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KUMASI, GHANA

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COLLEGE OF AGRICULTURE AND NATURAL RESOURCES

NUTRITIVE VALUE OF VARIOUSLY PROCESSED RUBBER SEED MEALS: GROWTH AND REPRODUCTIVE PERFORMANCE STUDIES USING THE LABORATORY RAT AS MODEL FOR PIGS

A THESIS PRESENTED TO THE SCHOOL OF GRADUATE STUDIES IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF PHILOSOPHY (MPhil) REPRODUCTIVE PHYSIOLOGY DEGREE

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SEPTEMBER, 2015

DECLARATION

I hereby certify that this research was carried out by me and that this thesis is entirely my own account of the research. The work has not been submitted to any other University for a degree. However, works of other researchers and authors which served as sources of information have duly been acknowledged.

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DEDICATION

Dedicated to the memory of my late father, Mr. William E. A. Farr, for his love, toil and sweat.

ACKNOWLEDGEMENTS

First and foremost, my indebtedness and sincere thanks are extended to my supervisors, Prof. Armstrong Donkoh and Dr. Michael Boateng for their guidance, assistance and encouragement throughout this study, Special thanks to Prof. D.B. Okai, Prof, S.A. Osei, Prof. (Mrs,) Comfort C. Atuahene, Prof. E.L.K. Osafo, all of the Department of Animal Science, and Dr. J. SarkodieAddo of the Crop and Soil Sciences Department, KNUST for their enthusiastic support throughout my studies in Ghana.

Appreciative thanks are also due to: Mr. Joseph Bentil of the Department of Animal Science, Mr. Samuel Joe Acquah of the Department of Crop and Soil Sciences and Mr. Prince Manu-Barfo of EVONIK Industries for their assistance with chemical analyses of the rubber seed meals. Mr. Gariba G. A. Laarey and Mr. F.A. Kwarteng for their help in blood sampling and analyses of the cellular and biochemical indices. Dr. Kwasi Boadu Mensah and the staff of the KNUST Faculty of Pharmacy and Pharmaceutical Sciences Animal House, especially Mr. Thomas Ansah, for provision of facilities and assistance with the care of the laboratory rats. Prof. P.K. Karikari is appreciated for his assistance in the statistical analyses of data.

The assistance of Mr. Bright Kwame Amponsah (a.k.a Obvious) and other post-graduate students, and members of staff of the Department of Animal Science is gratefully acknowledged.

I acknowledge with gratitude the scholarship provided by the RTI/USAID Excellence in Higher Education for Liberian Development (EHELD) programme. Furthermore, I am indebted to the Donkoh family in Ghana especially Mr. Ebenezer Quartey (Achaana), and to Mr. Thomas Cisco in Liberia, and the Farr family in Liberia and all my friends for their encouragement and support during these years of graduate study. Finally, I thank God for giving my life its direction.

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LIST OF ABBREVIATIONS

ACIAR	Australian Centre for International Agricultural Research
AOAC	Association of Official Analytical Chemists
BRSM	Boiled Rubber Seed Meal
FAO	Food and Agriculture Organization
Hb	Haemoglobin
HCT	Haematocrit
MCH	Mean Cell Haemoglobin
MCHC	Mean Cell Haemoglobin Concentration
MCV	Mean Cell Volume
NRC	National Research Council
PLT	Platelet
RBC	Red Blood Cells
RRSM	Raw Rubber Seed Meal
RoRSM	Roasted Rubber Seed Meal
SDRSM	Sun-Dried Rubber Seed Meal
SRSM	Soaked Rubber Seed Meal
UNIDO	United Nations Industrial Development Organisation
UFAW	Universities Federation for Animal Welfare
WBC	White Blood Cells

ABSTRACT

The study involved evaluating methods of processing raw rubber seeds and the nutritional quality of the processed rubber seed meals as dietary ingredients using the laboratory rat, with specific

application to the growing pig in terms of growth and reproductive performance. The work was conducted in four separate experiments.

The first experiment aimed at evaluating four simple methods of processing in terms of chemical compositions and energy values of the resultant rubber seed meals. The raw rubber seeds were de-hulled, partially sun-dried for 24 h and divided into five lots, each lot receiving one of four processing methods, namely; sun drying, soaking in water, boiling in water and roasting in addition to the raw or unprocessed rubber seed. The method of processing significantly ($P< 0.05$) affected the proximate compositions, the mineral contents, amino acid profiles and the metabolizable energy concentrations of the resultant rubber seed meals. Despite its potential as a source of protein for animals, fresh rubber seed meal was shown to contain a toxic factor, hydrogen cyanide (60 mg/100g DM). Imposition of the various processing methods, however, significantly reduced the hydrogen cyanide contents in the resultant rubber seed meals, with the boiled RSM registering the lowest HCN content. The second experiment determined the growth performance and physiological parameters of laboratory rats fed the variously processed rubber seed meals (RSM). Thirty six growing rats (18 males and 18 females) were randomly allotted into 6 groups and were fed a control diet with no RSM and 5 other diets containing 100 g of the raw RSM and the 4 resultant processed rubber seed meals. The feeding of diets incorporating 100 g of the various types of rubber seed meals had no significant ($P > 0.05$) effect on feed intake, body weight gain and feed conversion efficiency, as compared to rubber seed meal-free
(control) diet. However, water consumption by rats was significantly ($P< 0.05$) influenced by the dietary treatments. No deaths or health-related problems were recorded during the course of the study. Dietary treatments had significant ($P< 0.05$) impact on relative weights of the liver, heart and lungs but not on the kidney, spleen and intestinal weights. Treatment differences in blood

cellular elements and biochemical indices were not significant (P> 0.05), except the WBC count, MCV value and blood sugar levels. With the exception of the RoRSM diet, cost per gram feed and feed cost per gram live weight gain were slightly reduced when the rubber seed meals were used, Seasonal variations in the prices of feedstuffs such as maize and soyabean meal would make the use of alternative feedstuffs such rubber seed meal in animal diets more attractive In experiment three, the use of rat as a model animal for allowing the determination of the reproductive performance in the pig, was evaluated. Eighteen female rats with an average initial body weight of 153.9 g were randomly allotted into 6 groups and fed a control diet with no RSM and 5 other diets containing 100 g each of the raw RSM and the 4 resultant processed rubber seed meals. With the exception of rats on the RoRSM diet, all other rats recorded successful mating and pregnancies. There were no significant (P> 0.05) difference in the gestation length and the pregnant rats delivered normally. No external malformations were observed in any of the pups delivered. No significant (P> 0.05) differences in the maternal body weight, litter size, pup birth weight and number of pups weaned were observed. However, dietary treatments significantly (P< 0.05) influenced weaning weight and post-natal mortality of pups, with those on the control and the SDRSM diets recording the highest mortalities. The present findings indicate that the various types of rubber seed meals evaluated in this study, do not pose any significant reproductive toxicity or complications in pregnancy and delivery in rats.

The final experiment generated data on the effect of graded levels of the boiled-RSM (BRSM), identified to be the best quality rubber seed meal from the three initial studies, on growth performance and economy of gain of rats. Twenty four rats (12 males and 12 females) were randomly selected and allocated to 4 dietary treatments containing varying amounts of BRSM (0, 50, 100 and 150 g RSM kg^{-1}) in a complete randomized design such that there were 6 rats (3 males

and 3 females) with one rat per replicate. Feed and water were provided *ad libitum* for 4 weeks. The addition of the graded levels of the BRSM to rat diets significantly ($P< 0.05$) influenced feed intake and water consumption. However, the inclusion of BRSM in diets had no significant ($P> 0.05$) effect on body weight gain and efficiency of feed utilisation. In addition, there were no health-related problems nor mortalities attributable to level of BRSM in the diet. Examination of several organs at the termination of the 4-week study revealed no macroscopic deviation from the normal in terms of gross tissue changes. Also, dietary BRSM had no significant effect on the relative organ weights of the experimental animals. The cost per gram of feed declined as more BRSM was included to replace soya bean meal. The diet that contained the highest amount of BRSM was cheaper. Furthermore, feed cost per gram live weight gain was lowest for rats on the 150 g kg^{-1} diet and highest for the 50 g kg^{-1} diet. Inclusion of 150 g BRSM kg^{-1} diet might be beneficial in terms of cost effectiveness.

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CHAPTER ONE

1.0 GENERAL INTRODUCTION

The ultimate goal of animal production is to supply consumers with reasonably priced meat and meat products. Human beings require protein for growth, development, reproduction and to be able to fight and resist infection, because animal protein contains the essential amino acids needed for these purposes. However, inadequate food supply is a major challenge in the developing countries of the world especially in the humid tropics, where the level of animal protein consumption is estimated to be lower than the recommended daily requirement. According to the Food and Agriculture Organization (FAO, 2002), a third of humanity suffers from quantitative malnutrition. This is critical in Africa where the daily protein intake of eleven (11) grams is far from the physiological minimum of thirty (30) grams as recommended by the FAO (2002). Proteins of animal origin are known to be superior in quality than those of plant origin.

Pig production is one of the animal production sectors which have the potential to alleviate this shortage of protein intake, however, there are many factors limiting production. One of these factors is inadequate supply of feed. The cost of feeding has been reported to represent 70 - 80% of the total variable cost of commercial swine production (Adesehinwa, 2007). The economics of swine feeding are largely dependent on the local condition of feedstuff availability and competition for the same feedstuff for use by either humans or animals. In a bid to combat or reduce the competition between man and animals for the same feed resources, agro-industrial by-products and crop residues have been recommended for incorporation into swine diets (Adesehinwa, 2007; Trujillo, 2009). Agro-industrial by-products and crop residues represent a vast animal feed resource, which are yet to be exploited. One of such agro-industrial by-products or non-conventional feedstuffs is rubber seed meal which is currently under-exploited or

underutilized particularly in countries in West Africa such as Liberia and Ghana where large quantities of rubber seeds are produced annually and generally allowed to go to waste.

There is, however, paucity of information on the nutritive quality of the rubber seeds available in West Africa particularly, Liberia and Ghana. In addition, new non-conventional feedstuffs can be and are included in formulated feed, although the necessary information on nutritional composition and deleterious factors may not be clearly understood. The use of these materials may therefore produce unpredictable, often negative effects, on performance either in terms of nutrient digestion or in terms of anti-nutritive effects on growth and reproductive performance.

1.1 PROBLEM STATEMENT.

Processing and utilization of available local non-conventional feedstuffs have an especially important significance in reducing anti-nutritive factors and enhancing the nutritional value. Rubber seed meal is a suitable choice for the above-mentioned purpose. Rubber seed is abundant in Liberia and other West African countries where thousands of tons are produced and only a tiny fraction is used for nursery purposes, and abandoning the rest to rot or germinate under plantation trees. This underutilized product could be used as valuable protein source for animal production in areas where the seeds are readily available.

Nwokolo (1990) indicated that rubber seed meal is a protein-rich feed ingredient which can supply essential amino acids for pigs. Studies on the use of rubber seed meal to replace high quality protein sources such as soybeans have been undertaken. In conclusion to their study, Stosic and Kaykay (1981) reported that rubber seed meal was a very useful substitute in broiler chickens and higher in total nutrients compared to maize. Babatunde and Pond (1990) worked on the nutritive value of rubber seed meal, feeding semi-purified diets of rubber seed meal mixed with soybeans to swine. It was concluded at the end of their study that rubber seed meal can be used to provide up to 10% of the dietary protein without adversely affecting body weight gain or nitrogen utilization. The reasons for the limited use of rubber seed was attributed to its low

content of methionine and lysine and the presence of the anti-nutritional factor, hydrogen cyanide.

1.2 JUSTIFICATION OF THE STUDY

Because of its hydrogen cyanide content, raw rubber seed must be processed before it can be incorporated into pig diets. Current processing methods, such as the enzymatic method (Okafor and Anyanwu, 2006), are complicated and often expensive. In Liberia and Ghana, where the adoption of rubber seed meal is almost certain because conventional feeding-stuffs are either scarce or expensive, funds for investment in sophisticated facilities may not be available. These points to the need for identifying simple procedures for processing rubber seed meal to be used in pig diets.

1.3 OBJECTIVES OF THE STUDY

1.3.1 General Objectives

The main objective of the study was to evaluate four simple methods of processing – sundrying, soaking in water, steam heating and roasting – in terms of the chemical composition of the resultant meals and the growth and reproductive performance of pigs fed diets incorporating the variously processed meals, using rats as models.

1.3.2 Specific objectives

1. Determine the chemical composition of the unprocessed (raw) rubber seed.
2. Determine the chemical composition of variously processed rubber seeds.
3. Assess the growth performance and physiological parameters of laboratory rats fed diets containing the variously processed rubber seed meals.
4. Assess the reproductive performance of laboratory rats fed diets containing the variously processed rubber seed meals.

5. Determine the growth performance, physiological parameters and economics of production of laboratory rats fed diets containing graded levels of the best identified processed rubber seed meal.

1.4 EXPECTED OUTPUTS OF THE PROJECT

The expected outputs realized will facilitate the effective growth and reproductive performance of pigs being fed diets containing rubber seed meal for production in Ghana, Liberia and other West African countries. This will subsequently lead to improvement in meat supply and pig producers' income and livelihoods.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Non-Conventional Feed Resources and Agro-Industrial By-Products for Feeding Swine

Devendra (1985) defined Non-Conventional Feed Resources (NCFR) or Non-Traditional Feedstuffs as those feeds that have not traditionally been used in animal feeding and or are not normally used in commercially produced rations for livestock. They are not consumed by humans but are suitable for feeding animals such as pigs and transforming pigs into pork which is a human edible product (Trujillo, 2009). Defined in this manner, the NCFR embrace a wide diversity of feeds that are typical of, and abundant, in most developing countries. Myer and Hall (2004) also indicated that NCFR may include materials available locally that can be economical substitutes for expensive or not readily available traditional feedstuffs. Adesehinwa *et al.* (1998) reported that alternative feedstuffs must be ingredients with less competition by other secondary industrial users and producers which are readily available in commercial quantities and affordable prices. Myer and Hersom (2010) again emphasized some important characteristics of such alternative feed ingredients to include accurate identification of alternative feed, availability and consistency of availability, nutrient composition and nutrient availability, consistency of composition, suitability for the class of animal to be fed, perishability, freedom from health hazard, special handling, processing and storage requirements, effect on end product, storage space and legality to feed to animals and cost. A feature of NCFR is that whereas the traditional feeds of crop origin tend to be mainly from annual crops, the NCFR include commonly, a variety of feeds from perennial crops and feeds of animal and industrial origin. In this sense, the NCFR could really be more appropriately termed “new feeds”

The non-conventional and AIBPs can be grouped according to their nutritional content such as energy rich sources (i.e. molasses, rejected banana, pineapple waste, cassava by-products);

protein rich sources (i.e. oil seeds cake, fish and meat and pulses); minerals sources (i.e. bone meals and oyster shell) and miscellaneous by-products which supply energy and protein such as by-product from the brewery, fruit and vegetable industries (Ranjhan, 1997).

Devendra (1985) observed that it is not easy to draw a distinct demarcation between traditional feeds and NCFR. This is because in some countries what may now be classified as NCFR may in fact be traditional depending upon the fact that it may have been used as a feed for a long time.

2.1.1. Characteristics of non-conventional feed resources

Devendra (1985) reported that NCFR have a number of characteristics that are worth documenting. These include the following:

(a) They are the end-products of production and consumption that have not been used, recycled or salvaged.

(b) They are mainly organic and can be in a solid, slurry or liquid in form.

(c) Their economic value is often less than their cost of collection and transformation for use, and consequently, they are discharged as wastes.

(d) The feed crops which generate valuable NCFR are excellent sources of fermentable carbohydrates, for example, cassava and sweet potato, and this is an advantage to ruminants because of their ability to utilise inorganic nitrogen.

(e) Fruit wastes such as banana rejects and pineapple pulp by comparison, have nutrients which are energetically very beneficial.

(f) Concerning the feeds of crop origin, the majority are bulky poor-quality cellulosic roughages with a high crude fibre and low nitrogen contents, suitable for feeding ruminants

(g) Some of the feeds have deleterious effects on animals, and not enough is known about the nature of the active principles and ways of alleviating the effects.

(h) They have considerable potential as feed materials, and for some, their value can be increased if there were economically justifiable technological means for converting them into some usable products.

(i) More information is required on chemical composition, nutritive value, toxic factors and value in feeding systems.

Many of the NCFR are currently designated as wastes, and this is an inaccurate description. They are wastes to the extent that they have not been shown to have an economic value so that if these wastes can be utilized and converted by animals into valuable products for human benefits, they then become new feed resources. Additionally, they can alleviate the existing limited feed resources. Myer and Hall (2004) have reported that the suitability of an alternative feedstuffs or NCFR for a particular or physiological stage of the pig depends, among other factors, on its legality of use, availability on the local market, cost (including transportation, storage, processing and labour), palatability, consistency, nutrient composition and availability, presence of potential health hazards (toxic or disease factors) or anti-nutritive factors, and potential effects on pork quality and perishability including spoilage and rancidity.

Recycling, processing and utilization of all, or a portion of the wastes generated, offers the possibility of returning these to beneficial use, as opposed to the traditional methods of disposal and relocation of the same residues. The demonstration of potential value can therefore make many of the wastes, new feeds of value and importance. One of such potential new feeds is rubber seed meal obtained from the rubber tree as a by-product.

2. 2 The Rubber Tree (*Hevea brasiliensis*)

The para rubber tree (*Hevea brasilensis*), (Plate 1) which, belongs to the family *Euphorbiaceae,* is a perennial crop, generally cultivated for its industrially important latex which is used in the production of natural rubber (Columbia Encyclopedia, 2015). This is the most important

commercial source of natural rubber, a product of vital importance which is recovered from the latex. Natural rubber, however, has been found in the latex of over 895 species of plants belonging to 311 genera of 79 families. In the wild, the rubber tree will grow to heights of 100 to 130 feet and can live up to 100 years. Rubber tree can be tapped for latex (Plate 2) once it reaches approximately six years of age (Columbia Encyclopedia, 2015).

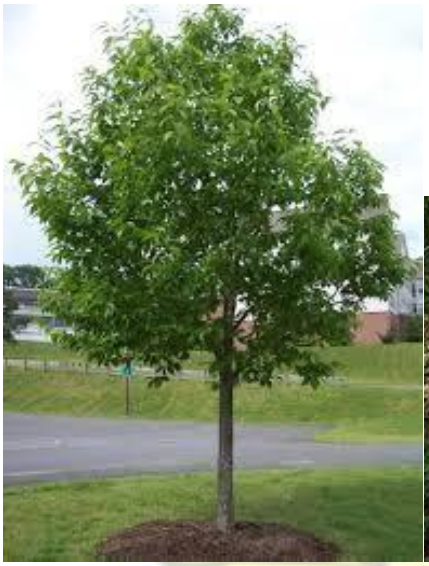

Plate 1: Rubber tree

Plate 2: Tapping of rubber tree for latex

2.2.1 *Overview of Rubber Plantations in the World*

The FAO in 1985 estimated that, on a worldwide basis, over 12 million hectares were planted with rubber trees. Malaysia, Indonesia and Thailand had about 5.0, 3.0 and 1.3 million hectares, respectively planted with rubber trees (FAO, 1985), and these together with large hectarages in other Southeast Asian countries, accounted for over 80% of world rubber production. According to the Australian Centre for International Agricultural Research

(ACIAR, 2006), Vietnam had 420,000 ha of rubber trees with density of 500 trees/ha. In Cambodia, rubber plantations cover 54,858 ha (GDCRP, 2002) and divided into two sectors one of which is the State companies and the other is private.

Current estimates provided by FAO (2014) indicate that five major rubber producers, namely Indonesia, Thailand, Malaysia, India and Vietnam account for more than 78% of the world's cultivation area of 9.82 million ha in 2011. Indonesia had the largest natural rubber planted area in the world with over 3.45 million hectares as compared to Thailand with 2.04 million ha.

In Africa, the FAO (2014) report indicated that rubber average declined in Nigeria throughout the decades (1967 – 1987) yet had the largest area under cultivation (247,000 ha) in 2011. Similar situation occurred in the Democratic Republic of Congo and Liberia, mainly as a result of political instability. However, recent changes in Liberia have been a factor leading to the rehabilitation of natural rubber plantation in the country. Cameroon, on the other hand, has more than doubled the 1960 planted area (from 11,000 ha in 1961 to about 54,000 ha by 2011). Cote d' Ivoire, now the third largest area under rubber in Africa, recorded the most remarkable expansion (up to 135,000 ha in 2011 from 10,406 ha in the early 1970's as a result of large rubber production. A few new comers to the industry are Gabon and Guinea, where production started in the 1990's (FAO, 2014).

2.2.2 *Production of Rubber Seeds*

In addition to the latex, the rubber tree also produces hundreds of seeds yearly and is also a source of lumber when the older trees are removed for replanting. Seed is produced from trees that are in the age group of three years and older. Rubber seed fall is seasonal and occurs at most for a period of two months (Eka *et al*., 2010). The best quality seeds are obtained if collected soon after seed-fall, with minimum duration of contact with the ground. The possibility of lipase activity and fungal infestation increases with longer duration of seed on the ground (Eka *et al.,* 2010).

The seeds are large (usually weighing between 3.5 and 6.0 g) and ovoid in shape with the ventral surface slightly flattened (Plate 3). The seed coat (husk) is hard and shiny, brown or grey to brown with numerous darker mottles or streaks on the dorsal surface, but few or none on the ventral side (Webster and Baulkwill, 1989).

According to Siriwardene and Nugara (1972), the rubber tree produces an average of 800 to 1200 kg of seeds per hectare. Vogt (1987) estimated the worldwide annual potential of rubber seed meal in 1983 to be 1.68 million tons.

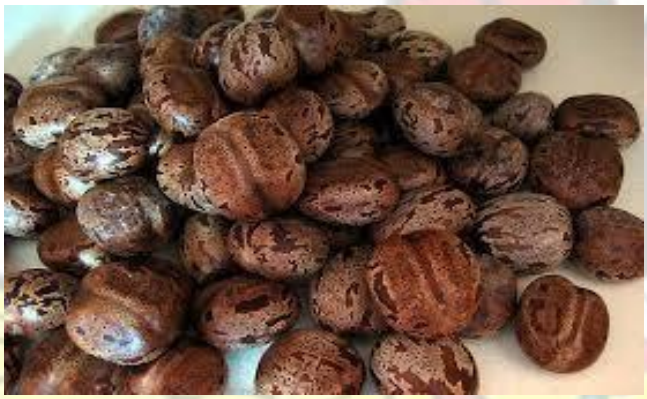

Plate 3: Rubber seeds

In Vietnam, based on an estimated production of approximately 300 kg rubber seeds/ha, it is possible to collect nearly 130,000 metric tons rubber seed/ha equivalent to 65,000 metric tons of rubber seed meal without hulls (ACIAR, 2006) every year from the level of rubber production. In Thailand, there are 590 million kg of para rubber seeds available each year.

After removing the shell and reducing the hydrocyanic acid in the seeds, there are some 300 million kg of para rubber seed kernel (Anonymous, 2007). Annual production of para rubber seeds is estimated at 43, 000 tons (Igeleke and Omorusi, 2007) in Nigeria. This quantity of seeds is largely wasted, as only a fraction is utilized in growing rootstocks for budding.

2.2.3 Nutrient Content of Rubber Seeds and Meal

Rubber seeds are potentially available feed raw materials which have received scanty research attention in recent years (Madubuike *et al*., 2006). Rubber seeds have to be dehulled before it can be utilized as animal feed and are usually dehulled immediately after they are collected from the field.

2.2.3.1 Proximate composition of rubber seed meals

According to Vogt (1987), the kernel contains 40 to 50% of a highly unsaturated vegetable oil, 20% protein and 40 to 50% carbohydrates. Dehulled fresh rubber seeds have high moisture content; this however, decreases rapidly in storage. Seeds stored for a month before dehulling have been reported to have a moisture content of about 30%. Full-fat rubber seed meal has a dry matter content of 84.5 to 94.2%. The proximate composition of various dehulled rubber seed meals as reported by Fetuga *et al*. (1977) and Nwokolo *et al*. (1987) are presented in Table 1.

With fat extraction, there is an increase in the content of various nutrients. The protein content of rubber seed meal has been shown to vary from 22.5% in whole rubber meal to 38.7% in meals that have been exhaustively solvent-extracted (Nwokolo *et al*., 1987). Crude fibre content is low in the whole meal, increasing to 12.2 and 13.9% in pressed and defatted meals, respectively. Similarly, ash content increases from 3.5% in the whole meal, to 8.4% in the deffated meal. Digestible energy values of full-fat rubber seeds and defatted rubber seed meals for swine are estimated at 5560 kcal/kg and 3900 kcal/kg, respectively (Nwokolo *et al* 1987).

Table 1: Proximate composition of various dehulled rubber seed meals (%)

	Full-Fat[1]	Solvent Extracted[1]	Mechanically Pressed[2]	Defatted[2]
Dry matter	84.5	91.4	90.4	94.2
Crude protein	22.5	36.5	28.8	38.7
Crude fibre	3.8	4.4	12.2	13.9
Ether extract	49.5	8.5	18.6	1.0

Ash	3.5	5.3	8.4	8.4
NFE	27.7	45.3	32.0	38.0

Source: [1]Fetuga *et al*. (1977); [2]Nwokolo *et al*. (1987)

According to Bressani *et al*. (1983), the rubber seed kernel (hull removed) contains 29.6% fat and 11.4% protein. Stosic and Kayaky (1981) have earlier reported that extracted rubber seed meal (after partial extraction of the oil) and rubber seed kernels contain fairly high amounts of protein and are also high in dietary energy. Values reported by Stosic and Kayaky (1981) are presented in Table 2. Both feeds are higher in total digestible nutrients than maize grain, which is considered one of the high energy feeds.

Table 2: Composition of meal from partially extracted rubber seed kernels and unextracted kernels

	Unextracted	Extracted
Dry matter	92.4	91.0
Crude protein	21.7	29.4
Ether extract	39.0	14.7
Crude fibre	2.8	6.6
Ash	3.1	5.1
NFE	25.9	35.3
TDN	112.0	85.0

Source: Stosic and Kaykay (1981)

Proximate analysis carried out by Njoku (1994) on rubber seeds (Table 3) shows the relative amounts of dietary components that could be derived when it is used in formulating animal and poultry feed.

Table 3: Composition (%) of rubber seed cake

Moisture	3.89
Carbohydrate	29.0
Crude protein	22.3
Ether extract	42.5
Ash	2.6

Source: Njoku (1994)

Siriwathanannukul and Tantikapong (2002) reported that after treatment (sun drying, followed by hot air incubation) para-rubber seed kernel contained 3.45% moisture, 17.16 crude protein, 42.6% fat, 16.7% fibre and 3.45% ash.

Proximate analysis of rubber seed reported by Eka e*t al*. (2010) also indicated a moisture content of 3,99%, protein content of 17.41%, fat content of 68.53%, and ash content of 3.08%.

2.2.3.2 Amino acid content and availability in rubber seed meal

Research results (Oluyemi *et al*., 1975) indicate that rubber is rich in essential amino acids like lysine (3.60%) and methionine (1.4%), while Selle *et al*. (1983) reported that rubber seeds are good sources of valine, isoleucine, phenylalanine and tyrosine. Eka *et al*. (2010) reported that amino acid in rubber seed is high in glutamic acid (16.13%) and low in cystine (0.78%). The content of most amino acids is about 20% lower in defatted rubber seed meal than in defatted soybean meal (Table 4). Like, many oilseed meals, defatted rubber seed meal has a low content of tyrosine, threonine, isoleucine, phenylalanine and lysine and a very low content of cystine and methionine.

The availabilities of amino acids reported herein have been determined with young broiler chicks (Table 4). Average availability of amino acids in solvent-extracted rubber seed meal is between 75 and 86%, with a mean availability of 80.3%.

Table 4: Amino acid content (%) and availability (%) in rubber seed meal

	Amino acid content		Amino acid availability	
	Defatted RSM	Defatted SBM	Defatted RSM	Defatted SBM
Arginine	4.31	4.08	88.9	95.5

Histidine	1.78	1.58	59.5	86.2
Isoleucine	1.38	2.12	75.3	97.6
Leucine	2.69	3.71	85.5	97.4
Lysine	1.66	3.25	66.2	96.6
Methionine	0.64	0.68	83.3	96.9
Cystine	0.34	0.46	68.4	70.1
Phenylalanine	1.52	2.23	89.3	96.9
Threonine	1.30	1.80	77.6	90.1
Valine	2.57	2.17	90.1	97.1

Source: Nwokolo *et al*. (1987)

Histidine is poorly available, while threonine, cystine, lysine and isoleucine are moderately available in rubber seed meal. In spite of this, defatted rubber seed meal is a satisfactory source of dietary amino acids, when compared to other vegetable protein sources, such as sunflower seed, cotton seed or rapeseed meal.

2.2.3.3 Mineral composition of rubber seed meal

Eka *et al*. (2010) have reported that rubber seed is a poor source of both calcium and iron. Comparative mineral analyses of completely defatted rubber seed meal and soybean meal indicate that rubber seed meal has about twice the phosphorus and magnesium content, onehalf times the potassium content and the same content of calcium and sodium as soybean meal (Table 5). Content of zinc and iron is three to four times higher in solvent-extracted rubber seed meal than in soybean meal, while copper is twice as high. All meals have a high content of potassium. While the mineral content of the soil may affect the concentration of minerals in rubber seed meal (Nwokolo *et al*., 1987), in general, rubber seed meals have a higher content of most minerals than other common protein concentrates.

Table 5: Mineral composition of various rubber seed meals

	Full Fat[1]	Solvent Extracted[1]	Mechanically Pressed[2]	Defatted[2]
Calcium (%)	0.48	0.88	0.20	0.27
Chloride (%)	0.07	0.18	-	-
Magnesium (%)	0.28	0.34	0.35	0.20
Phosphorus (%)	0.64	0.94	0.67	0.52
Potassium (%)	0.96	1.54	1.38	1.34

Sodium (%)	0.09	0.21	0.55	0.76
Iron (mg/kg)	93	147	225	86
Manganese (mg/kg)	23	25	42	35
Zinc (mg/kg)	78	112	102	36
Copper (mg/kg)	25	32	+ 31	16

Source: [1]Fetuga *et al*. (1977); [2]Nwokolo *et al*. (1987)

2.2.4 Anti-Nutritional Factors and other Undesirable Constituents in Rubber Seeds and Meal

Anti-nutritional factors (ANFs) may be defined as those substances generated in natural foodstuffs by the normal metabolism of species and by different mechanisms (e.g. inactivation of some nutrients, diminution of the digestive process, or metabolic utilization of feed) which exert effects contrary to optimum nutrition (Kumar, 1992).

2.2.4.1 Cyanogenic glycosides

Some agronomic crops contain anti-metabolites, which reduce the nutritional value (Ukpebor *et al*., 2007). These 'inhibitors', bind tightly to the proteolytic enzymes, thus causing indigestion and poor utilization of the nutrients. Cyanogenic glycosides are phytotoxins which occur in at least 2000 plant species, of which a number of species are used as food in some areas of the world. Cassava and sorghum are especially important staple foods containing cyanogenic glycosides (Vennesland *et al.,* 1982; Rosling, 1987). There are approximately 25 cyanogenic glycosides known. According to Conn (1979 a,b), the major cyanogenic glycosides found in the edible parts of plants used for human or animal consumption include amygdalin (in almonds), dhurrin (in sorghum), linamarin (in cassava and Lima beans), taxiphyllin (in bamboo shoots), prunasin (in stone fruits) and lotaustralin (in cassava) . The potential toxicity of a cyanogenic plant depends primarily on the potential that its consumption will produce a concentration of HCN that is toxic to exposed animals or humans (Nartey, 1980). Several factors are important in this toxicity: The first aspect is the processing of plant products containing cyanogenic glycosides. The second aspect is the direct consumption of the cyanogenic plant. The third aspect is that the cyanogenic glycosides taken up intact with the food are (partly) hydrolyzed

by the ß-glucosidase activity of the bacteria of the gut flora of animals or humans (Conn, 1979 a,b; Nartey, 1980; Rosling, 1987; Gonzales and Sabatini, 1989).

According to their chemical composition and nutritive value, rubber seed kernels can be considered as a very good feedstuff (Babatunde *et al*., 1990:1991; Ly *et al*., 2001). However, they contain a toxic factor that is a problem in the use as animal feed. Narahari and Kothandaraman (1983) found that the kernels of rubber seeds contained 749 mg HCN kg^{-1}. The toxic factor of rubber seeds is a cyanogenic glycoside that decomposes either as a result of enzyme action or from being in a very slightly acidic medium (Stosic and Kaykay, 1981). Cyanogenic glycosides on hydrolysis yield toxic hydrocyanic acid (HCN). The cyanide ions inhibit several enzyme systems; depress growth through interference with certain essential amino acids and utilization of associated nutrients. They also cause acute toxicity, neuropathy and death (Fernando, 1987). The toxic level of HCN is reported to be 1.4 mg/kg of body weight (Pongpeajan, 1996).

2.2.4.2 Other anti-nutritional factors in rubber seed meals

Achinewhu (1982) reported the existence of 1.2% of crude saponin in rubber seed but concluded that soyabean meal containing a higher saponin level (2.4%) has successfully been used over the years in monogastric nutrition without adverse effects. Ononogbo (1998) also reported the presence of anti-nutritional factors such as trypsin inhibitor (0.422 units), phytate (3.7×10^4 g/ml) and tannin (4.23%) in rubber seed meal. Narahari and Kothandaraman (1983) found the tannin levels in rubber seed to be low (0.42 – 0.53%) and within the safety levels for incorporation in livestock feeds. Moreover, the tannins were confined to the shell portion of the rubber seeds. Thus, decortications appeared to be a satisfactory method for eliminating the tannins in rubber seeds, but increased the HCN levels slightly

These anti-nutritional factors reported to be contained in rubber seeds diminish animal productivity and may also cause toxicity during periods of scarcity or confinement when the feed ingredient rich in these substances is consumed by animals in large quantities (Kumar, 1992)

Saponins reportedly cause hypocholesterolaemia by binding cholesterol, making it unavailable for absorption. They also cause haemolysis of red blood cells and are toxic to rats (Johnson *et al*., 1986).

Trypsin (protease inhibitor) cause pancreatic enlargement and growth depression (Aletor and Fetuga, 1987) whilst phytates bind minerals such as, calcium and magnesium, and interfere with their metabolism. They also cause muscular weakness and paralysis (Blood and Radostits, 1989)

Tannins cause decreased feed intake in animals, bind dietary protein and digestive enzymes to form complexes that are not readily digestible (Aletor, 1993). They are also reported to cause decreased palatability and reduced growth rate (Roeder, 1995).

Offiong and Olumu (1990), however, had confirmed that these anti-nutritional factors are heat labile and are reduced to insignificant non-toxic levels when the rubber seeds are either toasted (105^0C) or stored for 4 -6 months before processing (Anonymous, 1975).

2.2.4.3 Aflatoxins

Besides containing cyanogenic glycosides and other anti-nutrional factors, rubber seed meal has also been found to be a medium for moulds such as *Aspergilus flavus, Aspergilus foetidus*, and *Rhizopus sarrhizus* (Nguyen The Vinh, 1990). Among these fungal species, *Aspergilus flavus* generates the most problems since its metabolites, called aflatoxins, may cause vast economic losses to livestock producers (Vincelli *et al.,* 1995). The compounds of aflatoxin are closely related to aflatoxin B_1, aflatoxin B_2 ,aflatoxin G_1 and aflatoxin G_2 (Humphreys, 1988).

Among these, aflatoxin B_1 is the most toxic and the toxin level in feeds are thus referred to B_1 (Mount, 2001). Pigs are affected differently by aflatoxins depending on their age, dietary levels of the toxins, length of exposure, and health conditions (Humphreys, 1988; Horn *et al.,* 1992; Vincelli *et al.,* 1995).

2.2.5 Rubber Seed Meal as Animal Feed

The use of rubber seed meal (RSM), which is the residue from the kernel after oil extraction, has been studied thoroughly in many tropical countries such as Sri Lanka, Malaysia and many experiments have been conducted. From the experimental results, Buvanendran and Siriwardene (1970) recommended that RSM was a very useful substitute for coconut meal in broiler rations up to level of 20%. The results showed no significant differences in egg production when layer hens were fed with rations containing 55% of rubber seed meal. Thuoc (1968) indicated that dried rubber seed kernel could be incorporated at 10% level in diets of finishing meat-type chickens and at 20% level in diets for finishing pigs without adverse effect on palatability of diet, body weight gain, feed conversion efficiency or health.

Experimental diets containing 10, 20, 30 and 40% RSM were studied in Sri Lanka. This experiment was conducted by Rajaguru (1971) raising pullets from the third month of age. It was found that the inclusion rates of RSM in the diet did not affect the egg production, but egg size, shell thickness, hatchability and chick weight were reduced. These adverse effects were attributed to the amino acid imbalance of RSM. In Malaysia, Yeong and Syed Ali (1979) found the body weight and feed efficiency of 5-10 weeks chicks to be adversely affected by RSM supplemented with 0.15% crystalline methionine in the diet.

Pham Thi Lam Phoung (1990), in Vietnam, included 5, 10 and 15% RSM in broiler rations and found that the chickens grew best when fed 10% RSM supplemented with 0.15% crystalline methionine in the diet. In another study, Trieu Tuyet Mai (1991) fed commercial laying hens

diets containing 10% rubber seed cake and concluded that the inclusion of the meal in the rations did not adversely affect the egg production, egg weight and feed efficiency. At household level, Le Teo (1999) reported that scavenging chickens can be fed up to 30% RSM in the diet.

Orok and Bowland (1974) found the rate of gain, energy and nitrogen digestibility and carcass composition of rats fed diets containing RSM to be comparable to those fed diets without the meal. However, the feed consumption of rats fed RSM-supplemented diet was lower than that of rats on a soybean-supplemented diet.

Ong and Yeong (1978) fed six groups of growing finishing pigs one of six experimental diets containing 0, 5, 10, 15, 20 and 25% RSM, and observed a growth depression trend in pigs consuming the meal, though the difference was not significant until the 25% level was reached. Additionally, the feed efficiency was poorer when more than 15% of the meal was incorporated into the rations. Ong and Radem (1981) fed four groups of growing-finishing pigs diets with 0, 10, 20 and 30% RSM, and reported that average daily gain and daily feed intake were similar among the pigs for all diets. They then suggested that rubber seed meal could be included up to 30% in pig diets. Ravindran (1983) found impaired performance traits with pigs fed more than 10%. This effect was, however, attributed to a deficiency of lysine and methionine rather than to cyanogenic glucoside present in the meal. Devendra (1985) also considered 20% as optimum. Fuller (1988) suggested that with correctly processed meal, the diet could contain up to 40%.

Rubber seeds have been used as feed supplement for sheep in Cameroon (United Nations Industrial Development Organisation, UNIDO, 1987; Njwe *et al*., 1988) and have also been suggested as having good potential as feeds for livestock in Cambodia (Pech, 2002).

2.2.6 Methods of Processing Rubber Seeds for Animal Feeding

There are a wide variety of different methods of processing the rubber seeds to reduce their content of cyanogenic glycosides and hence their toxicity. These methods comprise of different or combinations of drying, soaking, boiling and fermentation of whole seeds. All of these reduce the total hydrogen cyanide content of the seeds.

In Liberia, rubber seeds are routinely detoxified by boiling and soaking in water for 12 hours, or roasting at 50^{o}C for 15 minutes, procedures which are reported to be quite effective in detoxifying rubber seeds for poultry and swine feeding (Stosic and Kaykay, 1981).

Selle *et al*. (1983) observed that 20 hours of soaking rubber seeds in water combined with 1 hour of cooking was the most effective treatment for reducing the cyanide content. Fuller (1988) reported that since rubber seeds slowly lose hydrocyanic acid, storage for a minimum of four months, or detoxification by roasting (50^{o}C for 15 minutes) or soaking in water or in a 2.5% ash solution for 12 hours, would eliminate the anti-nutritional factors and improve palatability.

On the other hand, ACIAR (2006) reported that heat processing studies have shown that a number of high temperature processing methods, which utilized wet or dry heat for period between 10 – 35 minutes, on both decorticated seed and un-decorticated rubber seed meal failed to significantly reduce the cyanide content of the rubber seed. Preliminary results reported by ACIAR (2006), using combinations of treatments in laboratory scale studies showed that if seeds were first either homogenised with water or crushed to extract the oil and moisture followed by sun-drying then the cyanide content declined significantly with increased time. It was further observed that after the water wash and one day sun-drying, there was a significant reduction in HCN in chopped seeds, whereas after one day sun-drying there was only a lesser reduction in crushed seeds without the wash step. The removal of the hull prior to these processes enhanced

the cyanide reduction response. The greatest rubber seed cyanide reduction was achieved in seeds that were initially decorticated then crushed to remove oil and water followed by a homogenisation (wash) in water with a period of 2 – 5 days of sun-drying to yield a dry meal product with almost complete elimination of the HCN content.

Fresh para-rubber seed kernel (PRKS) is reported by Siriwathanannukul and Tantikapong (2002) to contain about 770 ppm of HCN which is toxic to animals. They, however, further reported that the HCN can be reduced to a level of 38.20 ppm by exposing the PRKS to the sun for 6 days, followed by 24 hours of hot air incubation at 70^{o}C.

Okafor and Anyanwu (2006) evaluated an enzymatic method (i.e. the use of endogenous Bglucosidase) together with oven drying in the detoxification of cyanogenic glycosides as a means of processing rubber seed for animal feed. The results showed that crushing or grating of the rubber seeds and allowing interaction of the endogenous B-glucosidase with the cyanogenic glycosides content for a period of 60 minutes or more resulted in about 90 - 95% hydrolysis of the cyanogenic glycoside by the B-glucosidase as measured by the release of HCN. The cyanide release by the endogenous B-glucosidase showed higher degree of hydrolytic activity when compared to that of cassava linamarase under the assay conditions. Oven drying of the mashed seeds at 60^{o}C for a period of 30 – 180 minutes resulted in about 81% total cyanogens removal.

Eka *et al*. (2010) reported that storage and heat treatment can reduce HCN content of rubber seeds. Storage at room temperature for a minimum period of two months has been shown to be effective in reducing the HCN content of rubber seeds to safe levels (Narahari and Kothandaraman, 1983). Moreover, the HCN content dramatically decreases under high temperatures during the oil extraction process (FAO, 1997). In addition, boiled and drained rubber seeds have been found to be valuable food for Indians in the Amazon Valley of South America (Njwe *et al*., 1988). Daulay *et al*. (2014) showed that combination of boiling rubber

seeds for 30 minutes followed by drying out for 12 hours with sun light was effective way in decreasing the HCN content.

2.3 The Growing Laboratory Rat as a Nutritional and Reproductive Model for other Monogastric Animals

There is no doubt about how research using animals, particularly laboratory rats, as experimental models has contributed to increasing the current knowledge about nutrition, physiology, reproduction and other areas in science (Perez-Cano *et al*., 2012). Furthermore, animal models have the advantage of allowing invasive tissue sampling to assess nutrient status and easily monitor compliance with the dietary protocols (Baker, 2008).

The laboratory rat belongs to the order Rodentia. Both rats and mice are in the family Muridae. The term murine refers to rats and mice. The laboratory rat is widely used in toxicological, nutritional, genetic, behavioural and environmental studies (Harleman and Seinen, 1979). Rats have also been long used as models of mammalian health and disease (Stahl *et al*., 2008). The small size of rats and the ease of housing and caring for them have made them preferable as pets and research animals. The use of humans and food animals in experiments is restricted for ethical and economic reasons, respectively.

Nutritional and reproductive studies with pigs are expensive both in terms of time and materials. Furthermore, small numbers of observations per treatment have been characteristics of pig experimentations (Low, 1980 a,b), so economic considerations dictate their replacement by simpler and cheaper methods. Nevertheless, it seems unlikely that animal experiments can be totally eliminated in the foreseeable future, but the dependence on pigs can be minimised by choosing a sensitive model animal. In this respect, the growing laboratory rat has proved a valuable model for investigations into basic processes of nutrition, metabolism (Waddell and Desai, 1981; Donkoh *et al*., 1993; Donkoh *et al*., 2012) and reproduction (Dey *et al*., 2004; Lee and DeMay, 2004; Hamid and Zuki, 2013), and tests with laboratory rats have been used in the

development of methods designed to replace the use of live animals, such as the growing pig. The rat has prominence over most other species in nutrition and reproduction research for many reasons. The rat is omnivorous and can be fed the same (nutritionally adequate) diet for most of its lifetime. It is easily handled and cared for, and makes minimal maintenance demands, being docile, handy and thriving well in small areas. Rats are also notable for high fecundity: they breed easily all year round, they are sexually mature at 6 – 7 weeks, and litter sizes average 14 (Universities Federation for Animal Welfare, 2010). As a result, several generations may be studied in a few weeks. The rat is intelligent and can learn quite complicated manipulations.

The rat, especially the male rat, is considered to be in a continuous of growth during its lifetime (Vinerean, 2015). The latter is probably the more important reasons for its widespread use in nutritional and reproductive studies which require the use of animals that continue to gain weight. The rat, at birth, weighs about 6 g: it is weaned at 3 weeks when it weighs about 7 times its birth weight (Yang and Mickelsen, 1974). At puberty, which occurs when the rat is about 6 weeks of age, it weighs more than 25 times its weight at birth.

2.3.1 Comparative Digestive Anatomy and Physiology of the Rat and Pig

The pig and rat are two examples of non-ruminants (monogastrics). The digestive processes of the rat and pig are anatomically very similar (Davenport, 1982) with the exception of the large intestines. The rat does not have a gall bladder. Bile is produced in the liver and passed directly to the duodenum via the bile duct. The process of protein digestion in rats is very similar to that in pigs. The pig has a long but simple small intestine, a moderate-sized caecum, and a sacculated large intestine. The pig and man are classified as colonic digesters (Stevens, 1977). In comparison, the rat has a relatively shorter but simple small intestine, an enlarged caecum, and an unsacculated large intestine (classed as a caecal fermentor). Both of these species depend on

hindgut fermentation to varying degrees, the pig having fermentation in both the caecum and colon, while most of the fermentation in the rat's gastrointestinal tract occurs in the caecum.

Kennedy *et al*. (1974) compared rats and pigs on diets containing increasing concentrations of meat and bone meal, and collagen. Both animal species reacted in a similar negative fashion to these increases. The results of the study showed a linear ($P< 0.01$) reduction in the average daily gain and feed conversion efficiency in growing pigs and rats as the levels of meat and bone meal in the diet increased. In work with 9 Nigerian protein feeds, Fetuga *et al*. (1974) obtained no consistent differences in biological values between rats and pigs. Studies (Picard *et al*. 1984; Moughan *et al*., 1984; Moughan *et al*., 1987; Smith *et al*., 1990; Donkoh *et al*., 1993) indicate overall, the growing rat is a suitable model for the pig for the determination of protein (amino acid) digestibility at the terminal ileum. Where species differences have been reported, it is possible that they have resulted from insufficiently similar experimental conditions being applied to both species, rather than intrinsic differences in the way that pigs and rats digest protein.

2.3.2 Characteristics of the Female Rat's Reproductive System

Rats have made valuable contributions to many fields, especially reproduction (Dey *et al*., 2004). This section provides the basic facts about female rat reproduction and highlights the reproductive characteristics which make the rat an appropriate animal model for research on pig and human reproduction.

According to Hamid and Zuki (2013), the female reproductive system consists of the two ovaries and the female genital tract. The genital tract includes the oviducts, uterus, cervix and vagina. The oviducts are small, highly coiled tubes. The uterus consists of two separated uterine horns, enabling the rat to have multiple offspring. The vagina of the rat opens directly to the exterior (Kent and Carr, 2001).

2.3.2.1 Estrous cycle

The rat estrous cycle is short, lasting four to five days. It occurs throughout the year, with no seasonal effect. The first regular estrous cycle occurs about one week after the opening of the vaginal orifice, usually 33 to 42 days after birth (Maeda *et al*., 2000). The cycle length increases slightly with age and lasts about 6 days near the end of the reproductive life span (Lu *et al*., 1979).

2.3.2.2. Estrous cycle phases

The estrous cycle in the rat consists of four stages known as proestrus, estrus, metestrus and diestrus. Proestrus lasts approximately 12 h ; estrus, 9 to 15 h; metestrus, 21 h; and diestrus (the longest phase), over 57 h (Lohmiller and Swing, 2006).

Hormones, such as gonadotrophins, luteinizing hormone (LH), follicle stimulating hormone (FSH), progesterone, estradiol-17β and estrogen play critical roles in the estrous cycle (Freeman, 1988).

2.3.2.3 Identification of estrous cycle stages

Phases of the estrous cycle can be detected by observing behavioural changes or examining vaginal cytology (Lohmiller and Swing, 2006). The latter method is widely used and considered as a rapid and practical way to determine the phases of the estrous cycle (Marcondes *et al*., 2002). Behaviour and vaginal smear morphology during the different phases of estrous cycle as well as the duration of each phase are presented in Table 6.

Estrus is defined as the period when the female accepts the male and allows copulation. Many behavioural changes occur during this phase, including increased running activity, lordosis and ear quivering. During estrus, dry vaginal wall and a swollen vulva can also be observed (Baker,

1979). The female accepts the male at the end of proestrus, while during metestrus and diestrus, the female does not accept the male (Lohmiller and Swing, 2006).

Table 6: Behaviour and vaginal cytology with estrous cycle phases

Cycle phase	Duration (h)	Behaviour	Vaginal smear morphology
Proestrus	12	Male acceptance at end of phase	Nucleated epithelial cells
Estrus	12	Lordosis; male acceptance	75% nucleated cells; 25% cornified cells
Metestrus	21	No male acceptance	Many leukocytes with nucleated and cornified cells
Diestrus	57	No male acceptance	Leukocytes

Source: Lohmiller and Swing (2006).

2.3.2.4 Environmental factors affecting the estrous cycle

The estrous cycle in the rat can be affected by various environmental factors, such as temperature, photoperiod, noise, restraint, immobilization, handling and research procedures.

High ambient temperatures (35°C) increase the duration of the cycle and thus reduce the number of estrous cycles occurring in a given period of time (Sod-Moriah, 1971).

Changes in photoperiod can also alter estrous-cycle length. Extending the light period from 12 to 16 h a day increased the estrous cycle from four days to five days (Clough, 1982), but constant light disturbed the estrous cycle in female rats (Hardy, 1970) and resulted in persistent estrus. Noise also influences the estrous cycle in rats. Exposure of female rats to ultrasound in the sensitive hearing range of the rats (range near 40 Hz) alters the estrous cycle (Clough, 1982).

Acute stress or immobilization of female rats suppresses LH pulses, independent of estrogen (Maeda *et al*., 2000). Handling and research procedures (that is, restraint and subcutaneous (SC) and tail intravenous (IV) injection) can induce stress but have little effect on the estrous cycle, regardless of the stage (Sharp *et al*., 2002).

2.3.2.5 Mating and reproductive behaviour

Mating behaviour in females is controlled by both estrogen and progesterone; in males, it is controlled by testosterone (Maeda *et al*., 2000). Lordosis is a characteristic mating behaviour of female rats, whereas auditory stimuli play crucial roles in the reproductive behaviour of both male and female rats (Maeda *et al*., 2000). In addition, olfactory cues from pheromones are very important to the sexual behaviour of the male rat (Nelson, 1995). Copulation in rats mostly occurs during the last third of the dark cycle (Mercier *et al*., 1987).

2.3.2.6 Pregnancy detection

The presence of sperm in the vaginal smear or observation of a vaginal plug indicates the occurrence of mating. In rats, the vaginal plug does not persist as long as in mice; thus, the absence of the vaginal plug is not a reliable indicator that copulation did not occur. On the other hand, detection of sperm in vaginal smear is an excellent predictor of pregnancy in rats (Baker, 1979). The day that sperm is detected in the vaginal smear is designated as day 1 of gestation. After 10 days of gestation, the fetuses can be palpated, but palpation is more accurate after day 12. By day 13 of gestation, the abdominal enlargement is visible, and mammary development and nipple enlargement can be observed on day 14 of gestation.

2.3.2.7 Fertilization and early embryonic development

Fertilization in mammals takes place in the oviduct. Successful fertilization requires complex spermatozoa-ova interactions. Fertilization involves many sequential steps, beginning with the binding of spermatozoa to the zona pellucida, followed by the acrosome reaction and penetration of spermatozoa through the zona pellucida, and then the spermatozoa bind to and fuse with the egg, leading to egg activation (Yanagimachi, 1994). Sperm migration through the rat oviduct depends on both estradiol and progesterone (Orihuela *et al*., 1999).

Fertilization steps in mammals are thought to be regulated by proteins located in the acrosome of the spermatozoa (Cohen *et al*., 2000). After fertilization, a single cell embryo (zygote) doubles to two cells, then undergoes a series of mitotic divisions into four cells, eight cells, and a morula. Several more rounds of mitotic division form the blastocyst. The blastocyst becomes competent for implantation after shedding the zona pellucid (Lee and DeMay, 2004).

In rats, fertilization takes place in the morning at 04:00 to 05:00 h. The zygote develops into two and four cells on the first day, to eight cells on the second day, and to a sixteen-cell embryo on the third day after fertilization. The embryo develops to the morula stage on day four and to the blastocyst stage on day five of pregnancy (Agca and Critser, 2006).

2.3.2.8 Maternal recognition of pregnancy

Maternal recognition of pregnancy in rodents involves activation of the non-functional CL of the estrous cycle into the functional CL of pregnancy. This functional CL must be maintained until day 17. The formation and maintenance of CL and production of progesterone require two events (Hamid and Zuki, 2013).

First, mating induces the release of prolactin (PRL) from the anterior pituitary, which increases LH receptors on luteal cells to form the CL and suppress 20α-hydroxysteroid dehydrogenase activity; this transition prevents the conversion of progesterone to 20α-hydroxyprogesterone, which will not support pregnancy.

Second, the lactogenic hormones that are produced by the uterine decidua and placenta act through prolactin receptors on the luteal cells to maintain their function and the production of

progesterone throughout gestation. Thus, PRL is the initial luteotrophic signal for CL formation and progesterone production (Soares, 2004).

2.3.2.9 Embryo implantation

Implantation is divided into three stages; apposition, adhesion (attachment), and invasion (Enders and Schlafke, 1967). In rodents, the embryo that enters the uterus attaches to the uterine epithelium immediately. After loss of the zona pellucida, closure of the uterine lumen brings the blastocyst into close apposition to the luminal epithelium (Parr and Parr, 1989). The blastocyst attaches to the anti-mesometrial side of the endometrium, and the inner cell mass is directed to the mesometrial side. Rodents demonstrate rapid implantation, as apposition, attachment and invagination of the uterine epithelium occur within 6 h (Lee and DeMay, 2004).

Bazer *et al*. (2010) have reported that implantation may also be divided into three categories based on the type of blastocyst–uterine cell interaction: centric, eccentric and interstitial. Implantation in rats is eccentric; the luminal epithelium forms an invagination to surround the trophoblast.

The first sign of implantation, according to Psychoyos (1986), is the increase in uterine vascular permeability at the site of blastocyst apposition. Increases in vascular permeability coincide with the attachment reaction between the blastocyst and uterine epithelium (Psychoyos, 1986). Implantation in rats is initiated on day 5 and completed by day 7 of pregnancy (Garside *et al*., 1996; Hamid *et al*., 2012).

2.3.2.10 Gestation, parturition and weaning

Gestation in rats takes 21 to 23 days from copulation to parturition. Placentation in rats is discoidal and hemochorial (Kaufmann and Burton, 1994). Delivery in rats takes from 55 min to 4 hours depending on the litter size, with an average of 1.5 h (Baker, 1979). Weaning in rats occurs at around 21 days of age. At this age, pups are able to eat and drink.

In general, rats have been used in researches for almost two centuries. Short estrous cycle and gestation period make the rat an ideal and good animal for research on reproduction.

2.4 NUTRITION – REPRODUCTION INTERACTIONS IN ANIMALS

The role and effects of nutrition on reproductive events in farm animals is well documented. Kamalzadeh *et al*. (2009) reported that the nutrition characteristic and toxicity index of a diet alters the time course of development, reproductive ability and survival of organisms.

Reproductive performance of livestock in the tropics as elsewhere is determined by four factors — genetic merit, physical environment, nutrition and management. Evidence from the literature and practical experiences suggests that nutritional factors are perhaps the most crucial, in terms of their direct effects on the reproductive phenomenon, and the potential to moderate the effects of other factors (Smith and Akinbamijo, 2000). Thus, adequate nutrition could encourage mediocre biological types to reach their genetic potential, alleviate the negative effects of a harsh physical environment, and minimise the effects of poor management techniques. Poor nutrition on the other hand, will not only reduce performance below genetic potential, but also exacerbate detrimental environmental effects. Moreover, nutritional factors more than all others, readily lend themselves to manipulations to ensure positive outcomes. Hence, there is a need to pay particular attention to the interactions between nutrition and reproduction particularly in the tropics, where, for a variety of reasons, nutritional inadequacies in terms of quantitative feed intake and qualitative nutrient imbalances remain prevalent. Failure to properly understand these interactions in order to minimize the negative, and enhance the positive impacts will adversely affect livestock production efficiency, since this depends largely on reproductive performance.

Several studies (Hamra and Bryant, 1982; Kirkwood *et al*., 1987; Manspeaker *et al.*, 1989 and reviews (den Hartog and van Kempen, 1980; Short and Adams, 1988; Smith and Somade, 1994) have adequately examined the effects of quantitative feed and energy, as well as qualitative protein and macronutrients intake on livestock reproductive performance. In general, the results

of such studies suggest that poor nutrition caused by inadequate, excess or imbalanced nutrient intake may adversely affect the various stages of the reproductive event, going from delayed puberty, reduced ovulation and lower conception rates, through high embryonic and foetal losses to excessively long post-partum anoestrus, poor lactation, high perinatal mortality and poor neonatal performance.

2.5 BLOOD INDICES OF LABORATORY RATS

Haematological values and blood biochemical constituents reflect the physiological responsiveness of the animal to its internal and external environments, which include feed and feeding (Esonu *et al.,* 2001; Iheukwamere and Okoli, 2002). The determination of blood component values in laboratory examination is an important procedure to establish diagnostic baseline of blood characteristics to assist in routine management practices of farm animals (Bounous *et al*., 2000; Tambuwal *et al*., 2002). Normal haematological and biochemical reference ranges for the laboratory rat are presented in Tables 7 and 8, respectively.

Table 7: Rat Blood Haematological Reference Ranges

Parameter	Values
Red blood cell ($x10^6$/ mm^3)	6.76 – 9.75
Packed cell volume (%)	37.6 - 50.6
White blood cells ($x10^3/mm^3$)	6.6 - 12.6
Haemoglobin (g/dL)	11.5 - 16.1
Neutrophils ($x10^3/mm^3$)	1.77 - 3.38
Lymphocytes ($x10^3/mm^3$)	4.78 – 9 .12
Eosinophils ($10^3/mm^3$)	0.03 - 0.08
Moncytes ($10^3/mm^3$)	0.01 - 0.04
Basophils ($10^3/mm^3$)	0.00 - 0.03
Platelets (10^3/ml)	150 – 460

Source: Johnson-Delaney (1996)

Table 8: Rat Blood Biochemistry Reference Ranges

Parameter	Values
Total protein (g/dL)	5.6 – 7.6
Albumin (g/dL)	3.8 – 4.8
Glucose (mg/dL)	50 – 135
Blood urea nitrogen, BUN (mg/dL)	15 – 21
Creatinine (mg/dL)	0.2 – 0.8
Sodium (mEq/L)	143 – 156
Potassium (mEq/L)	5.4 - 7.0
Chloride (mEq/L)	100 – 110
Phosphorus (mg/dL)	3.11 – 11
Calcium (mg/dL)	5.3 – 13
Alanine aminotransferase, ALT (U/L)	17.5 – 30.2
Aspartate aminotransferase, AST (U/L)	45.7 – 80.8
Alkaline phos (U/L)	56.8 – 128
Cholesterol (mg/dL)	40 – 130
Total bilirubin (mg/dL)	0.2 – 0.55
Amylase (SU/dL)	128 – 313

Source: Queensbury and Carpenter (2012)

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CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 STUDY ONE: Evaluation of Methods of Processing Rubber Seed Meal in Terms of Chemical Composition and Energy Value

This study aimed at evaluating four simple methods of processing raw rubber seeds – sun drying, soaking in water, boiling in water and roasting – in terms of chemical composition and energy value of the resultant rubber seed meals.

3.1.1 Experimental Site

The study was carried out at the Department of Animal Science, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

3.1.2 Source of Rubber Seeds and Processing Methods

The rubber seeds used in the study were obtained from the Rubber Plantations Section of the Department of Crop and Soil Sciences, KNUST in the Ashanti Region of Ghana. The seeds were de-hulled and partially sun-dried for 24 hours at an ambient temperature of about 30ºC. The raw rubber seeds were divided into five lots, each lot receiving one of five processing methods:

A. Raw rubber seed meal – this lot was not subjected to any processing method but ground in a hammer mill and stored in polythene sacs.

B. Soaking in water – the partially dried, de-hulled rubber seeds were soaked in water for 3 days with a Rubber Seed to Water ratio of 1:3. The water was replaced daily. After 3 days, the water was decanted and seeds sun-dried for 3 days at ambient temperatures ranging from 30ºC to 35ºC. The rubber seeds were turned over periodically and collected overnight to protect the seeds from being moistened by dew.

C. Sun-drying – this treatment of consisted of spreading out a portion of the partially dried raw rubber seeds in the open and sun-drying for 3 days at ambient temperatures ranging from 30ºC to 35ºC. The rubber seeds were turned over periodically and collected overnight to protect the seeds from being moistened by dew.

D. Boiling in water - the partially dried, de-hulled rubber seeds were placed in a 20 – litre aluminum bowl containing water and subjected to heating at a temperature of about 100ºC for 30 minutes with a Rubber Seed to Water ratio of 1:3. After heating for about 30 minutes, the water was decanted and seeds sun-dried for 3 days at ambient temperatures ranging from 30ºC to 35ºC. The rubber seeds were turned over periodically and collected overnight to protect the seeds from being moistened by dew.

E. Roasting – the fifth lot was subjected to dry heat for 30 minutes in an open pan at a temperature of about 80ºC. The seeds were stirred constantly to avoid burning.

Each processed lot was ground using a hammer mill and stored in polythene sacs.

3.1.3 Chemical Analysis

The crude protein contents of triplicate (100 mg) samples of the raw and the variously processed rubber seed meals were determined using the Kjeldahl method. Amino acids were determined following acid hydrolysis using a Beckman 119 BL amino acid analyzer. Duplicate samples (5 – 7 mg) were hydrolysed in 500 µl of 6 ml HCl with 1% added phenol for 24 h at 110 ± 1^0C in glass tubes sealed under vacuum. For the determination of methionine and cystine, separate duplicate samples were oxidized with performic acid prior to hydrolysis. Tryptophan was not determined. The ash contents of the samples were obtained after heating triplicate 10 g samples in a furnace at 500°C for 24 h. Ether extract (fat) and Weende crude fibre were determined according to AOAC (2005) standard methods. The mineral contents were determined by dry-ashing the samples at 550°C in a furnace and dissolving in 10% HCl, and filtered. Calcium and magnesium were determined by Atomic Absorption Spectrophotometer (AOAC, 2005) while flame photometer was used to determine phosphorus and potassium. The hydrogen cyanide contents of the samples were determined using the enzymatic assay described by Essers *et al.* (1993). The metabolizable energy contents of the

RSMs were calculated from proximate compositions following the NRC (1994) equation: ME (kcal/kg) = (21.26 X % dry matter) + (47.13 X % fat) + (35.85 X % crude fibre).

3.1.4 Statistical Analysis

Data collected were subjected to Analysis of Variance (ANOVA) using the GenStat Statistical Software to identify significance of main effects (GENSTAT, 2007). Where significant differences were found among treatments, specific effects were tested by the least significant difference procedure. All tests for significance were based on the 5% probability.

3.2 STUDY TWO: Assessment of the Nutritional Quality of Variously-Processed Rubber Seed Meals as Dietary Ingredients Using the Laboratory Rat as Model for Pigs This study was conducted to assess the effects of including the variously processed rubber seed meals in diets on growth performance, physiological parameters and economy of gain using the laboratory rats as model for pigs.

3.2.1 Experimental Site

The rat growth performance trial was conducted at the Animal House of the Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

3.2.2 Dietary Treatments

A control diet and five treatment diets (produced by adding 10% of one of the raw and the 4 processed RSMs to the control to replace fishmeal and soyabean meal) were formulated (Table 9). The experimental diets were formulated to be isoproteic and isoenergetic.

Table 9: Ingredient composition of the experimental diets fed to rats

	Dietary treatments					
Ingredients (g kg^{-1})	Control	RRSM	SRSM	SDRSM	BRSM	RoRSM
Maize	550	500	500	500	500	500
Fishmeal	120	120	120	120	120	120
Soyabean meal	160	110	110	110	110	110
RSM	0	100	100	100	100	100
Wheat Bran	150	150	150	150	150	150
Vitamin/mineral premix	5	5	5	5	5	5
Salt (NaCl)	5	5	5	5	5	5
Dicalcium phosphate	5	5	5	5	5	5
Oyster shell	5	5	5	5	5	5

Chemical analysis (g kg-1 DM)						
Crude protein	216.8	206.9	208.8	208.9	209.2	209.7
Crude fibre	37.83	47.21	42.12	46.26	42.43	43.63
Ether extract	39.35	53.15	56.65	50.15	54.15	53.65
Calcium	8.75	8.82	8.77	8.81	8.80	8.76
Phosphorus	8.34	8.13	8.16	8.30	8.30	8.27
ME (MJ kg_{-1}) [b]	12.78	12.58	12.70	12.58	12.66	12.69

RRSM: raw rubber seed meal, SRSM: soaked rubber seed meal; SDRSM: sundried rubber seed meal; BRSM: boiled rubber seed meal; RoRSM: roasted rubber seed meal.

[a]Vitamin/mineral premix specified to provide the following kg^{-1} diet: vitamin A 10,000 IU; D 2000 IU; K 3 mg; riboflavin 2.5 mg; niacin 12.5 mg; cobalamin 0.05 mg; pantothenic acid 5 mg; choline 175 mg; folic acid 0.5 mg; zinc 25 mg; iron 0.5 mg; copper 50 mg; cobalt 625mg; iodine 0.5 mg; selenium 0.3 mg; chlorine 1.6 g; sodium 1.3 g; magnesium 2mg; sulphur 0.4 g; potassium 3.0 g.

[b]Calculated from data of NRC (1998) and the estimated metabolizable energy value of RSM.

3.2.3 Experimental Animals and Management

Thirty six Sprague-Dawley growing rats (18 males and 18 females) which were 4 weeks old were kept individually in raised stainless steel cages with wire mesh floors, in a room with a 12 h light/dark cycle. The rats were randomly allocated to the six experimental diets such that there were 6 rats (3 male and 3 female) per diet. The rats were dewormed before the start of the trial. Each rat had free access to its respective diet for a 28-day period. Water was available *ad libitum*.

3.2.4 Parameters Measured

3.2.4.1 Growth Parameters

Rat growth performance were assessed weekly by measuring feed intake, body weight gain, water consumption, and feed conversion efficiency. The economy of gain was computed as the cost of feed per unit weight gain after feed cost per kg had been calculated from the market prices of the individual ingredients.

3.2.4.2 Physiological parameters

At the end of the feeding trial, 2 rats (1 male and 1 female) in each of the six (6) dietary treatment groups were randomly selected for blood collection and organ weight determination. The rats from each treatment were anesthetized by chloroform asphyxiation followed by decapitation and

10 ml of blood was collected into two sample tubes. Blood collection was done in the morning before feeding. The blood samples for the haematological studies were collected in sample bottles with EDTA before being analyzed. Haematological attributes were estimated in whole blood just after bleeding, using the KX21N Sysmex Haematology Analyzer (Sysmex, Corporation, 2006) for its haemoglobin (Hb), red blood cells (RBC), packed cell volume (PCV), and white blood cells (WBC) contents. The blood samples for serum analysis were allowed to clot before centrifuging to obtain serum. The separated sera were decanted into bijoh bottles and stored at -20^{0}C until analyzed. Blood glucose levels were determined using the One Touch Select Glucometer System of LifeScan Inc., USA. A test strip was fitted into the glucometer and a drop of blood sample was applied at the appropriate test area. The results were read on the screen of the meter after 5 seconds. The other serum metabolites (total protein, albumin, globulins, and cholesterol) were estimated using the Flexor Junior Chemistry

Auto-Analyzer (Vital Scientific Dierer, the Netherlands).

For organ weights and histological studies, the abdomen was opened by an incision along the mid-ventral line and the skin and musculature folded back to expose the internal organs. The heart, liver, kidney, spleen, lung and intestines were excised, weighed immediately and expressed as g g^{-1} liveweight to ensure uniformity in comparison. The heart, liver, kidney, spleen, lung and intestines were examined to determine whether the diets had resulted in gross pathological changes.

3.2.5 Statistical Analysis

Data collected were subjected to Analysis of Variance (ANOVA) using the GenStat Statistical Software to identify significance of main effects (GENSTAT, 2007). Where significant differences were found among treatments, specific effects were tested by the least significant difference procedure. All tests for significance were based on the 5% probability.

3.3 STUDY THREE: Effect of Variously-Processed Rubber Seed Meals on Rat Reproductive Performance

The study determined the effects of unprocessed rubber seed meal and variously processed rubber seed meals (sun-drying, soaking in water, boiling in water and roasting) supplementation in diets on reproductive performance of laboratory rats.

3.3.1 Experimental Site

The rat reproductive performance trial was conducted at the Animal House of the Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

3.3.2 Dietary Treatments

A control diet and five treatment diets (produced by adding 10% of one of the raw and 4 processed RSMs to the control to replace maize and soyabean meal) were formulated (Table 9). The experimental diets were formulated to be isoproteic and isoenergetic.

3.3.3 Experimental Animals and Management

Eighteen Sprague-Dawley growing female rats (154 g body weight) which were 8 weeks old were kept individually in raised stainless steel cages with intact floors, in a room with a 12 h light/dark cycle. The rats were randomly allocated to the six experimental diets such that there were 3 female rats per diet. The rats were dewormed before the start of the trial. Each rat had unlimited access to its respective diet for a 28-day period. Water was available *ad libitum*.

3.3.4 Parameters Measured

Female rats for the reproductive trial were weighed prior to mating. The mating ratio was three females to one male. The male rats were placed with the females for 3 weeks, after which the females were individually housed. Upon parturition, the litters were individually counted, weighed and examined for external malformations and again weighed at weaning at 4 weeks of age. Other parameters studied included: number of fertile females, pregnancy index (no. of

females delivering young offspring/no. of females examined X 100), gestation length (length of pregnancy in days), number of pups born, number of pups born alive, number of still births and live birth index (no. of live offspring/ no.of offspring delivered X 100).

3.3.5 Statistical Analysis

Data collected were subjected to Analysis of Variance (ANOVA) using the GenStat Statistical Software to identify significance of main effects (GENSTAT, 2007). Where significant differences were found among treatments, specific effects were tested by the least significant difference procedure. All tests for significance were based on the 5% probability.

3.4 STUDY FOUR: Utilization of Diets in which Rubber Seed Meal Partially Replaced Soyabean Meal using the Laboratory Rat as Model for Pigs

The rat growth performance trial was based on the results of the rat growth and reproductive trials. The processed rubber seed meal identified as the best feed material was used in the conduct of the rat growth trial. The present study was conducted to determine the effects of incremental replacement of soya bean with rubber seed meal in diets on growth performance of rats.

3.4.1 Experimental Site

The rat growth performance trial was conducted at the Animal House of the Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

3.4.2 Source of Rubber Seed and Processing Method

The source of the boiled rubber seed meal and the processing method employed are as described in Section 3.1.2 D of this thesis.

3.4.3 Dietary Treatments

Four experimental diets were formulated: a control diet containing no rubber seed meal and three other treatment diets in which the selected processed RSM (boiled rubber seed meal, BRSM) was incorporated at 50, 100 and 150 g kg^{-1} to replace soyabean meal (Table 10). The experimental diets were formulated to be isoenergetic.

3.4.4 Experimental Animals and Management

Twenty four (12 males and 12 females) 4 week-old Sprague Dawley growing rats were kept individually in raised stainless steel cages with intact floors, in a room with a 12 h light/dark cycle. The rats were randomly allocated to the four experimental diets such that there were 6 rats (3 males and 3 females) per diet. Animals in the treatments were balanced for sex and weight. The rats were dewormed before the start of the trial. Each rat had free access to its respective diet for a 28-day period. Water was available *ad libitum*.

3.4.5 Parameters Measured

Rat growth performance was assessed by measuring: Initial body weight, feed intake, body weight gain, feed conversion efficiency (feed: gain), mortality, cost/g feed, and feed cost/g weight gain.

Table 10: Composition of experimental rat diets

	Level of dried rubber seed meal, g kg^{-1}			
	0	50	100	150
Ingredients, g kg^{-1}				
Maize	550	550	550	550
Fishmeal	120	120	120	120

Soyabean meal	160	110	60	10
Rubber seed meal	0	50	100	150
Wheat bran	150	150	150	150
Oyster shell	5	5	5	5
Dicalcium phosphate	5	5	5	5
NaCl	5	5	5	5
Vitamin/trace mineral premix[a]	5	5	5	5
Chemical analysis, g kg^{-1} DM				
Crude protein	216.8	204.1	191.4	179.2
Crude fibre	37.83	39.36	40.88	42.41
Ether extract	39.35	47.35	54.53	63.35
Calcium	8.75	8.74	8.66	8.71
Phosphorus	8.34	8.23	8.11	8.00
ME (MJ kg^{-1}) [b]	12.78	12.75	12.71	12.68

[a]Vitamin/mineral premix specified to provide the following kg^{-1} diet: vitamin A 10,000 IU; D 2000 IU; K 3 mg; riboflavin 2.5 mg; niacin 12.5 mg; cobalamin 0.05 mg; pantothenic acid 5 mg; choline 175 mg; folic acid 0.5 mg; zinc 25 mg; iron 0.5 mg; copper 50 mg; cobalt 625mg; iodine 0.5 mg; selenium 0.3 mg; chlorine 1.6 g; sodium 1.3 g; magnesium 2mg; sulphur 0.4 g; potassium 3.0 g.

[b]Calculated from data of NRC (1998) and the estimated metabolizable energy value of RSM.

After the experimental period of 4 weeks, the possible effects of BRSM on the weights of some body organs were assessed. Two rats (1 male and 1 female) from each treatment were anesthetized by chloroform asphyxiation followed by decapitation. The abdomen was opened by an incision along the mid-ventral line and the skin and musculature folded back to expose the internal organs. The heart, liver, kidney, spleen, lung and intestines were excised, weighed immediately and expressed as g g^{-1} live weight to ensure uniformity in comparison. The heart, liver, kidney, spleen, lung and intestines were examined to determine whether the diets had resulted in gross pathological changes.

3.4.6 Statistical Analysis

The dietary treatment effects for all the traits measured were statistically analysed. The computations were performed using the general linear models procedure of SAS (2003).

Differences between means were determined by the use of the Duncan's multiple range test (Steel *et al*., 1997) and considered significant if $P<0.05$.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 STUDY ONE: Evaluation of Methods of Processing Rubber Seed Meal in Terms of Chemical Composition and Energy Value

The proximate compositions and the energy values of the raw and the variously-processed rubber seed meals are as shown in Table 11. The method of processing significantly ($P<0.05$) influenced the chemical compositions of the variously-processed rubber seed meals. The proximate compositions were quite variable between meals, particularly for dry matter, crude protein, crude fibre and ether extract.

The moisture contents of the various rubber seed meals as determined in this study ranged from 9.0% for the roasted rubber seed meal (RoRSM) to 26.0% for the unprocessed rubber seed meal (RRSM). Moisture contents for rubber seed meals reported from other studies are 3% (Ukhun and Uwatse, 1988): 3.99% (Eka *et al*., 2010); 5.8% (Madubuike *et al*., 2006) and 9% (Oyekunle and Omode, 2008).

When compared with the raw or unprocessed rubber seed meal (RRSM), the crude protein contents of the processed meals (SRSM, SDRSM, BRSM and RoSM) were enhanced as a result of the various methods employed. The different types of rubber seed meals in the present study showed protein contents ranging from 16.1% for the RRSM to 18.9% for the RoRSM. These values are lower than the value reported by Gick *et al*. (1967) (27%) but are higher than that found by Bressani *et al*. (1983) (11.4%). The values, however, are in good comparison with 17.41% reported by Eka *et al*. (2010).

The crude fibre content was significantly higher ($P<0.001$) in RRSM (13.03%) than the variously processed rubber seed meals (SRSM, SDRSM, BRSM and RoRSM) which registered values which ranged from 7.58% (SDRSM) to 9.45% (RoRSM).

The ether extract (fat) contents of the variously processed rubber seed meals in this study, varied from 13.5% for SDRSM to 20.0% SRSM and are lower than 37.30% – 66.53% quoted in earlier studies (Ukpebor *et al*., 2007; Oyekunle and Omode, 2008; Eka *et al*., 2010).

Table 11: Effect of processing method on the chemical compositions and energy values of rubber seed meal[1]

Parameter	RRSM	SRSM	SDRSM	BRSM	RoRSM	P-value and level of significance
Dry matter (%)	74.0^{d}	88.0^{c}	89.5^{b}	88.5^{c}	91.0a	0.001***
Moisture (%)	26.0^{a}	12.0^{b}	10.5^{c}	11.5^{b}	9.0^{d}	0.001***
Crude protein (%)	16.1^{c}	18.0^{b}	18.3^{b}	18.4^{ab}	18.9^{a}	0.001***
Crude fibre (%)	13.03^{a}	7.94^{c}	7.58^{c}	8.25^{c}	9.45^{b}	0.001***
Ether extract (%)	16.5^{b}	20.0^{a}	13.5^{c}	17.5^{b}	17.0^{b}	0.001***
Ash (%)	2.0^{a}	1.75^{b}	1.75^{b}	1.25^{c}	2.50^{a}	0.001***
NDF (%)	16.0^{d}	36.5^{a}	21.0^{cd}	31.0^{ab}	25.5^{bc}	0.001***
Calcium	0.31^{a}	0.26^{d}	0.30^{b}	0.29^{c}	0.25^{e}	0.002***
Phosphorus	0.25^{c}	0.28^{c}	0.42^{a}	0.42^{a}	0.39^{b}	0.001***
Potassium	1.03^{d}	1.21^{c}	1.59^{a}	1.58^{a}	1.50^{b}	0.001***
Magnesium	0.28^{a}	0.29^{a}	0.27^{a}	0.25^{a}	0.26^{a}	0.037NS
Hydrogen cyanide (mg/100g)	60.95^{a}	10.90^{c}	7.10^{d}	4.6^{e}	14.30^{b}	
ME (MJ/kg)[2]	11.79^{c}	12.97^{a}	11.76^{c}	12.56^{b}	12.87^{a}	0.001***

RRSM: raw rubber seed meal, SRSM: soaked rubber seed meal; SDRSM: sundried rubber seed meal; BRSM: boiled rubber seed meal; RoRSM: roasted rubber seed meal. [1]The values are means of three samples
[2]Estimated using the formula of NRC (1994)
NS, non-significant (P≥0.05); *P≤0.05; **P≤0.01; ***P≤0.001

The ash content of a feedstuff is an indication of the inorganic elements in the sample (Oyekunle and Omode, 2008). In this study, the ash contents ranged from 1.25% for BRSM to 2.50% for RoRSM. Reported ash contents in rubber seed meals varied between 3.08% – 5.0% (Ukhun and Uwatse, 1988; Oyekunle and Omode, 2008; Eka *et al*., 2010).

The results of the elemental analysis are also tabulated in Table 9. The data presented shows that calcium varied from 0.25% in RoRSM to 0.31% in RRSM. Phosphorus was significantly (P<0.001) higher in SDRSM and BRSM (0.42%) followed by RoRSM (0.39%), SRSM

(0.28%) and RRSM (0.25%). Similarly, potassium contents were higher (P<0.001) in BRSM (1.58%) and SDRSM (1.59%). However, there were not much variations (P>0.05) in the magnesium contents of the different types of processed rubber seed meals which ranged from 0.25% (BRSM) to 0.29% (SRSM). It appears the various rubber seed meals can make contributions to the dietary requirements of farm animals.

Animals have a dietary requirement for certain inorganic elements (NRC, 1998). Minerals are essential elements that the animal body requires to function properly. In animals, calcium and phosphorus play a major role in the development and maintenance of the skeletal system and perform many other physiologic functions, including their roles in muscle contraction, blood vessel contraction and expansion, the activation of hormones and enzymes, and transmission of messages through the nervous system whilst magnesium is a co-factor in many enzyme systems and is also a constituent of bone (NRC, 1998). Potassium is involved in electrolyte balance and neuromuscular functions. It also serves as the mono-valent cation to balance anions intra-cellularly, as part of the sodium-potassium pump physiological mechanism (NRC, 1998).

In the present investigation, the presence of HCN has been identified in the raw and variouslyprocessed rubber seed meals (Table 11). The detection of HCN in the rubber seed meals is in good agreement with reports that rubber seeds contain toxic hydrocyanic glycosides (Narahari and Kothandaraman, 1983; Fuller, 1988; George *et al*., 2000; Ukpebor *et al*., 2007; Eka *et al*., 2010; Daulay *et al*., 2014; Sharma *et al*., 2014). It is worth noting that intact glucosides are not toxic, but become so after the compound is hydrolysed to release free HCN. When active in plant tissues, the enzymes involved (glucosidases) may release HCN by autolysis, which is enhanced by moisture (Montgomery, 1980). The HCN contents of the samples in this study, ranged from 4.6 mg/100 g for BRSM to 60.95 mg/100 g for the fresh (unprocessed) rubber seed meal (RRSM). The BRSM recorded the largest percentage reduction in HCN (92.5%) content while the least (76.5%) was for the R_oRSM. The results obtained in this study indicate that boiling

of rubber seeds followed by sun-drying gave a rubber seed meal of the lowest HCN content. This is in agreement with the conclusion of Daulay *et al*. (2014) who reported that the combination of boiling rubber seeds for 30 minutes and drying out for 12 hours with sunlight gave a better quality of rubber seed for animal feeding.

There are various processing techniques for hydrogen cyanide containing ingredients such as cooking, sun-drying, oven-drying, roasting, soaking, ensiling or fermentation and pulping . No single processing technique will completely eliminate the HCN content of the products. For example, while sun-drying may substantially reduce the HCN content of cassava tuberous roots, Omole (1977) suggested that the heating process during rapid drying may degrade the hydrolytic enzymes of glucosides and thereby prevent the release of free HCN. These views have been confirmed by Gomez *et al*. (1984) who indicated that more than 86% of the HCN present in cassava was lost during sun-drying, and also Devendra (1977) who indicated a reduction of HCN by about 50% in oven-drying at 36°C for 24 hours. In a recent study, Sharma *et al*. (2014) reported that economical ways of detoxification can remove 85% of the toxicant.

The detailed gross amino acid compositions of the different types of rubber seed meals are presented in Table 12. Whiles the amino acid profiles in the rubber seed meals are presented in Table 11. The variously-processed rubber seed meals were variable in their amino acid compositions. For example, the gross lysine (an indispensable amino acid) contents ranged from 0. 434 g/100 g DM for the raw rubber seed meal (RRSM) to 0.590 g/100 g DM for the sundried rubber seed meal (SDRSM).

Proteins are composed of amino acids, and it is actually the amino acids that are the essential nutrients. Therefore, the dietary provision of amino acids in correct amounts and proportions determines the adequacy of a dietary protein ingredient (NRC, 1998).

Table 12: Mean gross amino acid compositions (g/100 g DM) of the various rubber seed meals

Amino acid	Type of rubber seed meal					P-value and level of significance
	RRSM	SRSM	SDRSM	BRSM	RoRSM	
Lysine	0.434^{c}	0.512^{b}	0.590^{a}	0.569^{a}	0.504^{b}	0.001***
Methionine	0.199^{b}	0.153^{c}	0.216^{a}	0.208^{ab}	0.199^{b}	0.001***
Cystine	0.313^{ab}	0.258^{c}	0.330^{a}	0.322^{a}	0.291^{b}	0.001***
Histidine	0.329^{a}	0.294^{a}	0.329^{a}	0.321^{a}	0.307^{a}	0.053NS
Phenylalanine	0.622^{b}	0.509^{c}	0.685^{a}	0.659^{ab}	0.647^{ab}	0.001***
Threonine	0.519^{a}	0.422	0.553^{a}	0.530^{a}	0.519^{a}	0.001***
Leucine	1.002^{a}	0.796^{b}	1.061^{a}	1.022^{a}	1.012^{a}	0.001***
Isoleucine	0.512^{a}	0.437^{b}	0.528^{a}	0.503^{a}	0.496^{a}	0.006
Valine	1.139^{a}	0.953^{b}	1.163^{a}	1.131^{a}	1.115^{a}	0.003**
Alanine	0.781^{a}	0.599^{b}	0.803^{a}	0.824^{a}	0.781^{a}	0.001***
Aspartic acid	1.758^{a}	1.419^{b}	1.811^{a}	1.745^{a}	1.723^{a}	0.001***
Arginine	1.718^{ab}	1.268^{c}	1.819^{a}	1.784^{a}	1.657^{b}	0.001***
Serine	0.732^{a}	0.582^{b}	0.771^{a}	0.740^{a}	0.727^{a}	0.001***
Glutamic acid	2.563^{a}	2.027^{b}	2.722^{a}	2.567^{a}	2.601^{a}	0.001***
Glycine	0.698^{a}	0.575^{b}	0.709^{a}	0.681^{a}	0.674^{a}	0.002**
Proline	0.889^{a}	0.563^{b}	0.897^{a}	0.901^{a}	0.858^{a}	0.001***

RRSM: raw rubber seed meal, SRSM: soaked rubber seed meal; SDRSM: sundried rubber seed meal; BRSM: boiled rubber seed meal; RoRSM: roasted rubber seed meal. ` ^{abc}Means within a row with the same superscripts are not significantly different NS, non-significant (P≥0.05); *P≤0.05; **P≤0.01; ***P≤0.001

Amino acids in the protein of different types of rubber seeds (Table 12) were generally high in glutamic acid (mean value of 13.70 g/100 g protein) and low in methionine (mean value of 1.07 g/100 g protein). The values for aspartic acid were also generally high. Glutamic acid is considered to be a conditionally essential amino acid in some species (Lacey and Wilmore, 1990), because it prevents intestinal atrophy under certain conditions. Study conducted by Wu *et al*. (1996) showed that addition of 1% glutamine to corn-soybean meal diet prevented jejunal atrophy in pigs weaned at 21 days during the first week post-weaning and increased feed efficiency during the second week post-weaning.

Table 13: Amino acid profiles of the various rubber seed meals (g/100g protein)

Amino acid	Type of rubber seed meal					P-value and level of significance
	RRSM	SRSM	SDRSM	BRSM	RoRSM	
Lysine	2.448^{c}	3.249^{a}	3.041^{b}	3.047^{b}	2.591^{c}	0.001***
Methionine	1.126^{a}	0.968^{b}	1.114^{a}	1.111^{a}	1.021^{a}	0.001***
Cystine	1.768^{a}	1.633^{b}	1.700^{a}	1.726^{a}	1.497^{c}	0.001***
Histidine	1.855^{a}	1.863^{a}	1.695^{a}	1.715^{a}	1.580^{a}	0.053NS
Phenylalanine	3.515^{b}	3.230^{c}	3.529^{a}	3.528^{a}	3.327^{c}	0.001***
Threonine	2.448^{c}	2.687^{b}	2.849^{a}	2.837^{a}	2.669^{b}	0.001***
Leucine	5.658^{a}	5.057^{c}	5.471^{a}	5.474^{a}	5.211^{b}	0.001***
Isoleucine	2.894^{a}	2.771^{ab}	2.721^{ab}	2.693^{b}	2.552^{c}	0.006*
Valine	6.436^{a}	6.050^{b}	5.998^{b}	6.057^{b}	5.741^{c}	0.003*
Alanine	4.412^{a}	3.805^{c}	4.140^{b}	4.414^{a}	4.019^{b}	0.001***
Aspartic acid	9.935^{a}	9.008^{b}	9.335^{a}	9.345^{a}	8.867^{c}	0.001***
Arginine	9.706^{a}	8.046^{c}	9.374^{a}	9.549^{a}	8.425^{b}	0.001***
Serine	4.135^{a}	3.690^{c}	3.972^{b}	3.963^{b}	3.739^{bc}	0.001***
Glutamic acid	14.483^{a}	12.868^{c}	14.032^{a}	13.748^{b}	13.381^{b}	0.001***
Glycine	3.945^{a}	3.648^{b}	3.657^{a}	3.646^{b}	3.469^{c}	0.002**
Proline	4.995	3.569^{d}	4.623^{bc}	4.823^{ab}	4.411^{c}	0.001***

RRSM: raw rubber seed meal, SRSM: soaked rubber seed meal; SDRSM: sundried rubber seed meal; BRSM: boiled rubber seed meal; RoRSM: roasted rubber seed meal. `

^{abc}Means within a row with the same superscripts are not significantly different; NS, non-significant (P≥0.05); *P≤0.05; **P≤0.01; ***P≤0.001

The variation in the nutrient compositions and the hydrogen cyanide contents of the different types of rubber seed meals may have resulted from the different processing employed in this study. Based on the results of this study, the variously-processed rubber seed meals contain relative good potential amounts of dietary components that could be derived when utilized in formulating animal feeds.

4.2 STUDY TWO: Assessment of the Nutritional Quality of Variously-Processed

Rubber Seed Meals as Dietary Ingredients Using the Laboratory Rat as Model for Pigs

The summary of the growth performance characteristics and organ weights of growing laboratory rats is presented in Table 14.

Table 14: Effect of variously-processed RSM on rat growth performance and organ weights

Parameter	Dietary treatments						
	Control	RRSM	SRSM	SDRSM	BRSM	RoRSM	P-value and level of significance
Total feed intake, g	359.1^{a}	340.0^{a}	353.7^{a}	332.4^{a}	359.5^{a}	305.3^{a}	0.178NS
Daily feed Intake, g	12.83^{a}	12.14^{a}	12.63^{a}	11.87^{a}	12.84^{a}	10.90^{a}	0.178NS
Initial body weight, g	61.84^{a}	59.17^{a}	65.00^{a}	61.67^{a}	62.50^{a}	62.50^{a}	0.106NS
Final body weight, g	162.0^{a}	152.0^{a}	166.16^{a}	142.0^{a}	166.82^{a}	134.16^{a}	0.120NS
Total weight gain, g	100.2^{a}	92.8^{a}	101.20^{a}	80.30^{a}	104.30^{a}	71.70^{a}	0.164NS
Daily weight gain, g	3.58^{a}	3.32^{a}	3.61^{a}	2.87^{a}	3.73^{a}	2.56^{a}	0.164NS
Feed:gain	3.58^{a}	3.67^{a}	3.50^{a}	4.14^{a}	3.45^{a}	4.26^{a}	0.124NS
Total water intake, ml	454.7^{a}	414.9^{a}	427.32^{a}	340.7^{b}	446.8^{a}	325.0^{b}	0.007**
Daily water intake, ml	16.24^{a}	14.82^{a}	15.26^{a}	12.17^{b}	15.96^{a}	1161^{b}	0.007**
Mortality, %	0	0	0	0	0	0	-
Organ weights, g g^{-1}LBW							
liver	0.496^{c}	0.506^{bc}	0.604^{a}	0.588^{a}	0.567^{ab}	0.490^{c}	0.009**
heart	0.041^{b}	0.061^{a}	0.047^{a}	0.044^{b}	0.040^{b}	0.036^{b}	0.017*
kidney	0.089^{a}	0.109^{a}	0.093^{a}	0.104^{a}	0.100^{a}	0.093^{a}	0.165NS
lung	0.079^{c}	0.113^{ab}	o.099^{abc}	0.123^{a}	0.093^{bc}	0.111^{ab}	0.030*
spleen	0.072^{a}	0.087^{a}	0.098^{a}	0.083^{a}	0,100^{a}	0.074^{a}	0.205NS
intestines	0.506^{a}	0.487^{a}	0.470^{a}	0.534^{a}	0.457^{a}	0.453^{a}	0.194NS
Cost/g feed, GH¢	0.020	0.0185	0.0188	0.0187	0.0189	0.0188	-
Feed cost per g weight gain, GH¢	0.072^{a}	0.0679^{a}	0.0658^{a}	0.0774^{a}	0.0652^{a}	0.0801^{a}	0.016NS

RRSM: raw rubber seed meal, SRSM: soaked rubber seed meal; SDRSM: sundried rubber seed meal; BRSM: boiled rubber seed meal; RoRSM: roasted rubber seed meal. `

^{abc}Means within a row with the same superscripts are not significantly different; NS, non-significant (P≥0.05); *P≤0.05; **P≤0.05

Feed intake by rats for the 4-week period was not significantly (P≥0.05) affected by the various dietary treatments. The daily feed intake varied from 10.9 g (RoRSM diet) to 12.83 g (control

and BRSM diets). The non-significant effect of the various rubber seed meals inclusion in diets on feed intake suggests that rats will consume diets containing processed rubber seed meals. A major factor affecting feed consumption in animals is the dietary energy content because animals eat to satisfy their inner metabolic need for energy. In this study, the experimental diets were formulated to be isocaloric.

There was not much difference ($P \geq 0.05$) in average rat weight prior to the conduct of the feeding trial. Final body weight also had little effect ($P \geq 0.05$) on the dietary treatments. Consequently, there was little influence ($P \geq 0.05$) of the dietary treatments on the mean daily live weight gain. Nevertheless, compared with rats on the BRSM diet and the control and the other rubber seed meal containing diets, in absolute terms, the BRSM diet registered slightly faster growth. Similarly, the dietary treatments had little impact ($P \geq 0.05$) on efficiency of feed utilization (feed: gain) of the rats. However, feed: gain ratio was slightly better for the BRSM diet than the control diet and other the rubber seed-containing diets in absolute terms.

There were no indications of ill health and no mortalities were recorded attributable to the experimental treatments during the conduct of the trial. The structural and size of organs such as the liver, heart and gastro-intestinal tract are often indication of the physiological state of the body. In this study, dietary treatments had significant ($P < 0.05$) influence on the relative weights of the heart, liver and lung, however, kidney, spleen and empty intestinal weights were unaffected ($P \geq 0.05$). Rats on the rubber seed meals, which on chemical analysis were found to contain certain amounts of HCN, in general, registered significantly higher liver weights in comparison with the control diet. Palmer and Olson (1979) reported an increase in liver weight in animals exposed to 4 mg cyanide/kg body weight/day. At the termination of the 4-week trial, examination of several organs (heart, liver, kidney, spleen, lung and intestines) from all the rats, however, revealed no macroscopic deviation from the normal in terms of gross tissue changes.

The results of the haematological and blood biochemical indices of rats fed diets which contained the raw and the variously-processed rubber seed meals are shown in Table 15. Haematology and blood biochemistry are routinely used to evaluate the health status of animals (Mafuvadze and Erlwahger, 2007). The degree to which blood constituents changes occur in apparently healthy animals indicate to what extent they can make physiological adjustments to stresses due to pathological, environmental, hormonal and nutritional factors, as well as the actions of drugs and toxic substances. Nutrition, particularly dietary protein intake, reportedly affects the liveweight and haematological indices of animals (Makinde *et al.,* 1991). The effects of diets on haematological parameters have also been reported by other researchers (Ologhobo *et al*., 1993; Otesile *et al*., 1991).

Table 15: Haematological and blood biochemical indices of rats fed diets containing variouslyprocessed RSMs

Parameter	Dietary treatments						
	Control	RRSM	SRSM	SDRSM	BRSM	RoRSM	P-value and level of significance
Haemoglobin, g/dl	12.45^{a}	15.00^{a}	14.65^{a}	15.25^{a}	14.20^{a}	14.75^{a}	0.263NS
RBC X 10^{6}/µl	7.00^{a}	8.34^{a}	8.22^{a}	8.53^{a}	8.10^{a}	8.22^{a}	0.481NS
WBC X 10^{3}/µl	14.50	11.40	7.95	11.30	6.30	14.20	0.001***
Haematocrit, %	39.30^{a}	47.00^{a}	46.30^{a}	46.50^{a}	43.30^{a}	45.20^{a}	0.482NS
MCHC, g/dl	31.85^{a}	32.00^{a}	31.65^{a}	32.05^{a}	32.85^{a}	32.65^{a}	0.588NS
MCH, pg	17.95^{a}	18.00^{a}	17.85^{a}	17.90^{a}	17.60^{a}	18.00^{a}	0.977NS
MCV, fL	56.30	56.35	53.85	56.00	53.50	55.05	0.001***
Lymphocytes, X 10^{3}/µl	9.25^{ab}	9.15^{ab}	6.35^{b}	13.00^{a}	5.20^{b}	11.90^{a}	0.009**
Neutrophils, X 10^{3}/µl	1.05^{a}	0.45^{a}	0.30^{a}	0.90^{a}	0.20^{a}	0.30^{a}	0.075NS
PLT, X 10^{3}/µl	422.0^{a}	713.0^{a}	890.0^{a}	654.0^{a}	934.0^{a}	802.0^{a}	0.055NS
Blood sugar, mmol/l	4.50^{a}	3.35^{bc}	2.85^{c}	2.80^{c}	3.60^{b}	3.30^{bc}	0.001***
Globulins, g/l	46.0^{a}	43.0^{a}	46.50^{a}	49.0^{a}	47.0^{a}	56.0^{a}	1.130NS
Albumin, g/l	36.05^{a}	40.65^{a}	38.50^{a}	39.50^{a}	41.30^{a}	41.80^{a}	0.585NS
Total protein, g/l	82.15^{a}	83.35^{a}	84.80^{a}	88.35^{a}	88.00^{a}	97.85^{a}	0.935NS
Total bilirubin, µmol/l	2.50^{a}	2.00^{a}	2.50^{a}	3.00^{a}	3.50^{a}	5.00^{a}	0.637NS
Cholesterol, mmol/l	1.25^{a}	1.50^{a}	1.40^{a}	1.70^{a}	1.55^{a}	2.05^{a}	2.219NS

Hb- haemoglobin; RBC- Red blood cells; WBC- white blood cells; HCT- haematocrit; MCHC- mean cell haemoglobin concentration; MCH- mean cell haemoglobin ; MCV- mean cell volume; PLT- platelet ^{abc}Means within a row with different superscripts are significantly different. NS, non-significant (P≥0.05); *P≤0.05; **P≤0.01; ***P≤0.001. RRSM: raw rubber seed meal, SRSM: soaked rubber seed meal; SDRSM: sundried rubber seed meal; BRSM:
boiled rubber seed meal; RoRSM: roasted rubber seed meal. `

In the present study, the dietary treatment had little influence ($P \geq 0.05$) on haematological and blood biochemical indices of rats, with the exception of the white blood cell count, MCV and lymphocyte values as well as the blood sugar levels.

The serum total protein, albumin and globulins of the rats in this study were little affected ($P > 0.05$) by the different types of rubber seed meals inclusion in the diets. This is an indication that the protein levels of the experimental diets were able to support the protein reserves of the rats. The insignificant differences in the total protein and albumin values between the control group and rubber seed meal fed groups suggests that there was not much depression in hepatic synthesis and or degradation of protein (Akanya *et al*., 2015).

In general, rats on the rubber seed meal diets, registered lower ($P < 0.05$) blood sugar levels in comparison with those on the control diet devoid of rubber seed meal. This observation may be attributed to the high fibre contents of the rubber seed meal diets and supported by the assertion that high fibre diets have been associated with the reduction in blood sugar contents of animals (Dodson *et al*., 1981).

The absence of significant variations in the serum metabolites, but for blood sugar levels, could also be attributed to the comparable protein and feed intakes among the rats on the various diets. This is in support of the study conducted by Adesehinwa *et al*. (2008). Jain (1986) reported that protein deficiency reduces most haematological and serum biochemical indices through reduction or impairment of the synthesis of blood cells which are proteinaceous in nature. The levels of haematological and blood biochemical indices observed in this study were within the normal ranges reported by Research Animal Resources (2015).

The cost per g of feed and the feed cost per g weight gain are presented in Table 14. Although the rubber seeds were obtained free of charge from the Plantation Section of the Department of

Crop and Soil Sciences, the variously-processed rubber seed meals were assigned values of GH¢1.00; GH¢1.15; GH¢1.25; and GH¢1.10 per kg for SDRSM, SRSM, BRSSM and RoRSM, respectively being the cost of picking the seeds, transportation and processing. The cost per kg maize and soyabean meal, which the variously-processed rubber seed meals replaced in the experimental diets were GH¢1.04 and GH¢3.00, respectively. Consequently, the cost per g of the control diet was higher (GH¢ 0.020) than all the various rubber seed mealcontaining diets. This was due solely to the price disparities between soyabean meal and the different types of the rubber seed meals. Feed cost per gram live weight was lowest for rats on the BRSM-containing diet and highest for the RoRSM diet ($P<0.05$). The lowest cost of feed conversion registered by rats on the BRSM diet may be attributed to the slightly better efficiency of feed utilization and vice versa for rats on the RoRSM diet.

Based on the results of this study, processed rubber seed meal could be harnessed as a supplement for formulating animal or poultry feed. Seasonal variations in the prices of feedstuffs such as maize and soyabean meal would make the use of alternative feedstuffs such as rubber seed meal in animal diets more attractive.

4.3 STUDY THREE: Effect of Variously-Processed Rubber Seed Meals on Rat Reproductive Performance

The number of fertile females, gestation length, number of pubs born, number of pups born alive, still births, weight at birth, number weaned and weaning weight of pups as determined in this study are presented in Table 16.

Table 16: Effect of variously-processed RSM on rat reproductive performance

Parameter of	Control	RRSM	SRSM	SDRSM	BRSM	R_oRSM	P-value and level significance
No. of fertile rats examined		3	3	3	3	3	3
No. of fertile females	3	3	3	3	3	1	
Pregnancy index, %	100	100	100	100	100	33.3	
Gestation length, days	22.67^{a}	22.67^{a}	23.0^{a}	22.33^{a}	22.0^{a}	22.0^{a}	0.108NS
No. of pups born	8.67^{a}	7.33^{a}	10.0^{a}	5.67^{a}	9.67^{a}	4.01a	0.036NS
No. of pups born alive	8.67^{a}	7.00^{a}	10.0^{a}	5.67^{a}	9.33^{a}	4.01^{a}	0.061NS
Live birth index, %	100	95.5	100	100	96.5	100	
No. of still birth	0^{a}	0.333^{a}	0^{a}	0^{a}	0.333^{a}	0^{a}	0.658NS
Weight of pups at birth, g	4.88^{a}	6.00^{a}	5.59^{s}	7.07^{a}	5.68^{a}	6.25^{a}	0.062NS
Weaning weight, g	34.06^{bc}	33.73^{bc}	34.68^{b}	29.60^{cd}	32.33^{c}	48.25^{a}	0.001***
No. of pups weaned	6.0^{a}	7.0^{a}	9.67^{a}	3.33^{a}	8.67^{a}	4.01	0.015NS
Mortality of pups	2.67^{a}	0.00^{b}	0.33^{b}	2.33^{a}	0.67^{b}	0.00^{b}	0.001***
Malformations	nil	nil	nil	nil	nil	nil	

^{abc}Means within a row with different superscripts are significantly different. NS, non-significant (P≥0.05); *P≤0.05; **P≤0.01; ***P≤0.001. RRSM: raw rubber seed meal, SRSM: soaked rubber seed meal; SDRSM: sundried rubber seed meal; BRSM: boiled rubber seed meal; RoRSM: roasted rubber seed meal.

The results indicate that the inclusion of the various types of rubber seed meals in diets did not alter the general health of rats. Also, there were no significant changes in the maternal body weights of rats, which averaged 199.8 g

Conception occurred in all the females fed the various dietary treatments, except those on roasted rubber seed meal (Ro.RSM)-containing diet. Apparently, no conception occurred in two of the three female rats fed diets which contained the RoRSM. The reasons for this observation are not clear.

The dietary treatments did not have any significant (P≥0.05) influence on the gestation lengths or length of pregnancy of the animals, the average observed in this study being 22.45 days. This observation is in agreement with reports that gestation in rats takes 21 to 23 days from copulation to parturition (Hamid and Zuki, 2013). All pregnant rats delivered normally with no evidence of

prematurity or abortion suggesting that the various rubber seed meals were not abortificatient when consumed. No external malformations were observed in any of the pups delivered.

The average litter size and the number of pups born alive both varied from 4.01 for those on RoRSM-containing diet to 10.0 for the soaked rubber seed meal (SRSM), whiles the number of still births varied from 0 for animals on the control, the SRSM and SDRSM-containing diets to 0.333 for the RRSM and BRSM diets but the values obtained were not statistically ($p>0.05$) different.

Birth weights of the pups were little affected by the various dietary treatments. The weaning weights of pups were significantly ($P< 0.05$) influenced by the dietary treatments and the values varied from 29.6 g for SDRSM diet to 48.25 g for RoRSM diet. However,the results obtained in this study is in agreement with observation of Yang and Mickelsen (1974) who reported that the rat, at birth, weighs about 6 g and it is weaned at 3 weeks when it weighs about 7 times its birth weight.

Post-natal mortalities were significantly ($P> 0.05$) affected by the dietary treatments. The values recorded varied from 0 for the RRSM and RoRSM diets to 2.67 for those on the control devoid of any of the rubber seed meals.

Studies conducted by Tewe and Maner (1981) indicated that reproductive performance of rats fed diets containing 500 mg CN^-/kg throughout gestation and lactation was unaffected.

Furthermore, litter size, weight of the pups at birth and feed consumption and growth rate of pups after birth were not significantly different from the controls.

The present findings indicate that the variously-processed rubber seed meals do not pose any significant reproductive toxicity or complications in pregnancy, delivery and early pup growth in rats.

4.4 STUDY FOUR: UTILIZATION OF DIETS IN WHICH BOILED RUBBER SEED MEAL PARTIALLY REPLACED SOYABEAN MEAL USING THE LABORATORY RAT AS MODEL FOR PIGS

The general performance of the experimental population is presented in Table 17. Average feed consumption per rat for the 4-week period ranged from 262.8 g to 353.7 g. Feed intake was significantly (P≤0.05) affected by the inclusion of BRSM in the diet. Feed consumption of rats fed on the control diet was not different (P≥0.05) from those given the diet containing 50 g kg^{-1} diet. However, rats fed on the diets containing higher concentrations of BRSM (100 and 150 g BRSM kg^{-1} diets) registered significantly (P≤0.05) lower feed intakes.

Table 17: Effect of varying amounts of BRSM on growth performance, organ weights, and economy of gain of rats

Parameter	Level of BRSM (g kg^{-1} diet)					
	Control	50	100	`150	SEM	P-value and level of significance
Growth performance						
Feed intake, g	353.7[a]	341.0[ab]	262.8[c]	309.5[b]	11.45	0.0001****
Initial body weight, g	71.67[a]	76.83[a]	68.17[a]	73.50[a]	8.95	0.16NS
Final body weight, g	152.17[a]	150.83[a]	131.33[a]	141.0[a]	12.50	0.60NS
Body weight gain, g	80.5[a]	74.0[a]	63.2[a]	67.5[a]	7.93	0.91NS
Feed: gain	4.49[a]	4.89[a]	4.71[a]	4.63[a]	0.46	0.14NS
Water intake, ml	470.7[a]	483.8[a]	406.2[b]	488.5[a]	12.05	0.0003****
Mortality, %	0	0	0	0	-	-
Organ weights, g g^{-1}LBW						
liver	0.049[a]	0.053[a]	0.050[a]	0.054[a]	0.0071	0.46NS
heart	0.004[a]	0.005[a]	0.004[a]	0.004[a]	0.0008	0.67NS
kidney	0.012[a]	0.008[a]	0.009[a]	0.010[a]	0.0016	1.95NS
lung	0.011[a]	0.010[a]	0.009[a]	0.007[a]	0.0016	1.69NS
spleen	0.006[a]	0.007[a]	0.008[a]	0.006[a]	0.0025	0.14NS
intestines (empty)	0.055[a]	0.048[a]	0.050[a]	0.057[a]	0.0086	0.57NS
Economy of gain						
Cost/g feed, GH¢	0.020	0.0193	0.0184	0.0175	-	
Feed cost per g weight gain, GH¢	0.0898	0.0944	0.0867	0.0810		

SEM – Standard error of the mean; NS – non-significant (P≥0.05); *P≤0.05; ****P≤0.0001
[abc]Means within a row with different superscripts are significantly different

There was little difference (P>0.05) in average rat weight at the start of the feeding trial for rats fed on diets containing 0, 50, 100 and 150 g BRSM kg^{-1} in place of soya bean meal. Body weight gain was relatively low for the BRSM-containing diets and was not significantly (P≥0.05) affected by the dietary levels of BRSM. Weight gains of rats fed on the diet containing no BRSM were not different (P≥0.05) from those given different amounts of BRSM, though rats on the BRSM-containing diets registered slightly lower weight gains.

The efficiency with which feed was converted to gain (feed:gain ratios) did not show any difference (P>0.05) though animals fed on the control diet (devoid of BRSM) were slightly more efficient in converting feed to weight gain, followed by those on the 150 g kg^{-1} diet.

High dietary fibre reportedly negatively affects the digestibility of proteins and energy (Fernandez and Jorgensen, 1986; Chebeauti *et al*., 1991; Noblet and Perez, 1993). Cyanogenic glycosides on hydrolysis yields toxic hydrocyanic acid (HCN). The cyanide ions inhibit several enzyme systems; depress growth through interference with certain essential amino acids and utilization of associated nutrients (Fernando, 1987; Kumar, 1992; Ukpebor *et al*., 2007). Ravindran (1983) found impaired performance traits with pigs fed more than 10% rubber seed meal. This effect was, however, attributed to a deficiency of lysine and methionine rather than to cyanogenic glycoside present in the meal.

The amount of water drunk by rats was significantly (P≤0.05) affected by dietary treatments. Rats on the 100 g BRSM kg^{-1} diet consumed significantly (P≤0.05) lower amount of water compared with the control diet and the other BRSM-containing diets. This might be due to significantly (P≤0.05) lower quantity of feed consumed by the rats on the100 g BRSM kg^{-1} diet. This observation corroborates with studies that have shown a relationship between feed and water consumption under *ad libitum* conditions (Harris and Van Horn, 2003; Donkoh *et al*., 2012). These studies and the present study herein reported confirm the observation that absolute water intake of animals is positively correlated with feed intake.

There were no indications of ill-health among the rats on the various dietary treatments and no mortalities were recorded during the study. The relative organ weights of the experimental

animals are given Table 15. The liver, heart, kidney, lungs, spleen and intestinal weights for rats fed on diets containing graded levels of BRSM were not significantly (P≥0.05) different from those fed the control diet devoid of BRSM. At the termination of the 4-week study, gross organ (liver, heart, kidney, lungs, spleen and intestines) changes were not observed at necroscopy in all the experimental rats.

The cost per gram of feed declined as more BRSM was included to replace soyabean meal. The diet that contained the highest amount of BRSM was cheaper, that is, GH¢ 0.020, GH¢ 0.0193, GH¢ 0.0184 and GH¢ 0.0175, respectively. This was solely attributed to the price disparities between BRSM and soya bean meal. Feed cost per gram live weight gain was lowest for rats on the 150 g kg^{-1} diet and highest for the 50 g kg^{-1} diet. The highest cost of feed conversion registered by rats on the 50 g kg^{-1} diet may be attributed to the poorer efficiency of feed utilization.

From the results of this study, 150 g kg^{-1} diet inclusion rate of BRSM might be beneficial in terms of cost effectiveness.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 *Conclusion*

Based on the results of the studies conducted, rubber seed, which is normally considered as a waste product of rubber plantation, can be utilized as a by-product. Chemical analysis of rubber seed indicates that it can be considered as a good source of animal feed. The protein, mineral and amino acid contents of the RSMs evaluated in this study revealed the potential relative amounts

of dietary components that could be derived when it is used in formulating animal and poultry feed.

Samples of rubber seed used in this study, however, contained the anti-nutritional factor, hydrogen cyanide. Various processing methods employed in this study (sun-drying, soaking in water, boiling in water followed by sun-drying and roasting) can reduce the HCN content to tolerable levels. The results showed that the combination of boiling rubber seeds for 30 minutes and sun-drying for 12 hours was the most effective way of decreasing the HCN concentration and also resulted in a better quality rubber seed meal for animal feed based on the growth performance and reproductive trials.

The findings of the studies indicated that the various types of rubber seed meals did not have any significant detrimental effects on growth and physiolocal paramters as well as any significant reproductive toxicity or complications in pregnancy in rats compared to those fed the control diet.

It appears rubber seed meal has the potential to be a protein source to the diets of animals and that partial replacement of other protein sources, such as soyabean meal was possible and even confers economic benefits.

5.2 *Recommendations*

From the results of the studies conducted, further studies with rubber seed are justified. Consequently, it is recommended that further studies be conducted to determine;

1. The length of storage on the nutrient quality and hydrogen cyanide concentrations of unprocessed rubber seed meal.
2. The duration of boiling rubber seed on the nutrient quality and hydrogen cyanide concentrations of the resultant seed meal.

3. The effect of methionine supplementation on growth performance and economy of gain of animals fed diets containing rubber seed meal.

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APPENDICES

STUDY 1

APPENDIX 1: ANOVA table for %DM

Source of Variation	Df	S.S	M.S	V.r	F. Pr
Treatments	4	573.90	143.48	717.37	< 0.001
Residual	10	2.00	0.20		
Total	14	475.900			

APPENDIX 2: ANOVA table for % Moisture

Source of Variation	Df	S.S	M.S	V.r	F. Pr
Treatments	4	573.90	143.48	717.37	< 0.001
Residual	10	2.00	0.20		
Total	14	575.900			

APPENDIX 3: ANOVA table for %CP

Source of Variation	Df	S.S	M.S	V.r	F. Pr
Treatments	4	13.96 0.78	3.49	44.73	< 0.001
Residual	10	14.74	0.08		
Total	14				

APPENDIX 4: ANOVA table for % CF

Source of Variation	Df	S.S	M.S	V.r	F. Pr
Treatments	4	59.66 2.61	14.91	57.08	< 0.001
Residual	10	62.27	0,26		
Total	14				

APPENDIX 5: ANOVA table for %E.E

Source of Variation	Df	S.S	M.S	V.r	F. Pr
Treatments	4	65.10 9.50	16.28	17.13	< 0.001
Residual	10	74.60	0.95		
Total	14				

APPENDIX 6: ANOVA table for % ASH

Source of Variation	Df	S.S	M.S	V.r	F. Pr
Treatment	4	2.48	0.62	16.50	< 0.001
Residual	10	0.36	0.04		
Total	14	2.84			

APPENDIX 7: ANOVA table for %NDF

Source of Variation	Df	S.S	M.S	V.r	F. Pr
Treatments	4	781.50	195.38	14.69	< 0.001
Residual	10	133.00	13.30		
Total	14	914.50			

APPENDIX 8: ANOVA table for M.E

Source of Variation	Df	S.S	M.S	V.r	F. Pr
Treatments	4	229141.4	57285.4	64.02	< 0.001
Residual	10	8948.7	894.9		
Total	14	238090.1			

APPENDIX 9: ANOVA table for Ca

Source of Variation	Df	S.S	M.S	V.r	F. Pr
Treatments	4	0.008	0.0020	10.05	0.002
Residual	10	0.002	0.0002		
Total	14	0.010			

APPENDIX 10: ANOVA table fo · P

Source of Variation	Df	S.S	M.S	V.r	F. Pr
Treatments	4	0.080	0.0200	60.12	< 0.001
Residual	10	0.003	0.0003		
Total	14	0.083			

APPENDIX 11: ANOVA table fo : K

Source of Variation	Df	S.S	M.S	V.r	F. Pr
Treatments	4	0.7602	0.1900	509.04	< 0.001
Residual	10	0.0037	0.0004		
Total	14	0.7639			

APPENDIX 12: ANOVA table fo : Mg

Source of Variation	Df	S.S	M.S	V.r	F. Pr
Treatments	4	0.0030	0.00075	3.88	0.037
Residual	10	0.0019	0.00019		
Total	14	0.0049			

STUDY 2

APPENDIX: GROWTH PARAMETERS

APPENDIX 13: ANOVA table for Initial weight

Source of Variation	Df	S.S	M.S	V.r	F. Pr
Treatments	5	52.772	10.554	2.33	0.106
Residual	12	54.271	4.523		
Total	17	107.043			

APPENDIX 14: ANOVA table fo : final weight

Source of Variation	Df	S.S	M.S	V.r	F. Pr
Treatments	5	2608.4	521.7	2.22	0.120
Residual	12	2819.7	235.0		
Total	17	5428.1			

APPENDIX 15: ANOVA table fo : total weight gain

Source of Variation	Df	S.S	M.S	V.r	F. Pr
Treatments	5	2417.2	483.4	1.92	0.164
Residual	12	3018.4	251.5		
Total	17	5435.6			

APPENDIX 16: ANOVA table fo · weekly weig ıt gain

Source of Variation	Df	S.S	M.S	V.r	F. Pr
Treatments	5	151.07	30.21	1.92	0.164
Residual	12	188.65	15.72		
Total	17	339.72			

APPENDIX 17: ANOVA table fo · Daily weight gain

Source of Variation	Df	S.S	M.S	V.r	F. Pr
Treatments	5	3.0831	0.6166	1.92	0.164
Residual	12	3.8500	0.3208		
Total	17	6.9331			

APPENDIX 18: ANOVA table fo · total feed int ıke

Source of Variation	Df	S.S	M.S	V.r	F. Pr
Treatments	5	6509.2	1301.8	1.85	0.178
Residual	12	8454.1	704.5		
Total	17	14963.3			

APPENDIX 19: ANOVA table for weekly feed intake

Source of Variation	Df	S.S	M.S	V.r	F. Pr
Treatments	5	406.82	81.36	1.85	0.178
Residual	12	528.38	44.03		
Total	17	935.20			

APPENDIX 20: ANOVA table for Daily feed intake

Source of Variation	Df	S.S	M.S	V.r	F. Pr
Treatments	5	8.3025	1.6605	1.85	0.178
Residual	12	10.7833	0.8986		
Total	17	19.0858			

APPENDIX 21: ANOVA table for FCR

Source of Variation	Df	S.S	M.S	V.r	F. Pr
Treatments	5	1.5781	0.3156	2.18	0.124
Residual	12	1.7355	0.1446		
Total	17	3.3136			

APPENDIX 22: ANOVA table fo · feed cost/kg ıveight gain

Source of Variation	Df	S.S	M.S	V.r	F. Pr
Treatments	5	5.1894	1.0379	1.98	0.155
Residual	12	6.2971	0.5248		
Total	17	11.4865			

APPENDIX 23: ANOVA table fo · total water c nsumption

Source of Variation	Df	S.S	M.S	V.r	F. Pr
Treatments	5	45848.0	9170.0	5.62	0.007
Residual	12	19580.0	1632.0		
Total	17	65428.0			

APPENDIX 24: ANOVA table fo · Daily water onsumption

Source of Variation	Df	S.S	M.S	V.r	F. Pr
Treatments	5	58.480	11.696	5.62	0.007
Residual	12	24.975	2.081		
Total	17	83.454			

ORGAN WEIGHTS

APPENDIX 25: ANOVA table for Viscera

Source of Variation	Df	S.S	M.S	V.r	F. Pr
Treatments	5	178.51	35.70	1.44	0.279
Residual	12	197.30	24.77		
Total	17	475.81			

APPENDIX 26: ANOVA table fo · Heart

Source of Variation	Df	S.S	M.S	V.r	F. Pr
Treatments	5	0.40571	0.08114	3.55	0.034
Residual	12	0.27455	0.02288		
Total	17	0.68026			

APPENDIX 27: ANOVA table fo · Lungs

Source of Variation	Df	S.S	M.S	V.r	F. Pr
Treatments	5	0.48130	0.09626	1.70	0.209
Residual	12	0.67870	0.056		
Total	17	1.16000			

APPENDIX 28: ANOVA table fo
· Kidneys

Source of Variation	Df	S.S	M.S	V.r	F. Pr
Treatments	5	0.77590	0.15518	1.83	0.181
Residual	12	1.01610	0.08468		
Total	17	1.79200			

APPENDIX 29: ANOVA table fo
· Spleen

Source of Variation	Df	S.S	M.S	V.r	F. Pr
Treatments	5	1.54810	0.30962	3.42	0.038
Residual	12	1.08650	0.0954		
Total	17	2.63460			

APPENDIX 30: ANOVA table fo
· Liver

Source of Variation	Df	S.S	M.S	V.r	F. Pr
Treatments	5	40.921	8.184	6.21	0.005
Residual	12	15.822	1.319		
Total	17	56.743			

APPENDIX 31: ANOVA table for GIT Full

Source of Variation	Df	S.S	M.S	V.r	F. Pr
Treatments	5	20.04	4.01	0.36	0.867
Residual	12	134.31	11.19		
Total	17	154.35			

APPENDIX 32: ANOVA table fo
· GIT Empty

Source of Variation	Df	S.S	M.S	V.r	F. Pr
Treatments	5	20.540	4.108	2.60	0.081
Residual	12	18.973	1.581		
Total	17	39.513			

RELATIVE WEIGHTS OF ORGANS

APPENDIX 33: ANOVA table for Relative weight of Viscera

Source of Variation	Df	S.S	M.S	V.r	F. Pr

Treatments	5	11.977	2.395	0.94	0.490
Residual	12	30.612	2.551		
Total	17	42.589			

APPENDIX 34: ANOVA table fo · Relative wei ght of Heart

Source of Variation	Df	S.S	M.S	V.r	F. Pr
Treatments	5	0.112048	0.022410	4.33	0.017
Residual	12	0.062069	0.005172		
Total	17	0.174117			

APPENDIX 35: ANOVA table fo · Relative wei ght of Lungs

Source of Variation	df	S.S	M.S	V.r	F. Pr
Treatments	5	0.37759	0.07552	3.69	0.030
Residual	12	0.24586	0.02049		
Total	17	0.62345			

APPENDIX 36: ANOVA table for Relative weight of Kidneys

Source of Variation	df	S.S	M.S	V.r	F. Pr
Treatments	5	0.087837	0.017567	1.92	0.165
Residual	12	0.109950	0.009162		
Total	17	0.197786			

APPENDIX 37: ANOVA table for Relative weight of Spleen

Source of Variation	df	S.S	M.S	V.r	F. Pr
Treatments	5	0.20529	0.04106	1.72	0.205
Residual	12	0.28666	0.02389		
Total	17	0.49195			

APPENDIX 38: ANOVA table fo · Relative wei ght of Liver

Source of Variation	df	S.S	M.S	V.r	F. Pr
Treatments	5	3.8411	0.7682	5.25	0.009
Residual	12	1.7563	0.1464		
Total	17	5.5974			

APPENDIX 39: ANOVA table fo · Relative wei ght of GIT Full

Source of Variation	df	S.S	M.S	V.r	F. Pr

Treatments	5	17.321	3.464	2.38	0.101
Residual	12	17.452	1.454		
Total	17	34.773			

APPENDIX 40: ANOVA table fo · Relative wei ght of GIT Empty

Source of Variation	df	S.S	M.S	V.r	F. Pr
Treatments	5	1.4661	0.2932	1.77	0.194
Residual	12	1.9889	0.1657		
Total	17	3.4550			

BLOOD PARAMETERS

APPENDIX 41: ANOVA table for Blood sugar

Source of Variation	df	S.S	M.S	V.r	F. Pr
Treatments	5	5.7750	1.1550	9.50	< 0.001
Residual	12	1.4500	0.1208		
Total	17	7.2250			

APPENDIX 42: ANOVA table fo · HCT_%

Source of Variation	df	S.S	M.S	V.r	F. Pr
Treatments	5	128.68	25.74	0.95	0.482
Residual	12	323.57	26.96		
Total	17	452.26			

APPENDIX 43: ANOVA table for Hb (g/dl)

Source of Variation	Df	S.S	M.S	V.r	F. Pr
Treatments	5	15.325	3.065	1.50	0.263
Residual	12	24.600	2.050		
Total	17	39.925			

APPENDIX 44: ANOVA table fo · Lymphocyte_x103_ul

Source of Variation	Df	S.S	M.S	V.r	F. Pr
Treatments	5	137.511	27.502	5.22	0.009
Residual	12	63.195	5.266		
Total	17	200.706			

APPENDIX 45: ANOVA table fo · MCHC_g_dl

Source of Variation	Df	S.S	M.S	V.r	F. Pr
Treatments	5	3.3263	0.6653	0.77	0.588
Residual	12	10.3450	0.8621		
Total	17	13.6713			

APPENDIX 46: ANOVA table fo · MCH_pg

Source of Variation	df	S.S	M.S	V.r	F. Pr
Treatments	5	0.3400	0.0680	0.15	0.977
Residual	12	5.5100	0.4592		
Total	17	5.8500			

APPENDIX 47: ANOVA table fo · MCV_fl

Source of Variation	df	S.S	M.S	V.r	F. Pr
Treatments	5	23.7112	4.7422	12.12	<0.001
Residual	12	4.6950	0.3912		
Total	17	28.4062			

APPENDIX 48: ANOVA table fo · Neutrophil_x103_ul

Source of Variation	df	S.S	M.S	V.r	F. Pr
Treatments	5	1.8850	0.3770	2.68	0.075
Residual	12	1.6900	0.1408		
Total	17	3.5750			

APPENDIX 49: ANOVA table fo · PLT_X103_ ıl

Source of Variation	df	S.S	M.S	V.r	F. Pr
Treatments	5	519375	10.3875	3.00	0.055
Residual	12	415486	34624		
Total	17	934861			

APPENDIX 50: ANOVA table for RBC_103_ul

Source of Variation	df	S.S	M.S	V.r	F. Pr
Treatments	5	4.4626	0.8925	0.96	0.481
Residual	12	11.2077	0.9340		
Total	17	15.6703			

APPENDIX 51: ANOVA table fo · WBC_X103_ul

Source of Variation	df	S.S	M.S	V.r	F. Pr
Treatments	5	162.336	32.467	11.34	<o.oo1
Residual	12	34.365	2.864		
Total	17	196.701			

AMINO ACID PROFILE

APPENDIX 52: ANOVA table for Vlanine

Source of Variation	df	S.S	M.S	V.r	F. Pr
Treatments	4	0.097682	0.024421	20.26	<0.001
Residual	10	0.012054	0.001205		
Total	14	0.109737			

APPENDIX 53: ANOVA table fo
: Arginine

Source of Variation	df	S.S	M.S	V.r	F. Pr
Treatments	4	0.591310	0.147827	26.15	<0.001
Residual	10	0.056527	0.005653		
Total	14	0.647836			

APPENDIX 54: ANOVA table for Aspartic-acid

Source of Variation	df	S.S	M.S	V.r	F. Pr
Treatments	4	0.290438	0.072610	12.15	<0.001
Residual	10	0.059738	0.005974		
Total	14	0.350176			

APPENDIX 55: ANOVA table fo
: Cystine

Source of Variation	df	S.S	M.S	V.r	F. Pr
Treatments	4	0.0102114	0.0025528	13.49	<0.001
Residual	10	0.0018925	0.0001893		
Total	14	0.0121039			

APPENDIX 56: ANOVA table for Glutamic -acid

Source of Variation	df	S.S	M.S	V.r	F. Pr
Treatments	4	0.87468	0.21867	16.78	<0.001
Residual	10	0.13047	0.01305		
Total	14	1.00514			

APPENDIX 57: ANOVA table fo
: Glycine

Source of Variation	df	S.S	M.S	V.r	F. Pr
Treatments	4	0.0347031	0.0086758	9.33	0.002
Residual	10	0.0092950	0.0009295		
Total	14	0.0439981			

APPENDIX 58: ANOVA table for Histidine

Source of Variation	df	S.S	M.S	V.r	F. Pr
Treatments	4	0.0027969	0.0006992	3.41	0.053
Residual	10	0.0020535	0.0002054		
Total	14	0.0048504			

APPENDIX 59: ANOVA table fo · Isoleucine

Source of Variation	df	S.S	M.S	V.r	F. Pr
Treatments	4	0.0144975	0.0036244	7.06	0.006
Residual	10	0.0051350	0.0005135		
Total	14	0.0196325			

APPENDIX 60: ANOVA table fo · Leucine

Source of Variation	df	S.S	M.S	V.r	F. Pr
Treatments	4	0.130522	0.032630	16.15	<0.001
Residual	10	0.020206	0.002021		
Total	14	0.150727			

APPENDIX 61: ANOVA table fo · Lysine

Source of Variation	df	S.S	M.S	V.r	F. Pr
Treatments	4	0.0453201	0.0113300	19.52	<0.001
Residual	10	0.0058050	0.0005805		
Total	14	0.0511251			

APPENDIX 62: ANOVA table for Methionine

Source of Variation	df	S.S	M.S	V.r	F. Pr
Treatments	4	0.00730740	0.00182685	23.24	<0.001
Residual	10	0.00078600	0.00007860		
Total	14	0.00809340			

APPENDIX 63: ANOVA table forMethionine-Cystine

Source of Variation	Df	S.S	M.S	V.r	F. Pr
Treatments	4	0.0339444	0.0084861	16.31	<0.001
Residual	10	0.0052025	0.0005202		
Total	14	0.0391469			

APPENDIX 64: ANOVA table for Phenylalanine

Source of Variation	df	S.S	M.S	V.r	F. Pr

Treatments	4	0.0558609	0.0139652	17.09	<0.001
Residual	10	0.0081710	0.0008171		
Total	14	0.0640319			

APPENDIX 65: ANOVA table for Proline

Source of Variation	df	S.S	M.S	V.r	F. Pr
Treatments	4	0.254623	0.063656	47.34	<0.001
Residual	10	0.013445	0.001345		
Total	14	0.268068			

APPENDIX 66: ANOVA table fo · Serine

Source of Variation	df	S.S	M.S	V.r	F. Pr
Treatments	4	0.065421	0.016355	15.45	<0.001
Residual	10	0.010602	0.001060		
Total	14	0.076023			

APPENDIX 67: ANOVA table fo · Threonine

Source of Variation	df	S.S	M.S	V.r	F. Pr
Treatments	4	0.0302079	0.0075520	13.77	<0.001
Residual	10	0.0034835	0.0005484		
Total	14	0.0356914			

APPENDIX 68: ANOVA table for Valine

Source of Variation	df	S.S	M.S	V.r	F. Pr
Treatments	4	0.085088	0.021272	8.39	0.003
Residual	10	0.025363	0.002536		
Total	14	0.11045			

APPENDIX 69: ANOVA table fo · Ammonia

Source of Variation	df	S.S	M.S	V.r	F. Pr
Treatments	4	0.0135744	0.0033936	8.71	0.003
Residual	10	0.0038960	0.0003896		
Total	14	0.0174704			

STUDY 3

REPRODUCTIVE PERFORMANCE

APPENDIX 70: ANOVA table for Weight at birth

Source of Variation	df	S.S	M.S	V.r	F. Pr

Treatments	5	8.0761	1.6152	3.06	0.062
Residual	10	5.2825	0.5282		
Total	15	13.1064			

APPENDIX 71: ANOVA table fo · Gestation length

Source of Variation	df	S.S	M.S	V.r	F. Pr
Treatments	5	2.4410	0.4882	2.44	0.108
Residual	10	2.0000	0.2000		
Total	15	4.0000			

APPENDIX 72: ANOVA table fo · Mortality of ɔups

Source of Variation	df	S.S	M.S	V.r	F. Pr
Treatments	5	21.3245	4.2649	15.99	<0.001
Residual	10	2.6667	0.2667		
Total	15	21.7500			

APPENDIX 73: ANOVA table fo · Number of still birth

Source of Variation	df	S.S	M.S	V.r	F. Pr
Treatments	5	0.4436	0.0887	0.67	0.658
Residual	10	1.3333	0.1333		
Total	15	1.7500			

APPENDIX 74: ANOVA table for Number of pups weaned (rat)

Source of Variation	df	S.S	M.S	V.r	F. Pr
Treatments	5	94.340	18.868	4.97	0.015
Residual	10	38.000	3.800		
Total	15	119.000			

APPENDIX 75: ANOVA table for Number of p ɹps born alive (rat)

Source of Variation	df	S.S	M.S	V.r	F. Pr
Treatments	5	80.236	16.047	3.09	0.061
Residual	10	52.000	5.200		
Total	15	105.750			

APPENDIX 76: ANOVA table fo · Pups born (r t)

Source of Variation	df	S.S	M.S	V.r	F. Pr
Treatments	5	83.667	16.733	3.75	0.036
Residual	10	44.667	4.467		
Total	15	100.000			

APPENDIX 77: ANOVA table fo · Weaning wei ght per litter

Source of Variation	df	S.S	M.S	V.r	F. Pr
Treatments	5	100167	20033	3.70	0.037
Residual	10	54117	5412		
Total	15	151936			

APPENDIX 78: ANOVA table fo · Weaning wei ght per pup

Source of Variation	df	S.S	M.S	V.r	F. Pr
Treatments	5	639.393	127.879	44.55	<0.001
Residual	10	28.707	2.871		
Total	15	299.379			

APPENDIX 79: ANOVA table for Weight of pups at birth per litter

Source of Variation	df	S.S	M.S	V.r	F. Pr
Treatments	5	1735.4	347.1	1.51	0.271
Residual	10	2299.3	229.9		
Total	15	3297.9			

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APPENDIX 80: ANOVA table for Initial Weight

Source of Variation	Df	S.S	M.S	V.r	F. Pr
Treatments	3	235.458	78.486	0.16	0.9198
Residual	20	9612.50	480.625		
Total	23	9847.958			

APPENDIX 81: ANOVA table fo · final Weight

Source of Variation	Df	S.S	M.S	V.r	F. Pr
Treatments	3	1696.3	565.4	0.60	0.6207
Residual	20	18759.0	937.95		
Total	23	20455.3			

APPENDIX 82: ANOVA table fo · Total Weight Gain

Source of Variation	Df	S.S	M.S	V.r	F. Pr

Treatments	3	1035.125	345.0416	0.91	0.4517
Residual	20	7545.83	377.292		
Total	23	8580.96			

APPENDIX 83: ANOVA table fo : Feed Intake

Source of Variation	df	S.S	M.S	V.r	F. Pr
Treatments	3	29462.83	9820.94	12.50	<.0001
Residual	20	15719.08	785.95		
Total	23	45181.92			

APPENDIX 84: ANOVA table fo : Water Intake

Source of Variation	Df	S.S	M.S	V.r	F. Pr
Treatments	3	26226.46	8742.2	10.04	0.0003
Residual	20	17420.48	871.1		
Total	23	43646.93			

APPENDIX 85: ANOVA table fo : FCR

Source of Variation	Df	S.S	M.S	V.r	F. Pr
Treatments	3	0.51223	0.1707	0.14	0.9371
Residual	20	25.0229	1.2511		
Total	23	25.5351			

APPENDIX 86: ANOVA table for Viscera

Source of Variation	Df	S.S	M.S	V.r	F. Pr
Treatments	3	50.9682	16.9894	1.49	0.3449
Residual	20	45.58755	11.396887		
Total	23	96.555787			

APPENDIX 87: ANOVA table fo : Liver

Source of Variation	df	S.S	M.S	V.r	F. Pr
Treatments	3	2.2197375	0.7399125	0.46	0.7260
Residual	20	6.451950	1.6129875		
Total	23	8.6716875			

APPENDIX 88: ANOVA table fo : Spleen

Source of Variation	df	S.S	M.S	V.r	F. Pr

Treatments	3	0.10170	0.03390	0.14	0.9306
Residual	20	0.96450	0.2411250		
Total	23	1.06620			

APPENDIX 89: ANOVA table fo
: Heart

Source of Variation	df	S.S	M.S	V.r	F. Pr
Treatments	3	0.03883750	0.01294583	0.67	0.6121
Residual	20	0.076950	0.01923750		
Total	23	0.11578750			

APPENDIX 90: ANOVA table fo
: Lung

Source of Variation	df	S.S	M.S	V.r	F. Pr
Treatments	3	0.52853750	0.17617917	1.69	0.3059
Residual	20	0.41735000	0.10433750		
Total	23	0.94588750			

APPENDIX 91: ANOVA table fo
: Kidney

Source of Variation	df	S.S	M.S	V.r	F. Pr
Treatments	3	0.41863750	0.13954583	1.95	0.2635
Residual	20	0.28625000	0.07156250		
Total	23	0.70488750			

APPENDIX 92: ANOVA table for GIT Full

Source of Variation	Df	S.S	M.S	V.r	F. Pr
Treatments	3	40.44373750	13.48124583	3.14	0.1491
Residual	20	17.18235000	4.29558750		
Total	23	57.62608750			

APPENDIX 93: ANOVA table for GIT Empty

Source of Variation	df	S.S	M.S	V.r	F. Pr
Treatments	3	3.80613750	1.26871250	0.57	0.6659
Residual	20	8.96355000	2.24088750		
Total	23	12.76968750			