LSD test:0.7 $t_{0.05}$ (t=2.179, df =12, r= 5 &0.26 MSE) and 0.98 $t_{0.01}$ (t=3.055, df =12, r= 5 & 0.26 MSE).

	Treatment	Mean Total Protein concentration[b] g/dl	Difference from treatment 3[d] g/dl
1	K_1E_0	2.458	0.89[*]
4	K_0E_1	2.516	0.83[*]
2	K_1E_1	3.616	0.27[ns]
1 and 2[a]	(K_1E_0 & K_1E_1)	3.037[c]	0.31[ns]
4 and 2[a]	(K_0E_1 & K_1E_1)	3.066[c]	0.28[ns]
3	K_0E_0	3.348	-

[a]Difference compared with LSD of 0.49 $t_{0.05}$ and 0.69 $t_{0.01}$ calculated by sd = $(s^2/r)^{1/2}$. [b]Average of five replications. [c]Average of two means. [d] *significant at 5% level [ns] = not significant at 5% level

XX ANOVA FOR GROWING PIGS TOTAL PROTEIN AT 8 HOURS POST INJECTION

(a) Blood Total Protein concentrations (g/dl).

	Block/Litter					Treatment	
	Red	White	Yellow	Orange	Green	Total	Mean
	Rep I	Rep II	Rep III	Rep IV	Rep V	(T)	( )
Treatment							
1 $K_1 E_0$	3.26	1.48	3.26	3.11	2.52	13.63	2.726
2 K_1E_1	3.26	2.81	2.37	3.85	2.81	15.1	3.02
3 $K_0 E_0$	2.22	1.93	5.63	2.52	2.07	14.37	2.874
4 $K_0 E_1$	1.63	1.78	2.67	2.22	1.78	10.08	2.016
Block total	10.37	8	13.93	11.7	9.18	53.18	
Block Mean	2.5925	2	3.4825	2.925	2.295		2.659

(b) The ASTFP X Endotoxin table of totals for blood total protein (g/dl).

Endotoxin	Total Protein Totals (A X B)		Endotoxin total
	K_0	K_1	(B)
E_0	14.37	13.63	28.00
E_1	10.08	15.10	25.18
ASTFP Total (A)	24.45	28.73	53.18

(c) Analysis of variance for blood total protein.[a]

Source of variance	Degree of freedom	Sum of Squares	Mean Squares	Computed F[b]	Tabular F 5%	Tabular F 1%
Total	19	16.95818				
Blocks (liter)	4	5.28043	1.320107	1.81972309[ns]	3.26	5.41
Treatment	3	2.97242	0.990807	1.36579314[ns]	3.49	5.95
ASTFP (A)	1	0.91592	0.91592	1.26256443[ns]	4.75	9.33
Endotoxin(B)	1	0.39762	0.39762	0.54810559[ns]	4.75	9.33
A X B	1	1.65888	1.65888	2.28670941[ns]	4.75	9.33
Error	12	8.70533	0.725444			

[a] cv = 35.53%

[b] [ns] = not significant

XXI ANOVA FOR GROWING PIGS TOTAL PROTEIN AT 24 HOURS POST INJECTION

(a) Blood Total Protein concentrations (g/dl).

	Block/Litter					Treatment	
	Red	White	Yellow	Orange	Green	Total	Mean
	Rep I	Rep II	Rep III	Rep IV	Rep V	(T)	( )
Treatment							
1 $K_1 E_0$	2.52	2.96	2.22	2.96	3.85	14.51	2.902
2 K_1E_1	2.81	1.78	2.81	1.93	1.63	10.96	2.192
3 $K_0 E_0$	1.33	1.48	2.52	2.22	2.81	10.36	2.072
4 $K_0 E_1$	1.78	1.78	1.93	3.26	2.52	11.27	2.254
Block total	8.44	8	9.48	10.37	10.81	47.10	
Block Mean	2.11	2	2.37	2.5925	2.7025		2.355

(b) The ASTFP X Endotoxin table of totals for blood total protein (g/dl).

Endotoxin	Total Protein Totals (A X B)		Endotoxin total
	K_0	K_1	(B)
E_0	10.36	14.51	24.87
E_1	11.27	10.96	22.23
ASTFP Total (A)	21.63	25.47	47.10

(c) Analysis of variance for blood total protein.[a]

Source of variance	Degree of freedom	Sum of Squares	Mean Squares	Computed F[b]	Tabular F 5%	Tabular F 1%
Total	19	8.2203				
Blocks (liter)	4	1.45375	0.363437	0.93065612[ns]	3.26	5.41
Treatment	3	2.08034	0.693447	1.77571214[ns]	3.49	5.95
ASTFP (A)	1	0.73728	0.73728	1.88795637[ns]	4.75	9.33
Endotoxin(B)	1	0.34848	0.34848	0.89235438[ns]	4.75	9.33
A X B	1	0.99458	0.99458	2.54682569[ns]	4.75	9.33
Error	12	4.68621	0.390518			

[a] cv = 27.93%

[b] [ns] = not significant

XXII. ANOVA FOR GROWING PIGS ALBUMIN AT 0 HOURS POST INJECTI N.

(a) Blood Albumin concentrations (g/dl).

	Block/Litter					Treatment	
	Red	White	Yellow	Orange	Green	Total	Mean
	Rep I	Rep II	Rep III	Rep IV	Rep V	(T)	( )
Treatment							
1 $K_1 E_0$	4.58	4.17	3.36	4.08	3.17	19.36	3.872
2 K_1E_1	4.17	3.92	3.50	3.33	3.50	18.42	3.684
3 $K_0 E_0$	3.50	3.36	4.17	3.92	3.33	18.28	3.656
4 $K_0 E_1$	2.08	3.50	3.17	3.21	3.17	15.13	3.026
Block total	14.33	14.95	14.2	14.54	13.17	71.19	
Block Mean	3.5825	3.7375	3.55	3.635	3.2925		3.5595

(b) The ASTFP X Endotoxin table of totals for blood albumin (g/dl).

Endotoxin	Albumin Totals (A X B)		Endotoxin total
	K_0	K_1	(B)
E_0	18.28	19.36	37.64
E_1	15.13	18.42	33.55
ASTFP Total (A)	33.41	37.78	71.19

(c) Analysis of variance for blood albumin.[a]

Source of variance	Degree of freedom	Sum of Squares	Mean Squares	Computed F[b]	Tabular F 5%	Tabular F 1%
Total	19	5.655695				
Blocks (liter)	4	0.43717	0.109293	0.412027[ns]	3.26	5.41
Treatment	3	2.035455	0.678485	2.557851[ns]	3.49	5.95
ASTFP (A)	1	0.954845	0.954845	3.599713[ns]	4.75	9.33
Endotoxin(B)	1	0.836405	0.836405	3.153201[ns]	4.75	9.33
A X B	1	0.244205	0.244205	0.92064[ns]	4.75	9.33
Error	12	3.18307	0.265256			

[a] cv = 15.33%

[b] [ns] = not significant

XXIII ANOVA FOR GROWING PIGS ALBUMIN AT 2 HOURS POST INJECTI N

(a) Blood Albumin concentrations (g/dl).

	Block/Litter					Treatment	
	Red Rep I	White Rep II	Yellow Rep III	Orange Rep IV	Green Rep V	Total (T)	Mean ( )
Treatment							
1 $K_1 E_0$	3.57	3.67	3.67	3.67	2.83	17.41	3.482
2 K_1E_1	4.33	4.25	4.50	4.83	3.42	21.33	4.266
3 $K_0 E_0$	2.00	2.41	2.41	2.33	3.58	12.73	2.546
4 $K_0 E_1$	2.92	2.83	2.83	3.67	3.67	15.92	3.184
Block total	12.82	13.16	13.41	14.5	13.5	67.39	
Block Mean	3.205	3.29	3.3525	3.625	3.375		3.3695

(b) The ASTFP X Endotoxin table of totals for blood albumin (g/dl).

	Albumin Totals (A X B)		Endotoxin total (B)
Endotoxin	K_0	K_1	
E_0	12.73	17.41	30.14
E_1	15.92	21.33	37.25
ASTFP Total (A)	28.65	38.74	67.39

(c) Analysis of variance for blood albumin.[a]

Source of variance	Degree of freedom	Sum of Squares	Mean Squares	Computed F[b]	Tabular F 5%	Tabular F 1%
Total	19	11.5201				
Blocks (liter)	4	0.39592	0.09898	0.341357^{ns}	3.26	5.41
Treatment	3	7.644655	2.548218	8.788172^{**}	3.49	5.95
ASTFP (A)	1	5.090405	5.090405	17.55554^{**}	4.75	9.33
Endotoxin(B)	1	2.527605	2.527605	8.717082^{*}	4.75	9.33
A X B	1	0.026645	0.026645	0.091892^{ns}	4.75	9.33
Error	12	3.47952	0.28996			

[a] cv = 23.11% [b]** = significant at 1% level, * = significant at 5% le l, [ns] = not significant

(d) Comparison between mean blood Albumin for treatment 3 (K_0E_0) and the other treatments (1, 2 and 4) using the LSD test: 0.74 $t_{0.05}$ (t=2.179, df=12, r=5 and 0.29 MSE) and 1.04 $t_{0.01}$ (t=3.055, df=12, r=5 and 0.29 MSE).

Treatment		Mean Albumin concentration[b] mg/dl	Difference from treatment 3[d] mg/dl
1	K_1E_0	3.48	0.936^{*}
4	K_0E_1	3.18	0.638^{ns}
2	K_1E_1	4.27	1.720^{**}
1 and 2[a]	(K_1E_0 & K_1E_1)	3.87[c]	1.328^{**}
4 and 2[a]	(K_0E_1 & K_1E_1)	3.72[c]	1.179^{**}
3	K_0E_0	2.55	-

[a]Difference compared with LSD of 0.52 $t_{0.05}$ and 0.74 $t_{0.01}$ calculated by $sd = (s^2/r)^{1/2}$. [b]Average of five replications. [c]Average of two means. [d]** = significant at 1% level, [ns] = not significant

XXIV ANOVA FOR GROWING PIGS ALBUMIN AT 4 HOURS POST INJECTION

(a) Blood Albumin concentrations (g/dl).

	Block/Litter					Treatment	
	Red Rep I	White Rep II	Yellow Rep III	Orange Rep IV	Green Rep V	Total (T)	Mean ( )
Treatment							
1 $K_1 E_0$	3.67	3.67	3.36	3.93	3.25	17.88	3.576
2 K_1E_1	3.79	3.75	3.42	3.58	2.71	17.25	3.450
3 $K_0 E_0$	2.14	3.36	2.83	3.58	3.42	15.33	3.066
4 $K_0 E_1$	2.42	3.42	2.42	3.58	3.42	15.26	3.052
Block total	12.02	14.2	12.03	14.67	12.8	65.72	
Block Mean	3.005	3.55	3.0075	3.6675	3.2		3.286

(b) The ASTFP X Endotoxin table of totals for blood albumin (g/dl).

	Albumin Totals (A X B)		Endotoxin total (B)
Endotoxin	K_0	K_1	
E_0	15.33	17.88	33.21
E_1	15.26	17.25	32.51
ASTFP Total (A)	30.59	35.13	65.72

(c) Analysis of variance for blood albumin.[a]

Source of variance	Degree of freedom	Sum of Squares	Mean Squares	Computed F[b]	Tabular F 5%	Tabular F 1%
Total	19	4.87528				
Blocks (liter)	4	1.51663	0.379157	1.988684^{ns}	3.26	5.41
Treatment	3	1.07076	0.35692	1.872048^{ns}	3.49	5.95
ASTFP (A)	1	1.03058	1.03058	5.405400^{*}	4.75	9.33
Endotoxin(B)	1	0.0245	0.0245	0.128503^{ns}	4.75	9.33
A X B	1	0.01568	0.01568	0.082242^{ns}	4.75	9.33
Error	12	2.28789	0.190658			

[a] cv = 15.42% [b] * = significant at 5% level, ns = not significant

(d) Comparison between mean blod albumin for treatment 3 (K_0E_0) and the other treatments (1, 2 and 4) using the LSD test: 0.60 $t_{0.05}$ (t=2.179, df=12, r=5 and 0.19 MSE) and 0.84 $t_{0.01}$ (t=3.055, df=12, r=5 and 0.19 MSE).

Treatment		Mean Albumin concentration[b] g/dl	Difference from treatment 3[d] g/dl
1	K_1E_0	3.58	0.51^{ns}
4	K_0E_1	3.05	0.01^{ns}
2	K_1E_1	3.45	0.38^{ns}
1 and 2[a]	(K_1E_0 & K_1E_1)	3.51[c]	0.45^{*}
4 and 2[a]	(K_0E_1 & K_1E_1)	3.25[c]	0.18^{ns}
3	K_0E_0	3.07	

[a]Difference compared with LSD of 0.42 $t_{0.05}$ and 0.60 $t_{0.01}$ calculated by $sd = (s^2/r)^{1/2}$. [b]Average of five replications. [c]Average of two means. [d]** = significant at 1% level, ns = not significant

XXV ANOVA FOR GROWING PIGS ALBUMIN AT 8 HOURS POST INJECTI N

(a) Blood Albumin concentrations (g/dl).

	Block/Litter					Treatment	
	Red	White	Yellow	Orange	Green	Total	Mean
	Rep I	Rep II	Rep III	Rep IV	Rep V	(T)	( )
Treatment							
1 $K_1 E_0$	3.83	2.33	2.93	3.67	3.17	15.93	3.186
2 K_1E_1	4.17	2.92	3.67	3.50	3.17	17.43	3.486
3 $K_0 E_0$	3.67	3.00	2.92	2.79	3.50	15.88	3.176
4 $K_0 E_1$	2.00	2.75	2.93	2.75	3.83	14.26	2.852
Block total	13.67	11.00	12.45	12.71	13.67	63.50	
Block Mean	3.4175	2.75	3.1125	3.1775	3.4175		3.175

(b) The ASTFP X Endotoxin table of totals for blood albumin (g/dl).

Endotoxin	Albumin Totals (A X B)		Endotoxin total
	K_0	K_1	(B)
E_0	15.88	15.93	31.81
E_1	14.26	17.43	31.69
ASTFP Total (A)	30.14	33.36	63.5

(c) Analysis of variance for blood albumin.[a]

Source of variance	Degree of freedom	Sum of Squares	Mean Squares	Computed F[b]	Tabular F 5%	Tabular F 1%
Total	19	5.6793				
Blocks (liter)	4	1.2086	0.30215	1.046455^{ns}	3.26	5.41
Treatment	3	1.00586	0.335287	1.161220^{ns}	3.49	5.95
ASTFP (A)	1	0.51842	0.51842	1.795477^{ns}	4.75	9.33
Endotoxin(B)	1	0.00072	0.00072	0.002494^{ns}	4.75	9.33
A X B	1	0.48672	0.48672	1.685688^{ns}	4.75	9.33
Error	12	3.46484	0.288737			

[a] cv = 17.22%

[b] ns = not significant

XXVI ANOVA FOR GROWING PIGS ALBUMIN AT 24 HOURS POST INJECTION

(a) Blood Albumin concentrations (g/dl).

	Block/Litter					Treatment	
	Red Rep I	White Rep II	Yellow Rep III	Orange Rep IV	Green Rep V	Total (T)	Mean ( )
Treatment							
1 $K_1 E_0$	2.83	2.36	2.75	3.17	2.75	13.86	2.772
2 K_1E_1	4.14	2.64	3.43	5.21	2.71	18.13	3.626
3 $K_0 E_0$	1.83	2.75	3.92	2.75	3.17	14.42	2.884
4 $K_0 E_1$	1.79	3.33	2.33	2.67	2.67	12.79	2.558
Block total	10.59	11.08	12.43	13.8	11.3	59.2	59.2
Block Mean	2.6475	2.77	3.1075	3.45	2.825	59.2	2.96

(b) The ASTFP X Endotoxin table of totals for blood albumin (g/dl).

Endotoxin	Albumin Totals (A X B)		Endotoxin total (B)
	K_0	K_1	
E_0	14.42	13.86	28.28
E_1	12.79	18.13	30.92
ASTFP Total (A)	27.21	31.99	59.2

(c) Analysis of variance for blood albumin.[a]

Source of variance	Degree of freedom	Sum of Squares	Mean Squares	Computed F[b]	Tabular F 5%	Tabular F 1%
Total	19	11.7516				
Blocks (liter)	4	1.65535	0.413837	0.723403^{ns}	3.26	5.41
Treatment	3	3.2314	1.077133	1.882867^{ns}	3.49	5.95
ASTFP (A)	1	1.14242	1.14242	1.996990^{ns}	4.75	9.33
Endotoxin(B)	1	0.34848	0.34848	0.609155^{ns}	4.75	9.33
A X B	1	1.7405	1.7405	3.042455^{ns}	4.75	9.33
Error	12	6.86485	0.572071			

[a] cv = 26.57%

[b] ^{ns} = not significant

XXVII. ANOVA FOR GROWING PIGS GLUCOSE AT 0 HOURS POST INJECTI N

(a) Blood Glucose concentrations (mg/dl).

	Block/Litter					Treatment	
	Red	White	Yellow	Orange	Green	Total	Mean
	Rep I	Rep II	Rep III	Rep IV	Rep V	(T)	( )
Treatment							
1 $K_1 E_0$	18.18	18.18	22.73	22.73	18.18	100.00	20.00
2 K_1E_1	22.73	22.73	22.73	18.18	18.18	104.55	20.91
3 $K_0 E_0$	18.18	09.09	22.73	22.73	18.18	90.91	18.18
4 $K_0 E_1$	18.18	18.18	13.36	18.18	18.18	86.08	17.22
Block total	77.27	68.18	81.55	81.82	72.72	381.54	
Block Mean	19.3175	17.045	20.3875	20.455	18.18		19.077

(b) The ASTFP X Endotoxin table of totals for blood glucose (mg/dl).

Endotoxin	Glucose Totals (A X B)		Endotoxin total
	K_0	K_1	(B)
E_0	90.91	100.00	190.91
E_1	86.08	104.55	190.63
ASTFP Total (A)	176.99	204.55	381.54

(c) Analysis of variance for blood glucose above.[a]

Source of variance	Degree of freedom	Sum of Squares	Mean Squares	Computed F[b]	Tabular F 5%	Tabular F 1%
Total	19	234.6858				
Blocks (liter)	4	34.43107	8.607767	0.654277^{ns}	3.26	5.41
Treatment	3	42.38082	14.12694	1.073789^{ns}	3.49	5.95
ASTFP (A)	1	37.97768	37.97768	2.886684^{ns}	4.75	9.33
Endotoxin(B)	1	0.00392	0.00392	0.000298^{ns}	4.75	9.33
A X B	1	4.39922	4.39922	0.334385^{ns}	4.75	9.33
Error	12	157.8739	13.15616			

[a] cv = 18.42%

[b] ns = not significant

XXVIII ANOVA FOR GROWING PIGS GLUCOSE AT 2 HOURS POST INJECTION

(a) Blood Glucose concentrations (mg/dl).

	Block/Litter					Treatment	
	Red Rep I	White Rep II	Yellow Rep III	Orange Rep IV	Green Rep V	Total (T)	Mean ( )
Treatment							
1 $K_1 E_0$	13.64	22.73	18.18	22.73	13.64	90.92	18.184
2 K_1E_1	13.64	9.09	4.54	18.18	18.18	63.63	12.726
3 $K_0 E_0$	18.18	22.73	13.64	22.73	18.18	95.46	19.092
4 $K_0 E_1$	22.73	18.18	18.18	22.73	22.73	104.55	20.91
Block total	68.19	72.73	54.54	86.37	72.73	354.56	354.56
Block Mean	17.0475	18.1825	13.635	21.5925	18.1825	354.56	17.728

(b) The ASTFP X Endotoxin table of totals for blood glucose (mg/dl).

Endotoxin	Glucose Totals (A X B)		Endotoxin total (B)
	K_0	K_1	
E_0	95.46	90.92	186.38
E_1	104.55	63.63	168.18
ASTFP Total (A)	200.01	154.55	354.56

(c) Analysis of variance for blood glucose.[a]

Source of variance	Degree of freedom	Sum of Squares	Mean Squares	Computed F[b]	Tabular F 5%	Tabular F 1%
Total	19	491.9555				
Blocks (liter)	4	130.2529	32.56323	2.224837^{ns}	3.26	5.41
Treatment	3	186.0678	62.0226	4.237607*	3.49	5.95
ASTFP (A)	1	103.3306	103.3306	7.059916*	4.75	9.33
Endotoxin(B)	1	16.562	16.562	1.131575^{ns}	4.75	9.33
A X B	1	66.17522	66.17522	4.521329^{ns}	4.75	9.33
Error	12	175.6348	14.63623			

[a] cv = 28.70% [b] * = significant at 5% level, ^{ns} = not significant

(d) Comparison between mean blood glucose for treatment 3 (K_0E_0) and the other treatments (1, 2 and 4) using the LSD test: 5.27 $t_{0.05}$ (t=2.179, df=12, r=5 and 14.64 MSE) and 7.39 $t_{0.01}$ (t=3.055, df=12, r=5 and 14.64 MSE).

Treatment		Mean Glucose concentration[b] mg/dl	Difference from treatment 3[d] mg/dl
1	K_1E_0	18.18	0.91^{ns}
4	K_0E_1	20.91	1.82^{ns}
2	K_1E_1	12.73	6.37*
1 and 2[a]	(K_1E_0 & K_1E_1)	15.46[c]	3.64^{ns}
4 and 2[a]	(K_0E_1 & K_1E_1)	16.82[c]	2.27^{ns}
3	K_0E_0	19.09	-

[a]Difference compared with LSD of 3.73 $t_{0.05}$ and 5.22 $t_{0.01}$ calculated by sd = $(s^2/r)^{1/2}$. [b]Average of five replications. [c]Average of two means. [d]* = significant at 5% level, ^{ns} = not significant

XXIX ANOVA FOR GROWING PIGS GLUCOSE AT 4 HOURS POST INJECTION.

(a) Blood Glucose concentrations (mg/dl).

	Block/Litter					Treatment	
	Red Rep I	White Rep II	Yellow Rep III	Orange Rep IV	Green Rep V	Total (T)	Mean ( )
Treatment							
1 $K_1 E_0$	54.54	73.73	68.18	77.27	77.27	350.99	70.198
2 K_1E_1	63.64	54.54	54.54	59.09	68.18	299.99	59.998
3 $K_0 E_0$	63.64	63.63	50	59.09	68.18	304.54	60.908
4 $K_0 E_1$	40.91	54.54	50	59.09	68.18	272.72	54.544
Block total	222.73	246.44	222.72	254.54	281.81	1228.24	1228.24
Block Mean	55.6825	61.61	55.68	63.635	70.4525	1228.24	61.412

(b) The ASTFP X Endotoxin table of totals for blood glucose (mg/dl).

Endotoxin	Glucose Totals (A X B)		Endotoxin total (B)
	K_0	K_1	
E_0	304.54	350.99	655.53
E_1	272.72	299.99	572.71
ASTFP Total (A)	577.26	650.98	1228.24

(c) Analysis of variance for blood glucose.[a]

Source of variance	Degree of freedom	Sum of Squares	Mean Squares	Computed F[b]	Tabular F 5%	Tabular F 1%
Total	19	1738.628				
Blocks (liter)	4	609.5783	152.3946	3.687211*	3.26	5.41
Treatment	3	633.0832	211.0277	5.10585*	3.49	5.95
ASTFP (A)	1	271.7319	271.7319	6.574598*	4.75	9.33
Endotoxin(B)	1	342.9576	342.9576	8.297916*	4.75	9.33
A X B	1	18.39362	18.39362	0.445037ns	4.75	9.33
Error	12	495.9669	41.33057			

[a] cv = 15.58% [b] * = significant at 5% level, ns = not significant

(d) Comparison between mean blood glucose for treatment 3 (K_0E_0) and the other treatments (1, 2 and 4) using the LSD test: 8.86 $t_{0.05}$ (t=2.179, df=12, r=5 and 41.33 MSE) and 12.42 $t_{0.01}$ (t=3.055, df=12, r=5 and 41.33 MSE).

Treatment		Mean Glucose concentration[b] mg/dl	Difference from treatment 3[d] mg/dl
1	K_1E_0	70.198	9.29*
4	K_0E_1	54.544	6.36ns
2	K_1E_1	59.998	0.91ns
1 and 2[a]	(K_1E_0 & K_1E_1)	65.098[c]	4.19ns
4 and 2[a]	(K_0E_1 & K_1E_1)	57.271[c]	3.64ns
3	K_0E_0	60.908	

[a]Difference compared with LSD of 6.26 $t_{0.05}$ and 8.78 $t_{0.01}$ calculated by sd = $(s^2/r)^{1/2}$. [b]Average of five replications. [c]Average of two means. [d]* = significant at 5% level, ns = not significant

XXX ANOVA FOR GROWING PIGS GLUCOSE AT 8 HOURS POST INJECTI N

(a) Blood Glucose concentrations (mg/dl).

	Block/Litter					Treatment	
	Red Rep I	White Rep II	Yellow Rep III	Orange Rep IV	Green Rep V	Total (T)	Mean ( )
Treatment							
1 $K_1 E_0$	54.54	59.09	54.54	54.54	68.18	290.89	58.178
2 K_1E_1	54.54	68.18	68.18	40.91	54.54	286.35	57.27
3 $K_0 E_0$	54.54	68.18	54.54	45.45	40.91	263.62	52.724
4 $K_0 E_1$	68.18	68.18	90.91	40.91	31.82	300.00	60.00
Block total	231.8	263.63	268.17	181.81	195.45	1140.86	
Block Mean	57.95	65.9075	67.0425	45.4525	48.8625		57.043

(b) The ASTFP X Endotoxin table of totals for blood glucose (mg/dl).

Endotoxin	Glucose Totals (A X B)		Endotoxin total (B)
	K_0	K_1	
E_0	263.62	290.89	554.51
E_1	300	286.35	586.35
ASTFP Total (A)	563.62	577.24	1140.86

(c) Analysis of variance for blood glucose.[a]

Source of variance	Degree of freedom	Sum of Squares	Mean Squares	Computed F[b]	Tabular F 5%	Tabular F 1%
Total	19	3490.634				
Blocks (liter)	4	1522.609	380.6523	2.503827^{ns}	3.26	5.41
Treatment	3	143.6868	47.89561	0.315044^{ns}	3.49	5.95
ASTFP (A)	1	9.27522	9.27522	0.06101^{ns}	4.75	9.33
Endotoxin(B)	1	50.68928	50.68928	0.33342^{ns}	4.75	9.33
A X B	1	83.72232	83.72232	0.550703^{ns}	4.75	9.33
Error	12	1824.338	152.0282			

[a] cv = 23.76%

[b] ^{ns} = not significant

XXXI ANOVA FOR GROWING PIGS GLUCOSE AT 24 HOURS POST INJECT ON

(a) Blood Glucose concentrations (mg/dl).

	Block/Litter					Treatment	
	Red Rep I	White Rep II	Yellow Rep III	Orange Rep IV	Green Rep V	Total (T)	Mean ( )
Treatment							
1 $K_1 E_0$	54.54	36.36	50	54.54	63.64	259.08	51.816
2 K_1E_1	50	63.64	63.64	68.18	77.27	322.73	64.546
3 $K_0 E_0$	50	50	40.91	45.45	59.09	245.45	49.09
4 $K_0 E_1$	50	54.54	77.27	36.36	63.64	281.81	56.362
Block total	204.54	204.54	231.82	204.53	263.64	1109.07	
Block Mean	51.135	51.135	57.955	51.1325	65.91		55.4535

(b) The ASTFP X Endotoxin table of totals for blood glucose (mg/dl).

	Glucose Totals (A X B)		Endotoxin total (B)
Endotoxin	K_0	K_1	
E_0	245.45	259.08	504.53
E_1	281.81	322.73	604.54
ASTFP Total (A)	527.26	581.81	1109.07

(c) Analysis of variance for blood glucose.[a]

Source of variance	Degree of freedom	Sum of Squares	Mean Squares	Computed F[b]	Tabular F 5%	Tabular F 1%
Total	19	2587.096				
Blocks (liter)	4	686.2633	171.5658	1.694881^{ns}	3.26	5.41
Treatment	3	686.1223	228.7074	2.259377^{ns}	3.49	5.95
ASTFP (A)	1	148.7851	148.7851	1.469833^{ns}	4.75	9.33
Endotoxin(B)	1	500.1	500.1	4.940437^{*}	4.75	9.33
A X B	1	37.2372	37.2372	0.367863^{ns}	4.75	9.33
Error	12	1214.71	101.2259			

[a] cv = 21.04% [b] * = significant at 5% level, [ns] = not significant

(d) Comparison between mean blood glucose for treatment 3 (K_0E_0) and the other treatments (1, 2 and 4) using the LSD test: 13.86 $t_{0.05}$ (t=2.179, df=12, r=5 and 101.22MSE) and 19.44 $t_{0.01}$ (t=3.055, df=12, r=5 and 101.22MSE).

Treatment		Mean Glucose concentration[b] mg/dl	Difference from treatment 3[d] mg/dl
1	K_1E_0	51.816	2.73^{ns}
4	K_0E_1	56.362	7.27^{ns}
2	K_1E_1	64.546	15.46^{*}
1 and 2[a]	(K_1E_0 & K_1E_1)	58.181[c]	9.09^{ns}
4 and 2[a]	(K_0E_1 & K_1E_1)	60.454[c]	11.36^{*}
3	K_0E_0	49.09	-

[a]Difference compared with LSD of 9.80 $t_{0.05}$ and 13.75 $t_{0.01}$ calculated by $sd = (s^2/r)^{1/2}$. [b]Average of five replications. [c]Average of two means. [d]* = significant at 5% level, [ns] = not significant

XXXII. ANOVA FOR GROWING PIGS CHOLESTEROL AT 0 HOURS POST INJECTION.

(a) Blood Cholesterol concentrations (mg/dl).

	Block/Litter					Treatment	
	Red	White	Yellow	Orange	Green	Total	Mean
	Rep I	Rep II	Rep III	Rep IV	Rep V	(T)	( )
Treatment							
1 $K_1 E_0$	171.32	171.32	114.29	114.29	100.00	671.22	134.244
2 K_1E_1	137.50	157.14	114.29	125.00	137.50	671.43	134.286
3 $K_0 E_0$	142.86	114.29	157.14	100.00	114.29	628.58	125.716
4 $K_0 E_1$	137.5	157.14	128.57	114.29	125.00	662.5	132.5
Block total	589.18	599.89	514.29	453.58	476.79	2633.73	
Block Mean	147.295	149.9725	128.5725	113.395	119.1975		131.6865

(b) The ASTFP X Endotoxin table of totals for blood cholesterol (mg/dl).

Endotoxin	Cholesterol Totals (A X B)		Endotoxin total
	K_0	K_1	(B)
E_0	628.58	671.22	1299.8
E_1	662.5	671.43	1333.93
ASTFP Total (A)	1291.08	1342.65	2633.73

(c) Analysis of variance for blood cholesterol above.[a]

Source of variance	Degree of freedom	Sum of Squares	Mean Squares	Computed F[b]	Tabular F 5%	Tabular F 1%
Total	19	9234.537				
Blocks (liter)	4	4313.017	1078.254	2.768608[ns]	3.26	5.41
Treatment	3	248.0343	82.6781	0.212291[ns]	3.49	5.95
ASTFP (A)	1	132.9732	132.9732	0.341432[ns]	4.75	9.33
Endotoxin(B)	1	58.24285	58.24285	0.149549[ns]	4.75	9.33
A X B	1	56.81821	56.81821	0.145891[ns]	4.75	9.33
Error	12	4673.486	389.4572			

[a] cv = 16.74%

[b] [ns] = not significant at 5% level

XXXIII ANOVA FOR GROWING PIGS CHOLESTEROL AT 2 HOURS POST INJECTION

(a) Blood Cholesterol concentrations (mg/dl).

	Block/Litter					Treatment	
	Red	White	Yellow	Orange	Green	Total	Mean
	Rep I	Rep II	Rep III	Rep IV	Rep V	(T)	( )
Treatment							
1 $K_1 E_0$	157.14	71.43	114.29	128.57	114.29	585.72	117.14
2 K_1E_1	114.29	142.86	187.50	137.50	125.00	707.15	141.43
3 $K_0 E_0$	128.57	100.00	137.50	157.14	100.00	623.21	124.64
4 $K_0 E_1$	128.57	128.57	175.00	137.50	114.29	683.93	136.79
Block total	528.57	442.86	614.29	560.71	453.58	2600.01	
Block Mean	132.1425	110.715	153.5725	140.1775	113.395		130.0005

(b) The ASTFP X Endotoxin table of totals for blood cholesterol (mg/dl).

Endotoxin	Cholesterol Totals (A X B)		Endotoxin total
	K_0	K_1	(B)
E_0	623.21	585.72	1208.93
E_1	683.93	707.15	1391.08
ASTFP Total (A)	1307.14	1292.87	2600.01

(c) Analysis of variance for blood cholesterol.[a]

Source of variance	Degree of freedom	Sum of Squares	Mean Squares	Computed F[b]	Tabular F 5%	Tabular F 1%
Total	19	13389.38				
Blocks (liter)	4	5245.887	1311.472	2.501975^{ns}	3.26	5.41
Treatment	3	1853.398	617.7993	1.178614^{ns}	3.49	5.95
ASTFP (A)	1	10.18165	10.18165	0.019424^{ns}	4.75	9.33
Endotoxin(B)	1	1658.931	1658.931	3.164845^{ns}	4.75	9.33
A X B	1	184.2852	184.2852	0.351572^{ns}	4.75	9.33
Error	12	6290.094	524.1745			

[a] cv = 20.42%

[b] ^{ns} = not significant

XXXIVANOVA FOR GROWING PIGS CHOLESTEROL AT 4 HOURS POST INJECTION

(a) Blood Cholesterol concentrations (mg/dl).

	Block/Litter					Treatment	
	Red Rep I	White Rep II	Yellow Rep III	Orange Rep IV	Green Rep V	Total (T)	Mean ( )
Treatment							
1 $K_1 E_0$	142.86	185.71	157.14	157.14	114.29	757.14	151.43
2 K_1E_1	75.00	75.00	157.14	114.29	185.71	607.14	121.43
3 $K_0 E_0$	128.57	85.71	85.71	114.29	114.29	528.57	105.71
4 $K_0 E_1$	100.00	114.29	112.50	112.50	87.50	526.79	105.36
Block total	446.43	460.71	512.49	498.22	501.79	2419.64	
Block Mean	111.601	115.18	128.12	124.56	125.45		120.98

(b) The ASTFP X Endotoxin table of totals for blood cholesterol (mg/dl).

Endotoxin	Cholesterol Totals (A X B)		Endotoxin total (B)
	K_0	K_1	
E_0	528.57	757.14	1285.71
E_1	526.79	607.14	1133.93
ASTFP Total (A)	1055.36	1364.28	2419.64

(c) Analysis of variance for blood cholesterol.[a]

Source of variance	Degree of freedom	Sum of Squares	Mean Squares	Computed F[b]	Tabular F 5%	Tabular F 1%
Total	19	21483.86				
Blocks (liter)	4	821.0689	205.2672	0.180575^{ns}	3.26	5.41
Treatment	3	7021.895	2340.632	2.059071^{ns}	3.49	5.95
ASTFP (A)	1	4771.578	4771.578	4.197593^{ns}	4.75	9.33
Endotoxin(B)	1	1151.858	1151.858	1.013298^{ns}	4.75	9.33
A X B	1	1098.458	1098.458	0.966322^{ns}	4.75	9.33
Error	12	13640.9	1136.742			

[a] cv = 27.79%

[b] ^{ns} = not significant

XXXV ANOVA FOR GROWING PIGS CHOLESTEROL AT 8 HOURS POST INJECTION.

(a) Blood Cholesterol concentrations (mg/dl).

	Block/Litter					Treatment	
	Red Rep I	White Rep II	Yellow Rep III	Orange Rep IV	Green Rep V	Total (T)	Mean ( )
Treatment							
1 $K_1 E_0$	128.57	157.14	162.5	114.29	100.00	662.5	132.5
2 K_1E_1	85.71	57.14	128.57	85.71	100.00	457.13	91.426
3 $K_0 E_0$	150.00	114.29	100.00	185.71	171.43	721.43	144.286
4 $K_0 E_1$	100.00	128.57	128.57	171.43	75.00	603.57	120.714
Block total	464.28	457.14	519.64	557.14	446.43	2444.63	2444.63
Block Mean	116.07	114.28	129.91	139.28	111.61	2444.63	122.23

(b) The ASTFP X Endotoxin table of totals for blood cholesterol (mg/dl).

Endotoxin	Cholesterol Totals (A X B)		Endotoxin total
	K_0	K_1	(B)
E_0	721.43	662.50	1383.93
E_1	603.57	457.13	1060.70
ASTFP Total (A)	1325.00	1119.63	2444.63

(c) Analysis of variance for blood cholesterol.[a]

Source of variance	Degree of freedom	Sum of Squares	Mean Squares	Computed F[b]	Tabular F 5%	Tabular F 1%
Total	19	23880.91				
Blocks (liter)	4	2255.046	563.7615	0.486342[ns]	3.26	5.41
Treatment	3	7715.623	2571.874	2.218689[ns]	3.49	5.95
ASTFP (A)	1	2108.842	2108.842	1.819243[ns]	4.75	9.33
Endotoxin(B)	1	5223.882	5223.882	4.506507[ns]	4.75	9.33
A X B	1	382.9	382.9	0.330318[ns]	4.75	9.33
Error	12	13910.24	1159.186			

[a] cv = 29.00%

[b] [ns] = not significant 5% level

XXXVI ANOVA FOR GROWING PIGS CHOLESTEROL AT 24 HOURS POST INJECTION.

(a) Blood Cholesterol concentrations (mg/dl).

	Block/Litter					Treatment	
	Red	White	Yellow	Orange	Green	Total	Mean
	Rep I	Rep II	Rep III	Rep IV	Rep V	(T)	( )
Treatment							
1 $K_1 E_0$	100	171	142.86	125	100	638.86	127.772
2 K_1E_1	100	71.14	114.29	157.14	85.71	528.28	105.656
3 $K_0 E_0$	142.57	128.557	125	157.14	100	653.267	130.6534
4 $K_0 E_1$	85.71	142.86	128.57	112.5	100	569.64	113.928
Block total	428.28	513.557	510.72	551.78	385.71	2390.047	
Block Mean	107.07	128.3893	127.68	137.945	96.4275		119.5024

(b) The ASTFP X Endotoxin table of totals for blood cholesterol (mg/dl).

Endotoxin	Cholesterol Totals (A X B)		Endotoxin total
	K_0	K_1	(B)
E_0	653.267	638.86	1292.127
E_1	569.64	528.28	1097.92
ASTFP Total (A)	1222.907	1167.14	2390.047

(c) Analysis of variance for blood cholesterol.[a]

Source of variance	Degree of freedom	Sum of Squares	Mean Squares	Computed F[b]	Tabular F 5%	Tabular F 1%
Total	19	13933.8				
Blocks (liter)	4	4691.977	1172.994	1.964764^{ns}	3.26	5.41
Treatment	3	2077.639	692.5464	1.160015^{ns}	3.49	5.95
ASTFP (A)	1	155.4979	155.4979	0.260459^{ns}	4.75	9.33
Endotoxin(B)	1	1885.818	1885.818	3.158743^{ns}	4.75	9.33
A X B	1	36.32321	36.32321	0.060841^{ns}	4.75	9.33
Error	12	7164.183	597.0152			

[a] cv = 22.66%

[b] ^{ns} = not significant at 5% level

APPENDIX D *KIGELIA AFRICANA* IDENTIFICATION CERTIFICATE.

THE UNIVERSITY OF ZAMBIA
DEPARTMENT OF BIOLOGICAL SCIENCES

Telephone: 291777/8 or Direct: 291531
Telegrams: UNZA LUSAKA
Telex:UNZALUZA44370
Fax + 260-1-253952

P.O. BOX 32379
Lusaka.
Zambia.

Your Ref:
Our Ref:

IDENTIFICATION RESULTS

DATE: 7/05/09

Name of specimen(s):

1. *Kigellia Africana*, Family. Biginoniaceae

Number of specimens: One (1).

Identified by: Mr. H. Zulu.

Designation: Herbarium Technician.

Signature: .....

Stamp