KAMPALA INTERNATIONAL UNIVERSITY – WESTERN CAMPUS

SCHOOL OF PHARMACY

ANALYSIS OF DIFFERENT ANIMAL FEEDS IN UGANDA MARKETS AND THEIR HAEMATOLOGICAL AND BIOCHEMICAL ROLES IN MALE WISTAR RATS

BY

ATIM SHARON

BPH/0003/81/DU

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SUPERVISED BY

MR. EZEONWUMELU JOSEPH

DECLARATION

I Atim Sharon hereby declare that this information compiled has not been used at the university or any other higher institution of learning to the best of my knowledge.

NAME: ATIM SHARON

Registration no: BPH/0003/81/DU

Signature: Atim.

SUPERVISOR: .....

Signature: .....

DEDICATION

I would like to dedicate my research to my family especially my mother, Mrs. Margaret Lukermoi and friends, near and far, thank you for showing me your support in so many diverse yet tremendous ways and I will always be grateful.

God bless you all!!

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LIST OF ABBREVIATIONS:

WBC	White Blood Cells
RBC	Red blood Cells
MCH	Mean Cell Haemoglobin
PLT	Platelet
CK	Creatine Kinase
ALP	Alanine Phosphatase
AST	Aspartate Aminotransferase
ALT	Alanine Aminotransferase
GGT	Gamma Glutamyl Transpeptidase
ALB	Albumin
CHOL	Cholesterol
TRIG	Triglycerides
CREA	Creatinine
LDH	Lactate Dehydrogenase
Na^+	Sodium ions

K^-	Potassium ions
Cl^-	Chloride ions
HM	Hound Meal
FF	Formulated Feed
SW	Sow and Weaner
RP	Rabbit Pellets
WHO	World Health Organisation
SEM	Standard Error Mean

ABSTRACT

The utilization of animal feeds and diets in Uganda prepared from available and affordable plants such as; soya bean, groundnut, maize, and pumpkin green leaves, sunflower seeds and animal sources such as; milk, catfish or silverfish, and bones or shells, in the alleviation of poor nutritional status is to be studied, to determine comparative dietary efficiencies of some fortified weaning formulae. This study was carried out in two places; Kampala International university-Western campus Pharmacy laboratory in Ishaka-Bushenyi Uganda and Kabale Regional Referral Clinical Laboratories. This is an experimental study that was carried out between November 2012 and March 2013 at the places. Male wistar rats weighing not less than 90g were used. Selection was done by simple random sampling. Effects on body weights, Organ weights, haematological and biochemical parameters were analysed and recorded. The different types of feed in Ugandan Markets had varying amounts of proteins, carbohydrates, fats, fibre and minerals, thus affected all biochemical parameters such as ALP, AST, ALT, GGT, cholesterol, urea, and creatinine variably but did not cause pronounced effects on the electrolytes (sodium ions, potassium ions and chloride ions). The different types of feed did not affect the Hb, WBC, RBC, PCV, Platelets, MCH, MCHC, MCV levels and differential counts (lymphocytes, neutrophils, monocytes, eosinophils and basophils). However, they caused body weight gain. Suggested recommendations include: Further studies and experiments regarding histological effects of different components of various types of animal feed in Uganda Markets on male wistar rats should be carried out. The regulatory agencies in charge of animal feeds in Uganda markets need to make a set of guidelines ensuring compulsory quantification of feed components to ensure animal health and appropriate growth.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Nutrition in living organisms is one of the most fundamental requirements that is essential for their growth and development of tissues and organs. It is also vital because through feeding, various medicines and drugs are easily absorbed, distributed, metabolized and eliminated through respective organs such as the kidney, the lungs and the skin.

The relevance of Wistar rats in the measurement of the nutritional quality, as a means of scientific investigations, correlate to the human physiological condition, and is founded on the fact that Wistar rats have a dietary requirement for the same essential nutrients as humans. Variations in performance characteristics could occur, as a result of disease conditions such as malnutrition (Obimba, 2006).

Animal feeds in Uganda majorly consist of various components that are formulated and blended together to provide well balanced and healthy meals. The major constituents include; Maize brand, sunflower seeds, fish meal, calcium, enzymes and premix.

Various formulations are prepared for as many omnivorous animals as possible however the quantities of subsequent ingredients depend abundantly on the nutritional needs of a particular animal.

Poor nutrition is a range of pathological conditions arising from co-incident lack, in varying proportions, of proteins and calories occurring most frequently in infants and young children and commonly associated with infections (Roulet, 1994). Under nutrition, is an energy deficit due to chronic deficiency of all macronutrients (Morley, 2007). It can be sudden and total (starvation) or gradual. Severity ranges from subclinical deficiencies to obvious wasting (with edema, hair loss, and skin atrophy) to starvation. Multiple organ systems are often impaired. Diagnosis usually involves laboratory testing, including serum

albumin. Treatment consists of correcting fluid and electrolyte deficits with intra-venous solutions, then gradually replenishing nutrients, orally if possible (Morley, 2007).

(Etukudo et al, 1999) described the broad spectrum nature of malnutrition, which ranges from marasmus through marasmic kwashiorkor to kwashiorkor, and is characterized by low weight for age, oedema, dermatitis, hair changes, mental changes, hepatomegaly and diarrhea.

Nutritional status assessment methods used in detecting and monitoring recovery of animals and patients suffering from malnutrition include: clinical methods, morphological methods, haematological and biochemical methods, dietary survey, and anthropometry.

A major factor among the dietary causes of malnutrition is deficiency in energy intake. Patients have slightly reduced fasting blood sugar and reduced glucose tolerance (Becker, 1983). Fatty liver and/or atrophy of the liver resulting in increased level of Aspartate amino transferase enzyme in the blood, occurs in protein and carbohydrate malnourished subjects (Islam et al., 2007). Hormonal changes, especially in insulin and thyroid hormone secretions, occur as well (Abrol et al., 2001).

1.2 The problem statement

Insufficient components of animal feeds influence growth and development of animal organs and tissues, resulting into malnourishment features thereby hindering responsiveness to absorption, distribution, metabolism and elimination of various medicines yet it is preventable.

1.3 Broad objective

The main objective of the study was to analyse the components of different rodent feeds in Uganda markets, prepare suitable formulae for sufficient nutritional needs of male wistar rats and evaluate their hematological and biochemical roles.

1.4 Specific objectives

The specific objectives of this study were:

1. To determine the amounts of components of various animal feeds in Uganda markets.

2. To determine appropriate amounts of various components needed for standard animal feeds.

3. To formulate a standard rat feed of the required quality.

4. To ascertain the effects of these animal feeds on the hematological and biochemical parameters of male wistar rats.

1.5 Hypothesis

Components of different animal feeds in Uganda markets had varying effects on the haematological and biochemical parameters of male wistar rats.

1.6 Study justification

The direct and indirect effects of feeding male wistar rats with various animal feeds and related complications on organs and tissues are not fully understood in Uganda. The effects on the hormones, enzymes and blood parameters of male rats are variable. Therefore the study was expected to produce results, conclusions and recommendations upon which;

i) good animal feeds can be produced or alternatives could be found

ii) health planners, beneficiaries and implementers may base their efforts to manage animal growth and production and

iii) there would be enhanced drug potency and efficacy depending on the state of the respective animal physiological functions.

CHAPTER TWO

2.0 LITERATURE REVIEW

Nutrition is the science of food, the nutrients, and components in various foods and how the body uses those nutrients. It includes the processes of ingestion, digestion, absorption, metabolism, storage and excretion of those nutrients. The six classes of nutrients include: Carbohydrates, Proteins, Fats, Vitamins, Minerals and water. Considering the Chinese proverb, "He that takes medicine and neglects diet wastes the skills of the physician." Nutrition is therefore an inevitable factor of health and life (Ladau, 1980).

Feed, also called animal feed is food grown or developed for livestock and poultry. Modern feeds are produced by carefully selecting and blending ingredients to provide highly nutritional diets that both maintain the health of the animals and increase the quality of such end products as meat, milk, or eggs. Ongoing improvements in animal diets have resulted from research, experimentation, and chemical analysis by agricultural scientists.

The first scientific effort to evaluate feeds for animals on a comparative basis was probably made in 1809 by the German agriculturist Albrecht von Thaer, who developed "hay values" as measures of the nutritive value of feeds. Tables of the value of feeds and of the requirements of animals in Germany followed and were later used in other countries.

A 20 to 30% addition of animal protein to a 7: 3 (weight to weight) cereal to legume combination improves the nutritive value of foods and induces good and consistent biological responses in experimental animals. The protein advisory group recommends that the protein contents of weaning foods should be at least 20%, on a dry weight basis (FAO/WHO, 1971; WHO, 2001, 2002).

The efficiency of different types of feeds given to the rats are analysed on the basis of consumption index, growth rate and efficiency of conversion of ingested feed as described by (Waldbauer, 1968) and (Dawodu, 2012).

Feed cost account for more than 70% of the total production costs for most types of animal feed, so it is important that returns are maximized through use of adequate diets. Feed formulation is a central operation in animal feed production, ensuring that feed ingredients are economically used for optimum growth of rats and it requires a good knowledge of animal and feed ingredients, so it is essential that formulations are accurate to ensure that large numbers of animal feeds are not adversely affected. For the animal production scientists, the manipulation of animal diet ingredients is the most effective way of regulating not only the animal growth rate but their reproduction and survival rates.

In Nigeria, inadequate availability of ingredients for animal feed production is a major problem especially in the livestock industry. More than half the cost of raising meat farm animals is accounted for by the feed cost (Oyenuga, 1969).

In an attempt to keep peace with increasing demand for livestock for human consumption feed ingredient, feed scientist and nutritionists are always looking for cheap alternatives sources of feed ingredient that can raise livestock production to their desirable level. (Dawodu, 2012)

Laboratory rats such as Wistar rats are the most commonly used animals in preclinical trials in Africa; including species such as *rattus*. Animal experiments have contributed much to our understanding of mechanisms of disease (Hackam, 2006). Systematic review and meta-analysis of animal studies may aid in the selection of the most promising treatment strategies for clinical trials (Vanderwop, 2010).

2.1 Animal feed types, and varying feed components

Animal feeds are classified as;

1. Concentrates; these are feeds that are high in energy value, including fat, cereal grains and their by-products (barley, corn, oats, rye, wheat), high-protein oil meals or cakes (soybean, canola, cottonseed, peanut [groundnut]), and by-products from processing of sugar beets, sugarcane, animals, and fish.

2. Roughages; these are feeds that include pasture grasses, hay, silage, root crops, straw, and Stover (cornstalks).

1.1 Compound Feed

These are blends of various additives and raw materials that are formulated to specifically suit the intended animal. They are often produced as pellets or crumbles. Like modern vitamins with humans, they can be used to either satisfy the complete nutritional requirements of their target animals or as a supplement to other staples of the animals' diets. They're often complemented with extra vitamins and minerals.

1.2 Fodder

Fodder is typically composed of plant matter like hay, straw and grains and is the term is used to describe these plants being given to the animals after the plants have been harvested, which contrasts with forage.

1.3 Forage

Forage is composed of ingredients such as legumes, grasses, corn, oats, alfalfa and other edible plants. The act of eating or grazing upon the plant matter is known as foraging.

Feed additives are pharmaceutical or nutritional substances which are not of natural origin and are added to prepare and store feeds. The different functions of food additives include: growth promotion, prevention of infectious diseases, and enhancement of feed digestibility. Epidemic diseases such as bird flu as well as foot-and mouth-disease have led to an increase in concern over animal health round the globe, due to which meat producers have increased their focus on feed quality and certification. Feed

additives include; Antibiotics, Vitamins, Antioxidants, Amino Acids, Feed Enzymes, Acidifiers, preservatives, prebiotics, and probiotics.

2.2 Roles of various feed components

Proteins, fat, carbohydrates, vitamins, and minerals can all have pharmacological as well as physiological effects on a biological system. The control of vitamin D metabolism, calcium and calcitonin are important factors subject to dietary modification. A conditioned marginal or frank deficiency of folic acid can result from oral contraceptives and administration of anticonvulsants in humans; if studies are done in animals using these types of chemicals, dietary folate will be highly significant. Newer information about the role of ascorbic acid in activation of lipase and lipid mobilization is of direct concern to those using research animals requiring a source of dietary ascorbic acid. (Newberne PM, 2005)

2.3 Formulation of various types of feed

Feed formulation is the process of quantifying the amount of feed that is required to be put together to form a single uniform (diet) for animals that supplied their entire nutrient requirement. It is one of the main operations of the feed industry in view of its role in ensuring good nutrition. (Dawodu, 2012)

Rats are extremely curious and explorative animals and prefer diversity in their nutrition. They are very flexible and will thrive on almost any kind of food provided their nutritional needs are met.

It has been proposed that rats can also acquire their preferences for novel foods from other rats. The mechanisms behind this information transfer are not known. (Berdoy, Macdonald 1991)

Chemical analysis of feeds provides information on the amount of fat, fiber, minerals, vitamins, dry matter and protein that is contained in the feed. (Feed Additives Handbook)

Formulated feed consists of minerals: 2.30g ' 100g of feed, carbohydrates: 63.70g 100g of feed, proteins: 16.00g / 100g of feed, fat: 9.00g /100g of feed, moisture: 2.30g 100g of feed (Obimba, 2006)

Table 1.a:

Conventional and alternative feedstuffs in non-ruminant dietary formulations

Nutrient	Conventional feedstuffs	Percent ration	Alternative feedstuffs	Maximum inclusionn rate
Protein	Groundnut cake	15	Palm kernel cake	15
	Soybean meal	15	Cottonseed cake	10
			Jackbean	10
			Poultry offal	10
Energy	Maize	55	Sorghum	55
			Cassava	45
			Sweet potato	15
Fibre	Brewer's dry grains	15	Maize offal	10
	Rice bran	15	Wheat offal	2.5
			Sorghum offal	10
			Rice husk/bran	5
			Cassava peel	10
Minerals	Oyster shell	7.5	Periwinkle shell	7.5
	Bone meal	2.5	Limestone	5
	Dicalcium phosphate	2.5	Malt dust	2
			Common salt	2

2.4 Animal care

Investigation of scientific progress by developing and testing novel hypotheses involves using appropriately and robustly designed experiments necessary for scientific research using animals. (Russell et al, 2009)

Some of the guidelines include:

Nutrition

Animals should feed on palatable, non-contaminated, and nutritionally adequate food daily unless the experimental protocol requires otherwise (Maneka, 2005)

Beddings

Animal beddings should be absorbent, free of toxic chemicals and other substances that may cause harm to animals or personnel in charge of animal care.

Sanitation

Animal cages should be cleaned and dried before animals are placed in them. Cleaning should also be done washed frequently to keep animals clean and contamination free.

Anaesthesia and euthanasia (animal ethics)

Painful procedures should be conducted under appropriate anaesthesia procedures as recommended for each species of animals. The following requirements should be met.

(a) Death without causing anxiety, pain or distress with minimum time lag phase.

(b) Minimum physiological and psychological disturbances.

2.2 BIOCHEMICAL STUDIES

These include the studies of enzymes and other biological factors such as urea, bilirubin, creatinine and potassium plasma levels that are physiologically important for normal functioning of the body systems. A large number of enzymes are synthesized in the cell and are continuously released in circulation in small amounts as a result of the normal wear and tear of cells. They are removed from circulation by degradation or excretion. They are present in circulation in very minute amount. The liver function tests (LFTs) give information about the activity or concentration of enzymes and compounds in serum rather than quantifying specific hepatic functions, and they are divided into two:

Functional plasma enzymes or plasma specific enzymes; These enzymes are purposely secreted into circulation to perform specific catalytic functions, and include lipoprotein, lipase, blood coagulation factors, complement proteins and others.

Non-functional plasma enzymes or non-plasma specific enzymes: these enzymes do not perform their catalytic function in plasma. They are intracellular enzymes that enter circulation when cells in which they are synthesized disintegrate. When the cell breakdown is at normal rate, the enzymes are found to be every minute in the circulation, and if the cell destruction increases due to pathological conditions, these enzymes will be released into circulation in large amounts and their concentration in plasma will rise many times above normal. However, if the enzyme has a selective tissue distribution or if it is present in far higher concentration in some tissues than elsewhere in the body, it can pinpoint the site of the disease, and this makes these non functional plasma enzymes having a selective tissue distribution to be of diagnostic importance. The following enzymes have become of diagnostic importance (Calbreath, 1992):

Lactate dehydrogenase (LDH): This enzyme catalyses the interconversion of pyruvate and lactate. Its tissue distribution is very wide; however, its concentration is much higher in myocardium, muscle and liver than in any other tissues. Therefore, LDH plasma levels rises in myocardial infarction, viral hepatitis and muscles injuries. There are five isoenzymes, LDH_1 and LDH_2 appear primarily in the heart, LDH_3 appear primarily in the lungs, LDH_4 and LDH_5 are also primarily in the liver and skeletal muscles (Calbreath, 1992).

Transaminase: The serum transaminases are increased if cells are damaged and enzymes released into the circulation. The two most common transaminases are Aspartate amino transferase (ASP) and Alanine aminotransferase (ALT), and they are present in high concentrations in myocardium, liver and muscles. ASP is more concentrated in the myocardium than ALT, and ALT is more concentrated in the liver than ASP.

Creatine kinase (CK): is present in myocardium, muscles, and brain. It catalyses the reaction, creatine + ATP $\longrightarrow$ creatine-P + ADP. Plasma CK rises in myocardial injuries, it is an early indicator in myocardial infarction.

There are three isoenzymes of Creatine kinase:

BB or CK_1 or CK-BB is found in brain, which is the only ck isoenzyme present, bladder, stomach and colon.

MB or CK_2 or CK-MB found in cardiac tissue, which is a cardinal sign of myocardial infarction, if its level is risen in serum.

MM or CK_3 or CK-MM found in skeletal muscle

Alkaline Phosphatase (ALP): this is a group of enzymes that hydrolyze organic phosphate esters at an alkaline pH. It is released into circulation mainly from bones and liver. Its level indicates or estimates the amount of bile flow impedance.

Amylase: this is a digestive enzyme, synthesized in the pancreas and the parotid gland. In acute pancreatitis there is a sharp rise of plasma amylase.

Gamma glutamyl transpeptidase (GGT): Is found in hepatobiliary, pancreatic, and kidney cells. This enzyme catalyses the transfer of the gamma-glutamyl residue of glutathione to other substrates. Its plasma concentration rises in most of the hepatocellular and hepatobiliary disease, although elevations correlate with obstructive disease than with pure hepatocellular damage. An elevated GGT level is often one of the early indicators of alcoholic liver disease (Calbreath, 1992).

Lipases: this lipolytic enzyme is release into circulation from the pancreas. Plasma lipase rises in acute pancreatitis.

2.3 HEMATOLOGICAL STUDIES

Hematological studies deal with the scientific studies of blood and its diseases.

Blood is a connective tissue in a fluid form. It is made up of three kinds of cells which are:

Red blood cells (ERYTHROCYTES)

White blood cells (LEUKOCYTES) and

Platelets (THROMBOCYTES)

A complete blood count (CBC) or full blood count (FBC) comprises;

Hemoglobin (Hb): the Hb test measures the grammes of Hb contained in 100ml (1dl) or 1L of whole blood and provide an estimation of the oxygen-carrying capacity of the red blood cells. The Hb values depend on the number of red blood cells and the amount of Hb in each red blood cell. The normal values are 14-18g/dl for men and 12-16g/dl for women. Low values indicate anaemia (Calbreath. 1992).

Hematocrit (Hct) or packed cell volume (PCV): this measures the percentage by volume of packed red blood cells in a whole blood sample after centrifugation. Its value is three times the Hb value, and it's given in percentage or fraction.

Total white blood cells (TWBCs): report the number of white blood cells in a cubic millimeter of whole blood. The normal value range from 4000WBC/mm3 to 11,000WBC/mm3, an increase in WBC count (LEUKOCYTOSIS) usually indicates infection, and may also result from leukemia or from tissue necrosis, most found in bacterial infection and a decreased WBC count (LEUKOPENIA) indicates bone marrow depression, that may result from metastatic carcinoma, lymphoma or toxic reaction to substance, such as antineoplastic agents. The TWBCs also include white blood cell differential that comprises granulocytes and non-granulocytes.

Total red blood cells; reports the number of RBCs in a cubic millimeter of whole blood, provides an indirect estimate of the blood's Hb content. Normal values are: 4.3-5.9million/mm3of blood for men and 3.5-5.0million/mm3 of blood for women. It comprises Hb, Hct, MCV and erythrocyte sedimentation rate (ESR), RBC indices, mean cell Hb (MCH), RBC distribution width (RDW) and Reticulocytes counts. Mean cell volume (MCV); is the ratio of the Hct to the RBC count. It essentially assesses average RBC size and reflects any anisocytosis.

Platelet count; expresses the number of platelets (thrombocytes) per liter, the normal values are 150,000/mm3 — 300,000mm3. A decreased platelet count is referred to as Thrombocytopenia and increased platelet count as Thrombocytosis. Below is a table showing normal ranges of hematological data of laboratory rats determined from previous studies (Ghandhi, 2009).

Table 1.b:

Parameters:	Values:
RBC(x106/mm³)	7 –10
PCV(%)	36 – 18
Hb(g/dl)	11 – 18
BBC(X10³/mm³)	6 – 17
Neutrophils(%)	9 – 34
Lymphocytes(%)	65 – 85
Eosinophils(%)	0 – 6
Monocytes(%)	0 – 5
Basophils(%)	0 – 1.5
Platelets(X10³/mm³)	500 – 1300

CHAPTER THREE

3.0 METHODOLOGY

3.1 Study design

The study was experimental and involved male wistar rats.

3.2 Setting of the study

The experiments were conducted in Kampala international University –Western Campus. and Kabale Regional Referral Clinical Laboratories. The animal feeds were purchased from Kampala and Ishaka markets and rats were acquired from School of Pharmacy Animal House. KIU-WC Ishaka.

3.3 Selection of study population

The studies were conducted on 30 laboratory male wistar rats, above 8 and 10 weeks of age and not weighing less than 90g.

3.4 Determination of sample size

30 male wistar rats were used, with a minimum of 5 experimental rats in each group. Therefore there were 6 rats in each of the 5 cages.

3.4.1 Exclusion and inclusion criteria

Young and healthy wistar male rats were used for this study. The mature and sickly ones were not used for the study.

3.4.2 .1 Independent Variables

The feeds and water.

3.4.2.2 Dependent Variables

Biochemical parameters: ASP, ALT, ALP, GGT, creatinine, albumin, Urea.

Electrolytes: Na^+, K^+, Cl.

Lipids: triglycerides and total cholesterol.

Hematological parameters: RBC, WBC: Neutrophils, Basophils, Eosinophils, Monocytes, Lymphocytes. Hb, PCV, MCV, MCHC, platelets.

3.5 Sampling techniques

Sampling of rats was done randomly.

3.6 Data collection methods

Data were collected by the use of data collection forms which had different parameters of the study.

This involved observation and quantification as follows:

3.7 Animal feed composition quantification (composition analysis)

Proximate analysis:

Each type of feed was chemically analysed for the following according to Kirk of 1999.

3.7.1. Crude Fat

40g of each powdered sample was placed in a soxhlet apparatus and 100ml of diethyl ether (extracting solvent) was used to extract fat from each feed type. Fat was extracted; the sample was placed in a water bath to evaporate off any remaining solvent. To the sample, 5ml of acetone was added to evaporate to completeness, placed in a desiccator and weighed.

Calculation for all feed types:

Crude fat (g) = (weight of crude fat (g) ÷ weight of sample (g)) x 100

3.7.2 Crude Fibre

To 3g of fat free bone dry sample of each feed type, 200ml of hot dilute sulphuric acid was added and filtered with a Buchner funnel set under light vacuum by suction. The samples were then washed with hot water until acid free, tested by methyl orange indicator.

The residue was added to 200ml of hot dilute sodium hydroxide, boiled, filtered using the Buchner set and washed with hot water until sodium hydroxide free. The filtrate was tested with phenolphthalein indicator.

The residue was dried at 100°C to bone dryness in a hot air oven and weighed.

The residue was transferred to a silica crucible and ignited at 250°C in a hot air oven for 2 hours.

The silica crucible was cooled in a desiccator and ash values of different feed types were weighed.

Calculation for crude fibre of all feed types:

Crude fiber (g) = (Residue – Ash) (g) / Sample (g) x (100-F)

3.7.3 Crude Protein

Each feed type was blended and sieved to obtain fine powder particles and 1g of each sample was weighed and placed in a blender. To 1g of each sample, 700mg of cupric carbonate, 2ml of isopropyl solution, 100ml of 0.5M NaOH and 30% Isopropyl solution were added and blended and filtered using a Buchner set under suction. 5ml of the filtrate of each sample was placed in a test tube and absorbance read at 550nm using a spectrophotometer.

10µL, 2µL, 3µL, 4µL and 5 µL of standard albumin reagent was pipette and used as standard concentrations, and evaluated using the above procedures.

The concentration and weight of each sample of feed type were determined using the formula:

$y = mx + c$: $y = 0.014(x) + 0.012$

Where: m = gradient and c = intercept.

3.7.4 Ash values

10g of each feed type were weighed before ashing and placed in a dry weighed silica crucible. The different animal feeds were burned in a hot air oven at 250°C for 24 hours and the sums of residual minerals were determined. After ashing, the hot air dried samples were weighed to determine the concentration of ash present (Christian. 1994).

Calculation:

The ash content was expressed as:

$$\% \text{ Ash (dry basis)} = \frac{M_{ASH}}{M_{DRY}} \times 100$$

Where; M_{ASH} refers to the mass of the ashed sample, and M_{DRY} refers to the original mass of the dried samples before burning.

3.7.5 Moisture content

2.5g of each feed type were weighed and placed in a dried silica crucible. The various samples were placed in a hot air oven at 100°C for 6 hours to evaporate off any available moisture in the food samples. The various weights of food samples after evaporation were determined and recorded.

Calculation of moisture content in each feed type:

Moisture content (g) = weight of samples before evaporation (g) - weight of samples after evaporation (g).

3.7.6 Crude Carbohydrates

Calculation of crude carbohydrate of each feed type was determined by:

Crude carbohydrate (g) = 100 – (crude fat + crude fibre + crude protein + moisture content)

3.7.7 Reagents and Materials used

Reagents:

- Diethyl ether or petroleum ether
- Acetone
- Sulphuric acid (H2SO4) (1.25 g/100 ml)
- Sodium Hydroxide (NaOH) (1.25 g/100 ml)
- Phenolphthalein indicator
- Methyl orange indicator
- Hydrochloric acid (1 to 2.5 v/v)
- Cupric Carbonate
- Albumin standard reagent
- Isopropyl alcohol

Materials:

- Soxhlet assembly
- Desiccator
- Sample
- Thimble
- Heating arrangement
- Balance

- Buchner filter assembly
- Whatman filter papers
- Hot air over
- Filter papers
- Dishes, porcelain and silica crucibles
- Spectrophotometer
- Blender

3.8.0 Laboratory Animal Acquisition and Maintenance

Only male wistar rats were used for this study. These animals have been bred and housed in the Animal Facility Centre of the School of Pharmacy, Kampala International University-Western Campus. The animals were kept in a cage lined with sawdust, at room temperature with adequate ventilation, under a naturally illuminated environment with 12 hours of light and 12 hours of darkness. They were fed on a standard diet (Nuvita® Animal Feed Ltd, Jinja Uganda). Other animal feeds such as Sow and weaner® (pig feed), Hound meal® (dog feed), Rabbit Pellets® feed and formulated feed that were weighed, and the rats had access to clean drinking water *ad libitum*. The rats were therefore weighed twice a week throughout the period of the study. Animal care protocols were used according to the National Institute of Health Guide for the care and use of laboratory animals (NIH, 1996) and ethical considerations during investigation were adhered to.

3.8.1 Animal Experiments

The body weight of each rat was determined using digital chemical balance before and after the experimental period (as initial and final body weights, respectively), and the mean body weight for each group calculated. Weight changes were expressed as percentage weight increase where:

(i) Percentage weight increase was calculated from the formula:

$$\frac{Wy-Wx}{Wx} \times 100$$

After 90 days of feeding the rats with respective diets, they were euthanised and sacrificed according to the method of (Klaunberg et al, 2004). Their blood samples were collected using cardiac puncture via needle and syringe into heparinised tubes for hematological analysis and some were collected into lithium bottles for other analyses. Non-clotted whole blood was centrifuged to obtain plasma for haematological analyses and clotted blood was centrifuged at 3000g to obtain serum for biochemical analyses. All chemicals and reagents were strictly of analytical grade. The lean body mass of (liver, kidneys, pancreas, lungs, testes, stomach, intestines and spleen) were recorded.

3.8.2. Biochemical assays

After dissecting the rats, blood samples for biochemical assays were collected in requisite blood sample bottles, and stored in a refrigerator at 4°C. Commercial test kits were obtained and the Humaster 180 machine –Germany used for all biochemical parameters measured. Standard methods were used to estimate Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Gamma Glutamyl Transferase (GGT), ALP, creatinine, albumin, urea, triglycerides and total cholesterol. Commercial kits as well as the Humalyte plus[3] human machine were used to determine electrolytes such as Na^+, K^+ and Cl^-

3.8.3. Hematological assays

Blood samples were collected into heparinized tubes and used for the estimation of Complete Blood Count: RBC (Erythrocyte) Count, total WBC Count, Differential Leukocyte Count, Hemoglobin, Mean Cell Volume, Mean Cell Hemoglobin, Mean Cell Hemoglobin Concentration, Hematocrit (PCV) and Blood Platelet Count. The sysmex –Japan, machine was used for analysis.

3.9. Outcome measures

At the end of the study, different components of 4 various animal feeds in Uganda markets were analysed, suitable formulae for sufficient nutritional needs of wistar rats were formulated, analysed and their biochemical and hematological roles were ascertained.

3.10 Data analysis

The data collected from the study were analysed by using Excel data spread sheet to obtain t-test p values and the results were presented in form of tables, graphs and figures. A statistician was consulted during data analysis. Results were also expressed as Mean±SEM and statistical significance level was set at $p<0.05$.

3.11 Ethical Considerations

The laboratory rats were used under minimal stress during the induction of euthanasia and sacrificed as necessary and humanly as possible and ethical considerations during investigation were adhered to. Permission to conduct the study was obtained from the relevant authorities:

the School of Pharmacy KIU and the Research and Post graduate Committee KIU- Western Campus.

3.12 Dissemination of findings

Presentation of the research findings were made to a cross section of KIU staff and students and people selling feeds. Also a copy of the research was submitted to the office of the Dean of School of Pharmacy.

Another copy was deposited in the Library of KIU-WC for reading and consultation by interested readers.

3.13 Study limitations

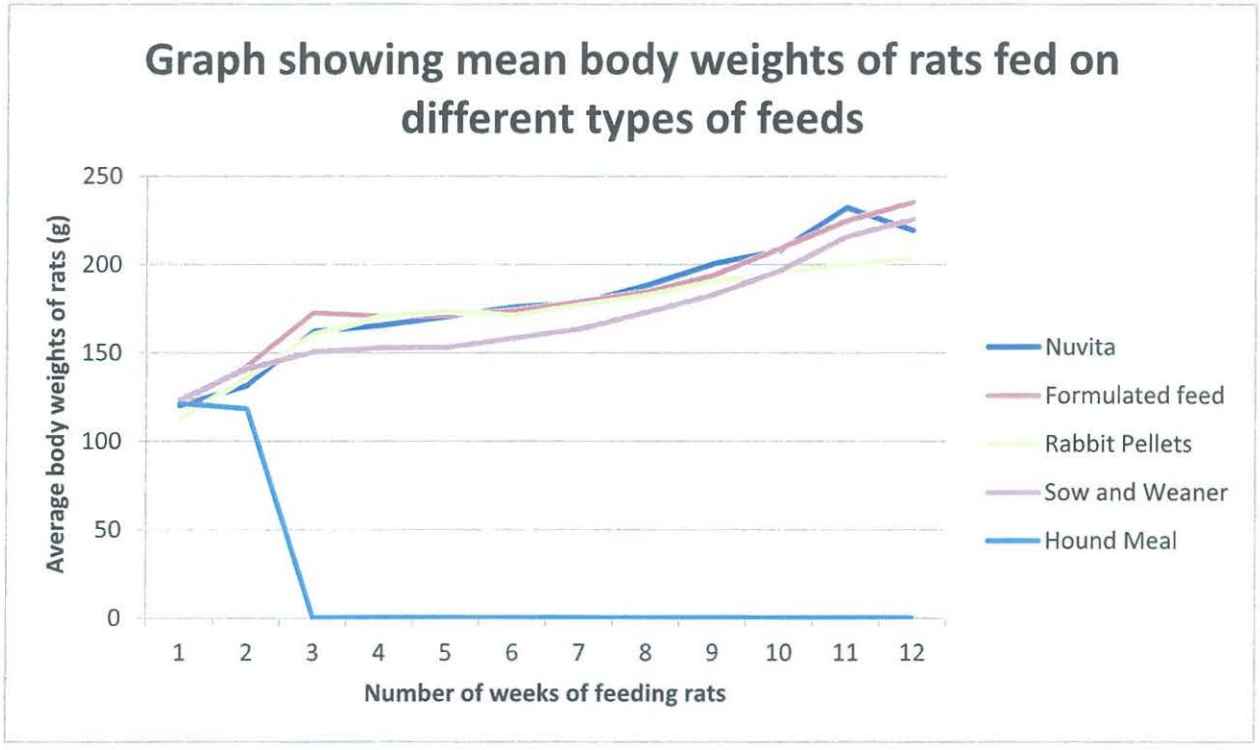
a) Frequent power black outs.

b) Lack of equipment

4.2 Animal body weights

There was an exponential weight gain between week 1 and week 4 in groups of rats fed on Formulated feed, Rabbit Pellets® and Sow and Weaner® -tested groups compared to the control group fed on Nuvita®, with a very slight decrease in weight of rats fed on Hound Meal® feed for the first week and a very steep drop in body weights of these rats between weeks 2 and 3; of which they died during week 3. Between week 4 and week 10, weight increased gradually in groups of rats fed on all other types of feed, except Hound meal® when compared to the control group that were fed on Nuvita® standard feed. However, between weeks 10 and 12, there was a slightly exponential increase in weight gain of rats fed on Formulated Feed and Sow and Weaner® compared to the control group, followed by a continued gradual weight gain of rats fed on Rabbit pallets®. A decrease in weight of the control group was also observed from week 11 to week 12.

Graph 1: A graph showing mean body weights of rats fed on different types of feeds in Uganda Markets



CHAPTER FOUR

4.0 RESULTS

4.1 Proximate analysis

All feed types were analysed and quantified to determine composition of the various components such as crude fat, crude fibre, crude protein, ash values, and moisture content as shown in Table 1 below. Carbohydrates were the highest portion of each type of feed analysed, with the greatest amount observed as 60.50g in Hound Meal® feed (HM), followed by 57.90g in Sow and Weaner® feed (SW), 53.70g in Formulated feed (FF), 51.60g in Rabbit Pellets® feed (RP) -tested feed as compared to the standard feed Nuvita® feed having 42.20g which had the lowest amount of carbohydrates. Moisture content was the second highest, followed by crude fibre content, protein content and crude fat being the smallest portion of each type of feed. There was preponderant decreased amount of protein in Sow and weaner® feed, as compared to the standard feed.

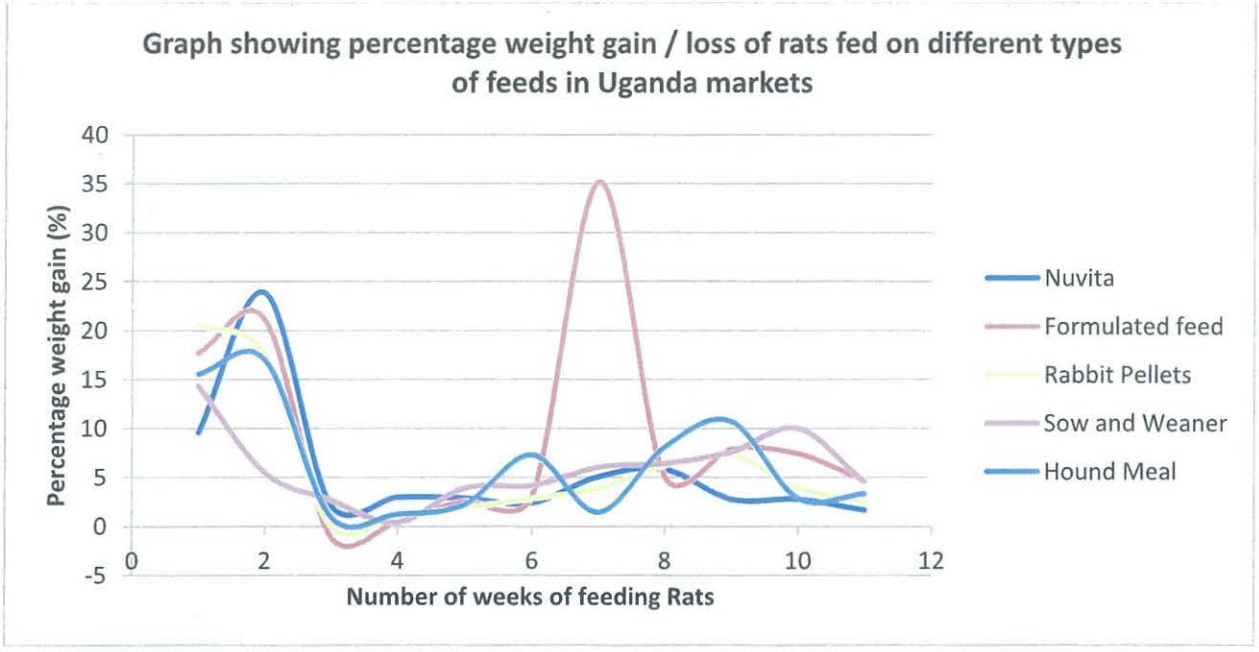
Table 1: Component Amounts of Different Feeds in Uganda Market

Per 100g of feed	Nuvita® (control)	FF (test)	H M® (test)	SW® (test)	RP® (test)
Fat(g)	7.50	7.00	2.00	1.10	4.50
Fibre(g)	10.00	6.70	6.70	16.70	16.70
Moisture(g)	25.00	22.00	21.00	23.00	23.00
Protein(g)	8.30	10.60	9.80	1.30	4.20
Carbohydrate(g)	49.20	53.70	60.50	57.90	51.60
Ash value(g)	68.00	61.00	39.00	22.00	32.00

4.4 Percentage Weight gain

There was a slight increase in percentage weight between week 1 and week 2 observed in all the rats in the tested groups and control group, except those that fed on Sow and Weaner® feed that showed a gradual decrease in percentage weight between week 1 and week 4, followed by gradual decrease in percentage weight in all the tested groups. A drastic increase in percentage weight gain was observed in rats fed on Formulated feed between week 6 and week 7, followed by a drastic decrease in percentage weight. However, subsequent increase and decrease in percentage weight was observed in all rats that fed on the tested feeds and the standard feed, Nuvita® between week 3 and week 12.

Graph 2: A graph showing percentage weight gain / loss of rats fed on different types of feed in Uganda markets.



4.3 Relative organ weights

There was a significant increase in relative organ weight of intestines of rats fed on Rabbit Pellets® feed (6.04±0.65*) compared to the control group (4.36±0.28), with a decrease in relative organ weight of intestines of rats fed on Formulated feed (3.85±0.10). Sow and Weaner ® (3.43±0.77) and Hound Meal® (1.90±0.82) -tested groups compared to the control group. Relative organ weight of liver significantly decreased as observed in rats fed on Rabbit Pellets® (4.67±0.24*) as compared to the control group (5.55±0.54), and decreased in rats fed on Sow and Weaner® and Hound Meal® -tested groups followed by a slight increase in relative organ weight of the liver of groups of rats fed on Formulated feed compared to the control group. There was a significant increase in relative organ weight of pancreas of rats fed on Rabbit Pellets® (0.74±0.08*) compared to the control group (1.24±0.49), with a slight general increase in relative organ weights of rats fed on Formulated feed, Sow and Weaner® and Hound Meal® -tested groups as compared to the control group. Relative organ weights of the spleen significantly increased in the Sow and Weaner® (0.48±0.08*) -tested group compared to the control group (0.40±0.04).

Table 2: A Table showing Relative Organ Weights of rats fed on different types of feeds in Uganda

Organs of Rats	Type of feed				
	Nuvita®	Formulated feed	Rabbit Pellets®	Sow and Weaner®	Hound Meal®
Stomach (g)	1.08±0.08	1.01±0.04	0.87±0.04	0.98±0.18	0.71±0.17
Intestines (g)	4.36±0.28	3.85±0.10	6.04±0.65*	3.43±0.77	1.90±0.82
Liver (g)	5.55±0.54	5.82±0.14	4.67±0.24*	5.13±1.03	2.89±1.03
Pancreas (g)	1.24±0.49	0.54±0.08	0.74±0.08*	0.46±0.12	0.33±0.11
Spleen (g)	0.40±0.04	0.41±0.02	0.44±0.08	0.48±0.08*	0.27±0.11
Kidney (g)	0.83±0.21	0.98±0.06	0.88±0.05	1.11±0.17	0.83±0.21
Lungs (g)	0.86±0.10	1.00±0.07	0.79±0.04	0.91±0.16	0.67±0.15
Heart (g)	0.53±0.05	0.55±0.05	0.37±0.05	0.40±0.09	0.45±0.14
Testes (g)	2.53±0.14	2.06±0.51	2.53±0.19	2.92±0.52	1.64±0.54

Results were expressed as Mean±SEM. Statistical significance done with student t-test was set at $p<0.05$.

4.5 Hematological Parameters

All hematological parameters of other rats were compared to the control rats that were fed on Nuvita® standard feed as shown in Table 2 below.

WBC count significantly increased in rats fed on Formulated feed (7.03±0.37*) and Sow and weaner® feed (10.57±0.91*). A general increase in WBC count was also observed in Rabbit pellet® feed (8.90±1.58), and Hound meal® feed (9.41±0.45) -tested groups as compared to the control group (5.28±0.48). There was a normal steady increase in RBC count in all rats fed on all types of feed in the tested groups as compared to the control group. Haemoglobin (Hb) levels increased slightly in rats on feeds in the tested groups such as Formulated feed, Rabbit pellet® feed, Sow and weaner® feed and Hound meal® feed as compared to the control group. Hematocrit percentage was found to be higher in the tested groups compared to the control group. Mean cell volume increased in Formulated feed, Rabbit pellet® feed, Sow and weaner® feed -tested groups with a slight decrease in Hound meal®. Mean cell haemoglobin was found to be higher in Formulated feed, Rabbit pellet® feed, Sow and weaner® feed -tested groups than that of the control group. There was no significant difference in mean cell haemoglobin concentration in the tested groups including control group. Platelet count increased in all tested feeds compared to the control group, with a slight decrease in Sow and weaner® -tested group. Neutrophil percentage increased in all tested groups compared to the control group (2.86±0.42) with a statistically significant increase in Sow and weaner® (6.34±0.54*) -tested group. Lymphocyte percentage increased in all tested groups compared to the control group (2.56±0.33), with a significant increase in Sow and weaner® (4.23±0.36*) and Rabbit pellet® feed

(3.73±0.55*) -tested groups. Monocyte percentage was found to be higher in all the tested groups than in the control group, and there was no significant change in basophil and eosinophil count.

Table 3: Haematological Parameters of rats fed on different feeds in Uganda Markets

Hematological value	Groups of rats fed on different types of feeds				
Hematogram	Nuvita® (Control)	Formulated feed (Test)	Rabbit pellets® (Test)	Sow and weaner® (Test)	Hound Meal® (Test)
WBC $10^3\mu L$	5.28±0.48	7.03±0.37*	8.90±1.58	10.57±0.91*	9.41±0.45
RBC $10^6\mu L$	9.28±0.15	9.02±0.29	9.52±0.59	9.30±0.50	9.24±0.24
HGB g/dl	15.36±0.19	15.56±0.36	16.60±0.97	16.55±0.70	15.40±0.23
HCT %	52.36±0.81	52.66±1.48	56.98±3.47	52.68±2.89	50.30±0.95
MCV FL	56.48±0.96	58.42±0.71	59.90±41.24	56.78±2.45	54.40±0.77
MCH pg	16.62±0.23	17.28±0.25	17.46±0.22	17.85±0.36	16.70±0.13
MCHC g/dl	29.38±0.19	29.60±0.18	29.16±0.30	31.50±0.79	30.60±0.37
PLT $10^{3\mu L}$	653.43±1.83	755±49.66	662.4±75.61	452.75±150.43	828±5.02
NEUT %	2.86±0.42	4.22±0.23	5.00±1.18	6.34±0.54*	4.20±0.27
LYMP %	2.56±0.33	2.81±0.15	3.73±0.55*	4.23±0.36*	1.88±0.17
MONO %	0.50±0.07	0.62±0.12	0.70±0.09	0.92±0.11	0.53±0.05
EO %	0.21±0.02	0.20±0.02	0.26±0.04	0.28±0.08	0.29±0.16
BASO %	0.002±0.002	0.01±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Results were expressed as Mean±SEM. Statistical significance done with student t-test was set at $p < 0.05$.

4.6 Biochemical parameters

There was a slight increase in levels of Alkaline Phosphatase (ALP) in male rats fed on Formulated feed, a slight decrease in rats fed on Sow and weaner®, Rabbit Pellets® and Hound meal® compared to the control group. Aspartate Aminotransferase (AST) levels fairly increased in rats fed on Formulated feed (139.80±44.83) when compared to the control group (126.40±42.46), and decreased in rats fed on Sow and Weaner® (55.00±0.81) and Hound Meal® (8.00±18.16), with a remarkably significant reduction in AST of rats fed on Rabbit Pellets® (2.80±1.16*) as compared to the control group. ALT levels were

highest in groups of rats fed on Sow and weaner® feed compared to the control, and lower in other groups of rats fed on Formulated feed, Rabbit pellets® and Hound meal® feed. GGT levels were decreased in all groups of rats that were fed on feed types other than the standard feed, Nuvita®. Albumin levels were not significantly increased in all the treated groups compared to the control group. Cholesterol levels significantly increased in rats fed on Sow and weaner® compared to the standard whereas levels did not significantly change in all other groups of rats when compared to the control group. Creatinine levels significantly decreased in rats fed on Sow and weaner® (1.04±0.17*) compared to the control group (0.78±0.06). Na^+, K^+ and Cl^- levels did not show significant increase in all the tested groups as compared to the control group as displayed in Table 5 below.

Table 4: Biochemical Parameters of rats fed on different types of feeds in Uganda Markets

Biochemical Parameters	Types of feed				
	Nuvita® (Control)	Formulated feed (Test)	Rabbit pellets® (Test)	Sow and Weaner® (Test)	Hound meal® (Test)
ALP U/L	765.40±64.40	782.81±89.54	369.00±106.99	517.00±109.59	226.00±157.21
AST U/L	126.40±42.46	139.80±44.83	2.80±1.16*	55.00±0.81	8.00±18.16
ALT U/L	160.80±51.54	142.00±76.68	126.20±55.60	235.00±36.27	21.00±74.32
GGT U/L	50.00±11.95	30.20±13.06	18.80±5.62	12.00±5.45	14.00±3.41
ALB g/dl	4.42±0.22	4.06±0.15	3.88±0.10	4.70±0.99	4.50±1.36
CHOL mg/dl	105.00±11.66	102.20±9.23	112.00±29.50	157.00±33.35	99.00±47.70
TRIG mg/dl	200.20±48.08	189.20±19.63	162.20±49.6	219.00±47.83	146.00±66.26
UREA mg/dl	46.00±23.50	49.20±3.80	31.40±8.05	35.00±9.39	62.00±0.33
CREA mg/dl	0.78±0.06	0.65±0.07	0.79±0.23	1.04±0.17*	1.09±0.32

Results were expressed as Mean±SEM. Statistical significance done with student t-test was set at $p < 0.05$.

Table 5: Electrolytes of rats fed on different types of feeds in Uganda Markets

	Types of feed				
Electrolytes	Nuvita	Formulated feed	Rabbit Pellets	Sow and weaner	Hound meal
K^+ mmol/L	13.63±2.83	22.01±9.30	84.18±4.71	3.97±27.27	24.25±8.41
Na^+ mmol/L	147.60±4.87	141.30±2.56	115.24±28.95	139.70±34.52	144.70±41.79
Cl^- mmol/L	129.62±13.68	105.42±35.1	84.18±21.14	102.50±25.19	106.70±30.62

Results were expressed as Mean±SEM. Statistical significance done with student t-test was set at $p<0.05$.

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

The safety, efficiency and quantification of different components of feed for animals, more so male wistar rats used for laboratory experiments regarding pre-clinical and clinical trials can be determined by composition (proximate) analysis of various feeds in Uganda Markets. This is done to predict amounts of essential nutrients and individual food ingredients, so as to provide guidelines for selecting an appropriate and healthy feed formulation for laboratory wistar rats and animals. The proximate analysis studies showed that the standard rat feed –Nuvita®, and the formulated feed had the highest and most sufficient nutritional satisfaction regarding essential components such as Carbohydrates, Proteins, Fat, Fibre and Minerals per 100g of feed. However, in general all the feeds in Uganda markets can be categorized as relatively well formulated based on the scale proposed by (Feed Additives Handbook, 2012).

Growth of animals, weight gain and weight loss are factors that are majorly dependent on the amount of Protein in any given diet or feed (Guyton, 2006). Considering that general body weight gain was observed throughout the feeding period, with increase in physical activity showed that all the different types of feed had sufficient Protein content for growth and sufficient carbohydrate content for provision of energy. However, death of rats fed on hound meal was not as a result of malnutrition but it most likely occurred due to stress and preying.

Increased and decreased organ weights have been observed as a sensitive indicator of organ function. The different types of feeds did not produce any pronounced effects to the relative organ weights of kidneys, lungs, heart, testes and stomach. The Rabbit Pellets® decreased the relative organ weights of liver and pancreas, while Rabbit Pellets® and Sow and Weaner® increased the relative organ weights of intestines and spleen of tested rats.

Subsequent increase and decrease in percentage body weight gain or weight loss is an observation that most likely occurred due to differences in quantity of feed weighed daily during the feeding period.

Analysis of blood parameters is relevant in nutritional evaluation as changes in the haematological system have higher predictive value for studies (Olson, *et al* 2000). The effect on haemoglobin concentration (Hb), Red blood cell count, packed cell volume (PCV), MCH, MCV, and MCHC indicated the unlikelihood of the different types of feed to induce anaemia within the feeding period and the results were in the normal ranges as shown in previous experiments carried out in rats (Gandhi, 2009). The elevated levels of red blood cells may be due to the high rate of erythropoiesis occurring in the bone marrow of the rats (Guyton, 2006). From the observed values of WBC, it is clear that an increase in the number of WBC is a normal reaction of rats to new feeds and foreign substances contained in various feed formulation (Guyton, 2006). Leucocytosis observed in the study indicates stimulation of immune system which protects the rats against infection that might have been caused by chemical and secondary infections. Leucocytosis which may be directly proportional to the severity of the causative stress condition may be attributed to an increase in leukocyte mobilization.

The Alkaline Phosphatase test (ALP) is used to detect liver disease or bone disorders. Any condition that affects bone growth or causes increased activity of bone cells can affect ALP levels in the blood, such as vitamin D deficiency in diets. ALT is a cytoplasm enzyme found in very high concentration in the liver, and indicates hepatocellular damage, while AST is less specific than ALT as an indicator of liver function. A GGT test and/or a test for 5'-nucleotidase may also be done to differentiate between liver and bone disease. GGT and 5'-nucleotidase levels are increased in liver disease but not bone disorders. Cholesterol is transported by specific carriers, within the blood, to the body's cells. The major carrier is low-density lipoprotein (LDL) and another carrier is high-density lipoprotein (HDL). High levels of LDL level are not healthy, while a high HDL level is considered healthy. The ratio of LDL to HDL is the best indicator of cardiovascular disease susceptibility. The concentration of urea depends on protein content in diets. Rats that fed on feeds with high protein content had a higher serum urea concentration than those

that took feeds with low protein content (Chronolab, 2009). Increased levels of creatinine in blood during the study revealed decreased kidney function; creatinine tests give an estimate of glomerular filtration rate (Mayo Clinic, 2013). The different types of feeds did not significantly affect the electrolytes (Na^+, K^+ and Chloride ions (Cl^-)).

5.2 Conclusion

In conclusion, these results provide evidence that different feeds in Uganda markets consist of various quantities of different components, mixed during feed formulation and various feed types have varying roles on male wistar rats. All the feeds cause a general increase in body weight of rats, especially Formulated feed and the standard feed which contains higher values of essential nutrients and ingredients as compared to the rest of the feeds. The general increase and decrease in organ weights of rats is majorly dependant on protein content in the feeds. Considering the fact that hematological parameters of all the rats were relatively improved and within the normal range, it was an indication that all the feeds contained necessary vitamins, minerals and ingredients required. Biochemical parameters of male wistar rats were either increased or decreased accordingly depending on the chemical composition of each type of feed. However, electrolytes were unchanged and lied within the normal ranges.Therefore the different types of tested feeds and the standard feed, Nuvita® have varying roles on the body weights, relative organ weights, percentage weight gain, hematological and biochemical parameters of male wistar rats.

5.3 Recommendations

Further studies and experiments regarding histological effects of different components of various types of animal feeds in Uganda Markets on male wistar rats need to be carried out.

The regulatory agencies in charge of animal feeds in Uganda markets need to make a set of guidelines ensuring compulsory quantification of feed components to ensure animal health and growth.

CHAPTER SIX

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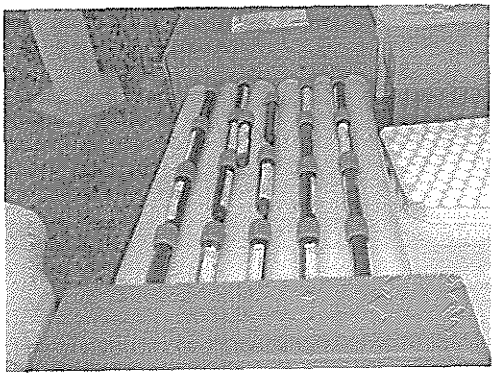
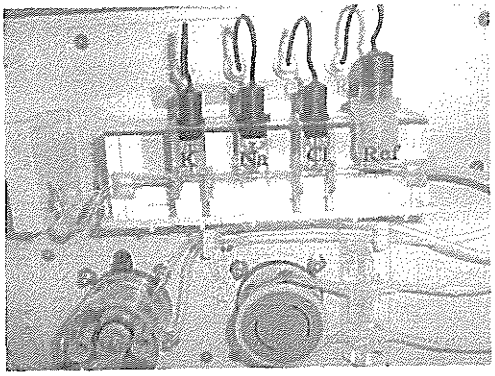
CHAPTER SEVEN

7.0 APPENDICES

7.1 Hematological and Biochemical analysis pictures



7.2 Clinical chemistry analytical machines



7.3 Histological tissues in storage