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COMPARATIVE EFFECT OF THREE DIFFERENT ENERGY SOURCES ON HEMATOLOGY AND SERUM BIOCHEMISTRY OF RABBITS

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Abstract

Purpose: This study examined the effect of three different energy sources on hematology and serum biochemistry of rabbits.

Methodology: Eighteen seven-weeks old rabbits where randomly divided into three groups (A, B and C), each consisting of six rabbits. Group A, B and C were given maize, sorghum and millet respectively as their sources of energy and were fed for a period of five weeks before sample collection and analysis.

Results: The results showed, that there was no significant different ($P>0.05$) in the hematology and serum biochemistry. Nevertheless, the red blood cells in all the treatment fell below the normal range. It was observed that the alkaline phosphatase of all the experimental rabbits were above the normal range. This study suggests that our test materials were not toxic to the muscle, kidney and liver of rabbits at 52.21% inclusion level.

Unique Contributions to Theory Practice and Policy: This implies that sorghum or millet can completely replace maize in rabbit diet with no adverse effect on the hematology and serum biochemistry parameters of rabbits.

Keywords: *Hematology, Serum Biochemistry, Rabbits.*

1.0 INTRODUCTION

A study of the accessible statistics of food supplies consumed in different part of developing countries including Nigeria shows that the per caput protein intake is comparatively low. Onyimonyi & Ene (2003), reported that the average animal protein intake of average Nigerian is 4.5 g/head/day instead of the minimum requirement of 35 g/head/day recommended by the Food and Agricultural Organization of the United Nations (1992). This has been attributed to the insufficient animal protein supply as well as high cost of animal products due to high cost of production and low level of animal productivity. Inadequate supply of feeds, nutritionally poor rations, adulterated ingredients or stale feeds are some of the factors responsible for the low supply of animal protein as a result of low productivity of livestock in tropics (Ogundipe *et al.*, 2003). When it comes to livestock management, nutrition is perhaps the most important consideration. Cattle, sheep, goats, pigs and poultry are the conventional sources of animal protein, particularly for urban dwellers. However, rabbits are beginning to gain popularity as promising food animal in many developing countries. The demand for protein of animal origin in Nigeria is greater than the supply (Akinmutimi & Onwukwe, 2002). There is therefore acute shortage of animal protein in the diet of many Nigerians, demanding that effort should be directed to livestock that are more prolific and have short gestation interval such as rabbit.

The domestic rabbit when compared with other livestock is characterized by early sexual maturity, high prolificacy, relatively short gestation period, short generation interval, high productive potential and rapid growth. In addition, they have better ability to utilize forages and fibrous plant materials and agricultural by-products. They are efficient feed converters, have low cost per breeding female and are profitable for small-scale system of production and in backyards (Cheeke *et al.*, 1986; Finzi & Amici, 1991). In addition, the rabbit meat is nearly white, fine grained, palatable, mildly flavored and high in good quality protein content. They are however low in fat and caloric contents, with a higher percent of minerals than other meats, nearly of the same nutritive value as beef and comparable to that of broiler chicken. Rabbit yields good meat-to-bone ratio and is generally acceptable in most countries of the world (Reddy *et al.*, 1977).

Statement of the Problem

Maize is the chief source of energy to human and livestock, which leads to its high demand, higher competition and increase cost of the product. Furthermore, Vyas *et al*. (2015) & Sanchis-Gomar *et al*., (2016) opined that the quantity of cholesterol in meat products could cause hypercholesterolemia a predisposing factor for cardiovascular diseases like atherosclerosis and myocardial infarctions, which could cause morbidity and mortality in humans. Consequently, the majority of people tend to limit consumption of meat due to the fear of high serum cholesterol concentrations (Ravnskov, 2014).

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2.0 LITERATURE REVIEW

2.1 General Overview of Rabbit

Rabbits are small mammals belonging to the family "*Laporidae*". They were generally found in the wild before their domestication. In the wild, they live in groups in furrows feeding mainly on vegetables like carrots. The male animal is called buck, the female is doe, while the young one is kitten or kit. Rabbit habitats include meadows, woods, forests, grasslands, deserts and wetlands (www.wikipedia.com/rabbit). Mostly, their natural predators such the fox, the birds of prey and the badger check their population (www.wikipedia.com/rabbit). The rabbit is an animal that is endowed with various good qualities like high prolificacy, short gestation period, high growth rate, high feed conversion efficiency, low cost of production when compared to other livestock, with highly nutritious meat that contains low fat, sodium and cholesterol (Mailafia *et al.,* 2010).

The meat has a high protein content of about 26.8% and its consumption is not prohibited by religious and cultures (Biobaku & Oguntona, 1997). The efficiency of the rabbit to digest large amount of fibrous feed when compared to other monogastric has been linked with the presence of numerous microbes in its caecum (Taiwo *et al.*, 1999).

Table 2.1. Nutritional value of meat products from different animals

Animal	Protein (%)	Fat (%)	Moisture (%)	Calories (/1b)
Rabbit	20.8	10.2	67.8	795
Chicken	20	11	37.6	810
Veal	19.1	12	68	840
Beef	16.3	28	55	1440
Pork	11.9	45	42	2050
Lamb	16.7	27.7	55.8	1420

Source: (Lane, 1999)

The growth rate of the Nigerian agricultural sector is below the potentials of natural and human resources due to high cost of agricultural inputs, poor funding of agriculture, inadequate functional infrastructural facilities, inconsistencies of government agricultural policies, inadequate private sector participation, poor mechanized farming and little or no adoption of some simple agricultural technologies developed by scientists (Nworgu, 2007). In Nigeria, consumption of animal protein remains low at about 6.0 – 8.4 g/head/day which are far below the 13.5 g/day prescribed by the WHO (Egbunike, 1997). Rabbit production is a veritable way of alleviating animal protein deficiency in Nigeria (Ajala & Balogun, 2004).

2.2.Taxonomy

Taxonomy is process of classifying organisms according to their biological relationship (i.e. similarities, differences) from Kingdom up to Species level.

Kingdom: Animalia

Phylum: Chodata

Class: Mammalia

Order: Lagomorpha

Family: Leporidae

Genus: *Oryctolagus*

Species: *O. cuniculus*

Sources: (Linnaeus, 1758)

2.3. Breed of Rabbits

According to the United States Department for Agriculture (USDA) (1972), rabbit has been classified according to size, weight and type of pelt. Small rabbits weigh about 1.4 – 2 kg at maturity, medium breeds 4 – 5.4 kg and large breeds 6.4 – 7.3 kg. Based on this classification, two most popular breeds for meat production include the New Zealand White and the Californian. These breeds are most popular because they combine white fur and good growth characteristics. The New Zealand rabbits are slightly larger than the Californian, 4 – 5.9 kg and 3.6 – 4.5 kg respectively. The New Zealand rabbit has a completely white, red or black body, whereas the Californian is white with colored nose, ears and feet. The two most popular rabbits for fur production are the Rex and the American Chinchilla. The Rex is slightly smaller than the American Chinchilla, 3.2 kg versus 4.5 kg (USDA, 1972).

In Nigeria, the commonest breeds include the New Zealand White, Californian, Angora, Rex amongst others (Aduku & Olukosi, 1990). All of these breeds of domestic rabbits are descendants of the European wild rabbit, *Oryctolagus cuniculus* (Aduku & Olukosi, 1990). Although many of these are breeding successfully in various countries, the most popular breeds are the New Zealand White and the Californians. These two breeds are also the most popular in commercial rabbit industries in the developed countries. The various production traits such as fertility, growth and feed conversion rates when considered, under commercial conditions, New Zealand Whites and Californians are amongst the better breeds available for meat production (Bombeke *et al*., 1975).

2.4 Housing rabbits

Housing constitutes an important factor for a successful rabbit production. Housing types include the cages, pens, paddocks, underground and insulated housing. They are made from locally sourced raw materials such as bamboo and rabbits kept on free range make for themselves houses in holes. In many rural areas of developing countries, the materials for building cages and insulated housing and electricity to power fans and ventilators that are used in commercial rabbitries in the developed countries are often not available. When available the cost may be too high to justify their use in anything but highly developed commercial rabbit industries. It is possible, however, to construct rabbit housing from locally available materials such as old packing cases, intermeshed branches or bamboo strips (Action for Food Production, 1974), or local hard wood or bamboo-like material (Owen *et al*., 2008). Housing made of wood would have to be renewed more regularly due to gnawing than that constructed from wire for example. Rabbit housing in the tropics should be designed and situated to keep the rabbits as cool and quiet as possible, to keep out predators such as python and cats, and to keep out dogs and children that may disturb the rabbit leading to general unthriftiness (Owen *et al*., 2008). Rabbit housing should be built under trees or such natural shelter as exists and, if possible, sited to take advantage of breezes (Bewg, 1974). Flooring can be made of hard bamboo-like material slatted together. Bamboo flooring of this type is recommended for adult rabbits only, as young rabbits tend to slip on the smooth slates and can cause leg deform. Splitting and weaving bamboo strips into a mat provides much better footing for all rabbits. The outer surface of the bamboo should face upwards in order to minimize damage from gnawing and to facilitate cleaning (Owen *et al*., 2008). Nesting boxes can be constructed from thinner material or even from clay. Wire has many advantages when used for rabbit housing, especially for floors and the fronts of cages. It should be noted, however, that this material are prone to rapid rusting in warm humid climates if not galvanized or if the galvanized coating is damaged. Possibly its most important use would be in making ties (Ministry of Agriculture Fisheries and Food, 1973).

For water supply, sprays and the installation of sprinklers in the rabittary is very useful. In arid areas where the water supply is restricted, the construction of underground compartments with inspection hutches could be important (Templeton, 1968). This would assist in keeping rabbits cool in hot climates though, would be relatively difficult to clean and may lead to increased parasites or disease spread. Therefore, this type of housing has been discouraged in Nigeria.

2.5 Feeding of Rabbits

Feeding of the rabbit can be made to be either cheap or expensive depending on the farmer (Kalaba, 2012). According to Schiere (2004), the main feed of the rabbit can be prepared at no cost using roadside grass, kitchen and garden waste, although supplementation with grains and concentrates will prove beneficial and enhance growth rates. Utilizing dried tomatoes pomace in feeding rabbits up to 20% level has proven efficient and safe with no adverse effect on the animal performance and carcass qualities (Sayed & Abdul-Azeem, 2009). Feeding of rabbits can be done utilizing garden waste (e.g. cabbage leaves, carrot and bananas) and kitchen waste from home or nearby restaurants (Kalaba, 2012), although using garden waste should be done carefully because of the herbicides/pesticide residues used during cultivation (Schiere, 2004). Normally rabbits should be given free access to clean water daily (Kalaba, 2012). The daily requirement of a doe and her litter is 3.79 liters of water in a warm condition (Shaeffer & Harper, 2008). Generally, the feeding material depending on the locality may vary greatly. Feeds normally given in tropical Africa include; grasses such as guinea grass (*Pinicum maximum*) and stargrass (*Cynodon dactylon*); legumes include Kudzu (*Pueraria phaseoloides*), groundnut haulms and cowpea haulms; the root crops include sweet potatoes leaves, and cassava chips; and herbs like *Tridax procumbens*, *Euphorbia* and *Aspilla* (Aduku & Olukosi, 1990).

Rabbits may be maintained solely on green feeds together with household vegetable waste. However, careful management and balancing of diets is necessary (Aduku & Olukosi, 1990). The two most common deficiencies encountered in such diets are of energy and protein rather than minerals or vitamins. Although, the rabbit is by nature herbivorous, growth rates on forage based diets containing high fiber levels will be increasingly curtailed with increasing fiber level. This is due to the animal's inability to obtain sufficient digestible material to satisfy its energy demands. The nature of the fibrous components is also important; the greater the degree of lignification, the greater the reduction in the digestibility (Mailafia *et al*., 2010). In general, tropical species of grasses are less digestible than temperate species at the same stage of maturity and are often of low protein content (Mailafia *et al.,* 2010). The feeding of forage legumes, cut at an early stage, could help to increase protein supply. Alternatively, a protein supplement may be provided, such as vegetable oil seeds or oil seed residues (Blattachary & Taylor, 1975).

2.6 Hematological Components and their Functions

Blood which is a vital special circulatory tissue is composed of cells suspended in a fluid intercellular substance (plasma) with the major function of maintaining homeostasis (Isaac *et al.*, 2013). Hematological components, which consist of red blood cells, white blood cells or leucocytes, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration are valuable in monitoring feed toxicity especially with feed constituents that affect the blood as well as the health status of farm animals (Oyawoye & Ogunkunle, 2004). Red blood cells (erythrocytes) serve as a carrier of haemoglobin. It is this haemoglobin that reacts with oxygen carried in the blood to form oxyhemoglobin during respiration (Johnston & Morris, 1996; Chineke *et al.,* 2006). According to Isaac *et al.* (2013) red blood cell is involved in the transport of oxygen and carbon dioxide in the body. Thus, a reduced red blood cell count implies a reduction in the level of oxygen that would be carried to the tissues as well as the level of carbon dioxide returned to the lungs (Ugwuene, 2011; Soetan *et al.,* 2013; Isaac *et al*, 2013). The major functions of the white blood cell and its differentials are to fight infections, defend the body by phagocytosis against invasion by foreign organisms and to produce or at least transport and distribute antibodies in immune response. Thus, animals with low white blood cells are exposed to high risk of disease infection, while those with high counts are capable of generating antibodies in the process of phagocytosis and have high degree of resistance to diseases (Soetan *et al*., 2013) and enhance adaptability to local environmental and disease prevalent conditions (Kabir *et al.,* 2011; Okunlola *et al.,* 2012; Iwuji & Herbert, 2012; Isaac *et al.,* 2013).

Blood platelets are implicated in blood clotting. Low platelet concentration suggests that the process of clot-formation (blood clotting) will be prolonged resulting in excessive loss of blood in the case of injury. Packed Cell Volume (PCV) which is also known as hematocrit (Ht or Hct) or erythrocyte volume fraction (EVF), is the percentage (%) of red blood cells in blood (Purves *et al.,* 2003). According to Isaac *et al.* (2013) packed cell volume is involved in the transport of oxygen and absorbed nutrients. Increased packed cell volume shows a better transportation and thus results in an increased primary and secondary polycythemia. Haemoglobin is the iron-containing oxygen-transport metalloprotein in the red blood cells of all vertebrates (Maton *et al.*, 1993) with the exception of the fish family, channichthyldae (Sidell & O'Brien, 2006) as well as tissues of invertebrates. Hemoglobin has the physiological function of transporting oxygen to tissues of the animal for oxidation of ingested food so as to release energy for the other body functions as well as transport carbon dioxide out of the body of animals (Ugwuene, 2011; Omiyale *et al.,* 2012; Soetan *et al.,* 2013; Isaac *et al.,* 2013).

According to Peters *et al.* (2011), previous reports stated that packed cell volume, hemoglobin and mean corpuscular hemoglobin are major indices for evaluating circulatory erythrocytes, and are significant in the diagnosis of anemia and also serve as useful indices of the bone marrow capacity to produce red blood cells as in mammals (Awodi *et al.,* 2005; Chineke *et al.,* 2006). Furthermore, Chineke *et al.* (2006) posited that high PCV reading indicated either an increase in number of RBCs or reduction in circulating plasma volume. Mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration indicate blood level conditions. A low level is an indication of anemia (Aster, 2004).

2.6.1 Factors Influencing Hematological Parameters of Farm Animals

The genetic and non-genetic factors affecting haematological parameters of farm animals have been observed (Agaie & Uko, 1998; Kleinbeck & McGlorie, 1999; Svoboda *et al.*, 2005; Xie *et al.*, 2013). Several factors including physiological (Alodan & Mashaly, 1999), environmental condition (Vecerek *et al.*, 2002; Graczyk *et al.*, 2003), dietary content (Odunsi *et al.*, 1999; Yeong, 1999; Kurtoğlu *et al.*, 2005; Iheukwumere & Herbert, 2002), fasting (Lamošová *et al.*, 2004), age (Forlan *et al.*, 1999; Seiser *et al.*, 2000), administration of drugs (Khan *et al.*, 1994), anti-aflatoxin treatment (Oguz *et al.*, 2000) and continuous supplementation of vitamin (Tras *et al.*, 2000) affect the blood profile of healthy animal. Swenson (1970); Addass *et al.* (2012) also observed that factors such as age, nutrition, health of the animal, degree of physical activity, sex and environmental factors affect blood values of animals. According to Daramola *et al.* (2005), age and sex of farm animals affect haematological parameters. Schalm *et al.* (1975) reported that blood pictures of animals might be influenced by certain factors such as nutrition, management, breeds of animal, sex, age, diseases and stress factors. Dukes (1955); Afolabi (2010) posited that hematological values of farm animals are influenced by age, sex, breed, climate, geographical location, season, day length, time of day, nutritional status, life habit of species, present status of individual and other factors. Carlson (1996); Johnston & Morris (1996) reported that besides physiological and environmental factor that might affect blood values as: age of the animal, factors such as oestrus cycle, pregnancy and parturition, genetics, method of breeding, breeds of animal, housing, feeding, fasting, extreme climatic conditions, stress, exercises, transport, castration and diseases have been identified.

2.7 Source of Energy

2.7.1 Maize

Livestock consume more than a third of all the world's grains particularly maize (Thompson & Weber, 1981). Maize has remained the chief source of dietary energy in compound feed and constitutes about 50 – 60% in most poultry nutrition. Despite its worldwide production, a stiff competition for the usage of maize by humans, livestock and industries persist. This is simply because, maize is high in energy and forms the standard (100) against which other cereals grains is compared (Atteh, 2002). Maize has a fat content of about 4% and this fat is high in linolleic acid (about 50%) making it an excellent source of this essential fatty acid. Olomu (1995) reported that metabolizable energy (ME) and crude protein (CP) of maize stand at 3510 kcal/kg and 8.80% respectively. The ever-increasing competition between man and animals for available grains (Tegbe *et al*., 1984; Egbunike *et al.*, 2002), the inadequate production of farm crop to meet the needs of man and his livestock (Babatunde *et al*., 1990), and ever-increasing cost of maize had made it necessary to critically re-evaluate some other grains like sorghum, millet and for alternative energy source in livestock production.

2.7.2 Millet

Millet grain could potentially be successfully incorporated into animal diets. The protein content of millet, although variable, is higher than maize and the essential amino acid profile is more balanced than maize ((Burton *et al*., 1972; Adeola & Rogler, 1994; Amato & Forrester, 1995). Millet also has higher oil content than other common cereal grains (Rooney, 1978; Hill & Hanna, 1990; Adeola & Rogler, 1994) and is a better source of linolenic acid (Rooney, 1978). At has also been indicated that millet is superior to maize and sorghum in protein content and quality, as well as protein efficiency ratio (PER) values. According to Olomu (1995), millet has a lower metabolizable energy (2555 kcal/kg), but higher percent crude fibre (4.30), ash (3.00) and crude protein (12.0). Millet does not contain any condensed polyphenols such as the tannins in sorghum that can interfere with or slow down digestibility.

Millet is native to the western edges of the Sahara Desert. The agronomic characteristics that allow millet to be successfully grown in the Sahelian region of West Africa would allow it to grow well in the coastal plain of the southern United States where the soils are also acidic and low in natural fertility, and droughts are common. In addition, millet matures quickly, which makes it a potentially ideal component of traditional double cropping and rotational cropping systems.

2.7.3 Sorghum

Sorghum can be successfully grown on poorer soils and in drier conditions than maize. Olomu (1995) gave the Metabolizable Energy and percent crude protein as 3270 kcal/kg and 9.5% respectively. The percent ash 1.20% and fibre 2.70% are higher than that of maize. Millet and sorghum are relatively cheaper and easy to cultivate than maize. However, an in-depth understanding of the nutrochemical characteristics of varieties of millet (white and red millet) and sorghum (white and red sorghum) with their consequent effects on performance of broiler chickens would ensure a more judicious utilization of these ingredients, hence there may be a fall in demand for maize which may eventually bring down its price. Against the foregoing, there is paucity of information on the nutritional values of different varieties of millet and sorghum.

2.8 Rabbit Slaughter and Processing

One of the important aspects of any rabbit meat production enterprise is the efficient and hygienic slaughter and handling of the animals and the hygienic handling of the carcasses. This applies to both the large-scale commercial enterprises and small-scale enterprises alike. Problems concerning these aspects are common to meat production from all types of livestock in developing countries, particularly in rural areas. The relatively small size of rabbit presents advantages of easy transportation and consumption is easier by a few people (Aduku & Olukosi, 1990). Rabbits should be starved for about six to ten hours before slaughter, to empty the gut as much as possible. They should be well watered during this period to prevent dehydration and weight loss especially in warm weather. It is better to slaughter rabbits in an area fenced or walled off from other people and domestic animals such as dogs. It is also preferable to have a roof of some kind over the area and a water supply for cleaning purposes (Aduku & Olukosi, 1990).

Rabbits are best slaughtered by stunning or dislocating the neck before severing the head at the atlas joint using a sharp knife. The hind legs should be held firmly in the left hand, with the right hand holding the rabbit's head directly behind the ears. Pulling sharply on the head with a downward and backward twist of the hand will effectively break the neck. This operation should be followed immediately by bleeding, which is best carried out by severing the head with a knife in one smooth cut (Aduku & Olukosi, 1990).

Rabbit carcasses are mostly consumed fresh; where this is not the case, processing and preservation becomes necessary. Facilities such as refrigeration are not always available in rural areas of developing countries or where available, power supply may be inefficient, so in these areas traditional methods of processing have to be considered. The oldest and most widely used methods of processing and preservation of meat in rural communities include drying, smoking and sometimes salting (Pellett & Miller, 1963).

2.9 Problems of Rabbit Production in Nigeria

2.9.1Rabbit feed and feed ingredients

Apart from unfavorable climate, infectious and non-infectious diseases inadequate feed and feed ingredients constitute parts of major impediments to efficient rabbit production in Nigeria (Mailafia *et al.,* 2010). In spite of the exceptional attributes and advantages of keeping rabbits, its production in Nigeria is still comparatively rudimentary (Onifade *et al.*, 1999). One of the major problems of rabbit production in Nigeria is high cost of concentrates, relatively smaller weight gain during the dry season, non-readily available market when the farmers are ready to sell their stock and inadequate knowledge and information about the advantages of eating rabbit meat (Nworgu, 2006). Also, energy source sometimes is the limiting factor in successful rabbit production especially maize in which there is competition between the human and animals. In view of this it becomes necessary to find out alternative source of energy such as millet and sorghum for the rabbit.

2.10 Prospects of Rabbit Production in Nigeria

2.10.1 Rabbit skin

Rabbit skins, when properly processed and made into other products, are very attractive and command a high price. While it is not suggested that the production of fully processed skins is within the capacity of small-scale breeders, dried or salted skins can easily be produced and, if properly handled during their preparation, they can give a good return (Aduku & Olukosi, 1990). The first essential with all fur skin, whether from a rabbit or any other animal, is to prevent putrefaction from taking place. Unless checked, decay will set in within a few hours of flaying and one of the first results, loss of hair, makes the skin totally unsuitable for use in fur garments. Therefore, all skins should be cured or preserved properly immediately after flaying, using salting or sun drying (or both) and simple rules will ensure that a top quality cured skin is produced.

2.10.2. Rabbit manure

Rabbit manure is very useful, its dry matter content being about twice that of horse manure and over three times that of dairy cow manure under conventional farming systems (Sandford, 1979). Moreover, rabbit manure is relatively rich in phosphorous and nitrogen when compared to the manure of other livestock on a dry matter basis (Casady, 1975).

2.10.3 Marketing

The single most important step before building a rabittary or starting a commercial rabbit production is to develop a market for the rabbits. In most cases, producers must develop their own markets. Rabbits produced for their meat must have good loins, shoulders, hips and pelts. The rabbit meat industry will not buy unhealthy rabbits; therefore, rabbit producers must furnish healthy, high-quality, disease free rabbits to the processors. It is therefore advisable to engage the services of a veterinarian at every stage of the production process (Mailafia *et al.*, 2010).

3.0 MATERIALS AND METHODS

3.1Location and Duration of the Study

The study was carried out at the Laboratory Animal Unit of the Veterinary Teaching Hospital, Usmanu Danfodiyo University Sokoto, Sokoto State, Nigeria. The State is located in the North-West political zone of Nigeria. The National Population Commission reported that the State is between longitude 4°8E and 6°54E, and latitude 12°N and 13°58N. The State is bordered by Niger republic to the north, Kebbi state to the west and Zamfara state to the East (NPC, 2006). The State is made up of 23 Local Government Areas. The State has a total land area of 25,937 square kilometer, with an estimated population of 3,696,999 people (NPC, 2006).

The study was undertaken from the period of 11th July, 2019 to 15th August, 2019, a time span of five weeks i.e. 35 days.

3.2 Experimental Animals

Eighteen young mixed rabbit breeds (Dutch black and New Zealand White) made up of 13 New Zealand White and 5 Dutch black aged six to seven weeks old were used for this study. The rabbits were purchased from Katsina and some from Sokoto markets, they were divided into 3 groups of 6 rabbits per group and housed according to their groups. The rabbits were raised on deep litter, and metallic feeders and plastic drinkers were provided. The rabbits were allowed to acclimatize to the environment for seven days before commencing the study. These animals were given ivermectin injection subcutaneously to treat both external and internal parasite that may affect the growth of the animals.

3.3 Collection of Feed Ingredients

White maize, white millet, red sorghum, fish meal, soya bean meal, wheat bran, bone meal, vital premix and salt were purchased from the market.

3.4 Experimental Diet

The feed was formulated and compounded using three different sources of energy (white maize, white millet and red sorghum) in the diets 1, 2 and 3 respectively. White millet and red sorghum were used as test ingredient by completely replacing maize (in control diet) in diets 2 and 3 respectively.

Table 2: Composition of the experimental diets

Ingredients	Group A Level (kg)	Group B Level (kg)	Group C Level (kg)

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Maize	52.21	-	-
Sorghum	-	52.21	-
Millet	-	-	52.21
Fish meal	4.45	4.45	4.45
Soya bean meal	13.34	13.34	13.34
Wheat bran	26	26	26
Bone meal	3	3	3
Salt	0.5	0.5	0.5
Premix	0.5	0.5	0.5
Total	100	100	100
CP (%)	17.38	18.47	19.58
ME (kcal/kg)	2569.48	2477.59	2571.34

CP = Crude protein, ME = Metabolizable energy

3.5 Materials

- Micro-hematocrit centrifuge machine
- Micro-hematocrit reader
- Hematocrit tube
- Micropipette
- Test tube
- Microscope
- Test tube rake
- Hematocrit reader
- Neabauer counting chamber
- EDTA bottle
- Plane bottle
- Digital colorimeter
- Cuvette
- Glass slide
- Cover slip
- Plasticine
- Cotton wool

3.6 Blood Collection and Analysis

At the end of the feeding period (5 weeks), three rabbits were randomly sampled from each group and blood was collected from the ear vein of each rabbit using a sterilized disposable syringe and needle (5 ml syringe with 21gauge needle). An initial 2 ml blood was collected into labelled sterile vacuum tube containing ethylene-diamine-tetra-acetic acid (EDTA) as anticoagulant which was used for hematological analysis. Another 3 ml of blood was collected

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into labelled sterile sample bottles without anticoagulant and used for the serum biochemical analysis.

The red blood cell (RBC) counts, total white blood cell (WBC) counts, hemoglobin (Hb) concentration and packed cell volume (PCV) parameters were determined as describe in Ewuola & Egbunike (2008). Blood constants mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) were determined using appropriate formulae as follows:

Mean corpuscular volume (MCV) = PCV × 10/RBC

Mean corpuscular hemoglobin (MCH) = Hb × 10/RBC

Mean corpuscular hemoglobin concentration = RBC × 100/PCV

3.6.1 Procedure

3.6.1.1 Erythrocyte count

The counting of erythrocytes was carried out in a standard hemacytometer (Model-JSQA) following the method of Svobodava *et al.*, (2003). The 4 ml of Haymen's Fluid was taken into the test tube plus 0.02 ml of blood, Red blood cells were counted in 5 out of 25 small squares which contained 16 smaller squares of $1/400mm^3$ size as contained in improved Neubauer counting chamber.

3.6.1.2 Hematocrit (PCV) determination

Blood filled 2/3 of heparinized micro-hematocrit capillary tube and the tube was centrifuged at12000 rounds per minute for 5 minutes, using a micro hematocrit reader, the Percentage of the hematocrit mean was recorded.

3.6.1.3 Hemoglobin estimation

The hemoglobin concentration was measured by the cyan-methemoglobin method using the method of Wickham *et al.*, (1990). About 0.02 ml of blood was mixed with 5 ml of Drabkins reagent (20 mg Potassium ferricynide; 50 mg Potassium cyanide and 11ml of distilled water). Few minutes was allowed for hemoglobin to be oxidized by Potassium ferricynide to methemoglobin and be fully converted to cyanmethemoglobin by Potassium cyanide. Thereafter, the absorbance at 540 nm wavelenght of the cyanmethaemoglbin equivalent to the maximum hemoglobin was read off using a standard colorimeter.

3.6.1.4 Leucocyte count

About 0.02 ml of blood was dissolved in 0.32 ml of Tureks solution. White blood cells were counted 4 out of 16 squares in the Neubauer chamber of a standard hemacytometer.

3.6.1.5 Differential count of WBC sub population

The differential count of leucocyte sub-populations was estimated using the method of Svobodava *et al.*, (2003). A drop of blood was smeared on a glass slide and allowed to dry. Blood cells were then stained with leishman stain. Differential cells of leucocyte were counted with the low power of microscope.

Biuret method of serum total protein determination was employed in this assay as described by Kohn & Allen (1995). Liver function test (i.e. alkaline phosphates (ALP), alanine amino transferase (ALT), aspartate amino transferase activity (AST) were measured using the colorimetric method of Reitman & Frankel (1957), and total protein (TP), albumin was determined using Bromocresol Green (BCG) method as described by Peter *et al*., (1982). Total bilirubin and direct bilirubin were determined using spectrophotometric method. Also, the creatinine and blood urea nitrogen was determined by biuret reaction (Meyer *et al*., 1992).

3.7 Statistical Method

Results were subjected to one-way Analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS Version 17) to determine significant difference between the three groups.

4.0 RESULTS

4.1 Hematological Analysis of Rabbits Fed with Three Different Energy Sources

As shown in Table 3, the dietary treatments had no significant (P>0.05) effect on the hematology of experimental rabbits and were all found to be within normal ranges, except RBC which was observed to be lower in all groups. MCHC was below normal in groups A and C, while Hb and MCV were also lower than normal for groups C. in addition the neutrophils of rabbits under groups A and C were subnormal. However, rabbits under group A had the highest values for PCV (37.67%), Hb (10.34 g/dl), MCV (94.33 fl) and neutrophils (25.00%), with lowest values for MCHC (27.45 g/dl) and lymphocyte (74%). Group B rabbits had the highest MCH (28.46 pg), MCHC (30.39 g/dl), lymphocyte (83.00%) and monocyte (2.00%), while it had the lowest readings for RBC (3.52 $\times10^6/mm^3$), WBC (5.73 $\times10^3/mm^3$) and neutrophils (15.00%). On the other hand, rabbits in group C were highest for RBC (4.61 $\times10^6/mm^3$) and eosinophils (1.00%), while least for Hb (9.72 g/dl), MCV (71.54 fl) and MCH (21.08 pg). A PCV of 33.00% was recorded for both groups B and C.

Table 3 Mean hematology of experimental rabbits fed three different energy sources

Parameters	Group A	Group B	Group C	SEM	Ref. ranges
PCV (%)	37.67	33.00	33.00	1.56	30 – 50
Hb (g/dl)	10.34	10.03	9.72	0.18	10 – 15
RBC ($\times10^6/mm^3$)	3.99	3.52	4.61	0.32	*5 – 8
MCV (fl)	94.33	93.67	71.54	7.49	78 – 95
MCH (pg)	25.89	28.46	21.08	2.17	#19 – 22
MCHC (g/dl)	27.45	30.39	29.46	0.87	#30 – 35
WBC ($\times10^3/mm^3$)	8.75	5.73	8.30	0.94	4.5 – 11
Neutrophils (%)	25.00	15.00	20.00	2.89	!22 – 38
Lymphocytes (%)	74.00	83.00	78.00	2.60	40 – 80
Monocytes (%)	1.00	2.00	1.00	0.33	1 – 4
Eosinophils (%)	0.00	0.00	1.00	0.33	0 – 4
Basophils (%)	0.00	0.00	0.00	0.00	1 – 7

Reference range: Adapted from: Research Animal Resources [RAR] (2009) cited by Etim *et al.*, (2014). Nse Abasi N. Etim, Mary E. Williams, Uduak Akpabio and Edem E. A. Offiong. (2014). Haematological Parameters and Factors Affecting Their Values**.** *Agricultural Science*, 2 (1): 37-47.

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#Ref. ranges obtained from Medirabbit.com (http://www.medirabbit.com/EN/haematology/bloodchemistry.htm)

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4.2Serum Biochemical Analysis of Experimental Rabbits Fed Three Different Energy Sources

Table 4 shows the result for serum biochemical analysis of rabbits fed three different energy sources. This feeding trials had no significant (P>0.05) effect on the serum biochemistry of experimental rabbits. It was however observed that group C had the least creatinine (1.63 mg/dl), total protein (6.00 g/dl), albumin (3.27 g/dl) and AST (45.57 U/L) and highest values for direct bilirubin (0.14 mg/dl), ALT (55.57 U/L) and AST (218.57 U/L). Urea (3.76 mmol/l), ALT (44.90 U/L) and ALP (182.67 U/L) were least in group B, while total bilirubin (0.98 mg/dl) and AST (62.03 U/L) were highest in this group. Group A had the lowest total bilirubin (0.88 mg/dl) and highest creatinine (1.67 mg/dl) and albumin (3.67 g/dl).

Table 4 Mean serum biochemical analysis of experimental rabbits fed three different energy sources

Parameters	Group A	Group B	Group C	SEM	Ref. ranges
Creatinine (mg/dl)	1.67	1.66	1.63	0.01	0.5 – 2.2
Urea (mmol/l)	4.63	3.76	4.63	0.29	
Total Protein (g/dl)	6.10	6.10	6.00	0.03	*5.4 – 7.5
Albumin (g/dl)	3.67	3.46	3.27	0.12	#2.43 – 4.5
Total Bilirubin (mg/dl)	0.88	0.98	0.92	0.03	0 – 1
Direct Bilirubin (mg/dl)	0.11	0.11	0.14	0.01	
AST (U/L)	52.57	62.03	45.57	4.77	*35 – 130
ALT (U/L)	55.33	44.90	55.57	3.52	*45 – 80
ALP (U/L)	202.00	182.67	218.57	10.37	4 – 20

*A. Melillo (2007). Rabbit Clinical Pathology. Journal of Exotic Pet Medicine, 16: 135-145.

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5.0 DISCUSSION AND CONCLUSION

5.1 Hematology

Hematology refers to the numerical and morphological study of the cellular elements of the blood, as well as the usage of these results in the diagnosis and monitoring of disease (Merck Manual, 2012). Moreover, hematological parameters are good markers of the physiological status of animals (Khan & Zafar, 2005). This study revealed that all experimental diets had no significant effect on the hematology of experimental rabbits. This is an indication that neither the maize, sorghum nor millet had at 52.21% had any negative impact on the hemopoietic centers of rabbit. Nevertheless, the red blood cells in all treatments fell below the normal range as reported by Merck Sharp & Dohme (2011), this may have been attributed to incidence of mange encountered in all the animals at the early stage of the experiment. It was noted that MCV and MCHC of rabbits fed millet-based diet were non-significantly below normal, this may be due to reduced or lack of dietary iron.

White blood cell counts are a rough indication of immune status of an animal. It was observed that the WBC of all experimental rabbits were within normal range and it signifies that both the maize, sorghum and millet had no depressing effect on the immune response of rabbits. However, the neutrophils and animals under groups B (sorghum) and C (millet) fell below normal, although not significant. This may be as a result of persistent or overwhelming bacterial infection.

5.2 Serum Biochemistry

The serum biochemical analysis and especially the liver function test of experimental rabbits fed three different energy sources (maize: group A; sorghum: group B and millet: group C) were within normal ranges except alkaline phosphatase which is above normal range. This may be as a result of gall stone and this signifies that our test materials were not toxic to the muscle, kidney and liver of rabbit at 52.21% inclusion level.

5.3 Conclusion and Recommendation

This study revealed that, replacing maize with sorghum or millet in rabbit diet had no negative effect on the hematological and serum biochemical parameters of rabbits.

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